Applied Soil Ecology 48 (2011) 38–44

Contents lists available at ScienceDirect

Applied Soil Ecology

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

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

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ARTICLE INFO

Article history:
Received 6 July 2010
Received in revised form 11 February 2011
Accepted 12 February 2011

Keywords:
Drought effects
Rhizodeposition
Carbon cycle
Grassland species
Plant community composition
Rhizosphere
Extracellular enzyme activity

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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doi:10.1016/j.apsoil.2011.02.004

(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

Table 1
The substrates for estimation of enzymes activities and the specific compounds degraded by these enzymes (Stemmer et al., 1999; Saiya-Cork et al., 2002; Caldwell, 2005).

Enzymes	Substrates	Compounds
Carbon cycle		
Oligomer producing enzymes		
Xylanase	4-MUF-β-D-xylopyranoside	Hemicellulose, xylan
β-Cellobiosidase	4-MUF-β-D-cellobioside	Cellulose
Monomer producing enzymes		
β-Glucosidase	4-MUF-β-D-glucopyranoside	Cellulose, cellobiose
Chitinase	4-MUF-N-acetyl-β-D-glucosaminide	Chitin
Nitrogen cycle		
Leucine-aminopeptidase	L-Leucine-7-AMC	Peptides

4-MUF = 4-methylumbelliferone and 7-AMC = 7-amino-4-methyl coumarin.

were determined in preliminary experiments. The microplates were incubated at 22 °C for 1 h for enzymes releasing monomers (β-glucosidase, chitinase and leucine amino peptidase) and 3 h for enzymes releasing oligomers (β-cellobiosidase, xylanase). The calibration solutions were prepared using soil suspension (0.5 ml) and MUF or AMC of different concentrations (0–100 μmol, 1.5 ml). Deep Well Plates with the soil-substrates and soil-calibration solutions were centrifuged (3000 rpm for 10 min). Thereafter, 1 ml of supernatant was transferred to 24-well microplates (Becton Dickinson, USA). Inhibition of the reaction and maximization of the fluorescence intensity through alkalinisation of the medium was found unnecessary because of the high sensitivity and fast processing power of the analytical equipment used to measure fluorescence (Marx et al., 2001). Fluorescence was measured in microplates within 2–3 min after pipetting at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, slit width of 25 nm, with a Victor^3 1420-050 Multilabel Counter (PerkinElmer, USA). Calibration curves as well as the controls for the autofluorescence of the substrate and for quenching effect (Marx et al., 2001; Pritsch et al., 2004) were included in every series of enzyme measurements. Enzyme activities were expressed as MUF or AMC release in μmol per g soil and hour (μmol g^{-1} h^{-1}).

2.5. Calculations and statistics

The differences induced by the two treatments (optimum conditions or drought) were tested for significance using the Mann and Whitney test. The level of significance of statistical tests was $p < 0.05$. These analyses were done with Microsoft Excel.

The contribution of independent single factors "plant composition" and "drought" and their interactions (plant composition × drought) to various parameters was estimated by two-way ANOVA after testing the data for normal distribution by the Shapiro–Wilk test. This contribution was calculated by dividing sum of squares of the factors or their interactions by total sum of squares and multiplying by 100 to get percent contribution of these factors. The statistical software R (version 2.9.2) was used for this analysis.

