Geoderma 397 (2021) 115099

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Arbuscular mycorrhizal traits are good indicators of soil multifunctionality in drylands

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ARTICLE INFO

Handling Editor: Matthew Tighe

Keywords:
Arbuscular mycorrhizal fungi
Drylands
Native plant species
Soil microbial communities
Soil multifunctionality
Soil organic matter

ABSTRACT

Drylands are highly susceptible to degradation and climate change, which has important ecological and socio-economic consequences worldwide. To halt drylands degradation, plant species selection for restoration is starting to include also a functional approach, but does not integrate belowground functional traits yet. Therefore we tested the use of mycorrhizal traits to identify native plant species which host guilds of beneficial microbes and therefore enhance multiple soil functions simultaneously – soil multifunctionality. We used a soil organic matter (SOM) gradient (0.9–1.9%) and evaluated the effect of 14 common and abundant native herbaceous plant species (+bare soil) on soil functionality. We measured several soil functions (soil microbial biomass, metabolic quotient, and enzymatic activities – dehydrogenase, β-glucosidase and phosphatase) and built a soil multifunctionality index. Soil multifunctionality was strongly associated with mycorrhizal traits across the analysed SOM gradient. Bare soils and soils under non- or low-mycorrhizal plant species displayed the lower soil functionality (both individual functions and multifunctionality), while soils under Fabaceae species (*Medicago truncatula*, *Astragalus corrugatus* and *Lotus halophilus*) displayed the highest. For each plant species, the highest soil multifunctionality was observed at the SOM-richer site. Soil multifunctionality was strongly associated with all the mycorrhizal traits but mycorrhizal intensity and AMF spores abundance were more correlated with soil multifunctionality than mycorrhizal frequency. Our data show that: i) AM traits can be good indicators of simultaneous multiple soil functions in drylands; and ii) soil multifunctionality in drylands can be improved by management practices promoting SOM accumulation and favouring specific native plant species.

1. Introduction

Drylands, which include dry sub-humid, semiarid, arid and hyper-arid areas, cover about 40% of the Earth land surface (Maestre et al., 2012b). Besides hosting 38% of the global human population, drylands also host c.a. 20% of plant and 30% of bird biodiversity hotspots (Myers et al., 2000), while supporting 50% of the world's livestock (James et al., 2013). Further, faced with a global need to sequester more carbon, drylands may store up to 45% of the global terrestrial carbon (MEA, 2005b), further prioritising soil conservation in these areas. Despite their major regional and global importance, drylands are among the most susceptible biomes to land degradation and climate change (Maestre et al., 2012b) due to their characteristic low and variable rainfall and poor soils (Reynolds et al., 2007). Increasing grazing intensity, and changes in climate and land-use contribute to forest degradation, and lead to the regression and extinction of many dryland pasture and forage species (Martinez-Garcia et al., 2012). In 2005, > 10% of the drylands around the world were considered as degraded (MEA, 2005a) and another 12 million hectares are being degraded each year (James et al., 2013), which has important economic, ecological and social consequences. International programs widely recognize dryland restoration as instrumental to combat global dryland degradation and

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https://doi.org/10.1016/j.geoderma.2021.115099
Received 12 August 2020; Received in revised form 15 December 2020; Accepted 14 March 2021

ensure global sustainability (James et al., 2013).

As vegetation cover in drylands is a key variable to control degradation and desertification (Assouline et al., 2015), plant species selection is a critical step for improving restoration success (Vallejo et al., 2012). Restoration programs often make use of fast-growing commercial plant species, which under dryland conditions produce undesirable results (Bochet et al., 2010b) as most of the sowed commercial species disappear after the first growing season, and in unfavourable dry years these commercial plant species do not survive (Bochet et al., 2010a). Thus, native species are an attractive alternative to improve restoration success in drylands as they further contribute to local biodiversity conservation, the existence of ecotypes adapted to specific environmental conditions, the provision of compatible habitats for other native plants and animals, and the enhancement of natural colonization (Bochet et al., 2010b).

Besides the taxonomic diversity concerns (e.g. commercial *versus* native plant species), in the case of the Mediterranean Basin, the most successful restoration programs are those integrating also a functional diversity approach (Nunes et al., 2016). Although aboveground functional traits are widely considered in ecological restoration, the integration of belowground functional traits is still lacking though needed to better predict changes in plant biodiversity and consequently in ecosystem functioning (Laliberté, 2017). Further, apart from vegetation changes, belowground functional networks also need attention since, in most cases, ecological degradation starts with their disruption (Dias et al., 2017).

Plants interact with guilds of belowground functional groups (including beneficial microbes) living in their roots and the surrounding soil. These microbes establish the plant microbiome, modulating plant phenotype and consequently, plant fitness and ecosystem functioning (Smith and Read, 1997; van der Heijden et al., 2015). Mycorrhiza are probably one of the better known belowground functional groups associated with plants, and one of the proposed belowground functional traits to understand ecosystem-level consequences of plant traits (Laliberté, 2017). Arbuscular mycorrhiza (AM) are generally mutualistic, as soil resources and other benefits are traded for photosynthates (Smith and Read, 1997, 2008). Besides the well-known improvement in plant nutrition (Dias et al., 2015, 2018), other examples of AMF benefits to the host plant include pathogen suppression, pollination enhancement, herbivore protection and improved water relations (Verbruggen and Kiers, 2010). Therefore, AMF play a crucial role in terrestrial ecosystems functioning, especially in drylands (Mahmoudi et al., 2019, 2020).

Our objective was to test the use of mycorrhizal traits to identify native plant species which host guilds of beneficial microbes and therefore enhance multiple ecosystem soil functions simultaneously – soil multifunctionality (Delgado-Baquerizo et al., 2016; Maestre et al., 2012a). In drylands, where soil organic matter (SOM) is low (Cruz et al., 2008), plants constitute a source of organic carbon (up to 20–50% of the photosynthates are exuded through plant roots) for the soil microbes. As 80% of terrestrial plants establish AM (Smith and Read, 1997, 2008), AMF are a main recipient of the organic carbon, which is allocated to the roots through its intraradical structures. Similarly to plant roots, AMF hyphae are leaky and release primary metabolites into the hyphosphere (the soil volume influenced by the AMF hyphae) (Zhang et al., 2014) which selectively promote the development of certain microbes. As a result of this selective soil microbial recruitment (Cabral et al., 2019; Fonseca et al., 2017; Zhang et al., 2014), AMF play a crucial role in building belowground functional networks which modify plant performance and ecosystem functioning (Smith and Read, 1997; van der Heijden et al., 2015). Therefore, we hypothesized that AM traits are good indicators of soil multifunctionality in drylands. We tested our hypothesis by evaluating the effect of fourteen common and abundant native herbaceous plant species (including one non-mycorrhizal plant species) on soil functionality along a SOM gradient. We focused on a SOM gradient because land degradation and climate change can further impoverish dryland soils in SOM, which may constitute a tipping point leading to abrupt and possibly irreversible shifts between alternative ecosystem states, potentially incurring high societal costs (Dakos et al., 2019).

Table 1
Bare soil physical and chemical properties. Different letters show significant differences between sites ($p < 0.05$). Values are the mean ± SE (n = 3).

