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REGULAR ARTICLE

Unraveling the role of hyphal networks from arbuscular mycorrhizal fungi in aggregate stabilization of semiarid soils with different textures and carbonate contents

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Abstract
Aims Our study was intended to elucidate the involvement of three species of arbuscular mycorrhizal fungi (AMF) in the formation and stabilization of aggregates in semiarid soils with different textures and calcium carbonate contents.
Methods We used a root-hyphae compartment approach to compare the effect of three AMF (*Rhizophagus irregularis*, *Septoglomus deserticola*, and *Gigaspora gigantea*) on the structural stability of the hyphosphere (root-free hyphae) and mycorrhizosphere (hyphae + root) soil of *Olea europaea* plants grown in two soils differing in their texture (sandy loam and silty loam) and calcium carbonate content.
Results Only the *R. irregularis* strain significantly increased the percentage of stable aggregates in both types of soil, being the increases higher in the hyphosphere compartment (on average, about 30 % compared to non-inoculated soil). In the hyphosphere compartment of both soils, the hyphal length developed by plants inoculated with *R. irregularis* was 81 % greater than that of non-inoculated plants. The effect of the AMF on soil aggregation was mediated by mechanical entanglement of mycorrhizal fungal hyphae but without a contribution of labile carbohydrates.
Conclusion The ability of extraradical hyphae to improve soil structure was independent of the soil texture and content of carbonates.

Keywords Aggregate stability · AMF · Hyphosphere · Mycorrhizal hyphae · Semiarid soils

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Introduction

Semiarid and arid soils are often characterized by a poor structure, which increases their erodibility by water and anthropic actions (Caravaca et al. 2004). Among other effects, the degradation of structure leads to changes in the habitats, size, and composition of the microbial communities—which, in turn, affect soil functioning. Thus, the loss of soil structural stability can cause negative effects on biogeochemical and ecosystem processes such as soil infiltration, gas and water exchange, nutrient cycling, and protection against erosion (Six et al. 2002; Six et al. 2004; Rillig and Mummey 2006; Daynes et al. 2013). Furthermore, the degradation of this physical property will strongly impact the ability of soil to stabilize organic matter (OM) that acts as a C sink (Bronick and Lal 2005).

Soil aggregation is a dynamic, complex, and hierarchized process performed by several biotic and abiotic components of soil (Asano and Wagai 2014). It is generally recognized that fungi act as key biotic binding agents in the aggregation and stabilization of aggregates (Oades 1984). In particular, arbuscular mycorrhizal fungi (AMF) that form symbioses with the majority of land plants, actively participate in the formation and stabilization of soil macroaggregates (>250 μm) by means of biophysical and/or biochemical mechanisms (Miller and Jastrow 2000). In particular, AMF promote soil aggregation by entangling soil particles with their extraradical mycelium and/or by deposition of organic substances that act as cementing agents and bind soil particles together, forming microaggregates (<250 μm) (Tisdall and Oades 1982; Rillig and Mummey 2006). According to the hierarchical model of the aggregation process proposed by Tisdall and Oades (1982), the microaggregates are bound into macroaggregates mainly by fine roots, fungal hyphae, microbial and plant-derived polysaccharides, and bacterial cells. Different AM fungal species can differ in their contribution to soil aggregation due to their differing abilities to form an efficient extraradical mycelium and produce organic aggregating agents (Piotrowski et al. 2004; Leifheit et al. 2014). Meanwhile, specific root and mycorrhizal fungal traits, as described in Rillig et al. (2015), have been proposed that could influence the formation and stabilization of soil aggregates. Likewise, the specificity among AM fungal species and their host plants can result in variable inputs of above- and below-ground OM into soil, which is a prominent contributor to soil structure formation and stabilization (Tisdall and Oades 1982; Oades 1993). The interaction between AMF and the rhizosphere-dwelling microbial community can also cause significant effects on soil structure (Rillig et al. 2010).

