# Rhizosphere Carbon Turnover from Cradle to Grave: The Role of Microbe–Plant Interactions

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**Abstract**

Plant roots are the primary source of organic materials that become stabilized in soil. While most root carbon is decomposed into carbon dioxide ($CO_2$), the remainder typically undergoes multiple microbial transformations before it forms longer-term associations with soil minerals. However, the mechanisms by which roots affect microbial utilization of organic materials and subsequent mineral stabilization processes are poorly understood. It is well known that living roots increase the biomass of nearby microbial communities, and shape their population dynamics, diversity, and interactions. Community assembly and metabolic potential of these rhizosphere-enriched microorganisms are strongly influenced by the chemical composition of the exudates released by the host plant. The root exudate pools of plants undergo compositional changes as they grow, reproduce, and senesce. In the well-studied annual grasses *Avena barbata* and *Avena fatua*, this changing rhizosphere substrate pool and the "bloom" of organisms that respond are phylogenetically coherent; Acidobacteria and Actinobacteria are consistently depleted, whereas Alpha and Betaproteobacteria and Bacteroidetes are reliably enriched. When compared to non-root-influenced bulk soils, the responsive community is predictably less taxon-rich, yet forms more complex networks. These rhizosphere dynamics have significant downstream effects on the colonization of nearby soil minerals, degradation of prior season's root litters, and the balance of stabilized versus lost soil carbon.

## 2.1 Introduction

Complex interactions among roots, soil microbes, and soil mineral surfaces play key roles in soil carbon (C) cycling. Decades of research have illustrated that root–microbe interactions facilitate plant immune responses and the acquisition of nutrients, water, and trace metals (Jones and Dangl 2006; Pii et al. 2015; Berg 2009; Colombo et al. 2014). However, the fact that roots are also precursors for most soil organic matter (SOM) and play a critical role in the broader soil C cycling by shaping soil microbial community assembly and dynamics is not well recognized. A holistic understanding of the pathways by which C moves from root tissues to the surrounding soil and is ultimately stabilized is essential before efforts are taken to improve plant nutrition, soil health, and manage terrestrial C sinks.

Plant roots are the primary source of organic C in soil (Rasse et al. 2005; Clemmensen et al. 2013; Austin et al. 2017; Jackson et al. 2017; Pett-Ridge and Firestone 2017; Sokol et al. 2018). While the soil surrounding plant roots may comprise only 1–2% of the total soil volume, this zone can provide 30–40% of the total soil organic carbon input (Grayston et al. 1996) and is a nexus for microbial C transformations. Microbial densities and activities are frequently up to ten times higher in the rhizosphere compared to surrounding bulk soil (Herman et al. 2006; Hawkes et al. 2007). This bloom of activity and biomass plays multifaceted roles in the soil C cycle. Primarily, this rhizosphere bloom contributes microbial biomass

(or better put "necromass"—cellular material consisting of dead cells and hyphae) and stimulates a cascade of interactions (among bacteria, archaea, fungi, fauna, and viruses) that consume organic materials and move C from the root biomass pool into $CO_2$, the dissolved organic carbon pool, and into the surrounding mineral soil, thus regulating how soil C is ultimately stabilized.

In this chapter, we study a series of recent results that illustrate the mechanisms of C flow between growing plant roots, soil microbial communities, and the surrounding mineral matrix. These studies describe the rhizosphere dynamics for two annual grasses (*Avena barbata* and *Avena fatua*), common to many Mediterranean systems with cool wet winters and hot dry summers. Our discussion covers the following topics:

- Measuring carbon fluxes in the rhizosphere of wildland annual grasses
- Rhizosphere microbial community succession
- Increasing network complexity in rhizosphere microbes
- Roles of rhizosphere communities in soil carbon cycling
- Roles of root metabolites and exudates
- Effects of elevated $CO_2$ ($eCO_2$) and root metabolites
- Role of soil moisture
- Downstream effects on soil carbon stocks and fluxes

