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Moisture modulates rhizosphere effects on C decomposition in two different soil types

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Abstract

While it is well known that soil moisture directly affects microbial activity and soil organic matter (SOM) decomposition, it is unclear if the presence of plants alters these effects through rhizosphere processes. We studied soil moisture effects on SOM decomposition with and without sunflower and soybean. Plants were grown in two different soil types with soil moisture contents of 45% and 85% of field capacity in a greenhouse experiment. We continuously labeled plants with depleted ^{13}C, which allowed us to separate plant-derived CO_2-C from original soil-derived CO_2-C in soil respiration measurements. We observed an overall increase in soil-derived CO_2-C efflux in the presence of plants (priming effect) in both soils. On average a greater priming effect was found in the high soil moisture treatment (up to 76% increase in soil-derived CO_2-C compared to control) than in the low soil moisture treatment (up to 52% increase). Greater plant-derived CO_2-C and plant biomass in the high soil moisture treatment contributed to greater priming effects, but priming effects remained significantly higher in the high moisture treatment than in the low moisture treatment after correcting for the effects of plant-derived CO_2-C and plant biomass. The response to soil moisture particularly occurred in the sandy loam soil by the end of the experiment. Possibly, production of root exudates increased with increased soil moisture content. Root exudation of labile C may also have become more effective in stimulating microbial decomposition in the higher soil moisture treatment and sandy loam soil. Our results indicate that moisture conditions significantly modulate rhizosphere effects on SOM decomposition.

Keywords: Continuous ^{13}C labeling; Decomposition; Diffusion; Plant species; Priming; Rhizosphere effects; Root exudates; Soil moisture; Soil organic matter; Soil texture

1. Introduction

Soil moisture is one of the key factors influencing soil microbial activity and soil organic matter (SOM) decomposition (Brady and Weil, 2002). Most global circulation models predict an increase in precipitation by 5–10% over the 21st century in much of the Northern Hemisphere, although a decrease has been predicted for some regions such as north and west Africa and parts of the Mediterranean (Intergovernmental Panel on Climate Change, 2001). This change in precipitation is likely going to affect soil moisture and consequently SOM decomposition.

Rhizosphere processes are soil processes that are influenced by living plant roots and that have shown to influence SOM decomposition (see review by Cheng and Kuzyakov, 2005). A decrease in SOM decomposition up to 50% has been observed (Kuzyakov and Cheng, 2001), while in many other studies increased SOM decomposition was found, up to 380% (Cheng et al., 2003). These results indicate that the presence of plants cannot be ignored in SOM decomposition studies. Despite these large impacts on SOM decomposition, very little is known about the mechanisms behind rhizosphere effects on SOM decomposition, or how it is affected by soil moisture content.

Several mechanisms have been suggested that can cause rhizosphere effects on SOM decomposition (Cheng and Kuzyakov, 2005). A mechanism that could suppress SOM decomposition is the competition for nutrients, and nitrogen in particular, between plant roots and the

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microbial decomposer community in the soil (Schimel et al., 1989; Ehrenfeld et al., 1997; Wang and Bakken, 1997). Root uptake of N reduces the availability of N for microbial growth and metabolism, thereby suppressing SOM decomposition. Suppression of SOM decomposition by rhizosphere effects could also occur when microorganisms in the soil prefer to decompose rhizodeposits (that are very labile and energy rich), thereby temporally decreasing SOM decomposition (Sparling et al., 1982; Cardon et al., 2001).

Rhizosphere effects could stimulate SOM decomposition by increasing the destruction of soil aggregates because of root growth, thereby exposing physically protected SOM for microbial decomposition (Reid and Goss, 1981; Helal and Sauerbeck, 1984, 1986). The presence of plants intensifies drying–rewetting cycles in the soil that can increase SOM decomposition (Van Schreven, 1967; Lundquist et al., 1999) because of enhanced solubility of humic substances, increased microbial death/growth cycles, and increased destruction of soil aggregates (Magid et al., 1999). Finally, rhizosphere effects could increase SOM decomposition because exudation of labile energy-rich compounds by roots can stimulate microbial activity and decomposition (Helal and Sauerbeck, 1986; Sallih and Bottner, 1988; Cheng and Coleman, 1990).

Soil moisture could affect all of the mechanisms mentioned above: it could alter the competition for N between plants and soil microorganisms (Lodge et al., 1994; Lipson and Monson, 1998), it could affect aggregate stability (Cousen and Farres, 1984; Lavee et al., 1996), it could affect the intensity of drying–rewetting cycles, and it could affect root exudation (Gorissen et al., 2004). Here, we present results from a study where we tested whether rhizosphere effects on soil C decomposition are altered by soil moisture. We did this for soybean (*Glycine max*) and sunflower (*Helianthus annuus*), grown in a sandy loam and a clay loam soil. While this experiment was not designed to replicate truly natural conditions, it allowed us to better understand the impacts of soil moisture, soil type and plant species on priming of SOM.

2. Materials and methods

2.1. Experimental design

We did our experiment in a greenhouse at the University of California, Santa Cruz. We planted soybean (*Glycine max*, cultivar Envy) and sunflower (*Helianthus annuus*, cultivar Sunbright) seeds in PVC pots (diam. 15 cm, height 40 cm), capped at the bottom with an air outlet (Fig. 1). We placed a nylon bag filled with 2500 g playground sand at the bottom of each pot. We filled 24 pots with 7500 g air-dried soil (corresponding to a bulk density of 1.38 g cm^{-3}) that came from the organic farm of the Agroecology Center that has been organically farmed (without use of synthetic fertilizer) for over 30 years, located on the campus of the University of California, Santa Cruz

