Soil Biology & Biochemistry 69 (2014) 147–156

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Differential priming of soil carbon driven by soil depth and root impacts on carbon availability

Marie-Anne de Graaff [a,*], Julie D. Jastrow [b], Shay Gillette [a], Aislinn Johns [a], Stan D. Wullschleger [c]

[a] Department of Biological Sciences, Boise State University, 1910 University Drive, Boise, ID 83725-1515, United States
[b] Biosciences Division, Argonne National Laboratory, Argonne, IL 60439, United States
[c] Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States

ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form
28 October 2013
Accepted 29 October 2013
Available online 15 November 2013

Keywords:
Switchgrass cultivars
Specific root length
Soil depth
Decomposition
Priming
Carbon-13

ABSTRACT

Enhanced root-exudate inputs can stimulate decomposition of soil carbon (C) by priming soil microbial activity, but the mechanisms controlling the magnitude and direction of the priming effect remain poorly understood. With this study we evaluated how differences in soil C availability affect the impact of simulated root exudate inputs on priming. We conducted a 60-day laboratory incubation with soils collected (60 cm depth) from under six switchgrass (*Panicum virgatum*) cultivars. Differences in specific root length (SRL) among cultivars were expected to result in small differences in soil C inputs and thereby create small differences in the availability of recent labile soil C; whereas soil depth was expected to create large overall differences in soil C availability. Soil cores from under each cultivar (roots removed) were divided into depth increments of 0–10, 20–30, and 40–60 cm and incubated with addition of either: (1) water or (2) ^{13}C-labeled synthetic root exudates (0.7 mg C/g soil). We measured CO_2 respiration throughout the experiment. The natural difference in ^{13}C signature between C_3 soils and C_4 plants was used to quantify cultivar-induced differences in soil C availability. Amendment with ^{13}C-labeled synthetic root-exudate enabled evaluation of SOC priming. Our experiment produced three main results: (1) switchgrass cultivars differentially influenced soil C availability across the soil profile; (2) small differences in soil C availability derived from recent root C inputs did not affect the impact of exudate-C additions on priming; but (3) priming was greater in soils from shallow depths (relatively high total soil C and high ratio of labile-to-stable C) compared to soils from deep depths (relatively low total soil C and low ratio of labile-to-stable C). These findings suggest that the magnitude of the priming effect is affected, in part, by the ratio of root exudate C inputs to total soil C and that the impact of changes in exudate inputs on the priming of SOC is regulated differently in surface soil compared to subsoil.

1. Introduction

Root derived soil carbon (C) inputs, the soil microbial community, and the soil C cycle are intrinsically linked, because roots supply soil microbes with highly assimilable C-rich substrates that drive microbial decomposition processes (Lynch and Whipps, 1990; Kong and Six, 2010). As such, roots exert significant control on the rate at which C cycles between plants, soils and the atmosphere, making them an important component of the global C cycle (Norby and Jackson, 2000; Lal, 2004). Environmental perturbations, such as climate change and increasing atmospheric CO_2 concentrations can change the relative allocation of C to belowground organs in plants, and increase the production of fine roots (Meier and Leuschner, 2008; Iversen et al., 2008; Iversen, 2009; Phillips et al., 2011). Such changes enhance root-derived soil C input, and the availability of C to soil microbes (Drake et al., 2011; Carillo et al., 2011; Phillips et al., 2009, 2011, 2012). Despite a general consensus that root-derived C inputs are a key component of the soil C cycle, the effect of changes in root-derived C release on soil C cycling remains highly uncertain.

Greater root C inputs to soil, for example as a result of elevated atmospheric CO_2 concentrations, may not lead to measurable increases in soil C (Langley et al., 2009; van Kessel et al., 2006), although increased soil C sequestration has been observed in some cases (Jastrow et al., 2005; Hoosbeek and Scarascia-Mugnozza, 2009). One explanation for a lack of soil C accrual in response to greater root C inputs, is that increased C inputs can promote soil organic carbon (SOC) turnover rates (Hoosbeek et al., 2004; Phillips

* Corresponding author.
E-mail address: Marie-annedegraaff@boisestate.edu (M.-A. de Graaff).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.10.047

et al., 2012) via the priming effect (Kuzyakov et al., 2000). Priming is defined as the stimulation of microbial activity and overall decomposition rates by the addition of labile plant-derived substrates (Dalenberg and Jager, 1989; Kuzyakov et al., 2000). Indeed, increases in root-C inputs, such as observed under elevated atmospheric CO_2 concentrations, are predominantly driven by increased input of labile plant-derived substrates such as root exudates (i.e., the passive continuous release of low molecular weight organic compounds from living roots [Merckx et al., 1986]) and root turnover (Drake et al., 2011; Carillo et al., 2011; Phillips et al., 2009, 2011, 2012). This C is a preferred "food-source" for soil microbes and can stimulate microbial decomposition processes (Joslin et al., 2006; Rasse et al., 2005), including decomposition of native SOC (Phillips et al., 2012). A priming effect can be positive (increase in SOC decomposition) or negative (slowdown of SOC decomposition) and can vary in magnitude from very small to rather large (Cheng et al., 2003; Cheng and Kuzyakov, 2005; Hamer and Marschner, 2005; Blagodatskaya et al., 2007; De Graaff et al., 2010). Although priming appears to mediate climate change impacts on ecosystem C dynamics in important ways, the direction and magnitude of priming effects are highly variable among experiments, and the mechanisms responsible for these diverse responses are uncertain.

The variability in magnitude and direction of the priming effect observed in different experiments is likely caused by a myriad of factors, including the quantity and quality of short-term substrate input to soil, the active microbial community, C quality and availability in soil, and interactions among all of these factors (Hamer and Marschner, 2002; Fierer et al., 2003; Fontaine et al., 2003; De Graaff et al., 2010; Salome et al., 2010). Root characteristics that control the rate and quality of root-C input to soil over longer time periods may be key factors in regulating how short-term increases in exudation impact the magnitude and direction of SOC priming. Namely, in the long-term roots regulate C quality and availability across the soil profile through: (1) rooting depth, which controls soil C availability at corresponding depths, and (2) root branching, because increased root branching increases root exudation rates (Groleau-Renaud et al., 1998) and may promote fine root turnover rates (Guo et al., 2008b) thereby increasing soil C availability. Thus, root system characteristics including rooting depth and root architecture may be important traits that control soil C availability, the soil microbial community, and the response of SOC cycling to climate-induced changes in rhizodeposition.

With this work, we evaluated how subtle versus large differences in soil C availability, as induced by differences in root architecture and rooting depth, affect microbial processing of simulated root exudate inputs and the decomposition of SOC via the priming effect. To create subtle differences in soil C availability, we collected soil from beneath monocultures of six *Panicum virgatum* (hereafter: switchgrass) cultivars with a wide range of root architectures (De Graaff et al., 2013) as defined by specific root length (SRL). In addition, by sampling to a depth of 60 cm below each cultivar, we obtained soils at different depth increments with stark differences in C availability. These differences were due to a significant decline with depth in the abundance of roots and, thus, root-C inputs under switchgrass and throughout the site's preceding land-uses. We hypothesized that exudate-C inputs would have a greater effect on SOC decomposition (1) at greater depth and (2) in soil from cultivars with coarser root systems. In both cases, we inferred that a decrease in particulate or otherwise uncomplexed organic matter and microbial biomass resulting from a decline in root mass with depth (Fierer et al., 2003; Fontaine et al., 2007) would limit C availability to soil microorganisms and, therefore, that the addition of C-rich substrates would lead to greater increases in microbial biomass, microbial activity and SOC decomposition.

