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Carbon allocation in grassland communities under drought stress followed by ^{14}C pulse labeling

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ABSTRACT

Although extreme climatic events such as drought have important consequences for belowground carbon (C) cycling, their impact on the plant-soil system of mixed plant communities is poorly understood. Our objective was to study the effect of drought on C allocation and rhizosphere-mediated CO_2 fluxes under three plant species: *Lolium perenne*, *Festuca arundinacea* and *Medicago sativa* grown in monocultures or mixture. The conceptual approach included $^{14}CO_2$ pulse labeling of plants grown under drought and optimum water conditions in order to be able to follow above- and belowground C allocation. After ^{14}C pulse labeling, we traced ^{14}C allocation to shoots and roots, soil and rhizospheric CO_2, dissolved organic carbon (DOC) and microbial biomass.

Drought and plant community composition significantly affected assimilate allocation in the plant-soil system. Drought conditions changed the source sink relationship of monocultures, which transferred a relatively larger portion of assimilates to their roots compared to water sufficient plants. In contrast, plant mixture showed an increase in ^{14}C allocation to shoots when exposed to drought.

Under drought stress, root respiration was reduced for all monocultures except under the legume species. Microbial respiration remained similar in all cases showing that microbial activity was less affected by drought than root activity. This may be explained by strongly increased assimilate allocation to easily available exudates or rhizodeposits under drought. In conclusion, plant community composition may modify the impact of climatic changes on carbon allocation and belowground carbon fluxes. The presence of legume species attenuates drought effects on rhizosphere processes.

1. Introduction

The influence of climate on the plant soil system is complex and the interactions are poorly understood. Short-term events may have much stronger impacts on pools and/or fluxes in ecosystems compared with long-term trends (Kuzyakov and Gavrichkova, 2010). Climatic modification may concern mostly plant–soil interactions due to its impact on carbon (C) allocation and root activity (Cheng and Kuzyakov, 2005).

Roots of higher plants are key functional components of belowground systems and the zone of soil around roots – rhizosphere – plays an important role in the soil C cycle. The rhizosphere has been considered as one of the key fine scale components in global carbon cycle research (Coleman et al., 1992). In the rhizosphere, soil organic matter (SOM) decomposition and mineralization are controlled by root litter inputs, but also through root–microbial interactions i.e. rhizosphere effects influencing (1) nutrient availability, (2) physical and chemical environment, (3) availability of organic substrates and (4) priming (Hinsinger et al., 2009; Cheng and Kuzyakov, 2005). Moisture conditions may significantly alter rhizosphere effects on decomposition. While root exudation was increased following water limitation, priming effects were found to be reduced (Dijkstra and Cheng, 2007). In agricultural systems, as consequence of agricultural monocultures, most studies on rhizosphere effects have been limited to plants grown individually, although species mixtures were found to respond differently to environmental stress such as drought. For example, at low water levels, rhizosphere effects of plant species grown in mixture were found to reduce SOM decomposition and plant N uptake compared to monocultures (Dijkstra et al., 2010). Potential activity of enzymes involved in the C cycle tended to increase in soil under plant mixtures, while they were unchanged or decreased under monocultures (Sanaullah et al., 2011).

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CO_2 efflux from soil constitutes a major component of the global carbon cycle and is likely to be altered by climate change. The evolution of CO_2 is a sensitive indicator of crop residue decomposition, SOM turnover and ecosystem disturbance (Paul et al., 1999). Summer drought was found to decrease soil CO_2 efflux and to alter its sources (Joos et al., 2010). Under wheat plants, the amount of assimilates lost as CO_2 respired from soil was found to be enhanced following water limitations (Palta and Gregory, 1997). Soil CO_2 efflux originating from assimilates is the result of two distinct processes controlling rhizosphere respiration: (1) root respiration and (2) microbial respiration from the metabolism of rhizodeposits (Andrews et al., 1999; Cheng and Kuzyakov, 2005). These distinctions are important for interpreting the sources of CO_2 and the fate of carbon within soils and ecosystems. At present, we lack a detailed understanding of the rhizosphere processes of single species as well as plant mixtures that occur in response to drought stress.

Therefore, in this study, we tested the effect of drought on belowground C allocations and rhizosphere-mediated soil respiration in grassland soil under grassland species grown in monoculture or mixture. Our conceptual approach included pulse labeling of plants with $^{14}CO_2$ and partitioning of the labeled carbon in plant and soil carbon dioxide fluxes. The aim of this study was to evaluate the changes of rhizosphere processes occurring in response to drought.

2. Materials and methods

2.1. Soil

The soil samples were taken from the top 20 cm of a loamy Cambisol under flat temporary grassland established since more than 50 years. The site is part of the long-term observatory for environmental research (ORE-ACBB) of INRA, France. It is located near Lusignan in the south-west of France (46°25′12,91″ N; 0°07′29,35″ E). The soil is carbonate-free and has the following characteristics: pH 6.4, organic C (C) 1.4%, nitrogen (N) 0.16%, sand 11%, clay 17%, silt 72% (Chabbi et al., 2009). After sampling the soil was air-dried and passed through a 5 mm sieve.

2.2. Experimental design and growth conditions

In order to study interactive effects of plant community composition and water availability on belowground C allocation and root-derived CO_2, a two factorial experiment was established. We used seeds of *Lolium perenne*, *Festuca arundinacea* and *Medicago sativa*, which were grown for five days in petri dishes. Thereafter, the plants were planted in microcosms containing 500 g of soil. The experimental setup included planting (i) as 6 plants of the same species (monocultures) or (ii) as 2 × 2 × 2 plants (mixture) of each species. To assure 3 replicates for each treatment combination (individual plant species, mixture or unplanted soil and two different water levels), in total 24 microcosms with planted soil and 6 microcosms with unplanted soil (control) were incubated for 70 days. The plants were grown at 26–28 °C day and 22–23 °C night temperature with a day-length of 14 h and light intensity of 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 available field capacity) was maintained for all plants. After one month of plant development, the soils were adjusted to two water levels: (1) optimum conditions (70% of the field capacity) and (2) drought conditions (30% of the field capacity) for 40 days. The unplanted soil control was maintained for both moisture levels.

