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Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake

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ABSTRACT

Plants can affect soil organic matter decomposition and mineralization through litter inputs, but also more directly through root-microbial interactions (rhizosphere effects). Depending on resource availability and plant species identity, these rhizosphere effects can be positive or negative. To date, studies of rhizosphere effects have been limited to plant species grown individually. It is unclear how belowground resources and inter-specific interactions among plants may influence rhizosphere effects on soil C decomposition and plant N uptake. In this study, we tested the simple and interactive effects of plant diversity and water availability on rhizosphere-mediated soil C decomposition and plant N uptake. The study was conducted in the greenhouse with five semi-arid grassland species (monocultures and mixtures of all five species) and two water levels (15 and 20% gravimetric soil moisture content). We hypothesized that microbial decomposition and N release would be less in the low compared to high water treatment and less in mixtures compared to monocultures. Rhizosphere effects on soil C decomposition were both positive and negative among the five species when grown in monoculture, although negative effects prevailed by the end of the experiment. When grown in mixture, rhizosphere effects reduced soil C decomposition and plant N uptake compared to monocultures, but only at the low-water level. Our results suggest that when water availability is low, plant species complementarity and selection effects on water and N use can decrease soil C decomposition through rhizosphere effects. Although complementarity and selection effects can increase plant N uptake efficiency, plant N uptake in the mixtures was still lower than expected, most likely because rhizosphere effects reduced N supply in the mixtures more than in the monocultures. Our results indicate that rhizosphere effects on C and N cycling depend on water availability and inter-specific plant interactions. Negative rhizosphere effects on soil C decomposition and N supply in mixtures relative to monocultures of the component species could ultimately increase soil C storage and possibly influence how plant communities in semi-arid grasslands respond to global climate change.

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1. Introduction

There is considerable evidence that storage and cycling of soil C and N are affected by plant species composition and diversity (Hooper and Vitousek, 1997, 1998; Wardle et al., 1999; Craine et al., 2001; Zak et al., 2003; Dybzinski et al., 2008; Fornara and Tilman, 2008). Greater storage and cycling of soil C and N with increased plant species richness has been associated with increased plant litter inputs resulting from enhanced plant productivity (Zak et al., 2003; Dijkstra et al., 2005; Fornara and Tilman, 2008). However, Steinbeiss et al. (2008) observed a positive effect of plant diversity on soil C storage in a temperate grassland in Germany that could not be totally explained by greater plant biomass production. Similarly, greater plant productivity could not completely explain more rapid N mineralization with increased plant diversity in a tall grass prairie in Minnesota (Zak et al., 2003). These results indicate that plant diversity can affect ecosystem C and N cycling through mechanisms other than effects on plant litter production.

Plants can also affect soil organic matter (SOM) decomposition through direct root–microbe interactions (rhizosphere effects, Cheng and Kuzyakov, 2005). For instance, plant roots can enhance SOM decomposition by supplying the decomposer soil microbial community with labile C substrates (rhizosphere priming effect, Kuzyakov, 2002). This rhizosphere priming effect appears to be plant species-specific (Cheng et al., 2003; Cheng and Kuzyakov, 2005) and can be more pronounced in soils with greater water

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availability (Dijkstra and Cheng, 2007b) and smaller N availability (Liljeroth et al., 1990; Fontaine et al., 2004). With rhizosphere priming, N transfer from the inactive SOM pool into the active microbial pool may be enhanced, which ultimately may increase plant N availability (Hungate, 1999; Paterson, 2003). However, it has also been suggested that plant water uptake and competition for nutrients could reduce microbial activity, SOM decomposition and net N mineralization (Van Veen et al., 1989; Ehrenfeld et al., 1997; Wang and Bakken, 1997). Indeed, agricultural field studies from the 1960s and 70s have shown that decomposition of labeled plant material is markedly lowered in the presence of plants (Führ and Sauerbeck, 1968; Shields and Paul, 1973; Jenkinson, 1977).

Given that rhizosphere effects vary with species identity, water and N availability, they might also vary with plant diversity, and therefore influence effects of plant diversity on C and N cycling. Plant diversity could influence rhizosphere effects through species-specific differences in inputs of labile C and/or inter-specific competition for belowground resources. A greater diversity of organic compounds produced by more diverse plant communities could stimulate a more diverse microbial decomposer community (Lodge, 1997; Hooper et al., 2000; Stephan et al., 2000). A greater chemical diversity of organic compounds could also stimulate a greater diversity of extracellular enzyme production thereby increasing the probability of occurrence of a rhizosphere priming effect (Fontaine et al., 2003). On the other hand, Loreau (2001) suggested that a greater organic compound diversity could have negative effects on soil C decomposition and N cycling because of an increased likelihood that some of the organic compounds will not be consumed by decomposers. Plant diversity might also decrease belowground resource availability through complementarity and selection effects (Tilman et al., 1996, 1997; Hooper and Vitousek, 1997), thereby potentially reducing rhizosphere effects on microbial SOM decomposition and N mineralization. To our knowledge, rhizosphere effects on SOM decomposition (i.e., SOM decomposition measured in the presence of live roots) in plant species mixtures have never been examined, and empirical studies of rhizosphere effects on N cycling in non-N fixing plant species mixtures are rare (Saj et al., 2007; Nilsson et al., 2008).

Here we compared rhizosphere effects on soil C decomposition and plant N uptake between monocultures of five different semi-arid grassland species and mixtures of all five species grown in a greenhouse experiment at two water levels. We also included treatments without plants. Throughout the experiment we continuously labeled the plants with depleted $^{13}CO_2$. We used ^{13}C isotope analyses in respiration measurements to separate the soil-derived CO_2–C flux (soil C decomposition) from the plant-derived CO_2–C flux while plants were present. After 85 days of growth we analyzed plant material for N content (g pot^{-1}) as a measure of plant N uptake. We hypothesized that differences in the rhizosphere effect on soil C decomposition and plant N uptake between low and high water levels and between mixtures and monocultures would be driven by their effects on water and N availability. Specifically, we predicted that reduced water availability in the low-water treatments and reduced water and N availability in the mixtures (due to selection and complementarity effects) would reduce rhizosphere-mediated soil C decomposition and plant N uptake in these treatments.

2. Materials and methods

2.1. Experimental design

We filled 70 polyvinyl chloride (PVC) pots (diam. 20 cm, height 40 cm) with a sandy loam soil (Aridic Argiustoll, Ascalon series) from the USDA-ARS Central Plains Experimental Range in the shortgrass steppe region of north-eastern Colorado. The pots were closed at the bottom except for an air inlet (see Dijkstra et al., 2010). The soil was taken from the top 20 cm. The soil contained no carbonates based on the lack of effervescence with addition of 10% HCl, had a pH of 6.6, 0.95% C, 0.09% total N, and 23 mg kg^{-1} inorganic N ($NH_4^+ + NO_3^-$) at the start of the experiment. We placed a nylon bag filled with 3 kg sand at the bottom of each pot before filling the pots with 14 kg sieved (4 mm), air-dried soil. The pots were then watered to field capacity (30% gravimetric soil moisture content). We transplanted seedlings (grown in peat pellets for two weeks) of *Artemisia frigida* Willd. (sub-shrub), *Linaria dalmatica* [L.] Mill. (forb), *Bouteloua gracilis* [Willd. ex Kunth] Lag. Ex Griffiths (C_4 grass), *Hesperostipa comata* [Trin. & Rupr.] (C_3 grass), and *Pascopyrum smithii* [Rybd.] A. Love (C_3 grass) to the pots. All species are native to the shortgrass steppe, except for the invasive weed *L. dalmatica*. Each species was grown as a monoculture (five plants per pot) in 10 pots for each species. In another 10 pots, we grew all species together (All, one plant of each species per pot). We further included 10 pots without plants (control).

