

# Plant roots alter microbial functional genes supporting root litter decomposition

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## ABSTRACT

Decomposition of soil organic carbon is central to the global carbon cycle and profoundly affected by plant roots. While root “priming” of decomposition has been extensively investigated, it is not known how plants alter the molecular ecology of soil microbial decomposers. We disentangled the effects of *Avena fatua*, a common annual grass, on <sup>13</sup>C-labeled root litter decomposition and quantified multiple genetic characteristics of soil bacterial and fungal communities. In our study, plants consistently suppressed rates of root litter decomposition. Microbes from planted soils had relatively more genes coding for low molecular weight compound degradation enzymes, while those from unplanted had more macromolecule degradation genes. Higher abundances of “water stress” genes in planted soils suggested that microbes experienced plant-induced water stress. We developed a conceptual model based on Mantel analyses of our extensive data set. This model indicates that plant root effects on the multiple soil environmental and microbial mechanisms involved in root litter decomposition act through changing the functional gene profiles of microbial decomposers living near plant roots.

## 1. Introduction

Understanding the factors regulating the accumulation and persistence of soil organic carbon (SOC) is critical to predicting the response of global carbon (C) cycling to future environmental change (Luo et al., 2016; Harden et al., 2018). Plants provide the primary input of C that ultimately becomes soil organic matter (Clemmensen et al., 2013; Jackson et al., 2017); plants also impact soil organic C decomposition mediated by soil microbial processes (Cheng and Kuz'yakov, 2005; Finzi et al., 2015; Keiluweit et al., 2015). Previous studies have shown that the presence of plants can stimulate organic C decomposition rates multiple-fold (Bird et al., 2011; Cheng et al., 2014). Yet other studies suggest that plants can have negative or neutral impacts on organic C decomposition rates (Van der Krift et al., 2002; Loya et al., 2004; Cheng et al., 2014; Saar et al., 2016). These seemingly contradictory effects

insinuate a diversity of mechanisms by which plant roots impact SOC cycling. Because the impacts of plants on decomposition processes are of a similar magnitude to those of changing climate, Cheng et al. (2014) suggest that this phenomenon, commonly known as “rhizosphere priming”, merits significant attention, particularly in the context of climate-induced changes in plant ecology.

Predicting the consequences of plant modulated terrestrial decomposition processes requires a fundamental understanding of the mechanisms operative at the root-soil-microbe interface. A variety of hypotheses have been proposed to explain the effects of roots on decomposition processes in soil, including: root exudate enhancement of microbial growth and activity; root exudate extraction of pre-existing mineral-associated organic matter; plant depletion of soil N availability causing enhanced (or decreased) microbial attack on macromolecular C; soil decomposers preferentially using labile C compounds from roots;

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and stimulation of decomposition by soil drying and rewetting (Cheng and Kuzyakov, 2005; Kuzyakov, 2010; Keiluweit et al., 2015; Mason-Jones and Kuzyakov, 2017). Several of these possible explanations, such as plant-induced changes in soil environmental parameters (e.g., soil moisture, nutrient availability), have been intensively examined (Magid et al., 1999; Fontaine et al., 2004; Knorr et al., 2005; Zhu and Cheng, 2013; Castanha et al., 2018). However, relatively few studies have focused on how plant root effects on microbial function might alter rates of decomposition (Cheng et al., 2014; van der Wal and de Boer, 2017). Since soil microbes play a central role in soil C decomposition and stabilization (Schimel and Schaeffer, 2012; Liang et al., 2017), understanding the mechanisms through which plant roots impact microbial mediation of decomposition is critical.

Most of the investigations of root effects on decomposition that include microbial characterizations have relied on taxonomic identification of microbes involved (Bird et al., 2011; España et al., 2011; Su et al., 2017). However, taxonomy is not an ideal way to represent functional attributes in complex microbial communities (Louca et al., 2018). Because we expect taxonomy and functionality are only loosely related for broadly-based processes such as mineralization and decomposition, we chose to document changes in functionality, in addition to the changes in taxa present in rhizosphere soils.

The primary objective of our study was to explore the genetic bases for previously proposed priming mechanisms, using a range of molecular analyses that address microbial functionality as well as identity and quantity. We examined the effects of live *Avena fatua* roots on decomposition of  $^{13}\text{C}$ -labeled root litter in soil over two simulated growing seasons (Fig. 1), with extensive analysis of microbial community characteristics at the end of the experiment. By continuously monitoring the production of  $^{13}\text{CO}_2$ , we quantified the effect of plant roots on root litter decomposition rates; by following the fate of the  $^{13}\text{C}$  in soil, we assessed the form of root litter-derived C remaining; and finally, we assessed microbial abundance, community composition, and functional gene profiles to delineate how microbial attributes underpin root-induced changes in rates of decomposition.

## 2. Materials and methods

### 2.1. Root litter decomposition experiment

We conducted a greenhouse experiment containing planted and unplanted treatments (5 replicates each) at the University of California, Berkeley. Soil (0–10 cm of the mineral horizon) was collected from the Little Buck watershed at the UC Hopland Research and Extension Center, from a Mediterranean climate grassland site where *Avena* spp. is the dominant vegetation. The soil is classified as a coarse-loamy, mesic Ultic Haploxeroll ([websoilsurvey.nrcs.usda.gov](http://websoilsurvey.nrcs.usda.gov)), containing 55% sand, 31% silt and 14% clay with a pH of 5.6. Mean soil C and N contents were 1.89% and 0.16%, respectively. After sieving (< 2 mm), the soil was mixed with sand (V:V = 1:1) and packed to approximate field bulk density ( $1.21 \text{ g cm}^{-3}$ ) in mesh bags ( $20 \mu\text{m}$ ,  $10 \text{ cm} \times 10 \text{ cm} \times 10 \text{ cm}$ ) with a PVC tube (diameter: 54 mm, length: 12 cm with 9 cm located belowground) in the middle of each bag (Fig. 1). Four windows ( $6 \text{ cm} \times 4 \text{ cm}$ ) cut in the sides of each PVC tube allowed root access, if present, into the tube. During packing, the PVC tubes and windows were blocked by a polycarbonate insert (outer diameter 54 mm, height 15 cm). All ten mesh bags were placed in one large container, with the spaces between bags filled with a soil/sand mixture (Fig. S1). This design, with the mesh bags restricting roots to the planted treatment, allowed for some equilibration of soil water content between planted and unplanted treatments. Four *Avena fatua* seedlings per mesh bag were planted surrounding the PVC tube in five randomly selected mesh bags (planted treatment). The other five bags were left unplanted. After two weeks of plant growth, the insert inside each PVC tube was removed to allow developing roots to enter the PVC tubes and interact with isotopically labeled root detritus placed therein (see below).

