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Original article

Changes in biological activity of a degraded Mediterranean soil after using microbially-treated dry olive cake as a biosolid amendment and arbuscular mycorrhizal fungi

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ABSTRACT

A field experiment was carried out to assess the effect of a combined treatment involving addition of *Aspergillus niger*-treated dry olive cake (DryOC) in the presence of rock phosphate, plus pre-transplant inoculation of seedlings with the arbuscular mycorrhizal (AM) fungi *Glomus intraradices*, *Glomus deserticola* or *Glomus mosseae*, on the establishment of *Dorycnium pentaphyllum* L., in a degraded semiarid Mediterranean area. Associated changes in soil labile C fractions, enzyme activities and aggregate stability were also observed. One year after planting, the combined treatment of fermented DryOC addition and inoculation with AM fungi, particularly with *G. mosseae* (on average 328% greater than control plants), had the strongest effect on the shoot biomass of *D. pentaphyllum*. Only the fermented DryOC addition increased assimilable P, total N and aggregate stability, the greatest increase being in the soil available P content (about four-fold higher than in the non-amended soil). Both the addition of fermented DryOC and the mycorrhizal inoculation treatments significantly increased enzyme activities of rhizosphere soil (dehydrogenase, protease-BAA, acid phosphatase and β-glucosidase). The microbially-treated DryOC proved to be an effective amendment for improving the soil quality which, in turn, enhanced the success of revegetation with mycorrhizal *D. pentaphyllum* seedlings.

1. Introduction

The main areas of olive oil production are concentrated in the Mediterranean Basin countries, Spain being the greatest producer (about 990,400 tons annually). The main system of olive oil extraction used in Spain (two-phase centrifugation system) generates large quantities of a solid residue called dry olive cake (DryOC) or ''alperujo''. The agronomic utilisation of DryOC has increased steadily in recent years as an alternative nutrient and organic matter source, and as an acceptable method for its disposal. However, the use of DryOC has been seen to have a detrimental effect on seed germination, plant growth and microbial activity [17]. In fact, several studies have reported phytotoxic and antimicrobial effects of olive-mill residues due to the phenol, and organic acid contents [16,17]. Efforts to decrease the environmental impact of

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olive-mill wastes include biological fermentation with filamentous fungi, such as *Aspergillus niger* [30] or white-rot fungi, such as *Phanerochaete flavido-alba* [16]. Such bio-systems involving agrowastes and microorganisms, have been used for rock-phosphate (RP) solubilisation and improvement of crop plant growth and nutrition in agricultural soils [32]. The employment of microbially-treated DryOC as an organic soil amendment, in the presence of rock phosphate, may be a way to improve the quality and productivity of afforested soils of degraded lands in Mediterranean semiarid areas. However, no information is available on the use of such materials in revegetation programs.

Woody legumes are useful for revegetation of semiarid ecosystems that have low availability of N, P and other nutrients, because of their ability to develop symbiotic associations with both rhizobial bacteria and mycorrhizal fungi [3,29]. *Dorycnium pentaphyllum* L. is a low-growing leguminous shrub, which belongs to the natural succession in certain plant communities of semiarid Mediterranean ecosystems in southeast Spain. It is also well-adapted to water stress conditions and, therefore, potentially could be used in the revegetation of semiarid disturbed lands. Under water stress, the mycorrhizal colonisation has been demonstrated to be important for N-nitrate assimilation activity and for mitigating the detrimental effects of drought in *D. pentaphyllum* plants grown in relatively dry soils [4]. However, the knowledge of reafforestation strategies involving *D. pentaphyllum* is still very scarce.

The ability of plants to establish themselves in semiarid and disturbed soils can be improved by colonisation with arbuscular mycorrhizal (AM) fungi [12]. It has been demonstrated that mycorrhizal activity, among other biological processes within the soil-plant system, is clearly diminished by desertification in semiarid Mediterranean ecosystems [1]. Thus, successful reafforestation programs use mycorrhizal inoculum [12]. The establishment of the AM symbiosis also seems to depend on the concentration of phenolic compounds present in the soil [15]. Recently, it has been proven that the effect of phenolic compounds contained in DryOC residues on AM root colonisation can vary with the type of fungi and the time of inoculation [17].

