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Plant inter-species effects on rhizosphere priming of soil organic matter decomposition

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ABSTRACT

Living roots and their rhizodeposits can stimulate microbial activity and soil organic matter (SOM) decomposition up to several folds. This so-called rhizosphere priming effect (RPE) varies widely among plant species possibly due to species-specific differences in the quality and quantity of rhizodeposits and other root functions. However, whether the RPE is influenced by plant inter-species interactions remains largely unexplored, even though these interactions can fundamentally shape plant functions such as carbon allocation and nutrient uptake.

In a 60-day greenhouse experiment, we continuously labeled monocultures and mixtures of sunflower, soybean and wheat with ^{13}C-depleted CO_2 and partitioned total CO_2 efflux released from soil at two stages of plant development for SOM- and root-derived CO_2. The RPE was calculated as the difference in SOM-derived CO_2 between the planted and the unplanted soil, and was compared among the monocultures and mixtures.

We found that the RPE was positive under all plants, ranging from 43% to 136% increase above the unplanted control. There were no significant differences in RPE at the vegetative stage. At the flowering stage however, the RPE in the soybean–wheat mixture was significantly higher than those in the sunflower monoculture, the sunflower–wheat mixture, and the sunflower–soybean mixture. These results indicated that the influence of plant inter-specific interactions on the RPE is case-specific and phenology-dependent. To evaluate the intensity of inter-specific effects on priming, we calculated an expected RPE for the mixtures based on the RPE of the monocultures weighted by their root biomass and compared it to the measured RPE under mixtures. At flowering, the measured RPE was significantly lower for the sunflower–wheat mixture than what can be expected from their monocultures, suggesting that RPE was significantly reduced by the inter-species effects of sunflower and wheat. In summary, our results clearly demonstrated that inter-species interactions can significantly modify rhizosphere priming on SOM decomposition.

1. Introduction

Soil organic carbon (C) functions as both an important source and a sink of atmospheric CO_2. Many uncertain parameters of the global C cycle are associated with complex soil processes and biological communities, which are both difficult to measure and highly sensitive to disturbance (Moyes et al., 2010). Soil CO_2 mainly consists of (1) root-derived CO_2, including root respiration and microbial decomposition of rhizodeposits from living roots (rhizomicrobial respiration), and (2) CO_2 derived from microbial decomposition of soil organic matter (SOM) (Kuzyakov, 2006). Both sources are linked through the rhizosphere priming effect (RPE) which describes changes in the rate of SOM decomposition in the presence of living roots (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Plant roots can alter microbial activities by providing organic substances (rhizodeposits) (Paterson, 2003), by competing with microorganisms for mineral nutrients (Schimel et al., 1989; Wang and Bakken, 1997), and by changing the physical and chemical conditions in the rhizosphere (e.g. water, pH) (Shields and Paul, 1973; Jenkinson, 1977). These alterations can lead to either stimulation (positive RPE) or retardation (negative RPE) of SOM

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decomposition with rates ranging from 70% reduction to as high as 330% increase compared to an unplanted control (Cheng and Kuzyakov, 2005). The direction and magnitude of RPE on SOM decomposition depend on both the plant and the soil. The amount of decomposable organic C and the mineral nitrogen (N_{min}) content of the soil have been identified as two of the main soil factors that significantly influence RPE (Liljeroth et al., 1994; Cheng and Johnson, 1998; Bottner et al., 1999; Kuzyakov, 2002). On the other hand, plant species and their developmental stages (Fu and Cheng, 2002; Cheng et al., 2003) also strongly influence the RPE, possibly through differences in the quality and quantity of rhizodeposits (Van der Krift et al., 2001; Nguyen, 2003; Jones et al., 2004).

Since the RPE is plant-species specific (Fu and Cheng, 2002; Cheng et al., 2003), it may also vary with plant inter-species interactions. It was proposed that with higher plant diversity the diversity of root exudates may also increase (Lavelle et al., 1995; Hooper et al., 2000). This wider spectrum of root exudates from mixed plant species may support a higher microbial biomass and activity in the rhizosphere (Hooper et al., 2000; Spehn et al., 2000; Stephan et al., 2000) whereby, the production of extracellular enzymes can be enhanced. Consequently, the potential for a positive RPE may increase (Fontaine et al., 2003). In contrast, Dijkstra et al. (2010) suggested a plant-diversity-induced decrease in SOM decomposition for systems with low water availability. With higher plant richness, belowground resources, especially N, are being complementarily used (Hooper and Vitousek, 1997; Von Felten et al., 2009). This may result in a lower availability of belowground resources and thus, in a decline in the decomposition of SOM (Dijkstra et al., 2010).

In this study monocultures and mixtures of sunflower, soybean and wheat were continuously exposed to ^{13}C-depleted CO_2. The soil CO_2 efflux was measured at an early stage of plant development (day 29–30 after planting), and at flowering of sunflower and soybean (day 55–56 after planting). Based on its $\delta^{13}C$ values the total soil CO_2 efflux was separated into root- and SOM-derived CO_2. The RPE was calculated as the difference in SOM-derived CO_2 between planted and unplanted treatments. To our knowledge, up to now the experimental work of Dijkstra et al. (2010) is the only study investigating the effect of plant diversity on the RPE, although inter-specific effects on carbon allocation belowground (Sanaullah et al., 2012) and on the activity of microorganisms (Sanaullah et al., 2011) are known. Although the results of Dijkstra et al. (2010) provide evidence that plant–plant interactions modify RPE, no firm conclusions on general patterns could be drawn, which necessitates future research on this topic.

Therefore, the aim of this study was to gain a more comprehensive understanding of modified RPE due to plant inter-species interactions. We hypothesized that the modulation of RPE by inter-species interactions is specific to the plant species composition and dependent on plant developmental stage.