To evaluate how differences in soil C availability, as induced by differences in root architecture and soil depth, affect SOC decomposition following an increase in exudation, we applied a ^{13}C-labeled synthetic exudate cocktail to soils collected from beneath the six switchgrass cultivars. Our previous work has shown that the switchgrass cultivars used for this study have different root architectures to a depth of 15 cm, where three of the cultivars have coarse root systems, whereas the other three have finely branched root systems (De Graaff et al., 2013). The switchgrass cultivars (C_4 plants) were established on soils that supported a stand of C_3 grasses for 36 years, which enabled us to use the natural abundance C isotope ratio technique to (1) estimate the contribution of new root-derived C to the available SOC pool, (2) test whether differences in the architecture of the root systems were accompanied by differences in soil C availability, and (3) evaluate how differences in C availability among cultivar-soils and soil depth affect microbial processing of simulated root exudates and decomposition of SOC.

2. Materials and methods

2.1. Sample collection

We collected soils in October 2010 from field plots supporting six switchgrass cultivars grown as monocultures at the Fermilab National Environmental Research Park in Batavia, IL. The experimental field plots in this research facility were established in June 2008 on Grays silt loam (fine-silty, mixed, superactive, mesic Mollic Oxyaquic Hapludalfs) that previously supported a stand of perennial, cool-season Eurasian pasture grasses for 36 years. In the experimental area, switchgrass cultivars originating from different latitudes were grown in 2 × 3 m or 2 × 1 m replicated field plots ($n = 4$). The cultivars were (1) Alamo, (2) Kanlow, (3) Carthage, (4) Cave-in-Rock, (5) Forestburg, and (6) Blackwell. A single soil core (4.8-cm diameter) was collected to a depth of 60 cm from on top of the crown of one individual in each of the four replicate plots of each cultivar (total cores = 24).

Upon collection, the cores were divided into six depth increments of 10 cm. For the incubation study we used the following depth increments: 0–10 cm, 20–30 cm and 40–60 cm. These depth increments were selected for the incubation study because of their observed general correspondence to three soil horizons. On the basis of the United States Department of Agriculture, Natural Resources Conservation Service (NRCS) official soil series description for Grays silt loam (https://soilseries.sc.egov.usda.gov/OSD_Docs/G/GRAYS.html), the 0–10 cm depth increment lies within the Ap horizon (0–20 cm depth; silt loam), the 20–30 cm depth increment roughly corresponds to the BE horizon (20–28 cm depth; silt loam), and the 40–60 cm increment occurs within the Bt horizon (28–86 cm depth; silty clay loam). Representative distributions of sand-, silt- and clay-sized particles given in the NRCS Web Soil Survey, Soil Report (http://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm) for the Grays soil series vary minimally across these horizons: 9%, 70%, and 21% (Ap), 9%, 71% and 20% (BE), and 9%, 61% and 30% (Bt). Measured dry-combustion soil C concentrations (mean ± SE, $n = 6$) for each depth increment were: 2.6% ± 0.1 (0–10 cm), 1.3% ± 0.1 (20–30 cm), and 0.6% ± 0.03 (40–60 cm).

The soil cores were shipped to Boise State University and kept at 6 °C until further processing. The field-moist soils were sieved (2 mm), rhizomes were removed, and all visible roots were hand-picked from the soil. Soils were kept at 6 °C until further analyses. A subsample of sieved, root-free soil was dried (100 °C for 48 h), ground to a fine powder using a ball mill, and analyzed for total C, N and stable C isotope ratios ($^{13}C/^{12}C$) using a Thermo Delta V Plus isotope ratio mass spectrometer (IRMS) coupled with a Costech Elemental Analyzer in continuous flow mode. Analysis of root samples for C and N was conducted previously (De Graaff et al., 2013).

2.2. Root architecture analysis

The roots of each cultivar ($n = 4$) were analyzed for root diameter size class distribution using WinRhizo (Regent Instruments, Inc.: http://www.regentinstruments.com/products/rhizo/Rhizo.html). Roots were washed in deionized water and floated in acrylic trays (20 × 25 cm) on a flatbed scanner. The scanner was acquired from Regent Instruments and allows the roots to be lit from above and below while being scanned, thereby reducing shadows on the root image. Roots were arranged using plastic forceps and care was taken that roots did not overlap. Roots were scanned at a resolution of 200 dpi and the threshold for root diameter size class separation was set at 0.5 mm. Following scanning, the roots were dried (70 °C) and weighed. Specific root length was determined by dividing total root length by the total weight of the roots within each depth increment.

2.3. Synthetic root exudate solution

We prepared an exudate solution that mimicked observed root exudate C-release by including the most common C compounds found in root exudates. Each compound was purchased at 99.9 atom% ^{13}C and diluted with its unlabeled equivalent to achieve a final ^{13}C atom percent enrichment of 12 atom% ^{13}C. The relative concentrations of sugars, organic acids, and amino acids were kept constant and reflect ratios observed in the field (i.e. ~60% sugars, ~35% organic acids and ~2% amino acids) (Kraffczyk et al., 1984; Hutsch et al., 2002). The stock solution for this exudate "cocktail" contained: D-Glucose (28 mg ml^{-1}), D-fructose (9.4 mg ml^{-1}), D-xylose (8 mg ml^{-1}), oxalate (7.8 mg ml^{-1}), fumarate (14.6 mg ml^{-1}), succinate (1.2 mg ml^{-1}), acetate (4.0 mg ml^{-1}), L-proline (0.1 mg ml^{-1}), L-arginine (0.1 mg ml^{-1}), L-glycine (0.1 mg ml^{-1}), L-serine (0.1 mg ml^{-1}), and glutamate (0.3 mg ml^{-1}) (De Graaff et al., 2010). We added 0.7 mg of exudate-C per gram of soil, which amounted to a ratio of exudate C (mg) per unit of total soil C (mg) of 0.027 in soils from the 0 to 10 cm depth increment, 0.056 in soils from the 20 to 30 cm depth increment and 0.12 in soils from the 40 to 60 cm depth increment.

2.4. Incubation design

Subsamples of sieved soil from each core (equivalent to 60 g dry weight) were incubated at 20 °C in one of two treatments: (1) without artificial exudates (i.e., water only), and (2) with artificial root exudates. The water-only treatment was included to: (1) assess how cultivar impacts soil C availability by partitioning between C_4- and C_3-derived CO_2 respiration, and (2) quantify CO_2 evolution and its isotopic signature (i.e. a mixture of C_3 and C_4 $\delta^{13}C$ signatures) from the preexisting soil C, which is needed for further stable isotope calculations (i.e., calculation of exudate C derived respiration and the priming of SOC) on CO_2 samples derived from soils that received the artificial root exudates. The water-holding capacity (WHC) of the soils was determined by calculating the difference in soil weight at the water saturation point and the oven-dry weight (100 °C). Either water or the exudate solution was added to obtain 60% of WHC. Prepared soils were placed in specimen cups inside one-liter mason jars and 5 ml of water was added to the bottom of the jar to maintain humidity.

2.5. Microbial respiration

Soil CO_2 evolution was measured at days: 1, 2, 3, 5, 7, 15, 30 and 60. Headspace air samples (10 ml) were removed with a syringe through a septum in the lid of each mason jar. Blank jars without soil (three) were included to determine the background concentration and stable-isotope composition of headspace CO_2. Headspace gas samples were collected in 12 ml Exetainers (Labco Limited, Buckinghamshire, UK). Following each headspace sampling, the mason jars were opened, the soils were removed, and the jars were flushed with air for 30 min. Gas samples were analyzed for CO_2 and its ^{13}C signature with a continuous flow, isotope mass spectrometer (PDZ Europa TGII trace gas analyzer coupled with a Geo 20–20 isotope ratio mass spectrometer, Cheshire UK). For the CO_2 analyses the highest available 'certified' ^{13}C standard IAEA 309B (+535.3‰ VPDB) was used. Carbon mineralization data were expressed on the basis of oven-dry (100 °C) soil mass. Results of the C-isotope analyses are expressed in atom% ^{13}C. The ^{13}C values were determined in relation to Vienna-Pee Dee Belemnite (PDB) as follows:

$$\delta^{13}C = \left(\left(R_{sample}/R_{standard}\right) - 1\right)*1000 \quad (1)$$

where R is the ratio of heavy (^{13}C):light (^{12}C) isotopes in either the standard or the sample. $R_{standard}$ (PDB) is: 0.011180.

