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Elevated CO_2 increases the effect of an arbuscular mycorrhizal fungus and a plant-growth-promoting rhizobacterium on structural stability of a semiarid agricultural soil under drought conditions

Josef Kohler, Fuensanta Caravaca, María del Mar Alguacil, Antonio Roldán*

CSIC-Centro de Edafología y Biología Aplicada del Segura, Department of Soil and Water Conservation, P.O. Box 164, Campus de Espinardo, 30100 Murcia, Spain

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ABSTRACT

In arid and semiarid Mediterranean regions, an increase in the severity of drought events could be caused by rising atmospheric CO_2 concentrations. We studied the effects of the interaction of CO_2, water supply and inoculation with a plant-growth-promoting rhizobacterium (PGPR), *Pseudomonas mendocina* Palleroni, or inoculation with an arbuscular mycorrhizal (AM) fungus, *Glomus intraradices* Schenk & Smith, on aggregate stabilisation of the rhizosphere soil of *Lactuca sativa* L. cv. Tafalla. The influence of such structural improvements on the growth of lettuce was evaluated. We hypothesised that elevated atmospheric CO_2 concentration would increase the beneficial effects of inoculation with a PGPR or an AM fungus on the aggregate stability of the rhizosphere soil of lettuce plants. Leaf hydration, shoot dry biomass and mycorrhizal colonisation were decreased significantly under water-stress conditions, but this decrease was more pronounced under ambient vs elevated CO_2. The root biomass decreased under elevated CO_2 but only in non-stressed plants. Under elevated CO_2, the microbial biomass C of the rhizosphere of the *G. intraradices*-colonised plants increased with water stress. Bacterial and mycorrhizal inoculation and CO_2 had no significant effect on the easily-extractable glomalin concentration. Plants grown under elevated CO_2 had a significantly higher percentage of stable aggregates under drought stress than under well-watered conditions, particularly the plants inoculated with either of the assayed microbial inocula (about 20% higher than the control soil). We conclude that the contribution of mycorrhizal fungi and PGPR to soil aggregate stability under elevated atmospheric CO_2 is largely enhanced by soil drying.

1. Introduction

In arid and semiarid Mediterranean regions, an increase in the severity of drought conditions could be caused by rising atmospheric CO_2 concentrations under future climate change scenarios (Gregory et al., 2003). This has consequences for agricultural production in these regions, since water shortage limits the growth and production of crop plants (Harle et al., 2007).

In contrast to the effect of climate dryness, a continued rise in CO_2 may stimulate plant above-ground biomass production as well as root growth. This could result in greater below-ground carbon allocation due to higher rates of plant litterfall, root turnover and rhizodeposition (Denef et al., 2007). The organic C released into soil promotes a dense microbial community in the immediate environment of the root which, in turn, produces exocellular mucilaginous polysaccharide material that has the capacity to stabilise soil aggregates (Roldán et al., 2006). Then, any change in the amount and/or composition of soil available carbon in response to elevated CO_2 is likely to affect soil aggregate stability. In this sense, Rillig et al. (1999) provided evidence for three natural ecosystems that soil aggregation was increased by long-term CO_2 fumigation. Adequate soil structural stability favours water infiltration and C storage and protects the soil against water erosion. Clearly, a CO_2-mediated increase of soil aggregate stability could be of particular importance in Mediterranean agroecosystems with a poorly-developed soil structure and exposed to long drought periods. In this context, drying of soil can also affect macroaggregation directly through physical or chemical process and/or indirectly through its action on microbial activity (Cosentino et al., 2006). However, effects of drying on soil structure are still unclear, since both increases and decreases in water-stable aggregation have been observed following drying (Denef et al., 2001). Because of the critical role that soil aggregation plays in the functioning of an ecosystem, modifications of soil aggregation should be

* Corresponding author. Tel.: +34 968 396337; fax: +34 968 396213.
E-mail address: aroldan@cebas.csic.es (A. Roldán).

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examined in response to elevated CO_2 under drought conditions. However, there are still only a limited number of studies on the combined effect of the regime of watering and the atmospheric CO_2 concentration on soil structural stability (Rillig et al., 2001).