2.3. Plant ^{14}C labeling

After 40 days of growth under different moisture conditions, carbon allocation patterns were determined using ^{14}C labeling. The detailed procedure for plant ^{14}C labeling is given in previous studies (Kuzyakov and Siniakina, 2001; Kuzyakov et al., 1999). Briefly, the labeling apparatus consisted of two compartments. The lower compartment was used for soil and plant roots and the upper compartment for the shoots and $^{14}CO_2$ generation. One day before labeling, each hole in the lid of the lower compartment containing one plant was sealed with silicon paste. Three hours before labeling, pots were flushed with CO_2-free air to remove CO_2 evolved prior to labeling. Each species was labeled separately. 1480 kBq of ^{14}C as $Na_2^{14}CO_3$ solution was put in a test tube in the upper compartment of the chamber and the chamber was then closed. Three ml of 5 M H_2SO_4 was added to the $Na_2^{14}CO_3$ solution in the test tube through a Teflon tube. This allowed the complete evolution of $^{14}CO_2$ into the chamber atmosphere. Assimilation took place within 3 h after the pulsing of $^{14}CO_2$. After the labeling period of 3 h, trapping of CO_2 from the upper compartment was started to remove the remaining unassimilated $^{14}CO_2$ by pumping the air through 15 ml of 1 M NaOH solution. Thereafter, the top of the chamber was removed.

2.4. Carbon mineralization and analysis of plant material, soil as well as microbial biomass

Throughout the experiment, which lasted 120 h, CO_2 evolved from the soil–root compartment was trapped in 15 ml of 1 M NaOH solution by continuous pumping (100 cm^3 min^{-1}) with a membrane pump. The CO_2 trap was changed every 6 h starting immediately after the labeling. Total content of CO_2–C collected in the NaOH solution was measured by titration with 0.01 M HCl against phenolphthalein, after addition of 2 M $BaCl_2$ solution (Kuzyakov and Cheng, 2001).

Five days after the labeling, the soil–root chamber was destructively sampled. Shoot material was separated from roots and roots were separated from soil and washed by dipping them into water. Shoots, roots and soil were dried at 60 °C, homogenized and pulverized in a ballmill (Retsch) prior to further analysis. For total C analysis the plant shoots, roots and soil were combusted and the CO_2 evolved trapped in NaOH and the CO_2–C measured as described above.

A subsample of fresh soil was used for microbial biomass determination by $CHCl_3$ fumigation-extraction (Vance et al., 1987). After fumigation, 10 g of the fumigated and an unfumigated sample were extracted with 40 ml of 0.05 M K_2SO_4 solution. Briefly, the mixture was shaken for 30 min at 300 rev min^{-1}. Thereafter it was centrifuged (8000 × G, 10 min), the supernatant recovered by filtration and analyzed for TOC and ^{14}C. The microbial biomass C and ^{14}C were calculated as the difference between fumigated and non-fumigated soil samples after correcting for extraction efficiency ($k = 0.45$) (Vance et al., 1987).

Dissolved organic carbon contents were determined as the sum of C in the K_2SO_4 extract of unfumigated samples and C in water remaining after root washing. The ^{14}C activity of both fractions were accepted as ^{14}C in DOC (Kuzyakov and Domanski, 2002). The total C and N contents in water after root washing and in K_2SO_4 extracts were determined with Multi C/N 2100 (Analytik Jena, Germany).

2.5. ^{14}C activity measurements

^{14}C activities of DOC and CO_2 in NaOH from respiration as well as of bulk plant shoot, roots and soil were measured by mixing 1 ml of this solution with 2 ml of scintillation cocktail (Rotiszint EcoPlus, Carl

Roth, Germany) after decay of chemiluminescence (for NaOH). The ^{14}C measurements were done by a 1450 LSC & Luminescence Counter (MicroBeta TriLux, Perkin Elmer Inc., USA). The ^{14}C counting efficiency was at least 70% and the measurement error did not exceed 3.5%. The absolute ^{14}C activity was standardized by adding increasing amounts of NaOH as a quencher (Kuzyakov, 2002; Kuzyakov and Cheng, 2001; Kuzyakov and Domanski, 2002; Kuzyakov et al., 1999).

2.6. Partitioning of the root-derived CO_2 efflux

Partitioning of the root-derived CO_2 efflux from the soil into actual root respiration (RR) and rhizomicrobial respiration (RMR) in the rhizosphere was achieved by using a model to separate C flows in the rhizosphere (Kuzyakov, 2002; Kuzyakov and Domanski, 2002; Kuzyakov et al., 1999). In order to separate the processes of root respiration and microbial respiration of dead roots and root parts, a simple model for $^{14}CO_2$ efflux from soil after $^{14}CO_2$ pulse labeling of shoots was used. The model describes separately only the underground flows of labeled C after above ground assimilation and can divide total $^{14}CO_2$ efflux from soil into RR and RMR. The model consists of seven ^{14}C labeled compartments. The separation of CO_2 efflux coming from different flows is based on the assumption that these two processes occur at different rates: the most rapid process is CO_2 efflux from root respiration. The CO_2 evolution by microbial respiration of root exudates is a slower process than root respiration because it consists of a chain of successive processes: exudation from the root, intake by microorganisms and respiration of microorganisms. The monitoring of $^{14}CO_2$ efflux from soil after ^{14}C pulse labeling of plants, fitting the model parameters on the measured $^{14}CO_2$ efflux, and subsequently modeling of RR and RMR allowed the independent estimation of both flows.