3. Results

3.1. Plant biomass

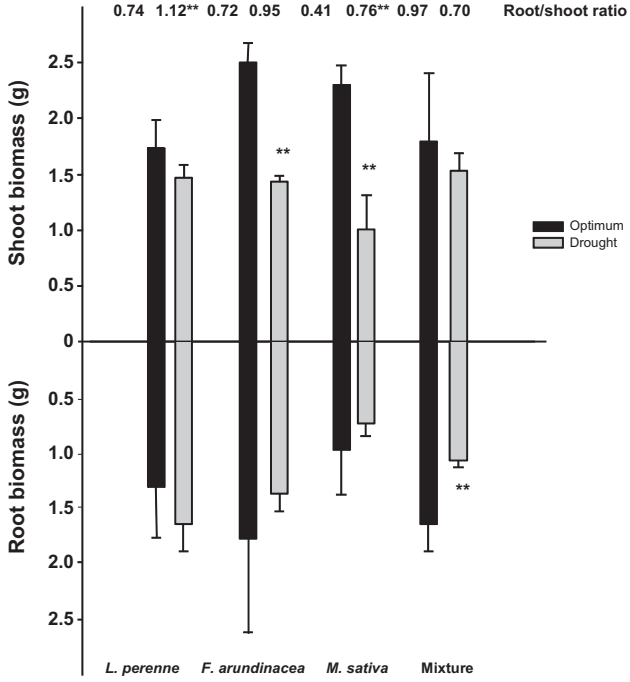
Shoot biomass of *F. arundinacea* and *M. sativa* decreased significantly due to drought compared to the same plants grown under optimum moisture conditions, whereas it was unaffected for *L. perenne* and the plant mixture (Fig. 1). Root biomass of plants grown in monoculture was unaffected by drought stress, whereas a significant decrease was noted for plants grown in mixture (Fig. 1). The root-to-shoot (R/S) ratio of all three individual plants increased under drought. This increase was significant for *L. perenne* and *M. sativa*. In contrast, the R/S ratio showed a decreasing trend when plants were grown in mixture (Fig. 1). For plant roots, shoots and their ratios, 53–80% of the variability could be related to drought and plant community composition (Fig. 2).

3.2. Microbial biomass

Microbial biomass carbon (MBC) was significantly higher in vegetated soils compared with the unplanted control (Fig. 3). Under optimal moisture, highest MBC in vegetated soils was recorded under *L. perenne*, and lowest under plant mixture. Drought had no effect on MBC under *L. perenne*, while in the presence of *M. sativa*, MBC decreased by 14% under drought compared to optimum conditions. Under drought conditions MBC increased compared to optimum conditions by 20 and 36% in soils under mixture and *F. arundinacea*.

3.3. Effect of plant community composition on extracellular enzyme activities

In planted soil, we observed in most cases increased extracellular enzyme activity compared to the unplanted control (Fig. 4).



**significantly different from optimum

Fig. 1. Root and shoot biomass at optimum moisture and drought conditions. Both parameters were analysed independently. The root-to-shoot ratios are given above each column. Data are presented as mean + standard deviation of three replicates ($n = 3$). **Significantly different from optimum ($p < 0.05$).

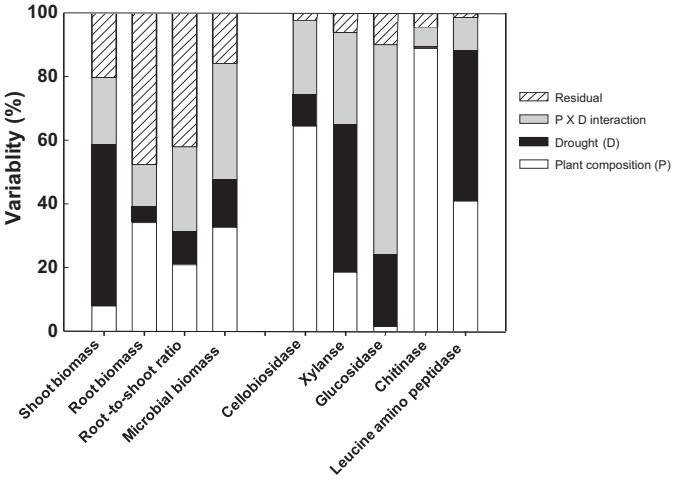


Fig. 2. Contribution of independent factors "plant composition" and "drought" and their interactions (plant composition × drought) to various parameters estimated by two-way ANOVA.

Under optimum moisture conditions, the enzyme activities were always higher for soil under plant species grown in monoculture compared to soil under mixture, except for glycosidase, where the opposite was true. Enzyme activities showed only slight differences in soils under the different monocultures except for chitinase activity, which was highest in soil under *M. sativa* compared to all other treatments.