The experiment was conducted in a greenhouse facility of the USDA-ARS Crops Research Laboratory in Fort Collins, Colorado. To label the plants with C depleted in ^{13}C we raised the atmospheric CO_2 concentration inside the greenhouse to a constant level of 780 ± 50 μL L^{-1} (average ± standard deviation) by adding pure CO_2 depleted in ^{13}C ($\delta^{13}C = -39.7‰$). We should note that rhizosphere effects on SOM decomposition (see below) may be different than under ambient CO_2 concentration (Cheng, 1999), but a doubling of atmospheric CO_2 concentration by the end of this century is not unlikely (Forster et al., 2007). The CO_2 was added through a ventilation system to ensure uniform distribution of the CO_2 concentration inside the greenhouse. The CO_2 concentration was continuously monitored and the CO_2 supply was computer-controlled (Argus Control Systems Ltd, White Rock, BC). This continuous labeling method has been tested successfully in other greenhouse and growth chamber experiments (Dijkstra and Cheng, 2007a,b). During the experiment, air temperature inside the greenhouse was kept between 27 and 29 °C during the day and between 16 and 18 °C during the night using computer-controlled air conditioners and heaters (York International, York, PA). Light inside the greenhouse was supplemented with 600 W lights (P.L. Light Systems, Beamsville, Ontario) that were on for 12 h during the day. The light intensity inside the greenhouse was ~200 W m^{-2} during the day. The relative humidity inside the greenhouse was 24 ± 5% during the day and 30 ± 5% during the night.

During the first week after transplanting, all pots were maintained at 30% soil moisture content. After the first week, half of all pots (or 5 pots of each monoculture and mixture, and 5 pots without plants) were allowed to dry down to 15% (low-water treatment), and the other half to 20% (high water treatment). Pots were then maintained at these water levels by watering the pots three times a week. Pots were weighed once every week and watered up to their target weights. The amount of water added during the other two times of the week was calculated based on previous water loss from each pot. We calculated total water use for each pot as the total amount of water added to each pot during the experiment. Inside the greenhouse the 70 pots were placed in five blocks (14 pots per block, each block containing one replicate of each treatment). Treatments within each block were randomly assigned.

2.2. Measurements and analyses

We measured pot respiration 48, 69, and 83 days after transplanting (DAT). At each time, we placed opaque PVC chambers (diam. 20 cm, height 45 cm for the planted pots and 15 cm for the pots without plants) on top of the pots. The chambers were fitted

with a septum (to pull gas samples, see below) and an air outlet. Chambers were sealed to the pot with 10 cm wide rubber bands (made out of inner tubes). We circulated air inside the pot/chamber by connecting an aquarium pump (Apollo AM-3, Apollo Enterprises, Ventura, CA, flow rate 2.8 L min^{-1}) to the air inlet at the bottom of the pot and to the air outlet of the chamber. In this way, air inside the pot/chamber was completely isolated from the air outside (see Dijkstra et al., 2010). During the first 2 h of air circulation we removed CO_2 inside the pot/chamber by installing an in-line CO_2 scrubber (PVC tube, diam. 3.5 cm, height 36 cm, filled with sodalime) between the chamber and the aquarium pump. The total volume of air inside the pot/chamber ranged between 8.7 and 20.8 L (volume of the pot/chamber minus volume taken by sand, soil and water), thus the air passed the CO_2 scrubber between 16 and 39 times, and we assumed negligible amounts of atmospheric CO_2 (in air and soil water) remained inside the pot/chamber after 2 h of scrubbing. After 2 h, we pulled a 30 ml gas sample from the chamber (sample A), and then removed the CO_2 scrubber (but maintained air circulation inside the pot/chamber). Once the CO_2 scrubber was removed, the CO_2 concentration inside the pot/chamber increased because of plant and soil respiration (note that there was no light inside the pot/chamber, and thus no photosynthesis). After 2 h we pulled two more 30 ml gas samples from the chamber (samples B and C). The chambers and pumps were removed thereafter. Gas samples A and B were analyzed for CO_2 concentration on a gas chromatograph (Varian 3800, Varian Inc., Palo Alto, CA), while sample C was analyzed for CO_2–^{13}C on a gas bench device online with a mass spectrometer (Finnigan Delta plus XP, Thermo Scientific, Waltham, MA).

We harvested all plants 85 days after transplanting. Plants were separated into shoots and roots (including crowns). Plant biomass was dried (60 °C) and weighed. Plant biomass was then ground and analyzed for ^{13}C, and N on a mass spectrometer (20-20 Stable Isotope Analyzer, Europa Scientific, Chesire, UK). We calculated the relative yield of species i (RY_i) in the mixtures with (De Wit and van den Bergh, 1965):

$$RY_i = O_i/M_i \quad (1)$$

where O_i is the shoot biomass of the single individual of species i in the mixture and M_i is the shoot biomass of the 5 individuals of species i in the monoculture. An RY_i greater than 0.2 indicates that the shoot biomass of species i in the mixture is greater than would be expected from the monoculture, and if RY_i is smaller than 0.2, then the shoot biomass of species i is less than expected from the monoculture. We extracted a 25 g representative soil sample from each pot with 60 ml of 2 M KCl. Samples were shaken for 1 h and filtered through pre-leached (with 2 M KCl) Whatman No. 1 filter paper. Extracts were frozen until analysis for NH_4^+ and NO_3^- on a flow injection analyzer (QuickChem FIA+, Lachat Instruments, Milwaukee, WI). The NH_4^+ and NO_3^- concentrations were expressed on oven-dry soil weight (105 °C) basis.

Pot (plant + soil) respiration (CO_2–C_{pot}, mg C h^{-1} pot^{-1}) was calculated based on the difference between the concentrations of the samples A and B, and the air volume of the pot/chamber system. The $\delta^{13}C$ value measured in sample C allowed us to separate the plant derived- from the soil-derived CO_2–C flux in the planted treatments. We calculated the soil-derived CO_2–C flux (CO_2–C_{soil}, mg C hr^{-1} pot^{-1}) in the planted treatments using the following equation:

$$CO_2 - C_{soil} = CO_2 - C_{pot}\left(\delta^{13}C_{plant} - \delta^{13}C_{pot}\right)\Big/\left(\delta^{13}C_{plant} - \delta^{13}C_{control}\right) \quad (2)$$

where $\delta^{13}C_{plant}$, $\delta^{13}C_{pot}$, and $\delta^{13}C_{control}$ are the $\delta^{13}C$ values of plant biomass, the CO_2 measured in sample C of the planted treatments, and the CO_2 measured in sample C of the non-planted control treatments respectively. We used the weighted average $\delta^{13}C$ value measured in shoots and roots for $\delta^{13}C_{plant}$. We assumed that the $\delta^{13}C$ value of the plant-derived CO_2–C flux would be the same as the measured $\delta^{13}C$ value in plant biomass (Cheng, 1996; Dijkstra and Cheng, 2007a). Because the $\delta^{13}C$ value in plant biomass differed among species, we further assumed that the specific rate of plant-derived CO_2–C per amount of plant biomass was the same for all five species to calculate CO_2–C_{soil} in the mixtures (see Results). Only if plant-biomass-specific rates of plant-derived CO_2–C are the same for each species does the $\delta^{13}C$ value of the total biomass in mixtures reflect the $\delta^{13}C$ value of the plant-derived CO_2–C flux. We expressed the rhizosphere effect on soil C decomposition as the difference in soil-derived CO_2–C flux between planted and non-planted control treatments ($RhizCO_2$–C).