2.7. Calculations and statistics

^{14}C data for each replicate were expressed as percentages of ^{14}C recovered in the plant/soil system:

$$^{14}C \text{ recovered in the plant/soil system} = {}^{14}C\,CO_2 + {}^{14}C \text{ soil} + {}^{14}C \text{ shoot} + {}^{14}C \text{ root} + {}^{14}DOC \quad (1)$$

Moreover we calculated the specific ^{14}C activity as the proportion of ^{14}C assimilated:

$$\text{Specific } {}^{14}C \text{ activity} = \%\,{}^{14}C \text{ assimilated x}/\%\,\text{carbon mass x}, \quad (2)$$

Where x is root or shoot of plant monocultures or mixture

The ^{14}C data as percentages of net ^{14}C recovered in the plant/soil system under optimum and drought conditions were tested for significant difference using the Mann and Whitney test. Significant difference was declared at the 5% level.

3. Results

3.1. Effect of drought and plant community composition on ^{14}C allocation to plant and soil

Plant shoots were the main sinks for assimilated CO_2 regardless the moisture treatment with an allocation of 30 to 40% of the ^{14}C activity recovered in the plant/soil system (Fig. 1A). This is likely to be underestimated as we did not measure shoot respiration, which could reach up to 17% of total assimilated ^{14}C (Kuzyakov and Siniakina, 2001). Under drought stress, ^{14}C allocation to plant shoot was decreased for plant monocultures compared to optimum

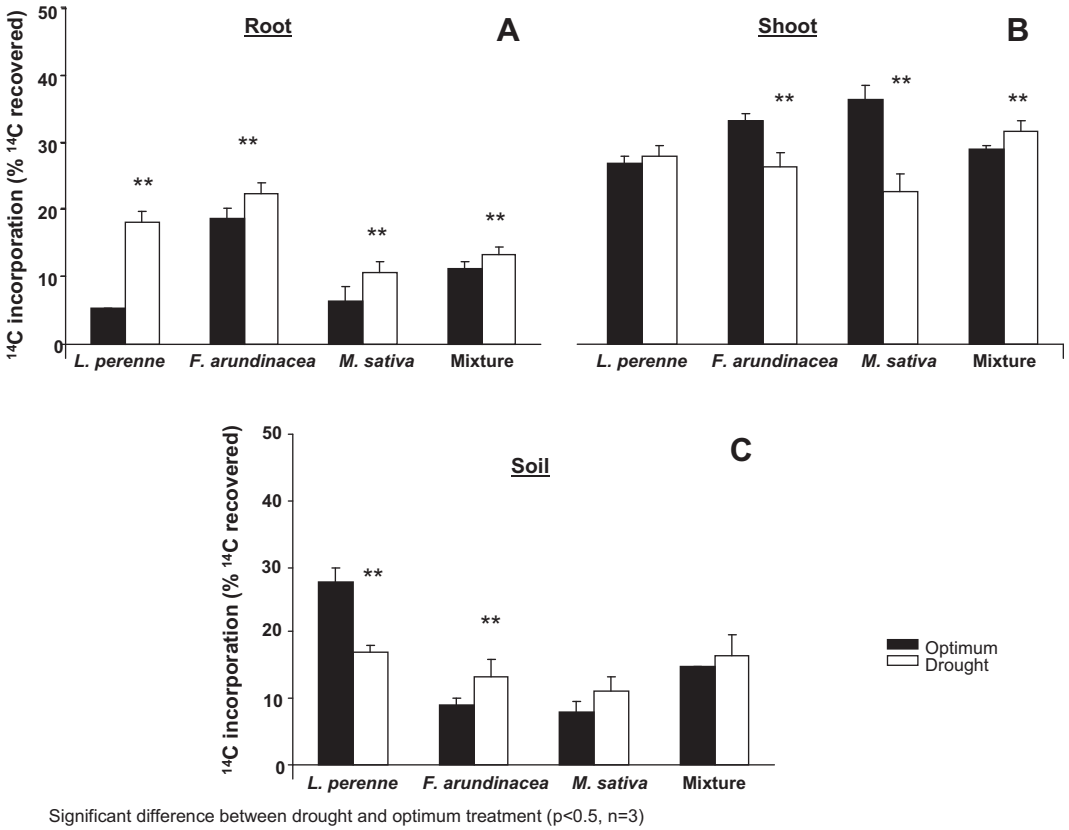

Fig. 1. Effect of drought on the amount of ^{14}C incorporated in the root (A), shoot (B), and in soil (C) in the presence of all three plants as monocultures or mixtures. The values represent means ± SE ($n = 3$).

Table 1
Root and shoot biomass as well as root/shoot ratio and root/shoot ratio of labeled ^{14}C allocation. All data, except ^{14}Croot/^{14}Cshoot from Sanaullah et al. (2011).

Plant species	Root	Shoot	Root/shoot	^{14}C root/ ^{14}C shoot
	g	g		
L. perenne				
Optimum	1.30 ± 0.45	1.73 ± 0.25	0.74 ± 0.15[a]	0.19 ± 0.01[a]
Drought	1.63 ± 0.25	1.47 ± 0.12	1.12 ± 0.21	0.64 ± 0.09
F. arundinacea				
Optimum	1.76 ± 0.85	2.50 ± 0.17[a]	0.72 ± 0.39	0.56 ± 0.06[a]
Drought	1.36 ± 0.15	1.43 ± 0.06	0.95 ± 0.08	0.84 ± 0.04
M. sativa				
Optimum	0.96 ± 0.40	2.30 ± 0.17[a]	0.41 ± 0.15[a]	0.18 ± 0.06[a]
Drought	0.73 ± 0.11	1.00 ± 0.30	0.76 ± 0.13	0.47 ± 0.13
Mixture				
Optimum	1.63 ± 0.25[a]	1.80 ± 0.61	0.97 ± 0.27	0.37 ± 0.05
Drought	1.06 ± 0.05	1.53 ± 0.15	0.70 ± 0.07	0.41 ± 0.03