Arbuscular mycorrhizal (AM) fungi are beneficial not only in providing plants with nutrients; they also improve the soil structure and aggregate stability (Jeffries and Barea, 2000). In addition to the evidence of mechanical entanglement by hyphae, extracellular polysaccharides of fungi or bacteria provide a cementing agent to large, transiently-stable aggregates (Chenu, 1993). AM fungi are thought to make their most important contributions to the stabilisation of macroaggregates (>250 μm), in which they are hypothesised to help stabilise aggregates via hyphal enmeshment (Miller and Jastrow, 2000) and by deposition of organic substances. In particular, AM fungal hyphae produce a glycoprotein, glomalin, which stabilises soil aggregates (Wright and Anderson, 2000). Rillig et al. (1999) described how the effect of CO_2 enrichment on water-stable aggregate stabilisation of natural grassland ecosystems was related to a significant increase in the length of AM fungal hyphae and an increase in the soil concentrations of the protein glomalin. When the different factors that affect aggregate stability are taken into account, it is clear that any improvement in the structure of semiarid soils will depend on microbial activity (Roldán et al., 2006). Oxidoreductases, such as dehydrogenase, are involved in oxidative processes in soils and their activity mainly depends on the metabolic state of the soil biota; thus, they are considered as good indicators of the soil microbial activity in semiarid areas (García et al., 1997).

Plant-growth-promoting rhizobacteria (PGPR) are a group of bacteria that can actively colonise plant roots and increase plant growth. They can act either directly (e.g. by enhancement of plant nutrient uptake or by production of phytohormones) or indirectly (e.g. by biological control of root pathogens and alteration of the balance of microbial populations) (Vessey, 2003). Because of the potential of PGPR for improving plant nutrition and health, the use of these rhizobacteria in low-input agriculture has been addressed in several investigations (Vessey, 2003). Specific strains of *Pseudomonas* have been shown to increase the growth and yield of some agricultural crops (Rodriguez and Fraga, 1999; Kohler et al., 2006). Likewise, we have provided the first evidence of the beneficial effect of a PGPR of the genus *Pseudomonas* on soil aggregates stabilisation under field conditions (Kohler et al., 2006). However, to date, there are no studies on the effect of *Pseudomonas* on soil structural stability under elevated CO_2.

In this study we hypothesised that (i) elevated CO_2 will increase the beneficial effects of inoculation with a PGPR or an AM fungus on the aggregate stability of the rhizosphere soil of lettuce plants; (ii) the improved aggregation will correlate with water-soluble carbon and carbohydrates, total microbial biomass and activity and glomalin; and (iii) the plant watering regime will affect soil aggregation mediated by the microbial inoculants under elevated CO_2. Thus, we tested the effects of a PGPR or an AM fungus on the rhizosphere soil structural stability to elevated CO_2 under well-watered or drought-stressed conditions.

2. Materials and methods

2.1. Soil and plant species

An agricultural soil, used to cultivate lettuce was collected near Murcia (SE Spain). 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 y^{-1}. The main characteristics of the agricultural soil used were: pH (1:5) 8.51; electrical conductivity 2.88 dS m^{-1}; clay 20.1%; silt 43.3%, sand 36.6%; total organic C 8.5 g kg^{-1}; total N 1.03 g kg^{-1}; available P, 42 μg g^{-1}; extractable K, 550 μg g^{-1}, cationic exchange capacity, 8 cmol kg^{-1} and water holding capacity, 32.8%.

The plant used in the experiment was lettuce (*Lactuca sativa* L. cv. Tafalla). Seeds of lettuce were grown for 15 days in peat substrate under nursery conditions, without any fertilization treatment.

2.2. Microorganisms

The mycorrhizal fungus used was *Glomus intraradices* Schenk & Smith obtained from the collection of the experimental field station of Zaidín, Granada. AM fungal inoculum consisted of a mixture of rhizospheric soil from pot cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments. The inoculum was subjected to a most probable number test (Sieverding, 1991) to determine potential infectivity. The source of inoculum had a potential infectivity of about 35 infective propagules g^{-1} inoculum.

The *Pseudomonas mendocina* Palleroni strain was obtained from Probelte, S.A., Murcia, which was selected on the basis of its ability to produce siderophores. *P. mendocina* was grown in a medium (nutrient broth, Scharlau Chemie, Spain) composed of meat and yeast extracts, peptone and sodium chloride, for 2 days at room temperature on a Heidolph Unimax1010 shaker. The bacterial culture was centrifuged at 2287 × g for 5 min at 2 °C and the sediment was re-suspended in sterilised tap water. The bacterial suspension contained 10^9 colony forming units (CFU) ml^{-1}.