The stability of aggregates is affected by soil texture, the content of carbonates, the predominant type of clay, extractable iron, extractable cations, the amount and type of OM present, and the composition of the microbial population. Soil OM and soil fine mineral particles are the main abiotic binding agents in aggregation processes (Chenu et al. 2000). Many primary silt- and clay-sized particles are actually strongly associated with OM and stabilized as aggregates. The carbonate content also influences soil aggregation in semiarid soils, as it is thought that Ca^{2+} ions serve as a cationic bridge between soil organic and inorganic components (Virto et al. 2011). Regarding soil texture, coarser soils generally have a lesser intrinsic ability to form stable aggregates than finer soils (Skidmore and Layton 1992). Lesser aggregation in sandy soils is associated with faster OM decomposition and lower soil fertility (Chivenge et al. 2011). In these nutrient-poor soils, AMF usually present a more profound influence on plant growth—which, in turn, could lead to a stronger effect on soil aggregation (Leifheit et al. 2014). Furthermore, sand-sized particles and aggregates are more susceptible to entanglement and mechanical retention by the hyphae for forming larger aggregates than primary silt- and clay-sized fine particles; thus, we hypothesize that the relative importance of entanglement by AM hyphae as an aggregate stabilizing mechanism is greater in coarse-textured soils than in fine-textured soils.

The effectiveness of mycorrhizal inoculation in the improvement of the structure of degraded semiarid soils has been previously tested under field conditions (Caravaca et al. 2002a, b; Roldán et al. 2006). In a revegetation experiment with four shrub species under semiarid conditions, the aggregating ability depended on AM fungal species, as well as the host plant (Caravaca et al. 2005), but the mechanisms underlying the difference in the stabilization of macroaggregates between the fungal species have not yet been investigated. Likewise, it is unknown whether soil characteristics like texture or carbonate content can influence the role of different AMF strains in soil aggregation. This information will assist the successful restoration of degraded soils and contribute to identification of the main drivers determining the development and stabilization of soil structure in semiarid ecosystems.

Our study was intended to elucidate the involvement of AMF in the formation and stabilization of aggregates in semiarid soils with different textures and calcium carbonate contents. We hypothesized that different AM fungal species differ in the formation of soil aggregate in terms of stability, which is also dependent on soil mineral particle and carbonate contents. In order to test this, we conducted a two-factor greenhouse experiment to compare the effect of three AMF on the structural stability of the hyphosphere and mycorrhizosphere soil of *Olea europaea* plants grown in two soils differing in texture and carbonate content. To disentangle the influence of AM fungal hyphae on soil aggregation from the

effects of plant roots, in the present study, we excluded the roots by using a root-hyphae compartment approach.

Materials and methods

Soils, plants, and mycorrhizal inocula

Two soils with different textures were selected in two semiarid Mediterranean areas: Mazarrón (coordinates 37°31′7.8″ N, 1°27′58.7″ W) and Rellano (coordinates 38°12′50.8″ N, 1°13′30.9″ W), both in the Province of Murcia, Spain. The soils are classified as a Lithic Torriorthent (SSS 2010) and Typic Torriorthent, respectively. The areas are under a typical Mediterranean climate with a mean annual precipitation of 280 mm, a mean annual temperature of 17.5 °C, and an average annual potential evapotranspiration (ETP) that reaches 1000 mm. Physico-chemical and chemical characteristics of both soils are shown in Table 1.

The plant used, *O. europaea* L. subsp. *sylvestris*, is a representative shrub species from semiarid scrublands in Southeast Spain. It is also well-adapted to water stress conditions and, therefore, frequently used in the reafforestation of semiarid disturbed lands (Caravaca et al. 2002a; Caravaca et al. 2005). Mature seeds were collected from the experimental areas. The surface-sterilized seeds were placed in darkness on Whatman No.1 filter paper moistened with distilled water in Petri dishes until germination. Once germinated, seedlings were grown for 1 year in nursery conditions with peat as substrate.

Table 1 Soil physicochemical and chemical characteristics of Mazarrón and Rellano sites ($N = 5$)

	Mazarrón	Rellano
pH (H_2O)	8.83 ± 0.03	8.51 ± 0.02
Electrical conductivity (1:5, μS/cm)	97 ± 1	176 ± 2
Texture (% sand, silt, clay)	Sandy loam (65, 26, 9)	Silty loam (32, 61, 7)
Available P (μg g^{-1})	9 ± 0	10 ± 0
Total N (g kg^{-1})	2.9 ± 0.4	2.6 ± 0.1
Total organic C (g kg^{-1})	12.9 ± 3.2	18.3 ± 5.3
Water-soluble carbon (μg g^{-1})	55 ± 9	76 ± 2
Water-soluble carbohydrates (μg g^{-1})	12 ± 1	11 ± 1
$CaCO_3$ (%)	0.3 ± 0	74.0 ± 1.0
Total P (%)	0.10 ± 0.01	0.09 ± 0.01