Soil (230 g), mixed thoroughly with 0.61 g of fragmented  $^{13}\text{C}$ -labeled root litter (32.9% C at 88.1 atom%  $^{13}\text{C}$  and 2.46% N, ~ 1 cm in length), was packed into each PVC tube in both treatments (Fig. 1); we refer to the area inside the tube as the 'root litter zone'. The amount of root litter added was based on the root biomass found in an average California grassland (~ 320  $\text{g m}^{-2}$  in top soil) (Dukes et al., 2005). The  $^{13}\text{C}$ -root litter used in this study was produced by growing *Avena fatua* seedlings under  $^{13}\text{CO}_2$  (99 atom%) for 12 weeks at the Environmental Plant Isotope Chamber facility at University of California, Berkeley (see SI for details). A no-litter control treatment (no root litter addition,

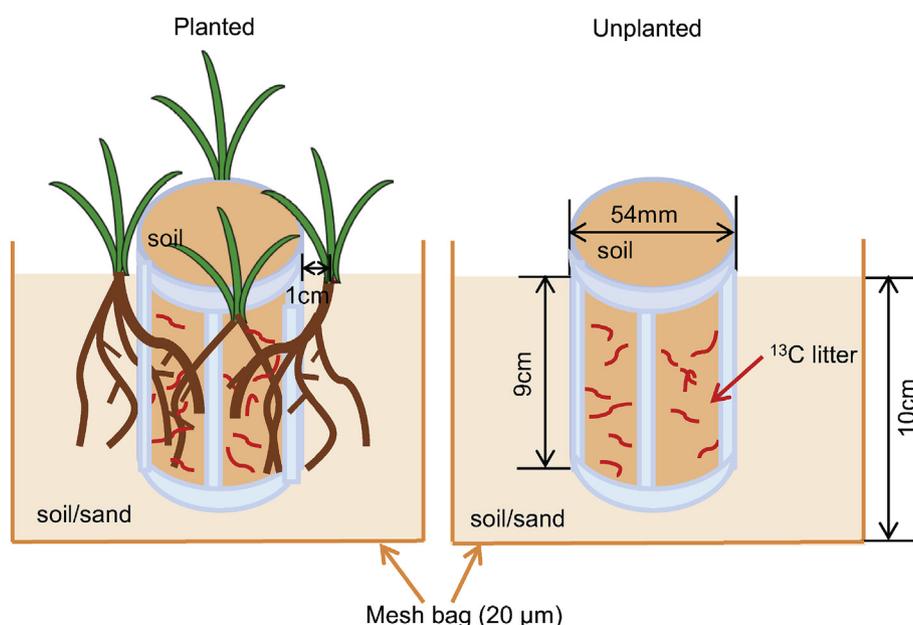


Fig. 1. Root litter decomposition experimental design. Planted and unplanted ( $n = 5$ ) treatments were placed in the same container using a completely randomized design (Fig. S1 for more detail).

n = 4) with similar design was also included in the middle of the experimental container (Fig. S1).

This experiment was conducted for two consecutive *Avena fatua* growing periods over 280 days (the addition of root litter was day 1 (d1)). Between the two growing periods, there was a 3-month dry period during which no water was added to the system. This dry period was intended to simulate the annual summer dry period characteristic of the California Mediterranean-type climate. During this very dry period, Mediterranean annual grasses normally persist as seeds. Bacteria and fungi indigenous to these systems are highly adapted to this annual dry period (Placella et al., 2012; Barnard et al., 2013). During our experimental growing periods (d1–d79; d179–d280), plants and soils were watered three times a week with tap water to maintain soil moisture at approximately 15%. This moisture level was selected based on the mean moisture of soil samples we collected from this field site during plant growth. Just before the plants began to senesce in the second growing season, the experiment was terminated (d280) and harvested for analyses.

## 2.2. Root litter zone CO<sub>2</sub> efflux rate measurement

Immediately after packing soil with root litter in the PVC tubes (d1), a PVC cylinder (15 cm height) with attached cap was affixed to the top of each existing soil tube using a connecting collar, creating a headspace for soil gas efflux measurements. CO<sub>2</sub> efflux from the root litter zone soil was trapped using 10 mL of CO<sub>2</sub>-free NaOH (0.25M) hanging inside the capped PVC cylinder. NaOH solutions were replaced every 3–5 days. The C content of the NaOH solution was measured with an inorganic-C analyzer (O-I Analytical Model 1010), and the <sup>13</sup>C enrichment of the CO<sub>2</sub> was analyzed using a Vario MICRO cube elemental analyzer coupled to an Isoprime 100 isotope ratio mass spectrometer (EA-IRMS; Elementar Americas, Mt. Laurel, NJ) after precipitating out carbonates with excess SrCl<sub>2</sub> and filtration (Harris et al., 1997). The <sup>13</sup>CO<sub>2</sub> efflux rate was calculated using the formula:

$$F_{13CO_2} = \frac{At\%M - At\%N}{At\%L - At\%N} \times F_{total}$$

where At%M is the measured atom% of CO<sub>2</sub>; At%N is the atom% of natural abundance CO<sub>2</sub>; At%L is the atom% of the added labeled root litter (88.1%); and  $F_{total}$  is the total CO<sub>2</sub> efflux rate calculated based on the C content in the trapping solution.

## 2.3. Leaching events

To test the effect of N-availability on root-priming of decomposition (Knorr et al., 2005), we imposed two major “leaching events” on d27 and d34 to reduce soil N to similar levels in the planted and unplanted soil. The entire container of soil (containing both treatment types) received 1.2 cm of tap water three times, 4 h apart on each occasion; in total, this summed to 3.6 cm of “simulated rainfall” over a 12 h period. This procedure was intended to remove the standing pool of soil solutes, in particular NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>, and approximates the magnitude of large precipitation events that occasionally occur at the Hopland field site. CO<sub>2</sub> efflux was not collected on leaching days. On d17 (before leaching), d28 and d35 (post leaching), small soil samples (< 2% of total soil in root litter zone) were removed from randomly chosen root litter zones with a corer (8 mm diameter, 9 cm depth) for inorganic N analysis. The sampling site was refilled with the same soil used in the original setup. Samples were extracted immediately with 20 mL of 2M KCl, and NO<sub>3</sub>-N and NH<sub>4</sub>-N analyses were performed on a QC8000 flow injection analyzer (Zellweger Analytics, Milwaukee, Wisconsin, USA).

## 2.4. Soil sampling and analysis

On day 280, soils from the root litter zones were retrieved from the PVC tubes and sieved (< 2 mm) to remove fresh plant roots. After

collection, subsamples were stored at –80 °C for molecular analysis. Dissolved organic C (DOC) was extracted from the harvested soils using 0.05 M K<sub>2</sub>SO<sub>4</sub>, and assays for microbial biomass C (MBC) were performed using chloroform fumigation (Herman et al., 2003) on the day of sampling. The C content of extracted solutions was analyzed on a model 1010 C analyzer (OI Analytical, College Station, TX). <sup>13</sup>C enrichment of MBC and DOC was determined by EA-IRMS of lyophilized extracts. Bulk soil C and <sup>13</sup>C enrichment were measured on subsamples of oven-dried (100 °C), milled soils by EA-IRMS. Soil pH was electrometrically determined using 1:1 (mass: 0.01 M CaCl<sub>2</sub> volume) soil slurries (Herman et al., 2003). Within 5 days of the harvest, soils (stored at 4 °C) were separated into three fractions: free light fraction (fLF), occluded light fraction (oLF) and heavy fraction (HF) using a fractionation process modified from Golchin et al. (1994) and Bird et al. (2011) (details in SI).