The objectives of this study were: (1) to assess the effectiveness of the combined treatment involving addition of *A. niger*-treated DryOC residue, in the presence of rock phosphate, plus mycorrhizal inoculation of seedlings, for improving the soil quality from a degraded semiarid Mediterranean area, and (2) to determine the influence of such improvements on the establishment of *D. pentaphyllum* seedlings. Soil quality was assessed through measuring labile C fractions (water-soluble C and water-soluble carbohydrates), enzyme activities (dehydrogenase, protease-BAA, acid phosphatase and β-glucosidase) and soil aggregate stability.

2. Materials and methods

2.1. Study sites

The experimental area was located in Los Cuadros in the Province of Murcia (southeast Spain) (coordinates: 1°05′ W and 38°10′ N). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2 °C; the potential evapo-transpiration reaches 1000 mm year^{-1}. The loam soil used was a Typic Haplocalcid [26] developed from Quaternary sediments (Table 1).

2.2. Materials

Dry olive cake (DryOC), a lignocellulosic material obtained from an olive-mill located in Granada (Spain), was dried at 60 °C and then ground to pass a 2-mm-pore screen. The DryOC characteristics were: total N, 1.7%; total organic C, 54%; phenols, 6.5 g kg^{-1}; lipids, 90 g kg^{-1}. Portions of 15 g of DryOC were mixed with 40 ml of Czapek solution (agar 15.0 g L^{-1}; dipotassium hydrogen phosphate 1.0 g L^{-1}; iron(II) sulphate heptahydrate 0.01 g L^{-1}; potassium chloride 0.5 g L^{-1}; magnesium sulphate heptahydrate 0.5 g L^{-1}; sodium nitrate 3.0 g L^{-1}; sucrose 30.0 g L^{-1}; pH 7.3) for static fermentation in 250 ml Erlenmeyer flasks. Rock phosphate (Morocco fluorapatite, 12.8% P, 1 mm mesh), was added at a rate of 0.75 g per flask. This medium was sterilised by autoclaving at 120 °C for 30 min. A spore suspension of *Aspergillus niger* NB2 (1.2×10^7) was spread carefully over the surface of the media. The mixture was allowed to ferment at 30 °C for 20 days without shaking. The characteristics of the DryOC after fermentation were: pH, 4.0; electrical conductivity (1:10), 1231 μS cm^{-1}; total P, 0.38%; total N, 0.62%; total organic C, 22.2%; water soluble C, 1146 μg g^{-1}; phenols, 1.5 g kg^{-1}; lipids, 30 g kg^{-1}.

One representative leguminous shrub from this area, found generally in semiarid shrublands in southeastern Spain, namely *D. pentaphyllum*, was used for the reafforestation experiment.

2.3. Mycorrhizal inoculation of seedlings

The mycorrhizal fungi used in the experiment, *Glomus intraradices* Schenck & Smith (EEZ 1), *Glomus deserticola* (Trappe, Bloss. & Menge) (EEZ 45) and *Glomus mosseae* (Nicol & Gerd.)

Table 1 – Chemical, biochemical, microbiological and physical characteristics of the soil used in the experiment

pH (H_2O)	8.5 ± 0.0[a]
EC (1:5, μS cm^{-1})	225 ± 2
Texture	Loam
Total organic C (g kg^{-1})	10.3 ± 0.3
Total carbohydrates (μg g^{-1})	552 ± 20
Water soluble C (μg g^{-1})	100 ± 1
Water soluble carbohydrates (μg g^{-1})	8 ± 0
Total N (g kg^{-1})	0.95 ± 0.02
Available P (μg g^{-1})	7 ± 0
Extractable K (μg g^{-1})	222 ± 4
Microbial biomass C (μg g^{-1})	396 ± 11
Dehydrogenase (μg INTF g^{-1})	51 ± 1
Urease (μmol NH_3 g^{-1} h^{-1})	0.31 ± 0.03
Protease-BAA (μmol NH_3 g^{-1} h^{-1})	0.60 ± 0.04
Phosphatase (μmol PNP g^{-1} h^{-1})	0.28 ± 0.02
β-Glucosidase (μmol PNP g^{-1} h^{-1})	0.46 ± 0.01
Aggregate stability (%)	11.5 ± 0.4

a Mean ± standard error ($n = 6$).

Gerd. & Trappe (EEZ 43), were obtained from the collection of the experimental field station of Zaidín, Granada.

AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments. Once germinated, seedlings were transplanted into the growth substrate, consisting of peat and cocopeat (1:1, v/v). The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v). The same amount of an autoclaved mixture of the inocula was added to control plants, supplemented with a filtrate (Whatman no. 1 paper) of culture to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and non-inoculated seedlings were grown for 8 months under nursery conditions without any fertiliser treatment. The plants were kept at 30.0 °C/10 °C maximum/minimum temperatures, respectively, and at 85%/40% maximum/minimum relative humidity, respectively. Midday photosynthetically active radiation averaged 240 $\mu E\, m^{-2}\, s^{-1}$. The plants were watered regularly with tap water.

2.4. Experimental design and layout

A factorial design in randomised blocks was established with two factors and five-fold replication. The first factor was the addition of fermented DryOC residue to the soil, and the second was the inoculation of *D. pentaphyllum* plants with three AM fungi (*G. intraradices*, *G. deserticola* or *G. mosseae*) in the nursery.

Each replication block was 180 m^2 (12.5 × 14.5 m). Planting holes 40 × 40 cm^2 and 30 cm deep were dug manually. In early February 2006, fermented DryOC residue was added to half of the holes (0–20 cm depth) and mixed manually with the soil coming from hole at a rate of 5% (w/w). The seedlings (inoculated and non-inoculated) were planted at least 1 m apart, one in each hole, with 3 m between blocks. At the very least 64 seedlings per block were planted (eight plants × eight treatments in each block).

2.5. Sampling procedures

One year after planting, five soil samples (one per block) were collected from each treatment (40 soil samples in total). Each sample consisted of eight bulked subsamples (200 cm^3 soil cores), collected randomly at 0–20 cm in the rhizospheres of eight individual plants. To collect the rhizosphere soil the root system with rhizosphere soil adhered was introduced into a plastic bag, shook and separated the rhizosphere soil from the root system. The sampling was carried out in early April (before the dry season) when the highest microbial activity would be expected [14]. Five plants of each treatment (one per block) were harvested 1 year after planting, thus coinciding with the end of the flowering stage.

2.6. Plant analyses

One year after planting, basal stem diameters and heights of plants were measured, fresh and dry (105 °C, 5 h) mass of shoots and roots were recorded. The percentage of root length colonised by arbuscular mycorrhizal fungi was calculated by the gridline intersect method [11] after staining with trypan blue [21].

2.7. Soil physico-chemical, chemical and biochemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. In soil aqueous extracts, water-soluble carbon was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm [25]. Water-soluble carbohydrates and total carbohydrates were determined by the method of Brink et al. [2]. Total nitrogen was determined by ammonia distillation after Kjeldahl digestion. Available P, extracted with 0.5 M $NaHCO_3$, was determined by colorimetry according to [18].

Dehydrogenase activity was determined according to García et al. [10]. For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through Whatman no. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

N-α-Benzoyl-L-argininamide (BAA) hydrolysing protease activity was determined in 0.1 M phosphate buffer at pH 7; 0.03 M BAA was used as substrate. Two ml of buffer and 0.5 ml of substrate were added to 0.5 g of sample, which was incubated at 39 °C for 90 min. The activity was determined as the NH_4^+ released in the hydrolysis reaction [19].

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two millilitres of 0.5 M sodium acetate buffer at pH 5.5 using acetic acid [20] and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm [27]. Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. This assay is based on the release and detection of *p*-nitrophenyl (PNP). Two millilitres of 0.1 M maleate buffer pH 6.5 and 0.5 ml of substrate was added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with Tris-hydroxymethyl aminomethane (THAM) according to [28]. The amount of *p*-nitrophenol was determined by spectrophotometry at 398 nm [27].

2.8. Physical analysis

The percentage of stable aggregates was determined by the method described in [13]. A 4 g aliquot of soil sieved between 0.2 and 4 mm was placed on a small 0.250 mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 ml with energy of 270 $J\, m^{-2}$. The remaining soil on the sieve was placed in a previously weighed capsule (T), dried at 105 °C and weighed (P1). Then the soil was soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve,

which was made up of plant debris and sand particles, was dried at 105 °C and weighed (P2). The percentage of stable aggregates with regard to the total aggregates was calculated by (P1 − P2) × 100/(4 − P2 + T).