2.3. Design of the experiment

The experiment was a mesocosm assay, conducted as a randomised factorial design with three factors. The first factor had three levels: control soil, soil inoculated with the AM fungus *G. intraradices*, and soil inoculated with the bacteria *P. mendocina*, the second one had two regimes of watering: adequate and inadequate water supply and the third factor had two concentrations of CO_2: ambient CO_2 and elevated CO_2. Six replicates per treatment were set up, making a total of 72 pots.

Non-sterile substrate (700 g), consisting of soil and vermiculite with a particle size of 6 mm at a ratio of 2:1 (v:v) were placed in 1.5-litre square pots (13 × 13 × 13 cm). The vermiculite was used to avoid soil compaction in the pots at the first stage of the plantation and so to facilitate the growth and adequate aeration of the roots. In April 2007, *L. sativa* seedlings were transplanted to the pots (one per pot). The AM inoculum was mixed with the potting substrate at a rate of 5% (v/v). The same amount of the autoclaved inoculum was added to non-AM plants, supplemented with a filtrate (Whatman no. 1 paper) of the culture to provide the microbial populations accompanying the mycorrhizal fungus. *P. mendocina* was inoculated two times during the growth period. The dose of inoculum applied corresponded to 10^{10} CFU per plant. All seedlings were grown for 9 weeks without any fertiliser treatment. Plants were grown under controlled environmental conditions for 9 weeks in two growth chambers, located in the SACE service at the Campus of Espinardo (Murcia, Spain), one exposed to ambient CO_2 (380 parts/10^6) and the other exposed to elevated CO_2 (760 parts/10^6). Within each of the growth chambers, one half of the pots were watered regularly with decalcified water maintaining substrate water potential equivalent to field capacity (−0.03 MPa) and the other half of pots were cultivated under a soil water potential of −0.3 MPa. During the experiment, the lettuce plants were subjected to a photoperiodic cycle of 13 h light with a light intensity of 2 K Lux and 11 h dark. The average day/night temperature was 24 °C/18 °C, and relative humidity was hold constant at 60%. Soil moisture was monitored gravimetrically before each watering.

2.4. Soil water potential

Soil substrate water potential was determined by a pressure plate apparatus and soil water content was measured by weighing the soil before and after drying at 110 °C for 24 h (Richards, 1941). A characteristic soil moisture curve was constructed and used to correlate soil water content and soil water potential (Ψ) by gravimetric measurement of soil water content in the pots.

2.5. Plant analyses

Nine weeks after planting, six plants per treatment were harvested and soil samples were taken from the pots. One subsample was sieved through 2 mm mesh and stored at 2 °C for biological and biochemical analyses and another one was separated by dry sieving in a particle size fraction ranging from 0.25 to 4 mm and allowed to dry at room temperature for physical and physical–chemical analyses. Both particle size fractions of 2 and 0.25–4 mm were free of vermiculite because it was removed during the sieving.

Fresh and dry (105 °C, 5 h) weight of shoots and roots were recorded. Roots were subsampled in three 2-cm cross-sections of the upper, middle, and lower root system. To assess colonisation, roots were cleared with 10% KOH and stained with 0.05% trypan blue (Phillips and Hayman, 1970). The percentage of root length colonised by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980). Positive counts for AM colonisation included the presence of vesicles or arbuscules or typical mycelium within the roots.

2.6. Soil analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. In a 1:5 (w/v) aqueous solution, water-soluble carbon was determined with an automatic Carbon Analyser for liquid samples (Shimadzu TOC-5050A) and water-soluble carbohydrates were determined by the method of Brink et al. (1960).

The percentage of stable aggregates was determined by the method described in Lax et al. (1994). Replicate 4 g soil samples of 0.25–4 mm particle size fraction was placed on a small 250 μm sieve and wetted by spraying with water. After 15 min the soil was subjected to an artificial rainfall of 150 ml with energy of 270 Jm^{-2}, which is equivalent to a rain of 75 mm h^{-1}. The remaining soil on the sieve was placed in a capsule, dried at 105 °C and weighted (P1). The soil was then soaked in distilled water and, after 2 h, passed through the same 250 μm sieve with the assistance of a spatula to break the remaining aggregates. The residue remaining on the sieve, made up of plant debris and sand particles, was dried at 105 °C and weighed (P2). The mass of stable aggregates as a percentage of the total aggregates was calculated by $(P1 - P2) \times 100/(4 - P2)$, where 4 are the grams of soil samples of 0.25–4 mm particle size fraction.

Dehydrogenase activity was determined according to García et al. (1997). 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.

