Soil Biology & Biochemistry 112 (2017) 191–203

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Seasonal switchgrass ecotype contributions to soil organic carbon, deep soil microbial community composition and rhizodeposit uptake during an extreme drought

Catherine E. Stewart [a, b, *], Damaris Roosendaal [a], Karolien Denef [c], Elizabeth Pruessner [a], Louise H. Comas [d], Gautam Sarath [e], Virginia L. Jin [f], Marty R. Schmer [f], Madhavan Soundararajan [g]

[a] Soil Management and Sugar Beet Research Unit, United States Department of Agriculture-Agricultural Research Service, Suite 100, 2150 Centre Avenue, Building D, Fort Collins, CO 80526-8119, USA
[b] Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523- 1499, USA
[c] Central Instrument Facility (CIF), Department of Chemistry, Colorado State University, Fort Collins, CO 80523-1872, USA
[d] Water Management and Systems Research Unit, USDA-ARS, Suite 320, 2150 Centre Avenue, Building D, Fort Collins, CO 80526-8119, USA
[e] Wheat, Sorghum and Forage Research Unit, USDA-ARS, 251 Filley Hall, Univ. of Nebraska, Lincoln, NE 68683-0937, USA
[f] Agroecosystems Management Research Unit, USDA-ARS, 251 Filley Hall, Univ. of Nebraska, Lincoln, NE 68583-0937, USA
[g] Department of Biochemistry, University of Nebraska, Lincoln, NE 68588-0664, USA

ARTICLE INFO

Article history:
Received 30 September 2016
Accepted 10 April 2017
Available online 22 May 2017

Keywords:
Switchgrass
Soil microbial biomass
PLFA
Soil C sequestration
^{13}C stable isotope probing

ABSTRACT

The importance of rhizodeposit C and associated microbial communities in deep soil C stabilization is relatively unknown. Phenotypic variability in plant root biomass could impact C cycling through belowground plant allocation, rooting architecture, and microbial community abundance and composition. We used a pulse-chase ^{13}C labeling experiment with compound-specific stable-isotope probing to investigate the importance of rhizodeposit C to deep soil microbial biomass under two switchgrass ecotypes (*Panicum virgatum* L., Kanlow and Summer) with contrasting root morphology. We quantified root phenology, soil microbial biomass (phospholipid fatty acids, PLFA), and microbial rhizodeposit uptake (^{13}C-PLFAs) to 150 cm over one year during a severe drought. The lowland ecotype, Kanlow, had two times more root biomass with a coarser root system compared to the upland ecotype, Summer. Over the drought, Kanlow lost 78% of its root biomass, while Summer lost only 60%. Rhizosphere microbial communities associated with both ecotypes were similar. However, rhizodeposit uptake under Kanlow had a higher relative abundance of gram-negative bacteria (44.1%), and Summer rhizodeposit uptake was primarily in saprotrophic fungi (48.5%). Both microbial community composition and rhizodeposit uptake shifted over the drought into gram-positive communities. Rhizosphere soil C was greater one year later under Kanlow due to turnover of unlabeled structural root C. Despite a much greater root biomass under Kanlow, rhizosphere δ^{13}C was not significantly different between the two ecotypes, suggesting greater microbial C input under the finer rooted species, Summer, whose microbial associations were predominately saprotrophic fungi. Ecotype specific microbial communities can direct rhizodeposit C flow and C accrual deep in the soil profile and illustrate the importance of the microbial community in plant strategies to survive environmental stress such as drought.

Published by Elsevier Ltd.

1. Introduction

Deep soils, defined here as soils below 30 cm soil depth, contain a low SOC concentration but can comprise more than 50% of the total soil C stock (Jobbágy and Jackson, 2000). Despite the abundance of SOC below 30 cm, the mechanisms responsible for subsurface C stock changes, as well as their magnitude, are poorly

* Corresponding author. Suite 100, 2150 Centre Avenue, Building D, Fort Collins, CO 80526-8119, USA.
E-mail address: Catherine.Stewart@ars.usda.gov (C.E. Stewart).

http://dx.doi.org/10.1016/j.soilbio.2017.04.021
0038-0717/Published by Elsevier Ltd.

understood (Rumpel and Kögel-Knabner, 2011). Deep-rooted perennial grass species have a high potential to store C at depth where dead root biomass would be protected from decomposition through physico-chemical interactions or microbial inaccessibility. Root biomass turnover plus rhizodeposit-C (i.e., C derived from root exudation and sloughing of root cells), can contribute 2.4 times more to SOC compared to aboveground litter (Rasse et al., 2005, 2006). The relative importance of root morphology, architecture, and interactions with soil microbial community, however, is unclear. In a review comparing *in-situ* root growth experiments to soil incubations with added litter, Rasse et al. (2005) found that root biochemistry accounted for only ¼ of the increase in root-derived C mean residence time compared to shoot-derived C, with other mechanisms such as physico-chemical protection, physical protection through mycorrhiza and root-hair activities, and chemical interactions with metal ions, accounting for the rest. Since rhizodeposit-C is rapidly incorporated into microbial biomass, soil microbial community composition could influence the fate of plant-derived C and its stabilization in the soil (Six et al., 2006).

Plant-derived low molecular weight carbonaceous compounds, like rhizodeposits, may contribute proportionally more to SOC compared to more highly lignified compounds through higher microbial carbon use efficiency (Cotrufo et al., 2013; Parton et al., 2014). Although C sequestration is directly related to the quantity of C inputs to soils, recent work has shown that as more lignified plant residues decompose, they lose proportionally more C as CO_2 and thus, are converted to SOC at a lower rate (Stewart et al., 2015). More 'labile' products (i.e., dissolved organic C, root exudates) promote microbial biomass and are more efficiently converted to SOC due to lower respiration losses and physico-chemical protection through mineral association (Cotrufo et al., 2015). Microbially-processed C comprises the majority of C deep in the soil profile, and plant root morphology and interactions with the microbial community may impact C inputs in deep soils.

