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The effectiveness of arbuscular-mycorrhizal fungi and *Aspergillus niger* or *Phanerochaete chrysosporium* treated organic amendments from olive residues upon plant growth in a semi-arid degraded soil

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi and a residue from dry olive cake (DOC) supplemented with rock phosphate (RP) and treated with either *Aspergillus niger* (DOC-A) or *Phanerochaete chrysosporium* (DOC-P), were assayed in a natural, semi-arid soil using *Trifolium repens* or *Dorycnium pentaphyllum* plants. The effects of the AM fungi and/or DOC-A were compared with P-fertilisation (P) over eleven successive harvests to evaluate the persistence of the effectiveness of the treatments. The biomass of dually-treated plants after four successive harvests was greater than that obtained for non-treated plants or those receiving the AM inoculum or DOC-A treatments after eleven yields. The AM inoculation was critical for obtaining plant growth benefit from the application of fermented DOC-A residue. The abilities of the treatments to prevent plant drought stress were also assayed. Drought-alleviating effects were evaluated in terms of plant growth, proline and total sugars concentration under alternative drought and re-watering conditions (8th and 9th harvests). The concentrations of both compounds in plant biomass increased under drought when DOC-A amendment and AM inoculation were employed together: they reinforced the plant drought-avoidance capabilities and anti-oxidative defence. Water stress was less compensated in P-fertilised than in DOC-A-treated plants. DOC-P increased *D. pentaphyllum* biomass, shoot P content, nodule number and AM colonisation, indicating the greater DOC-transforming ability of *P. chrysosporium* compared to *A. niger*. The lack of AM colonisation and nodulation in this soil was compensated by the application of DOC-P, particularly with AM inoculum. The management of natural resources (organic amendments and soil microorganisms) represents an important strategy that assured the growth, nutrition and plant establishment in arid, degraded soils, preventing the damage that arises from limited water and nutrient supply.

1. Introduction

The main characteristics of arid and semi-arid zones are the limited vegetation cover and soil degradation. Thus, the establishment of plant species is considered an effective strategy for preventing soil disturbance and erosion and enhancing soil quality. Moreover, it has been shown that soil microorganisms play an important role in the establishment of vegetation cover and contribute to the improvement of soil/plant conditions (Barea and Jeffries, 1995).

In this respect, inoculation with arbuscular mycorrhizal (AM) fungi has been recommended for reclaiming degraded areas because they are important for improving plant establishment, promoting plant growth and nutrition (Azcón and Barea, 1997; Barea et al., 1990a, 1990b). Nevertheless, when the soil is highly degraded, the inoculation of plants with these beneficial microorganisms may not be enough to achieve the restoration of the vegetation cover. For this reason, in order to improve soil properties, the application of organic amendments to the soil, prior to the inoculation of AM fungi, has been recommended (Medina et al., 2004).

Large amounts of agrowastes such as dry olive cake (DOC) are produced during the extraction of oil from the olive fruit. This product poses serious environmental problems due to its content of phenolic compounds. Moreover, it has been shown that the direct application of DOC to the soil has detrimental effects on plant growth (Vassileva et al., 1998; Sampedro et al., 2008). Nevertheless, it can be used as an organic amendment after biological

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transformation. Because of its lignocellulosic composition, DOC can be mineralised during a fermentation process by specific lignocellulolytic microorganisms such as *Aspergillus niger* or *Phanerochaete chrysosporium*, resulting in a substrate rich in simple sugars and minerals (N and P particularly) that can be used as energy sources by heterotrophic microorganisms (Puppi et al., 1994). In addition, the fertiliser potential of such agrowastes can be increased if RP is supplied to the medium prior to the fermentation process (Rodríguez et al., 1999). The RP solubilisation is carried out by the acid produced by *A. niger* or *P. chrysosporium* growing on agro-waste residues such as DOC. *A. niger* NB2 has been shown to produce citric acid when growing on complex substrates (Vassilev et al., 1986) and to mineralise lignocellulosic materials (Vassilev et al., 1998). The addition of the fermented-DOC to soil results in an improvement of plant nutrition and growth, as well as soil fertility, especially when it is applied together with beneficial soil microorganisms such as AM fungi (Medina et al., 2005; Vassilev et al., 2006).