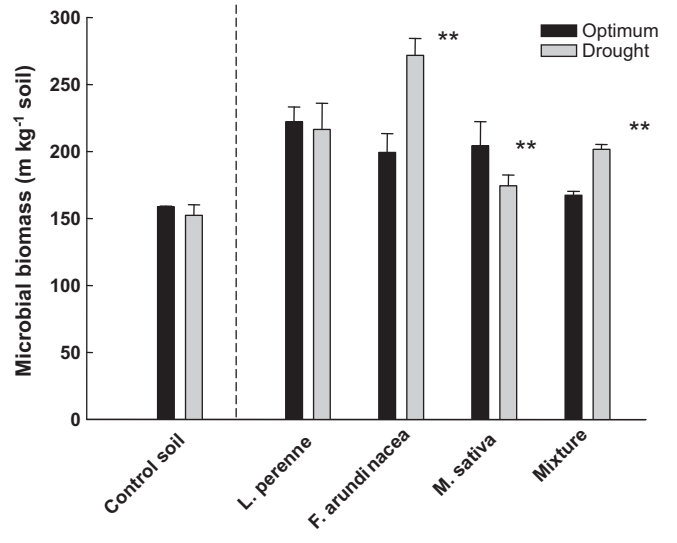
At both moisture levels the activity of the enzyme involved in the N-cycle (leucine-aminopeptidase) was higher than the activities of the enzymes involved in the C-cycle (xylanase, β-cellobiosidase and β-glucosidase).

3.4. Drought impact on extracellular enzymes

The enzyme activities in the unplanted control soil were lower under drought than under optimum moisture conditions, except for β-cellobiosidase and chitinase (Fig. 4). Increased enzyme activities were observed under drought versus optimum moisture conditions in soil under *L. perenne* (β-cellobiosidase, chitinase and leucine amino peptidase), and *M. sativa* (β-cellobiosidase, leucine amino peptidase). Enzyme activities in soil under *F. arundinacea* did not show significant differences. No significant decrease of enzyme activity was recorded for soil under monocultures subjected to drought conditions.

This was in contrast to soil under the plant mixture. Here, the activity of three enzymes was unchanged and the activity of glucosidase strongly reduced in response to drought. Only leucine amino peptidase showed enhanced activity under drought.

90–99% of the variability of activity of all five enzymes was due to plant composition and moisture.



** significantly different from optimum (0.05)

Fig. 3. Microbial biomass carbon in soil under plants grown at optimum moisture and drought conditions. Data are presented as mean + standard deviation of three replicates ($n = 3$). **Significantly different from optimum ($p < 0.05$).

4. Discussion

4.1. Plant and microbial biomass

Drought caused significant decrease in plant shoot biomass compared to optimum moisture conditions in two out of four treatments (Fig. 1). Plant growth can be limited by insufficient photosynthates during drought stress because of stomata closure (Pospíšilová and Čatský, 1999; Wertin et al., 2010). Drought stress however often results in larger root systems (Liu and Li, 2005) and frequently increased C allocation to the roots, which enhances water and nutrient uptake (Bryla et al., 1997; Huang and Gao, 2000; Liu and Li, 2005). Increase of the R/S ratio following drought under the plant monocultures indicate that plants under water stress had limited shoot growth and sustained root production by allocation of higher portion of assimilates to the roots (Liu et al., 2004). This was not the case when plants were grown in mixture. Here, root biomass as well as the R/S ratio decreased under drought stress compared with optimal moisture, indicating that plants of more complex communities limit root growth and sustain shoot production, most probably because they compete more strongly for light, than for water and nutrients. However, significant increase of MBC under drought stress compared to optimum conditions under plant mixture and under *F. arundinacea* (Fig. 3) indicate that plants increase rhizodeposition to facilitate soil water and nutrient transport (see below).

Microbial biomass always increased when soil was vegetated regardless the moisture treatment (Fig. 3). Moisture and plant community were responsible for 84% of total variability of MBC (Fig. 2). MBC increased in the vegetated soil probably due to rhizodeposition (Benizri et al., 2007). Rhizodeposition may occur by root exudation and root cell sloughing (Rasse et al., 2005). Root exudation seems to be unrelated to root biomass production (Roumet et al., 2008) and might have increased MBC in soils under *F. arundinacea* and plant mixture, despite similar or decreased root biomass (Fig. 1). This may be explained by energy input through water-soluble compounds and mucilaginous material by drought-stressed roots (Czarnes et al., 2000b; Dijkstra and Cheng, 2007; Henry et al., 2007). These compounds could play a major role in the maintenance of root–soil contact, which is especially important under drought conditions (Walker et al., 2003). They represent a source of labile C in soil, which is rapidly consumed by microorganisms (Jones et al., 2009), thereby stimulating microbial biomass production (Benizri et al., 2007).

4.2. Enzyme activities under drought stress

Activities of extracellular enzymes in the rhizosphere were sensitive to drought and plant community composition. This is illustrated by the high proportion of variability, which is explained by these two parameters (Fig. 2).