Parameters	Site 1	Site 2	Site 3	Site 4
Organic matter (%)	1.9 ± 0.2a	1.4 ± 0.1b	1.1 ± 0.3c	0.9 ± 0.1d
Clay (%)	11 ± 0b	9 ± 0c	8 ± 0c	15 ± 0a
Silt (%)	24 ± 2b	9 ± 0c	5 ± 1d	38 ± 3a
Sand (%)	65 ± 7b	82 ± 7a	87 ± 7a	47 ± 3c
pH	8.0 ± 0.1	8.0 ± 0.2	8.3 ± 0.1	8.1 ± 0.1
Electrical conductivity (s. m^{-1})	2.3 ± 0.3a	2.3 ± 0.1a	2.0 ± 0.1ab	1.7 ± 0.2b
Total N (mg kg^{-1})	182 ± 23a	151 ± 15ab	125 ± 10b	90 ± 10c
Phosphorus (mg kg^{-1})	7 ± 0.1b	5 ± 0.2c	8 ± 0.2b	14 ± 0.2a
Calcium carbonate (mg kg^{-1})	5 ± 0.1d	9 ± 0.2b	7 ± 0.1c	10 ± 0.1a

By measuring several soil microbial parameters (soil microbial biomass, metabolic quotient, and enzymatic activities – dehydrogenase, β-glucosidase and phosphatase), we calculated a soil functionality index based on the average approach (soil multifunctionality – Delgado-Baquerizo et al., 2016). We chose these microbial parameters to build our soil multifunctionality index because: i) soil microbial biomass is an integrative indicator of the microbial community; ii) metabolic coefficient is an indicator of the microbial community efficiency in using organic carbon as an energy source (Anderson, 2003); iii) dehydrogenase is an enzyme that occurs in all viable microbial cells and is therefore a measurement of the metabolic state of soil microbes (Jarvan et al., 2014); iv) β-glucosidase is involved in carbon cycling in the limiting step of cellulose degradation (Turner et al., 2002), being predominantly found among plants, animals, fungi, bacteria, and yeasts (Adetunji et al., 2017); and v) phosphatase activity represents a group of enzymes involved in phosphorus cycling, being derived predominantly from plants and microbes (including mycorrhiza) (Adetunji et al., 2017). Studying these microbial parameters, on the soil under the influence of a certain plant, and not only on its rhizosphere or hyphosphere, allowed us to assess structural and functional aspects of the soil microbial community influenced by, but not directly related with, AMF.

2. Materials and methods

2.1. Study area

This study was performed at the Bou-Hedma National Park, in a semi-arid area of Tunisia. The park was founded in 1980 and covers 16,488 ha with distinct degrees of protection (6,000 ha are fully protected). The climate is classified as rain-shadowed Mediterranean arid (Noumi et al., 2016) even in the semi-arid lower fresh variant. According to the records from the Tunisian National Institute of Meteorology (1996–2009), the monthly temperature was lowest in January (3.9 °C) and highest in August (36.2 °C). The mean annual temperature is 17.2 °C, while the mean annual rainfall varies between 100 and 200 mm.

According to the World Reference Base for Soil Resources (http://www.fao.org/soils-portal/data-hub/soil-classification/world-reference-base/en/), soil in the study area belongs to the order Alfisols, suborder Ustalfs and great group Rhodustalfs. Vegetation is mainly dominated by *Acacia tortilis* subsp. *raddiana* associated with several species of grasses and shrubs. Sampling was done in four sites along a SOM gradient (Table 1): three inside the Bou-Hedma National Park and one outside. Site 1 (34.48 N 9.46E; 100–150 m altitude) was an open area near an *Acacia* population, Site 2 (34.49 N 9.59E; 800 m altitude) was

Table 2
List of the sampled plant species in the four sites, respective families and abbreviations.

Family	Plant species	Abbreviation
Asteraceae	*Anacyclus clavatus* (Desf.) Pers.*	*Acla*
	Chrysanthemum coronarium L.*	*Ccor*
	Launaea angustifolia (Desf.) O.Kuntze*	*Lang*
Aizoaceae	*Aizoon canariense* L.*	*Acan*
Brassicaceae	*Diplotaxis simplex* Asch. ex Rohlfs*	*Dsim*
Caryophyllaceae	*Paronychia arabica* (L.) DC.*	*Para*
Fabaceae	*Argyrolobium uniflorum* (Decne.) Jaub. & Spach#	*Auni*
	Astragalus corrugatus Bertol.*	*Acor*
	Lotus halophilus Boiss.et Spruner#	*Lhal*
	Medicago truncatula Gaertn.*	*Mtru*
Malvaceae	*Malva aegyptiaca* L.*	*Maeg*
Plantaginaceae	*Plantago coronopus* L.#	*Pcor*
Polygonaceae	*Emex spinosa* (L.) Campd.*	*Espi*
Xanthorrhoeaceae	*Asphodelus tenuifolius* Cav.#	*Aten*

* indicates an annual plant species while.
# indicates those that are perennials.

located at the mountain summit and Site 3 (34.49 N 9.52E; ≤100 m altitude) was located near a river. The three sites inside the Park were subjected to light grazing (0.025 animal per ha) by Saharan antelopes (*Addax nasomaculatus* and *Oryx leucoryx*) and some ostriches (*Struthio camelus*). The site located outside the Park, Site 4 (34.45 N 9.58E; 100–150 m altitude), was subjected to more intensive grazing by domestic herds of sheep, goats and camels (2 animals per ha) (Abdallah and Chaieb, 2013; Fterich et al., 2012). The study sites were at least 2 km apart from each other.

2.2. Plant and soil sampling

We conducted an initial plant survey to identify the herbaceous plant species that were present in all the four sites along the SOM gradient. The sampling area of the study sites varied between 200 and 400 m^2. Then, in February 2012 (when plants exhibited vegetative growth – data not shown) we sampled fourteen herbaceous plant species (Table 2) that were abundant and common to all four sites: for each plant species, three individual plants were randomly selected and analysed at each site (14 × 3 × 4 = 168 plants). Plants were analysed for their AMF colonization: plant roots were carefully collected to include the fine active roots where mycorrhiza colonization occurs. Soil samples under each individual plant influence were also collected by digging around the root system (up to 20 cm deep). For each site, three soil samples were collected by digging a hole of 10 cm × 10 cm × 20 cm (depth) in an area without vegetation (designated as bare soil). These bare soil samples were used