It is accepted that symbiotic microorganisms such as AM fungi are very effective in enhancing the ability of plants to become established and to cope with stress situations like drought and nutrient limitation by improving nutrients uptake and water relations (Ruíz-Lozano and Azcón, 1995, 1996; Ruíz-Lozano et al., 1995). The effect of AM colonisation on drought tolerance may be ascribed to various mechanisms such as nutritional and physiological plant improvements (Ruíz-Lozano et al., 1995), direct water uptake (Ruíz-Lozano and Azcón, 1995) and changes in soil structure in terms of the quantity and quality of aggregate stability (Medina et al., 2004). Moreover, it has been shown that the combination of *Glomus intraradices* with DOC-A significantly increased the structural stability of the rhizosphere soil of *Juniperus oxycedrus* (Caravaca et al., 2006). One important mechanism affecting water availability in soil is aggregate stabilisation, which is based on the interconnection of soil particles by fungal mycelia, roots and polysaccharides (Bearden and Petersen, 2000). Then, an increase in soil aggregate stability could help the plant to tolerate drought conditions.

Previous studies have used DOC-A as an amendment, associated with AM fungi in reclamation strategies for sustainable systems (Vassilev et al., 2006; Medina et al., 2010). However, no information on the persistence of the efficiency of this amendment with time (through successive harvests) is available so far. Likewise, it is unknown whether, in addition to its ability to replace mineral fertilisers in nutritional terms, the application of AM fungi and/or DOC-A may increase water uptake and drought tolerance by plants. These aspects are the logical next step of interest for the practical application of this treated agro-waste and selected microbial groups in the revegetation of semi-arid sites.

In this study, two experiments were performed under similar experimental conditions. In both experiments we used the same natural soil from a semi-arid site and the same AM fungi inoculum. In the first experiment, the effectiveness of DOC-A, in the presence or absence of an indigenous AM fungi inoculum, was analysed along successive yields. The comparative effects of phosphorus fertilisation and DOC-A amendment, associated or not with AM inoculum, were studied as well. The aim of this first experiment was to determine if the positive effect of the applied treatments on plant growth persisted with time. For that *Trifolium repens* was used as a representative legume test plant, since it allows successive yields. In addition, we tested how the applied treatments performed under stress conditions. For this purpose, alternative well-watered (8th harvest) and drought-stress conditions (9th harvest) were imposed to determine the effectiveness of the applied treatments in alleviating drought stress. The osmolytes proline and total sugars were used as indexes of drought avoidance and of the antioxidant plant defence response. We also evaluated the plant capacity for recovery after the re-watering period (10th and 11th harvests).

In the second experiment, we were interested in determining the effect of DOC as an amendment when it was fermented with *P. chrysosporium.* This fungus has greater lignocellulolytic abilities than *A. niger* (Vassilev et al., 2006). *Dorycnium pentaphyllum,* a woody legume used in revegetation programmes, was the test plant in this subsequent study.

2. Materials and methods

2.1. Preparation of the fermented-DOC amendments

DOC was used as substrate in the fermentation trials. Its characteristics were: cellulose 18%, hemicellulose 16% and lignin 26%; total C, total N and total P, 464, 11 and 0.6 g kg^{-1} dw, respectively.

DOC was oven-dried at 60 °C and then ground to pass a 2-mm-pore sieve. Portions of 15 g of the resulting substrate were placed in 250-ml Erlenmeyer flasks giving a total of 20 flasks. Distilled water (40 ml) and RP (0.75 g) (Morocco fluorapatite, 12.8% soluble P, 1-mm mesh) were added to each flask. All flasks containing the described media were sterilised by autoclaving at 120 °C for 30 min.

The strain of *A. niger* NB2, used in the first experiment, was maintained on potato-dextrose agar slants at4 °C. For inoculum preparation, *A. niger* was grown on a slant at 30 °C for 7 days and spores were scraped in sterile, distilled water. A spore suspension of *A. niger* (1.2×10^7 spores ml^{-1}) was added to each flask. The amount of spores was determined by optical density measurement at 750 nm, following calibration of this data according to direct haemocytometer counting.

The strain of *P. chrysosporium*, used in the second experiment, was maintained on malt-extract plates at 4 °C. For the DOC treatment process, *P. chrysosporium* was incubated at 26 °C for 7 days, then a spore suspension for inoculation was prepared by dislodging spores from the plate surface in sterile, distilled water. The spore number was measured as described for *A. niger*. A spore suspension of *P. chrysosporium* (2.3×10^6 spores ml^{-1}) was spread carefully on the flask over the surface of the DOC.

Solid-state DOC fermentation [performed with DOC supplemented with RP inoculated with *A. niger (*DOC-A) (14 flasks, first experiment) or with *P. chrysosporium (*DOC-P) (six flasks, second experiment)] were carried out at 30 °C for 20 days.