Under drought stress, the activity of β-cellobiosidase was increase for soil under *L. perenne* and *M. sativa* (Fig. 4). Cellulose

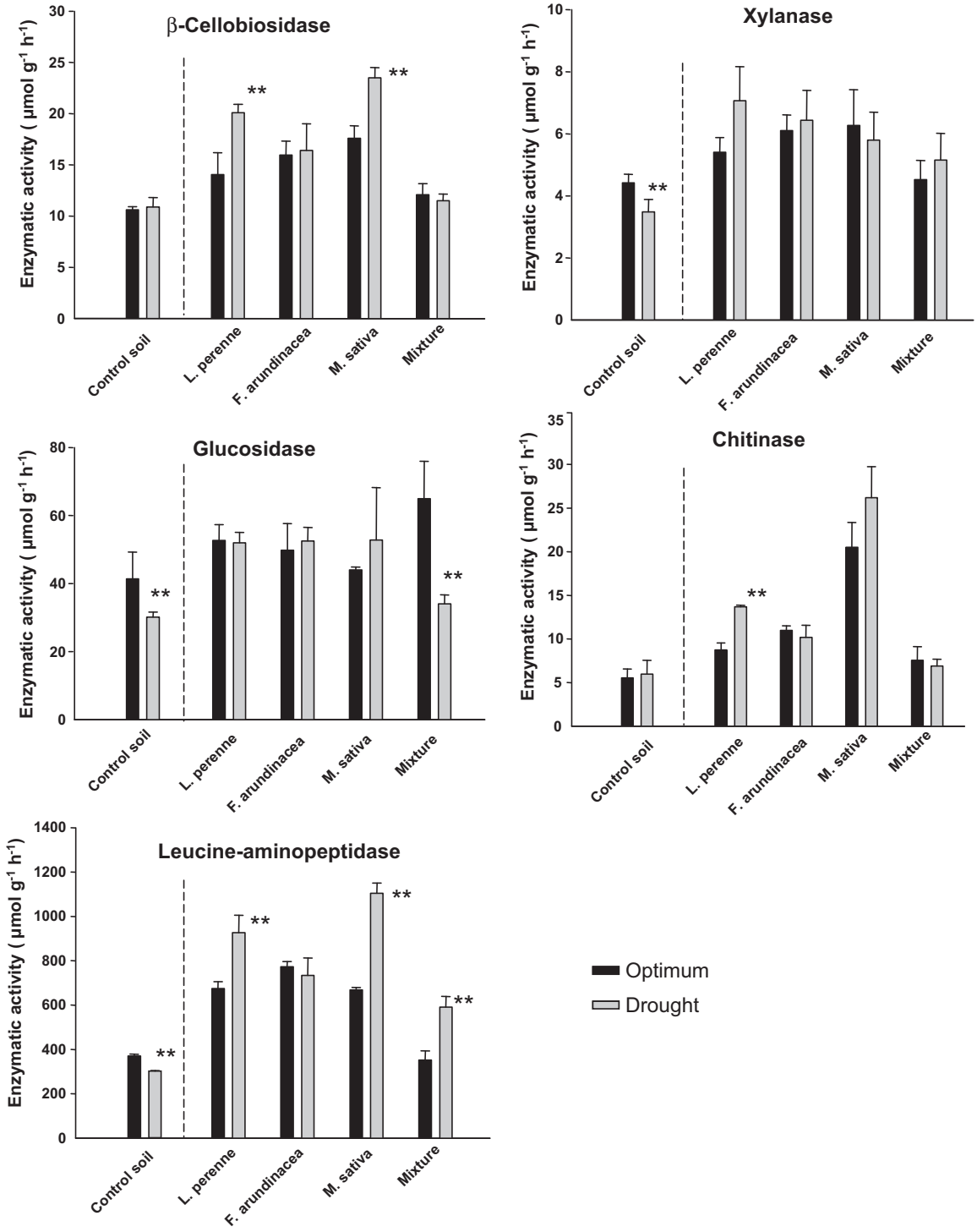


Fig. 4. Enzyme activities (μmol g^{-1} h^{-1}) in unplanted (control) soil and soil under plants grown in monoculture or mixture exposed to optimum or drought conditions. Data are presented as mean + standard deviation of three replicates ($n = 3$). Note, that different scales were used for the y-axes. **Significantly different ($p < 0.05$) from optimum.