## 2.2 Rhizosphere and Carbon Flux

In the past decade, it has become increasingly clear that microbial cells and their processes are central to the stabilization of soil carbon (Chenu and Stotzky 2002; Gleixner et al. 2002; Kögel-Knabner 2002; Kiem and Kögel-Knabner 2003; Dignac et al. 2005; Throckmorton et al. 2012). Typical carbon use efficiencies (the ratio of organic C allocated to growth versus the total amount assimilated) of soil microbes range from 0.1 to 0.8 (Steinweg et al. 2008; Manzoni et al. 2012; Blagodatskaya et al. 2014), indicating that for every C molecule consumed, a fraction is lost to respiration; the fraction that remains has the potential to become stabilized in the soil. Microbial communities play key roles in soil C stabilization: (1) they incorporate organic carbon into their cellular materials and products, which may subsequently become stabilized by mineral associations and (2) they supply enzymes that catalyze the decomposition and transformation of plant and soil C (Kögel-Knabner 2002). Due to the diversity of cell biomass composition and enzymatic strategies among soil microbial communities, it is likely that different microbial groups influence these two stabilization mechanisms in different ways. These factors are potentially amplified in the rhizosphere, where microbial taxa produce precursor molecules for stabilized SOM by transforming plant root exudates into large amounts of microbial biomass, and also mediate the breakdown of plant tissues and cell-derived macromolecules (Herman et al. 2006; DeAngelis et al. 2008; Sokol et al. 2018). In the rhizosphere and rhizoplane, dead root tissues are colonized by a succession of fungi, bacteria, and microfaunal communities, and commonly become

encased in protein- and polysaccharide-rich extracellular polymeric substances (Davidson et al. 2004). These materials, along with microbial cell necromass, are likely to be the molecular starting point for stabilized carbon. However, although the general importance of rhizosphere processes in soil C cycling is recognized (Finzi et al. 2015), we have a relatively poor understanding of how changes in rhizosphere microbial community composition and function ultimately affect C stabilization.

## 2.3 California Annual Grassland Soil Microbial Communities, an Ideal "Wild Model" System

The majority of rhizosphere studies have focused on model systems (e.g., *Arabidopsis thaliana*) and crop plants (maize, wheat, etc.). However, as a recent meta-analysis by Pérez-Jaramillo et al. (2017) indicates, the domestication process has significantly constrained the root microbiome of domesticated plants relative to their wild ancestors (Schlaeppi et al. 2014; Zachow et al. 2014; Bulgarelli et al. 2015; Pérez-Jaramillo et al. 2017). While the functional impact of these changes is not yet fully understood, focused studies suggest that the microbial taxa missing in crop plant rhizospheres may play critical roles, ranging from nutrient acquisition and plant growth promotion to disease protection (Kolton et al. 2012; Yin et al. 2013; Hartman et al. 2017). Thus, to study the mechanistic questions related to microbial community assembly or their functional roles in ecosystem processes, it is important to study wild plants, where evolutionary processes that have occurred in stable soil ecosystems are more likely to have developed adaptive soil microbial assemblages (Pett-Ridge and Firestone 2017). We find that California (CA) annual grasslands are an ideal "wild model system" to examine plant–microbe interactions.

For more than 20 years, our group has worked with annual grasses naturalized in CA grasslands, particularly the wild oat grasses *A. barbata* and *A. fatua*, and characterized these "wild model systems" along with the physical, chemical, and biological attributes of their soil habitat (Canals et al. 2003; Waldrop and Firestone 2004; DeAngelis et al. 2005; Hawkes et al. 2005, 2006; Waldrop and Firestone 2006a, b; DeAngelis et al. 2007, 2008, 2009; Eviner and Firestone 2007). The phenotypic and genotypic variabilities of both species are well described (Jain and Marshall 1967), and work by Nuccio et al. (2016) indicates that the rhizosphere bacterial communities of these two related grass species are extremely similar in CA grasslands. Both communities have an approximately 3-month growth period, occurring between January and April in the field. Additional studies have described the impact of climate on plant biochemistry, expression of genes coding for enzymes involved in photosynthesis, and plant N metabolism (e.g., rubisco *carboxylase/oxygenase*, pyruvate kinase, isocitrate dehydrogenase, glutamine and glutamate synthetase, and nitrate reductase) (Swarbreck et al. 2011a, b). Using seedstock of *Avena spp.* collected from wildland systems and combining the lineages with soils that have supported the growth of these wild plants for hundreds of years, our group has demonstrated that it is possible to conduct replicated, well-controlled experiments in a greenhouse setting (Fig. 2.1). To carry out multifactorial studies