[a] significant difference between drought treatment and optimum conditions.

conditions, whereas it increased for plant mixtures. This resulted in increased ^{14}Croot:^{14}Cshoot ratios under drought conditions for all three monocultures (Table 1). For plant mixture, the ^{14}Croot:^{14}Cshoot was unchanged. Root ^{14}C allocation was about half of that recorded for shoots (Fig. 1B). It increased significantly following drought stress in all treatments. Response of ^{14}C allocation to soil under drought conditions depended on plant species and community composition. *L. perenne* showed reduced C allocation to soil following drought (Fig. 1C), whereas it was increased under *F. arundinacea* and the plant mixture and remained unchanged under *M. sativa*.

To determine changes in the relative growth rate of roots and shoots, the % of C allocated to the plant organ was divided by the amount of dry C mass. Under drought stress, specific ^{14}C activity increased for both root and shoot material compared to optimum conditions (Table 2). The specific ^{14}C activity increased more in shoot material of the plant mixture compared to monocultures (Table 2).

3.2. Effect of drought and plant community composition on cumulative CO_2–C efflux, ^{14}C contribution to CO_2 efflux and microbial as well as root respiration

Cumulative CO_2–C efflux ranged between 3 mg g^{-1} for unplanted soil and 18 mg g^{-1} for planted soil (Fig. 2A). Drought had no significant effect on CO_2 efflux from unplanted soil and soil planted with *L. perenne*. For soil under the other two species as well as the species mixture we recorded significantly reduced CO_2 efflux when soils had experienced drought (Fig. 2A). Due to the non-significant differences between control and drought treatment of unplanted soil, we consider the pattern of total and rhizospheric CO_2 efflux to be similar.

The ^{14}C introduced by pulse labeling and recovered in the plant/soil system during the experimental period was found to be allocated between 25 and 50% to the CO_2 efflux from soil (Fig. 2B). Under optimum conditions highest ^{14}C allocation to soil respiration was found under *M. sativa*. Drought enhanced this tendency. Under

Table 2
Specific ^{14}C activity for monocultures and plant mixture under drought and optimum conditions.

	Monocultures		Mixture	
	Optimum	Drought	Optimum	Drought
Root	1.32 ± 0.56	2.47 ± 0.52	1.31 ± 0.30	2.23 ± 0.11
Shoot	3.09 ± 0.10[a]	3.71 ± 0.15[a]	2.90 ± 0.03[a]	4.13 ± 0.32[a]

[a] Significant difference between drought and optimum conditions.

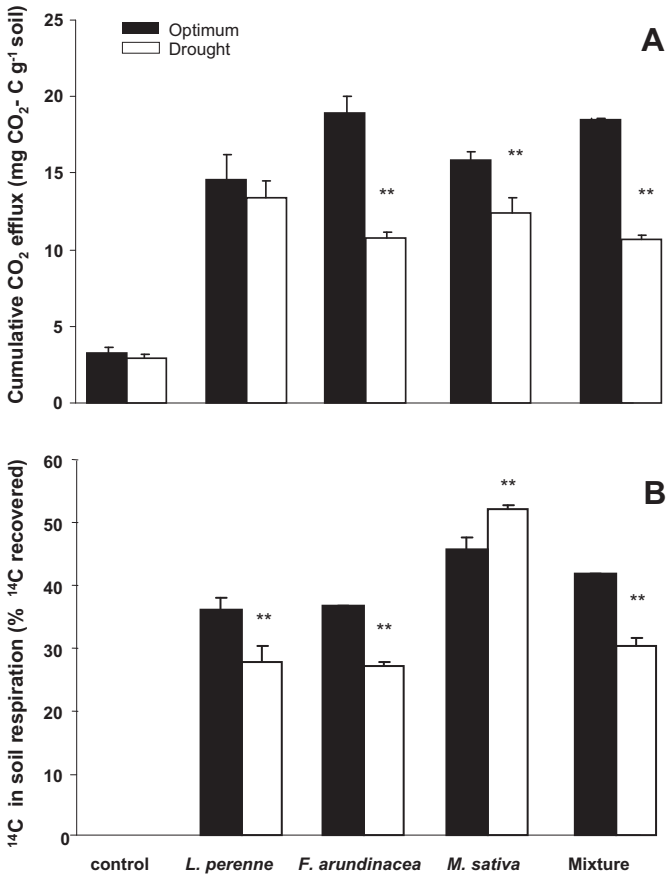

Fig. 2. Cumulative CO_2 efflux of all treatments (A) and ^{14}C in respired CO_2 of planted soil (B) at the end of the experiment. Data is represented as mean + SE ($n = 3$). **Significant difference between drought and optimum ($P < 0.05$, $n = 3$).

M. sativa, ^{14}C allocation to soil respiration increased in the drought treatment, whereas it decreased for all other plants as well as their mixture.

Using a model developed by Kuzyakov and Domanski (2002) we were able to separate cumulative $^{14}CO_2$ efflux rate into root respiration (RR) and rhizomicrobial respiration (RMR). These data are presented in Fig. 3. Our data show, that drought heavily affected root respiration in all treatments except under *M. sativa*, whereas ^{14}C efflux rate from microbial respiration remained similar in all treatments.

3.3. Effect of drought and plant community composition on microbial biomass and dissolved organic carbon

Drought induced significant increase in microbial biomass C in the presence of *F. arundinacea* and mixture (40 and 20% of optimum conditions) while under *L. perenne* and *M. sativa* we recorded a 5–10% decrease in MBC (Fig. 4A). Under drought stress, DOC increased greatly (more than 50%) in all planted treatments, except in soil planted with *M. sativa* monoculture, where a 10% increase was recorded (Fig. 4B).