## 2.5. Molecular analysis of microbial communities

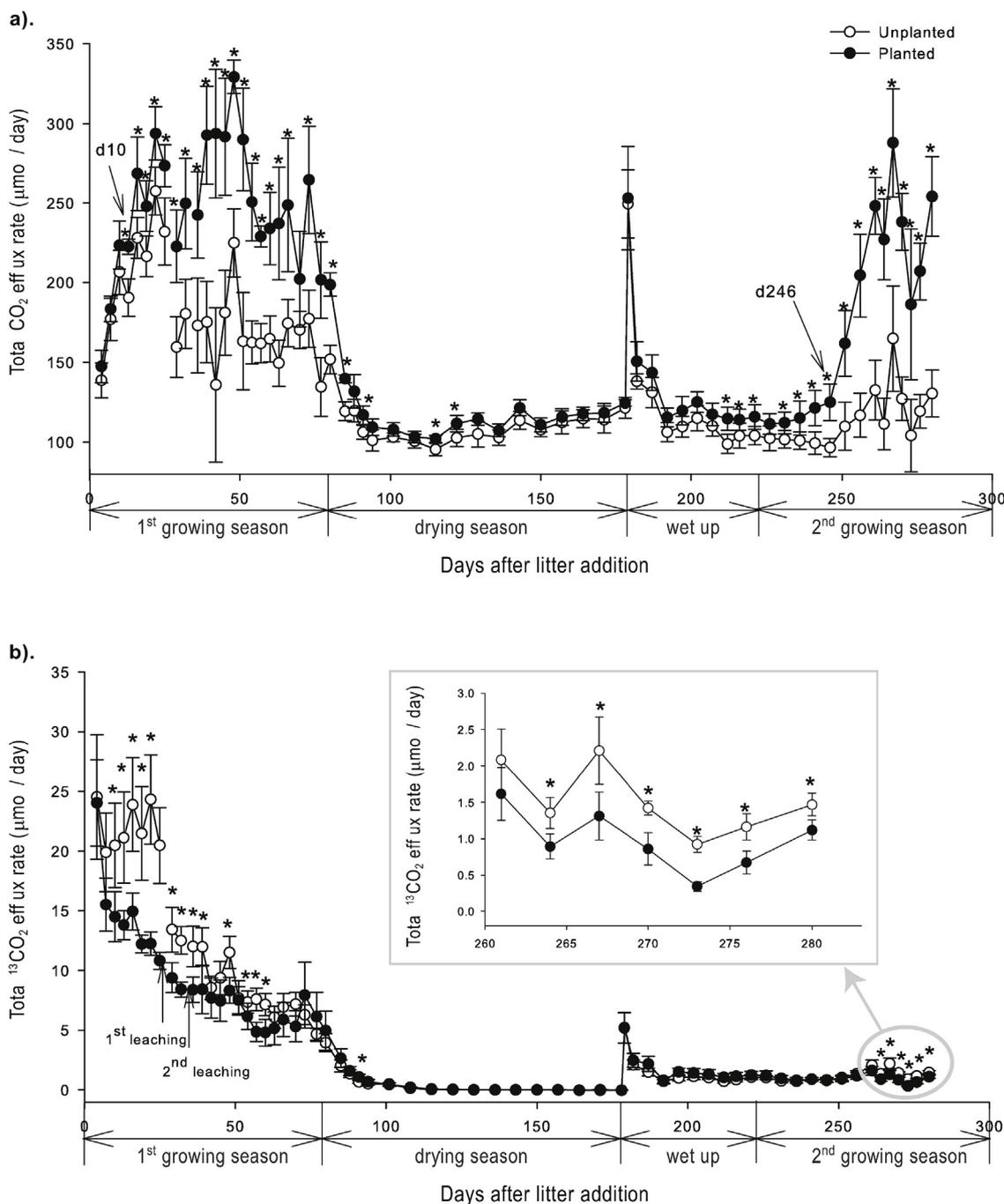
Soil microbial DNA was extracted from 0.5 g soil for each sample (stored at –80 °C) in a hexadecyl-trimethyl-ammonium bromide buffer using a phenol-chloroform purification protocol (DeAngelis et al., 2009). DNA concentrations were quantified by PicoGreen using a FLUOstar Optima (BMG Labtech, Jena, Germany).

The abundance of bacterial and archaeal 16S and fungal ITS rRNA gene copies was measured by qPCR with primer sets EUB338/EUB518 and ITS1/5.8S, respectively, following a protocol reported by Fierer et al. (2005) and described in the SI. Microbial community composition was analyzed using MiSeq sequencing of 16S and ITS gene amplicons on a MiSeq 2.0 platform (Illumina, San Diego, CA, USA) using a protocol described previously (Caporaso et al., 2012). The 16S rRNA gene was amplified using the primer pair 515F/806R (Caporaso et al., 2012) with attached Illumina flowcell adapter sequences. The ITS2 gene was amplified using the primer pair gITS7F and ITS4R (Ihrmark et al., 2012) with attached Illumina flowcell adapter sequences. The reverse amplification primer also contained a twelve base barcode sequence. Sample libraries were prepared according to the MiSeq Reagent Kit Guide (Illumina, San Diego, CA, USA); the sequencing protocol was modified from Caporaso et al. (2012) (details and sequence data analysis in SI).

The profile of soil microbial functional genes was analyzed with the functional gene array GeoChip 4.2 (He et al., 2007). Hybridization of 1.0 µg DNA from each sample was performed according to Tu et al. (2014). Briefly, Cy-3 labeled DNA samples, together with Cy5-labeled universal standard DNA, were hybridized to the GeoChip 4.2, (NimbleGen, Madison, WI) at 42 °C for 16 h on a MAUI hybridization station (BioMicro, Salt Lake City, UT). After washing, arrays were scanned for fluorescence (NimbleGen MS200, Madison, WI) at a laser power of 100%, and signal intensities of each spot were measured using ImaGene 6.0 (Biodiscovery, El Segundo, CA). Poor-quality spots were removed if signal intensity was < 2000 or signal/noise ratio was < 2.0. Signal intensity of each gene was normalized to Cy5-labeled universal standard DNA across samples as described previously (Xue et al., 2013). Genes were considered positive if detected in at least two out of five biological replicates. Signal intensities were natural log transformed.

## 2.6. Statistical analysis

Comparisons of CO<sub>2</sub> and soil biogeochemical data were analyzed by the Student's t-test (planted vs. unplanted, unplanted vs. control). Compositional and functional structure of microbial community data was ordinated by detrended correspondence analysis (DCA) based on the Bray-Curtis distance method (Hill and Gauch, 1980). Permutational multivariate analysis of variance (Adonis (Anderson, 2001)), based on a Bray-Curtis distance was used to evaluate significant differences compared to the null hypothesis. Differences in relative abundance (log 10 transformed) of each microbial phylum, or signal intensity of each gene family, were determined using a Student's t-test to compare treatments.



**Fig. 2.** Total CO<sub>2</sub> a) and <sup>13</sup>CO<sub>2</sub> b) daily efflux rate during the process of root litter mineralization in a California annual grassland soil over the course of two growing seasons. Soils were divided into planted (with *Avena fatua*) and unplanted treatments. All data are presented as mean  $\pm$  95% confidence interval (n = 5); significant differences ( $P < 0.05$ ) in daily efflux rates between the planted and unplanted treatment are indicated by stars. Leaching events are indicated by arrows in b). Live roots entered litter zones after d10 (season 1) and d246 (season 2) indicated by arrows in a). Low <sup>13</sup>CO<sub>2</sub> efflux rates in the 2<sup>nd</sup> growing season are shown as an insert within the graph, note the smaller scale.

Mantel analysis was performed to test for pair-wise correlation among root activity, environmental variables, microbial community variables and root litter decomposition rate as indicated by <sup>13</sup>CO<sub>2</sub> efflux rates on the last sampling day. For multivariate community analysis (i.e., GeoChip, 16S rRNA and ITS amplicon sequencing data), values of the first DCA ordination axis for each sample were used for Mantel analysis. Root activity was calculated as the mean total CO<sub>2</sub> efflux rate in the planted treatment on d280 minus the mean total CO<sub>2</sub> efflux rate in the unplanted treatment on d280. All analyses were conducted in the R environment (version 2.15.0) using the 'vegan' (Oksanen et al., 2013) and/or 'ade4' (Dray and Dufour, 2007) packages. Significant

differences were defined at a  $P$  value of  $< 0.05$  unless otherwise stated.