2. Materials and methods

2.1. Experimental set-up

Monocultures of sunflower (Sun) (*Helianthus annuus* L.), soybean (Soy) (*Glycine max* L. Merr.) and spring wheat (Wh) (*Triticum aestivum* L.) and mixed cultures of sunflower/soybean (Sun/Soy), sunflower/wheat (Sun/Wh), soybean/wheat (Soy/Wh) and sunflower/soybean/wheat (Sun/Soy/Wh) were grown in PVC pots (15 cm diameter, 40 cm height, equipped with an inlet tube at the bottom for aeration and soil CO_2 trapping) with four replicates of the monocultures and six replicates of the mixed cultures. Including two-species mixtures in our study allowed investigation of possible patterns of individual species' influence on RPE when they were grown in mixtures. In addition four unplanted pots were prepared. A nylon bag filled with 1500 g sand was placed at the bottom of each PVC pot to improve air circulation. The pots were then filled with 7.9 kg of air-dried, sieved (<4 mm) soil taken from the plough horizon (top 30 cm) of a sandy loam from a farm on the campus reserves of the University of California, Santa Cruz. Air drying and sieving allowed us to achieve a high degree of soil homogeneity and reduced the variability among the treatments and replicates. The soil contained 1.1% organic C and 0.1% N, had a $\delta^{13}C$ value of −26.0‰ and a pH value of 5.8. All filled pots were wetted to 20% gravimetric soil moisture content (equivalent of 80% of the water holding capacity) with deionized water.

Seeds were presoaked over night in deionized water before planting. For the mixed cultures we used one individual plant of each species. For all monocultures we used two individual plants per pot to establish comparable growing conditions for all treatments. To get one individual plant, six seeds of wheat, two of sunflower and three of soybean were planted and thinned to one after seedling emergence. The soil moisture content was measured gravimetrically and adjusted daily to 80% of the water holding capacity. To maintain homogeneous soil moisture and good soil structure, water was added through perforated tubes (inner diameter 0.32 cm, total length 180 cm, buried length approximately 140 cm) as described by Dijkstra and Cheng (2007). The location of the pots in the greenhouse was changed weekly by mixing them randomly to guarantee similar growing conditions for the plants.

The experiment was conducted from January to March 2011 in a greenhouse equipped with continuous labeling by ^{13}C-depleted CO_2 at the University of California, Santa Cruz. Plants were continuously, i.e. from the emergence of the first leaf till harvest, exposed to ^{13}C-depleted CO_2 (−15‰). The continuous labeling technique allows us to quantitatively differentiate root-derived CO_2 from native SOM-derived CO_2 since both C pools differ in their isotopic composition after labeling (Table 1). During plant growth the day time air temperature inside the greenhouse was maintained at 28 °C by two AC units. The night time temperature was kept above 18 °C. Artificial lighting (1100 W lights, P.L. Light Systems, Beamsville, ON) was used when the natural light intensity was less than 900 W m^{-2}. The photoperiod was set from 6 AM to 6 PM. The relative air humidity was kept at 45% by a dehumidifier (Kenmore Elite 70 pint, Sears, Chicago, IL, USA). We continuously labeled the plants with naturally ^{13}C-depleted CO_2 using the method described in detail by Cheng and Dijkstra (2007). Briefly, a constant CO_2 concentration of 400 ± 5 ppm and a constant $\delta^{13}C$ value (see below) was maintained inside the greenhouse by regulating the flow of pure, ^{13}C-depleted CO_2 (99.9% CO_2, −35‰) from a tank and setting CO_2-free air flow rate proportional to the leakage rate of the greenhouse (Zhu and Cheng, 2012). The CO_2-free air was produced from compressed air passed through six soda lime columns (20 cm diameter, 200 cm length) filled with approximately 40 kg soda lime each. The CO_2-free air flow was set at 120 L/min.

Table 1
End member values (±SEM) used in two-source isotopic mixing models in order to calculate the contribution of SOM-derived CO_2 to total soil CO_2 of the planted treatments.

Treatment	Root-derived CO_2 [‰]	SOM-derived CO_2 of the unplanted soil [‰]
Sun	−39.6 ± 0.09	T1
Soy	−37.0 ± 0.3	−23.9 ± 0.2
Wh	−39.4 ± 0.4	T2
Sun/Soy	−39.2 ± 0.1	−23.7 ± 0.1
Sun/Wh	−39.6 ± 0.06	
Soy/Wh	−39.4 ± 0.2	
Sun/Soy/Wh	−39.1 ± 0.07	

The leakage rate of the greenhouse (300 L/min) was determined without plants shortly before the start of the experiment. This was done, after closing all inputs (CO_2-free air, tank CO_2), by raising the CO_2 concentration inside the greenhouse to a certain level and monitoring the decrease of the concentration which is proportional to the leakage rate. The CO_2 concentration inside the greenhouse was continuously monitored by an infra red gas analyzer (Model LI-820, Li-COR, Lincoln, NE, USA) and stabilized at 400 ± 5 ppm by computer controlled CO_2 injection from the tank. A fan was used to ensure a uniform distribution of the CO_2 inside the greenhouse. For the duration of the experiment the $\delta^{13}C$ value of the greenhouse air was measured every three days during the light period by pumping air through a glass airstone immersed in 50 mL of 0.5 M NaOH solution. The CO_2 trapping efficiency was nearly 100% as checked by an infra red gas analyzer (Model LI-6262, Li-COR, Lincoln, NE, USA). An aliquot of the sample was precipitated with $SrCl_2$ as $SrCO_3$ using the method described by Harris et al. (1997) and analyzed for $\delta^{13}C$ (relative to PDB standard) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer. The mean $\delta^{13}C$ value of the greenhouse air was −15.2‰ with a day-to-day variability of <0.7‰.

The inlet tube at the bottom of each pot was connected to an aquarium pump (Elite 802, Hagen Corp., Mansfield, MA, USA) to aerate the pots. This was done two times during the dark period (from 6:30 PM–7 PM and from 12 AM–1 AM) to avoid contamination of the greenhouse $\delta^{13}C$ signal with that of soil-derived CO_2 during the assimilation period. Before the start of each photoperiod the isotopic composition of the greenhouse CO_2 returned to equilibrium.