^{14}C incorporation into the soil microbial biomass and dissolved organic matter was below 5% for all treatments under optimum conditions (Fig. 5). Following drought, ^{14}C partitioning to microbial biomass did not show a clear trend (Fig. 5A). It was decreased in two out of four treatments and increased for the others. In contrast, drought treatments showed increased ^{14}C allocation to dissolved organic matter in three out of four cases (Fig. 5B), an exception being the legume species *M. sativa*, which showed similar allocation of ^{14}C to DOC under drought and optimum conditions.

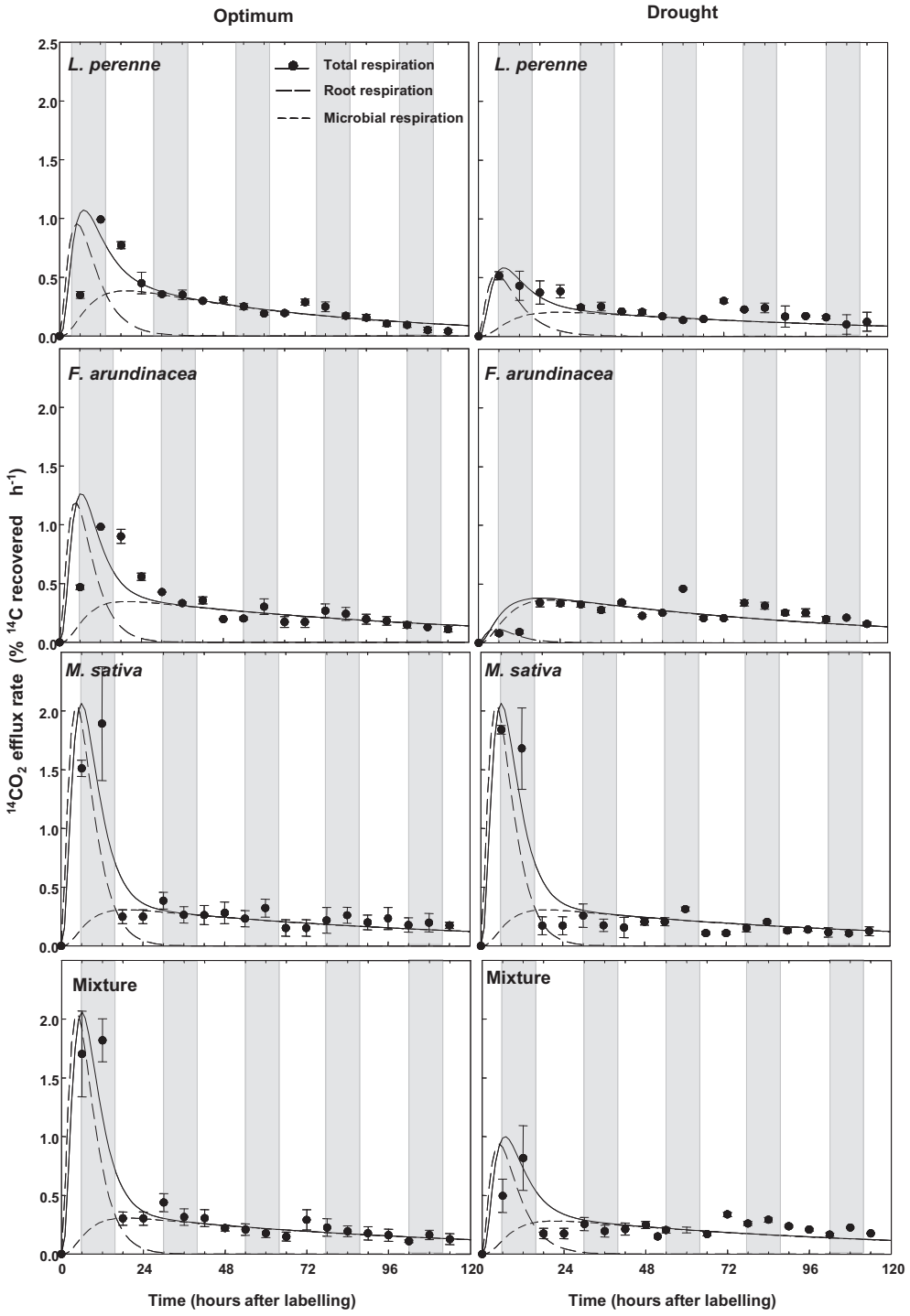

Fig. 3. Total $^{14}CO_2$ efflux rate, and simulated root- and rhizomicrobial- respiration. Data is presented as mean ± SE ($n = 3$).

4. Discussion

4.1. Effect of drought on assimilate allocation to root and shoot biomass

Drought had significant effect on plant shoot and root biomass but it was species specific as well as depending on plant community composition. Shoot biomass of all plants grown in monocultures decreased due to drought stress as compared with optimal moisture conditions (Table 1). This decrease was significant only for *F. arundinacea* and *M. sativa*, while there was no significant change in shoot biomass for plants grown in mixture (Table 1, Sanaullah et al., 2011). In contrast, root biomass significantly decreased following drought stress when plant were grown in mixture (Table 1), while no significant change was found for monocultures of all three species.