Our experimental design allowed us to measure CO_2 respiration derived from a combination of: (1) SOC derived from previous land-use (hereafter, old-SOC) + switchgrass derived SOC (hereafter, SG-SOC) in soils receiving the water treatment only, and (2) old-SOC + SG-SOC + exudate-C in soils amended with the exudate solution. Then, we were able to partition respired CO_2 into: (1) SG-SOC in the soils receiving the water treatment only (Eq. (2)), and (2) exudate derived-C, and primed SOC in the soils receiving the exudate solution (Eqs. (3)–(7)).

To quantify CO_2-C respiration that was derived from SG-SOC during the incubation, the following mass balance was used (Cheng, 1996):

$$Q_{SG} = Q_t*(\delta^{13}C_t - \delta^{13}C_s)/(\delta^{13}C_{SG} - \delta^{13}C_s) \quad (2)$$

where Q_t is the total amount of CO_2-C, $\delta^{13}C_t$ is its isotopic composition, Q_{SG} is the amount of CO_2-C derived from SG-SOC, $\delta^{13}C_{SG}$ is the isotopic composition of switchgrass plant material (−12.4‰ ± 0.21) (De Graaff et al., 2013), and $\delta^{13}C_s$ is the isotopic composition of original brome soils adjacent to the switchgrass field site (−24.09‰ ± 0.33, −17.26‰ ± 0.33, and −16.68‰ ± 0.77 ($n = 6$) for the 0–10 cm, 20–30 cm, and 40–60 cm depth increments, respectively). The relatively higher $\delta^{13}C$ values at greater depth result from C inputs derived from the original native prairie, which included C_4 grasses, and many years of corn (C_4) cropping before land-use conversion to cool-season C_3 grassland.

The CO_2-C derived from exudate C ($Q_{exudate}$) during the incubation and the priming effect of SOC in soils supplied with the exudate C was quantified using the following mass balance (Denef et al., 2001; De Graaff et al., 2013):

$$Q_{sb} = Q_{control} - Q_{bla} \quad (3)$$

$$\delta^{13}C_{sb} = (Q_{control}*\delta^{13}C_{control}) - ((Q_{bla}*\delta^{13}C_{bla})/Q_{sb}) \quad (4)$$

$$Q_{sc} = ((Q_{tc}*(\delta^{13}C_{tc} - \delta^{13}C_{exudate})) - (Q_{bla}*(\delta^{13}C_{bla} - \delta^{13}C_{exudate})))/(\delta^{13}C_{sb} - \delta^{13}C_{exudate}) \quad (5)$$

$$Q_{exudate} = Q_{tc} - Q_{sc} - Q_{bla} \quad (6)$$

$$\text{Priming} = Q_{sc} - Q_{sb} \quad (7)$$

where $Q_{control}$ is the measured total amount of C respired from the control soil (i.e. soil with water added only) including the blanks,

and $\delta^{13}C_{control}$ is its measured isotopic composition; Q_{bla} is the total amount of measured C respired from the blank jars, and $\delta^{13}C_{bla}$ is its measured isotopic composition; Q_{tc} is the measured total amount of C respired from soils amended with the exudate solution and including the blanks, and $\delta^{13}C_{tc}$ is its measured isotopic composition; Q_{sb} is the calculated total amount of C respired from the control soil minus the C derived from the blanks (i.e. no soil) (equation (3)) and $\delta^{13}C_{sb}$ is its calculated isotopic composition (equation (4)); Q_{sc} is the calculated total amount of C respired from soils amended with the exudate solution minus the blanks and the exudate C (equation (5)); $Q_{exudate}$ is the total amount of C respired from the exudate solution, and $\delta^{13}C_{exudate}$ is its isotopic composition (equation (6)). The priming effect of SOC was quantified for each treatment and cultivar combination by subtracting Q_{sb} from Q_{sc} (equation (7)).

2.6. Statistical analyses

To evaluate differences among cultivars and soil depths in SRL and CO_2 respiration rates, two-way analysis of variance (ANOVA) was conducted with treatments (i.e., cultivar and soil depths) as fixed effects, using GLM in SPSS Statistics 17.0. In addition, to assess differences among cultivars, one-way ANOVA comparisons were made using the Univariate GLM in SPSS Statistics 17.0 for each of the soil depth increments, and means were compared by the Tukey test after confirmation that the ANOVA was significant. We used regression analyses to relate SRL to CO_2 respiration rates at each depth and at each time point during the incubation. To assess if the differences among cultivars in SRL and CO_2 respiration were consistent across the depth increments, we conducted a multivariate Profile Analysis, using the GLM-repeated measures function in SPSS. When the assumption of sphericity was met, we assessed parallelism using the Pillai's Trace, Wilks' Lambda, Hotelling's Trace and Roy's Largest Root tests, and when the assumption of sphericity was not met, we used the Greenhouse-Geisser test. The level of significance for all statistical tests was $P \leq 0.05$.

3. Results

3.1. Differences in specific root length and root-derived CO_2 respiration among cultivars

Standing root biomass was equivalent for all cultivars within each depth, although root biomass declined significantly with depth (Table 1). Specific root length differed significantly among cultivars ($P < 0.001$), and the relative differences among cultivars were consistent across the depth increments to a soil depth of 40 cm (Fig. 1a–d), although there were some inconsistencies in the upper 30 cm. Specifically, Kanlow and Alamo had a lower SRL to a depth of 20 cm relative to Forestburg (0–10 cm depth) and Carthage (0–20 cm depth) (Fig. 1a,b). In addition, Kanlow continued to have a significantly lower specific root length to a depth of 40 cm compared to Carthage (Fig. 1c,d). Specific root length of Cave-in-Rock and Blackwell did not significantly differ from the other cultivars at each of the depth increments (Fig. 1a–d). The patterns persisted past a depth of 40 cm depth, but at those depths differences were not significant at the $P \leq 0.05$ level (Fig. 1e,f).

The cultivars differentially affected soil C availability, which is revealed by differences in SG-SOC CO_2 respiration rates from the different cultivar soils to a depth of 10 cm (Fig. 2). Cultivars may have also differentially affected the availability of SOC at greater depths, as would be suggested by differences among cultivars in SG-SOC + old-SOC respiration rates of soils from depths of 20–30 cm and 40–60 cm (Table 2). However, the $\delta^{13}C$ signal of SG-SOC inputs at the 20–30 cm and 40–60 cm depths was insufficiently different from that of the control soils to partition between SG-SOC and old-SOC respiration at those depths. To ensure that observed differences among cultivars in SG-SOC + old-SOC derived CO_2 respiration rates were not the result of pre-existing differences in soil C stocks among soils, we normalized the CO_2 respiration data on the basis of soil C (Table 2). Using multivariate Profile Analysis, we confirmed that the observed pattern of relative differences among cultivars were consistent at each depth increment whether normalized or not normalized for soil C stocks. Upon regression analysis, however, we found that differences in CO_2 respiration rates derived from SG-SOC or from SG-SOC + old-SOC were not related to standing root biomass or to differences in SRL among the cultivars (data not shown).

3.2. Cultivar impacts on microbial processing of exudates and priming of SOC

The addition of ^{13}C-labeled artificial exudates to soils enhanced microbial CO_2 respiration rates, but the magnitude of increase differed among cultivars across the three soil depths (Fig. 3a–c). Specifically, on day three of the incubation, CO_2 respired from soils amended with the exudate cocktail differed significantly among cultivars across each of the soil depths, with more total CO_2 (i.e., CO_2 derived from SG-SOC + old-SOC + exudate-C) respired from Blackwell compared to Alamo soils (Fig. 3a–c). In addition, cultivar affected total respiration rates on days 5 and 7 in soils from depths of 20–30 cm and 40–60 cm, with more CO_2 respired from Cave-in-Rock and Blackwell soils, compared to Alamo soils (Fig. 3b,c).