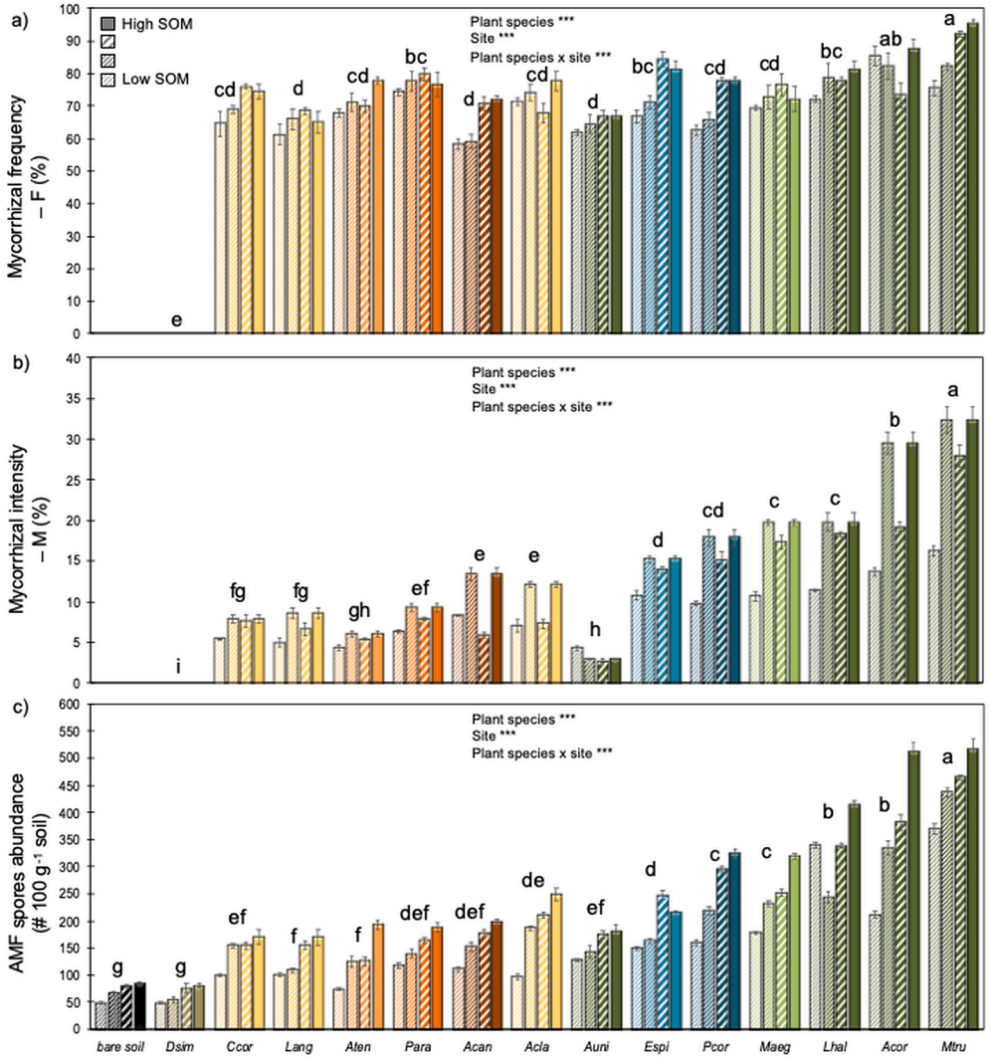

Fig. 1. Effect of plant species (see Table 2) and site on mycorrhizal traits: mycorrhizal frequency (a) and intensity (b), and AMF spores abundance in the soil (c). Plant species were ordered according to soil multifunctionality (Fig. 4) and bars with different colours represent different plant families. *** shows significant effects ($p < 0.01$). Different letters show significant differences between plant species ($p < 0.05$). Bars are the mean ± 1SE (n = 3).

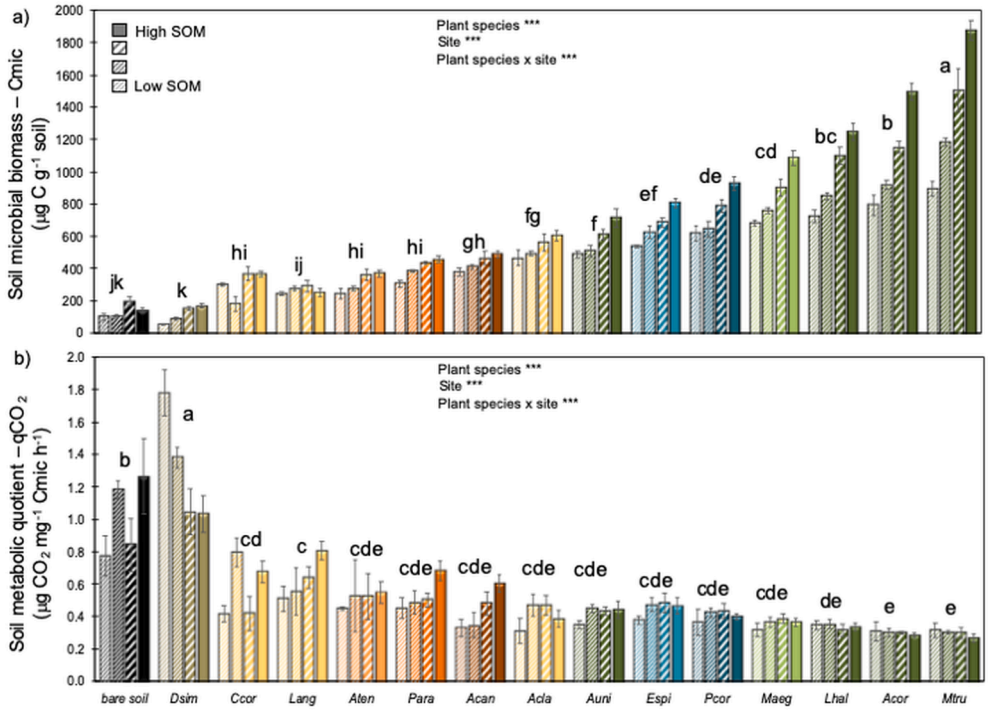

Fig. 2. Effect of plant species (see Table 2) and site on soil microbial biomass (a) and metabolic quotient (b). Plant species were ordered according to soil multifunctionality (Fig. 4) and bars with different colours represent different plant families. *** shows significant effects ($p < 0.01$) and different letters show significant differences between plant species ($p < 0.05$). Bars are the mean ± 1SE ($n = 3$).

as control for the plant influence, and plants degree of mycorrhization, on the dynamic soil characteristics. Soils (bare soil and soil under plants influence) were sieved (2 mm) to remove the plant remains, gravel and earthworms, and stored at 4 °C for further analysis.

2.3. AM fungal colonization status and spore isolation and quantification

Arbuscular mycorrhizal colonization was evaluated by staining 30 root fragments per plant (Phillips and Hayman, 1970): root segments of 1–2 cm length were submerged in 10% KOH at 90 °C for 45 min, bleached in H_2O_2 for 3 min and acidified in 1% HCl. Then, root segments were stained for 90 min in 0.05% Trypan Blue at 60 °C. The duration of staining varied among plant species according to the respective root diameter and surface root characteristics. The root fragments were preserved in lactoglycerol. All stained roots were viewed through a microscope at 400x magnification and the presence of hyphae, vesicles, and arbuscules inside the root was determined according to Trouvelot et al. (1986): Mycorrhizal (arbuscules and vesicles) frequency (F) was calculated as $F\ (\%) = Myc / N \times 100$, where Myc is the number of mycorrhized fragments and N is the number of observed root fragments. Mycorrhizal intensity (M – proportion of AMF colonization) was calculated by assigning an index of mycorrhization from 0 to 5 as follows: $M\ (\%) = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1) / N$, where n is the number of fragments assigned to n_1 (trace colonization, <1% of the root segment), n_2 (<10% of the root segment), n_3 (11–50% of the root segment), n_4 (51–90% of the root segment) and n_5 (>91% of the root segment); and N is the number of observed root fragments.

AMF spores occurring in soil samples were extracted following the wet sieving method described by Gerdemann and Nicolson (1963). Samples of 100 g of soil were submerged in 1 L of tap water. After 1 min of stirring and 30 s of settling, the supernatant was sieved through three nested sieves with meshes of 1000, 100, and 32 µm. The filtrate of each soil suspension was collected and sieved again. The spores retained on the sieves were recovered in 25 mL tubes. A viscosity gradient was created by adding 25 mL of a 60% (w/v) aqueous sucrose solution to each tube (Walker et al., 1982). After centrifugation at 3000 rpm for 2 min, the supernatant was sieved (32 µm) and the retained fraction, the spores, was rinsed with distilled water to remove sucrose. After extraction, AMF spores were counted under a stereomicroscope (40x magnification) and average numbers were expressed per 100 g of dry soil.