2.9. Statistical analysis

Data were log transformed to achieve normality. The effects of fermented DryOC addition, mycorrhizal inoculation and their interactions on measured variables were tested by a two-way analysis of variance, and comparisons among means were made using Tukey's test, calculated at $P < 0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

3. Results

3.1. Physico-chemical parameters

Only the addition of fermented DryOC slightly decreased soil pH and increased soil electrical conductivity (Tables 2 and 3). However, neither mycorrhizal inoculation nor the interaction of fermented residue x mycorrhizal inoculation had any significant effect on soil physico-chemical parameters (Table 3).

Assimilable P and total N contents were higher in the amended soil than in the soils from the mycorrhizal inoculation treatments and the control soil (Table 2). The greatest increase in response to the addition of the amendment was observed in the soil available P content (about four-fold higher than in the non-amended soil). It is worth noting that the addition of amendment increased the percentage of stable aggregates by about 66% regardless of AM.

3.2. Biochemical parameters

Water-soluble C and water-soluble carbohydrate values were increased only with the addition of the amendment (Tables 3 and 4), the greatest increases being observed in the water-soluble C fraction (on average, about 43% greater than non-amended soil).

Rhizosphere soil from the amendment and mycorrhizal inoculation treatments had significantly higher enzyme activities (dehydrogenase, protease-BAA, acid phosphatase and β-glucosidase) than the control soil (Table 4). All the biochemical parameters were similar (for dehydrogenase, acid phosphatase and protease-BAA activities) or even higher (for β-glucosidase activity) in the soils from the mycorrhizal inoculation treatments, compared to amended soil. In general, the combined treatments, involving mycorrhizal inoculation of seedlings with either of the AM fungi and addition of fermented DryOC to soil, changed the biochemical parameters of the rhizosphere soil to an equal or lesser extent than each treatment applied separately.

3.3. Growth and mycorrhizal infection of *D. pentaphyllum*

At the time of planting, the shoot dry weight of inoculated *D. pentaphyllum* plants was slightly greater than for non-inoculated plants (Fig. 1A). One year after planting, both addition of fermented DryOC and the mycorrhizal inoculation treatments had stimulated significantly the production of shoot biomass of *D. pentaphyllum* with respect to the control plants (Fig. 1A and Table 3). Mycorrhizal inoculation treatments increased plant growth to a greater extent than addition of the fermented residue, although their combination promoted even higher plant growth (Table 3). The combined treatment of fermented DryOC addition and inoculation with *G. mosseae* had the strongest effect on shoot biomass (on average, 328% greater than control plants).

The inoculated plants had an average of 77%, 60% and 61% of their short lateral roots colonised by *G. intraradices*, *G. deserticola* and *G. mosseae*, respectively, prior to planting in the field (Fig. 1B). The roots of the non-inoculated plants were not colonised by mycorrhizal fungi. One year after planting, inoculation with *G. intraradices*, *G. deserticola* or *G. mosseae* had significantly enhanced AM colonisation, similar levels of root colonisation being reached in all the mycorrhizal inoculation treatments. The natural colonisation produced about 25% of mycorrhizal colonisation in the roots of non-inoculated seedlings.

Table 2 – Physical-chemical properties of rhizosphere soil of *D. pentaphyllum* in response to mycorrhizal inoculation treatments and fermented DryOC addition 1 year after planting ($n = 5$)

	pH (H_2O)	EC (1:5, μS cm^{-1})	Total N (g kg^{-1})	Avail. P (μg g^{-1})	Aggregate stability (%)
C	8.73 ± 0.04d	302 ± 6a	0.70 ± 0.01a	1.5 ± 0.1a	17.8 ± 0.8a
A	8.53 ± 0.01abc	361 ± 6b	1.06 ± 0.02d	5.5 ± 0.1c	30.3 ± 0.5b
G1	8.68 ± 0.01cd	323 ± 7ab	0.73 ± 0.00a	1.6 ± 0.1a	14.0 ± 0.6a
AG1	8.59 ± 0.01abcd	346 ± 3ab	0.97 ± 0.03bcd	4.0 ± 0.1b	30.6 ± 0.4b
G2	8.64 ± 0.02bcd	324 ± 6ab	0.82 ± 0.01ab	1.9 ± 0.1a	14.0 ± 0.4a
AG2	8.52 ± 0.01ab	340 ± 5ab	0.96 ± 0.03bcd	4.7 ± 0.3bc	27.9 ± 0.6b
G3	8.66 ± 0.01bcd	326 ± 7ab	0.83 ± 0.01abc	1.8 ± 0.1a	15.4 ± 0.4a
AG3	8.47 ± 0.01a	353 ± 4ab	1.02 ± 0.02cd	4.4 ± 0.1bc	29.5 ± 0.6b