Using the ^{13}C-enrichment of the added solution, we were able to partition between primed SOC and exudate-derived C respiration, which allowed us to calculate microbial processing of the exudate-derived C (Fig. 3d–f). We found that on day three in soils from a depth of 20–30 cm and 40–60 cm, cultivar significantly impacted the rate of exudate-derived C decomposition, with more exudate-derived CO_2 respired from Cave-in-Rock, Blackwell and Kanlow soils, compared to Alamo soils (Fig. 3e,f). In addition, we were able to assess whether cultivar mediated the impact of exudate additions on priming of SOC. In general the impact of cultivar on priming was marginal (Fig. 3g–h). On day three of the incubation, priming of SOC was affected by cultivar in soils from a depth of 20–30 cm with Alamo being the only cultivar that caused negative priming (Fig. 3h). In soils from a depth of 40–60 cm, priming was

Table 1
Root biomass (g dry weight) of six different switchgrass cultivars across 6 soil depth increments. Values are means ± SE ($n = 4$).

Standing root biomass (g dry weight)						
Cultivar	0–10 cm	0–20 cm	20–30 cm	30–40 cm	40–50 cm	50–60 cm
Alamo	0.58 ± 0.12	0.36 ± 0.14	0.11 ± 0.02	0.12 ± 0.04	0.08 ± 0.02	0.06 ± 0.01
Blackwell	0.36 ± 0.03	0.21 ± 0.04	0.18 ± 0.04	0.09 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
Carthage	0.51 ± 0.11	0.16 ± 0.06	0.15 ± 0.02	0.12 ± 0.03	0.53 ± 0.45	0.07 ± 0.02
Forestburg	0.33 ± 0.03	0.22 ± 0.06	0.10 ± 0.03	0.09 ± 0.007	0.08 ± 0.02	0.06 ± 0.02
Kanlow	0.50 ± 0.07	0.45 ± 0.07	0.26 ± 0.09	0.20 ± 0.11	0.12 ± 0.03	0.09 ± 0.04
Cave-in-rock	0.53 ± 0.07	0.35 ± 0.09	0.16 ± 0.04	0.14 ± 0.02	0.11 ± 0.01	0.07 ± 0.01

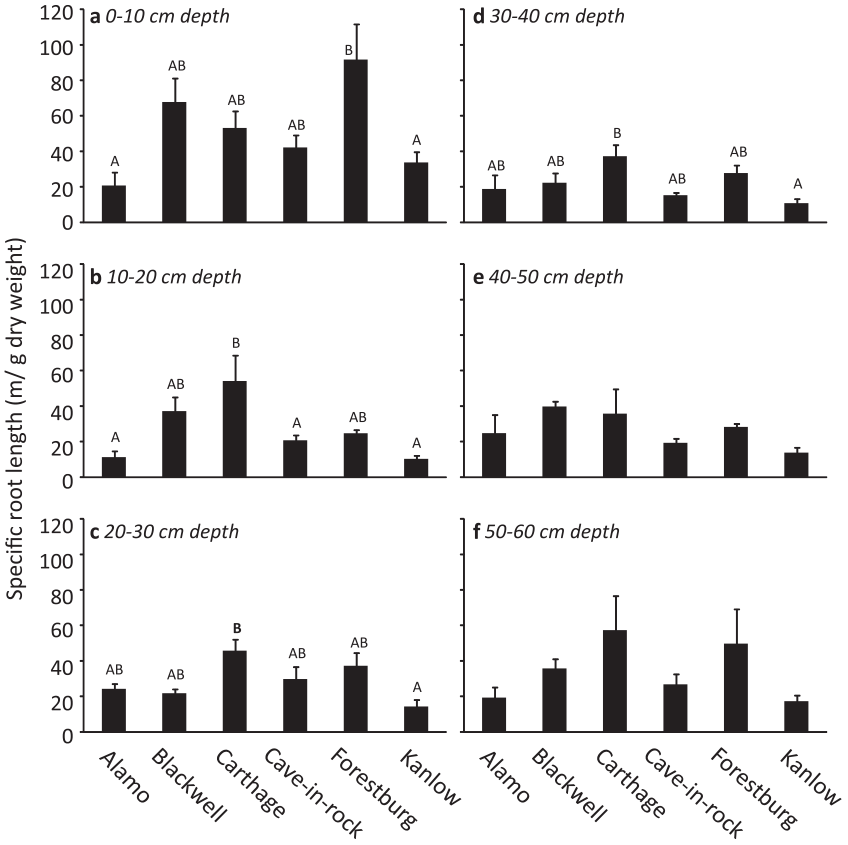

Fig. 1. Specific root lengths (m/g DW) of six different switchgrass cultivars across 6 soil depth increments. Values are means ± SE ($n = 4$). Different letters indicate significant differences in SRL among different cultivars at each depth.

affected by cultivar on day one, but not on any other days (Fig. 3i). Nevertheless, upon regression analysis, differences in the processing of exudate-derived C addition were not related to differences in SRL or root biomass among the cultivars (data not shown).

3.3. Soil depth impacts on microbial processing of exudate-C inputs and priming of SOC

Addition of exudate-C to the soils led to a significant increase in respiration rates for all depths, and this increase was significantly greater on day one for soils from 0 to 10 cm depth, than for soils from greater depths (Fig. 3a–c). On day three, however, total respiration rates were similar for soils from a depth of 0–10 cm and 20–30 cm (Fig. 3a–c). The difference in exudate impacts on soil C respiration rates among soils from different depths, was the result of differential respiration of the exudate C by microbes; in soils from a depth of 0–10 cm microbes respired the majority of exudate C on day one of the incubation (Fig. 4a), whereas microbes in soils from greater depths respired greater amounts of exudate C on subsequent days of the incubation (Fig. 4a).

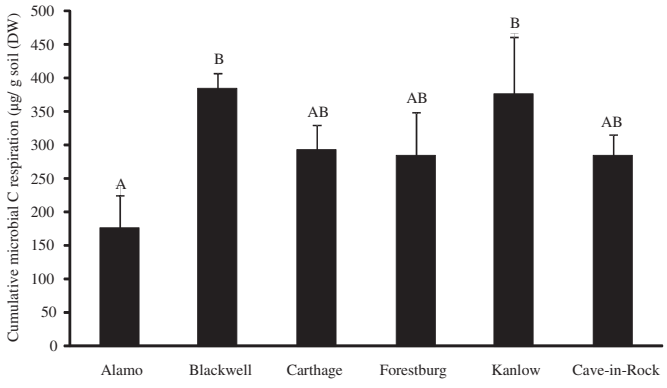

Fig. 2. Switchgrass-SOC derived microbial CO_2 respiration rates from soils previously planted with six switchgrass cultivars to a depth of 10 cm. These soils received the water treatment only. Values are means ± SE ($n = 4$). Different letters indicate significant differences in SG-SOC derived CO_2 respiration among soils from different cultivars.

Although the absolute amount of exudate-C respiration was greater on incubation day one of soils from a depth of 0–10 cm relative to those from other depths, when normalized for differences in total C respiration rates among depths (i.e., exudate-derived C respiration relative to total respiration for a particular depth and day), the percent exudate-derived C released from the 0 to 10 cm soils, was significantly smaller than the percent exudate-C released from deeper soils (Fig. 4b), indicating preferential use of exudate-C by microbes in soils from greater depths. This finding is supported by differences in the magnitude of cumulative exudate-C respiration among soil depths, where the absolute amount of exudate-C release was greater in soils from depths of 20–30 cm and 40–60 cm, compared to those from 0 to 10 cm (Fig. 4c). In addition, cumulative exudate-C respired as a percent of total C respiration, increased with increasing depth of soil origin (Fig. 4d).

The impact of exudate-C inputs on priming of SOC was also strongly regulated by soil depth. On day one of the incubation, addition of exudate-C induced strong negative priming effects (i.e., suppression of SOC-derived respiration) in soils from depths of 20–30 cm and 40–60 cm, whereas there was no impact on SOC decomposition in soils from a depth of 0–10 cm (Fig. 5a). Positive priming was induced by exudate C addition for all depth

Table 2
Cumulative microbial SG-SOC + old-SOC derived CO_2 respiration for treatments receiving water only normalized for soil weight and for initial differences in soil C for soils previously planted with six switchgrass cultivars at three depths (0–10 cm; 10–20 cm; 40–60 cm). Values are means ± SE ($n = 4$). Different letters indicate significant differences in respiration among soils from different cultivars.