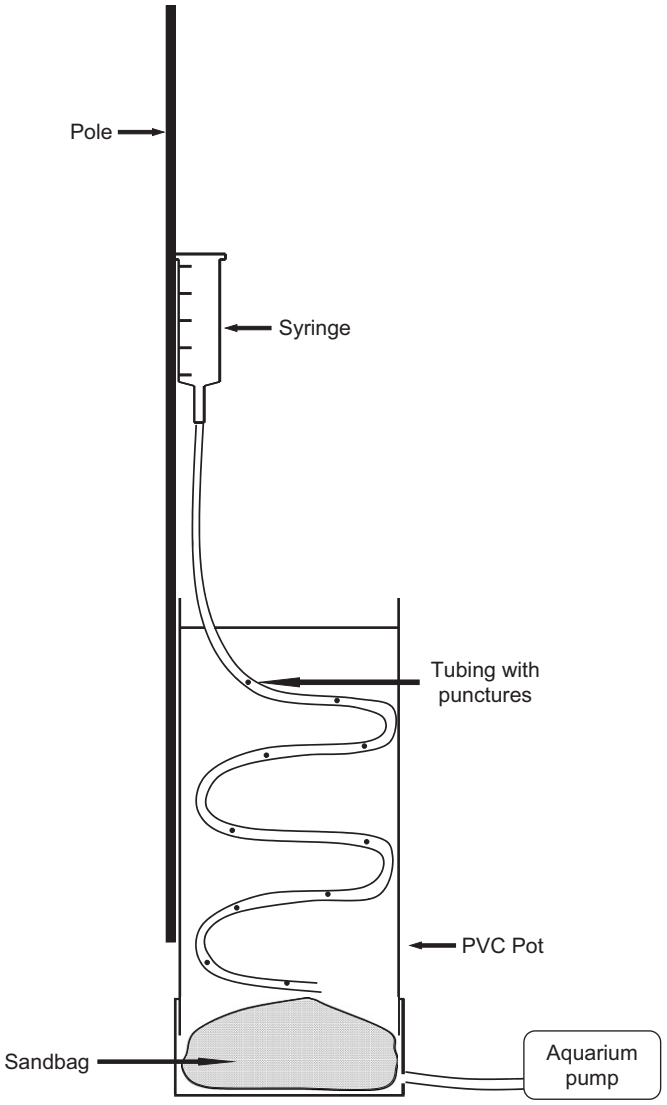

Fig. 1. Setup of the pots. The perforated tubing allows for homogeneous watering throughout the soil profile.

(Mollisol, sandy loam). We filled another 24 pots with 6000 g air-dried soil (corresponding to a bulk density of 1.10 g cm^{-3}) that came from a Kansas tall grass prairie (Mollisol, clay loam). We sieved (4 mm) to homogenize the soil, and removed most of the roots from both soil types before filling the pots. The soils experience frequent and severe disturbances in their natural settings (tillage in the organic farm soil and gopher activity in the Kansas prairie soil). We will refer to the organically farmed soil as the "sandy loam soil" and to the Kansas tall grass prairie soil as the "clay loam soil". The sandy loam and clay loam soil had a pH of 5.8 and 7.1, a total C content of 10 and 17 g kg^{-1} with a δ^{13}C value of −25.4‰ and −15.2‰, and a total N content of 1.2 and 1.8 g kg^{-1}, respectively. We buried a plastic watering tube (inner diam. 0.32 cm, total length 70 cm, buried length 50 cm), punctured with holes every 5 cm, while filling the pot with soil. The tube was buried in a spiral inside the pot, with a 100 ml reservoir

at the top end of the tube that protruded from the soil (Fig. 1). Water added to the reservoir dripped slowly out of the holes in the tube, thereby maintaining uniform soil moisture content throughout the pot. In eight pots of each soil type we planted five seeds of soybean inoculated with *Bradyrhizobium japonicum*, LiphaTech, Inc., Milwaukee, WI, and in another eight pots of each soil type we planted five seeds of sunflower. The remaining eight pots of each soil type were not planted, and will be referred to as the control pots. After germination we thinned each pot to one plant. In half of all the pots we kept soil moisture content at 45% and in the other half at 85% of field capacity by weighing and watering the pots with DI water every day during the experiment. During the experiment, soil moisture never deviated more than 3% from the target value. We connected the air inlet at the bottom of each pot to an aquarium pump and aerated the pots every 6 h for 15 min during the period of the experiment. We rotated pots inside the greenhouse each time plants were watered.

To label plants with ^{13}C we added ^{13}C-depleted CO_2 ($\delta^{13}C = -37.6‰$) to the greenhouse from a gas tank (manufactured from natural gas). We kept the CO_2 concentration constant at 760 ppm. The advantage of using natural gas rather than enriched ^{13}C to label plants is that it is less expensive. The CO_2 concentration inside the greenhouse was monitored and controlled with an infrared gas analyzer (IRGA, LI-COR 820). When the CO_2 concentration fell below 760 ppm, the IRGA opened a solenoid valve to let ^{13}C-depleted CO_2 into the greenhouse. The valve closed at 765 ppm. We sampled the CO_2 inside the greenhouse eight times during the experiment by pumping air for 8 h through a 4 M NaOH solution. Samples were analyzed for $\delta^{13}C$ (see below). The $\delta^{13}C$ signature of the CO_2 inside the greenhouse was relatively constant throughout the experiment at $-21.2 \pm 0.6‰$ (mean $\pm$ SD), or about 13.2 units more negative than that of ambient CO_2. While we were inside the greenhouse, we breathed out through a tube connected to the outside air to avoid disturbances in the CO_2 concentration and ^{13}C signature inside the greenhouse.

2.2. Sampling and measurements

We measured soil respiration 43 and 69 days after planting (DAP). The first soil respiration measurements occurred when plants were still in vegetative stage, the second occurred after flowering. Before measurement, we sealed the top of the pots by placing two half-moon-shaped Plexiglass pieces on top of the soil inside the pot around the base of the plant, and pouring two-component silicone rubber (Circle K Products, Temecula, CA) on top of the Plexiglass. An air inlet was created by attaching plastic tubing through the Plexiglass. We circulated air inside the pot by attaching an aquarium pump to the air in- and outlet. The initial CO_2 inside the root–soil system was removed with a soda lime column and subsequent CO_2 evolved inside the root–soil system was trapped in a CO_2 trapping column (a PVC tube filled with 350 g burnt and acid-washed sand and 35 ml of 4 M NaOH) during a 48 h period (Cheng et al., 2003). During this period, the pumps circulated air every 6 h for 15 min. The CO_2 trapping efficiency of this system was >99.9% as checked by an infrared gas analyzer (Model LI-6262, LI-COR, Lincoln, NE. Sub-samples of the solution containing CO_2 trapped from soil respiration and greenhouse air (see above) were measured for inorganic C on a TOC analyzer (Shimadzu TOC-5050A). A 0.3 M $SrCl_2$ solution was added to another sub-sample to form $SrCO_3$ precipitate. The $SrCO_3$ precipitate was repeatedly rinsed with deionized water until a solution pH $\approx$ 7, then dried at 80 °C (Harris et al., 1997).