C, control; A, fermented DryOC addition; G1, inoculation with *G. intraradices*; AG1, fermented DryOC addition and inoculation with *G. intraradices*; G2, inoculation with *G. deserticola*; AG2, fermented DryOC addition and inoculation with *G. deserticola*; G3, inoculation with *G. mosseae*; AG3, fermented DryOC addition and inoculation with *G. mosseae*.
Mean ± standard error. Values in columns followed by the same letter are not significantly different (Tukey, $P < 0.05$).

Table 3 – Two factor ANOVA (mycorrhizal inoculation treatments and fermented DryOC addition) for all parameters studied in the rhizosphere soil of *D. pentaphyllum* seedlings 1 year after planting

	Amendment (A)	Mycorrhiza (M)	Interaction (A × M)
	df = 1	df = 3	df = 3
pH	45.7 (<0.001)	2.5 (0.082)	1.3 (0.283)
Electrical conductivity	4.5 (0.045)	2.3 (0.105)	1.1 (0.102)
Total N	65.6 (<0.001)	1.2 (0.332)	2.9 (0.057)
Available P	203.7 (<0.001)	2.4 (0.095)	3.2 (0.043)
Aggregate stability	324.8 (<0.001)	2.6 (0.074)	1.2 (0.342)
Water soluble C	124.0 (<0.001)	1.2 (0.339)	0.9 (0.424)
Water soluble carbohydrates	5.5 (0.028)	2.8 (0.064)	4.1 (0.018)
Dehydrogenase	83.0 (<0.001)	22.3 (<0.001)	4.0 (0.019)
Protease	7.1 (0.014)	7.1 (0.001)	17.9 (<0.001)
Phosphatase	4.6 (0.041)	7.5 (0.001)	7.0 (0.001)
β-Glucosidase	17.8 (<0.001)	13.3 (<0.001)	14.6 (<0.001)
Shoot	718.2 (<0.001)	699.9 (<0.001)	5.4 (0.002)
Colonisation	0.2 (0.649)	82.3 (<0.001)	0.3 (0.853)

F values (*P* significance values).

4. Discussion

4.1. *Effectiveness of fermented DryOC residue addition*

The results of this study demonstrate the viability of applying the fermented DryOC residue in the presence of rock phosphate in order to improve the growth of *D. pentaphyllum*. Likewise, the absence of a negative effect of this fermented residue on the growth of *D. pentaphyllum* could be related to the elimination of the phytotoxic substances contained in the DryOC during the fermentation process. In this same area, we have recently reported that the addition of sugar beet, rock phosphate and *Aspergillus niger* directly into the planting hole was less effective (about 33% greater than control plants) for enhancing the performance of *D. pentaphyllum* than the fermented DryOC residue (about 113% greater than control plants) used in this study [6]. The effectiveness of fermented DryOC with respect to stimulation of plant growth could be due to an improvement in the available nutrient supply in soil. During the course of *A. niger* fermentation, the rock phosphate solubilises, increasing the level of bio-available P in the DryOC [31]. Thus, the use of the fermented DryOC improved soil productivity, increasing the soil nutrient status for several potentially limiting nutrients, such as N and P. However, the benefits of organic amendments are due also to the improvement of the physical characteristics of the soil, which in turn favours the establishment and viability of a stable plant cover [5,8,24]. In this regard, fermented DryOC improved the structural stability of rhizosphere soil of *D. pentaphyllum* to a statistically significant extent. Roldán et al. [24] found that the restoration of soil structure may depend on the amount and nature of the organic matter added. The biological transformations that the DryOC underwent during fermentation can increase the quantity of aggregate-stabilising agents, such as water-soluble C and water-soluble carbohydrates. In particular, we recorded noticeable increases in the contents of water-soluble C as consequence of the addition of fermented DryOC. The water-soluble organic matter fraction consists of a heterogeneous mixture of components of varying molecular weight, such as