	CO_2 respiration (mg/g soil)			CO_2 respiration normalized for soil C (mg CO_2/mg C)		
Cultivar	0–10 cm	20–30 cm	40–60 cm	0–10 cm	20–30 cm	40–60 cm
Alamo	$0.88^{a} \pm 0.07$	$0.32^{a} \pm 0.02$	$0.19^{ab} \pm 0.03$	$0.037^{a} \pm 0.002$	$0.030^{ab} \pm 0.003$	$0.036^{a} \pm 0.005$
Blackwell	$0.99^{a} \pm 0.05$	$0.31^{a} \pm 0.03$	$0.22^{ab} \pm 0.02$	$0.035^{a} \pm 0.002$	$0.026^{ab} \pm 0.007$	$0.034^{a} \pm 0.002$
Carthage	$1.00^{a} \pm 0.05$	$0.35^{a} \pm 0.04$	$0.18^{ab} \pm 0.01$	$0.044^{a} \pm 0.003$	$0.032^{b} \pm 0.001$	$0.036^{a} \pm 0.001$
Forestburg	$0.87^{a} \pm 0.03$	$0.27^{a} \pm 0.03$	$0.14^{a} \pm 0.01$	$0.036^{a} \pm 0.007$	$0.023^{a} \pm 0.002$	$0.027^{a} \pm 0.004$
Kanlow	$1.00^{a} \pm 0.07$	$0.30^{a} \pm 0.02$	$0.15^{ab} \pm 0.01$	$0.040^{a} \pm 0.005$	$0.026^{ab} \pm 0.002$	$0.030^{a} \pm 0.004$
Cave-in-rock	$0.91^{a} \pm 0.05$	$0.38^{a} \pm 0.05$	$0.23^{b} \pm 0.02$	$0.030^{a} \pm 0.002$	$0.022^{a} \pm 0.002$	$0.032^{a} \pm 0.002$

increments on days five and seven of the incubation (Fig. 5a). Ultimately, cumulative SOC decomposition in response to exudate additions, was much greater in soils from a depth of 0–10 cm, and became progressively smaller in soils from greater depths whether calculated in absolute values, or as percentage of total C respired (Fig. 5b). By day 15 of the incubation, the ^{13}C exudates had been largely respired or allocated to microbial biomass and enzymes, hence we show the impact of ^{13}C exudates additions on SOC decomposition for the first 15 days of the incubation only.

4. Discussion

Our experiment led to three main findings: (1) root morphology, soil C availability, and microbial processing of simulated root exudation varied among different cultivars of switchgrass across the soil profile, and these differences among the cultivars were greater in soils from deeper than from shallower depths; (2) small differences in C availability among the soils derived from different cultivars did not significantly mediate the impact of exudate additions on priming; but (3) large differences in available soil C, associated differences in microbial biomass C and microbial demand for C among soils from different depths led to differences in priming effects, where greater cumulative priming effects were observed for shallow-relative to deep-origin soils. These results suggest that genetic variation within plant species, as captured across the six cultivars examined here, can modify belowground C cycling processes and indicate that changes in root exudate inputs might alter SOC decomposition differently across the soil profile.

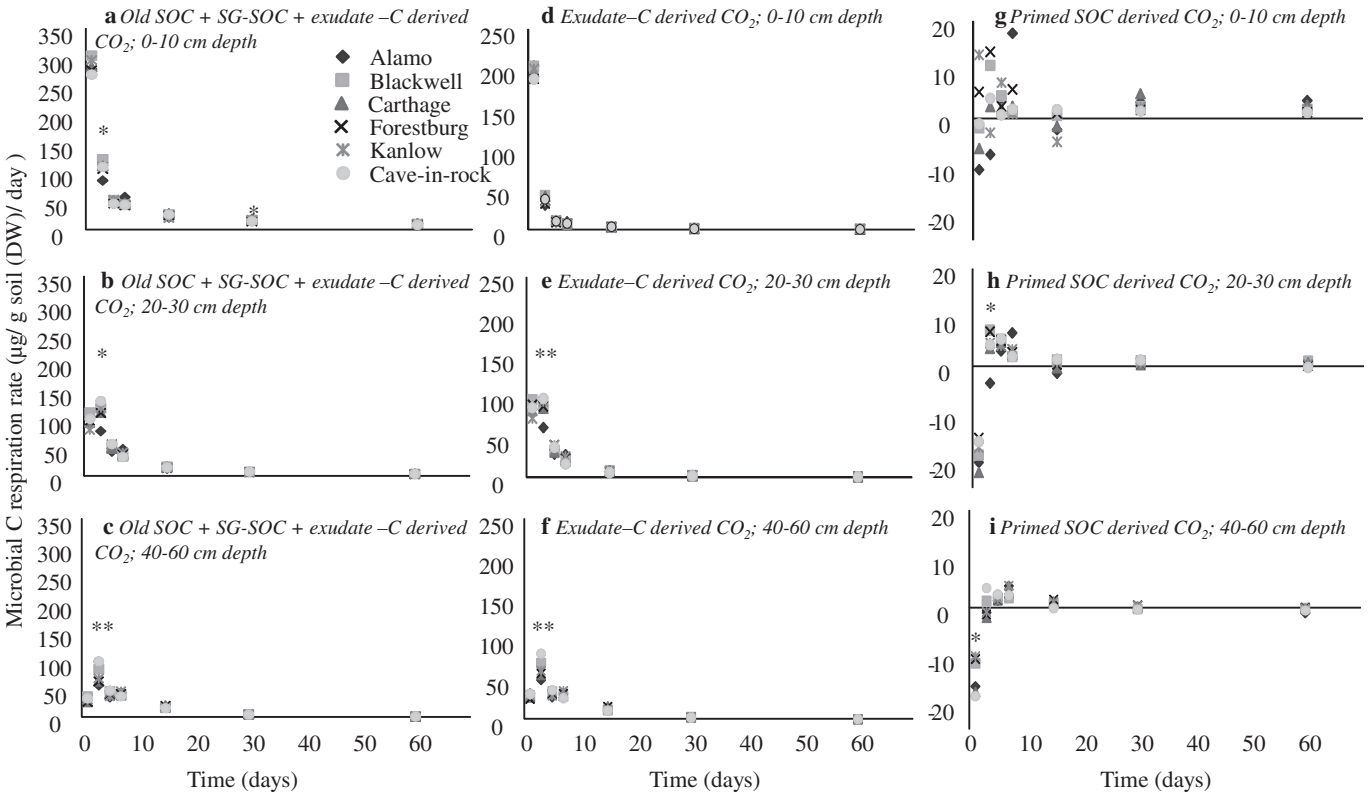

Fig. 3. Microbial CO_2 respiration rates from soils amended with ^{13}C labeled synthetic exudates during a 60-day incubation. The soils were previously planted with six switchgrass cultivars and were derived from three different depths. (a, b, c) measured old-SOC + SG-SOC + exudate-C derived CO_2 respiration rates at a depth of 0–10 cm, 20–30 cm and 40–60 cm, respectively; (d, e, f) calculated exudate C-derived CO_2 respiration rates at a depth of 0–10 cm, 20–30 cm and 40–60 cm, respectively (g, h, i) calculated primed SOC-derived CO_2 respiration rates at a depth of 0–10 cm, 20–30 cm and 40–60 cm, respectively. Values are means of the cultivars, error bars are not shown, one asterisk positioned above a group of data points at a particular day in the incubation indicates a difference among cultivars at the $P \leq 0.05$ and two asterisks indicate a difference at the $P \leq 0.01$ level ($n = 4$).