All plants were harvested after the second soil respiration measurement (70 DAP). Plants were separated into stems, leaves, reproductive organs, and roots, were then dried (65 °C) and weighed. Stems, leaves, and reproductive organs were then combined (shoot biomass) for each pot and ground. All $SrCO_3$ precipitate samples, and ground plant material samples were analyzed for $\delta^{13}C$ on a Hydra 20–20 continuous flow isotope mass spectrometer (PDZ Europa, Chesire, UK).

2.3. Calculations and statistical analyses

We calculated water use efficiency (WUE) by dividing the final total plant biomass with the cumulative amount of transpiration loss (the difference in the cumulative amount of water added to the non-planted control treatments and the planted treatments throughout the experiment). We separated soil respiration into plant-derived CO_2-C (root respiration, and microbial respiration of root associated materials) and soil-derived CO_2-C (microbial respiration of SOM) in the planted treatments using the following:

$$C_s = C_t(\delta^{13}C_p - \delta^{13}C_t)/(\delta^{13}C_p - \delta^{13}C_s), \quad (1)$$

where C_s is the efflux of CO_2-C derived from soil, C_t is the total efflux of CO_2-C from soil respiration (plant-derived plus soil-derived), and $\delta^{13}C_t$, $\delta^{13}C_s$, and $\delta^{13}C_p$ are the $\delta^{13}C$ values of the total efflux of CO_2-C from soil respiration, the efflux of soil-derived CO_2-C and plant-derived CO_2-C respectively. We used the $\delta^{13}C$ value (averaged by treatment) measured from soil respiration in control pots (no plants) for $\delta^{13}C_s$, and from plant biomass (averaged by treatment) for $\delta^{13}C_p$. In the control pots C_s equals the measured value of C_t. We assume that the soil-derived CO_2-C in the planted treatments has the same $\delta^{13}C$ signature as the soil-derived CO_2-C in the control treatments.

The $\delta^{13}C$ values measured in the NaOH CO_2 traps were corrected for contamination from carbonate in the NaOH stock solution and from sample handling using the following (Cheng et al., 2003):

$$\delta^{13}C_j = (C_t\delta^{13}C_t - C_c\delta^{13}C_c)/(C_t - C_c), \quad (2)$$

where $\delta^{13}C_j$ is the $\delta^{13}C$ value of a sample after correction, $\delta^{13}C_t$ is the $\delta^{13}C$ value of a sample before correction, $\delta^{13}C_c$ is the $\delta^{13}C$ value of the contaminant C (−6‰), C_t is the

total amount of *C* in the sample solution including contaminant *C*, C_c is the amount of *C* in blank control solutions. Because the pots with the clay loam soil contained less soil than the pots with the sandy loam soil, we calculated soil-derived CO_2-C per kg (oven-dry) soil. We then calculated the rhizosphere effect on soil *C* decomposition ("primed soil C") by subtracting the soil-derived CO_2-C per kg soil in the planted treatment from the non-planted control treatments.

We used analyses of variance (ANOVA) to test for plant, soil, and soil moisture ("water") effects and their interactions on plant biomass, plant biomass $\delta^{13}C$, WUE, plant- and soil-derived CO_2-C from soil respiration, and primed soil C. We also used analyses of covariance (ANCOVA) to test for plant, soil, and water effects and their interactions on primed soil C with plant-derived CO_2-C and plant biomass as a covariate to account for plant-derived CO_2-C and plant biomass differences among treatments. All statistical analyses were done with JMP (version 4.0.4).

3. Results

Plant biomass harvested after 70 days was significantly greater for sunflower than for soybean (except leaf biomass), significantly greater in the sandy loam than in the clay loam soil, and significantly greater in the wet than in the dry soil treatment (Table 1). Sunflower showed particularly greater biomass (except leaf biomass) in the sandy loam soil (significant plant × soil interaction terms). Root biomass particularly increased with increased soil moisture content in the sandy loam soil (significant soil × water interaction term). The plant, soil, and water treatments showed no significant effect on the root/shoot ratio, but the root/shoot ratio increased with increased soil moisture content in the sandy loam soil, but decreased with increased soil moisture in the clay loam soil (significant soil × water interaction term). The WUE was on average significantly greater in the dry than in the wet soil. The WUE was on average significantly greater for sunflower than for soybean, and significantly greater in the sandy loam than in the clay loam soil.

We successfully depleted the plant C in ^{13}C (Table 2), on average the plant $\delta^{13}C$ value was −41.7‰, or 16.3 units more negative than the C in the sandy loam soil, and 26.5 units more negative than the C in the clay loam soil. Plant biomass $\delta^{13}C$ was significantly more negative in sunflower than in soybean, significantly more negative for the clay loam than for the sandy loam soil (except root biomass $\delta^{13}C$), but only for root biomass significantly more negative in the wet than in the dry soil treatment.