We compared observed values of $RhizCO_2$–C in the mixtures to expected values of $RhizCO_2$–C ($RhizCO_2$–C_E). We calculated expected values in two different ways. We first calculated expected values as the average $RhizCO_2$–C of all five species when grown as monocultures. Because $RhizCO_2$–C can be positively related to plant biomass (Dijkstra et al., 2006), differences between observed and expected values of $RhizCO_2$–C in the mixtures could be due to shifts in the relative abundance of plant species in the mixtures. We therefore also calculated expected values of $RhizCO_2$–C ($RhizCO_2$–C_E) based on $RhizCO_2$–C measured in the monocultures weighted by plant biomass of each species in the mixtures:

$$RhizCO_2 - C_E = \left(\sum TotBio_{mix,i} * RhizCO_2 - C_{mono,i}\right)\Big/TotBio_{mix} \quad (3)$$

where $TotBio_{mix,i}$ is the total biomass of species i in the mixture, $RhizCO_2$–$C_{mono,i}$ is the $RhizCO_2$–C measured for species i in the monoculture, and $TotBio_{mix}$ is the total biomass of all five species in the mixture. We were unable to separate roots by species in the mixtures, and therefore do not have direct measurements of $TotBio_{mix,i}$. Instead, we estimated root biomass of species i in the mixtures ($Root_{mix,i}$) based on root/shoot ratios observed in the monocultures ($Root_{mono,i}/Shoot_{mono,i}$), shoot biomass of species i in the mixtures ($Shoot_{mix,i}$), and total root biomass in the mixtures ($TotRoot_{mix}$). If root/shoot ratios measured in the monocultures remained the same in the mixtures then $Root_{mix,i}$ can be calculated by multiplying $Root_{mono,i}/Shoot_{mono,i}$ with $Shoot_{mix,i}$. The sum of the root biomass of all five species in mixtures ($TotRoot_{mix,E}$) should then equal the total amount of root biomass measured in the mixtures ($TotRoot_{mix}$). However, this was not the case ($TotRoot_{mix}/TotRoot_{mix,E}$ ratios ranged between 1.00 and 1.46, and were on average 1.24 for the low water and 1.18 for the high water treatment), indicating that root/shoot ratios of the species in mixtures tended to be higher than in monoculture. We then assumed that the deviation of the root/shoot ratio in the mixtures from the monocultures was the same for each species, by using the correction factor α:

$$\sum\left(Root_{mono,i}/Shoot_{mono,i}\right) * Shoot_{mix,i} * \alpha = TotRoot_{mix} \quad (4)$$

where α is $TotRoot_{mix}/TotRoot_{mix,E}$ and $Root_{mix,i}$ now equals $(Root_{mono,i}/Shoot_{mono,i})*Shoot_{mix,i}*\alpha$. To test if using a single factor α to correct root/shoot ratios in the mixtures is realistic, we compared expected values of $\delta^{13}C$ in $TotRoot_{mix}$ ($\delta^{13}CTotRoot_{mix,E}$) with observed values of $\delta^{13}C$ in $TotRoot_{mix}$ ($\delta^{13}CTotRoot_{mix}$). We calculated $\delta^{13}CTotRoot_{mix,E}$ based on $\delta^{13}C$ values of root biomass

measured in the monocultures ($\delta^{13}CRoot_{mono,i}$) weighted by $Root_{mix,i}$:

$$\delta^{13}CTotRoot_{mix,E} = \left(\sum Root_{mix,i} * \delta^{13}CRoot_{mono,i}\right) \Big/ TotRoot_{mix} \quad (5)$$

If the expected values of the root biomass $\delta^{13}C$ value were similar to the observed values, we concluded that using a single correction factor was reasonable. We calculated the total biomass of species i in the mixtures ($TotBio_{mix,i}$) by adding $Root_{mix,i}$ to $Shoot_{mix,i}$.

We compared observed values of total plant N content (g N pot^{-1}), soil inorganic [N] (mg N kg^{-1} soil), and total water use (the sum of water added during the experiment, L pot^{-1}) in the mixtures to expected values, where expected values were simply calculated as the average values of all five species grown in monoculture. As with $RhizCO_2$–C, differences between these observed and expected values in the mixtures could be due to shifts in the relative abundance of plant species in the mixtures. We therefore also calculated expected values based on observed values of total plant N content, soil inorganic [N], and total water use in the monocultures weighted by plant biomass of each species in the mixtures (as in equation (3)).

We used repeated measures ANOVA to test for main effects of species presence (2 levels: planted and non-planted) and water (2 levels: low and high water), and their interaction on the soil-derived CO_2–C flux, and to test for main effects of species composition (6 levels: *A. frigida*, *L. dalmatica*, *B. gracilis*, *H. comata*, *P. smithii*, and all species) and water, and their interaction on $RhizCO_2$–C. The repeated measures ANOVA included random effects of date (48, 69, and 83 days after transplanting) and block. We also used ANOVA to test for main effects and interactions of species composition and water for each date separately. We used ANOVA to test for main effects and interactions of species composition and water on plant biomass, plant biomass $\delta^{13}C$, RY_i, plant N content, soil inorganic [N], and water use. We used two-tailed *t*-tests to test if the difference between observed and expected values of $RhizCO_2$–C, plant biomass, N%, plant N content, soil inorganic [N], and total water use significantly deviated from zero. We used linear regression to relate the plant-derived CO_2–C flux to plant biomass. When necessary, data were log-transformed to reduce heteroscedasticity. All statistical analyses were done with JMP (version 4.0.4; SAS Institute, Cary, North Carolina, USA).

3. Results

3.1. Plant biomass and plant-derived CO_2–C

Shoot and total plant biomass at the end of the experiment were significantly higher in the high than in the low-water treatment (on average by 20 and 10% respectively), while root biomass was not significantly affected by the water treatment (Table 1). Species composition (five monocultures plus one mixture) significantly affected shoot, root, and total biomass, where *B. gracilis* had the highest shoot biomass and *L. dalmatica* the highest root biomass. The $\delta^{13}C$ values in shoot and root biomass varied significantly with species composition, but water and water*species composition effects were non-significant or tended to be small (Fig. 1). The plant biomass $\delta^{13}C$ values were much more negative than values from the soil-derived CO_2–C flux (the latter ranging between −20.4 and −23.7‰), which allowed us to accurately separate plant-derived from soil-derived CO_2–C in pot respiration measurements.

Table 1
Means ± standard error for plant biomass, relative yield (RY, based on shoot biomass), soil inorganic [N] ($NH_4^+ + NO_3^-$) at the end of the experiment, and total water use during the experiment.