The proportion of the ^{14}C recovered in the plant/soil system, allocated to shoot was different according to the plant species and the plant community composition (monocultures vs mixture, Fig. 1A). This shows, that the strategy chosen by the plants to adapt

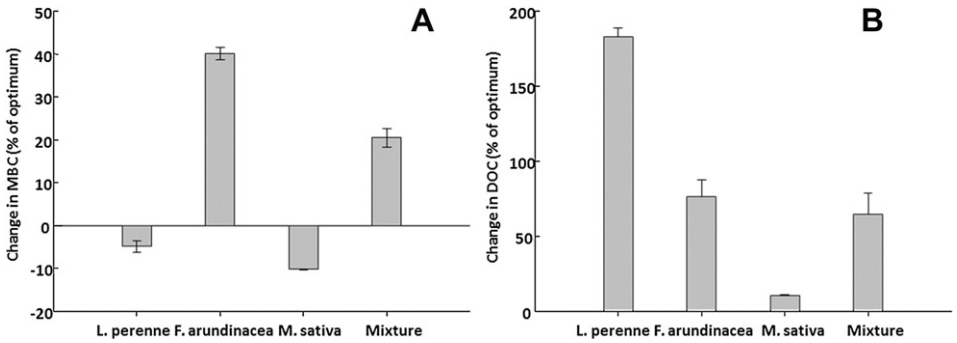

Fig. 4. Effect of drought on microbial biomass C (A) and DOC (B) in % of optimum conditions in the presence of all three plants and their mixture. The values represent means + SE ($n = 3$).

to drought conditions is species dependent. However, in all treatments, plants seemed to increase carbon allocation to root biomass (Fig. 1B). This increase was less pronounced for plant mixture, which allocated at the same time a higher amount of assimilates to shoots (Fig. 1A and B). This is in agreement with the reduced root to shoot ratio recorded for plant mixture, whereas this ratio was increased for plant monocultures (Table 1, Sanaullah et al., 2011). Greater C allocation to roots than shoots in plant monocultures under drought conditions resulted from lower reductions in root growth compared to shoot growth (Palta and Gregory, 1997). In our experiment, there was no significant effect of drought on assimilate accumulation in shoot biomass of *L. perenne*. This plant in contrast increased greatly carbon allocation to its root biomass. This is in line with higher root biomass and root C contents in roots of *L. perenne* under drought stress compared to optimum conditions (Liljeroth et al., 1994; Sanaullah et al., 2011). *M. sativa* and *F. arundinacea* showed decreased assimilate allocation to shoots after drought whereas the plant mixture showed an increase. Overall, drought conditions changed the source sink relationship of all plants.

4.2. Effect of drought on rhizosphere processes

Total CO_2 efflux from soil decreased in all planted treatments under drought compared to optimum water content (Fig. 2A). This is in line with results from a field experiment showing that CO_2 efflux from soil under grassland was reduced after a drought period even if rewetting events are included (Joos et al., 2010). The authors hypothesized that this reduction may be the result of reduced heterotrophic respiration in agreement with the study of Borken et al. (2006) in a forest ecosystem. In our study, no reduction of CO_2 efflux was noted for the unplanted treatment (Fig. 2A). This is in contrast to other studies, suggesting that drought reduces microbial activity, enzyme activity and hence SOM degradation (Dijkstra and Cheng, 2007), but in agreement with modeled results of $^{14}CO_2$ efflux from the planted treatments (see below), suggesting that the water limitation applied to the soil in this study was not strong enough to influence microbial decomposition of assimilates. When soils become dry, this usually reduces the thickness of the water film on soil surfaces and the rate of diffusion of substrates to microbes (Stark and Firestone, 1995), thereby reducing SOM decomposition. As we did not observe significant differences for SOM decomposition of unplanted soil, we suggest that rhizosphere processes are most important determinants of soil respiration in response to drought. The amount of CO_2 released from planted soil was about 3–4 times higher compared to unplanted soil. The magnitude of this response was plant species dependent (Fig. 2A). Respiration fluxes from the rhizosphere may be related to enhanced root respiration as well as enhanced microbial respiration of root exudates, which could generate a rhizosphere effect of SOM decomposition (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). The $^{14}CO_2$ fluxes related to these processes were monitored under

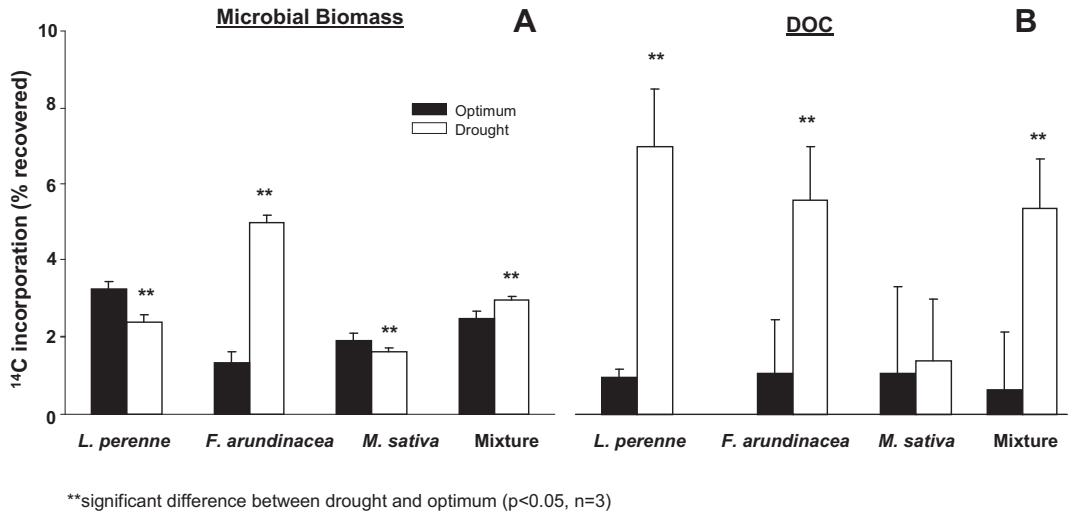

Fig. 5. ^{14}C incorporation (% recovered in the plant/soil system) in the microbial biomass (A) and in DOC (B) in the presence of all three plants. The values represent means + SE ($n = 3$).