Because the C assimilated by plants was much more depleted in ^{13}C than the soil C, we were able to separate plant-derived CO_2-C from soil-derived CO_2-C in soil respiration measurements according to Eq. (1). We used the weighted average $\delta^{13}C$ value of the shoot and root for $\delta^{13}C_p$. Using the $\delta^{13}C$ value of the shoot or the root for $\delta^{13}C_p$ did not substantially change our results because of the large difference in the $\delta^{13}C$ value between plant- and soil-derived C. Plant-derived CO_2-C was significantly greater in the sandy loam than in the clay loam soil and significantly greater in the wet than in the dry soil at 43 and

Table 1
Average plant biomass, root/shoot ratio, and water use efficiency (WUE) ± standard error with ANOVA results

Soil type	Soil moist.	Plant biomass (g pot^{-1})						Root/shoot	WUE (g L^{-1})
		Leaf	Stem	Reprod. organs	Root	Shoot	Total biomass		
Soybean									
Sandy loam	45%	8.4 ± 1.1	3.2 ± 0.3	6.6 ± 1.9	1.7 ± 0.2	18.2 ± 1.7	20.0 ± 1.8	0.10 ± 0.01	5.67 ± 0.23
	85%	10.7 ± 1.7	6.3 ± 0.3	9.3 ± 1.5	3.6 ± 0.2	26.2 ± 0.7	29.8 ± 0.9	0.14 ± 0.01	5.27 ± 0.51
Clay loam	45%	0.9 ± 0.1	0.4 ± 0.1	2.8 ± 0.4	0.4 ± 0.1	4.1 ± 0.6	4.5 ± 0.6	0.10 ± 0.01	5.55 ± 0.55
	85%	3.1 ± 0.3	1.3 ± 0.2	5.0 ± 0.6	0.7 ± 0.1	9.3 ± 0.9	10.0 ± 1.0	0.07 ± 0.01	4.39 ± 0.15
Sunflower									
Sandy loam	45%	8.2 ± 0.3	22.7 ± 2.1	18.9 ± 1.1	4.2 ± 0.4	49.7 ± 1.4	53.9 ± 1.5	0.08 ± 0.01	6.56 ± 0.12
	85%	10.3 ± 0.3	24.5 ± 1.1	19.4 ± 0.7	7.1 ± 1.4	54.2 ± 1.2	61.2 ± 2.3	0.13 ± 0.02	6.12 ± 0.16
Clay loam	45%	1.2 ± 0.4	1.6 ± 0.8	1.3 ± 0.6	0.4 ± 0.2	4.0 ± 1.8	4.5 ± 2.0	0.13 ± 0.02	6.09 ± 0.33
	85%	3.5 ± 0.2	5.3 ± 0.5	4.0 ± 0.1	1.1 ± 0.1	12.7 ± 0.6	13.9 ± 0.7	0.09 ± 0.01	5.33 ± 0.20
ANOVA (*P*-values)									
Plant		0.97	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.53	0.002
Soil		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.10	0.02
Water		0.0003	0.001	0.01	0.0006	<0.0001	<0.0001	0.49	0.006
Plant × soil		0.54	<0.0001	<0.0001	0.001	<0.0001	<0.0001	0.11	0.79
Plant × water		0.96	0.56	0.57	0.30	0.99	0.72	0.99	0.70
Soil × water		0.97	0.87	0.54	0.02	0.68	0.58	0.0003	0.25
Plant × soil × water		0.88	0.14	0.36	0.67	0.05	0.13	0.77	0.64

Table 2
Average plant biomass $\delta^{13}C$ values ± standard error with ANOVA results

Soil type	Soil moist.	$\delta^{13}C$‰		
		Shoot	Root	Whole plant[a]
Soybean				
Sandy loam	45%	−39.44 ± 0.44	−39.47 ± 0.32	−39.44 ± 0.43
	85%	−38.69 ± 0.52	−38.61 ± 0.54	−38.68 ± 0.53
Clay loam	45%	−41.22 ± 0.35	−37.52 ± 0.11	−40.91 ± 0.33
	85%	−41.48 ± 0.25	−39.06 ± 0.27	−41.32 ± 0.23
Sunflower				
Sandy loam	45%	−42.59 ± 0.34	−42.75 ± 0.15	−42.60 ± 0.33
	85%	−43.22 ± 0.07	−43.43 ± 0.13	−43.24 ± 0.06
Clay loam	45%	−43.29 ± 0.59	−43.21 ± 0.16	−43.26 ± 0.53
	85%	−44.58 ± 0.10	−43.89 ± 0.09	−44.53 ± 0.10
ANOVA (*P*-values)				
Plant		<0.0001	<0.0001	<0.0001
Soil		<0.0001	0.43	<0.0001
Water		0.19	0.01	0.14
Plant × soil		0.03	0.003	0.04
Plant × water		0.03	0.37	0.03
Soil × water		0.13	0.003	0.09
Plant × soil × water		0.74	0.003	0.60

[a]Weighted average of shoot and root.

69 DAP (Table 3). At 69 DAP plant-derived CO_2-C was also significantly greater for sunflower, particularly in the clay loam soil (significant plant × soil interaction) and in the wet soil (significant plant × water and plant × soil × water interaction). At 69 DAP plant-derived CO_2-C increased more in the clay loam than in the sandy loam soil with increased soil moisture content (significant soil × water interaction).

In the non-planted control pots we observed significantly greater soil-derived CO_2-C in the clay loam (on average by 27% after 43 days, $P = 0.003$, and by 37% after 69 days, $P = 0.0001$, ANOVA) than in the sandy loam soil, and not surprisingly, greater soil-derived CO_2-C in the wet (on average by 31% after 43 days, $P = 0.002$, and by 46% after 69 days, $P < 0.0001$, ANOVA) than in the dry soil. Including all pots in the ANOVA, we observed significant greater soil-derived CO_2-C in the wet than in the dry soil at 43 and 69 DAP (Table 3). On average, soil-derived CO_2-C was significantly greater for soybean than for sunflower, although at 69 DAP soil-derived CO_2-C in the clay loam soil was greater for sunflower than for soybean. At 43 DAP soil-derived CO_2-C increased more for soybean than for sunflower with increased soil moisture (significant plant × water interaction, $P < 0.0001$), but at 69 DAP soil-derived CO_2-C increased more for sunflower than for soybean with increased soil moisture ($P = 0.0004$). We observed no

Table 3
Plant- and soil-derived CO_2-C (±1 SE)

