Soil Biology & Biochemistry 41 (2009) 1710–1716

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

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

ARTICLE INFO

Article history:
Received 23 September 2008
Received in revised form
18 May 2009
Accepted 21 May 2009
Available online 17 June 2009

Keywords:
Aggregate stability
Elevated CO_2
Glomalin
Mycorrhiza
Pseudomonas
Water stress

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).

0038-0717/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2009.05.014

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.