Water treatm.	Species.	Shoot g pot^{-1}	Root g pot^{-1}	Total g pot^{-1}	RY	Inorg. N mg kg^{-1}	Water use L pot^{-1}
Monocultures							
Low	*A. frigida*	23.2 ± 2.1	17.4 ± 1.4	40.6 ± 3.2		1.09 ± 0.10	13.4 ± 0.4
	L. dalmatica	24.0 ± 2.5	43.5 ± 1.6	67.5 ± 3.1		0.55 ± 0.07	18.0 ± 0.6
	B. gracilis	24.5 ± 2.7	24.6 ± 1.3	49.2 ± 3.8		1.13 ± 0.09	13.3 ± 0.4
	H. comata	18.6 ± 1.0	11.4 ± 1.0	30.0 ± 1.3		1.43 ± 0.11	9.2 ± 0.1
	P. smithii	17.8 ± 2.0	26.6 ± 1.8	44.4 ± 3.5		1.09 ± 0.12	14.1 ± 0.4
High	*A. frigida*	25.9 ± 1.2	15.4 ± 1.6	41.4 ± 1.4		1.30 ± 0.12	16.3 ± 0.3
	L. dalmatica	24.7 ± 2.7	35.8 ± 4.1	60.4 ± 6.5		0.74 ± 0.11	22.2 ± 0.7
	B. gracilis	34.1 ± 0.8	28.6 ± 1.9	62.8 ± 1.2		1.36 ± 0.11	18.2 ± 0.4
	H. comata	21.7 ± 2.1	10.4 ± 1.6	32.2 ± 2.2		1.53 ± 0.13	12.8 ± 0.5
	P. smithii	22.5 ± 1.2	33.0 ± 2.6	55.5 ± 3.2		1.27 ± 0.16	20.4 ± 0.6
Mixtures							
Low	*A. frigida*	5.4 ± 0.7			0.23 ± 0.03		
	L. dalmatica	3.5 ± 0.8			0.15 ± 0.03		
	B. gracilis	6.2 ± 1.1			0.25 ± 0.05		
	H. comata	0.6 ± 0.3			0.03 ± 0.02		
	P. smithii	4.4 ± 0.7			0.25 ± 0.04		
	All	20.1 ± 1.2*	29.6 ± 1.3	49.7 ± 1.9		0.91 ± 0.13	14.5 ± 0.4
High	*A. frigida*	6.0 ± 0.7			0.23 ± 0.03		
	L. dalmatica	5.0 ± 1.2			0.20 ± 0.05		
	B. gracilis	6.1 ± 1.0			0.18 ± 0.03		
	H. comata	1.2 ± 0.4			0.06 ± 0.02		
	P. smithii	6.8 ± 0.4			0.30 ± 0.02		
	All	25.1 ± 1.1*	31.9 ± 2.5	57.1 ± 3.6		1.26 ± 0.16	18.8 ± 0.4
ANOVA *P*-values**							
Water		**0.0002**	0.77	**0.01**	0.53	**0.0002**	**<0.0001**
Sp		**<0.0001**	**<0.000**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Water*Sp		0.27.	**0.01**	**0.02**	0.23	0.72	**0.01**

* Sum of five species in mixtures.
** ANOVA includes 6 species levels: the biomass of the five monocultures and the total biomass of the mixtures, except for RY where the ANOVA only includes 5 species levels. Block effects were never significant ($P > 0.1$).

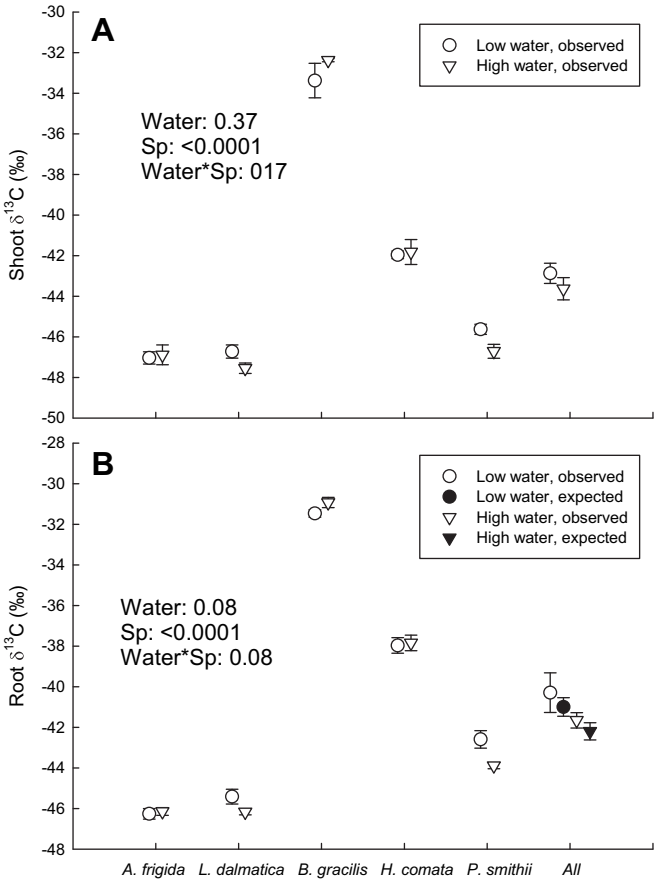

Fig. 1. Shoot (A) and root $\delta^{13}C$ (B) values averaged by species composition and water treatments (low water: 15% soil moisture, high water: 20% soil moisture). For the mixtures (All) the expected root $\delta^{13}C$ values are also shown (observed values in mixtures were calculated as the weighted average of all five species (shoots) or were directly measured (roots), expected root values were calculated based on $\delta^{13}C$ values measured in the monocultures weighted by species-specific root biomass in the mixtures, see text for details). Sub-legend shows ANOVA *P*-values, Sp stands for species composition effects. Error bars denote standard errors.

To evaluate whether the $\delta^{13}C$ values measured in total plant biomass in the mixtures could be used to represent the $\delta^{13}C$ values of the plant-derived CO_2–C flux in the mixtures, we compared the plant-derived CO_2–C flux with plant biomass in the monocultures. Only if plant-biomass-specific rates of plant-derived CO_2–C are the same for each species does the $\delta^{13}C$ value of the total biomass in mixtures reflect the $\delta^{13}C$ value of the plant-derived CO_2–C flux. The two measurements were taken 83 and 85 days after transplanting respectively. When using the total plant biomass $\delta^{13}C$ value as the $\delta^{13}C$ value of the plant-derived CO_2–C flux in the monocultures, the specific rate of plant-derived CO_2–C per unit of plant biomass did not significantly differ among all five species (Fig. 2). Although the plant-derived CO_2–C flux differed among species and water treatments (ANOVA, species: $P < 0.0001$, water: $P = 0.08$), the main effects of species and water disappeared when we used total plant biomass as a covariate, while interactions with plant biomass were not significant (ANCOVA, species: $P = 0.27$, water: $P = 0.71$, species*plant biomass: $P = 0.56$, water*plant biomass: $P = 0.32$). Using linear regression, total plant biomass explained 61% of the variability in the plant-derived CO_2–C flux among all treatments (including the mixtures). Thus, a similar plant-biomass-specific rate of plant-derived CO_2–C for all species indicates that in the mixtures the proportional plant-derived CO_2–C flux reflected the same proportional plant biomass of each species. We therefore

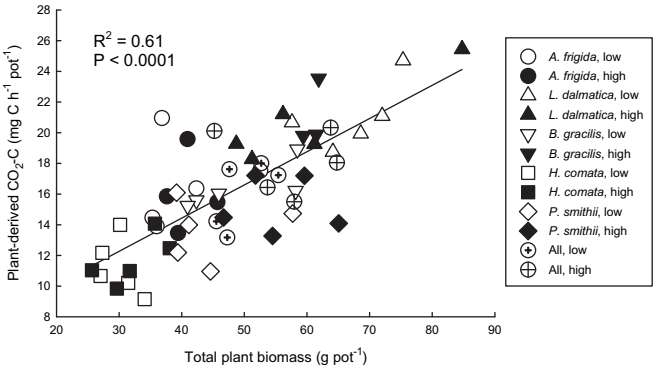

Fig. 2. Plant-derived CO_2–C measured 83 days after transplanting as a function of total plant biomass measured 85 days after transplanting (low: 15% soil moisture, high: 20% soil moisture). Line represents linear regression using all data points.

are confident in using the total plant biomass $\delta^{13}C$ value in the mixtures as the plant end-member in the linear mixing model ($\delta^{13}C_{plant}$, equation (2)) to separate pot respiration into soil- and plant-derived CO_2–C fluxes in the mixtures.