2.2. Soil-plant experiments

Treatments in the first experiment were as follows: (i) C (control, original soil without amendment), (ii) DOC-A (soil amended with *A. niger*-treated DOC), (iii) P (soil fertilised with P). These treatments were inoculated or not with 3 indigenous AM fungi. Each treatment had 5 replicates giving a total of 30 pots. Treatments in the second experiment were as follows: (i) C (control, original soil without amendment), (ii) DOC-P (soil amended with *P. crhysosporium*-treated DOC), (iii) DOC-P+M (soil amended with *P. chrysosporium*-treated DOC and inoculated with AM fungi). Each treatment had 5 replicates giving a total of 15 pots.

One portion of soil was not amended and it was used as the control in both experiments, while the other part of the soil was amended with either DOC-A or DOC-P in the first and second experiment respectively.

The topsoil (0–20 cm) from a semi-arid zone in Murcia province (Spain) was used. The main soil characteristics were pH 8.90, P 1.36 μg^{-1} g (Olsen test), organic carbon 0.94%, total N 0.22% and an electric conductivity of 1.55 dS m^{-1} (Rhoades, 1982).

The products, *A. niger*- or *P. chrysosporium*-treated DOC (DOC-A and DOC-P respectively), from each Erlemmeyer flask, were mixed with 500 g soil-sand mixture (5:2, v/v) per pot and left for equilibration for 4 weeks at room temperature. These treated DOC/soil proportion was stated as the most accurate in previous experiments (Vassilev et al., 1998).

Non-amended control pots received an amount of RP equivalent to that added to the fermentation products (0.75 g/500 g soil). For P-treatments, phosphate was supplied as KH_2PO_4 at 0.01 M (34 mg P) in 65 ml per pot (5 ml × 17 times).

Four seedlings of *T. repens* (first experiment) or one seedling of *D. pentaphyllum* (second experiment) were transplanted in each pot (d = 12.2 cm; 500-g capacity; 5 pots per treatment).. In both experiments, pots were inoculated or not with a mixture of three indigenous AM fungi [*Glomus mosseae* (T. H. Nicolson & Gerd.) Gerd. & Trappe (EEZ-43); *Glomus coronatum* Giovann (EEZ-44) and *Glomus claroideum* N. C. Schenck & G. S. Sm. (EEZ-47)]. These mycorrhizal fungi were isolated and selected from the desertified soil of the Mediterranean area used in this study (Murcia province, Spain) and identified morphologically. They were bulked separately in an open-pot culture of red clover, and used as a stock culture. From a mixture (1:1:1) of the three stock cultures, the mycorrhizal inoculum was obtained. It consisted of spores, mycelia and mycorrhizal root fragments. Ten grams of inoculum mixture per pot, having similar characteristics (an average of 30 spores per g and roots with 75% AM colonisation), were applied to each of the corresponding pots (M treatments), in the bottom of a 5-cm-deep hole. Plants were grown in a greenhouse under a day/night cycle of 16/8 h, 21/15 °C and 50% relative humidity. The photosynthetic photon flux density (PPFD) was 503 μmol/m^2/s, as measured with a light-meter (LICOR, model LI-188B). Water loss was compensated by watering every day to reach 100% or 75% (only for the 9th harvest) of water-holding capacity (WHC). The level of water stress (75% WHC) was selected and applied as described in previous studies (Ruíz-Lozano et al., 1995, 1996).

2.3. Analytical methods

Shoots of *T. repens* were harvested for the first time, two months after transplanting the plants, and every month for the successive harvests. For *D. pentaphyllum*, only one harvest, two months after transplanting, was performed. Shoot dry weight was recorded after drying at 70 °C, at the end of the experiment, 12 months (*T. repens*, first experiment) or two months (*D. pentaphyllum*, second experiment) after the transplanting. At the end of the experiment root fresh weight was recorded.

The percentage mycorrhizal root length was determined by microscopic examination of stained root samples (Phillips and Hayman, 1970), using the gridline intersect method of Giovannetti and Mosse (1980). This AM colonisation was evaluated in an aliquot (central part of the whole root system) of the total root. And the nodule numbers were assessed visually on washed roots before to select the aliquot to determine AM root infection.