Table 4 – Carbon fractions and enzyme activities of rhizosphere soil of *D. pentaphyllum* in response to mycorrhizal inoculation treatments and fermented DryOC addition 1 year after planting ($n = 5$)

	Water soluble C ($\mu g\ g^{-1}$)	Water soluble carbohydrates ($\mu g\ g^{-1}$)	Dehydrogenase (μg INTF g^{-1} soil)	Protease ($\mu mol\ NH_3\ g^{-1}\ h^{-1}$)	Phosphatase (μmol PNP $g^{-1}\ h^{-1}$)	β-Glucosidase (μmol PNP $g^{-1}\ h^{-1}$)
C	141 ± 2a	9 ± 0a	67.8 ± 1.1a	0.26 ± 0.01a	0.48 ± 0.03a	0.31 ± 0.01a
A	206 ± 1b	12 ± 1b	85.5 ± 0.9bc	0.69 ± 0.01b	0.91 ± 0.03abc	0.42 ± 0.01abc
G1	137 ± 3a	11 ± 1ab	78.5 ± 2.0ab	0.53 ± 0.02b	1.10 ± 0.03bc	0.54 ± 0.02de
AG1	191 ± 2b	10 ± 0ab	97.0 ± 0.8c	0.70 ± 0.02b	0.79 ± 0.06ab	0.37 ± 0.02ab
G2	119 ± 2a	10 ± 1ab	83.5 ± 1.4b	0.70 ± 0.05b	1.32 ± 0.03c	0.50 ± 0.01 de
AG2	198 ± 4b	11 ± 1ab	90.5 ± 0.9bc	0.51 ± 0.02b	0.87 ± 0.02abc	0.43 ± 0.01bcd
G3	132 ± 1a	9 ± 0a	85.7 ± 1.2bc	0.71 ± 0.02b	1.22 ± 0.11bc	0.58 ± 0.01e
AG3	210 ± 4b	9 ± 0a	110.8 ± 1.8d	0.65 ± 0.01b	0.97 ± 0.07bc	0.43 ± 0.01bcd

C, control; A, fermented DryOC addition; G1, inoculation with *G. intraradices*; AG1, fermented DryOC addition and inoculation with *G. intraradices*; G2, inoculation with *G. deserticola*; AG2, fermented DryOC addition and inoculation with *G. deserticola*; G3, inoculation with *G. mosseae*; AG3, fermented DryOC addition and inoculation with *G. mosseae*.
Mean ± standard error. Values in columns followed by the same letter are not significantly different (Tukey, $P < 0.05$).