3.2. Soil-derived CO_2–C and RhizCO_2–C

The soil-derived CO_2–C flux was significantly higher in the high than in the low-water treatment (on average by 27%, repeated measures ANOVA $P = 0.007$, Fig. 3). The soil-derived CO_2–C flux was initially higher in the planted pots (on 48 and 69 DAT), but ended being slightly lower in the planted pots (on 83 DAT) than in the non-planted controls (significant plant presence*date interaction, $P = 0.01$). Plant species composition effects on the soil-derived CO_2 flux are not shown, but are similar to their effects on RhizCO_2–C (see below).

The repeated measures ANOVA showed that RhizCO_2–C (difference in soil-derived CO_2–C flux between planted and non-planted control) was significantly higher in the high than in the low-water treatment (on average by 87%, $P = 0.001$) and varied significantly with species composition ($P = 0.0002$). For each date, water and species composition effects were also significant, except for the last date (83 DAT) when the water treatment was not

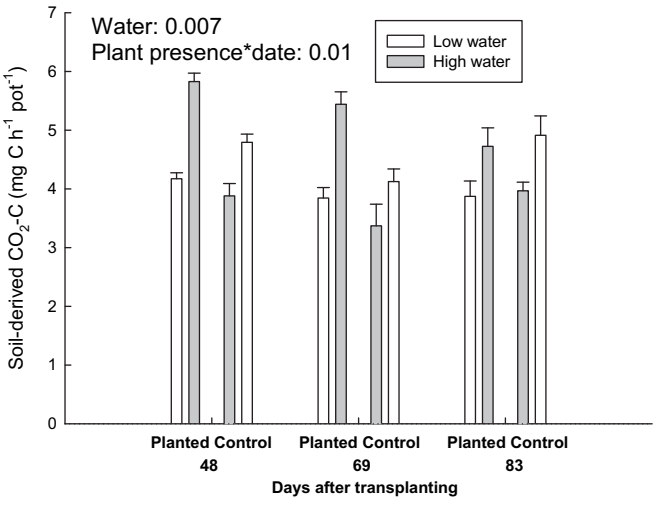

Fig. 3. Soil-derived CO_2–C measured 48, 69, and 83 days after transplanting, averaged by plant presence (planted and non-planted control) and water treatments (low water: 15% soil moisture, high water: 20% soil moisture). Sub-legend shows significant ANOVA *P*-values. Error bars denote standard errors.

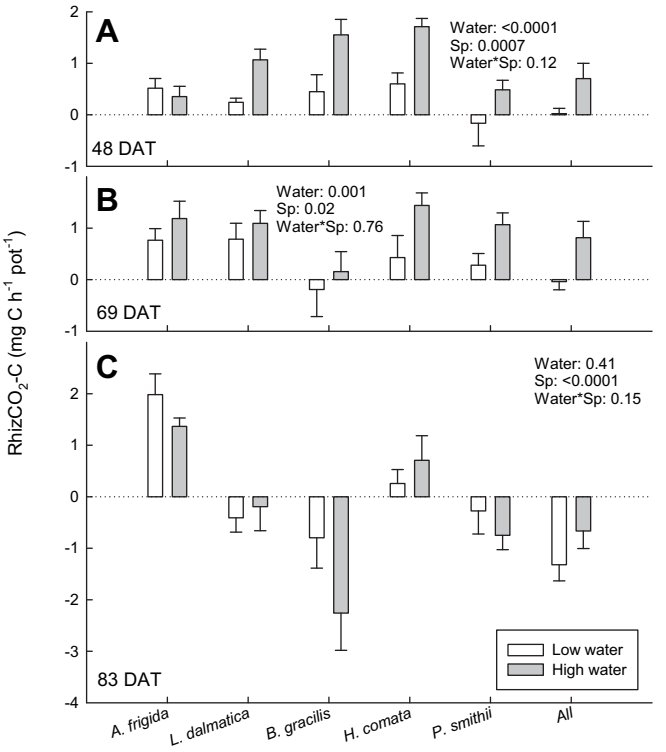

Fig. 4. Rhizosphere effect on soil-derived CO_2–C (difference in soil-derived CO_2–C between planted and non-planted control treatments, $RhizCO_2$–C) measured 48, 69, and 83 days after transplanting (DAT), averaged by species composition and water treatments (low water: 15% soil moisture, high water: 20% soil moisture). Sub-legend shows ANOVA *P*-values. Sp stands for species composition effects. Error bars denote standard errors.

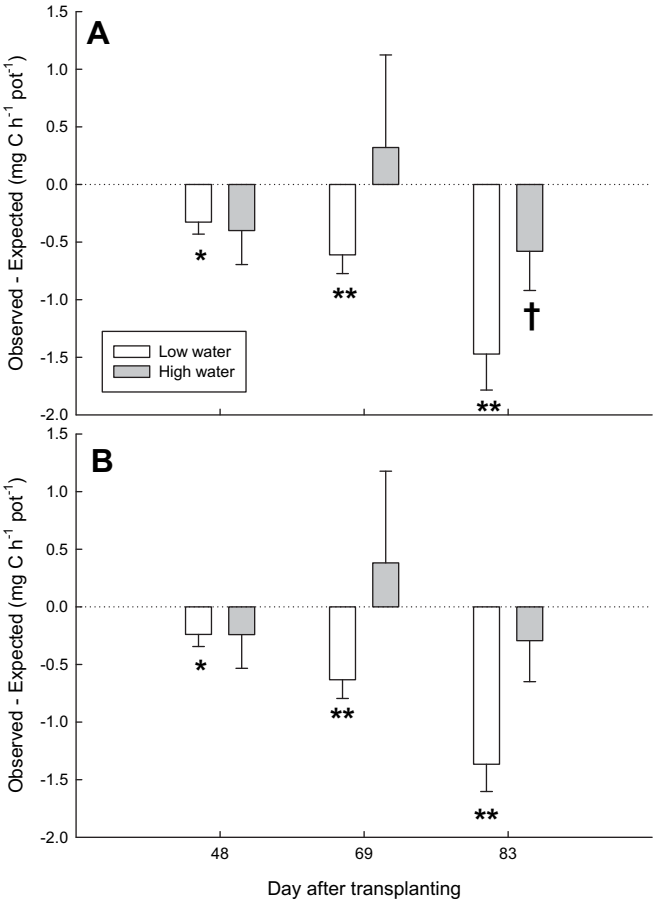

Fig. 5. Observed $RhizCO_2$–C in the mixture minus expected $RhizCO_2$–C based on the average $RhizCO_2$–C in the monocultures (unadjusted, A), and based on the average $RhizCO_2$–C in the monocultures adjusted for species-specific plant biomass (plant biomass adjusted, B, low water: 15% soil moisture, high water: 20% soil moisture). Two-tailed *t*-test testing for significant deviation from zero †: $P < 0.1$, *: $P < 0.05$, **: $P < 0.01$. Error bars denote standard errors.

significant (ANOVA, Fig. 4). Water*species composition (5 monoculture and 1 mixture) interactions were never significant. The species *A. frigida* and *H. comata* showed among the highest $RhizCO_2$–C during all three dates. During the last date, $RhizCO_2$–C became negative (indicating a lower soil-derived CO_2–C flux than in the control treatments) for *L. dalmatica*, *B. gracilis*, *P. smithii*, and all species. While *B. gracilis* had among the highest $RhizCO_2$–C on the first date (48 DAT), it had the most negative $RhizCO_2$–C on the last date. On the other hand, $RhizCO_2$–C for *A. frigida* increased with time.