Microbial biomass C was determined using the fumigation-extraction method (Vance et al., 1987). Ten g of soil at 60% of its field capacity were fumigated in a 125-ml Erlenmeyer flask with purified $CHCl_3$ for 24 h. After removal of residual $CHCl_3$, 40 ml of 0.5 M K_2SO_4 solution was added and the sample was shaken for 1 h before filtration of the mixture. The K_2SO_4-extracted C was measured as indicated for water-soluble carbon. Microbial biomass C was calculated as the difference between the carbon of fumigated and non-fumigated samples divided by the calibration factor $K_{EC} = 2.22$ (Joergensen and Brookes, 1990).

Easily-extractable glomalin was extracted of 0.25 g soil samples of 0.25–4 mm particle size fraction with 2 ml of 20 mM sodium citrate (pH 7.0). The extracts were autoclaved at 121 °C for 30 min, then centrifuged at 10,000 × g for 15 min to remove soil particles (Wright and Anderson, 2000). Protein in the supernatant was determined by the Bradford dye-binding assay using bovine serum albumin as the standard (Wright et al., 1996).

2.7. Statistical analysis

Aggregate stability and percentage colonisation were arcsin-transformed, and the other data were log-transformed to compensate for variance heterogeneity before analysis of variance. Microbial inoculation, water regime, CO_2 concentration and their interactions effects on measured variables were tested by a three-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. Growth, shoot water content and mycorrhizal colonisation

3.1.1. Ambient CO_2 conditions and water regime treatment

Under well-watered conditions (−0.03 MPa), the inoculation with *P. mendocina* or with *G. intraradices* increased significantly shoot fresh and dry biomass with respect to the control plants at ambient CO_2 (Table 1). The shoot fresh biomass of inoculated plants was about 34% higher than that of non-inoculated control plants. There was a significant microbial inoculation × water-stress interaction for shoot fresh biomass; it was enhanced by *P. mendocina* and *G. intraradices* in non-stressed plants but was not affected by the microbial inoculations in stressed plants. Water deficit caused a significant decrease in the shoot fresh and dry biomass and shoot water content of all plants (Tables 1 and 2). However, the microbial inoculation factor had no significant effect on the shoot water content (Table 2). Root dry biomass was increased by *P. mendocina*, for both watering regimes. In non-stressed plants, inoculation with the AM fungus led to its active colonisation of the root system of the *L. sativa* seedlings (Table 1). The percentage of roots colonised by AM fungi was not affected by bacterial inoculation, but decreased significantly with water stress.

3.1.2. Elevated CO_2 conditions and water regime treatment

The plants inoculated with *P. mendocina* presented higher shoot fresh and dry biomass than the control and *G. intraradices*-inoculated plants grown under both water regimes at elevated CO_2, particularly the stressed plants (Table 1). The root biomass of plants grown under well-watered conditions was significantly higher than that of the stressed plants, particularly the *G. intraradices*-inoculated plants. The root biomass decreased at elevated CO_2 but only in non-stressed plants. Under well-watered conditions, the *G. intraradices*-inoculated plants showed the highest percentages of mycorrhizal colonisation followed by the plants inoculated with the PGPR. Drought increased the colonisation of plants grown, particularly for the *G. intraradices*-inoculated plants under elevated CO_2.

3.1.3. Interaction between CO_2 and water regime

There was a significant CO_2 × water regime interaction for shoot and root dry biomass, shoot water content and percentages of

Table 1
Effect of inoculation with *G. intraradices* and *P. mendocina* on growth characteristics, shoot water content and colonisation of *L. sativa* seedlings grown at two concentrations of atmospheric CO_2 and two regimes of watering ($n = 6$).

