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Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition

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ABSTRACT

Little is known about the effect of drought on the interactions of roots and microorganisms in the rhizosphere under different plant communities. We compared drought effects on microbial biomass carbon (MBC) and on enzyme activities in the rhizosphere of two grasses (*Lolium perenne* and *Festuca arundinacea*) and one legume (*Medicago sativa*) grown individually or in mixture under controlled laboratory conditions. We analysed plant biomass production and extracellular enzyme activity as well as MBC in planted and unplanted soils with and without drought. We focused on three enzymes involved in the C cycle (xylanase, β-cellobiosidase and β-glucosidase), one involved in the nitrogen (N) cycle (leucine-aminopeptidase), and one enzyme involved in both cycles (chitinase). The aim of the study was to evaluate the importance of the plant community composition for the response of these parameters to drought.

Higher root-to-shoot ratio of all individual species under drought indicated that root growth was sustained under drought, whereas shoot growth was limited. Decrease of the root biomass and root-to-shoot ratio was observed for plants grown in mixture, showing that these plants competed more strongly for light than for water and nutrients compared to monocultures. MBC increased in response to drought in soil under the plant mixture, whereas it showed variable trends under monocultures. Our results further showed that drought and plant species composition were responsible for more than 90% of the variation of enzyme activities. Most enzyme activities decreased in unplanted soil in response to drought. The activity of the enzyme involved in the N cycle increased strongly under mixture and two out of three monocultures, indicating an increased N demand under drought conditions. The activities of enzymes involved in the C cycle in soil under mixture (1) generally were lower during drought compared to soil under monocultures and (2) were unchanged or tended to decrease, while they were more likely to increase under monocultures. This has an important ecological consequence: the decomposition of plant residues and soil organic matter will be slower under drought when plants are grown in mixture compared to monocultures.

1. Introduction

Global change is likely to increase drought periods which could alter global patterns of organic matter production and decomposition (Feyen and Dankers, 2009). Drought threat has significant consequences for belowground carbon (C) and nutrient cycling. It may affect soil processes through changes in C allocation to roots and foliage as well as C turnover in the rhizosphere.

The rhizosphere is subjected to specific processes due to the interaction of roots and root-associated microorganisms (Griffiths et al., 1999; Czarnes et al., 2000b). One of the main rhizosphere processes is rhizodeposition which is controlled by plant specific responses to various stresses (Czarnes et al., 2000a; Jones et al., 2004). In the rhizosphere, roots of different plants compete for space, water and mineral nutrients (Ryan et al., 2001). Strong intra- and inter-specific interactions can occur in this space. Intra-specific interactions occur between individual plants of the same species, while inter-specific interactions occur both at population level (plant species-specific interactions) and at community level

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(plant–microbial interactions). The plant–microbial interactions are controlled by bulk rhizosphere C flow and are essential for the functioning and maintenance of many ecosystems (Prosser et al., 2006). Changes in plant community composition can alter biomass production and hence rhizosphere processes (Paul et al., 2010).

Roots and shoots are interrelated in a functional equilibrium governed by optimal distribution of resources and biomass (Farrar and Jones, 2000). This equilibrium may be modified under changing environmental conditions. Water deficit induces a range of adaptations in plants that favor their growth or survival (Malinowski and Belesky, 2000). These adaptations include: (i) drought avoidance, which may be due to improved water uptake by an extensive root system, reduced transpiration losses following stomatal closure (Peñuelas et al., 2004) or water storage in plant tissues and (ii) drought tolerance and recovery from drought which includes accumulation and translocation of assimilates, osmotic adjustments or maintenance of cell wall elasticity (Malinowski and Belesky, 2000). Plant adaptations to drought stress may cause changes in belowground C input through higher root production and turnover. This may in turn influence the functional structure and activity of the microbial community in the rhizosphere (Bolton et al., 1992; Grayston et al., 1998). Drought also directly affects the soil microorganisms by creating osmotic stress, which leads to microbial death and cell lysis (Turner et al., 2003).

Soil extracellular enzyme activities as direct expression of the functioning of microbial communities are sensitive and respond rapidly to environmental stresses. The composition of organic substances entering the soil (proportion of easily available monomers and polymers, more difficult to degrade) varies significantly with plant community composition and in response to environmental stress. Therefore, we hypothesized a contrasting drought response of extracellular enzymes responsible for (1) the initial step of polymers degradation (release of oligomers from polymeric compounds) and (2) the last step of decomposition (production of monomers).

To investigate the impact of drought on microbial biomass and enzyme activities in the rhizosphere of different plant communities, we grew two grasses (*Lolium perenne* and *Festuca arundinacea*) and one legume – alfalfa (*Medicago sativa*) individually or in mixture during 70 days under controlled laboratory conditions. The aim of this study was to evaluate the effect of plant community composition on the activities of extracellular enzymes involved in the C – and the N cycle in response to drought stress.