2.2. Measurements

Soil CO_2 efflux was measured on day 29–30 after planting (T1) when the plants were still at vegetative stage and on day 55–56 after planting (T2) during the flowering of sunflower and soybean by means of a closed-circulation CO_2 trapping system (Cheng et al., 2003). Briefly, a Plexiglas lid with holes for the shoots was placed direct on the soil surface. A plastic tube was attached to the lid for CO_2 trapping. The holes around the shoots and between the lid and the rim of the pot were sealed with non-toxic silicone rubber (GI-1000, Silicones Inc., NC, USA). The pots were checked for airtightness. The CO_2 inside the pots was removed by circulating the isolated air through a soda lime column (3 cm diameter, 50 cm length) for 40 min. Then CO_2 produced in the sealed pot was trapped for 24 h in 400 ml of 0.5 M NaOH solution. Four blanks were included to correct the total inorganic C content for possible contamination from carbonate in the NaOH stock solution and from sample handling. An aliquot of each NaOH solution was analyzed for total inorganic carbon using a Shimadzu TOC-5050A Total Organic Carbon Analyzer. Another aliquot was precipitated as $SrCO_3$ (Harris et al., 1997) and analyzed for $\delta^{13}C$ by means of a continuous flow isotopic ratio mass spectrometer (see description above).

At the end of the experiment the pots were destructively sampled. The shoots were cut at the base, dried at 60 °C and weighed. Three out of the six replicates of the mixtures were used to determine the root dry weight of each individual plant. All the soil in each pot was pulled out and soaked in deionized water for 24 h. All soil was washed away and the roots of each single plant were carefully separated in a water bath with tweezers, dried at 60 °C and weighed. The shoot-to-root ratio was determined and used to calculate the root biomass of each individual plant for the three remaining replicates based on their shoot biomass. Careful and accurate separation of roots of individual plant species in the mixtures was required to compare RPE of the mixtures with expected RPE calculated for the respective monocultures in a reliable manner (see below). Furthermore, to obtain root-free soil the roots of the three remaining replicates of each mixed treatment as well as the roots of all monocultures were separated from soil by hand-picking. The monoculture roots were rinsed with deionized water, dried as described above and weighed. A part of the soil remaining after root-picking of all treatments as well as of the soil from the unplanted pots was dried at 60 °C for three days. All dried samples were ground in a ball mill and analyzed for C%, N% and $\delta^{13}C$ using a Carlo Elba 1108 elemental analyzer interfaced to a ThermoFinningan Delta Plus XP isotope ratio mass spectrometer.

Soil microbial biomass C (MBC) was determined on all remaining soil samples by the chloroform fumigation extraction method described by Vance et al. (1987) with the modification that fumigated and unfumigated soil samples (30 g fresh soil) were extracted for 2 h with 60 mL of 0.5 M K_2SO_4 solution. The samples were filtered and the extracts analyzed for total organic carbon by means of a Shimadzu TOC-5050A Total Organic Carbon Analyzer. The difference between the extracts of fumigated and unfumigated samples corrected for a k_{EC} factor of 0.45 (Wu et al., 1990) gives the total amount of microbial biomass C.

Furthermore, soil mineral N (N_{min}; $NO_3^- + NH_4^+$) was extracted from 30 g fresh soil with 60 ml of 2 M KCl solution. Samples were shaken for 2 h, filtered and the extracts were analyzed for NO_3^- and NH_4^+ by a flow injection analyzer (Lachat QuikChem 8000, Milwaukee, WI).

2.3. Calculations

The contribution of CO_2 derived from SOM decomposition ($C_{SOM-derived}$, mg C day^{-1} kg $soil^{-1}$) to total soil respiration was calculated using a linear two-source isotopic mixing model:

$$C_{SOM-derived} = C_{Total} \cdot \frac{\delta^{13}C_{Total} - \delta^{13}C_{Root-derived}}{\delta^{13}C_{SOM-derived} - \delta^{13}C_{Root-derived}} \quad (1)$$

$$C_{Root-derived} = C_{Total} - C_{SOM-derived} \quad (2)$$

where C_{Total} is the total CO_2 efflux of the planted treatment (mg C day^{-1} kg $soil^{-1}$) and $\delta^{13}C_{Total}$ the corresponding $\delta^{13}C$ value (‰). $\delta^{13}C_{SOM-derived}$ is the $\delta^{13}C$ value of CO_2 from SOM decomposition measured in the unplanted pots (‰). $C_{Root-derived}$ is the root-derived CO_2 in the planted pot (mg C day^{-1} kg $soil^{-1}$) with $\delta^{13}C_{Root-derived}$ as the corresponding $\delta^{13}C$ value (‰).

The separation of root- and SOM-derived CO_2, as a prerequisite to calculate RPE, often involves the assumption that the net isotopic fractionation during respiration processes is negligible (e.g. Buchmann and Ehleringer, 1998; Rochette et al., 1999; Søe et al., 2004). A recent study by Zhu and Cheng (2011), however, reported a ^{13}C-depletion of rhizosphere respiration compared to the isotopic composition of roots or shoots. Furthermore, a review of ^{13}C fractionation at the root–microorganisms–soil interface showed that the mean difference between $\delta^{13}C$ of root-derived CO_2 and that of roots was −2.3‰ for C_3 plants and −1.3‰ for C_4 plants, by variation of more than ±2.0‰ (Werth and Kuzyakov, 2010). Moreover, it has been discussed by Dijkstra et al. (2010) that a 1‰ deviation in the isotopic composition of the plant tissue may result in variations of up to 40% in the RPE in their particular experimental configuration.

In order to minimize the influence of isotopic fractionation on the RPE results, we investigated target species for which the fractionation between roots and root-derived CO_2 (f) was determined recently. Accounting for ^{13}C fractionations associated with rhizosphere respiration by using directly-measured species-specific data in our calculations of SOM-derived CO_2 and root-derived CO_2

eliminates a major uncertainty or possible erroneous conclusions. The *f* factors were taken from an earlier study by Zhu and Cheng (2011) and are −1.01‰ for sunflower, −1.71‰ for soybean and −0.87‰ for wheat. We kept the conditions as similar as possible by using the same seeds, soil, and equipment with similar growing conditions (Zhu and Cheng, 2011). By doing so we accounted for the effect of isotopic fractionation on the RPE results.