Mean ± standard error

The AMF species used were *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler, isolate EEZ 6, *Septoglomus deserticola* (Trappe, Bloss & Menge) G.A. Silva, Oehl & Sieverd., isolate CA113, and *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe, isolate MN922A. The *R. irregularis* strain was isolated from a semiarid Mediterranean soil also in the province of Murcia and deposited in the collection of the experimental field station of Zaidín, Granada (EEZ 6), whereas *S. deserticola* and *G. gigantea* strains came from the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM; http://invam.caf.wvu.edu/index.html). The *S. deserticola* species is common in semiarid soils, whereas *G. gigantea* is rarely found in those areas (Torrecillas et al. 2012). Each isolate was grown with a mixture of sepiolite:vermiculite (1:1, *v*/*v*) and *Sorghum bicolor* as trap plant. The final mycorrhizal inocula consisted of a mixture of substrate from the trap cultures containing spores, hyphae, and infected root fragments. The three species produced robust, strong, and abundant hyphae, ranging hyphal length between 5 and 6 m cm^{-3}, without significant differences among the strains.

Experimental design and setup

A mesocosm assay was conducted. The experiment was arranged in a randomized two-factorial design with five replicates, making a total of 40 mesocosms. The first factor was the inoculation or not with three different mycorrhizal inocula (*R. irregularis*, *S. deserticola*, and *G. gigantea*). The second factor consisted in the different soils tested (soil from Mazarrón and Rellano).

Two-compartment mesocosms were set up placing a cylinder (14 cm height, 12.2 cm diameter) constructed with walls of 42-μm stainless steel mesh (FEVAL filters, Barcelona, Spain) within a cylindrical pot of PVC (16 cm height, 17 cm diameter). The outer compartment was denominated as hyphosphere compartment, where only the AM fungal mycelium grows. The inner compartment where roots can grow was designated as mycorrhizosphere compartment (Johansson et al. 2004).

Soil was air-dried and sterilized by tindalization at 100 °C for 60 min in three consecutive days. Then 2 kg of soil were used to fill both compartments in each mesocosm. When appropriate, the arbuscular

mycorrhizal inoculum was mixed with the soil in the mycorrhizosphere compartment, at a rate of 5 % (*v*/*v*). The same amount of the autoclaved inoculum with an inoculum filtrate excluding fungi was added to the mycorrhizosphere compartment in the non-inoculated pots in order to maintain similar bacterial communities in all treatments. Seedlings were planted in the center of the mycorrhizosphere compartment (one plant per pot). The experiment was conducted in a greenhouse (SACE service at the Campus of Espinardo, Murcia, Spain), with temperatures ranging from 8 to 32 °C, and the relative humidity between 60 and 80 %. Midday photosynthetically active radiation (PAR) averaged 260 μE m^{-2} s^{-1}. Plants were watered regularly with decalcified water, without any fertilizer treatment. Eight months after planting, plants were harvested.

Plant analyses

Dry weight of shoots and roots (60 ºC, 48 h) were recorded.

The mycorrhizal colonization of roots was assessed by staining them with 0.05 % trypan blue after their clearing with 10 % KOH (Phillips and Hayman 1970). The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse 1980). Positive counts for AM colonization included the presence of vesicles or arbuscules or typical mycelium within the roots. Hyphal length was determined from 4.0 g of fresh soil per mesocosm by an aqueous extraction and membrane filter technique as described by Rillig et al. (1999). Hyphae of AMF and non-AMF were distinguished microscopically at 200 magnification according to Miller et al. (1995). However, we did not distinguish dead and living hyphae.

Soil analyses

Soil pH and electrical conductivity were measured in a 1:5 (*w*/*v*) aqueous solution. The total organic C and total N were determined by dry combustion using a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA). Total phosphorus (P) content was determined by ICP-OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL). Available P was extracted with sodium bicarbonate and determined by ICP-OES spectrometry. Total $CaCO_3$ was determined by Bernard's calcimetric method (Black et al. 1965). Soil texture was determined using the hydrometer method. The percentage of water-stable aggregates against the impact of an artificial rainfall of known energy (270 J m^{-2}) was measured in soil samples sieved between 0.25 and 4 mm according to the method described in Roldán et al. (1994). Water-soluble carbohydrates were determined in soil samples sieved between 0.25 and 4 mm as described in Brink et al. (1960).