### 3. Results

#### 3.1. Effects of plant roots on soil organic C decomposition

Rates of total CO<sub>2</sub> production were higher in the presence of live roots (indicated by significantly higher total CO<sub>2</sub> efflux rates between planted and unplanted treatments; Fig. 2a). However, mineralization rates of <sup>13</sup>C-root litter were significantly lower in planted soils than in unplanted control soils (Fig. 2b). This reduction in <sup>13</sup>C-root litter

mineralization rates disappeared when plants senesced (around d60). Efflux rates of  $^{13}\text{C}$  decreased rapidly with soil drying (after d80), and root litter decomposition was almost undetectable after two weeks of soil dry down. The first watering event after the dry period caused substantial  $\text{CO}_2$  pulses from both treatments and  $^{13}\text{C}$  production resumed at a rate similar to what had occurred prior to the dry period. No significant differences between planted and unplanted treatments were detected in either total  $\text{CO}_2$  or  $^{13}\text{C}$  efflux rates during the wet up period at the beginning of the 2nd season (Fig. 2); however, as the plants began to grow, rates of  $^{13}\text{C}$ -root litter decomposition ( $^{13}\text{C}$  efflux) again became significantly lower as active plant roots began to develop, just after day 246 (root presence was indicated by a substantial increase in total  $\text{CO}_2$  efflux rates, Fig. 2a). Although the decomposition rate of the residual  $^{13}\text{C}$ -root litter in the 2nd season was much reduced in both planted and unplanted treatments relative to the 1st season, the relative magnitude of plant impact on root litter decomposition ( $-23$  to  $-62\%$ ) was comparable to (or larger than) the impact during the 1st season ( $-16$  to  $-50\%$ ).

At the end of the experiment (d280), a significantly larger quantity of  $^{13}\text{C}$  remained in planted soils ( $\sim 39\%$  of added  $^{13}\text{C}$ ) relative to unplanted soils ( $\sim 33\%$  of added  $^{13}\text{C}$ ; Table 1). The difference in  $^{13}\text{C}$  remaining in the soil between the two treatments (11 mg) was mainly driven by the substantially lower amount of  $^{13}\text{C}$  present in the unplanted soil free light fraction (fLF) (10 mg), which is composed largely of partially-decomposed root litter fragments. The  $^{13}\text{C}$  soil data (Table 1), together with the  $^{13}\text{C}$  efflux rates (Fig. 2b), indicate that plant roots significantly suppressed the rate of root litter decomposition. Apart from the fLF and  $^{13}\text{C}$ -DOC, the other forms of root litter-derived  $^{13}\text{C}$  did not differ significantly between planted and unplanted treatments (Table 1). At the end of the 280-day experiment, the largest pool of  $^{13}\text{C}$  remaining in soil from both treatments was in the heavy fraction (HF), which is thought to be mineral-associated C (Table 1).

The focus of this study was on the effect of plant roots on root litter decomposition; it was not designed to test the effects of live roots on decomposition of indigenous, unlabeled soil organic C. However, we did find a significant increase in total soil C (TOC) and  $^{12}\text{C}$  content of the planted soils at the end of the experiment (Table 2), likely due to C inputs from growing plant roots. The addition of root litter to soil in the unplanted treatment did not appear to cause an increase in decomposition of the original soil C, as indicated by non-significant differences in both TOC and  $^{12}\text{C}$  soil C between the unplanted and control treatments which did not receive any root litter addition (Table 2, Fig. S1).

### 3.2. Effects of soil N on root litter mineralization

Active plant growth resulted in reduced soil nitrate concentrations (generally the dominant inorganic N form in this soil), in planted relative to unplanted soils (d17 data in Table S1). After the 1st leaching of the planted soil, the concentration of  $\text{NH}_4\text{-N}$  declined significantly from

**Table 1**

Total  $^{13}\text{C}$  and  $^{13}\text{C}$  associated with different soil fractions remaining in soil at the end of the experiment. Data are presented as means  $\pm$  standard errors ( $n = 5$ ). *P* values shown in bold indicate significant changes in different  $^{13}\text{C}$  between planted and unplanted treatments ( $P < 0.05$ ).

Source of $^{13}\text{C}$ <sup>a</sup>	Unplanted	Planted	<i>P</i> value
Total $^{13}\text{C}$ in soil (mg)	58.5 $\pm$ 0.5	69.6 $\pm$ 1.9	<b>&lt; 0.001</b>
$^{13}\text{C}$ fLF (mg)	11.6 $\pm$ 1.11	21.6 $\pm$ 2.7	<b>0.008</b>
$^{13}\text{C}$ oLF (mg)	0.77 $\pm$ 0.16	0.52 $\pm$ 0.07	0.191
$^{13}\text{C}$ HF (mg)	48.0 $\pm$ 1.8	47.2 $\pm$ 2.9	0.825
$^{13}\text{C}$ MBC (mg)	1.93 $\pm$ 0.12	1.93 $\pm$ 0.16	0.987
$^{13}\text{C}$ DOC (mg)	0.012 $\pm$ 0.001	0.028 $\pm$ 0.006	<b>0.031</b>

<sup>a</sup> Data presented here represent excess  $^{13}\text{C}$ ; all isotope label was derived from  $^{13}\text{C}$ -labeled litter.

0.91 to 0.44  $\mu\text{g g}^{-1}$  soil, but nitrate did not decrease (Table S1). No significant differences in  $^{13}\text{C}$  efflux were detected in the planted treatment due to the leaching event. However, concentrations of both nitrate and ammonium declined significantly in the unplanted soil after the 1st leaching. At the same time,  $^{13}\text{C}$  efflux rates declined by 35% ( $P < 0.05$ ) in unplanted soils (Table S1), resulting in a reduction in the difference between decomposition rate in the planted and unplanted soil, from  $-47\%$  to  $-30\%$  (Fig. 2b). Neither soil N nor  $^{13}\text{C}$  efflux rates were significantly changed after the 2nd leaching in any treatment (Table S1). Despite the fact that soil N concentrations were temporarily reduced to comparable levels in planted and unplanted soils by the leaching event(s), live roots continued to have a suppressive effect ( $-30\%$ ) on root litter decomposition (Fig. 2).

### 3.3. Impacts of live plant roots on soil properties

At the end of the experiment, soil moisture was 6.2% (equivalent to  $-1.31$  MPa water potential) in the planted and 12.6% ( $-0.40$  MPa) in unplanted soils; soil pH and DOC were significantly higher in the presence of plant roots (Table 2). No significant differences were measured in soil properties between unplanted and control (without root litter) soils.

### 3.4. Response of soil microbial communities to live plant roots in root litter zones

On d280, the copy numbers of both bacterial and archaeal 16S rRNA and fungal ITS genes were approximately 20% higher in planted than unplanted soils ( $P < 0.01$ , Table S2). Furthermore, the overall microbial community structure, both in terms of function (GeoChip) and composition (MiSeq 16S and ITS), differed significantly between the two treatments, as shown by DCA ordination (Fig. 3) and Adonis analyses ( $P < 0.01$ ; Table S3). Further analysis of sequence data showed that the relative abundances of nine bacterial phyla significantly changed between the two treatments (Fig. S2). Specifically, the relative abundance of *Actinobacteria*, *Bacteroidetes* and  $\alpha$ -*Proteobacteria* significantly increased in the presence of live roots, while the abundance of *Firmicutes*,  $\beta$ - and  $\delta$ -*Proteobacteria* significantly decreased. Amongst the fungi, relative abundances of *Zygomycota* and *Glomeromycota* increased ( $P < 0.05$ ) by 3.3–7.9-fold, respectively, in the presence of plant roots, while the abundance of *Tremellomycetes* significantly decreased (Table S4). Analysis of GeoChip functional gene profiles also indicated significant differences between the treatments, particularly for gene categories including C, N, P and S cycling and stress responses (Table S3). Adonis analyses of the bacterial phylogenetic marker *gyrB* (detected by GeoChip) showed a significant change in the phylogenetic structure of bacterial communities surrounding live roots, consistent with MiSeq-based 16S results (Table S3).