At the 8th (well-watered) and 9th (drought stress) harvests, free proline and total soluble sugars (TSS) were extracted from 1 g of fresh leaves as described by Bligh and Dyer (1959). The methanolic phase was used for quantification of both substances. Proline was estimated by spectrophotometric analysis at 515 nm, following the ninhydrin reaction, according to Bates et al. (1973). The TSS were analysed by reacting 0.1 ml of methanolic extract with 3 ml of freshly-prepared anthrone [200 mg anthrone + 100 ml of 72% (w/w) H_2SO_4] in a boiling-water bath for 10 min, according to Irigoyen et al. (1992). After cooling, the absorbance at 620 nm was determined in a Shimadzu UV-1603 spectrophotometer (Shimadzu, Kyoto, Japan). A calibration curve was prepared using glucose in the range of 20–400 μg/ml. The concentration of P in *D. pentaphyllum* shoots was measured according to Olsen and Dean (1965).

2.4. Statistical analysis

The data were subjected to an analysis of variance (ANOVA). The mean values of 5 replicates (in the first experiment and the second experiment) were compared using Duncan's multiple range test at a level of confidence of 95% (Duncan, 1955). Statgraphics Plus for Windows was used to perform the statistical test. Percentage values were arcsine-transformed before statistical analysis.

3. Results

In the first experiment, plant growth was affected positively by P-fertilisation and non −significantly affected by application of DOC-A (Fig. 1). In this natural arid soil, mycorrhizal inoculum alone resulted ineffective in improving plant biomass (Fig. 1). Nevertheless, AM inoculation was a critical factor for obtaining the maximum benefit from DOC-A or P-fertilizer application (Fig. 1). In fact, the greatest plant biomass was harvested from pots receiving DOC-A plus AM inoculum, and the effectiveness of this dual treatment was maintained over eleven successive harvests (Fig. 1). Moreover, the total plant biomass yielded in the first four harvests of these plants was greater than that obtained over the 11 successive yields for non-treated control, single AM-inoculated or DOC-A-amended plants.

In Fig. 2, the shoot weights of the 8th, 9th, 10th and 11th harvests are shown. Similar plant growth responses to the applied treatments were observed in each harvest, irrespective of the lower soil water content (75% WHC) at the 9th harvest. Moreover, in the last two harvests (the 10th and 11th), corresponding to re-watering after the drought period (9th harvest), DOC-A/AM- inoculated plants produced the greatest shoot biomass, contrasting with the previous 8th and 9th harvests where the interaction in AM- inoculated plants between DOC-A and P fertiliser produced similar growth responses (Fig. 2). In fact, at harvests 10 and 11, the shoot biomass of DOC-A/AM- inoculated plants was 66% and 65%, higher than for P-fertilised AM-inoculated plants respectively (Fig. 2), indicating a better plant recovery (in terms of plant growth) following the drought period (9th harvest) (Fig. 2). The comparative effects of these two treatments (DOC-A and P-fertilisation) regarding accumulation of proline and total sugars at 100% WHC (8th harvest) and 75% WHC (9th harvest) demonstrate that both types of osmoregulatory compounds were accumulated to a greater extent in DOC-A/AM-inoculated plants, independent of the water content of the medium (Fig. 3). Furthermore, under drought

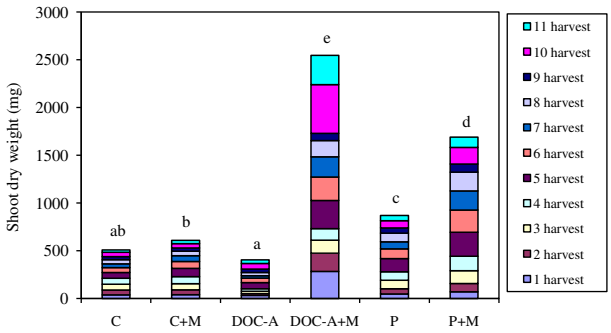

Fig. 1. Shoot dry weight (mg) of non-mycorrhizal fungal inoculated (C) or mycorrhizal fungal inoculated (M) *Trifolium repens* amended or not with *Aspergillus niger* treated dry olive cake (DOC-A) or PO_4^{3-}-fertilizer (P). Values after eleven successive harvests. Means (of five replicates) not followed by a common letter differ significantly ($P < 0.05$).