Basal soil respiration was assessed in moist (60 % of water holding capacity) soil samples using the μ-Trac 4200 system (SY-LAB, GmbH P.O. Box 47, A-3002 Neupurkersdorf, Austria). This system is based on the variation of electrical impedance of aqueous 0.2 % KOH (Fernández et al. 2004).

Statistical analysis

Prior to statistical analysis, data were tested for normality and log transformed when necessary in order to meet the assumptions of normality, excepting the percentage of stable aggregates that was arcsin transformed. The effects of the type of soil and mycorrhizal inoculation and their interaction on measured variables were analyzed by a two way ANOVA. All statistical analyses were performed using the software SPSS version 19.0 for Windows.

Results

The mycorrhizal inoculation had a significant effect on the percentage of stable aggregates in both the mycorrhizosphere and hyphosphere compartments (Fig. 1, $P < 0.001$). However, neither the type of soil nor the interaction between mycorrhizal inoculation and soil type had any effect on this physical property. Only the inoculation with *R. irregularis* significantly increased the percentage of stable aggregates in both types of soil. The increases produced by this AM fungus were higher in the hyphosphere compartment (on average, about 30 % compared to non-inoculated soil) than in the mycorrhizosphere compartment (on average, about 15 % compared to non-inoculated soil).

The length of the mycorrhizal hyphae was greater in the hyphosphere compartment than in the mycorrhizosphere compartment, irrespective of soil type and mycorrhizal inoculation (Fig. 2). In the mycorrhizosphere compartment, the length of the

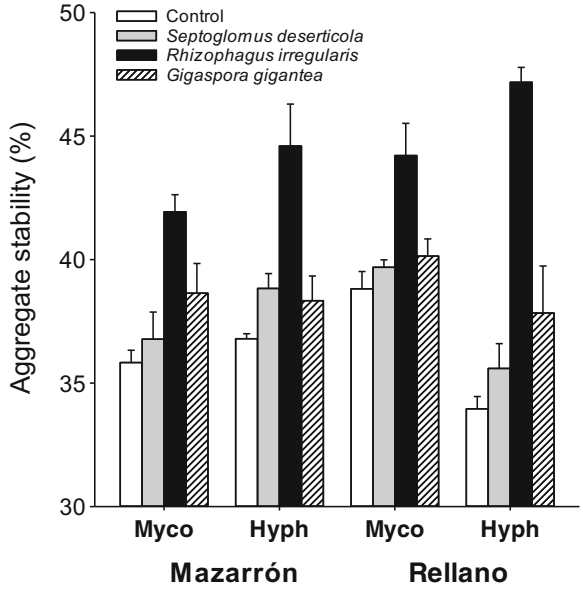

Fig. 1 Percentage of stable soil aggregates in the mycorrhizosphere and hyphosphere of *O. europaea* seedlings in response to the inoculation with three AMF in two soils differing in texture and calcium carbonate contents ($N = 5$). *Bars* represent standard errors