To better understand how live roots impact the genetic bases of microbial mediation of decomposition, we focused on key functional genes involved in C degradation. To increase confidence and minimize non-representative results, gene families with fewer than 3 positive individual probes detected across all 10 samples were excluded from this analysis. Fifteen out of 65 detected C degradation gene families significantly differed ( $P < 0.1$ ) between planted and unplanted treatments (Fig. 4). Although the number of probes targeting genes involved in low molecular weight (MW) compound degradation was fewer than those encoding extracellular enzymes for complex macromolecule decomposition (16 vs. 49) on the GeoChip, the abundance of four of them (formate dehydrogenase, glucose oxidase, vanillin dehydrogenase and malate synthase) was significantly increased by the presence of plant roots (by 2.5%–62%; Fig. 4). Signal intensities of nine gene families capable of degrading macromolecules (e.g., hemicellulose, lignin), including xylanase, glucoamylase and phenol peroxidase, were significantly suppressed by the plant roots. In contrast, two “macromolecular” gene families (i.e., serine protease and manganese

**Table 2**

Comparison of soil characteristics across treatments after two growing seasons (280 days). Data are presented as means  $\pm$  standard errors ( $n = 5$  for planted and unplanted,  $n = 4$  for control). *P* values shown in bold indicate significant changes in soil properties between planted and unplanted or unplanted and control treatments ( $P < 0.05$ ).

Soil property	Unplanted	Planted	Control	<i>P</i> value	
				Unplanted vs. Planted	Unplanted vs. Control
TOC (%)	1.62 $\pm$ 0.02	1.76 $\pm$ 0.05	1.58 $\pm$ 0.05	<b>0.036</b>	0.434
Soil <sup>12</sup> C (%) <sup>a</sup>	1.60 $\pm$ 0.02	1.73 $\pm$ 0.05	1.58 $\pm$ 0.05	<b>0.038</b>	0.740
Soil moisture (%)	12.60 $\pm$ 0.15	6.19 $\pm$ 0.37	12.50 $\pm$ 0.58	<b>&lt; 0.001</b>	0.868
Soil pH	5.13 $\pm$ 0.02	5.36 $\pm$ 0.03	5.18 $\pm$ 0.02	<b>&lt; 0.001</b>	0.165
DOC ( $\mu\text{g C g}^{-1}$ soil)	8.3 $\pm$ 0.83	15.6 $\pm$ 1.90	NA	<b>0.008</b>	–

NA: not measured.

<sup>a</sup> Soil<sup>12</sup>C: excluding the <sup>12</sup>C from added litter.

peroxidase) were significantly enriched in planted soils compared to unplanted soils (Fig. 4). These results suggest that microbes in the planted soils had a greater capacity for consuming low MW C compounds, while communities in unplanted soils appeared more capable of degrading macromolecular C.

Because of the significant difference in soil moisture between two treatments at harvest (Table 2), we also analyzed the osmotic stress genes detected by GeoChip. The presence of plant roots significantly increased the abundance of *proV* and *proW* (both encode proteins for glycine betaine transporters) by 5% and 22%, respectively (Fig. 4). Additionally, the abundance of these two osmotic stress genes was significantly correlated (Mantel analysis,  $r = 0.45$ ,  $P = 0.033$ ) with soil moisture, suggesting that the observed microbial osmotic stress may be due to water stress caused by plant evapotranspiration activity. In addition, osmotic stress genes correlated significantly with overall microbial functional potential ( $r = 0.658$ ,  $P < 0.01$ ), indicating this stress may impair microbial activity, and have consequently negative impacts on soil C decomposition.

#### 4. Discussion

Understanding the mechanisms through which plant roots affect decomposition in soil is critical to our ability to predict the response of these processes under changing environmental conditions and plant community composition. In this study, we addressed the decomposition of root litter, the primary source of organic C stabilized in soil (Jackson et al., 2017). We found that an actively growing common annual grass, *Avena fatua*, reduced rates of root litter decomposition in a soil to which it has been resident for about a century. If this pattern were to persist over multiple years, reduction in rates of root litter decomposition could cause an increase in soil C content, particularly when augmented by root C inputs. Functional gene analysis indicated that plants enhanced the soil microbiome's potential to decompose simple C substrates while simultaneously reducing microbial genetic potential to decompose macromolecular substrates.

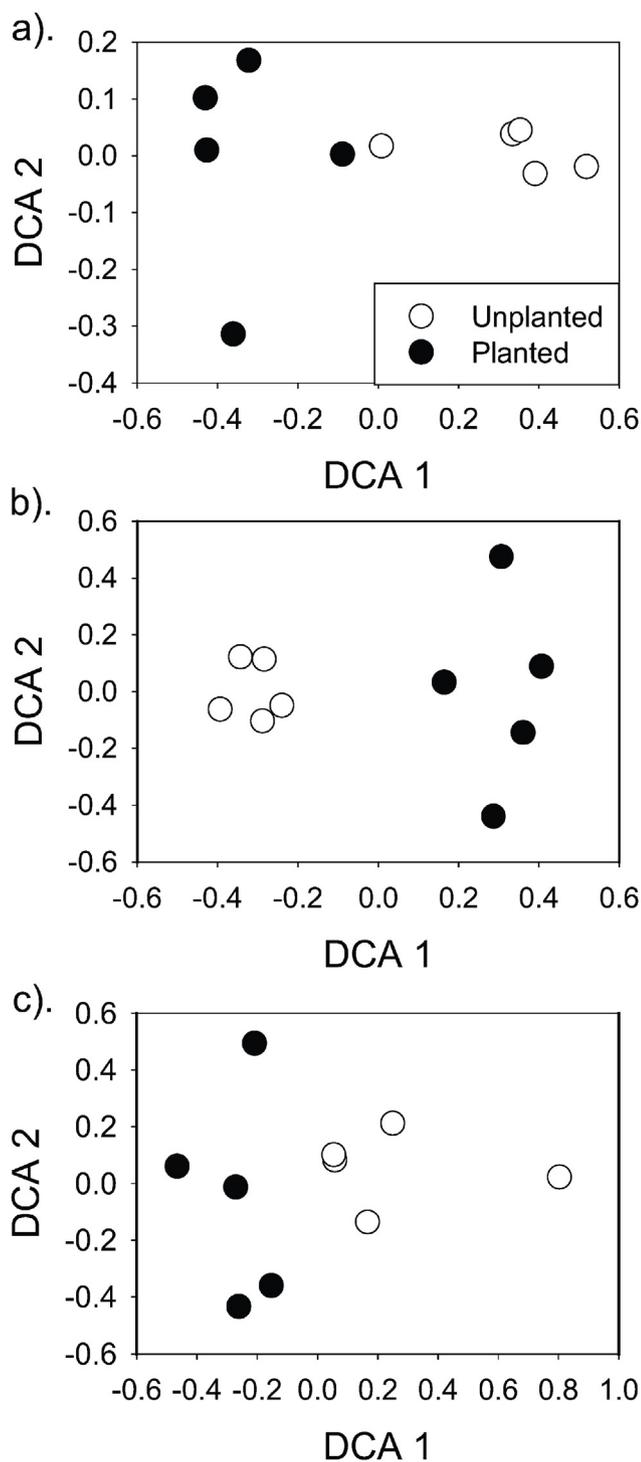
Increased rates of decomposition in the presence of roots have been reported a number of times (Cheng et al., 2014; Huo et al., 2017). Suppression of root litter decomposition by live root activities has also been reported, albeit less commonly (Loya et al., 2004; Cheng et al., 2014; Saar et al., 2016) and has been linked to plant species, litter type and soil nutrient levels (Van der Krift et al., 2002). Based on our previous work (Bird et al., 2011), we hypothesized that the presence of *Avena* sp roots would increase rates of root litter decomposition. However, to our surprise, these plant roots consistently suppressed rates of root litter decomposition throughout two full growing seasons, with a larger proportional reduction during the 2nd season relative to the first.

The seeming contradictions between our previous work and this study are consistent with the larger dichotomy between positive and negative effects reported in the literature (Cheng et al., 2014; Huo et al.,

2017). While there are some differences between the Bird et al. (2011) study and our current study (different soils, bacterial communities, root litter materials, and water status (Bird et al., 2011; Barnard et al., 2013), both studies used an *Avena* spp. growing in a California annual grassland soil. It is possible that differences in soil water content between the two studies are a key factor, but it is difficult to pinpoint the specific mechanisms responsible for the different outcomes of these two studies because the Bird et al. (2011) study did not include functional characterization of the operative microbial communities.