monocultures and plant mixture during 6 days after 3 h of pulse labeling. By this shorterm labeling we were be able to trace the fate of rhizodeposits derived from recent photoassimilates (i.e. root exudates, mucilage and border cells) (Jones et al., 2009). The proportion of ^{14}C recovered in soil respiration was significantly decreased in the drought treatments under two monocultures i.e. *L. perenne* and *F. arundinacea* and the plant mixture (Fig. 2b), whereas ^{14}C contribution to soil respiration increased under the legume species *M. sativa.* The total rhizosphere respiration contributes from 19 to 80 % of the total CO_2 efflux from planted soil (Kuzyakov and Cheng, 2001). Partitioning of the rhizophere respiration into actual root respiration (RR) and microbial respiration of exudates and root residues (RMR) was carried out by a modeling approach (Kuzyakov and Domanski, 2002). This model consists of seven pools and was developed and parameterized based on ^{14}C pulse labeling of *L. perenne*. It uses empirically determined parameters of C allocation in the plant-soil system (Kuzyakov and Domanski, 2002). It assumes that $^{14}CO_2$ respired from soil shortly after labeling is derived from RR, as it was shown that phloem transport of assimilated C is very rapid (Bahn et al., 2009; Carbone and Trumbore, 2007) and that plant roots will use assimilates for RR almost immediately (Kuzyakov and Gavrichkova, 2010). The model did not consider the storage of assimilated C and later use as was shown by Bahn et al. (2009). As use of assimilates by mycorrhizal fungi may be very rapid due to their close association with the roots, mycorrhiza activity may have contributed to RR (Kuzyakov and Gavrichkova, 2010). Despite of the short distance between roots and soil, exudation may be delayed due to the low permeability of cell membranes. The other processes related to rhizodeposition and subsequent use by rhizomicroorganisms such as sloughing off of cells, death of fine roots and root hairs as well as drying of mycorrhizal hyphes takes more time as special enzymes are required for their decay (Paterson et al., 2009). Thus our model assumes that rhizomicrobial respiration of ^{14}C labeled material thus occurs later than root and mycorrhiza respiration due to the time needed for synthesis of exudates, secretion, exudation (Warembourg and Billes, 1979) and enzyme production for their decay (Högberg and Read, 2006). Applied to the data recorded in this study, the modeling results suggest that drought may have decreased RR of all treatments except for *M. sativa,* whereas RMR was unaffected in all cases. Under drought stress, decreased RR for monocultures of *L. perenne* and *F. arundinacea* and plant mixture (Fig. 3) indicated a reduced root functioning. Under *M. sativa*, we noted similar RR indicating that root functioning was unaffected by drought stress (Liu and Li, 2005). Root derived CO_2 efflux is controlled by the rate of substrate supply to roots (Gavrichkova and Kuzyakov, 2010). Any alteration in environmental factors affecting photoassimilation may be expected to affect exudates release (Hodge and Millard, 1998; Kuzyakov and Cheng, 2001; Liu and Li, 2006). The results of our study suggest that exudation may increase following drought as assimilate allocation to DOC was increased in all the four treatments (Figs. 4 and 5). The increase could indicate, that exudation rates were high, or that the activity of root associated microorganisms, such as mycorrhizal fungi or root associated bacteria was greatly reduced following drought (e.g. Borken et al., 2006; Yuste et al., 2003) thus leading to accumulation of DOC. However, in our case the modeling results indicated that microbial activity was maintained. Drought stress may have significant effects on quantity and composition of root exudates and may also lead to release of increased amounts of mucilaginous material around drought-stressed roots (Dijkstra and Cheng, 2007; Henry et al., 2007). Such increased root exudation may have stimulated rhizomicrobial activity. We suggest that plant roots through production of labile compounds are able to maintain the rhizomicrobial activity.

4.3. What is the difference in belowground carbon fluxes under monocultures versus mixtures?

Under drought conditions, in contrast to monocultures, plant mixtures continued to allocate high amounts of assimilates to plant shoots as evidenced by the specific ^{14}C activity (Table 1), as well as root to shoot ratio below 1 for all treatments except *L. perenne* (Sanaullah et al., 2011). Our data suggest that plant mixtures attenuated extreme drought responses noted for individual species. This is especially evident, when comparing soil carbon fluxes of the legume species *M. sativa* with the other treatments. Under drought conditions, this species showed the highest reduction in ^{14}C allocation to shoots compared to all others, whereas most ^{14}C was recovered in soil respiration (Figs. 2 and 3). Moreover, the legume species was the only one able to maintain root respiration under drought conditions (Fig. 4) probably, because this species in contrast to the others did not increase exudation, as indicated by unchanged ^{14}C allocation to DOC compared to optimum conditions. Microbial respiration in the rhizosphere of *M. sativa* was likely maintained due to its capability to fulfill increased N demands by physiologically stressed soil microorganism (Tiemann and Billings, 2011). In plant mixtures, the presence of legume species may therefore be crucial for maintaining root activity and belowground processes (Breulmann et al., 2012) under drought conditions.

5. Conclusion

Drought and plant community composition significantly affected assimilate allocation in plant-soil system. Drought conditions changed the source sink relationship of the plants, which transferred in general a relatively larger portion of assimilates to roots compared to water sufficient plants. The translocated assimilates may be either incorporated in root tissue, as was observed in the majority of plant monocultures, or contribute to increased root exudation, in the case of plant mixtures. The latter also allocated more assimilates to plant shoots probably because of concurrence for light. Root respiration decreased following drought in all treatments, except for the legume species, which might be more drought tolerant than the others, due to its ability to provide rhizomicroorganisms with available N. In contrast, rhizomicrobial respiration of all treatments was unaffected by drought, indicating that plants preferred to maintain rhizomicrobial activity at the expense of root activity. In conclusion, plant community composition may modify the impact of climatic changes on belowground carbon allocations and root-derived respiration. Belowground processes under *M. sativa*, a legume species, were less affected by drought than those of non-legume species.