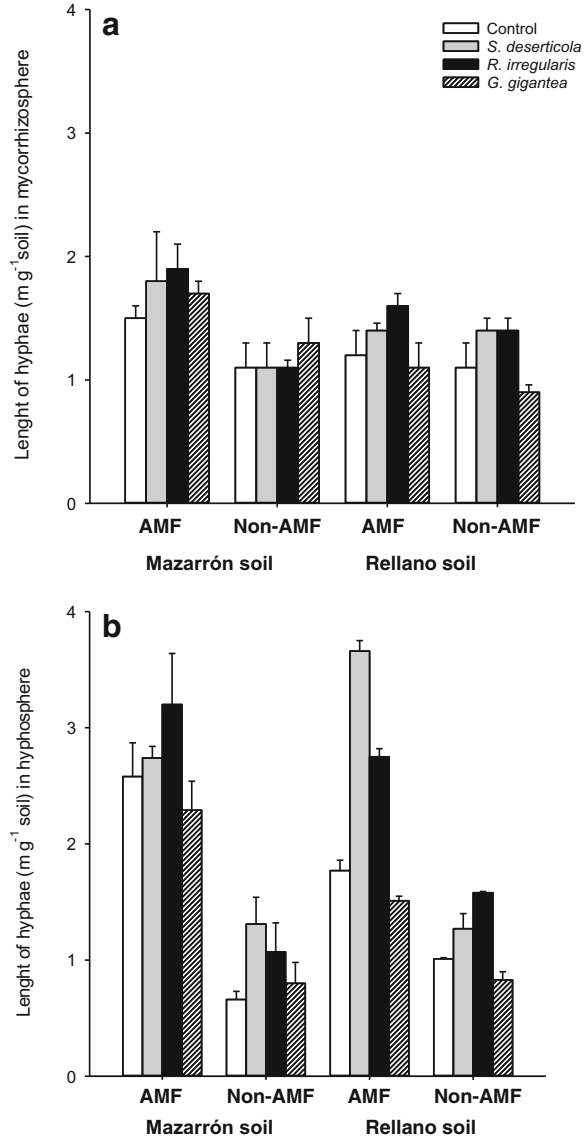

Fig. 2 Length of AMF and non-AMF hyphae in the mycorrhizosphere (**a**) and hyphosphere (**b**) of *O. europaea* seedlings in response to the inoculation with three AMF in two soils differing in texture and calcium carbonate contents ($N = 5$). *Bars* represent standard errors

mycorrhizal hyphae was significantly affected by the type of soil ($P = 0.009$), being greater in the coarse-textured soil (Mazarrón). In the hyphosphere compartment, the mycorrhizal inoculation significantly increased the length of the mycorrhizal hyphae ($P < 0.001$), especially in the fine-textured soil (Rellano). In this soil, the length of the mycorrhizal hyphae developed by the plants inoculated with *S. deserticola* or *R. irregularis* was 81 % greater than for control plants. For both compartments, neither the type of soil nor mycorrhizal inoculation had a significant effect on the length of the non-mycorrhizal hyphae.

The basal soil respiration did not vary with the mycorrhizal inoculation but did vary with the type of soil, being higher in the fine-textured soil (Rellano) in both compartments (Table 2). In the mycorrhizosphere compartment, the water-soluble carbohydrate (WSCH) concentrations of the soil aggregate fraction were affected by the type of soil, being higher in the Rellano soil (Table 2). In the hyphosphere compartment, all experimental factors significantly influenced the WSCH concentrations, particularly mycorrhizal inoculation. The soil aggregate fraction of the soil inoculated with AMF had lower levels of WSCH than that in non-inoculated soil.

Both types of soil and AMF inoculation significantly affected *O. europaea* shoot dry weight, while root dry weight and total root length were only affected by the type of soil (Table 3). In the Mazarrón soil, the inoculation with *R. irregularis* or *G. gigantea* significantly increased shoot biomass (by about 39 %, compared to control plants), without significant differences between these two AMF. In the Rellano soil, the three AMF were effective for enhancing the shoot biomass. The root biomass and total root length of plants grown in the Rellano soil were higher than in Mazarrón soil, but there

Table 2 Basal respiration and water-soluble carbohydrate concentration in the mycorrhizosphere and hyphosphere of *O. europaea* seedlings in response to the inoculation with three AMF in two soils differing in texture and calcium carbonate contents ($N = 5$)

	Basal respiration (mg C-CO_2 kg^{-1} h^{-1})		Water soluble CH (μg g^{-1})	
	Mycorrhiz	Hyphosphere	Mycorrhiz	Hyphosphere
Mazarrón soil				
Control	8.6 ± 0.5	8.9 ± 0.6	25 ± 2	27 ± 2
S. deserticola	8.9 ± 0.1	8.4 ± 0.3	30 ± 2	22 ± 3
R. irregularis	7.5 ± 0.5	8.6 ± 0.3	31 ± 2	22 ± 0
G. gigantea	7.8 ± 0.6	8.8 ± 0.2	27 ± 1	22 ± 2
Rellano soil				
Control	11.7 ± 0.6	11.5 ± 0.3	36 ± 4	31 ± 1
S. deserticola	11.2 ± 1.1	12.2 ± 0.1	35 ± 3	16 ± 2
R. irregularis	10.5 ± 0.7	11.9 ± 0.4	36 ± 0	16 ± 2
G. gigantea	11.5 ± 0.2	12.5 ± 0.2	32 ± 2	18 ± 1
Anova, *P* values				
Soil (S)	<0.001	<0.001	0.007	0.038
Mycorrhizal inoculation (I)	NS	NS	NS	<0.001
S × I	NS	NS	NS	0.045