Several mechanisms have been proposed to account for “priming” of SOC decomposition by plant roots (Kuzyakov, 2002; Cheng and Kuzyakov, 2005; Keiluweit et al., 2015). High nutrient availability, especially N, in soil can stimulate SOC decomposition (Rasmussen et al., 2007); however, under other conditions or in other ecosystems, N addition sometimes reduces SOC decomposition (Fontaine et al., 2004; Bird et al., 2011). A meta-analysis by Knorr et al. (2005) indicated that the direction and magnitude of N addition impacts on root litter decomposition may be soil N-level dependent. In our study, reduction of inorganic N availability in unplanted soil seemed to reduce root litter mineralization rates when concentrations of N were relatively high ( $\sim 5 \mu\text{g g}^{-1}$  soil), suggesting that a plant-mediated reduction in N-availability could have limited root litter decomposition rates in this soil. However, reducing soil N had no significant effect in planted soils where the inorganic N concentration was below  $2 \mu\text{g g}^{-1}$ ; it is possible that N availability was already limiting rates of decomposition in the presence of actively growing roots. Thus, N limitation may have been a component of root-suppression of root litter decomposition at the beginning of the growing season.

Relatively labile root exudate C that is continuously supplied by plant roots may act to support ‘co-metabolic’ activity of enzymes such as oxygenases, which are well suited for breaking down “recalcitrant” C compounds (Sylvia et al., 2004), leading to enhanced macromolecular C (e.g., root litter in this case) decomposition. Alternatively, soil microorganisms may preferentially use simple root exudates rather than more complex organic C compounds, resulting in decreased decomposition rates (Cheng and Kuzyakov, 2005; Kuzyakov, 2010). In our study, the availability of exudate compounds during plant growth altered the community composition of the rhizosphere, and the resulting rhizosphere populations had increased genetic functional capacity to use simple C compounds. Previous studies have attempted to test the “preferential substrate utilization” hypothesis by adding simple sugars or organic acids to soils containing litter, but with inconsistent results (Kuzyakov et al., 2007; Chigineva et al., 2009). We suggest that alteration of the functional gene capacity of a community is not accurately or precisely characterized as “preferential substrate utilization”. Our study demonstrates that growing plant roots may promote plant-associated microbial communities with a genetically-prescribed preference for simple substrates over macromolecular substrates. This mechanism provides a more precise conceptualization of why altered rates of organic C decomposition may be found in the presence of plant



**Fig. 3.** Detrended correspondence analysis (DCA) of soil microbial communities from planted and unplanted treatments based on a) normalized signal intensity of functional genes detected by GeoChip 4.2; and abundance of OTUs detected by b) MiSeq 16S rRNA and c) MiSeq ITS gene sequencing.

#### roots.

Plant modification of soil moisture impacts microbial activities and consequently can also influence the magnitude and direction of root effects on decomposition (Magid et al., 1999; Kuzyakov, 2002; Zhu and Cheng, 2013). Recently, Castanha et al. (2018) reported that presence of *Avena* plants stimulated decomposition of  $^{13}\text{C}$ -labeled root litter when soil moisture was relatively high early in the growing season; but later, when soil moisture was much lower, *Avena* plants suppressed

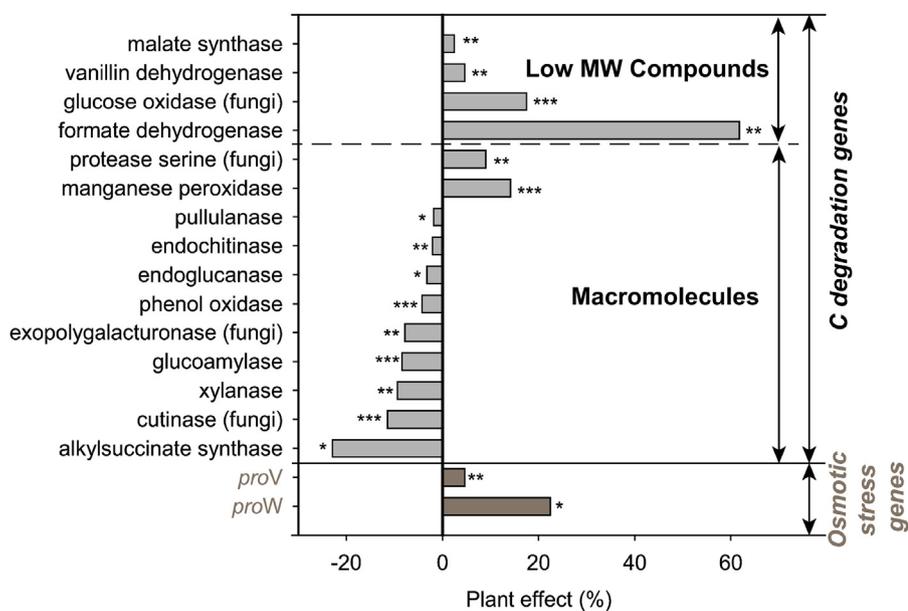
litter decomposition. In our experiment, although the design allowed water flow between treatments (Fig. S1), soils in the planted treatment experienced greater drying compared to the unplanted treatment due to plant evapotranspiration activities (at least until the next watering event). At the final harvest, the reduction in soil water content was apparent. Root-associated microbial communities may have experienced significant water stress, likely impairing their metabolic activities (Schimel et al., 1999; Manzoni et al., 2011); this is supported by the significant correlation between soil moisture and abundance of microbial stress genes, and correlation between stress genes with microbial functional potential in our Mantel analysis (Fig. 5).

It is likely that all the mechanisms we discuss above are in play, and with varying importance, during different stages of plant growth and the associated succession of rhizosphere microbial communities (Shi et al., 2015, 2016; Zhalnina et al., 2018), and as litter decomposition progresses (Blagodatskaya and Kuzyakov, 2008). In future studies, assessing the temporal dynamics of priming mechanisms will certainly be of value.