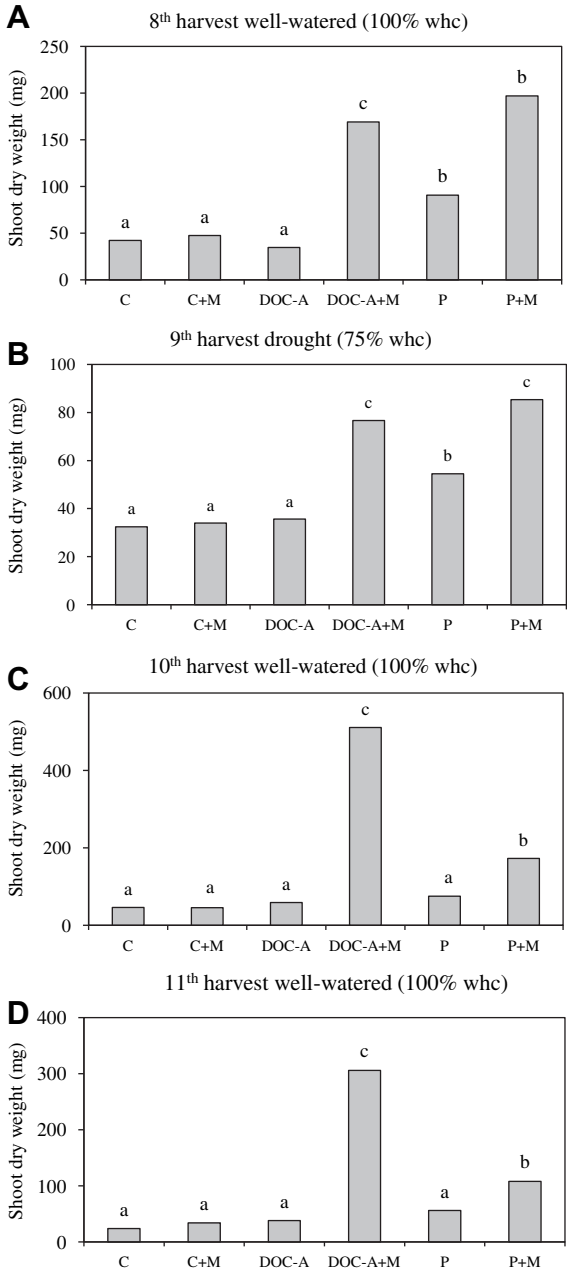

Fig. 2. Shoot dry weight (mg) of non-mycorrhizal fungal inoculated (C) or mycorrhizal fungal inoculated (M) *Trifolium repens* amended or not with *Aspergillus niger* treated dry olive cake (DOC-A) or PO_4^{3-}-fertilised (P). Values corresponding to 8th, 10th and 11th harvest under well-watered or under drought (9th harvest) conditions. Means (of five replicates) not followed by a common letter differ significantly ($P < 0.05$).