$\delta^{13}C_{Root-derived}$ was differently calculated for monocultures and mixed cultures. $\delta^{13}C_{Root-derived}$ of the monocultures was calculated by correcting the $\delta^{13}C$ value of the root ($\delta^{13}C_{Root}$) by a fractionation factor (*f*):

$$\delta^{13}C_{Root-derived} = \delta^{13}C_{Root} + f \quad (3)$$

For mixed cultures we calculated a root biomass weighted $\delta^{13}C_{Root-derived}$ value:

$$\delta^{13}C_{Root-derived} = \sum\left(\delta^{13}C_{Root,i} + f_i\right)\cdot\alpha_i \quad (4)$$

where α_i is the percentage of root dry weight of species *i* on total root dry weight per pot.

Note: Because root biomass was analyzed at the end of the experiment only, the calculation (Eq. (4)) involves the assumption that α_i does not change between T1 and T2. We examined the sensitivity of this assumption by changing α_i from 0 to 100% and found that variations of and $C_{Root-derived}$ did not exceed 15% and 19%, respectively for the treatments including soybean and were even smaller than 5% for the Sun/Wh treatment.

The isotopic composition of root-derived CO_2 and SOM-derived CO_2 from the unplanted soil used as end members of the linear two-source isotopic mixing models are given in Table 1.

The RPE on SOM decomposition (observed RPE) was calculated by subtracting the CO_2–C flux of the unplanted treatment ($C_{SOM-derived}(UP)$) from the SOM-derived CO_2–C flux of the planted treatment ($C_{SOM-derived}(P)$) and was denoted as mg C day^{-1} kg $soil^{-1}$.

$$RPE = C_{SOM-derived}(P) - C_{SOM-derived}(UP) \quad (5)$$

Since changes of root biomass may have occurred when plants were grown in mixed cultures and because the RPE as well as the MBC, N_{min} and the root-derived CO_2 can be positively related to root biomass (Dijkstra et al., 2006), we calculated expected values (EXP) for the mixtures:

$$EXP = \sum RB_{mix,i}\cdot\frac{OBS_{mono,i}}{RB_{mono,i}} \quad (6)$$

$RB_{mix,i}$ is the root dry weight of species *i* in the mixture (g pot^{-1}), $OBS_{mono,i}$ is the observed value of species *i* growing in monoculture, denoted as mg C day^{-1} kg $soil^{-1}$ for the RPE and the root-derived CO_2, as mg C kg $soil^{-1}$ for the MBC and as mg N kg $soil^{-1}$ for N_{min}. $RB_{mono,i}$ is the root dry weight of species *i* in the monoculture (g pot^{-1}).

To estimate the effect of plant inter-species interactions, the expected values were subtracted from the observed values. If the observed value is lower than the expected value, RPE, root-derived CO_2, MBC or N_{min} was negatively influenced by inter-species interactions.

2.4. Statistics

The values presented in the figures and tables are given as means ± standard errors of the means (±SEM). Significant differences in total microbial biomass C, shoot and root N content, soil mineral N, shoot and root dry matter and in their isotopic composition between the treatments were obtained by a one-way analysis of variance (ANOVA) in combination with a *post hoc* unequal N HSD test, a modification of the Tukey HSD test. A one-way ANOVA was also conducted to test for significant differences in root-derived and SOM-derived CO_2 and RPE between the treatments by calculating the ANOVA separately for each sampling date. The significance of differences between individual means was obtained by the unequal N HSD test. To test for significant differences in root- and SOM-derived CO_2 and RPE within each treatment but between T1 and T2 (phenological effects) a dependent (paired) *t*-test was used.

Before calculating the RPE, as difference in SOM-derived CO_2 between a planted treatment and the unplanted control, we further tested for significant differences in SOM-derived CO_2 between the unplanted control and each single treatment and sampling day using an independent *t*-test. The unplanted control always showed significant ($P \leq 0.05$) lower values in comparison to the planted treatments (statistics are not presented in the figures).

Moreover, observed minus expected values were tested for significant deviation from zero by a *t*-test. All statistical analyses were performed with the statistical package statistica for Windows (version 7.0; StatSoft Inc., OK, USA).

3. Results

3.1. Plant biomass, plant $\delta^{13}C$ and microbial biomass C

Sunflower grown in monoculture produced a significantly higher shoot biomass per pot than soybean and wheat, whereas wheat developed the highest root biomass (data not presented). The root biomass per wheat plant was significantly lower in the monoculture compared to that of wheat grown in the mixtures (Table 2). In contrast, sunflower as well as soybean produced similar root biomass in all treatments independently of the neighboring plants.

The plant biomass of sunflower and wheat was significantly depleted in ^{13}C compared to that of soybean (Table 2). Wheat and sunflower showed similar isotopic compositions of their plant tissue.

Planting increased the microbial biomass C (Table 2). The lowest microbial biomass C was found in the unplanted control with values of about 118 mg C kg $soil^{-1}$. While the wheat monoculture showed a significant higher microbial biomass C than the control, only a slightly but not significantly higher microbial biomass was detected for the monocultures of sunflower and soybean. Compared to the unplanted control, all mixed croppings had significantly higher microbial biomass C, with values ranging from 188 mg C kg $soil^{-1}$ for the Sun/Soy treatment to 212 mg C kg $soil^{-1}$ for the Sun/Wh treatment. The microbial biomass C of the three-species mixture (172 mg C kg $soil^{-1}$) was, however, significantly lower than that measured for the Sun/Wh treatment.

The legume soybean showed consistently higher root N concentrations than the non-legume species (Fig. 1). The N concentration of the wheat roots increased when grown in combination with soybean compared to the wheat monoculture. However, the root N concentration of sunflower was approximately 8 mg N g dw^{-1} regardless of treatments and did not increase with a neighboring soybean.

Because of plant uptake, the mineral N ($NH_4^+ + NO_3^-$) content in the soil was roughly one order of magnitude lower for all planted treatments compared to the unplanted control at harvest (Fig. 1C). There were no significant differences in soil mineral N content between any planted treatments.