2. Materials and methods

2.1. Soil

Soil samples were taken from the top 20 cm of a Cambisol at a flat temporary grassland site established since more than 50 years. This site is part of a long-term observatory for environmental research (ORE-ACBB, INRA, France). It is located near Lusignan in the southwest of France (46°25′12.91″ N; 0°07′29.35″ E). The soil is slightly acid and has a loamy texture with a carbon content of 1.4% and a C/N ratio of 9. Its water content at field capacity is 40%. After sampling, the soil was air dried, mixed and passed through a 5-mm sieve.

2.2. Experimental design and growth conditions

A two factorial experiment was established, including drought effect and plant species composition. We used seeds of *L. perenne*, *F. arundinacea*, and *M. sativa*, which were cultivated for five days in petri dishes. Five-day-old plants of each species were planted in microcosms containing 500 g of soil. Two treatments were established, consisting of (i) monocultures, i.e. 6 plants of the same species in each microcosm or (ii) mixtures, i.e. 2 + 2 + 2 plants (2 *L. perenne* + 2 *F. arundinacea* + 2 *M. sativa*) in each microcosm. To assure three replicates for each treatment combination, in total 24 microcosms with planted soil (three individual plant species and a mixture under two different water levels) and six microcosms with unplanted soil (control under two different water levels) were incubated for 70 days.

Incubation temperature was kept at 26–28 °C during daytime and at 22–23 °C during nighttime. Day-length was 14 h and light intensity approximately 400 μmol m^{-2} s^{-1} at the top of canopy.

During the first 30 days of plant growth, optimum water level (70% of the field capacity) was maintained for all microcosms. After one month, half of the vegetated microcosms and three microcosms with unplanted soil were maintained at 70% of the field capacity (optimum conditions) and the other half adjusted to 30% of the field capacity (drought conditions). After 40 days of growth under different moisture regimes, the plants were harvested. Each microcosm was emptied and roots were separated from the soil manually. Roots and shoots were dried at 60 °C. Fresh 2 mm sieved soil from each microcosm was used for the analysis of microbial biomass and extracellular enzyme activity. A subsample of soil from each microcosm was oven dried at 105 °C for 24 h in order to determine the moisture contents.

2.3. Microbial biomass

Microbial biomass C was determined by the Chloroform-fumigation–extraction method (Vance et al., 1987). Briefly, 10 g of chloroform fumigated and non-fumigated soil were extracted with 40 ml of 0.05 M K_2SO_4 for 30 min and filtered through ash-less filter paper '5893 Blue ribbon' (Schleicher and Schuell GmbH, Germany). The K_2SO_4 extracts were analysed for organic C with a TOC analyser (Dimatoc-100). The difference of fumigated and non-fumigated soils was taken as the microbial-C flush following chloroform fumigation and converted to microbial biomass C using Eq. (1) (Vance et al., 1987):

$$\text{Microbial C} = \text{C flush} \times 2.22 \quad (1)$$

2.4. Enzyme assays

Extracellular enzyme activities in soil were measured using fluorogenically labeled substrates (Pritsch et al., 2004; Sowerby et al., 2005). Four fluorogenic enzyme substrates based on 4-methylumbelliferone (MUF) were used for the assessment of enzyme activities: MUF-β-D-xylopyranoside (MUF-C; EC 3.2.1) for xylanase, MUF-β-D-cellobioside (MUF-C; EC 3.2.1) for β-cellobiosidase, MUF-β-D-glucopyranoside (MUF-G; EC 3.2.1.21) for β-glucosidase and MUF-N-acetyl-β-D-glucosaminide dihydrate (MUF-NAG; EC 3.2.1.14) for chitinase. L-Lencine-7-amino-4-methyl coumarin (AMC) substrate was used to study leucine-aminopeptidase activity involved in the hydrolysis of peptide bonds. The list of substrates, respective enzymes and the potentially degradable compounds is given in Table 1. The MUF-substrates were dissolved in 2 ml of 2-methoxyethanol (Hoppe, 1983). Pre-dissolved MUF-substrates were further diluted with sterile distilled water to obtain the desired concentrations (see below). All chemicals were purchased from Fluka (Germany).

The soils (1 g) were suspended in water (10 ml) and shaken on an overhead shaker for 30 min at room temperature and at maximum speed (500 rpm) to ensure thorough mixing. A sub-sample of the soil suspension (0.5 ml) was added to 1.5 ml of each substrate solution (containing either 200 μmol MUF or AMC), already pipetted in Deep Well Plates (24-wells × 10 ml, HJ-Bioanalytik GmbH, Germany). Saturation concentrations of fluorogenic substrates