Significance of effects of soil type, mycorrhizal inoculation, and their interaction on the measured variables is also shown. Mean ± standard error

NS not significance

were no differences in root biomass and length between non-inoculated and inoculated plants. Only the mycorrhizal inoculation had a significant effect on length of *O. europaea* roots colonized by AMF. *R. irregularis* inoculation resulted in higher colonized root lengths than either *S. deserticola* or *G. gigantea* inoculations.

Table 3 Growth parameters and AM fungal root colonization of *O. europaea* seedlings in response to the inoculation with three AMF in two soils differing in texture and calcium carbonate contents ($N = 5$)

	Shoot biomass (g)	Root biomass (g)	Total root length (m)	Colonized root length (m)
Mazarrón				
Control	5.06 ± 091	2.38 ± 0.19	10.4 ± 0.6	0 ± 0
S. deserticola	4.80 ± 1.20	3.47 ± 0.46	12.0 ± 1.5	3.4 ± 0.4
R. irregularis	6.91 ± 0.46	3.20 ± 0.07	15.2 ± 1.3	11.9 ± 1.0
G. gigantea	7.32 ± 0.78	3.32 ± 0.55	13.5 ± 2.9	4.0 ± 0.9
Rellano				
Control	4.48 ± 1.19	4.37 ± 1.12	14.1 ± 4.6	0 ± 0
S. deserticola	9.78 ± 1.07	4.65 ± 0.48	18.2 ± 0.7	6.2 ± 0.2
R. irregularis	11.08 ± 2.53	5.25 ± 0.58	17.0 ± 3.1	12.4 ± 2.3
G. gigantea	12.19 ± 1.11	5.05 ± 0.59	17.7 ± 0.4	4.8 ± 0.1
Anova, P values				
Soil (S)	0.004	<0.001	0.014	NS
Mycorrhizal inoculation (I)	0.001	NS	NS	<0.001
S x I	NS	NS	NS	NS

Significance of effects of soil type, mycorrhizal inoculation, and their interaction on the measured variables is also shown. Mean ± standard error

NS not significance

Discussion

This study demonstrated that different AM fungal species improve stability of soil macroaggregates differently. This finding reaffirms the species specificity of AMF in their potential as aggregators and/or stabilizers of soil structure (Schreiner et al. 1997; Bedini et al. 2009) and underlines the need of selecting the most effective isolate to perform successful tasks of restauration of soil structure. It is worth noting that the major contribution of *R. irregularis* to soil aggregation was independent of soil texture and carbonate contents. Moreover, the improvement in soil aggregate stability in response to inoculation with *R. irregularis* was accompanied by an improvement in the growth of *O. europaea*, compared to non-inoculated plants. Previous studies revealed that AMF species differ in their effectiveness for the stabilization of soil aggregates, presumably as a result of differences in their morphological and physiological traits involved in the aggregation process (Schreiner et al. 1997; Piotrowski et al. 2004; Bedini et al. 2009). In our study, only the inoculation with the *R. irregularis* strain was effective for increasing the percentage of stable aggregates, in spite of the fact that the other AMF strains assayed were also able to form extensive and dense mycelial networks in trap cultures. Furthermore, *R. irregularis* is a species that is highly infective and rapidly colonizes its host plants, which was confirmed by the fact that the levels of AM root colonization were highest in the *O. europaea* plants inoculated with this isolate.

The coexistence of a root system and mycorrhizal mycelial network in the mycorrhizosphere compartment would be expected to provide additive or synergistic effects on soil structural stability. However, the mycorrhizal inoculation with *R. irregularis* was more effective for improving soil aggregate stability in the hyphosphere compartment than in the mycorrhizosphere compartment. This could be due to a lack of significant growth of AM hyphae in the mycorrhizosphere compartment, a consequence of the high root biomass produced by *O. europaea* in all pots. It has been previously pointed out that the effect of AMF on soil aggregation may diminish or disappear with increasing root density (Piotrowski et al. 2004; Leifheit et al. 2014). Thus, the enmeshment of particles by fine roots and the release of root exudates appear to be the dominant mechanism by which aggregates were stabilized in the mycorrhizosphere compartment. The lack of significant hyphal development of *R. irregularis* in the presence of root system suggests that increased aggregate stabilization observed in this compartment is mediated by other mechanisms such as compounds produced by AMF and/or by mycelium-associated bacteria that may stabilize aggregates.