Soil type	Soil moist.	Plant-derived CO_2-C (mg C kg soil^{-1} day^{-1})		Soil-derived CO_2-C (mg C kg soil^{-1} day^{-1})	
		Days after planting		Days after planting	
		43	69	43	69
Control (no plants)					
Sandy loam	45%	—	—	3.21 ± 0.06	2.26 ± 0.08
	85%	—	—	4.77 ± 0.32	3.23 ± 0.19
Clay loam	45%	—	—	4.65 ± 0.30	3.03 ± 0.13
	85%	—	—	5.49 ± 0.39	4.52 ± 0.28
Soybean					
Sandy loam	45%	7.96 ± 0.66	5.15 ± 0.45	5.99 ± 0.27	4.07 ± 0.19
	85%	8.26 ± 0.71	6.22 ± 0.92	10.01 ± 0.59	7.29 ± 0.54
Clay loam	45%	2.23 ± 0.68	1.26 ± 0.19	4.55 ± 0.29	4.10 ± 0.12
	85%	5.40 ± 1.09	3.76 ± 0.75	7.81 ± 0.56	5.91 ± 0.42
Sunflower					
Sandy loam	45%	8.27 ± 0.47	3.85 ± 0.23	4.65 ± 0.23	2.81 ± 0.13
	85%	11.15 ± 0.81	6.18 ± 0.28	6.04 ± 0.24	5.62 ± 0.20
Clay loam	45%	1.08 ± 0.47	2.55 ± 1.61	5.49 ± 0.43	5.15 ± 0.29
	85%	1.58 ± 0.17	13.83 ± 1.24	6.89 ± 0.27	8.44 ± 0.51
ANOVA					
Plant		0.37	0.0004	<0.0001	<0.0001
Soil		<0.0001	0.99	0.86	<0.0001
Water		0.002	<0.0001	<0.0001	<0.0001
Plant × soil		0.0003	<0.0001	<0.0001	<0.0001
Plant × water		0.96	0.0004	<0.0001	0.0004
Soil × water		0.80	0.0003	0.25	0.69
Plant × soil × water		0.01	0.005	0.69	0.04

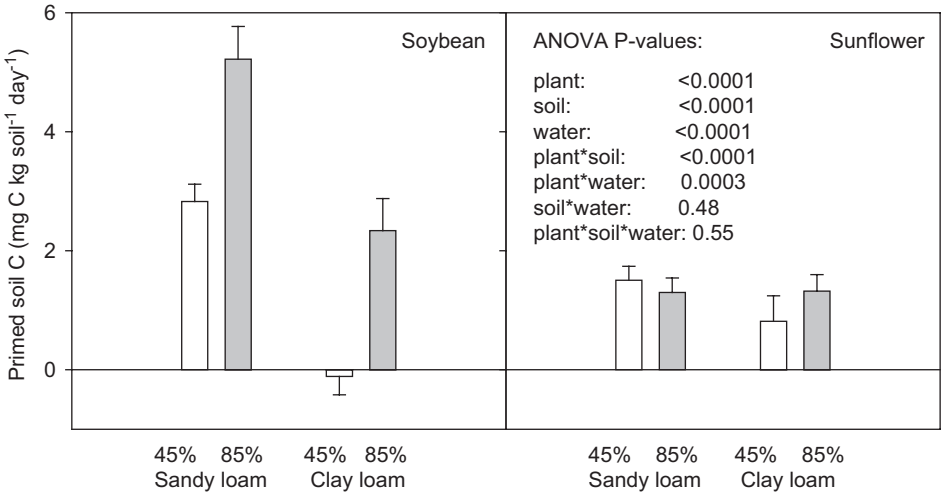

Fig. 2. Average primed C (difference in soil-derived CO_2-C between planted and control treatments) ± standard error at 43 days after planting.

significant soil × water interaction terms during both days of measurement.

In most cases, the presence of plants increased the efflux of soil-derived CO_2-C compared to the control treatments (Table 3), indicating a priming effect on soil C decomposition both at 43 and 69 DAP. At 43 DAP we observed primed C in all treatments, except for soybean in the clay loam soil at 45% field capacity (Fig. 2). The highest primed C rate occurred for soybean in the sandy loam soil at 85% field capacity (increase of 110% compared to control). On average soybean showed a greater priming effect on soil C decomposition (increase of 57% compared to control) than sunflower (increase of 27% compared to control, $P<0.0001$), particularly in the sandy loam soil (plant × soil interaction $P<0.0001$). On average increased soil moisture content increased the priming effect on soil C decomposition (increase of 32% and 50% compared to control for low and high soil moisture treatment respectively, $P<0.0001$), particularly for soybean (plant × water interaction, $P=0.0003$). We observed no significant soil × water interaction term.

At 69 DAP, we observed primed C for all treatments. By this time we observed no significant main plant or soil effects on primed C, but primed C significantly increased with increased soil moisture content (increase of 52% and 76% compared to control for low and high soil moisture treatment respectively, $P<0.0001$, Fig. 3A). Primed C was greater for soybean than for sunflower in the sandy loam soil, but greater for sunflower than for soybean in the clay loam soil (plant × soil interaction, $P<0.0001$, Fig. 4A). The response in primed C to increased soil moisture content was greater in the sandy loam than in the clay loam soil (soil × water interaction, $P=0.05$, Fig. 4A). We observed no significant plant × water interaction.

We tested whether treatment effects on primed soil C were related to differences in plant-derived CO_2-C among treatments. Plant-derived CO_2-C comes from root respiration and microbial respiration of root exudates and other labile root associated material that could stimulate the priming effect on soil C decomposition. When we used an ANCOVA with plant-derived CO_2-C as the covariate, then at 43 DAP the covariate was not significant ($P=0.40$), and treatment effects in the ANCOVA were not substantially different from the ANOVA. At 69 DAP the plant-derived CO_2 C was significant as a coviariate ($P=0.006$), but treatment effects in the ANCOVA were not substantially different from the ANOVA (Figs. 3B and 4B). The main water treatment effect became less significant, while the soil × water interaction became more significant than in the ANOVA results.