Phenotypic variability in plant root biomass could impact C cycling through belowground plant allocation, rooting architecture, and microbial community abundance and composition. Belowground biomass allocation impacts soil C sequestration by determining the overall belowground biomass contribution to SOC. Root architecture (root length versus mass) could impact the relative contribution of rhizodeposit versus root biomass to SOC (Adkins et al., 2016; Roosendaal et al., 2016). Fine roots, defined as the terminal two branches of the root system, contribute more surface area for root exudation (Guo et al., 2004), turn over more quickly than the rest of the root system (Xia et al., 2010), and form intimate associations with mycorrhizal fungi (Smith and Read, 2008).

Switchgrass ecotypes have a wide range in root biomass and architecture, making it an ideal species to test impacts of plant root traits on soil microbial communities and SOC (de Graaff et al., 2013; Roosendaal et al., 2016). Roosendaal et al. (2016) used a pulse-chase ^{13}C labeling experiment with compound-specific stable-isotope probing to investigate the importance of root-derived C to deep soil microbial biomass under two 3-yr old switchgrass ecotypes, Kanlow and Summer. We found that switchgrass ecotypes (*Panicum virgatum* L.) with contrasting root architectures supported different microbial communities that processed rhizodeposit C. Rhizodeposit C uptake in microbial biomass under the fine-rooted upland ecotype ('Summer') was associated with saprotrophic fungi, and the coarser-rooted lowland ecotype ('Kanlow') was associated with gram-negative bacteria. We report here results from sampling later in the season (anthesis and post-frost) when plant-microbial interactions would be expected to change. We measured plant biomass, microbial biomass (phospholipid fatty acids, PLFA) and rhizodeposit transformation (^{13}C-PLFAs), and soil C to a depth of 150 cm during the driest year on record. Due to the finer, smaller root architecture we observed during the initial sampling, we hypothesize that the upland ecotype, Summer, will be more resilient to drought and the associated microbial community to be smaller compared to the lowland ecotype Kanlow. Summer should also have proportionally greater contributions to soil C due to associations with fungal communities compared with Kanlow.

2. Materials and methods

2.1. Experimental site and treatments

The study site was located on the University of Nebraska-Lincoln's Agricultural Research and Development Center (ARDC), Ithaca, Nebraska, USA (41.151°N, 96.401°W) and the experiment is described in detail in Roosendaal et al. (2016). Soils are classified as silt-loam to silty clay loam (Yutan, fine-silty, mixed, superactive, mesic Mollic Hapludalf and Tomek, fine, smectitic, mesic Pachic Argiudoll). Soil C ranged from 199.0 to 20.5 g m^{-2} and N ranged from 17.7 to 2.5 g m^{-2} from the surface to 150 cm. Soil pH increased with depth from 5.9 to 6.9.

Switchgrass (*Panicum virgatum*, L) is a native, perennial C4 grass adapted to a broad geographic range in North America that has resulted in genetic differences in aboveground and below ground productivity, root architecture, and stress resistance (Monti, 2012). Upland ecotypes have a greater stress resistance with lower yields compared to lowland ecotypes which have a low freeze-tolerance, but greater yields. The experimental design was a randomized complete block with three field replicates of two switchgrass cultivars Summer (upland ecotype) and Kanlow (lowland ecotype). Each plot consisted of twelve switchgrass plants of the same ecotype arranged in a 4 × 3 plant grid for a planting density of 12 plants m^{-2}. Switchgrass was well-established and 3 years old when sampled in 2012. Prior to the 2012 growing season, residual biomass from the previous year was burned in the spring before switchgrass growth, as is typically done every year.

2.2. ^{13}C labeling

Switchgrass plants were labeled in May 2012 using a 1.0 m^3 customized portable $^{13}CO_2$ pulse-chase labeling system (Saathoff et al., 2014). Isotopically enriched CO_2 label (99 atom% ^{13}C (Sigma-Aldrich Co. St. Louis, MO)) was introduced into the chamber to raise chamber CO_2 concentrations between 1000 and 2000 ppm above atmospheric CO_2 concentration (420 ppm). Plants took up labeled CO_2 until chamber headspace concentrations decreased to 100 ppm below ambient CO_2 (LI-6200, LI-COR Biosciences, Lincoln, NE).

2.3. Plant and soil sampling

Switchgrass plants and soil from each plot were harvested two days following ^{13}C pulse-chase labeling (31 May 2012), at anthesis (11 July 2012 for Summer and 17 September 2012 for Kanlow), and post-killing frost the following year (2 April 2013). The aboveground biomass was removed by clipping at the soil surface. Plant samples were separated into stems, leaves, tillers and oven dried at 55 °C and ground for further analysis. Soil cores (10.16 cm diam.) were collected through the crown of the plant and divided in increments of 0–10, 10–30, 30–60, 60–90, 90–120, and 120–150 cm (0–15 cm Ap horizon, 15–110 cm Bt horizons, 110–150 cm C horizon). The 120–150 depth was not obtained at the anthesis sampling due to low soil moisture. Each depth increment was split in half length-wise, packed on ice, transported to the USDA-ARS laboratory in Ft. Collins, Colorado. Both half cores were weighed and the one for root separations was immediately