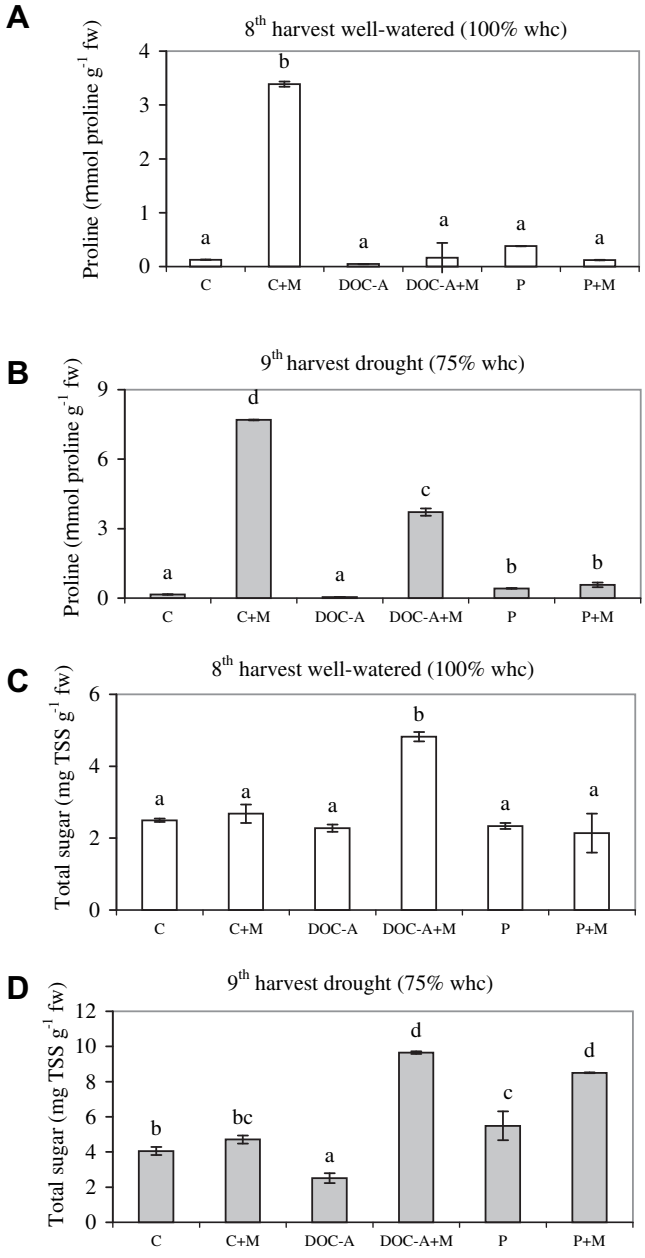

Fig. 3. Proline and total sugars accumulation of non-mycorrhizal fungal inoculated (C) or mycorrhizal fungal inoculated (M) *Trifolium repens* amended or not with *Aspergillus niger* treated dry olive cake (DOC-A) or PO_4^{3-}-fertilizer (P). Values corresponding to 8th harvest (under well-watered, 100% whc) and 9th harvest (under drought 75% whc, conditions). Means (of five replicates) not followed by a common letter differ significantly ($P < 0.05$).

conditions (9th harvest), the plants showed greater accumulation of proline and total sugars than under well-watered conditions (8th harvest). Moreover, there was a statistically significant correlation between shoot fresh weight and proline and sugar shoot content in plants grown under drought conditions.

Root growth was very limited in control plants (Fig. 4). Similar to the results obtained for shoot biomass, root growth was only stimulated by AM inoculation when plants also received DOC-A or PO_4^{3-}-fertiliser (Fig. 4).

Nodules were formed only in plants receiving the DOC-A amendment (with or without AM fungal inoculation) and in P-fertilised AM-inoculated plants (Fig. 4). Nodulation was zero in control plants and in plants receiving AM fungal inoculation or P-fertilisation alone (Fig. 4).

AM fungal colonisation by native endophytes was detected in non-inoculated plants (Fig. 4). Nevertheless, in AM-inoculated plants, AM fungal colonisation was increased by DOC-A amendment and particularly by P fertilisation. However, application of DOC-A alone reduced the colonisation in non-inoculated plants. The maximum AM colonisation (60% of the total root length) was observed in P-fertilised AM-inoculated plants (Fig. 4).

In the second experiment, DOC-P was very effective in increasing plant shoot biomass even in the absence of the AM fungal inoculum, as Fig. 5a shows. Related to this, the shoot P content was also enhanced greatly by this amendment. The greatest plant growth and

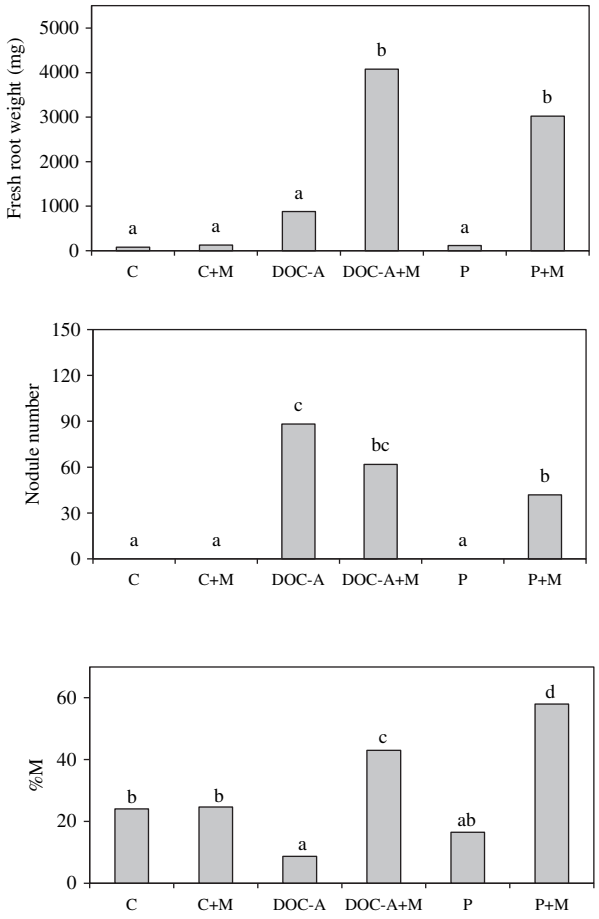

Fig. 4. Root fresh weight (mg), nodule number and percentage of mycorrhizal fungus colonisation (%M) of non-mycorrhizal fungal inoculated (C) or mycorrhizal fungal inoculated (M) *Trifolium repens* amended or not with *Aspergillus niger* treated dry olive cake (DOC-A) or PO_4^{3-}-fertilised (P). Means (of five replicates) not followed by a common letter differ significantly ($P < 0.05$).