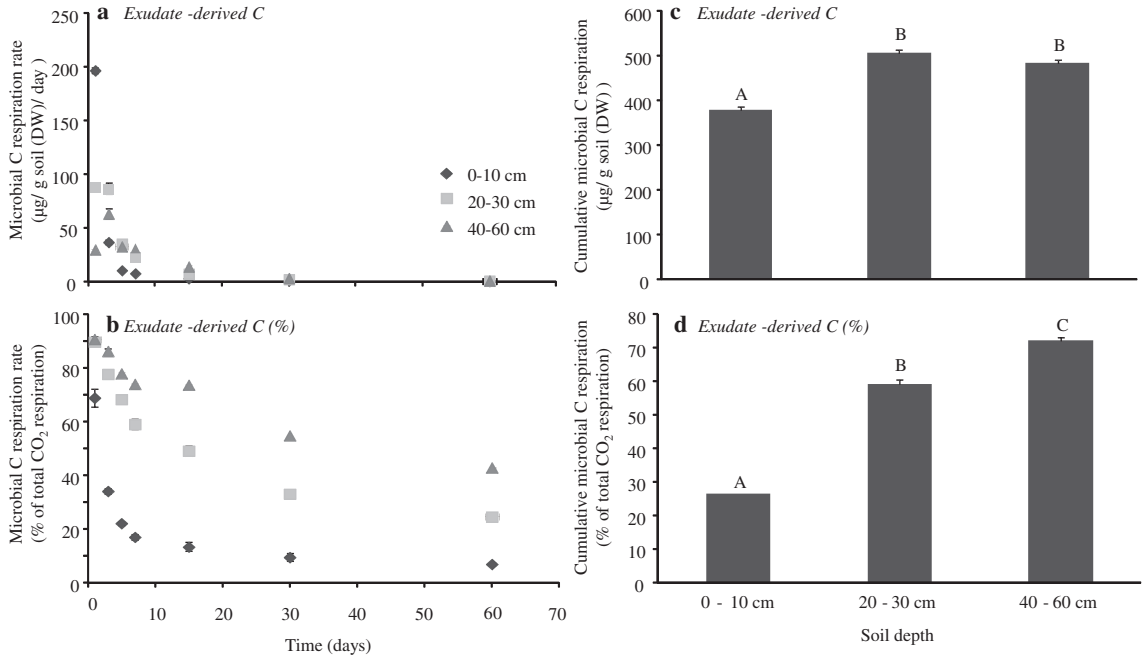

Fig. 4. Microbial CO_2 respiration rates derived from soils amended with a ^{13}C labeled synthetic exudate solution. Respiration rates are averaged across soils derived from six switchgrass cultivars and were calculated at three soil depths: (a) exudate-derived C respiration rates; (b) exudate derived C respiration rates as a percent of total (i.e. soil + exudate derived respiration) CO_2 respiration rates; (c) cumulative exudate-derived CO_2 respiration; (d) cumulative exudate-derived CO_2 respiration as a percent of cumulative total CO_2 respiration. Values are means of the cultivars ± SE ($n = 6$). Different letters indicate significant differences in respiration among soil depths.

4.1. Differences in specific root length and root-derived CO_2 respiration among cultivars

We have reported previously that the six switchgrass cultivars used in this study vary in SRL to a depth of 15 cm (De Graaff et al., 2013), and this study reveals that the differences in SRL persist to a depth of 60 cm. These differences may lead to differential soil C inputs and availability across the soil profile, because SRL, or the relative abundance of roots with very fine diameters in root systems (i.e. <0.5 cm), in-part controls root turnover (Guo et al., 2008a,b; Wells and Eissenstat, 2001), root decomposition (Fan and Guo, 2010; De Graaff et al., 2013) and root exudation rates (Groleau-Renaud et al., 1998). Using the natural difference in isotopic signature between C_4 plants and C_3 soils, we confirmed that the cultivars differentially affected soil C availability (assessed by microbial respiration rates in 60-day incubations) to a depth of 10 cm, which is likely the result of differential soil C inputs. Cultivars may have also differentially affected the availability of soil C at greater depths, but the $\delta^{13}C$ signal of root-derived C inputs at those depths was insufficiently different from that of the control soils to partition between SG-SOC and old-SOC derived CO_2 respiration. This lack of a significant switchgrass-derived signal was likely due to less biomass production at greater depths, and the fact that switchgrass had occupied the site for only 3 years at the time of sampling.

The sources of plant C inputs that lead to differences in soil C availability are uncertain, but we propose that differences in root exudation rates and sloughed cells among cultivars may be largely responsible for the observed variation in this study. Roots of C_4 prairie grasses, such as switchgrass, have a residence time of about 4 years (Weaver and Darland, 1949; Dahlman and Kucera, 1965), and at the time of sampling our plants were completing only their third growing season. Thus labile C input through root turnover was likely to have been relatively small. In addition, switchgrass-derived C in the soil appeared to be highly labile as the majority of switchgrass-derived C was respired during the first 15 days of the incubation experiment, suggesting a large proportion of this C was derived from recent rhizodeposits. Our measurements, however, failed to correlate SRL to soil C availability, suggesting that either SRL in our study cannot be linked to root-C input or that biophysical processes over time have uncoupled the link between SRL, root C input to soil, and soil C respiration rates. For example, we sampled in the fall, when photosynthetic and root exudation rates were likely diminished. Root exudates cycle through the microbial biomass quickly. Thus, missing peak root exudation rates, might explain the poor link between SRL and available soil C. In addition, SRL impacts on soil C availability may become more pronounced after the turnover of switchgrass roots increases over a greater number of growing seasons. To better understand the relationship between SRL, root C input to soil, and soil C availability, sequential sampling efforts throughout the growing season for several years are needed.

Because readily available C is the preferred substrate for the soil microbial community (Rasse et al., 2005; Joslin et al., 2006), the observed differences in C availability among cultivars might alter microbial communities and their function and, thereby, influence other soil C cycling processes. Indeed, our study shows that the cultivars differentially impacted the microbial consumption of added root exudates. However, relatively small differences in the isotopic composition of cultivars and relatively high variability among replicates precluded partitioning of SOC priming by cultivar. Interestingly, differences among cultivars in both soil C availability and microbial consumption of exudates became more pronounced in soils from greater depth (Table 2). For example, the greatest differences among cultivars in soil C respiration from that of control soils was 13% for 0–10 cm, 29% for 20–30 cm, and 39% for 40–60 cm. Although previous studies have reported on genetic variation in root architecture (Fischer et al., 2006; Jackson, 1995), and on the impact of genetic variation on belowground ecosystem processes (Hendrickson and Robinson, 1984; Hendricks et al., 1993;

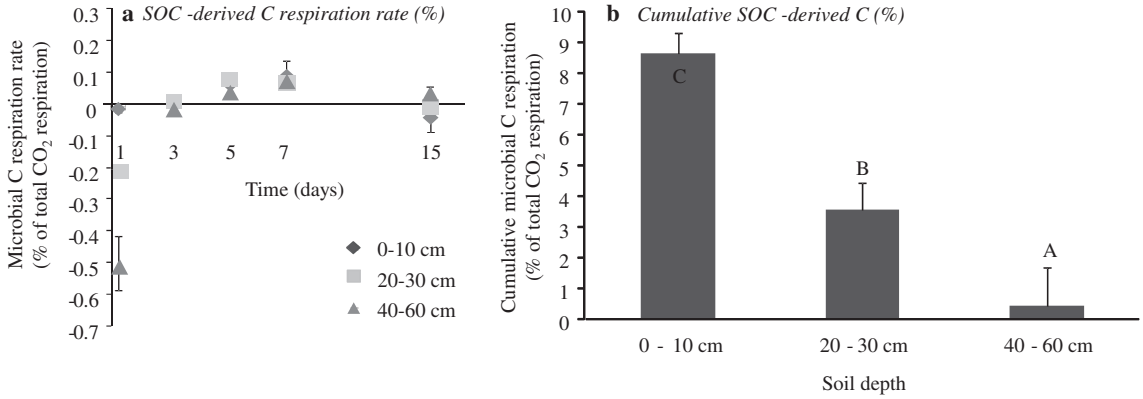

Fig. 5. Priming of SOC decomposition in soils amended with a ^{13}C labeled synthetic exudate solution (see equation (5) in text). Respiration rates are averaged across soils derived from six switchgrass cultivars and were calculated at three soil depths. (a) SOC derived C respiration rates as a percent of total (i.e. soil + exudate derived respiration) CO_2 respiration rates; (b) cumulative SOC-derived CO_2 as a percent of cumulative total CO_2 respiration. Values are means of the cultivars ± SE ($n = 6$). Different letters indicate significant differences in respiration among soil depths.