We tested if observed $RhizCO_2$–C in the mixtures differed from expected $RhizCO_2$–C calculated as the average $RhizCO_2$–C measured in all monocultures. Observed minus expected values of $RhizCO_2$–C in the mixtures were significantly smaller than zero in the low-water treatment, but were not significantly affected in the high water treatment (Fig. 5A). Thus, while $RhizCO_2$–C in monocultures and mixtures was sometimes positive and sometimes negative, rhizosphere effects always reduced soil C decomposition in the low-water treatment significantly more in mixtures than would be expected based on the average soil C decomposition in the component monocultures.

Differences between observed and expected values of $RhizCO_2$–C in the mixtures could have been due to shifts in the relative abundance of plant species in the mixtures. For instance, a smaller than expected $RhizCO_2$–C at the end of the experiment could have been caused by a relatively greater abundance of *B. gracilis* that showed a strong negative $RhizCO_2$–C in monoculture and/or by a relative decrease in the abundance of *A. frigida* or *H. comata* that showed a strong positive $RhizCO_2$–C in monoculture. Indeed, aboveground biomass of *H. comata* was relatively underrepresented in the mixtures, suggesting that we may have overestimated the expected values of $RhizCO_2$–C in the mixtures. The relative yield (RY) of *H. comata* was lower than the expected value of 0.2 (thus *H. comata* under-yielded, an RY of 0.2 indicates that aboveground plant biomass of a species in the mixture is the same as in the monoculture adjusted for plant density, Table 1). We therefore adjusted expected $RhizCO_2$–C in the mixtures by the total biomass of each plant species. Because we were unable to separate roots by species, we estimated root biomass of each species with equation (4). Expected values of total root biomass $\delta^{13}C$ in the mixtures were not significantly different from observed values (Fig. 1B), suggesting that our estimates of root biomass of each of the five species in the mixtures were reasonable. Note that the differences in root biomass $\delta^{13}C$ values among the five species grown in monoculture were large, indicating that the total root biomass $\delta^{13}C$ in the mixtures is sensitive to shifts in root biomass of the different species, further indicating that our species-specific root biomass estimates in the mixtures are realistic.

When we calculated expected $RhizCO_2$–C in the mixtures adjusted for species-specific plant biomass with equation (3), observed minus expected values of $RhizCO_2$–C in the mixtures were still significantly smaller than zero in the low-water treatment (Fig. 5B). Although *H. comata* under-yielded in the mixtures, adjusting for species-specific plant biomass hardly changed the expected values for $RhizCO_2$–C.

3.3. Plant N, soil inorganic N, and water use

Shoot N content (g N pot^{-1}) was significantly higher in the high than in the low-water treatment (average increase of 25%), and varied significantly with species composition (Fig. 6A). Notably, *A. frigida* and *H. comata* had the highest shoot N, the same species that on average also showed the largest RhizCO_2–C. Root N content varied significantly with species composition, but was not affected by the water treatment (Fig. 6B). Root N patterns were opposite of shoot N, with root N lowest in *A. frigida* and *H. comata* and highest in *P. smithii*. Because of these opposite patterns, species composition effects on total plant N were not significant (Fig. 6C). Total plant N was significantly higher in the high than in the low-water treatment (on average by 15%).

Observed minus expected values (average of all monocultures) of total plant N (g pot^{-1}) in the mixtures were lower than zero in both water treatments, but only significantly so in the low-water treatment (Fig. 7A). The lower than expected total plant N of the mixtures in the low-water treatment remained marginally significant after adjusting expected values for species-specific plant biomass in the mixtures (Fig. 7D).

Soil inorganic [N] ($NH_4^+ + NO_3^-$) at the end of the experiment was significantly lower in the low than in the high water treatment (by 20%), and varied significantly with species composition (Table 1). Not surprisingly, total soil water use was significantly higher in the high water than in the low-water treatment (by 32%), and also varied significantly with species composition (Table 1). The lowest soil inorganic [N] and highest total water use were observed in the *L. dalmatica* monocultures. Observed concentrations of soil inorganic [N] in the mixtures minus expected concentrations calculated as the average of the monocultures were slightly smaller than zero in the low-water treatment and did not differ from zero in the high water treatment (Fig. 7B). On the other hand, observed total water use in the mixtures minus expected total water use calculated as the average of the monocultures was significantly greater than zero in both water treatments (Fig. 7C). Thus, the mixtures utilized more water than expected based on water use in the monocultures. When expected values of soil inorganic [N] and total water use were adjusted for species-specific biomass in the mixtures, observed minus expected values of soil inorganic [N] remained similar (Fig. 7E), while observed minus expected values of total water use no longer differed significantly from zero (Fig. 7F).

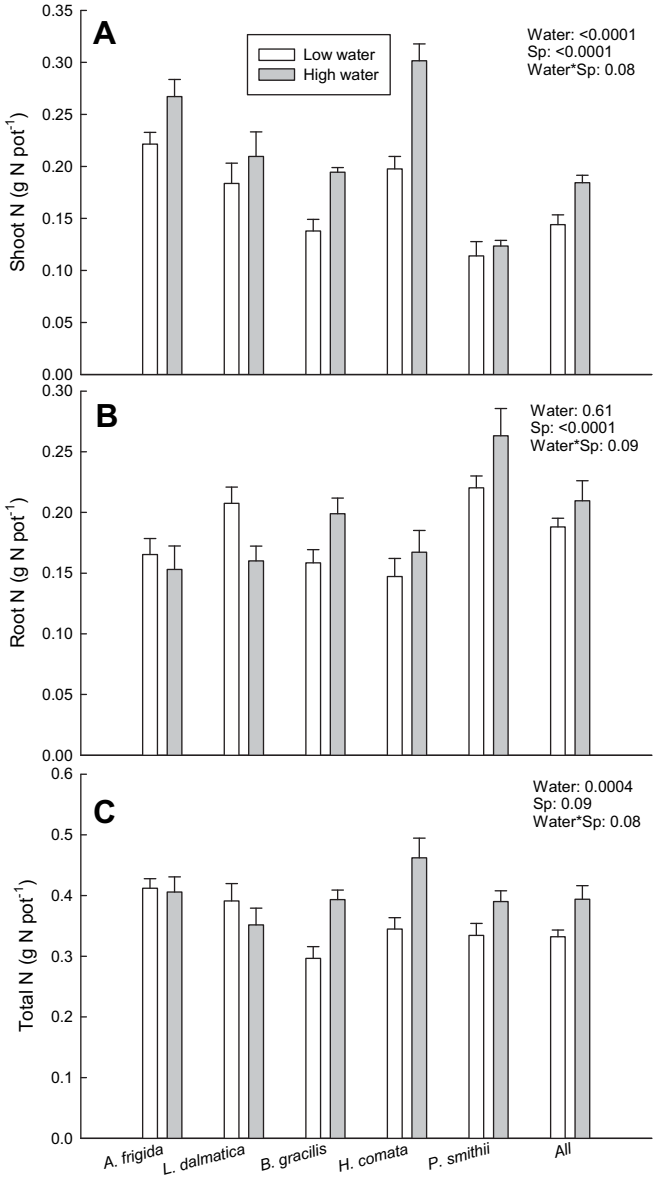

Fig. 6. Shoot N (A), Root N (B), and total plant N (C) averaged by species composition and water treatments (low water: 15% soil moisture, high water: 20% soil moisture). Sub-legend shows ANOVA *P*-values. Sp stands for species composition effects. Error bars denote standard errors.