2.4. Soil analysis

Soil physical and chemical properties were analysed for bare soil samples collected at each of the four sites (Table 1). Soil texture was determined using the Robinson's pipette method (Naanaa and Susini, 1988), and soil pH and electrical conductivity were measured in a 1:10 (w/v) water extract using a selective electrode for H^+ (Crison micro pH 2002) and a conductivity meter (Consort C562) respectively. The Soil analysis laboratory of the Regional Commissariat for Agricultural Development in Gabes (Tunisia) determined soil organic matter (ISO norm 10,694 by loss on ignition overnight at 600 °C), total nitrogen (ISO standard 13,878 by dry combustion using an elemental analyzer Leco CNS), phosphorus (modification of the Egner-Riehm method using plasma emission spectrophotometry with an optical detector ICP-OES, following extraction using ammonium lactate 0.1 M and acetic acid 0.4 M, pH 3.65–3.75), and calcium carbonate (ISO 10,693 by gravimetry).

Soil functioning was analysed for samples of bare soil and soil under plants influence collected at each of the four sites (Figs. 1-4). The carbon of the soil microbial biomass (Cmic) was determined using the fumigation-extraction method (Amato and Ladd, 1988). Briefly, the method consists in using ninhydrin-N reactive compounds extracted from soils with KCl after a 10-day fumigation period. Soil respiration was determined according to Ohlinger (1995), and the metabolic quotient (qCO_2) was calculated by dividing the C-CO_2 released from the sample by the microbial biomass carbon (Cmic) content.

Soil dehydrogenase activity was determined as described by Garcia et al. (1997), with the iodonitrotetrazolium formazan (INTF) formed being analysed colorimetrically (spectrophotometer Tecan Spectra

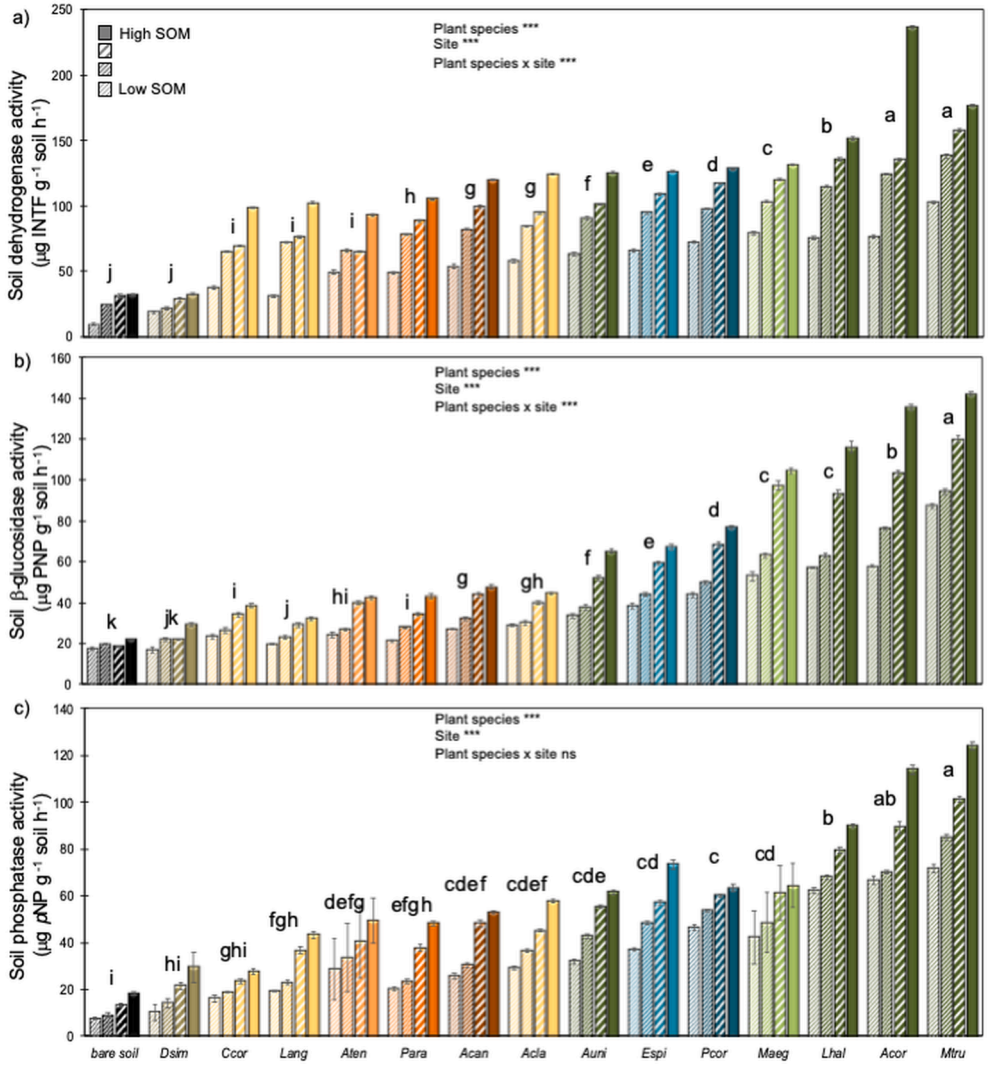

Fig. 3. Effect of plant species (see Table 2) and site on soil enzymatic activities: dehydrogenase (a), β-glucosidase (b) and phosphatase (c). Plant species were ordered according to soil multifunctionality (Fig. 4) and bars with different colours represent different plant families. *** shows significant effects ($p < 0.01$) and 'ns' means non-significant. Different letters show significant differences between plant species ($p < 0.05$). Bars are the mean ± 1SE (n = 3).

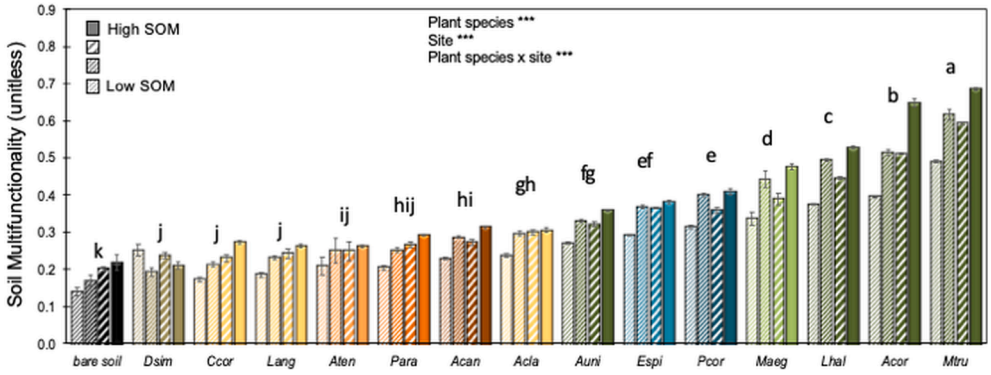

Fig. 4. Effect of plant species (see Table 2) and site on soil multifunctionality. Bars with different colours represent different plant families. *** shows significant effects ($p < 0.01$) and different letters show significant differences between plant species ($p < 0.05$). Bars are the mean ± 1SE (n = 3).