degradation is initiated by β-cellobiosidase which breaks cellulose chains into smaller units (Alef and Nannipieri, 1995). As cellulose comprises up to 40% of plant tissue, greater root litter production in the case of *L. perenne* and *M. sativa* in response to drought could have increased cellulose input into soil. In absence of significant root biomass increase under drought conditions (Fig. 1), the enhanced activity of β-cellobiosidase could indicate high root turnover in these two soils (Kreyling et al., 2008). Increased activity of cellulose degrading enzymes (β-cellobiosidase and β-glucosidase) in the presence of legumes (*M. sativa*) was also found in other studies (Salamon et al., 2004; Kreyling et al., 2008). Xylanase activity was unchanged following drought stress in planted soils (Fig. 4). Xylanase is one of the most important enzymes controlling the breakdown and decomposition of hemicellulose when readily available compounds are exhausted (Stemmer et al., 1999). The pattern of change for chitinase activity under different plant communities was similar to that of β-cellobiosidase. Higher chitinase activities may indicate the stimulation of fungi under drought (Parham and Deng, 2000; Kreyling et al., 2008), as chitinase degrades chitin from fungal or arthropod origin (Chung et al.,

2007) and converts it to easily degradable amino sugars (Ekenler and Tabatabai, 2002; Acosta-Martinez et al., 2008). The activity of chitinase may also indicate N-acquiring activities of microorganisms (Parham and Deng, 2000). It was interesting to note that for soils under mixture the activity of enzymes, involved in the C cycle remained unchanged or decreased following drought (Fig. 4). The only enzyme activity, which increased under drought stress in soil under the plant mixture was leucine-aminopeptidase, an enzyme involved in the N cycle.

Leucine-aminopeptidase is an enzyme involved in the degradation of proteins and we found its activity to be increased following drought in all vegetated soils, except the one under *F. arundinacea*. This could suggest that protein input from dead roots was enhanced following drought stress in most treatments. Faster microbial turnover due to drought might also have contributed to higher protein input.

Changing enzyme activities in soils submitted to low water potential may indicate that drought changes rhizodeposition, root litter production and ultimately substrate availability. Leucine-aminopeptidase, an enzyme involved in the N cycle responded more strongly to drought compared with enzymes involved in the C cycle. This may illustrate a high N demand due to microbial biomass production (Fig. 3) and could also indicate increased microbial N acquisition from peptide degradation (Parham and Deng, 2000; Sardans and Penuelas, 2005; Weintraub et al., 2007) in rhizodeposits.

With the exception of glucosidase under optimum conditions, the activity of all enzymes was lower in soil under mixture compared to monocultures for both water potentials (Fig. 4). Although the increase of leucine-aminopeptidase activity in response to drought was highest in soil under mixture (around 180% of the activity under optimum conditions) compared to most other treatments, the absolute activity of both enzymes involved in the N cycle was lower in drought-affected soil under plant mixture compared to soil under monocultures.

5. Conclusions

Drought stress affected differently root and shoot growth of monocultures and plant mixtures. Microbial biomass C was not correlated with root biomass and showed a significant increase due to drought only for soil under plant mixture and *F. arundinacea*. Responses of enzyme activities depend on plant community composition. We could not confirm the hypothesis that drought affected differently the activity of enzymes involved in the initial versus the last step of decomposition. Our results suggest that plant community composition had modified the drought effects in the rhizosphere. The presence of plants in mixture in grassland ecosystems may reduce drought effects by enhancing C sequestration due to reduced enzyme activities compared to monocultures. Thus, plant community composition can be used as a tool to meet drought stress not only because of different water use efficiency by plants, but also because of their effects on soil microbial activity affecting C and N cycles.

Acknowledgments

We acknowledge Higher Education Commission (HEC) of Pakistan and the Région Poitou-Charentes for financial support under the framework of the program 'Excellence Environnementale et Développement des Eco-Industries'. EGIDE-DAAD is acknowledged for funding of a PROCOPE exchange project (contract-20189ZB) and also the European Commission (Marie Curie IIF program, project MICROSOM). We are highly indebted to Dr. Sergey Blagodatsky for helpful comments. We are also grateful to Thaufelder Ilse for her technical assistance. In addition we thank three anonymous reviewers and the editor H. Insam for constructive comments on the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.apsoil.2011.02.004.

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