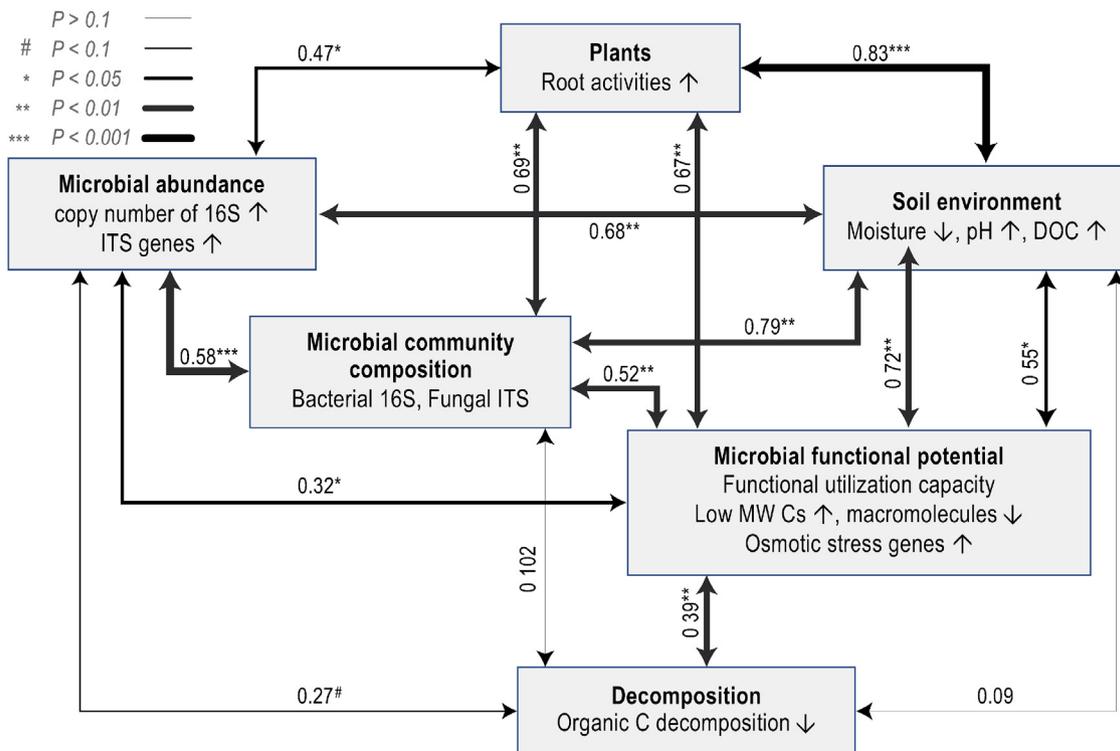
To better evaluate the variables and mechanisms affecting the patterns of root litter decomposition observed in this study, we developed a model of root control of root litter decomposition (Fig. 5) using Mantel analysis, which is designed to test the correlation between two distance or dissimilarity matrices. Plant root activity (as indicated by autotrophic respiration at the last sampling point) was significantly related to the surrounding soil physicochemical parameters (moisture, pH, and DOC) and microbial communities (abundance, composition and functional potential). Different characteristics of the microbial communities (abundance, composition and functional potential) were significantly correlated with each other; similarly, soil environmental parameters were correlated with all soil microbial community parameters. Mantel analysis identified four factors that were significantly related to microbial functional potential, these include: 1) soil environment ( $r = 0.55$ ,  $P < 0.05$ , and particularly soil moisture alone  $r = 0.72$ ,  $P < 0.01$ ); 2) plant root activity ( $r = 0.67$ ,  $P < 0.01$ ); 3) microbial community composition ( $r = 0.52$ ,  $P < 0.01$ ); and 4) microbial abundance ( $r = 0.32$ ,  $P < 0.05$ ). Interestingly, soil environment and microbial community composition did not have a significant direct correlation with rates of root litter decomposition (Fig. 5). Instead, the functional gene profile of the microbial community ( $r = 0.39$ ,  $P < 0.01$ ) and particularly genes related to C decomposition ( $r = 0.43$ ,  $P < 0.01$ ) were better predictors of root litter decomposition rates. Not surprisingly, our model indicates that plant roots directly impact microbial community characteristics and also modify the soil environment. However, the primary impact of live roots on decomposition appears to result from alteration of the soil microbial functional gene profiles. The model also suggests that the changing soil environment and microbial community characteristics impacted root litter decomposition primarily via alterations of the microbial functional potential.

Although the importance of soil microbes in decomposition processes is well recognized, microbial attributes are only rarely incorporated into decomposition models (Luo et al., 2016). Even when included, microbes are commonly considered as a C pool rather than central drivers of decomposition (Schimel and Schaeffer, 2012). In models that do include microbial functional characteristics, generally one or multiple microbial extracellular enzyme pools targeting C, N are included (Schimel and Weintraub, 2003; Sinsabaugh et al., 2008). Allison (2012) developed a trait-based model with microbial physiological and enzymatic traits, which determines resource availability and predicts litter decomposition rates. While our model is a data-driven model designed to identify dominant mechanisms, it highlights the importance of including microbial functional characteristics in modeling decomposition in the presence of plants.

The conceptual model we derived from our experimental results suggests that plants primarily modulate root litter decomposition processes by altering the genomic basis of the microbial mediation of decomposition. The model identifies two primary mechanisms by which



**Fig. 4.** Plant effect (%) on C degradation and osmotic stress genes. Data are shown as  $(\text{Signal}_{\text{planted}} - \text{Signal}_{\text{unplanted}}) / \text{Signal}_{\text{unplanted}} \times 100\%$ . Only genes with significantly abundance changes between planted and unplanted treatments are shown ( $n = 5$ ). Significant differences in gene signal intensity between planted and unplanted treatments were analyzed by Student's t-test, significance is indicated by stars (\* $P < 0.1$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ ).



**Fig. 5.** Graphical representation of the control of root litter decomposition by live roots. This depiction is derived from a quantitative model based on Mantel analysis of pair-wise correlations among root activity, environmental variables, microbial community variables and root litter decomposition rate. In addition, correlation between microbial functional potential and soil moisture alone was conducted. The thickness of connector lines indicates the significance (P value) of each correlation. Numerical values for each line indicate the Mantel correlation coefficients  $r$ ; correlation statistical significances are indicated by symbols (#:  $P < 0.1$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). Small arrows within text boxes indicate significant effects (increase or decrease) on environmental variables or microbial parameters by the presence of plant roots compared to unplanted treatment.

plant roots may decrease rates of root litter decomposition: 1) rhizosphere populations of bacteria and fungi may be more genetically capable of utilizing small molecular weight exudate materials and less likely to use complex macromolecules than populations not associated with roots; and 2) high rates of plant evapotranspiration and soil drying may diminish overall microbial functional potential and thus suppress decomposition activities. These mechanisms explain how the magnitude (and even direction) of plant-induced decomposition priming is

driven by root impacts on microbial functional genes. We suggest that a predictive understanding of root priming of decomposition processes in soil requires continued exploration and documentation of the relevant microbial functional genes.

**Conflicts of interest**

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2018.09.013>.