3.2. CO_2 efflux partitioning

The contributions of SOM- and root-derived sources to total soil CO_2 efflux were calculated based on a linear two source isotopic

Table 2
Plant biomass (±SEM), $\delta^{13}C$ values (±SEM) and microbial biomass C (±SEM) compiled at the end of the experiment. $N = 4$ for the monocultures and the unplanted soil; $N = 6$ for the mixed cultures except for root $\delta^{13}C$ and microbial biomass C of the mixed cultures for which $N = 3$.

Cultures	Treatment	Species	Plant biomass			$\delta^{13}C$		Microbial biomass C
			Shoot [g DW plant^{-1}]	Root [g DW plant^{-1}]	Shoot/Root	Shoot [‰]	Root [‰]	[mg C kg soil^{-1}]
	Unplanted soil							117.7 ± 7.9a
Monoculture	Sun	*H. annuus*	27.9 ± 3.7a[a]	2.7 ± 0.3abdf[a]	10.5 ± 1.1	−38.9 ± 0.1 ab	−38.6 ± 0.1a	136.7 ± 5.9 ab
	Soy	*G. max*	17.0 ± 1.7b[a]	2.0 ± 0.2abdf[a]	8.7 ± 1.0	−35.4 ± 0.2b	−35.1 ± 0.3b	140.0 ± 8.8ac
	Wh	*T. aestivum*	10.9 ± 0.7bc[a]	5.0 ± 0.5abg[a]	2.2 ± 0.1	−39.8 ± 0.2 acd	−38.6 ± 0.4a	165.7 ± 6.5bcd
Mixed Culture	Sun/Soy	*H. annuus*	35.8 ± 1.7a	5.0 ± 0.4a	7.3 ± 1.0	−38.8 ± 0.2ad	−38.9 ± 0.2a	187.5 ± 7.1def
		G. max	8.5 ± 0.9bc	2.0 ± 0.2bdf	4.2 ± 0.7	−36.3 ± 0.6b	−35.8 ± 0.1b	
	Sun/Wh	*H. annuus*	31.2 ± 1.1a	3.4 ± 0.3abdf	9.5 ± 1.4	−39.0 ± 0.2d	−38.8 ± 0.2a	211.5 ± 4.1f
		T. aestivum	8.9 ± 0.4bc	11.5 ± 1.0c	0.8 ± 0.1	−40.1 ± 0.0c	−38.7 ± 0.2a	
	Soy/Wh	*G. max*	10.6 ± 2.6bc	1.3 ± 0.4dfh	8.7 ± 1.5	−35.8 ± 0.1b	−35.9 ± 0.3b	207.0 ± 6.9 fg
		T. aestivum	13.3 ± 1.3b	9.6 ± 1.0ce	1.4 ± 0.1	−40.3 ± 0.1c	−38.8 ± 0.2a	
	Sun/Soy/Wh	*H. annuus*	28.9 ± 1.7a	2.9 ± 0.4ah	10.3 ± 1.7	−39.0 ± 0.0ad	−38.8 ± 0.1a	172.4 ± 5.5bceg
		G. max	5.0 ± 1.1c	1.2 ± 0.3f	4.1 ± 0.5	−36.1 ± 0.3b	−35.6 ± 0.2b	
		T. aestivum	7.7 ± 0.4bc	7.8 ± 0.8eg	1.0 ± 0.2	−40.2 ± 0.1ac	−38.3 ± 0.2a	

[a] Dry weight per pot divided by two because two individual plants were grown in these pots.

mixing model (Fig. 2). Since the $\delta^{13}C$ values of the roots differed among the species (Table 2), we assumed that the rate of root-derived CO_2 per unit of root dry weight for each species was the same in monoculture and in all mixtures in order to calculate the species-weighed $\delta^{13}C_{Root-derived}$ of the mixtures (Dijkstra et al., 2010). A significant positive correlation ($R^2 = 0.86$, $N = 12$, $P < 0.001$) between root-derived CO_2 and root biomass of the monocultures measured at T2 actually supported this assumption (data not shown).

Root-derived CO_2 varied between the treatments at T1 as well as at T2, probably due to varying root biomass (as indicated for T2 in Table 2) (Fig. 2A). A species effect was detected with low rates of root-derived CO_2 at T1 for the soybean and high rates for the sunflower monoculture. A combination of soybean and wheat resulted in a low rate of root-derived CO_2. At T2 wheat showed a very high rate of root-derived CO_2 when grown in monoculture, mainly because of its high root biomass (Table 2). The species composition effect was mainly influenced by the presence of wheat, leading to high rates of root-derived CO_2. When comparing T1 with T2, the root-derived CO_2 decreased for the sunflower and soybean monoculture as well as for the mixture of both species (Sun/Soy). In contrast, the root-derived CO_2 significantly increased for the wheat monoculture and the Soy/Wh treatment. The Sun/Wh and Sun/Soy/Wh treatments did no differ significantly between T1 and T2.

SOM-derived CO_2 did not differ between the planted treatments at T1 (Fig. 2B). Likewise, there is no statistically significant difference between the planted treatments in SOM-derived CO_2 at T2 with the exception of the Soy/Wh treatment showing higher values of about 15 mg C day^{-1} kg soil^{-1} compared to Sun/Soy and Sun/Wh mixtures, the sunflower monoculture and the unplanted control. When comparing T1 with T2 SOM-derived CO_2 decreased significantly for the Sun and the Sun/Wh treatment but increased for the Soy/Wh treatment. No statistically significant differences between T1 and T2 could be observed for the other treatments.

3.3. Rhizosphere priming effect

All planted treatments resulted in stimulation of SOM decomposition and hence, we found a consistently positive RPE (Fig. 3). The primed C at T1 ranges from 60% of SOM-derived CO_2 of the unplanted control for the Soy treatment to 98% for the Sun/Soy/Wh treatment. At T2 the values showed a broader range from 43% of SOM-derived CO_2 of the unplanted control for the Sun treatment to 136% for the Soy/Wh mixture. The RPE did not differ significantly between the planted treatments at T1 (Fig. 3). However, at T2 the Soy/Wh treatment showed a significantly higher RPE compared to Sun/Soy and Sun/Wh mixtures and sunflower monoculture. When comparing T1 with T2 no significant difference in the RPE could be detected for most treatments, except that the Soy/Wh treatment showed a significantly higher RPE at T2 compared to T1, and that the RPE decreased significantly for the sunflower monoculture and the Sun/Wh treatment from T1 to T2.