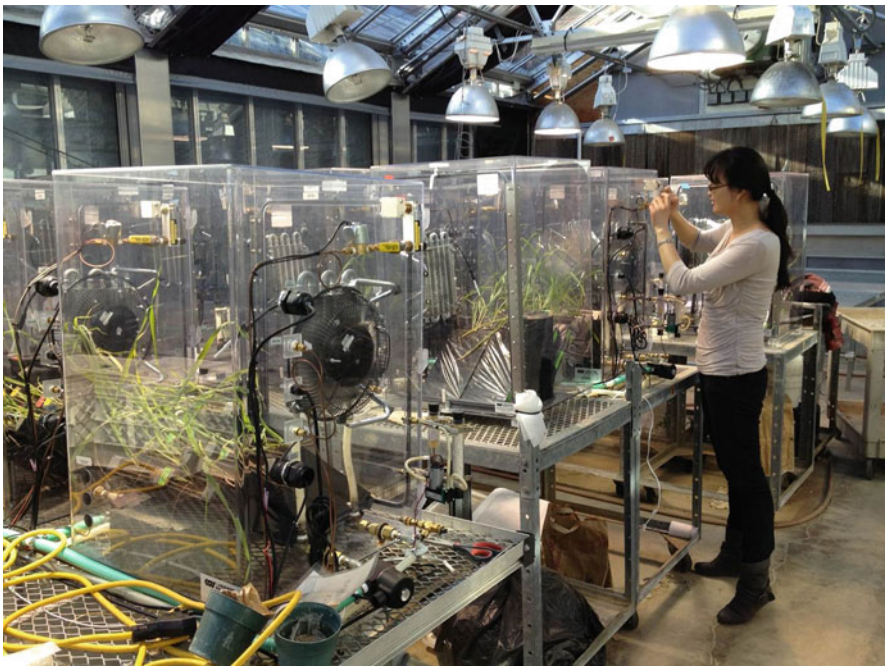

**Fig. 2.1** Plant $eCO_2$/isotope chambers at the EPIC facility at the University of California, Berkeley. The 16 replicate plant growth chambers pictured here are used for full-factorial experiments with controlled light, moisture, isotope, and atmospheric $CO_2$ concentrations

of rhizosphere dynamics, we typically use custom "rhizoboxes" with a removable, clear plexiglass sidewall that allows direct access to the rhizosphere (DeAngelis et al. 2009).

To fully understand the role of microbes in controlling soil C cycling, we have found stable isotope labeling to be a powerful tool that enables us to trace the trajectory of C transformation from "cradle to grave" (i.e., from atmospheric $CO_2$, to plant fixation, exudation, microbial uptake and turnover, and associations with mineral surfaces). Using multiple labeling chambers with automated controls for monitoring $^{13}CO_2$ or $^{12}CO_2$ concentrations, light intensities, temperature, moisture, and humidity is an ideal way to carry out such experiments and avoid pseudo-replication. We use a collection of 16 well-instrumented growth chambers (the Environmental Plant Isotope Chamber (EPIC) Facility at the University of California, Berkeley). Combined with the rhizobox containers mentioned above, this experimental system has enabled the replicated multifactorial studies of *Avena spp*. growth (with time, $eCO_2$, or litter/soil mineral additions) that we discuss below.

## 2.4 Rhizosphere Microbial Community Succession

Multiple studies indicate that microbial populations in the rhizosphere change dramatically and reproducibly as a plant grows, flowers, and senesces (Chaparro et al. 2014; Li et al. 2014; Donn et al. 2015; Edwards et al. 2015), implying that the root microbiome's relationship with its host plant is not static, but changes with time. However, the overarching significance of this functional and phylogenetic succession is not commonly recognized or understood. Particularly for annual plants, the distinction between rhizosphere and background bulk communities becomes more pronounced as the plants age. For example, Shi et al. (2015) observed a significant compositional succession with time in the rhizosphere microbiomes of *A. fatua* (Fig. 2.2); this pattern was remarkably consistent between one growing season and the next (Shi et al. 2015). Similar patterns have also been observed in *Zea mays*, *A. thaliana*, wheat, and rice (Chaparro et al. 2014, Li et al. 2014, Donn et al. 2015, Edwards et al. 2015). As plant roots develop, rhizosphere bacterial gene transcripts also change at different stages of plant development and in response to differences in the physicochemical environment (Nuccio et al. 2020; Shi et al. 2018; Yergeau et al. 2018; Chaparro et al. 2014). In *Avena,* for example, we find that microbial gene transcription changes more quickly than the overall community composition as roots grow (Nuccio et al. 2020). In addition, gene transcripts related to SOM decomposition and carbohydrate depolymerization are differentially affected in the rhizosphere versus bulk soil as the plant matures (Nuccio et al. 2020; Shi et al. 2018). The composition of both fungal communities and RNA viruses also changes as plant roots grow, although both appear to be more strongly affected by the presence of decaying roots than living roots (Nuccio et al. 2020; Starr et al. 2019).