Rainbow A-5082) at 490 nm. Phosphatase and β-glucosidase activities were measured according to Caravaca et al. (2005). The p-nitro-phenol (PNP) formed in alkaline phosphatase activity and the p-nitro-phenol glucopyranoside in β-glucosidase (PNG) activity were analysed colorimetrically (spectrophotometer Tecan Spectra Rainbow A-5082) at 398 nm. All analyses were performed in triplicate.

2.5. Soil multifunctionality

Similarly to other studies on dryland soils (e.g. Delgado-Baquerizo

Table 3
Pearson's correlations between soil multifunctionality and the studied belowground functional traits (AMF frequency, intensity and spores' abundance, soil microbial biomass, metabolic quotient, and soil dehydrogenase, phosphatase and β-glucosidase activities – since bare soil samples were not included, n = 168). All correlations were significant. **Correlation is significant ($p < 0.01$; 2-tailed).

	Soil multifunctionality	Mycorrhizal frequency (F)	Mycorrhizal intensity (M)	AMF spores abundance	Microbial biomass (Cmic)	Metabolic quotient (qCO_2)	Dehydrogenase activity	β-glucosidase activity	Phosphatase activity
Soil multifunctionality	1	0.492**	0.886**	0.926**	0.950**	−0.400**	0.858**	0.956**	0.925**
Mycorrhizal frequency (F)		1	0.602**	0.556**	0.577**	−0.760**	0.653**	0.494**	0.542**
Mycorrhizal intensity (M)			1	0.869**	0.866**	−0.518**	0.800**	0.833**	0.791**
AMF spores abundance				1	0.912**	−0.470**	0.860**	0.925**	0.891**
Microbial biomass (Cmic)					1	−0.568**	0.859**	0.956**	0.905**
Metabolic quotient (qCO_2)						1	−0.500**	−0.444**	−0.462**
Dehydrogenase activity							1	0.875**	0.871**
β-glucosidase activity								1	0.909*
Phosphatase activity									1

et al., 2016), we used a small (yet integrative) set of soil functions to assess soil functioning. We used two different approaches to assess soil functioning: i) individual soil functions assessed separately (soil microbial biomass, metabolic quotient and dehydrogenase, phosphatase and β-glucosidase activities); and ii) multifunctionality based on the average approach (Maestre et al., 2012b). Average multifunctionality, which is increasingly being used (Delgado-Baquerizo et al., 2016), calculates the average of the previously standardized multiple functions measured, thus providing a straightforward and easily interpretable measure of multifunctionality (Byrnes et al., 2014). To obtain our average multifunctionality index (from herein multifunctionality) for each soil (under the influence of the different plant species and bare soil) from the four different sites, we first standardized each of the five variables to a 0–1 scale by dividing each value by the maximum value for that particular variable. Following this, the standardized variables were averaged to obtain the multifunctionality value (Delgado-Baquerizo et al., 2016).

2.6. Statistics

The effect of the site on soil physico-chemical parameters was tested separately using a one-way analysis of variance, with site as fixed factor. The effect of the plant species on AMF (mycorrhizal frequency and intensity and AMF spores abundance) and soil parameters (soil microbial biomass, metabolic quotient, and enzymatic activities – dehydrogenase, β-glucosidase and phosphatase) was tested separately using a two-way analysis of variance, with site and plant species as fixed factors (Table S1). Bonferroni post hoc multiple comparisons tested for differences ($p < 0.05$) in AMF and soil parameters between plant species, including bare soil.

Linear correlations between soil multifunctionality and the studied belowground functional traits (mycorrhizal frequency and intensity, AMF spores abundance, and soil microbial biomass, metabolic quotient, dehydrogenase, phosphatase and β-glucosidase activities) were examined using Pearson's correlations (Table 3) for all the 14 plant species (since bare soil samples were excluded, n = 168). Correlation between soil multifunctionality and mycorrhizal parameters (mycorrhizal frequency and intensity and AMF spores abundance) were compared using the Steiger's Z test ($p < 0.05$). In all cases, preliminary analyses were performed to ensure that there was no violation of statistical assumptions (including the Levene's test to check for homogeneity of variances). SPSS (version 26·0, IBM, Inc., Chicago, IL, USA) was used for all the abovementioned analyses.

To determine whether site and plant species influence soil functioning, we performed a redundancy analysis (RDA) with the package "stats" using R version 4.0.1 (R Core Team, 2013) and executed on RStudio (IDE version 1.2.5033). The redundancy analyses (RDA) were performed on a correlation matrix of each dataset using the *rda()* function in the "vegan" package (Oksanen et al., 2013). The variables used in all models were: F %, M %, number of AMF spores, Cmic, qCO_2, soil enzymatic activities (dehydrogenase, ß-glucosidase and phosphatase) and soil multifunctionality. The RDA was then run using the factor variables for sites, plant species and both together. The *ordiellipse()* function was used on the plot site scores and on the plant species scores to create 95% confidence ellipses for the standard error of the average of each factor, site scores themselves were not plotted for better readability of the figures. The models and the first two axes were evaluated for significance against an unconstrained model using adjusted R^2 values in permutational significance tests (1000 permutations). For variance partitioning, the function *varpart()* from the "vegan" package was used.