Dungey et al., 2000; Hirsch et al., 2003; Phillips et al., 2003; Schweitzer et al., 2004, 2008; Lecerf and Chauvet, 2008), to our knowledge, this is the first study showing persistent genetic variation in root architecture and its potential effects on C cycling in soils from significant depths below the surface, suggesting that cultivars can differentially affect soil C inputs and microbial communities across a soil profile. Our results indicate that the impact of genetic variation in plants on soil C cycling may be underestimated if only the surface 15 cm of soil are considered and demonstrate the need for further research on this topic.

4.2. Variability in microbial consumption of exudates and SOC decomposition among soil depths

Large differences in soil C availability (Table 2) with depth in the soil profile, lead to large differences in microbial processing of added exudates and priming of SOC. Specifically, we found that the majority of exudates added to soils from a depth of 0–10 cm were consumed by microbes immediately upon addition on day one, after which consumption of the exudates quickly decreased. In contrast, consumption of exudates added to soils from depths of 20–30 cm and 40–60 cm was greater on day three than day one of the incubation, and depletion of exudates proceeded at a slower rate relative to that of soils from 0 to 10 cm. These data indicate that consumption of exudates in soils from greater depths may have been preceded by slower microbial biomass growth than at shallow depth; however, we do not have the microbial biomass-^{13}C data to confirm this speculation.

It appears that the ratio between exudate C input to soil and soil C (i.e. 0.027 for the 0–10 cm depth increment, 0.056 for the 20–30 cm depth increment, and 0.122 for the 40–60 cm depth increment) may explain differences in microbial respiration of exudates among depths, as well as differences in the direction and magnitude of priming effects. In soils from greater depths, addition of exudates induced negative priming of SOC (i.e., exudate-addition at a relatively large ratio of exudate-C to soil C suppressed SOC decomposition) during the first days of the incubation, whereas in soils from shallow depth, we found significant positive priming effects over time. In addition, cumulative SOC primed over the course of the experiment was much smaller in soils from greater than from shallower depths. Together, these results suggest that soil microbes preferentially used exudate-C in deeper-origin soils and that priming did not occur due to a smaller demand for C, owing to a presumably smaller microbial biomass (Killham and Firestone, 1984) relative to shallow soil.

Our results corroborate those of Salome et al. (2010), who found that a significant priming effect was induced by fructose addition (13.3 μg/mg soil) in topsoil (0–10 cm) but not in the subsoil (80–100 cm depth) of an Eutric Cambisol. Differences in priming responses to exudate inputs between surface soil and subsoil may be caused by differences in the structure and function of soil microbial communities across the soil profile (Lavahun et al., 1996; Blume et al., 2002; Fierer et al., 2003), resulting from variation in soil C availability (Fierer et al., 2003; Goberna et al., 2005). Our results suggest that the impact of changes in exudate inputs on cycling of SOC is regulated differently between surface soil and subsoil, following a gradient of soil C availability and, potentially, by differences in the soil microbial community across the soil profile.

Currently, the widely held perception is that an increase in root exudation stimulates decomposition of more stable SOC through the priming effect, but this study suggests that an increase in exudation at deeper depths might barely affect or even suppress decomposition of SOC. Fontaine et al. (2007) however, found that an increase in exudation at depth stimulated decomposition of old SOC. Our study does not contradict their results, since we did observe a small priming effect at greater depths. Although our study was conducted in a laboratory setting in the absence of living roots and not under field conditions, it does suggest that, in the short-term, increased exudation might enhance decomposition to a much greater extent at shallower depths, where the availability of labile C substrates is greater. If so, increases in exudation at deeper depths resulting from, for example, land-use change for bioenergy production could have vastly different impacts on SOC stabilization and turnover than in surface soil. However, longer-term sustained increases in root exudate inputs following land-use changes might enhance soil C availability (Wolters and Joergensen, 1991), and might lead to different responses in the subsoil than those prompted by short-term increases in exudation. Our study emphasizes the importance of root-C inputs in driving the belowground C cycle, and we suggest that future studies are greatly needed, particularly for deeper soils, to better assess root impacts on soil C cycling.

Acknowledgments

We thank Xochi Campos, Ian Duvall, Jaron Adkins and Jamie Hicks for assisting with root architecture and CO_2 respiration measurements and microbial analyses. Thanks to Timothy Vugteveen for collecting soil and root samples. This work was supported by the National Science Foundation EPSCoR Program and by the US Department of Energy, Office of Science, Office of Biological

and Environmental Research, Climate and Environmental Science Division under contract DE-AC02-06CH11357 to Argonne National Laboratory, which is managed for the U.S. Department of Energy by Chicago Argonne, LLC, and it was conducted in collaboration with Oak Ridge National Laboratory, which is managed by UT-Battelle, LLC, for the U.S. Department of Energy.

Appendix A. Supplementary material

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.soilbio.2013.10.047.

References

Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. Appl. Soil Ecol. 37, 95–105.

Blume, E., Bischoff, M., Reichert, J.M., Moorman, T., Konopka, A., Turco, R.F., 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Appl. Soil Ecol. 20, 171–181.

Carillo, Y., Pendall, E., Dijkstra, F.A., Morgan, J.A., Newcomb, J.M., 2011. Response of soil organic matter pools to elevated CO_2 and warming in a semi-arid grassland. Plant Soil 347, 339–350.

Cheng, W., Johnson, D.W., Fu, S., 2003. Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. Soil Sci. Soc. Am. J. 67, 1418–1427.

Cheng, W.X., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition. In: Zobel, R.W., Wright, S.F. (Eds.), Roots and Soil Management: Interactions between Roots and the Soil. Agronomy Monograph, WI, pp. 119–143.

Cheng, W.X., 1996. Measurement of rhizosphere respiration and organic matter decomposition using natural ^{13}C. Plant Soil 183, 263–268.

Dahlman, C., Kucera, C.L., 1965. Root productivity and turnover in native prairie. Ecology 46, 84–89.

Dalenberg, J.W., Jager, G., 1989. Priming effect of some organic additions to C-14 labeled soil. Soil Biol. Biochem. 21, 443–448.

De Graaff, M.A., Castro, H., Classen, A.T., Schadt, C.W., 2010. Root exudates mediate plant residue decomposition rates by regulating the microbial community structure. New Phytol. 188, 1055–1064.

De Graaff, M.-A., Jastrow, J.D., Six, J., Wullschleger, S.D., 2013. Variation in root architecture among switchgrass cultivars impacts root decomposition rates. Soil Biol. Biochem. 58, 198–206.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of wet–dry cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol. Biochem. 33, 1599–1611.

Drake, J.E., Gallet-Budynek, A., Hofmockel, K.S., Bernhardt, E.S., Billings, S.A., Jackson, R.B., Johnsen, K.S., Lichter, J., McCarthy, H.R., McCormack, M.L., Moore, D.J.P., Oren, R., Palmroth, S., Phillips, R.P., Pippen, J.S., Pritchard, S.G., Treseder, K.K., Schlesinger, W.H., DeLucia, E.H., Finzi, A.C., 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. Ecol. Lett. 14, 349–357.

Dungey, H.S., Potts, B.M., Whitham, T.G., Li, H.F., 2000. Plant genetics affects arthropod community richness and composition: evidence from a synthetic Eucalypt hybrid population. Evolution 54, 1938–1946.

Fan, P., Guo, D., 2010. Slow decomposition of lower order roots: a key mechanism of root carbon and nutrient retention in the soil. Oecologia 163, 509–515.