In a previous study, we observed that primed soil C could be positively related to plant biomass (Dijkstra et al., 2006), and thus treatment effects on primed soil C could have been related to treatment effects on plant biomass. We used ANCOVA with plant biomass as a covariate to test for plant, soil, water effects and their interactions on primed C, after adjusting for plant biomass effects. We observed that leaf biomass ($P=0.0001$) could explain more of the variation in primed soil C measured at 69 DAP than root ($P=0.11$) or total plant biomass ($P=0.01$). We therefore only presented the ANCOVA with leaf biomass as the covariate, although results of the ANCOVA do not change substantially when we use root or total biomass as the covariate. After correcting for leaf biomass effects, we still observed no significant main plant effect on primed C (Fig. 3C), while the plant × soil interaction remained significant ($P<0.0001$, Fig. 4C). Correcting for leaf biomass caused on average a significant greater priming effect on soil C in the clay loam than in the sandy loam soil ($P=0.0005$, Fig. 3C). It also caused a stronger soil × water interaction effect on primed C ($P=0.01$), where the response in primed C with increased soil moisture content was strong in the sandy loam soil, but weak in the clay loam soil (Fig. 4C).

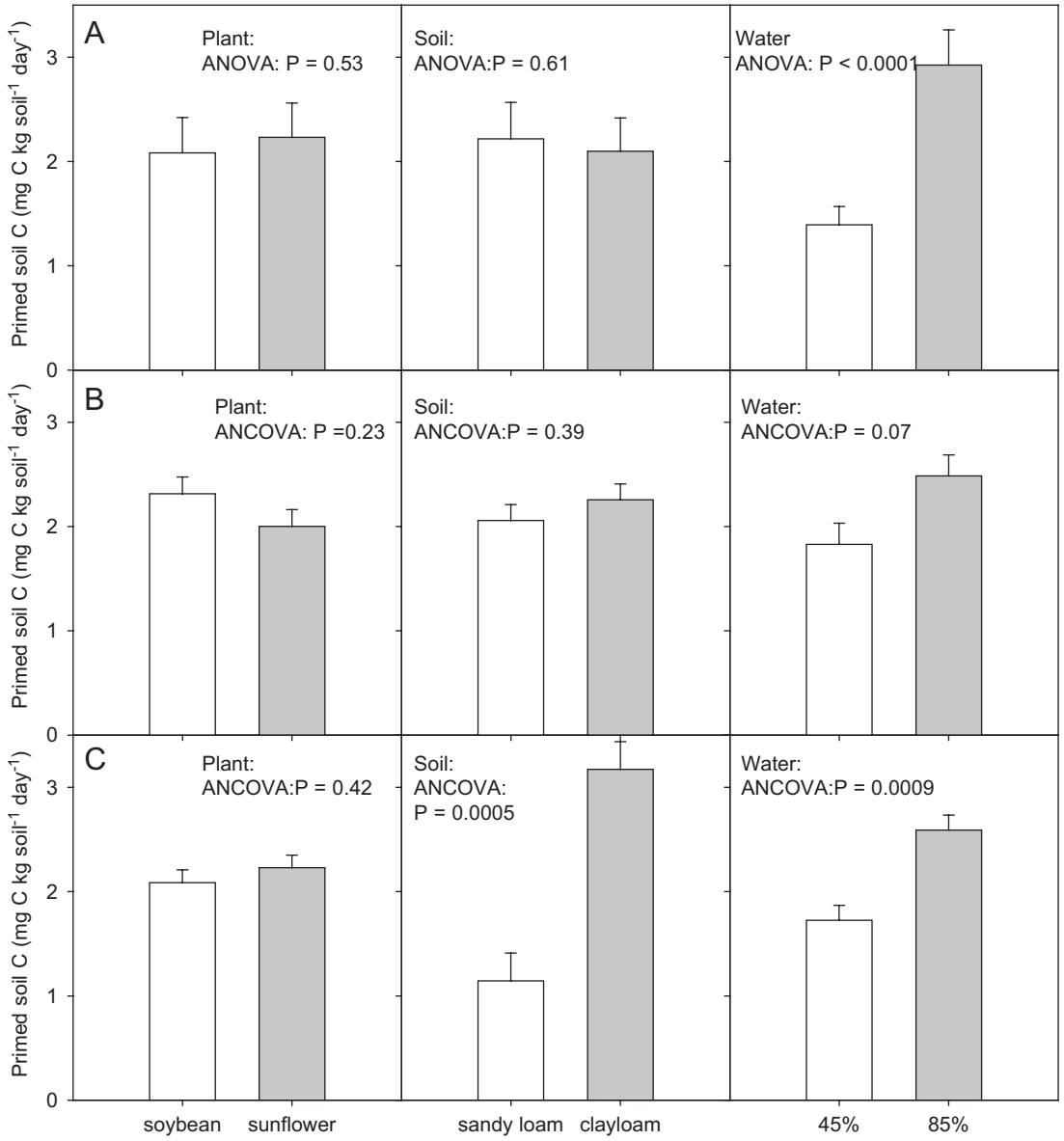

Fig. 3. Main treatment effects on primed C (difference in soil-derived CO_2-C between planted and control treatments) at 69 days after planting. (A) Means ± standard error and ANOVA *P*-values; (B) least-squares means ± standard error of ANCOVA with plant-derived-CO_2-C as the covariate and ANCOVA *P*-values; (C) least-squares means ± standard error of ANCOVA with leaf biomass as the covariate and ANCOVA *P*-values.

4. Discussion

Without plants (i.e., control treatments), soil C decomposition increased with soil moisture content. When soils become dry, it causes a decrease in enzyme activity (Skujins and McLaren, 1967; Sardans and Penuelas, 2005), and it reduces the thickness of water films on soil surfaces and therefore the rate of diffusion of substrates to microbes (Stark and Firestone, 1995) causing a decline in soil C decomposition. Because the soils were regularly aerated, there was enough oxygen in the soil to prevent adverse effects of soil moisture on SOM decomposition in the highest moisture treatments. Soil C decomposition without plants was on average higher in the clay loam than in the sandy loam soil. However, the clay loam soil also had a higher total soil C content. Expressed per unit of total soil C, the soil-derived CO_2-C efflux in the control pots were significantly higher in the sandy loam than in the clay loam soil (after 43 days 0.40 and 0.30 g C kg C^{-1} day^{-1} for sandy loam and clay loam soil respectively, $P = 0.0008$, and after 69 days 0.27 and 0.22 g C kg C^{-1} day^{-1} for sandy loam and clay loam soil respectively, $P = 0.003$). Greater soil C decomposition per unit of total soil C in the sandy loam soil suggests that the overall soil C of the sandy loam soil was more labile than that of the clay loam soil. It is also possible that physical differences (texture, bulk density) affecting oxygen supply and accessibility of soil C by microorganisms may have caused these differences in relative soil C decomposition between the two soil types.