3.4. Effect of inter-species interactions

The observed RPE was compared to an expected value calculated for the mixtures (Fig. 4). The expected RPE was always slightly higher compared to the observed, but significantly higher only for the Sun/Wh treatment (Table 3). Modulations of RPE by plant inter-species interactions were specific to the plant species composition and tended to inhibit the RPE. However, the replicates of the treatments showed high variations. All combinations that contained the legume soybean did not show a significant effect of inter-species interactions on the RPE suggesting that available N may be an important factor modulating RPE. In contrast to that, the rhizosphere induced decomposition of SOM was significantly inhibited when growing sunflower and wheat together.

Similar to the RPE we estimated the effect of plant inter-species interactions on MBC, N_{min} and root-derived CO_2 by comparing the observed values with the expected values (Table 3). The MBC was negatively affected by mixed-cropping with the exception of the Soy/Wh treatment where no influence could be detected (Table 3). A significantly negative effect on N_{min} by mixed cropping occurred for the Sun/Soy and the Sun/Wh treatments. Root-derived CO_2 was also significantly and negatively affected by most mixed-croppings (Table 3).

4. Discussion

4.1. Plant species and plant phenology effects on the RPE

The type of plant species did not significantly affect the RPE on SOM decomposition, as shown by the similar RPEs of the monocultures (Fig. 3). Even after normalization for root dry weight, an equivalent RPE was measured for the monocultures despite slight variations in the root biomass per pot (data not presented). However, the high variability between the replicates of one treatment (sunflower monoculture) might have masked the differences between plant species at T2. The absence of significant plant specific differences seemed contradictory to recent results which revealed a species specific effect on the RPE (Fu and Cheng, 2002;

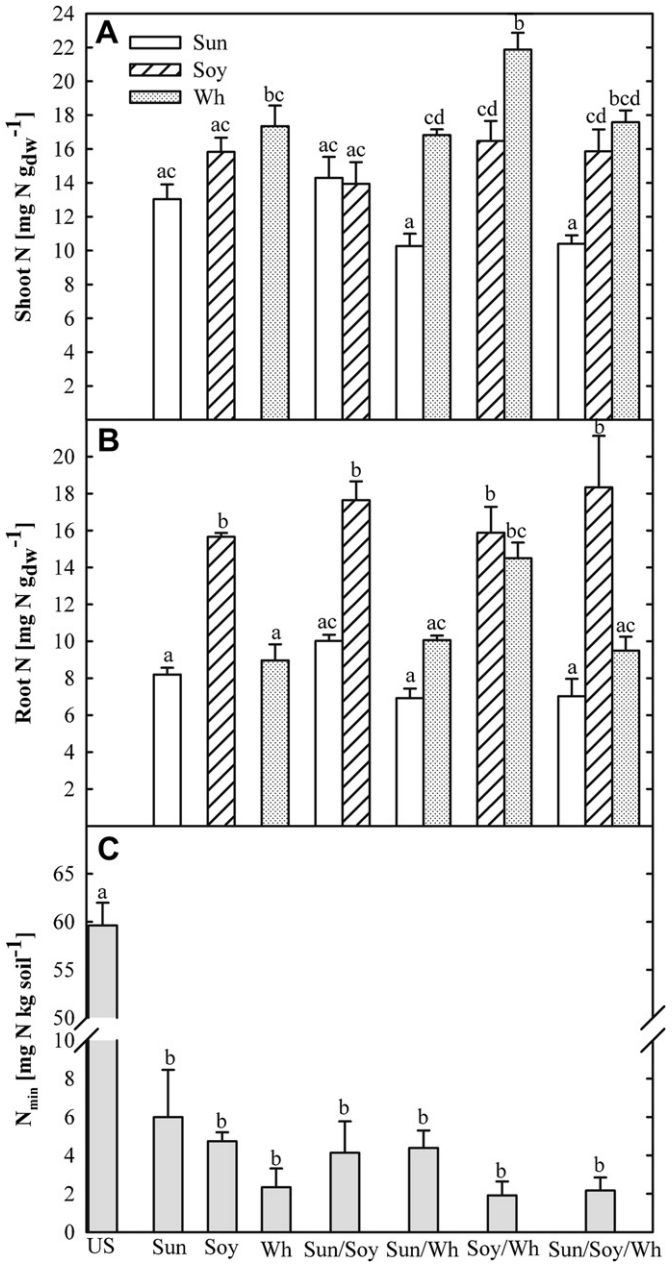

Fig. 1. N concentration (±SEM) of A: shoots ($N = 4$ for the monocultures; $N = 6$ for the mixtures) and B: roots ($N = 4$ for the monocultures; $N = 3$ for the mixtures). C: soil mineral N ($NH_4^+ + NO_3^-$) ($N = 4$ for the monocultures; $N = 3$ for the mixtures). Bars followed by the same lowercase letter are not significantly different at $P = 0.05$.

Cheng et al., 2003). Fu and Cheng (2002) reported a stronger priming effect under soybean, a N_2-fixing plant, compared to sunflower. A more pronounced RPE of soybean was also detected compared to wheat (Cheng et al., 2003). However, both studies compared the cumulative primed C over the whole growing period, which was, with more than 100 days, much longer than in our experiment. When considering only the first CO_2-trapping during the vegetative growth stage, the previous studies did not detect any effect of the plant species on RPE either. During the early stages of plant development, exudates, as a source of easily available C, may stimulate the growth and activity of rhizosphere microorganisms resulting in an increased rate of SOM decomposition ('Microbial activation hypothesis' Kuzyakov, 2002; Cheng and Kuzyakov, 2005). At later stages of plant development, other mechanisms controlling the RPE may gain increasing significance, such as the competition between roots and microorganisms for mineral N which may explain a negative RPE ('Competition hypothesis' Dormaar, 1990; Kuzyakov, 2002; Cheng and Kuzyakov, 2005).