4. Discussion

4.1. Rhizosphere effects on soil C decomposition: differences among plant species

Rhizosphere effects on soil C decomposition can be positive, because of stimulation of microbial activity through root exudates, or negative, because of plant competition for nutrients and water that could reduce microbial activity (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). We observed both positive and negative rhizosphere effects on soil C decomposition. Rhizosphere effects on soil C decomposition tended to become more negative with time. The soil-derived CO_2–C flux (a measure of microbial activity) decreased with time in the planted treatments (Fig. 3), while inorganic [N] also dropped from 23 mg kg^{-1} at the start of our experiment to 1.1 mg kg^{-1} by the end, averaged among all planted treatments. On the other hand, because of absence of plant N uptake, inorganic [N] increased with time in the non-planted control to 44 mg kg^{-1}, averaged between the two water treatments. Because of increased plant uptake, soil inorganic [N] in the planted treatments 83 DAT may have dropped below a certain threshold where microbes became N limited. Once microbial growth became N limited, this resulted in a decreased rate of SOM decomposition ("competition hypothesis", Cheng and Kuzyakov, 2005). The decrease in soil-derived CO_2–C flux with time was less pronounced in the planted treatments with low water than in the planted treatments with high water, possibly because the effect of a decline in soil inorganic [N] on microbial activity in the low-water treatment was constrained by soil water. Because of soil disturbance, soil inorganic [N] was relatively high at the start of the experiment. Therefore the mostly positive rhizosphere effects observed during the first 69 days of the experiment may have been caused by artificially high soil inorganic N levels, while rhizosphere effects on soil C decomposition measured at the end of the experiment may relate better to field conditions. Our results contrast with earlier reports in which lower N availability enhanced rhizosphere priming (Liljeroth et al., 1990; Fontaine et al., 2004). Increased rhizosphere priming with reduced soil inorganic [N] could occur because of microbial

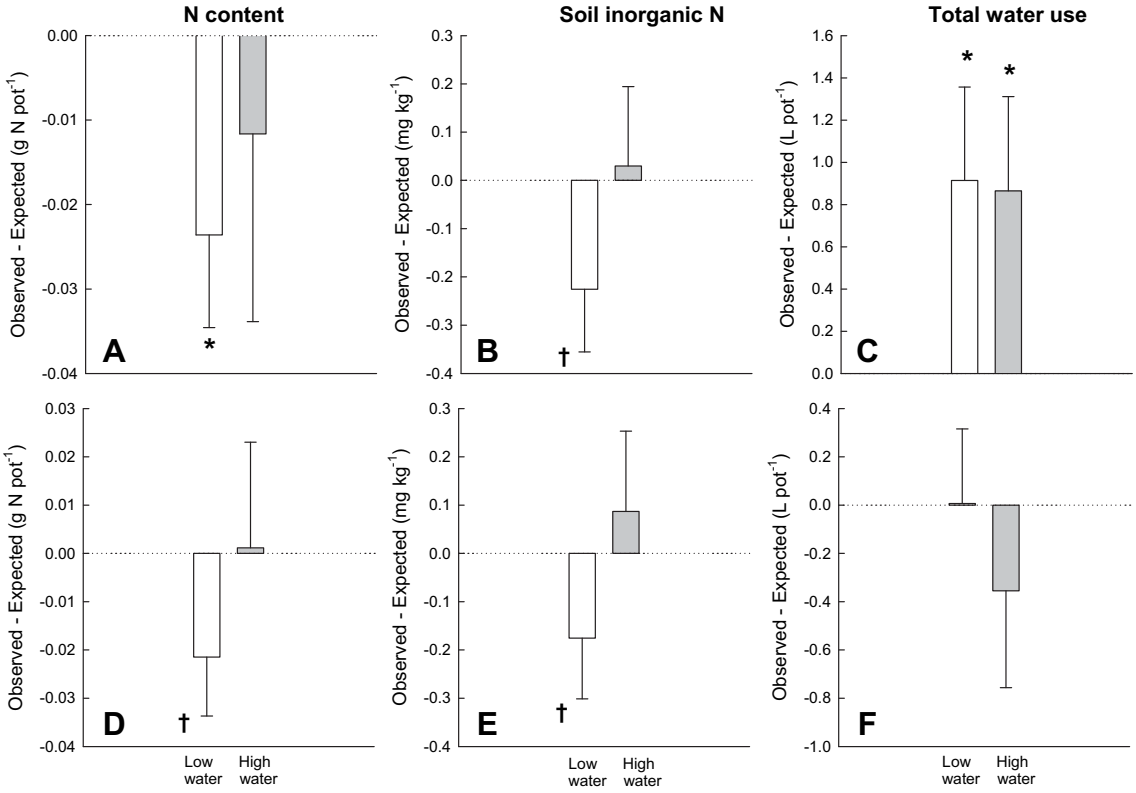

Fig. 7. Observed minus expected values of plant N content (A, D), soil inorganic N concentration (B, E), and total water use (C, F) in the mixtures (low water: 15% soil moisture, high water: 20% soil moisture). Expected values are calculated as the averages of the monocultures (A, B, C), and based on the average values of plant N content, soil inorganic N concentration, and total water use in the monocultures adjusted for species-specific plant biomass in the mixtures (D, E, F). Two-tailed *t*-test testing for significant deviation from zero †: $P < 0.1$, *: $P < 0.05$. Error bars denote standard errors.

preference of N-rich SOM to root-derived C when soil inorganic N is in short supply, while once soil inorganic N becomes abundant, microbial preference switches to energy-rich root-derived C ("preferential substrate utilization hypothesis", Cheng and Kuzyakov, 2005). Cheng and Kuzyakov (2005) reconciled this seeming contradiction by suggesting that at under the condition of severe N limitation (as in our study 83 DAT) the mechanism of competition dominates, while at high inorganic [N] the preferential substrate utilization mechanism dominates.

Plant phenology can also influence the magnitude of rhizosphere effects on soil C decomposition. Cheng et al. (2003) reported strong reductions in rhizosphere priming effects on soil C decomposition after flowering of wheat, but not of soybean. Morgan et al. (1998) reported strong increases in storage of non-structural carbohydrates in roots of *P. smithii* after 40 days of growth. In our study some of the *B. gracilis* and *L. dalmatica* plants were flowering by the end of the experiment, while *P. smithii* showed reduced aboveground growth during the last 30 days of the experiment (personal observation, unfortunately we have no information on root growth dynamics or storage of non-structural carbohydrates). Putting C resources into flowering or into roots may have reduced positive rhizosphere effects on soil C decomposition, but they cannot explain the negative rhizosphere effects by the end of the experiment for these species. In the sub-shrub *A. frigida*, rhizosphere effects on soil C decomposition were consistently positive (priming effect) and increased with time, suggesting that instead of reducing soil C decomposition through competition for resources, *A. frigida* increased soil C decomposition through inputs of labile C that stimulated microbial activity. A persistent priming effect that lasted more than 395 days has also been reported with tree seedlings of Ponderosa pine (*Pinus ponderosa*) and Fremont cottonwood (*Populus fremontii*), likely because of continuous rhizodeposition stimulating microbial activity (Dijkstra and Cheng, 2007a). In a review of priming studies, Cheng and Kuzyakov (2005) found that priming of SOM was greater in dicots than in monocots. Our results partly corroborate these results (greater soil C decomposition in *A. frigida* than in the grasses *B. gracilis*, *H. comata*, and *P. smithii*), although the low and ultimately negative rhizosphere effect on soil C decomposition in the invasive forb *L. dalmatica* does not follow this pattern. It is also not clear if any of these plant species can promote specific microorganisms that inhibit decomposition or that do not play a role in native SOM decomposition at the expense of microorganisms that do. Clearly, more research is needed on the relationship between plant species traits, phenology, resource availability, and rhizosphere effects on soil C decomposition.