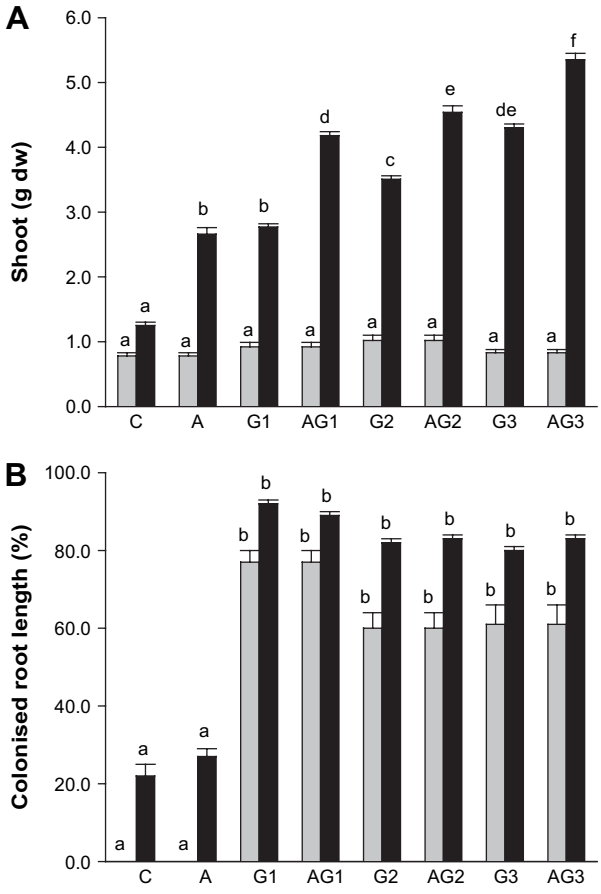

Fig. 1 – Shoot dry weight and AM colonisation of roots of *D. pentaphyllum* seedlings in response to mycorrhizal inoculation and fermented DryOC addition prior to planting and 1 year after planting. For each sampling date, values in columns followed by the same letter are not significantly different (Tukey, $P < 0.05$). C, control; A, fermented DryOC addition; G1, inoculation with *G. intraradices*; AG1, fermented DryOC addition and inoculation with *G. intraradices*; G2, inoculation with *G. deserticola*; AG2, fermented DryOC addition and inoculation with *G. deserticola*; G3, inoculation with *G. mosseae*; AG3, fermented DryOC addition and inoculation with *G. mosseae*. Bars represent standard errors.

mono- and polysaccharides, polyphenols, proteins and low molecular weight organic acids. This fraction can be used as carbon and energy sources by soil microflora [23] and can be related positively to microbial activity [9]. Increased biological activity was also revealed by the variations in dehydrogenase and hydrolases activities. Application of organic amendment to soil can increase dehydrogenase activity, which has been used frequently as an indicator of soil microbial activity [10], while the processes related to the degradation of organic matter by microbial activity may be followed by measuring hydrolases [7]. It is worth noting that this type of residue is, in the short term, more effective than other residues, such as sewage sludge and urban waste, widely used for improving soil structure under semiarid Mediterranean conditions [5]. The fermentation of DryOC with *A. niger* can be considered an effective method of preparation of this residue for its use as a soil amendment, since the application of non-fermented DryOC to the soil has been seen to have detrimental effects on the physical, chemical and microbiological quality of soil [22]. However, the fermentation process include difficulties related to operation control and scaling-up constraints and for this reason the use of the fermented DryOC in revegetation programs cannot be considered still as a viable alternative for its disposal.

4.2. *Effectiveness of the mycorrhizal inoculation treatments*

Inoculation with any of the AM fungi tested proved to be an effective means of encouraging *D. pentaphyllum* seedling growth. Mycorrhizae increase nutrient uptake, especially of P and N, by providing a larger absorbing surface, favour root system development and produce substances that promote seedling growth [12]. It is important to emphasise that mycorrhizal inoculation treatments with *G. deserticola* or *G. mosseae* on their own were even more effective than the addition of fermented DryOC alone in improving the performance of *D. pentaphyllum* plants under semiarid conditions (during the course of the experiment, only 130 mm of rainfall were recorded). These results reaffirm the key role of mycorrhizae in sustaining the plant cover in P-deficient soils, as well as showing the necessity of including mycorrhizal inoculation to guarantee plant performance in revegetation programmes. The mycorrhizal inoculation treatments showed different levels of effectiveness with respect to their ability to improve the performance of *D. pentaphyllum* seedlings. *G. mosseae* was the most effective to increase plant growth. However, this was not related to the extent of mycorrhizal infection, because all inoculated plants presented high infection rates in their roots and there were no differences between mycorrhizal treatments.

4.3. *Effectiveness of combined treatment*

This experiment shows that the combination of fermented DryOC and mycorrhizal inoculation can improve considerably the growth of *D. pentaphyllum* in semiarid conditions. This result contrasts with the widely accepted idea that mycorrhizae present little advantage to seedlings grown in fertilised soils [33]. The rapid growth of seedlings inoculated with AM fungi, as compared with the uninoculated seedlings, in the amended soil might be related to the capacity of the fungus to increase phosphate uptake from fermented DryOC. AM fungi also may facilitate the action or transfer of toxic substances to plants [17]. However, there was no negative effect of fermented DryOC on the growth of AM colonised plants. These results reinforce the hypothesis that the phytotoxicity of DryOC decreases during the fermentation process.

The addition of fermented DryOC, the inoculation with AM fungi and the combination of both treatments had no significant effect on plant survival. In the first stages of growth (1 year after planting), which are the most critical for revegetation in Mediterranean semiarid areas, plant survival ranged from 60% to 75% for all treatments and there were no significant differences between them.

We can conclude that the microbially-treated DryOC proved to be an effective amendment for improving the soil quality which, in turn, enhanced the success of revegetation carried out with *D. pentaphyllum*. The use of arbuscular mycorrhizal fungi in combination with the fermented DryOC increased seedling resistance to the inhospitable environmental conditions.

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