Interestingly, the results obtained in the hyphosphere compartment in the absence of a root system demonstrate that the action of fungal hyphae alone is able to enhance soil aggregate stability. The greater length of the *R. irregularis* hyphae compared to non-inoculated plants may be responsible for the increased aggregate stability in this compartment. However, the greatest production of extraradical mycelium by *S. deserticola* was not effective for increasing stability of macroaggregates. Piotrowski et al. (2004) also found that AMF species with greater production of hyphal biomass yielded lower percentages of stable aggregates. These authors suggested that the differences among AMF species in their influence on soil aggregate stability may be related to mycelium architecture rather than its length. The involvement of fungal hyphae in the formation and stabilization of soil aggregates can be related directly to the hyphal entanglement of loose soil particles and secretion of organic substances that act as glues. The absence of increased labile carbohydrate concentrations in the aggregates of soil inoculated with *R. irregularis*, compared with those of non-inoculated soil, indicates that the increase in soil aggregation was not due to increased secretion of such organic binding agent. In fact, the concentrations of labile carbohydrates were decreased in the aggregates formed in the inoculated soils, indicating that mycorrhizal fungi act as strong sinks for photosynthates (Muthukumar and Udaiyan 2000). Indirect effects of the AMF on soil aggregation may have occurred through changes induced by mycorrhizal inoculation in the composition and activity of rhizosphere microorganisms such as bacteria and non-AM fungi, which also contribute to soil aggregate stabilization (Tisdall 1994). In this study, there was no evidence that increased soil aggregate stability was the outcome of interaction between *R. irregularis* and the microbiota of the rhizosphere of inoculated plants, because soil basal respiration

did not vary with mycorrhizal inoculation compared to non-inoculated plants. Therefore, the improvement of soil structure after the inoculation with *R. irregularis* can be attributed mainly to the mechanical action of its extraradical hyphae. However, the participation of soil bacteria in aggregation process should not be completely discarded because of the AM fungus could have shifted bacterial community composition, which might have promoted a different effect on soil aggregate stability. The involvement of the mycorrhizal mycelium in aggregation processes of sandy soils has been previously evidenced in non-sterile conditions, by inoculation with *Scutellospora calospora* (Degens et al. 1996), and in sterile conditions, by inoculation with *R. irregularis* (Rillig et al. 2010).

Both the coarse- and fine-textured soils displayed similar increases in soil aggregate stability in response to the mycorrhizal inoculation with *R. irregularis*. In the coarse-textured soil, the mycorrhizal hyphae would have contributed mostly by entangling sand grains into aggregates, whereas in the fine-textured soil, the aggregating action of the AMF would have been based on hyphal enmeshment of microaggregates—in which bridging cations, such as Ca^{2+}, and OM participate in the binding of individual mineral particles. The high content of calcium carbonate in the fine-textured soil may also have contributed to its structural stabilization (Virto et al. 2011).

It can be concluded that the efficacy of mycorrhizal inoculation with respect to promoting the formation and stabilization of soil aggregates strongly depended on AMF species, being *R. irregularis* the most effective mycorrhizal fungus. The effect of the AMF on soil aggregation was stronger outside the rhizosphere environment, in the absence of a root system and its biota, being mediated mainly through mechanical entanglement by mycorrhizal fungal hyphae and without a contribution of labile carbohydrates to the aggregation process, although other aggregation mechanisms cannot be discarded. The ability of extraradical hyphae to improve soil structure was independent of soil texture and content of carbonates. The use of AMF species producing long hyphal network does not guarantee an improvement of soil structural stability in restoration programs of eroded semiarid ecosystems. In future investigations, it would be interesting to assess the durability of the aggregates formed by the action of the mycorrhizal fungal mycelium in soils subjected to wetting–drying cycles, simulating the climate conditions of semiarid environments.

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