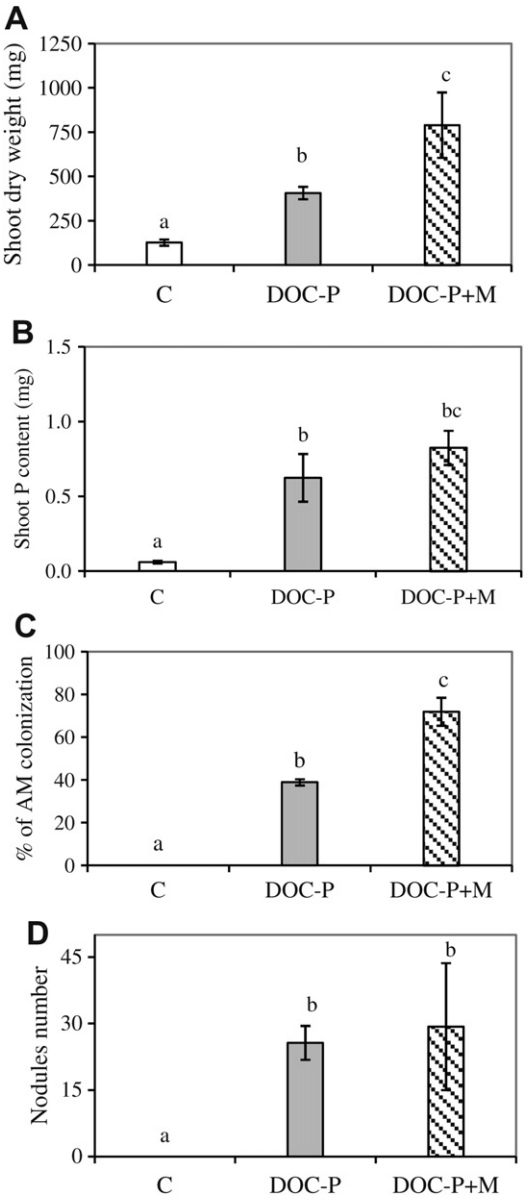

Fig. 5. Shoot dry weight (mg); P content (mg); nodule number and percentage AM-fungal colonisation of non-mycorrhizal fungal inoculated (C) or mycorrhizal fungal inoculated (M) *Dorycnium pentaphyllum* applied or not with *Phanerochaete chrysosporium* treated dry olive cake (DOC-P). Means (of five replicates) not followed by a common letter differ significantly ($P < 0.05$).

P content were observed in plants amended with DOC-P that were also inoculated with AM fungi (Fig. 5a, b). In these plants, the shoot P content was increased by 933% relative to the control plants (without AM fungal inoculation and amendment). The mycorrhizal and rhizobial populations in the soil were not able to colonise the roots of *D. pentaphyllum* growing in natural soil, but the application of DOC-P activated the development of both symbioses (Fig. 5c, d). Moreover, the inoculation of DOC-P amended soil with the AM fungi resulted in a more-extensive mycorrhizal development (Fig. 5c).

The effect of the AM fungal inoculum on nodule formation in plants growing in soil receiving the DOC-P was negligible (Fig. 5d).

4. Discussion

In our previous studies, we have shown that the selected strain of *A. niger* was able to grow on dry olive cake (DOC) and to solubilise rock-phosphate during the transformation process, giving an organic amendment of interest as a fertiliser (Vassilev et al., 2006; Medina, 2006).

As a following step, we wanted to assay the persistence of the effectiveness of this amendment along successive harvests, including drought stress periods, in order to use it in revegetation programmes for semi-arid areas.

Proline and sugars play a major role in the process of osmotic adjustment (Hasegawa et al., 2000) and most plant species can accumulate such compatible solutes. They act by lowering the cell osmotic potential, thus allowing higher water retention during drought (Yoshiba et al., 1997). A high proline accumulation, as occurred in plants of the DOC-A plus AM fungi treatment, could provide the leaf with osmotic mechanisms for preventing excessive water loss. Moreover, proline, as a nitrogenous compound, may be increased in AM-inoculated plants since it has been demonstrated that AM fungi are able to increase nitrate uptake, in particular under drought conditions (Tobar et al., 1994a, 1994b; Azcón et al., 1996; Azcón and Tobar, 1998).

The present results, like those of de Ronde et al. (2001), suggest the role of proline and sugars in the protection of plants against drought stress. Here, plants having the highest proline and sugar contents were also the least-damaged (in terms of plant growth) by drought; non-amended and non-AM inoculated plants suffered greater drought-imposed growth limitation. In addition, previous

studies have shown that the amendment DOC-A increases water-soluble carbohydrates as well as aggregate stability in the rhizosphere (Medina et al., 2004). Moreover, it is known that glomalin, produced by AM fungi, acts as an insoluble glue to stabilise aggregates (Wright and Anderson, 2000). This mechanism could also explain the enhanced development of DOC-A-amended AM- inoculated plants under drought-stress conditions.