3. Results

3.1. Sites characterization and native herbaceous plant species

The four sites differed in physical and chemical characteristics, but

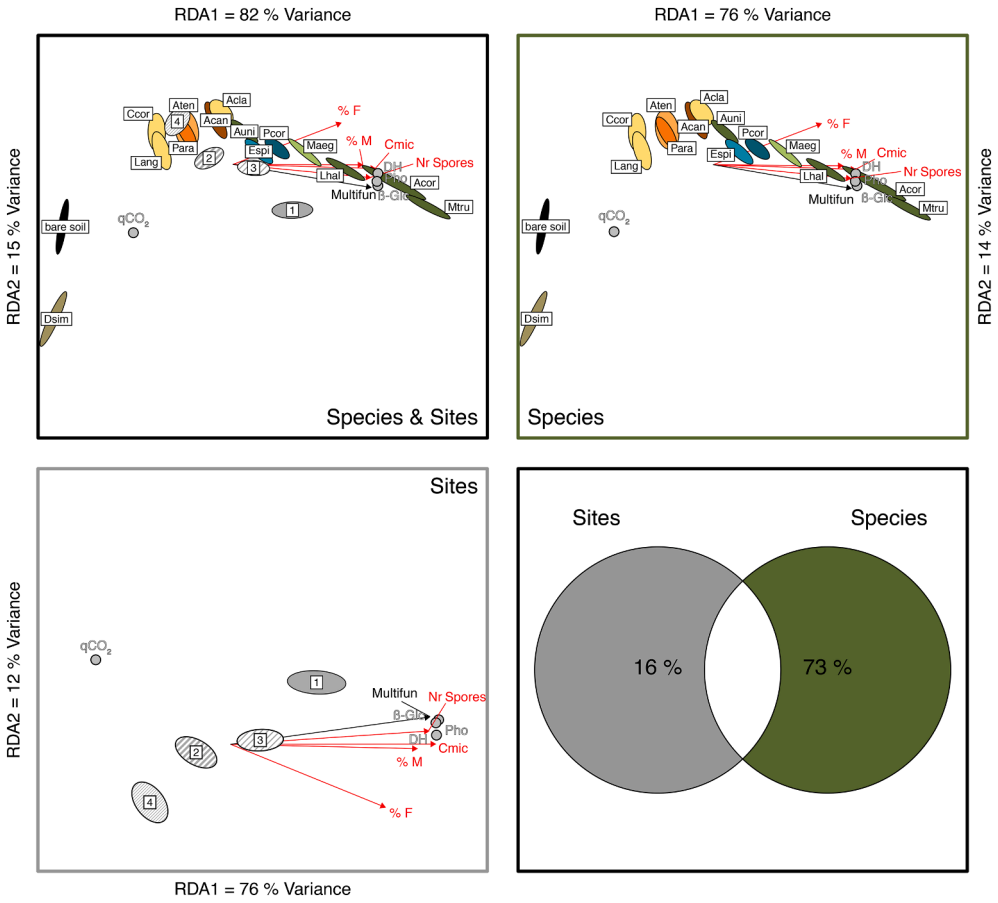

Fig. 5. Redundancy analysis (RDA) with variance partitioning, showing that both the plant species and sampling sites significantly changed AMF traits (mycorrizal frequency – % F; mycorrhizal intensity – % M; and AMF spores abundance – Nr spores), individual soil functions (microbial biomass Cmic; metabolic quotient - qCO_2, soil enzymatic activies of dehydrogenase – DH; ß-glucosidase – ßGlc; and phosphatase – Pho) and soil multifunctionality (Multifun). Response data were centered.

not in pH, which was alkaline (8.0–8.3 – Table 1). Our SOM gradient ranged between 0.9% and 1.9%: site 1 had the highest percentage of SOM (1.9%) and total nitrogen (N – 182 ppm), followed by site 2 (1.4% SOM and 151 ppm of total N) and site 3 (1.1% SOM and 125 ppm of total N). Site 4 had the lowest SOM (0.9%) and total N (90 ppm). Soil calcium carbonate concentrations varied from 5 (site 1) to 10 ppm (site 4), while those of available phosphorus varied from 5 (site 2) to 14 ppm (site 4).

The fourteen native plant species present in all the four sites (Table 2) belong to nine plant families: Asteraceae, Aizoaceae, Brassicaceae, Caryophyllaceae, Fabaceae, Malvaceae, Plantaginaceae, Polygonaceae and Xanthorrhoeaceae. Most plant families were represented by only one plant species, except for Asteraceae and Fabaceae which were represented by three and four plant species respectively. Ten plant species were annuals and four were perennials. The fourteen studied plant species provided both economic (grazing, medicinal, edible, etc.) and ecological (soil stability and fertility, etc.) services (Table S2).

3.2. AMF colonization and spores abundance

Root tips' direct microscopic observation showed that all plant species were AMF colonized, except *Diplotaxis simplex* (*Dsim* – Fig. 1-a, b). We observed all the characteristic structures of AMF root colonization (intracellular aseptate hyphae, vesicles and arbuscules – data not shown) in the roots of the 13 plant species. Mycorrhizal frequency (F%), intensity (M%) and AMF spores abundance varied according to the plant species, site and the interaction between plant species and site ($p < 0.001$ – Table S1 and Fig. 1). As *Dsim* plants were not AMF colonized, they presented the lower AMF spores abundances, while *Medicago truncatula* (*Mtru*) plants presented the higher mycorrhizal frequencies and intensities, and AMF spores abundances. AMF spores abundance in the soils under *Dsim* influence was as low as that detected in bare soil.

The plants with the highest AMF root colonization belonged to the Fabaceae family (*Mtru; Astragalus corrugatus* – *Acor;* and *Lotus halophilus* – Fig. 1). Further, in general, for each plant species, the highest mycorrhizal frequencies, intensities and AMF spores abundances were observed in plants occurring at the SOM-richer site (site 1), while the lowest values were observed in plants occurring at the SOM-poorer site (site 4).

3.3. Soil microbial communities

Soil microbial biomass (Cmic – Fig. 2-a) and metabolic quotient (qCO_2 – Fig. 2-b) varied according to the plant species, site and the interaction between plant species and site ($p < 0.001$ – Table S1 and Fig. 2). The soils under *Dsim* (no AMF colonization) influence presented the lower Cmic, even lower than those of bare soils, while soils under *Mtru* (higher AMF colonization) plants presented the higher. Again, the soils which presented the higher Cmic belonged to the Fabaceae family (*Mtru*, *Acor* and *Lhal*), and in general, for each plant species, the highest Cmic were determined under the influence of plants occurring at the SOM-richer site (site 1), while the lowest values were determined under the influence of plants occurring at the SOM-poorer site (site 4). qCO_2 varied in the opposite direction of Cmic, with soil under *Dsim* influence presenting the higher qCO_2 values and those under the three Fabaceae plant species' (*Mtru*, *Acor* and *Lhal*) influences presenting the lower. Further, in general SOM-richer soils presented lower qCO_2 values than SOM-poor ones.

Soil dehydrogenase (Fig. 3-a) and β-glucosidase (Fig. 3-b) activities

varied according to the plant species, site and the interaction between plant species and site ($p < 0.001$ – Table S1). Soil phosphatase activity (Fig. 3-c) also varied according to the plant species and site ($p < 0.001$) but the interaction between plant species and site was not significant ($p > 0.05$ – Table S1). Bare soils and soils under *Dsim* (no AMF colonization) influence displayed the lower enzymatic activities, while soils under the three Fabaceae plant species (*Mtru*, *Acor* and *Lhal* – higher AMF colonization) influences displayed the higher enzymatic activities. Soil enzymatic activities determined in soils under *Dsim* influence were as low as those detected in bare soils. Further, for each plant species influence, the highest soil enzymatic activities were determined in plants occurring at the SOM-richer site (site 1), while the lowest values were determined in plants occurring at the SOM-poorer site (site 4).

3.4. Soil multifunctionality

Soil multifunctionality (Fig. 4) varied according to the plant species, site and the interaction between plant species and site ($p < 0.001$ – Table S1). Bare soils displayed the lower multifunctionality. The soils under plant influence which displayed the lower multifunctionality were those under *Dsim* (no AMF colonization), *Chrysanthemum coronarium* and *Launaea angustifolia* (*Ccor* and *Lang* respectively – low AMF colonization), while soils under most Fabaceae species (*Mtru*, *Acor* and *Lhal*) displayed the higher values of multifunctionality. Except for *Dsim*, whose influence promoted the higher multifunctionality at the SOM-poorer site (site 4), when plants of the other plant species occurred at the SOM-poorer site, we determined the lowest multifunctionality. In general, the highest soil multifunctionality was determined when plants occurred at the SOM-richer site (site 1), but for some plant species (e.g. *Anacyclus clavatus* – *Acla*; *Plantago coronopus* – *Pcor*; *Malva aegyptiaca* – *Maeg*; *Lotus halophilus* – *Lhal*), high soil multifunctionality was also determined when plants occurred at intermediate sites along our SOM gradient. This means that the plant species which promote soil multifunctionality the most are not the same at all four sites.

Soil multifunctionality was correlated with all the analysed belowground functional traits (Table 3); those that were used to calculate multifunctionality (soil microbial biomass, metabolic quotient and soil enzymatic activities) and those that were excluded (mycorrhizal frequency and intensity and AMF spores abundance). Further, soil multifunctionality was more correlated with mycorrhizal intensity and AMF spores abundance, than with mycorrhizal frequency (Steiger's Z test; $p < 0.05$). The results of the redundancy analysis further corroborate that soil multifunctionality was strongly associated with mycorrhizal traits (especially mycorrhizal intensity and AMF spores abundance) across the analysed SOM gradient (Fig. 5). The constrained ordination had an adjusted $R^2 = 0.90$ for the RDA of sites, $R^2 = 0.92$ for the RDA of species, $R^2 = 0.95$ for the RDA of both sites and species. Using permutation tests, the constrained models were always significantly different from an unconstrained model by < 0.001 and both RDA1 and RDA2 of all models were found to be significant (<0.001).