	CO_2	Watering	Shoot fresh biomass (g fw)	Shoot water content (%)	Shoot dry biomass (g dw)	Root dry biomass (g dw)	Colonisation (%)
Control	380 ppm	−0.03 MPa	16.77 ± 0.28	92.9 ± 0.1	1.08 ± 0.03	0.21 ± 0.01	37.8 ± 0.8
P. mendocina			22.83 ± 0.28	92.0 ± 0.3	1.48 ± 0.02	0.26 ± 0.01	39.0 ± 1.5
G. intraradices			21.93 ± 0.72	93.9 ± 0.1	1.21 ± 0.05	0.18 ± 0.00	54.5 ± 0.6
Control		−0.3 MPa	6.71 ± 0.15	85.7 ± 0.3	0.63 ± 0.02	0.20 ± 0.01	18.4 ± 1.3
P. mendocina			6.91 ± 0.16	85.6 ± 0.2	0.69 ± 0.02	0.28 ± 0.00	32.5 ± 2.9
G. intraradices			6.51 ± 0.16	85.6 ± 0.2	0.63 ± 0.02	0.21 ± 0.01	37.7 ± 1.3
Control	760 ppm	−0.03 MPa	20.20 ± 0.54	94.5 ± 0.1	0.98 ± 0.03	0.12 ± 0.01	26.0 ± 0.7
P. mendocina			24.80 ± 0.36	94.0 ± 0.3	1.15 ± 0.04	0.14 ± 0.01	36.6 ± 1.2
G. intraradices			21.63 ± 0.43	94.4 ± 0.1	1.03 ± 0.01	0.09 ± 0.00	45.2 ± 1.3
Control		−0.3 MPa	9.18 ± 0.28	90.4 ± 0.2	0.64 ± 0.02	0.24 ± 0.01	31.2 ± 1.4
P. mendocina			11.62 ± 0.31	89.5 ± 0.3	0.89 ± 0.02	0.28 ± 0.02	45.2 ± 3.7
G. intraradices			9.52 ± 0.27	90.1 ± 0.2	0.77 ± 0.02	0.19 ± 0.01	65.7 ± 1.5

mycorrhizal colonisation (Table 2). Leaf hydration, shoot dry biomass and mycorrhizal colonisation were decreased significantly under water-stress conditions, but this decrease was more pronounced at ambient CO_2 than at elevated CO_2.

3.2. Soil biological properties

3.2.1. Ambient CO_2 conditions and water regime treatment

The microbial inoculants had a significant effect on the biological properties of the rhizosphere of lettuce plants, excepting the easily-extractable glomalin (Tables 2 and 4). Under well-watered conditions, inoculation with *P. mendocina* was the most effective treatment for increasing dehydrogenase activity (Table 4). However, under water-stress conditions, both inoculation treatments produced similar increases in dehydrogenase activity (about 18% higher than the non-inoculated control plants). Only the PGPR strain increased significantly the microbial biomass C of the rhizosphere of the lettuce plants – particularly under drought-stressed conditions (Tables 2–4), as indicated by the significant interaction of microbial inoculation and water stress. The microbial inoculation factor increased significantly the soluble C-fraction values (water-soluble C and carbohydrates) of the well-watered plants (Table 4). Drought strongly decreased water-soluble C and carbohydrates in plants inoculated with the AM fungus, but increased these C-fractions in non-inoculated control plants. Drought increased the concentrations of glomalin in the control plants and in the plants inoculated with the PGPR strain, but they were not significantly increased in the *G. intraradices*-colonised plants.

3.2.2. Elevated CO_2 conditions and water regime treatment

Only the dehydrogenase activity of soil inoculated with *G. intraradices* increased in response to elevated CO_2. There was a significant microbial inoculation × CO_2 interaction with respect to microbial biomass C (Table 2); this soil property decreased with elevated CO_2 for all plants and the differences between control soil and soil inoculated with the PGPR disappeared in both water regimes. The treatment with elevated CO_2 decreased the concentrations of water-soluble C and carbohydrates for all plants (Tables 2 and 4), but the plants inoculated with *P. mendocina* showed the highest values compared to the control soil and the soil inoculated with *G. intraradices*. The CO_2 factor had no significant effect on the easily-extractable glomalin concentration of the rhizosphere soil of *L. sativa* (Table 2).

3.2.3. Interaction between CO_2 and water regime

At elevated CO_2, the microbial biomass C of the rhizosphere of the *G. intraradices*-colonised plants depended on the water regime. Thus, these plants exhibited values of microbial biomass C lower than those recorded in the control plants under well-watered conditions, whereas the opposite trend was found under drought-stress. The concentrations of water-soluble C and carbohydrates of all stressed plants were lower at elevated CO_2 than at ambient CO_2. There was no a significant CO_2 × water regime interaction for dehydrogenase activity and easily-extractable glomalin (Table 2).

3.3. Soil aggregate stability

3.3.1. Ambient CO_2 conditions and water regime treatment

The percentage of water-stable aggregates was increased significantly by the microbial inoculation factor (about 33% higher than the control soil) (Table 2, Fig. 1). Desiccation caused a significant increase of the aggregate stability in the rhizosphere of control soil but it did not affect the percentages of stable aggregates in the two inoculated soils.

Table 2
Three factors ANOVA (microbial inoculation, water stress and CO_2) for all soil and plant properties studied. *P* significance values.