For the *Avena spp.,* the root microbiome is a subset of taxa which are stimulated from the background soil community; our work suggests that roots stimulate or inhibit about 8% of the resident soil bacterial and archaeal communities (DeAngelis et al. 2009). While many of the bacterial populations affected by *Avena spp*. roots occur within phyla (e.g., Proteobacteria and Firmicutes), which are generally characterized as fast-growing bacteria (Madigan et al. 2010), other major root-responding taxa are commonly associated with slow growth and/or macromolecular decomposition in soil (e.g., some Actinobacteria, Verrucomicrobia) (DeAngelis et al. 2009). We observed that *Avena spp*. roots affect only a portion of the resident soil bacteria and archaea (DeAngelis et al. 2009); rhizosphere microbiome patterns, however, are shaped by both climate and edaphic variables in the grassland ecosystems where *Avena* grows (Nuccio et al. 2016).

The "bloom" of microorganisms that respond to growing *Avena spp.* roots exhibits phylogenetic coherence, with groups of related organisms responding similarly over time (Fig. 2.3). From a study on the rhizosphere microbial community development during root growth, Shi et al. (2015) observed that the relative abundance of many taxa from the Alpha and Betaproteobacteria responded positively to the presence of a root, while most Actinobacteria and Acidobacteria responded negatively. There were important exceptions—within the Actinobacteria, for example, some populations belonging to *Microbacteriaceae*, *Streptomyces*, and