Therefore, the plant age itself governs the amount of primed C in the rhizosphere due to changes of the exudation intensity with the growth stages (Kuzyakov, 2002; Cheng et al., 2003). The stage of plant development controls C translocation belowground, in addition to the type of plant species (Kuzyakov and Domanski, 2000). Young plants translocate more carbon to the roots, whereas older plants preferably allocate the newly assimilated C to the shoots (Keith et al., 1986; Gregory and Atwell, 1991; Palta and Gregory, 1997) thus, leading to reduced C inputs per root biomass into the soil via exudation at older stages (reviewed by Nguyen, 2003). We found significantly higher ($P \leq 0.1$) root-derived CO_2 of the sunflower and the soybean monocultures at the vegetative stage (T1) compared to the flowering stage (T2; Fig. 2). In contrast, wheat was still at the vegetative stage at the second CO_2 trapping and showed a strong increase of root-derived CO_2 as compared to the first CO_2 trapping (Fig. 2). It has been reported that the rhizodeposition of annual plants increased until the end of tillering because the decrease of the exudation intensity with age is, at this stage of development, slower than the root growth (Kuzyakov, 2002). However, we found no phenological effect of wheat on the amount of primed soil C (Fig. 3), likely because an increased nutrients uptake intensifies the competition between roots and microorganisms. In an experiment where the plants had developed over a longer period, a strong reduction of RPE after flowering of wheat has been reported (Cheng et al., 2003). We detected a phenology effect on RPE only for sunflower (Fig. 3). The priming for sunflower was lower ($P \leq 0.1$) during flowering compared to the vegetative stage, likely due to a higher need of assimilates for flower development and hence, a lower C allocation belowground.

4.2. Plant inter-species interactions modify RPE

The RPE was consistently positive for all planted treatments (Fig. 3). The increase in the SOM decomposition rates in the planted treatments was likely induced by inputs of organic substances via rhizodeposition, which often stimulate, as a source of easily available C, the growth of microorganisms in the rhizosphere (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Moreover, it could be assumed that with higher species richness the types of organic compounds released by plants into the soil might have increased. This would further stimulate the microbial biomass and its activity, resulting in a greater diversity of extracellular enzyme production which subsequently contributed to positive priming (Hooper et al., 2000; Spehn et al., 2000; Stephan et al., 2000; Fontaine et al., 2003; Dijkstra et al., 2010). Our results partly support this line of reasoning since all planted treatments generally resulted in higher microbial biomass C than the unplanted control, and the two-species mixtures showed higher MBC values than the monocultures. However, the three-species mixture has lower MBC compared to the Sun/Wh treatment (Table 2).

Our results indicate that plant inter-species interactions can significantly modify the rhizosphere priming effect on SOM decomposition (Table 3) with a tendency of reducing the root-biomass-adjusted RPE than what could be expected from their monocultures. A similar trend was also reported for five semi-arid grassland species when grown in mixture compared to monocultures even though no significant treatment differences could be detected because of their high experimental variability (Dijkstra et al., 2010). Plant species may differ in their nutrient acquisition. More diverse plant communities may better utilize limited resources such as available N (Tilman et al., 1996; Hooper and

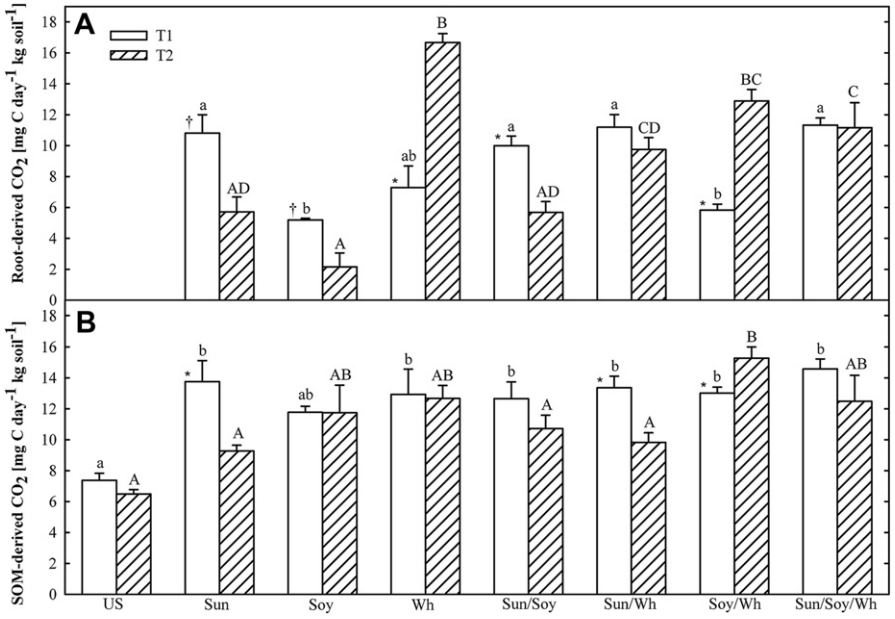

Fig. 2. Root-derived (A) and SOM-derived CO_2 (B) (±SEM) at T1 and T2. Significant differences between T1 and T2 within a treatment are presented as †: $P < 0.1$ and *: $P < 0.05$. Bars followed by different lowercase letters indicate significant differences between the treatments at T1 ($P < 0.05$). Significant differences between the treatments at T2 are marked by different uppercase letters ($P < 0.05$). $N = 4$ for the unplanted soil and the monocultures; $N = 6$ for the mixed cultures.