	Microbial inoculation (M)	Water stress (W)	CO_2 (C)	Interaction (M × W)	Interaction (M × C)	Interaction (W × C)	Interaction (M × W × C)
Shoot fresh biomass	<0.001	<0.001	<0.001	0.005	NS	NS	NS
Shoot water content	NS	<0.001	<0.001	NS	NS	<0.001	NS
Shoot dry biomass	0.008	<0.001	NS	NS	NS	0.001	NS
Root dry biomass	0.037	<0.001	0.005	NS	NS	0.004	NS
Colonisation	<0.001	NS	NS	NS	NS	<0.001	NS
Dehydrogenase	<0.001	0.004	0.045	NS	<0.001	NS	0.002
Biomass C	0.006	NS	NS	0.020	0.001	<0.001	<0.001
Water-soluble C	<0.001	NS	<0.001	<0.001	NS	<0.001	<0.001
Water-soluble CH	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
Glomalin	NS	0.001	NS	0.019	NS	NS	NS
Aggregate stability	<0.001	<0.001	0.008	0.001	0.015	<0.001	0.010

CH: carbohydrates.

Table 3
The *P*-values of differences among treatment means of microbial factor for all soil and plant properties studied.

	C vs P	C vs G	G vs P
Shoot fresh biomass	<0.001	0.010	0.012
Shoot water content	NS	NS	NS
Shoot dry biomass	<0.001	NS	0.007
Root dry biomass	NS	NS	0.028
Colonisation	0.027	<0.001	0.001
Dehydrogenase	<0.001	<0.001	NS
Biomass C	0.009	NS	0.003
Water-soluble C	<0.001	0.040	<0.001
Water-soluble CH	<0.001	NS	<0.001
Glomalin	NS	NS	NS
Aggregate stability	0.002	<0.001	0.022

CH: carbohydrates.

3.3.2. Elevated CO_2 conditions and water regime treatment

At elevated CO_2, the inoculated soils had the highest aggregate stability but the values were lower than those observed in the same soils at ambient CO_2.

3.3.3. Interaction between CO_2 and water regime

There was a significant water stress × CO_2 interaction for soil aggregate stability (Table 2). Plants grown at elevated CO_2 had a significantly higher percentage of stable aggregates under drought-stress than under well-watered conditions, particularly the plants inoculated with either of the assayed microbial inocula (about 20% higher than the control soil).

4. Discussion

4.1. Water regime and aggregate stability

The results demonstrate that the inoculation with an AM fungus or a PGPR was effective for increasing the aggregate stability of the rhizosphere soil of lettuce plants, when grown under well-watered conditions. However, soil desiccation only produced an improvement in the aggregate stability of the rhizosphere soil of control plants. Some studies have shown that soil drying may represent a significant stress for the soil microbiota, provoking a substantial loss of biomass and activity (Rosacker and Kieft, 1990). In the present experiment, microbial biomass C decreased in all soils but the dehydrogenase activity was not affected by the soil drying. Soil microorganisms have developed various mechanisms to survive desiccation in soil. For example, bacteria have been reported to change the structure of their membrane or to synthesise exopolysaccharide in order to increase their survival during periods of low external water potential. Polysaccharides are hygroscopic and therefore may maintain a higher water content in the colony microenvironment than in the bulk soil as water potential declines (Roberson and Firestone, 1992). This increase in water content could increase nutrient availability within the bacterial colony (Morse, 1990). In fact, the higher release of soluble carbohydrates into the rhizosphere soil of plants treated with PGPR probably increased the survival of microorganisms in the face of soil desiccation. Likewise, the extracellular polysaccharides of bacteria can form, with the surrounding mineral particles, an organo-mineral sheath around the cells (Chenu, 1993), which leads to an increase in formation of macroaggregates as an indirect additional effect. In this study, large differences in water-soluble carbon and carbohydrates between well-watered and stressed plants, accompanied by an increase in aggregate stability after soil drying, could support this idea in the control soil. However, the polysaccharides produced by the added microbial biomass during the drying period were not effective for improving aggregate stability of the *P. mendocina*-inoculated soil. On the other hand, the concentrations of water-soluble carbon and carbohydrates decreased in the rhizosphere soil of the plants inoculated with *G. intraradices* after the drought