4. Discussion

4.1. Plants and AMF are important modifiers of soil functioning

Our data corroborate that AMF extend plants influence in the soil by forming communication pathways between plants and the soil, modifying nutrient cycling, soil fertility and the microbial community. As hypothesized, AMF were validated as belowground functional traits useful to predict soil functioning across a SOM gradient. It is interesting that mycorrhizal frequency is perhaps the most widely studied AMF trait but, in our study, mycorrhizal intensity and AMF spores abundance were better indicators of soil functionality. Further, determining AMF spores abundance is simple, cheap and does not require expensive equipment.

However, AMF diversity and colonization depend on soil characteristics and management (Duponnois et al., 2005; Lekberg et al., 2007), on plant species and AMF spores availability (Zhu et al., 2000).

4.1.1. Soil characteristics constrain soil biological activity

Especially in drylands, soil characteristics modify soil microbial communities to a great extent. Although phosphorus availability is one of the abiotic factors that interferes the most with mycorrhization (Bouamri et al., 2006; Dickson et al., 1999; Smith and Read, 2008), soil phosphorus concentrations were low at all sites, ranging from 7 to 14 ppm. Therefore, phosphorus availability cannot be responsible for the distinct mycorrhizal degrees we observed.

Since clay and silt contents have been shown to favour microbial communities and activities (Hallett et al., 2009; Rillig, 2004), site 4, showing the highest clay and silt %, was expected to foster the highest AMF colonization and microbial activity; however in site 4 we observed the lowest AMF colonization and soil microbial activities, suggesting that soil management and other soil characteristics modify soil microbial structure and function (Ba et al., 2012).

Another soil characteristic which greatly modifies biological activity is SOM, which is the key to soil fertility because it: i) acts as a nutrient storage, gradually providing essential elements; ii) buffers plants against sudden environmental changes; iii) preserves moisture during drought periods; iv) keeps soil physical conditions compatible for seedlings growth; and v) supports a greater biodiversity (Garratt et al., 2018). Indeed, site 4 being at the lower end of our SOM gradient (<1%), and site 1 being at the upper end, it is not surprising that AMF colonization, microbial activity and soil multifunctionality were respectively the lowest and the highest, showing a positive response to SOM.

4.1.2. Plants species and AMF

Mycorrhized plants are the 'rule' rather than the exception (Smith and Read, 1997, 2008) and indeed we observed that 13 out of 14 of the studied plant species were AMF-colonized and only one (*Diplotaxis simplex* - *Dsim*, a Brassicaceae) was not. Brassicaceae are usually non-mycorrhizal species (Bagayoko et al., 2000; Smith and Read, 2008). By contrast, the legume species *M. truncatula* (*Mtru*), *A. corrugatus* (*Mtru*) and *L. halophilus* (*Lhal*) showed the highest mycorrhizal intensity, which may highlight their mycorrhizal dependency and their high demand for P in comparison with other plant families such as Poaceae (Bagayoko et al., 2000). The wide range of variation in AMF colonization rates and intensities between AMF-colonized plant species and within each plant species we observed may be related to several biotic and abiotic factors (Henriques and Hay, 1998), namely different levels of mycorrhizal dependence (Collier et al., 2003), and AMF spores availability (Li et al., 2005). In agreement with our results, as AMF are obligate biotrophs (Smith and Read, 1997, 2008), the number of spores and propagules tends to be higher under plants influence than in bare soil (Azcon-Aguilar et al., 2003; Eom et al., 2000; Lovelock et al., 2003), and higher under the influence of plants with higher AMF colonization. These differences are particularly evident in dryland soils with low SOM and high SOM turnover rates (Mohammad et al., 2003).

AMF sporulation is a highly carbon demanding process that occurs when the development of the AMF mycelium starts to be nutrient-limited. This may explain why the number of AMF spores in the soils under plant influence varied among plant species and within plant species between sites (Rodriguez-Echeverria et al., 2008). Our native plants growing in the SOM-poorer site (site 4) were most likely more nutrient-limited, resulting in less AMF spores than in SOM-richer sites.

4.2. Building belowground functional networks

Due to the excretion of catabolic enzymes into the surrounding medium, and to the direct access to the plant carbon, AMF increase the diversity of the carbon sources available to the soil microbes (Rillig, 2004). The low levels of microbial biomass (Cmic), enzymatic activities and soil multifunctionality observed in the soils without AMF (bare soil and the soil under the *D. simplex* – *Dsim* influence, non-mycorrhizal) and

with low mycorrhization (e.g. *C. coronarium* – *Ccor*; and *L. angustifolia* – *Lang*) show that AMF stimulated microbial community development, being a key player in building belowground functional networks in SOM-poor ecosystems. Further, the high metabolic coefficient (qCO_2 – low values reflect an efficient microbial use of the available organic substrates and *vice-versa* – Anderson, 2003) values we observed in the soils without AMF (bare soil and the soil under the non-mycorrhizal plant species influence, *Dsim*) show that mycorrhiza contribute to improve the availability of carbon substrates to the soil microbial community (Böhme et al., 2005). Our data support that AMF establish unique interactions with plant roots and the soil microbes, where several by-product-based symbiosis and microbial loops may be assembled, contributing to improved carbon use efficiency.

Phosphatase and β-glucosidase potential activities were higher in the soils under mycorrhized-plants influence than in the soils without AMF (bare soil and the rhizosphere of the non-mycorrhizal plant, *Dsim*). The importance of the soil microbial activity in association with enzyme activity was highlighted by the similarity in the activity patterns of the two hydrolytic enzymes (phosphatase and β -glucosidase) and those of dehydrogenase, an indicator of microbial activity (Garcia-Ruiz et al., 2008).

4.3. Modifying soil functioning in drylands

Drylands' high temperatures and seasonal drought accelerate soil degradation, constrain plant and microbial communities (Martinez-Garcia et al., 2012) and consequently soil functioning. Despite this unfavourable scenario, native plant species identity and SOM modified soil multifunctionality, which was shown to be highly correlated with mycorrhization traits (especially mycorrhization intensity and AMF spores abundance). Therefore, our study provides evidence that soil functioning may be improved by management options favouring certain plant species and enhancing SOM. Even though the native plant species which promoted soil multifunctionality the most were *M. truncatula* (*Mtru*), *A. corrugatus* (*Acor*), *L. halophilus* (*Lhal*) and *M. aegyptiaca* (*Maeg*) when growing in SOM-richer sites, the increments in soil multifunctionality driven by these plants when growing in SOM-poorer sites will be especially important in the most degraded biotopes to help restore soil functioning. Further, by stimulating soil functioning, management options promoting those plant species and enhancing SOM may help counterbalance the negative effects of climate change and dryland degradation (Maestre et al., 2012b).