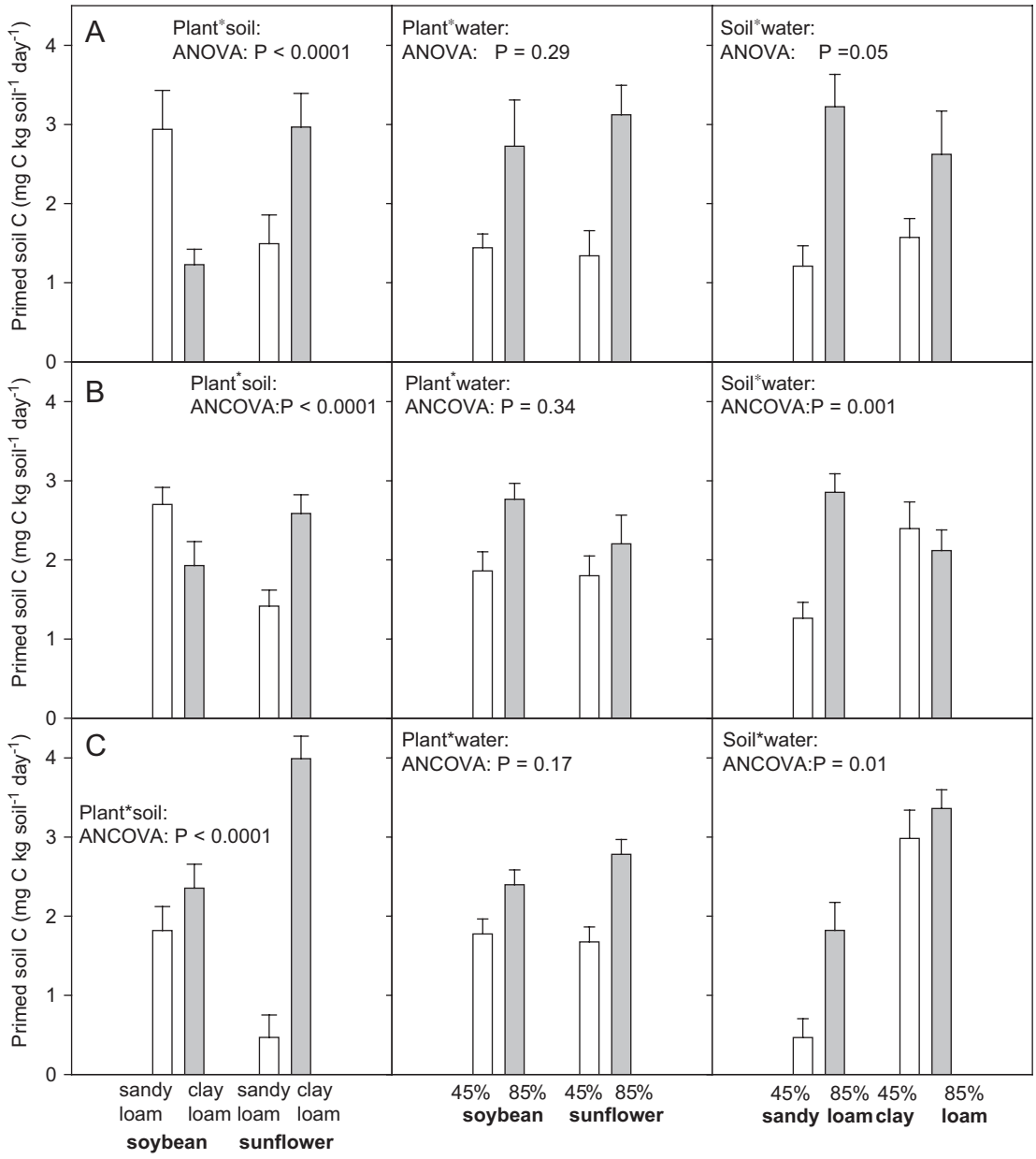

Fig. 4. Plant × soil, plant × water, and soil × water interaction effects on primed C (difference in soil-derived CO_2-C between planted and control treatments) at 69 days after planting. (A) Means ± standard error and ANOVA *P*-values; (B) least-squares means ± standard error of ANCOVA with plant-derived-CO_2-C as the covariate and ANCOVA *P*-values; (C) least-squares means ± standard error of ANCOVA with leaf biomass as the covariate and ANCOVA *P*-values.

We observed stimulation of soil C decomposition in the presence of plants in most of the treatments at 43 DAP, and in all treatments at 69 DAP, indicating significant priming effects. High soil moisture intensified the priming effect; particularly for the sandy loam soil at 69 DAP. We observed significant main plant, plant × water and plant × soil interaction effects at 43 DAP, but main plant and plant × water interaction effects on priming were absent at 69 DAP.

It has been suggested that an increase in soil aggregate destruction with root growth can cause a priming effect (Reid and Goss, 1981; Helal and Sauerbeck, 1984, 1986). SOM within aggregates is physically protected against microbial decomposition (Van Veen and Kuikman, 1990; Balesdent et al., 2000). Plant roots can penetrate aggregates and disrupt them, thereby exposing SOM to microbial decomposition. We observed only a weak positive relationship between root biomass and primed C at 69 DAP suggesting that the destruction of aggregates with root growth may not be the most important mechanism for the priming effect in our soils. Aggregates in our soils have partly been destroyed by sieving before filling the pots, which may have diminished a potential root growth effect on primed C. Further, root growth can also increase soil aggregation, particularly when they are mycorrhizal (Rillig and Mummey, 2006).

An increase in soil C decomposition in the presence of plants may also occur because of intensified drying–rewetting cycles in the soil (Van Schreven, 1967; Lundquist et al., 1999). Drying–rewetting cycles could enhance solubility of non-living SOM (Kieft et al., 1987; Van Gestel et al., 1993), increase microbial death during dessication and osmoregulatory shock (Kieft et al., 1987; Magid et al., 1999), and increase deterioration of soil aggregates (Van Veen and Kuikman, 1990). Because of daily watering, soil moisture never deviated more than 3% of the target value and caused only small intensity drying–rewetting cycles. The intensity of the drying–rewetting cycles were slightly higher in the planted treatments, but were most likely too small to cause a priming effect on SOM decomposition.