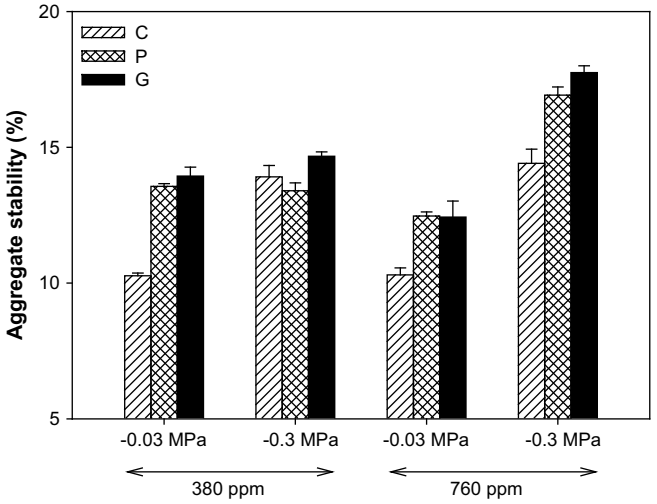

Fig. 1. Effect of inoculation with *G. intraradices* and *P. mendocina* on the percentage of stable aggregates of rhizosphere soil of *L. sativa* seedlings grown under two concentrations of atmospheric CO_2 and two regimes of watering ($n = 6$). C: control; P: inoculated with *P. mendocina* and G: inoculated with *G. intraradices*.

Table 4
Effect of inoculation with *G. intraradices* and *P. mendocina* on biochemical and biological properties of rhizosphere soil of *L. sativa* seedlings grown under two concentrations of atmospheric CO_2 and two regimes of watering ($n = 6$).

	CO_2	Watering	DHase (μg INTF g^{-1} soil)	Biomass C (μg g^{-1})	Water-soluble C (μg g^{-1})	Water-soluble CH (μg g^{-1})	Easily-extractable Glomalin (μg g^{-1})
Control	380 ppm	−0.03 MPa	41.9 ± 0.9	185 ± 11	132 ± 0	22 ± 1	707 ± 1
P. mendocina			49.9 ± 0.3	204 ± 3	182 ± 6	27 ± 3	722 ± 1
G. intraradices			41.1 ± 0.6	175 ± 13	197 ± 5	33 ± 1	721 ± 4
Control		−0.3 MPa	42.3 ± 0.5	139 ± 2	218 ± 2	35 ± 1	733 ± 7
P. mendocina			50.7 ± 0.8	188 ± 5	269 ± 8	70 ± 1	740 ± 2
G. intraradices			48.1 ± 0.7	125 ± 3	118 ± 4	14 ± 2	726 ± 1
Control	760 ppm	−0.03 MPa	39.3 ± 0.2	162 ± 6	92 ± 1	13 ± 1	725 ± 3
P. mendocina			47.3 ± 0.4	165 ± 4	174 ± 1	19 ± 0	706 ± 3
G. intraradices			52.7 ± 0.1	133 ± 5	96 ± 2	15 ± 1	731 ± 2
Control		−0.3 MPa	42.0 ± 0.4	175 ± 3	104 ± 6	15 ± 1	734 ± 7
P. mendocina			49.1 ± 0.3	177 ± 3	101 ± 1	15 ± 1	739 ± 4
G. intraradices			51.3 ± 1.1	220 ± 9	93 ± 2	13 ± 0	723 ± 4

DHase: dehydrogenase; CH: carbohydrates.

period, indicating that mycorrhizal fungi act as a strong sink for photosynthates and that these carbon fractions contribute in a lesser degree to the stabilisation of soil aggregates.

4.2. CO_2 enrichment and aggregate stability

Soil aggregate stability was unaffected by CO_2 enrichment under well-watered conditions. This could be due to a marked reduction in root biomass of all plants grown under elevated CO_2 with respect to those grown under ambient CO_2. According to Gavito et al. (2001), at elevated CO_2, although the plant photosynthesis rate increases and a larger amount of photosynthates could be sent below-ground, the additional C might not be sufficient to overcome an increased nutrient demand in both non-inoculated and inoculated plants; therefore, photosynthates could be accumulated in the leaves at the expense of root development. Moreover, the root biomass was also lower in mycorrhizal plants, possibly because shoot C was partitioned to the mycorrhizal fungi. Additionally, decreased root biomass could have caused a decrease in the microbial biomass, in turn diminishing the release of labile organic matter. In fact, the concentrations of water-soluble carbon and carbohydrates were significantly decreased in the rhizosphere soil of all plants under elevated CO_2. These results disagree with those of Rillig et al. (1999), who recorded increased soil aggregation in serpentine field plots at elevated CO_2. The lack of CO_2 response shown by aggregate stability could be due to the fact that the responses in the rhizosphere of a short-cycle crop such as lettuce are different to those in the rhizosphere of a long-cycle crop. However, Eviner and Chapin (2002) also found that elevated CO_2 in outdoor mesocosms, over a 4-year period, altered many of the factors known to influence aggregation, such as bacterial biomass, root biomass or fungal length, but did not affect water-stable soil aggregation of annual grassland species grown on a serpentine soil, as used by Rillig et al. (1999). These authors suggested that differences in aggregation responses between the serpentine field plots and the mesocosms may have resulted from a lesser fungal length in the mesocosms, due to the disturbance associated with their construction. In our experimental soil, this hypothesis needs to be confirmed.