Fischer, D.G., Hart, S.C., Rehill, B.J., Lindroth, R.L., Keim, P., Whitham, T.G., 2006. Do high-tannin leaves require more roots? Oecologia 149, 668–675.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167–176.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35, 837–843.

Goberna, M., Insam, H., Klammer, S., Pascual, J.A., Sanchez, J., 2005. Microbial community structure at different depths in disturbed and undisturbed semiarid Mediterranean forest soils. Microb. Ecol. 50, 315–326.

Groleau-Renaud, V., Plantureux, S., Guckert, A., 1998. Influence of plant morphology on root exudation of maize subjected to mechanical impedance in hydroponic conditions. Plant Soil 201, 231–239.

Guo, D., Li, H., Mitchell, R.J., Han, W., Hendricks, J.J., Fahey, T.J., Hendrick, R.L., 2008a. Fine root heterogeneity by branch order: exploring the discrepancy in root turnover estimates between minirhizotron and carbon isotopic methods. New Phytol. 177, 443–456.

Guo, D., Mitchell, R.J., Withington, J.M., Fan, P.P., Hendricks, J.J., 2008b. Endogenous and exogenous controls of root life span, mortality and nitrogen flux in a longleaf pine forest: root branch order predominates. J. Ecol. 96, 737–745.

Hamer, U., Marschner, B., 2005. Priming effects in soils after combined and repeated substrate additions. Geoderma 128, 38–51.

Hamer, U., Marschner, B., 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. J. Plant Nutr. Soil Sci. 165, 261–268.

Hendrickson, O.Q., Robinson, J.B., 1984. Effects of roots and litter on mineralization processes in forest soil. Plant Soil 80, 391–405.

Hendricks, J.J., Nadelhoffer, K.J., Aber, J.D., 1993. Assessing the role of fine roots in carbon and nutrient cycling. Trends Ecol. Evol. 8, 174–178.

Hirsch, A.M., Bauer, W.D., Bird, D.M., Cullimore, J., Tyler, B., Yoder, J.I., 2003. Molecular signals and receptors: controlling rhizosphere interactions between plants and other organisms. Ecology 84, 858–868.

Hoosbeek, M.R., Scarascia-Mugnozza, G.E., 2009. Increased litter build up and soil organic matter stabilization in a poplar plantation after 6 years of atmospheric CO_2 enrichment (FACE): final results of POP-EuroFACE compared to other forest FACE experiments. Ecosystems 12, 220–239.

Hoosbeek, M.R., Lukac, M., van Dam, D., Godbold, D.L., Velthorst, E.J., Biondi, F.A., Peressotti, A., Cotrufo, M.F., de Angelis, P., Scarascia-Mugnozza, G., 2004. More new carbon in the mineral soil of a poplar plantation under free air carbon enrichment (POPFACE): cause of increased priming effect? Global Biogeochem. Cycles 18, GB1040 http://dx.doi.org/10.1029/2003GB002127.

Hutsch, B.W., Augustin, J., Merbach, W., 2002. Plant rhizodeposition: an important source for carbon turnover in soils. J. Plant Nutr. Soil Sci. 165, 397–407.

Iversen, C.M., Ledford, J., Norby, R.J., 2008. CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. New Phytol. 179, 837–847.

Iversen, C.M., 2009. Digging deeper: fine root responses to rising atmospheric CO_2 concentration in forested ecosystems. New Phytol. 186, 346–357.

Jackson, L.E., 1995. Root architecture in cultivated and wild lettuce (*Lactuca* spp.). Plant Cell Environ. 18, 885–894.

Jastrow, J.D., Michael Miller, R., Matamala, R., Norby, R.J., Boutton, T.W., Rice, C.W., Owensby, C.E., 2005. Elevated atmospheric carbon dioxide increases soil carbon. Glob. Change Biol. 11, 2057–2064.

Joslin, J.D., Gaudinski, J.B., Torn, M.S., Riley, W.J., Hanson, P.J., 2006. Fine-root turnover patterns and their relationship to root diameter and soil depth in a ^{14}C-labeled hardwood forest. New Phytol. 172, 523–535.

Killham, K., Firestone, M.K., 1984. Salt stress control of intracellular solutes in Streptomycetes indigenous to saline soils. Appl. Environ. Microbiol. 47, 301–306.

Kong, A.Y.Y., Six, J., 2010. Tracing cover crop root versus residue carbon into soils from conventional, low-input, and organic cropping systems. Soil Sci. Soc. Am. J. 74, 1201–1210.

Kraffczyk, I., Trolldenier, G., Beringer, H., 1984. Soluble root exudates of maize: influence of potassium supply and rhizosphere microorganisms. Soil Biol. Biochem. 16, 315–322.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32, 1485–1498.

Lal, R., 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123, 1–22.

Langley, J.A., McKinley, D.C., Wolf, A.A., Hungate, B.A., Drake, B.G., Megonigal, J.P., 2009. Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. Soil Biol. Biochem. 41, 54–60.

Lavahun, M.F.E., Joergensen, R.G., Meyer, B., 1996. Activity and biomass of soil microorganisms at different depths. Biol. Fertil. Soils 23, 28–42.

Lecerf, A., Chauvet, E., 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic Appl. Ecol. 9, 598–605.

Lynch, J.M., Whipps, J.M., 1990. Substrate flow in the rhizosphere. Plant Soil 129, 1–10.

Meier, I.C., Leuschner, C., 2008. Belowground drought response of European beech: fine root biomass and carbon partitioning in 14 mature stands across a precipitation gradient. Global Change Biol. 14, 2081–2095.

Merckx, R., van Ginkel, J., Sinnaeve, J., Cremers, A., 1986. Plant-induced changes in the rhizosphere of maize and wheat. Part 1 Production and turnover of root-derived materials. Plant Soil 96, 85–93.

Norby, R.J., Jackson, R.B., 2000. Root dynamics and global change: seeking an ecosystem perspective. New Phytol. 147, 3–12.

Phillips, D., Ferris, A.H., Cook, D.R., Strong, D.R., 2003. Molecular control points in rhizosphere food webs. Ecology 84, 816–826.

Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol. Lett. 14, 187–194.

Phillips, R.P., Meier, I.C., Bernhardt, E.S., Grandy, A.S., Wickings, K., Finzi, A.F., 2012. Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. Ecol. Lett. 15, 1042–1049.

Phillips, R.P., Bernhardt, E.S., Schlesinger, W.H., 2009. Elevated CO_2 increases root exudation from loblolly pine (*Pinus taeda* L.) seedlings as an N-mediated response. Tree Physiol. 29, 1513–1523.

Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. Plant Soil 269, 341–356.

Salome, C., Nunan, N., Pouteau, V., Lerch, T.Z., Chenu, C., 2010. Carbon dynamics in topsoil and subsoil may be controlled by different regulatory mechanisms. Global Change Biol. 16, 416–426.

Schweitzer, J.A., Bailey, J.K., Fischer, D.G., LeRoy, C.J., Lonsdorf, E.V., Whitham, T.G., Hart, S.C., 2008. Soil microorganism–plant interactions: heritable relationship between plant genotype and associated microorgansims. Ecology 89, 773–781.

Schweitzer, J.A., Bailey, J.K., Rehill, B.J., Hart, S.C., Lindroth, R.L., Keim, P., Whitham, T.G., 2004. Genetically based trait in dominant tree affects ecosystem processes. Ecol. Lett. 7, 127–134.

van Kessel, C., Boots, B., de Graaff, M.A., Harris, D., Blum, H., Six, J., 2006. Total soil C and N sequestration in a grassland following 10 years of free air CO_2 enrichment. Global Change Biol. 12, 2187–2199.
Weaver, J.E., Darland, R.J., 1949. Soil–root relationships of certain native grasses in various soil types. Ecol. Monogr. 19, 303–338.
Wells, C.E., Eissenstat, D.M., 2001. Marked differences in survivorship among apple roots of different diameters. Ecology 82, 882–893.
Wolters, V., Joergensen, R.G., 1991. Microbial carbon turnover in beech forests soils at different stages of acidification. Soil Biol. Biochem. 23, 897–902.