AM fungal inoculation increased shoot biomass only in DOC-A-amended or P-fertilised plants, but the effectiveness of AM inoculation, in terms of plant growth, was greater in DOC-A-amended (10th and 11th harvests) than in P-fertilised plants. For the 8th and 9th harvests both treatments resulted similarly effective. These results suggest that DOC-A amendment has a greater potential than P-fertilisation for maintaining soil fertility and plant growth after a drought period, but only if AM inoculation is applied at the same time.

The growth responses of plants to the DOC amendment depended on the identity of the cellulolytic microorganism involved in the previous transformation process *A. niger* or *P. chrysosporium*: *A. niger*-treated DOC did not improve plant growth compared with the non-amended or AM- inoculated plants. These results suggest that the mineralisation of DOC by *A. niger* was only partially accomplished and that therefore there were phytotoxic phenolics remaining in the medium.

P. chrysosporium is characterised by its high lignocellulolytic potential (Tien and Kirk, 1983); thus, in the second experiment, we assayed the ability of *P. chrysosporium* to transform the DOC. The results obtained indicate that the P demand of *D. pentaphyllum* plants could be partially satisfied by DOC-P amendment and that *P. chrysosporium* was able to solubilise RP in the fermentation process using DOC, through organic acid production; this microbial process positively affected shoot growth and P nutrition.

The results from the two experiments may be comparable. Whereas in the first experiment DOC-A did not have any effect on *T. repens* growth, in the second experiment, DOC-P increased *D. pentaphyllum* shoot biomass. This indicates that the amendment obtained after the biotransformation of DOC by *P. chrysosporium* was more suitable for plant growth than the one obtained with *A. niger*.

Nevertheless, the AM fungal inoculum seemed to compensate the negative effect of DOC-A, and the association of DOC-A plus AM fungi resulted in the greatest plant growth in the first experiment (Vassileva et al., 1998).

The results show that both *A. niger*- and *P. chrysosporium*-treated DOC, used as an amendment, interacted with the AM fungal inoculum and positively affected growth and nutrient uptake by *T. repens* or *D. pentaphyllum* growing in pots using the same degraded, arid soil.

Besides, natural AM fungal colonisation increased when DOC amendment or P fertilisation was practiced. In previous studies, we found an increase in extraradical AM hyphal length and AM root colonisation when the *A. niger*-treated sugar beet waste was used as an amendment (Medina et al., 2005, 2007).

Of particular interest are the nutritional aspects of dual symbiosis: under these experimental conditions, AM fungal colonisation from natural soil and that formed by indigenous fungi plus AM fungal inoculum or P fertiliser were not able to promote nodule formation. Nodules were formed only in DOC-A amended plants and in AM-inoculated plants grown in P-fertilizer or DOC-A amended soil.. These results indicate that nodulation required phosphorus and only these treated plants posses the sufficient amount of this element (Barea and Jeffries, 1995). The important role of AM fungi in increasing nodule number, as observed here, and N_2-fixation is a well-documented subject (Barea et al., 2002). In addition, application of fermented-DOC strongly promoted nodule formation in both experiments. These results may be a consequence of the microbially-mediated processes resulting in the transformation of DOC into simple sugars that provide energy sources for the growth and metabolic activity of soil heterotrophic bacteria such as *Rhizobium*.

4.1. Conclusions

According to these results, we can conclude that management practices involving organic amendments and microbial inoculation seem a promising option for restoration of arid, degraded soil, since they positively affected soil fertility, plant growth and water-stress tolerance. Moreover, application of P fertiliser did not replace the effect of the microbiologically-treated DOC. Concerning revegetation, the most-important factor in plant establishment is the development of the root system. This was greatest for plants (*T. repens* or *D. pentaphyllum*) receiving the dual treatment, representing an important contribution to the establishment of plants, which protect soil against erosion.

The mycorrhizal effectiveness regarding accumulation of solutes (proline and total sugars) by plants grown in soil receiving the DOC-A amendment may be related to the improved physical characteristics of composted soil and the ability of these fungi to acquire water (Porcel et al., 2004, 2006; Ruíz-Lozano and Azcón, 1996). Such coordinated effects may have been the main mechanisms involved in the effectiveness of the DOC + AM treatment with time.

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