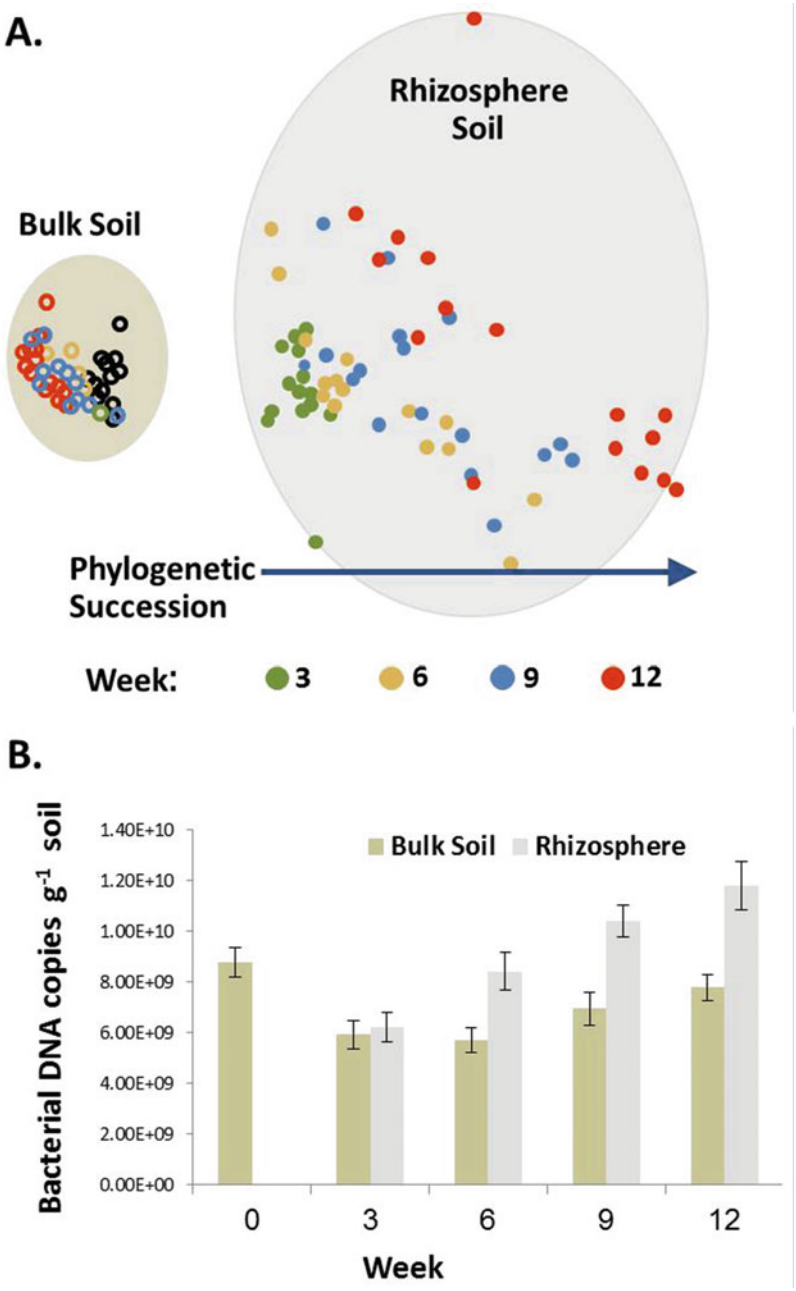


**Fig. 2.2** Compositional succession undergone by rhizosphere communities with the growth, senescence, and death of roots. (**a**) Ordination diagram illustrating temporal changes in bacterial community composition (Illumina sequencing, 16S rRNA gene) in rhizosphere versus bulk soils (based on data from Shi et al. (2015)). Microbial communities were assessed at 3, 6, 9, and 12 weeks during the lifespan of *A. fatua*. (**b**) Bacterial DNA copies per gram soil measured by qPCR (quantitative polymerase chain reaction)