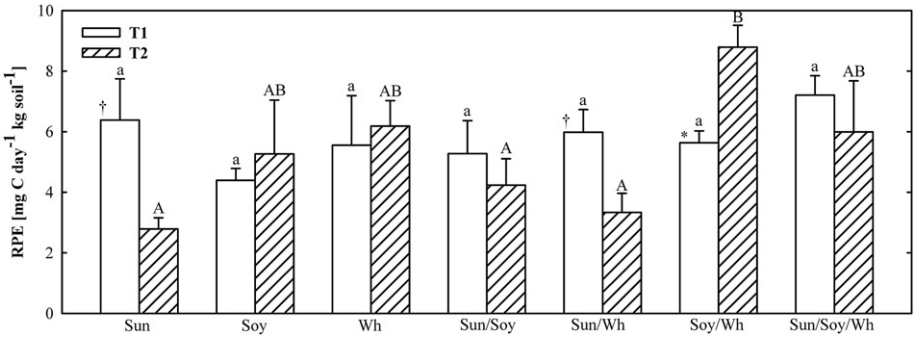

Fig. 3. Rhizosphere priming effect (±SEM) calculated for T1 and T2. Significant differences between T1 and T2 within a treatment are presented as †: $P < 0.1$ and *: $P < 0.05$. Bars followed by different lowercase letters indicate significant differences between the treatments at T1 ($P < 0.05$). Significant differences between the treatments at T2 are marked by different uppercase letters ($P < 0.05$). For the monocultures $N = 4$, for the mixed cultures $N = 6$.

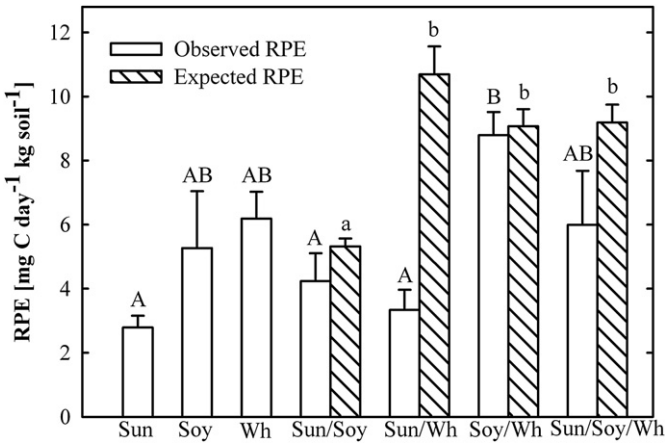

Fig. 4. Observed and expected RPE (±SEM). Significant differences between the observed values are marked by different uppercase letters ($P < 0.05$). Bars followed by different lowercase letters indicate significant differences between the expected RPE ($P < 0.05$).

Vitousek, 1997). Hence, the plant-microbial competition, especially for mineral N, may increase with higher plant diversity leading to partial reduction of microbial activity, which is accompanied with a decrease of the RPE (Dijkstra et al., 2010).

We suggest that the competition hypothesis applies for the mixture containing sunflower and wheat, the only treatment where a significantly lower root-biomass-adjusted RPE was observed than expected (Table 3). This is further supported by the lower N_{min} content of the Sun/Wh treatment than expected (Table 3). An increasing competition for mineral N between roots and microorganisms may also cause the lower observed microbial biomass C compared to the expected (Table 3). Moreover, the decreasing N_{min} content with time was accompanied with the reduced RPE at T2 compared to T1 (Fig. 3), despite the fact that the root-derived CO_2, reflecting exudation intensity, remained constant (Fig. 2). On the other hand, root-derived CO_2 was also significantly influenced by mixed cropping for all treatments containing wheat (Table 3). Therefore, the intensified competition for mineral N and the lower than expected exudation intensity together suppressed the RPE of the Sun/Wh mixture compared to the monocultures. However, the exact mechanisms behind these findings remain unknown.

Table 3
Observed minus expected values (±SEM) of RPE at T2 ($N = 6$), MBC ($N = 3$), mineral soil N ($N = 3$), and root-derived CO_2 ($N = 6$).

Observed minus expected values (±SEM)				
Treatment	RPE [mg C day^{-1} kg $soil^{-1}$]	MBC [mg C kg $soil^{-1}$]	N_{min} [mg N kg $soil^{-1}$]	Root-derived CO_2 [mg C day^{-1} kg $soil^{-1}$]
Sun/Soy	−1.1 ± 0.9	−21.6 ± 10.1[b]	−3.5 ± 0.3[a]	−0.9 ± 0.7
Sun/Wh	−7.4 ± 1.0[a]	−74.7 ± 21.9[a]	−5.4 ± 0.7[a]	−14.4 ± 1.8[a]
Soy/Wh	−0.3 ± 0.4	−4.1 ± 12.6	−1.3 ± 0.7	−4.8 ± 1.2[a]
Sun/Soy/Wh	−3.2 ± 1.9	−84.3 ± 15.6[a]	0.5 ± 0.7	−6.6 ± 2.4[a]

[a] Significant difference from zero: $P < 0.05$.
[b] Significant difference from zero: $P < 0.1$.

Our results demonstrated for the first time that mixed cropping of typical agricultural plants may reduce the decomposition of SOM compared to monocultures. Generally, this result indicates that on a longer-term C storage may be reduced through the cultivation of plants in monocultures. However, it has to be considered that the RPE strongly depends on soil properties, mainly on the organic C and mineral N content (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Agricultural soils are characterized by low contents of decomposable C and high mineral N contents through fertilization. It was hypothesized that microorganisms, not limited in N, can switch from the decomposition of SOM to the decomposition of rhizodeposits which provide easily available energy and C for microbial activity and growth (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Thus, RPE in agricultural soils are largely controlled by this preferential substrate utilization (Kuzyakov, 2002). However, rhizosphere priming will gain increasing importance in the future in the context of sustainable agriculture and organic farming. The shift toward systems with a low external input of fertilizers increases the dependence of plants on nutrient release from SOM due to RPE (Paterson, 2003).

Apart from the agricultural point of view, inter-species interactions have implications on C and N cycling in natural ecosystems with high plant diversity, not only through altered productivity and litter inputs but also through altered RPE. The reduced priming measured in this study may contribute to a long-term increase in SOC in mixed cultures compared to monocultures.

5. Conclusions

During the early stage of plant development the RPE was not specific to the plant species and was positive for all planted treatments. The modulation of RPE by plant inter-species interactions was specific to the species composition. The RPE was significantly reduced for the sunflower–wheat mixture compared to the monocultures. Our data provided clear evidence that plant species composition affects the RPE. Future research is needed to identify mechanisms and clarify the role of inter-species interactions, especially among plant functional groups, on RPE.

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