A third mechanism that could cause a priming effect is exudation of labile energy-rich compounds by roots that can stimulate microbial activity and decomposition (Helal and Sauerbeck, 1986; Sallih and Bottner, 1988; Cheng and Coleman, 1990). We observed a strong positive relationship between leaf biomass and primed C, suggesting that root exudation responsible for the priming effect is directly related to the photosynthetic rate of plants (Dijkstra et al., 2006). The increased priming effect in the wet soil compared to the dry soil may have been caused by greater production of root exudates (Gorissen et al., 2004). While we do not have direct estimates of root exudate production, the plant-derived CO_2-C flux that we measured includes CO_2 from microbial respiration of root exudates. With plant-derived CO_2-C as the covariate in the ANCOVA, primed C was still significantly greater in the wet soil at 43 DAP ($P = 0.002$), and marginally greater at 69 DAP ($P = 0.07$, Fig. 3B). Also, primed C remained significantly greater in the wet soil at 69 DAP after correcting for leaf biomass (Fig. 3C). These results suggest root exudation plays an important role in the priming effect but that soil moisture modulates the priming effect through mechanisms other than root exudate production alone.

It is possible that exudates were more effective in stimulating microbial activity and decomposition in the wet soil because root exudates diffused more rapidly away from the root, and as a result root exudates were less likely to be actively re-adsorbed by roots (Jones and Darrah, 1993; Muhling et al., 1993). Texture differences between the two soil types may also have affected the diffusion rate of root exudates, causing the significant soil × water interaction on primed C. Faunal grazing on microbes can enhance SOM decomposition (Clarholm, 1985; Freckman 1988; Kuikman et al., 1990), which may have increased more in the sandy loam soil than in the clay loam soil with increased soil moisture content, because high clay content tends to protect microbes from faunal grazing. Further, abiotic sorption of exudates by clay particles and SOM (van Hees et al., 2003) may also have increased more with increased soil moisture content in the clay loam soil than in the sandy loam soil.

The two soil types that we used did not only differ in texture, but also in other properties such as pH, C and N content, and most likely also in matric potential and microbial and faunal composition. It is possible that the significant main soil (at day 69 after correcting for leaf biomass), and plant × soil and soil × water interactions on primed C were caused by some or all of these factors. While the greater priming effect in the sandy loam than in the clay loam soil at 43 DAP may be related to plant biomass effects, the priming effect was greater in the clay loam than in the sandy loam soil at day 69 after correcting for leaf biomass. The sandy loam and clay loam soil that we used here came from very different sites (California grassland vs. Kansas tallgrass prairie), and their differences in fertility, microbial and faunal community may have contributed to the different responses in primed C between soybean and sunflower. We should further note that diffusion of root exudates and microbial activity may have been more affected by matric potential than by water holding capacity. The relationship between matric potential and percentage of water holding capacity may have differed between the two soil types, which may have contributed to the main soil, plant × soil and soil × water interaction effects on SOM decomposition and primed C.

Main plant and plant × water interaction effects on primed C were slightly different between the two dates of measurement. These differences between the two measurement dates may be related to plant biomass effects on primed C (e.g., sunflower appeared to be smaller than soybean in the clay loam soil after 43 days, but was of similar size to soybean in the clay loam soil after 70 days). We did not harvest plants after the first trapping session and therefore were unable to correct primed C for plant biomass effects. Different treatment effects in time on primed C may also have stemmed from differences in phenology (Cheng et al., 2003).

We are very confident that our estimates of primed C are precise. Plant biomass $\delta^{13}C$ values were 16.3 and 26.5 units more negative than the $\delta^{13}C$ values of soil respiration in the sandy loam and clay loam soil control treatments, respectively. To significantly deplete plant biomass in ^{13}C, we had to raise the CO_2 concentration inside the greenhouse to 760 ppm. Raising the atmospheric CO_2 concentration could alter the rhizosphere effects on soil C decomposition compared to the ambient CO_2 concentration (Cheng, 1999; Allard et al., 2006). We observed some variation in plant biomass $\delta^{13}C$ among the different plant tissues, but using these different values for $\delta^{13}C_p$ in Eq. (1) did not alter our estimates of primed C. The soil type and water treatment effects that we observed in plant biomass $\delta^{13}C$ may have been a result of differences in WUE. Plants with the C3 photosynthetic pathway discriminate against $^{13}CO_2$, particularly when the leaf stomata are fully opened (Farquhar et al., 1989). Therefore, plants grown in less water stressed conditions tend to have lower WUEs and tend to be more depleted in ^{13}C. Surprisingly, the soil moisture treatment did not significantly affect shoot biomass $\delta^{13}C$, despite a significantly lower WUE in the wet soil than in the dry soils (averaged across plant species

and soil type). Differences in WUE may not have been strong enough to cause significant effects on shoot biomass $\delta^{13}C$. On the other hand, the more negative $\delta^{13}C$ of plant biomass in the clay loam soil than in the sandy loam soil may have been caused by a significantly lower WUE in the clay loam soil than in the sandy loam soil. The significant plant species effects on plant biomass $\delta^{13}C$ may be related to differences in plant traits such as leaf thickness and N content. Differences in $\delta^{13}C$ of up to 4‰ have been observed among plant species grown under similar environmental conditions (Körner et al., 1991).

5. Conclusions

We observed an acceleration of the priming effect (i.e., increase in soil C decomposition caused by the direct effect of living roots) with increased soil moisture content, particularly in a soil that contained less clay. Possibly, exudates responsible for increased microbial decomposition diffuse away from roots more effectively with increased soil moisture content and with decreased clay content. Once diffused away from roots, these exudates have less of a chance to be re-adsorbed by roots again, thereby being more successful in stimulating microbial decomposition. While it has become clear that rhizosphere processes can cause significant effects on soil C decomposition, our results indicate that soil moisture content and soil type can substantially alter these effects.

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