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References

Andrews, J.A., Harrison, K.G., Matamala, R., Schlesinger, W.H., 1999. Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). Soil Science Society of America Journal 63, 1429–1435.

Bahn, M., Schmitt, M., Siegwolf, R., Richter, A., Brüggemann, N., 2009. Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale? New Phytologist 182, 451–460.

Borken, W., Savage, K., Davidson, E.A., Trumbore, S.E., 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Global Change Biology 12, 177–193.

Breulmann, M., Schulz, E., Weisshuhn, K., Buscot, F., 2012. Impact of the plant community composition on labile soil organic carbon, soil microbial activity and community structure in semi-natural grassland ecosystems of different productivity. Plant and Soil. http://dx.doi.org/10.1007/s11104-011-0993-6.

Carbone, M.S., Trumbore, S.E., 2007. Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. New Phytologist 176, 124–135.

Chabbi, A., Kögel-Knabner, I., Rumpel, C., 2009. Stabilised carbon in subsoil horizons is located in spatially distinct parts of the soil profile. Soil Biology & Biochemistry 41, 256–271.

Cheng, W., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition. In: Zobel, R.W., Wright, S.F. (Eds.), Roots and Soil Management: Interaction Between Roots and Soil. ASA-SSSA, Madison, Wasconsin, pp. 119–143.

Coleman, D.C., Odum, E.P., Crossley, D.A., 1992. Soil biology, soil ecology and global change. Biology and Fertility of Soils 14, 104–111.

Dijkstra, F.A., Cheng, W., 2007. Moisture modulates rhizosphere effects on C decomposition in two different soil types. Soil Biology & Biochemistry 39, 2264–2274.

Dijkstra, F.A., Morgan, J.A., Blumenthal, D., Follett, R.F., 2010. Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. Soil Biology & Biochemistry 42, 1073–1082.

Gavrichkova, O., Kuzyakov, Y., 2010. Respiration costs associated with nitrate reduction as estimated by $^{14}CO^2$ pulse labeling of corn at various growth stages. Plant and Soil 329, 433–445.

Henry, A., Doucette, W., Norton, J., Bugbee, B., 2007. Changes in crested wheatgrass root exudation caused by flood, drought, and nutrient stress. Journal of Environmental Quality 36, 904–912.

Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant and Soil 321, 117–152.

Hodge, A., Millard, P., 1998. Effect of elevated CO_2 on carbon partitioning and exudate release from Plantago lanceolata seedlings. Physiologia Plantarum 103, 280–286.

Högberg, P., Read, D.J., 2006. Towards a more plant physiological perspective on soil ecology. Trends in Ecology and Evolution 21, 548–554.

Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil 321, 5–33.

Joos, O., Hagedorn, F., Heim, A., Gilgen, A.K., Schmidt, M.W.I., Siegwolf, R.T.W., Buchmann, n, 2010. Summer drought reduces total and litter-derived soil CO_2 effluxes in temperate grassland – clues from a ^{13}C litter addition experiment. Biogeosciences 7, 1031–1041.

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33, 1915–1925.

Kuzyakov, Y., Domanski, G., 2002. Model for rhizodeposition and CO_2 efflux from planted soil and its validation by C-14 pulse labelling of ryegrass. Plant and Soil 239, 87–102.

Kuzyakov, Y., Gavrichkova, O., 2010. REVIEW: time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biology 16, 3386–3406.

Kuzyakov, Y., Siniakina, S.V., 2001. A novel method for separating root-derived organic compounds from root respiration in non-sterilized soils. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernährung und Bodenkunde 164, 511–517.

Kuzyakov, Y., Kretzschmar, A., Stahr, K., 1999. Contribution of Lolium perenne rhizodeposition to carbon turnover of pasture soil. Plant and Soil 213, 127–136.

Kuzyakov, Y., 2002. Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. Soil Biology & Biochemistry 34, 1621–1631.

Liljeroth, E., Kuikman, P., Veen, J.A., 1994. Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. Plant and Soil 161, 233–240.

Liu, H.S., Li, F.M., 2005. Root respiration, photosynthesis and grain yield of two spring wheat in response to soil drying. Plant Growth Regulation 46, 233–240.

Liu, H.S., Li, F.M., 2006. Effects of shoot excision on in situ soil and root respiration of wheat and soybean under drought stress. Plant Growth Regulation 50, 1–9.

Palta, J.A., Gregory, P.J., 1997. Drought affects the fluxes of carbon to roots and soil in ^{13}C pulse-labelled plants of wheat. Soil Biology & Biochemistry 29, 1395–1403.

Paterson, E., Midwood, A.J., Millard, P., 2009. Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. New Phytologist 184, 19–33.

Paul, E.A., Harris, D., Collins, H.P., Schulthess, U., Robertson, G.P., 1999. Evolution of CO_2 and soil carbon dynamics in biologically managed, row-crop agroecosystems. Applied Soil Ecology 11, 53–65.

Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. Applied Soil Ecology 48, 38–44.

Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218–221.

Tiemann, L.K., Billings, S.A., 2011. Changes in variability of soil moisture alter microbial community C and N resource use. Soil Biology & Biochemistry 43, 1837–1847.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass-C. Soil Biology & Biochemistry 19, 703–707.

Warembourg, F.R., Billes, G., 1979. Estimating carbon transfers in the plant rhizosphere. In: Harley, J.L., Scott Russel, R. (Eds.), The Soil–Root Interface. Academic Press, London, pp. 183–196.

Yuste, J.C., Janssens, I.A., Carrara, A., Meiresonne, L., Ceulemans, R., 2003. Interactive effects of temperature and precipitation on soil respiration in a temperate maritime pine forest. Tree Physiology 23, 1263–1270.