4.3. CO_2 enrichment, water stress and aggregate stability

Elevated CO_2 had a marked effect on the aggregate stability of all soils only under water-stress conditions. In disagreement with the results obtained by Rillig et al. (2001), we recorded a significant interaction between the water regime and CO_2 concentration regarding the percentage of stable aggregates. Likewise, the positive effect of both microbial inoculants on soil structural stability at elevated CO_2 was more pronounced after soil drying. To the best of our knowledge, this is the first study showing evidence of the effect of a plant-growth-promoting rhizobacterium and an AM fungus on soil aggregates stabilisation in response to CO_2 under drought conditions. The mechanisms involved in aggregate stabilisation are based on the enmeshment of soil particles by hyphae and roots, and on the exudation of polysaccharides (Bearden and Petersen, 2000). According to Roldán et al. (1994), the binding effect of roots and hyphae is long-lived (months), while that of polysaccharides is transient because they are decomposed rapidly by microbes. In our study, the root exudates did not seem to be involved in the soil structural improvement because the concentrations of water-soluble carbon and carbohydrates were not altered in response to elevated CO_2 under water-stress conditions. The high percentages of stable aggregates under such conditions might be due to increased root biomass, which binds soil particles together mechanically. In the case of soil inoculated with an AM fungus, the improvement in aggregate stability can be attributed also to the greater stimulation of the rhizosphere microbial population and, particularly, to the proliferation of fungal hyphae (Roldán et al., 1994; Jeffries and Barea, 2000). The contribution of AM fungal hyphae in the formation of stable aggregates during soil drying was also revealed in this study due to the increase in root colonisation. The reactivation of the rhizosphere microbial biomass at elevated CO_2 is clearly demonstrated in our case, since the mycorrhizal inoculation treatment increased the microbial biomass C in the rhizosphere soil after soil drying. AM fungi also produce a glycoprotein, glomalin, which acts as an insoluble glue to stabilise aggregates (Wright and Anderson, 2000). In this study, there was no evidence for the major participation of glomalin produced by exotic AM fungi, with respect to that produced by local indigenous AM fungi from the soil, in the improvement of soil structural stability.

4.4. CO_2 enrichment, water stress and plant growth

Soil structure is one of the most important properties controlling plant growth (De Freitas et al., 1996). CO_2 enrichment produced a greater relative stimulation of plant growth under drought than under well-watered conditions. Recent experiments on the interactive effects of CO_2 enrichment and water supply on the growth and yield of wheat mostly indicated a relatively higher CO_2 response when water was limited (Manderscheid and Weigel, 2007). As mentioned above, we also observed a stronger CO_2 response of soil aggregate stability under drought as compared with well-watered conditions. Soil aggregation determines the distribution of soil pore sizes, and thus soil moisture retention properties (Alami et al., 2000). Thus, improved soil aggregation can be expected to increase absorption of water by plants, which can also enhance plant growth. In fact, drought-stressed plants were more hydrated under elevated CO_2 than at ambient CO_2, counteracting the negative effect of water deficit on growth. This suggests that the observed increase in *L. sativa* seedling growth may be related to the increase in rhizosphere aggregate stability in response to elevated CO_2 and water stress. However, other factors may be responsible for determining CO_2 and water-stress effects on plant growth because the highest aggregate stability of soil inoculated with an AM fungus was not accompanied by a greater growth of the lettuce plants.

5. Conclusions

In conclusion, elevated CO_2 produced a greater relative increase in the structural stability of the control soil and the soil inoculated with an AM fungus or a rhizobacterium (PGPR) under drought than under well-watered conditions, contributing to a better plant growth. The higher CO_2 response of soil structure under limited water supply was due to an increase in root biomass. The greater effectiveness of mycorrhizal plants in increasing aggregate stability was also based on the proliferation of AM fungi. According to our results, in a context of climate change we can consider the inoculations with PGPR and/or AM fungus as effective tools for improving soil structure and promoting plant growth of semiarid soils, often subjected to long dry periods and highly susceptible to water erosion.

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