4.4. Integrating belowground functional traits in drylands restoration

Our data show that native plant species are not equally good at promoting soil multifunctionality. Even though our study was not designed to screen for plant families which promote or hamper soil functioning, one plant family stood up due to its generalized positive effect: Fabaceae. Indeed, wild Fabaceae, better known as legumes (herbs, shrubs or trees), play a critical role in natural ecosystems, agriculture, and agroforestry, where their ability to fix nitrogen in symbiosis makes them excellent colonizers of nitrogen-limited environments, and hence an economic and environmentally friendly species. The diversity and effectiveness of the nitrogen-fixing wild legumes are of major significance to soil fertility dynamics in drylands, having been shown to have positive effects on soil enzyme activities, microbial biomass and respiration (Rejili et al., 2012). However, if Fabaceae species are included in restoration projects, the inoculation of seeds and seedlings with appropriate native rhizobia resistant to salinity and acidity may be necessary to guarantee root nodulation, enhance plant performance, and reintroduce these microbes in the soil (Rejili et al., 2012).

By contrast, as plant species belonging to Brassicaceae, Chenopodiaceae and Amaranthaceae families and the genus *Lupinus* (a Fabaceae) (Harley and Harley, 1987; Smith and Read, 1997) are considered as non-mycorrhizal, these plant species are not likely to enhance soil functioning. Although certain native plant species are not as effective at promoting soil functioning and ecosystem services provision as others, we consider that non-mycorrhizal and 'low-mycorrhizal' plant species may be important elements to improve restoration success in drylands as they further contribute to the conservation of local biodiversity, the existence of ecotypes adapted to specific environmental conditions, the provision of compatible habitats for other native plants and animals, and the enhancement of natural colonization (Bochet et al. 2010a, 2010b).

4.5. Future perspectives

For dryland populations, whose livelihoods are often tied to subsistence agriculture and livestock production (James et al., 2013), the use of native plant species providing relevant ecological and economic services can contribute to build resilience and decrease vulnerability to multiple threats (Stringer et al., 2009). A major challenge of restoration is therefore the selection of native species that are adapted to drylands harsh conditions, successfully colonize degraded areas and provide relevant ecological and economical services. In agreement, native plant species may contribute poorly to soil functioning but provide important additional income/uses for local populations. For instance, *C. coronarium* (*Ccor*) has not been shown to provide any ecological service, and contributed poorly to increase soil multifunctionality, but the intake of this daisy plant enhances fatty acids content (rumenic and vaccenic acid) in sheep dairy products (Cabiddu et al., 2006). *A. uniflorum* (*Auni*) also contributed poorly to increase soil multifunctionality, but provides several ecological and economic services, namely as a persistent and highly palatable legume for grazing (Visser et al., 2012) that remains green and photosynthetically active along the year (Chaieb et al., 1992). *L. angustifolia* (*Lang*) has also been used in folk medicine in bitter stomach, skin diseases, and reported to have antitumor, insecticide and cytotoxic activities (Zellagui et al., 2012), and *A. clavatus* (*Acla*) volatile oil has antibacterial and anti-insect effects (Pascual-Villalobos and Robledo, 1999). Finally, it is important to consider that some native species may be characterized by high levels of innate dormancy such as *A. canariense* (*Acan*) (El-Keblawy and Gairola, 2017) which may hamper its positive effects in promoting soil multifunctionality. The trade-offs between plant species effects on soil functioning and its services require that new ways be developed to efficiently articulate knowledge between restoration actors (restorers, policy-makers, practitioners, local populations, etc.).

5. Conclusions

Studying native herbaceous plant species and soils under dryland conditions provided strong evidence that AM traits (especially mycorrhizal intensity and AMF spores abundance) are good indicators of simultaneous multiple soil functions in these ecosystems across a SOM gradient. The ecological functional role of a plant goes far beyond its aboveground role, as mycorrhization traits depended on the plant host; the higher the mycorrhization, the more AMF hyphae create a privileged space for microbial development beyond the root surface, and the more a plant sustains and promotes soil multifunctionality. We suggest that soil multifunctionality in drylands can be improved by management practices that promote soil carbon sequestration and favour specific native plant species such as *Medicago truncatula*, *Astragalus corrugatus*, *Lotus halophilus*, *Malva aegyptiaca*, *Plantago coronopus*, etc.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

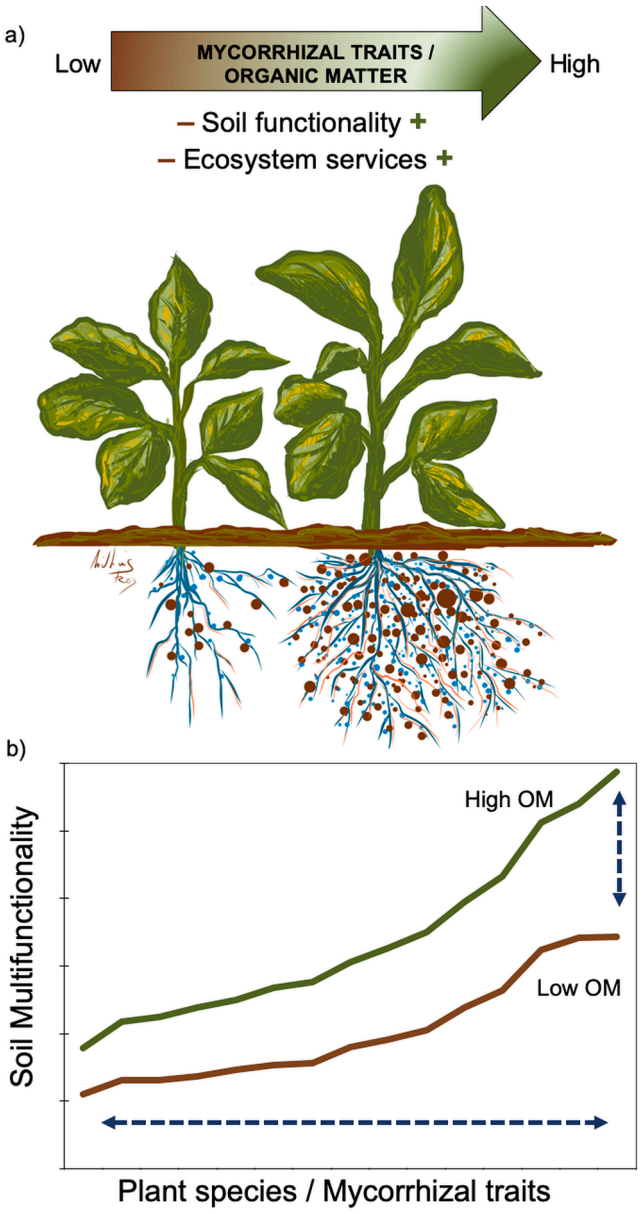

Fig. 6. Conceptual representation of how native plant species mycorrhizal traits and SOM modify soil multifunctionality. In SOM-poor soils, the more AMF (represented in orange) spreads its hyphae beyond the root surface (represented in blue) creating a privileged space for microbial development, the more a plant sustains and promotes soil multifunctionality (a). Improvement range of soil multifunctionality in drylands by SOM accumulation and favouring specific native plant species (b); the graph showing the improvement range of soil multifunctionality was built with our data.

Acknowledgements

This work was supported by the Tunisian Ministry of Higher Education and Research Development and by Portuguese funds through Fundação para a Ciência e a Tecnologia through the projects UIDB/00329/2020 and UIDP/50017/2020+UIDB/50017/2020 and postdoc grant to Teresa Dias (SFRH/BPD/85419/2012). We are grateful to Bou-Hedma National Park for making the experimental site available. The authors would like to thank Milhais Reis for drawing the plant-soil systems in Fig. 6-a.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115099.

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