## References

- Allison, S.D., 2012. A trait-based approach for modelling microbial litter decomposition. *Ecology Letters* 15, 1058–1070.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32–46.
- Barnard, R.L., Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *The ISME Journal* 7, 2229–2241.
- Bird, J.A., Herman, D.J., Firestone, M.K., 2011. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. *Soil Biol Biogeochem* 43, 718–725.
- Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45, 115–131.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal* 6, 1621–1624.
- Castanha, C., Zhu, B., Hicks Pries, C.E., Georgioul, K., Torn, M.S., 2018. The effects of heating, rhizosphere, and depth on root litter decomposition are mediated by soil moisture. *Biogeochemistry* 137, 267–279.
- Cheng, W., Parton, W.J., Gonzalez-Meler, M.A., Phillips, R., Asao, S., McNickle, G.G., Brzostek, E., Jastrow, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. *New Phytologist* 201, 31–44.
- Cheng, W.Y., 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*. ASA-CSSA-SSSA, Madison, WI, pp. 119–143.
- Chigineva, N.I., Aleksandrova, A.V., Titunov, A.V., 2009. The addition of labile carbon alters litter fungal communities and decreases litter decomposition rates. *Applied Soil Ecology* 42, 264–270.
- Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* 339, 1615–1618.
- DeAngelis, K.M., Brodie, E.L., DeSantis, T., Andersen, G., Lindow, S., Firestone, M.K., 2009. Selective progressive response of soil microbial community to wild oat roots. *The ISME Journal* 3, 168–178.
- Dray, S., Dufour, A.B., 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* 22, 1–20.
- Dukes, J.S., Chiariello, N.R., Cleland, E.E., Moore, L.A., Shaw, M.R., Thayer, S., Tobeck, T., Mooney, H.A., Field, C.B., 2005. Responses of grassland production to single and multiple global environmental changes. *PLoS Biology* 3, e319.
- España, M., Rasche, F., Kandeler, E., Brune, T., Rodriguez, B., Bending, G.D., Cadisch, G., 2011. Identification of active bacteria involved in decomposition of complex maize and soybean residues in a tropical Vertisol using 15N-DNA stable isotope probing. *Pedobiologia* 54, 187–193.
- Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology* 71, 4117–4120.
- Finzi, A.C., Abramoff, R.Z., Spiller, K.S., Brzostek, E.R., Darby, B.A., Kramer, M.A., Phillips, R.P., 2015. Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Global Change Biology* 21, 2082–2094.
- Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. *Ecology Letters* 7, 314–320.
- Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994. Study of free and occluded particulate organic matter in soils by solid state <sup>13</sup>C Cp/MAS NMR spectroscopy and scanning electron microscopy. *Australian Journal of Soil Research* 32, 285–309.
- Harden, J.W., Hugelius, G., Ahlström, A., Blankinship, J.C., Bond-Lamberty, B., Lawrence, C.R., Loisel, J., Malhotra, A., Jackson, R.B., Ogle, S., Phillips, C., Ryals, R., Todd-Brown, K., Vargas, R., Vergara, S.E., Cotrufo, M.F., Keiluweit, M., Heckman, K.A., Crow, S.E., Silver, W.L., DeLonge, M., Nave, L.E., 2018. Networking our science to characterize the state, vulnerabilities, and management opportunities of soil organic matter. *Global Change Biology* 24, e705–e718.
- Harris, D., Porter, L.K., Paul, E.A., 1997. Continuous flow isotope ratio mass spectrometry of carbon dioxide trapped as strontium carbonate. *Communications in Soil Science and Plant Analysis* 28, 747–757.
- He, Z., Gentry, T., Schadt, C., Wu, L., Liebich, J., Chong, S., Huang, Z., Wu, W., Gu, B., Jardine, P., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *The ISME Journal* 1, 67–77.
- Herman, D.J., Halverson, L.J., Firestone, M.K., 2003. Nitrogen dynamics in an annual grassland: oak canopy, climate, and microbial population effects. *Ecological Applications* 13, 593–604.
- Hill, M.O., Gauch Jr., H.G., 1980. Detrended correspondence analysis: an improved ordination technique. *Vegetatio* 42, 47–58.
- Huo, C., Luo, Y., Cheng, W., 2017. Rhizosphere priming effect: a meta-analysis. *Soil Biology and Biochemistry* 111, 78–84.
- Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* 82, 666–677.
- Jackson, R.B., Lajtha, K., Crow, S.E., Hugelius, G., Kramer, M.G., Piñeiro, G., 2017. The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. *Annual Review of Ecology Evolution and Systematics* 48, 419–445.
- Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K., Kleber, M., 2015. Mineral protection of soil carbon counteracted by root exudates. *Nature Climate Change* 5, 588.
- Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. *Ecology* 86, 3252–3257.
- Kuzyakov, Y., 2002. Review: factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science* 165, 382–396.
- Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organic matter. *Soil Biol Biogeochem* 42, 1363–1371.
- Kuzyakov, Y., Hill, P., Jones, D., 2007. Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. *Plant and Soil* 290, 293–305.
- Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology* 2, 17105.
- Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O'Connor, M.I., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. *Nature Ecology & Evolution* 2, 936–943.
- Loya, W.M., Johnson, L.C., Nadelhoffer, K.J., 2004. Seasonal dynamics of leaf- and root-derived C in arctic tundra mesocosms. *Soil Biol Biogeochem* 36, 655–666.
- Luo, Y., Ahlström, A., Allison, S.D., Batjes, N.H., Brovkin, V., Carvalhais, N., Chappell, A., Ciais, P., Davidson, E.A., Finzi, A., Georgiou, K., Guenet, B., Hararuk, O., Harden, J.W., He, Y., Hopkins, F., Jiang, L., Koven, C., Jackson, R.B., Jones, C.D., Lara, M.J., Liang, J., McGuire, A.D., Parton, W., Peng, C., Randerson, J.T., Salazar, A., Sierra, C.A., Smith, M.J., Tian, H., Todd-Brown, K.E.O., Torn, M., van Groenigen, K.J., Wang, Y.P., West, T.O., Wei, Y., Wieder, W.R., Xia, J., Xu, X., Xu, X., Zhou, T., 2016. Toward more realistic projections of soil carbon dynamics by Earth system models. *Global Biogeochemical Cycles* 30, 40–56.
- Magid, J., Kjærsgaard, C., Gorissen, A., Kuikman, P.J., 1999. Drying and rewetting of a loamy sand soil did not increase the turnover of native organic matter, but retarded the decomposition of added <sup>14</sup>C-labelled plant material. *Soil Biol Biogeochem* 31, 595–602.
- Manzoni, S., Schimel, J.P., Porporato, A., 2011. Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology* 93, 930–938.
- Mason-Jones, K., Kuzyakov, Y., 2017. “Non-metabolizable” glucose analogue shines new light on priming mechanisms: triggering of microbial metabolism. *Soil Biology and Biochemistry* 107, 68–76.
- Oksanen, J.F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P.M., Stevens, H.H., Wagner, H., 2013. *Vegan: Community Ecology Package*. R Package Version 2.0-10.
- Placella, S.A., Brodie, E.L., Firestone, M.K., 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *Proceedings of the National Academy of Sciences of the U S A* 109, 10931–10936.
- Rasmussen, C., Southard, R.J., Horwath, W.R., 2007. Soil mineralogy affects conifer forest soil carbon source utilization and microbial priming. *Soil Science Society of America Journal* 71, 1141–1150.
- Saar, S., Semchenko, M., Barel, J.M., De Deyn, G.B., 2016. Legume presence reduces the decomposition rate of non-legume roots. *Soil Biology and Biochemistry* 94, 88–93.
- Schimel, J., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. *Frontiers in Microbiology* 3.
- Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry* 35, 549–563.
- Schimel, J.P., Gullledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. *Soil Biol Biogeochem* 31, 831–838.
- Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., Firestone, M.K., 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. *Ecology Letters* 19, 926–936.
- Shi, S., Nuccio, E.E., Herman, D.J., Rijkers, R., Estera, K., Li, J., da Rocha, U.N., He, Z., Pett-Ridge, J., Brodie, E.L., Zhou, J., Firestone, M., 2015. Successional trajectories of rhizosphere bacterial communities over consecutive seasons. *mBio* 6.
- Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C.,

- Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. *Ecology Letters* 11, 1252–1264.
- Su, P., Lou, J., Brookes, P.C., Luo, Y., He, Y., Xu, J., 2017. Taxon-specific responses of soil microbial communities to different soil priming effects induced by addition of plant residues and their biochars. *Journal of Soils and Sediments* 17, 674–684.
- Sylvia, D.M., Fuhrmann, J.J., Hartel, P., Zuberer, D.A., 2004. *Principles and Applications of Soil Microbiology*. Prentice Hall.
- Tu, Q., Yu, H., He, Z., Deng, Y., Wu, L., Van Nostrand, J.D., Zhou, A., Voordeckers, J., Lee, Y.-J., Qin, Y., Hemme, C.L., Shi, Z., Xue, K., Yuan, T., Wang, A., Zhou, J., 2014. GeoChip 4: a functional gene array-based high throughput environmental technology for microbial community analysis. *Mol Ecol Resour* 14, 914–928.
- Van der Krift, T.A.J., Kuikman, P.J., Berendse, F., 2002. The effect of living plants on root decomposition of four grass species. *Oikos* 96, 36–45.
- van der Wal, A., de Boer, W., 2017. Dinner in the dark: illuminating drivers of soil organic matter decomposition. *Soil Biology and Biochemistry* 105, 45–48.
- Xue, K., Wu, L., Deng, Y., He, Z., Van Nostrand, J., Robertson, P.G., Schmidt, T.M., Zhou, J., 2013. Functional gene differences in soil microbial communities from conventional, low-input, and organic farmlands. *Applied and Environmental Microbiology* 79, 1284–1292.
- Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., da Rocha, U.N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B.P., Firestone, M.K., Northen, T.R., Brodie, E.L., 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology* 3, 470–480.
- Zhu, B., Cheng, W., 2013. Impacts of drying–wetting cycles on rhizosphere respiration and soil organic matter decomposition. *Soil Biol Biogeochem* 63, 89–96.