Calculations of the soil-derived CO_2–C flux in the planted treatments were based on the assumption that there was no C isotopic fractionation during plant respiration. Although recent studies have shown that C respired from leaves tends to be enriched in ^{13}C relative to C in whole leaf biomass (see review by Bowling et al., 2008), the opposite pattern has been observed in roots (Badeck et al., 2005; Schnyder and Lattanzi, 2005; Bathellier et al., 2008), which can lead to very small differences in $\delta^{13}C$ between whole-plant respiration and whole-plant biomass (Klumpp et al. 2005; Schnyder and Lattanzi, 2005). It is not clear if and how the $\delta^{13}C$ in whole-plant biomass differed from whole-plant respiration in the plant species that we used. We tested the sensitivity of CO_2–C_{soil} to changes in $\delta^{13}C_{plant}$ in equation (2). A deviation of 1‰ in $\delta^{13}C_{plant}$ caused the largest deviation of

0.9 mg C h^{-1} pot^{-1} (or a 40% change) in $RhizCO_2$–C in *B. gracilis* under high water, and the lowest deviation of 0.2 mg C h^{-1} pot^{-1} (or a 10% change) in $RhizCO_2$–C in *A. frigida* under low water at 83 DAT. Thus, we cannot rule out the possibility that apparent differences in rhizosphere effects on soil C decomposition among plant species were partly a result of fractionation during plant respiration.

4.2. Rhizosphere effects on soil C decomposition: differences between water treatments

The high water treatment increased the rhizosphere effect on soil C decomposition at 48 and 69 DAT, at times when rhizosphere effects on soil C decomposition were mostly positive (priming effect), but not at 83 DAT, when rhizosphere effects were mostly negative. Priming effects on soil C decomposition were also larger in soils kept at 85% field capacity than in soils kept at 45% field capacity in a study with sunflower and soybean (Dijkstra and Cheng, 2007b). It was suggested that root exudates may be more effective in stimulating microbial activity and decomposition in wet than in dry soils, because of more rapid diffusion of exudates away from the root, and therefore reduced chances of exudates being actively re-adsorbed by roots (Jones and Darrah, 1993; Muhling et al., 1993). It is also possible that low soil moisture in the low-water treatment simply limited microbial activity, regardless of the availability of root exudates. The disappearance of this water effect at the end of the experiment, and the mostly negative rhizosphere effects on soil C decomposition, may have been caused by a switch in resource limitation from water to N (and possibly other nutrients).

4.3. Rhizosphere effects, plant N uptake, and water use: differences between monocultures and mixtures

As we predicted, the observed rhizosphere effect on soil C decomposition in mixtures was lower than expected based on results in the monocultures. In mixtures, plant N utilization tends to be more complete than in the monocultures of the component species because of complementarity and selection effects (Tilman et al., 1996, 1997; Hooper and Vitousek, 1997). Complementarity and selection effects on plant N uptake may also increase plant-microbial competition for N in mixtures more than in monocultures of the component species, which in turn could have reduced microbial SOM decomposition (Van Veen et al., 1989; Ehrenfeld et al., 1997; Wang and Bakken, 1997). In our study, soil inorganic [N] at the end of the experiment was lower in the mixtures than expected from the monocultures, suggesting that low C decomposition in mixtures may have been due to low N availability. Water use was also significantly higher in the mixtures than expected from the monocultures, however, mostly because of changes in the relative abundance of plant species in the mixtures (Fig. 7C, F). Furthermore, inter-specific competition decreased C decomposition most with low water. Therefore, we suggest that complementarity in plant water use (Silvertown et al., 1999; Nippert and Knapp, 2007; Verheyen et al., 2008), in addition to complementarity in plant N use, can reduce microbial activity and lead to lower than expected rhizosphere effects on soil C decomposition. Note that soil water and N availability are highly correlated in semi-arid grasslands (Burke et al., 1997).

Because some species showed positive and others negative rhizosphere effects on soil C decomposition when grown in monoculture, lower than expected rhizosphere effects in the mixtures may have been a result of an increase in dominance of species that showed negative rhizosphere effects, particularly given a significant under-yielding of *H. comata* in the mixtures. However, adjusting rhizosphere effects on soil C decomposition for species-specific plant biomass revealed patterns very similar to those observed with unadjusted rhizosphere effects, despite a significant under-yielding of *H. comata* in the mixtures. Thus, rhizosphere effects on soil C decomposition were rather insensitive when they were adjusted for plant biomass, possibly because the positive rhizosphere effect of the under-yielding *H. comata* was offset by positive rhizosphere effects of over-yielding species (*A. frigida* and *P. smithii* during the first two dates) and by negative rhizosphere effects of other under-yielding species (*L. dalmatica* during the last date).

Total plant N uptake in mixtures was also lower than expected based on plant N uptake in monocultures, possibly because rhizosphere effects reduced soil C decomposition more than expected. Thus, complementarity and selection effects, by reducing water and N availability and therefore SOM decomposition, may also result in reduced N supply. This seems somewhat counterintuitive, since complementarity and selection effects in mixtures cause more complete utilization of available N and thus could increase plant N uptake compared to plant N uptake in monocultures of the component species. This would be true if plant N and water uptake did not affect N supply. However, if rapid plant N uptake and increased water use reduce decomposition and therefore N supply in mixtures more than in monocultures it may also reduce total plant N uptake. It should be noted that in the long-term, increased N retention due to complementarity/selection effects (Tilman et al., 1996) may overcome the negative rhizosphere effects on N supply. Indeed, increased N retention could ultimately help maintain soil fertility and plant N supply (Fargione et al., 2007; Dybzinski et al., 2008).

Our results suggest that soil moisture and plant diversity modulate root-microbial interactions affecting SOM decomposition and plant N uptake. Further, while plant diversity usually enhances soil C and N cycling because of increased plant productivity and litter inputs (Hooper and Vitousek, 1997, 1998; Wardle et al., 1999; Craine et al., 2001; Zak et al., 2003), we observed that rhizosphere effects reduced SOM decomposition and plant N uptake in the mixtures compared to the component monoculture species under dry soil conditions. Complementarity and selection effects that result in increased plant water and N use in mixtures compared to the monocultures (Tilman et al., 1996; Verheyen et al., 2008) may also reduce microbial activity, thereby reducing microbial SOM decomposition and N supply in the mixtures more than expected from the monocultures. These rhizosphere effects on SOM decomposition and N mineralization may be particularly important for semi-arid grasslands where soil moisture and N limit biological activity during much of the year. Our results further suggest that rhizosphere effects causing reduced SOM decomposition could contribute to long-term increases in soil C storage in systems with limited water and/or high plant diversity, irrespective of effects on plant litter production. Indeed, rhizosphere effects could help explain observations of greater soil C storage with increased plant diversity that could not be attributed solely to an increase in plant productivity (Steinbeiss et al., 2008). Our results also show stark contrasts in rhizosphere effects on soil C decomposition among individual plant species. It remains to be seen what role rhizosphere effects play on SOM decomposition and N mineralization in different plant species mixtures.

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