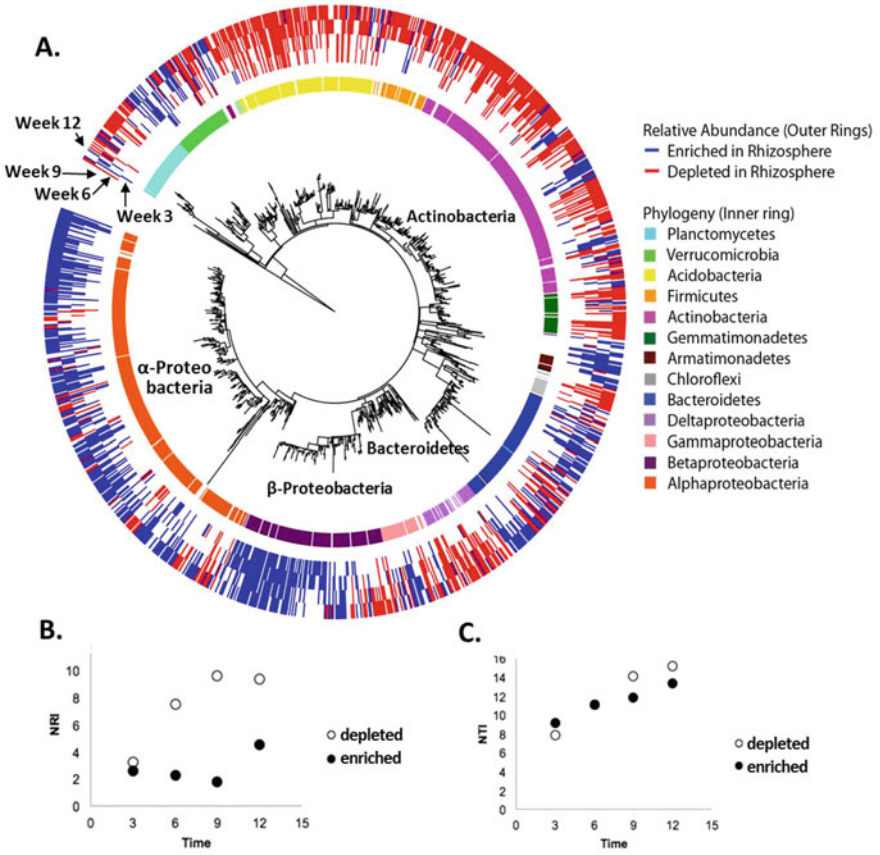


**Fig. 2.3** The rhizosphere-responsive communities of a wild annual grass are phylogenetically coherent. (**a**) Maximum likelihood tree depicting bacteria that were significantly enriched (blue) or depleted (red) in the *A. fatua* rhizosphere over time (outer rings: 3–12 weeks). The inner ring signifies the phylogenetic affiliation of operational taxonomic units (OTUs) organized by phylum. (**b**, **c**) Phylogenetic clustering of the rhizosphere-enriched and -depleted OTUs for each time point (3–12 weeks), as measured by net relatedness index (NRI) and nearest taxon index (NTI). Samples were collected during one growing season in an *A. fatua* greenhouse study with 16 replicates to document rhizosphere succession (Shi et al. 2015). Tree topology was calculated using Fasttree (Price et al. 2010) based a constraint tree as per Nuccio et al. (2016). The tree was visualized using the Interactive Tree of Life (ITOL) tool (Letunic and Bork 2011). The NRI and NTI for each time point were calculated using the R package picante (Kembel et al. 2010) and are presented in units of standard deviation (values >1.96 indicate significant phylogenetic clustering) (Vamosi et al. 2009)

*Catenulispora* appeared to prefer the rhizosphere to background soil. Overall, positive and negative responses to the root were phylogenetically clustered based on the net relatedness index (NRI) and nearest taxon index (NTI) (Webb et al. 2002), which likely reflect the phylogenetic evenness and clustering within community data. In this study, both indices were significantly positive at all time points (NRI, NTI $\geq$ 1.96), indicating clustering within both deep (NRI) and shallow (NTI)

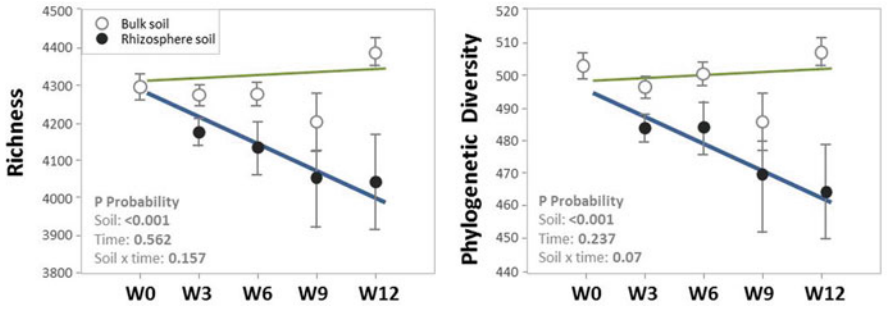


**Fig. 2.4** The diversity in bulk soil and rhizosphere microbial community associated with *A. fatua* are indicated by (**a**) OTU richness and (**b**) phylogenetic diversity (a measure of biodiversity which incorporates the phylogenetic differences between species) in rhizosphere and bulk soils across the stages of plant growth. Data are presented as mean ± standard errors ($n = 16$). The $P$ values calculated using ANOVA are shown in each figure. Data based on the large phylogenetic tree (Fig. 2.3) were used to calculate phylogenetic diversity (Faith's PD) using the generalized time reversible model in FastTree with a gamma branch-length correction (Price et al. 2010). The tree topology was constrained using a smaller tree composed of representatives for each family, where an OTU with a closely related full-length 16S sequence (97% similar) was selected for each family in the dataset (Nuccio et al. 2016). Faith's PD was calculated using alpha_diversity.py (QIIME 1.5dev) for the rhizosphere and bulk soils at weeks 0 (bulk only), 3, 6, 9, and 12

branches of the phylogenetic tree (Vamosi et al. 2009). Both the Shi et al. (2015) study and the Nuccio et al. (2016) study suggest that this phylogenetic coherence between the net positive and net negative root responses indicates an evolutionary adaptation of soil bacteria and the development of traits in individual populations that confer rhizosphere competence.

Community ecological factors, such as community assembly, diversity, and interactions, may also be affected by the growth of plant roots. In our studies of *Avena spp.*, we have found that rhizosphere bacterial community assembly coincides with increases in network size and complexity, and a concurrent decrease in richness and diversity (Shi et al. 2016). The positive change in bacterial co-occurrence network complexity indicates that root growth may progressively stimulate interactions within microbial communities or induce the development of shared niches as a plant matures (Shi et al. 2016). We saw some evidence for such interactions in our early *Avena spp.* studies, which suggest that co-occurring groups (modules) of Alphaproteobacteria interact via quorum signaling with homoserine lactone compounds near mature (12-week-old) roots (DeAngelis et al. 2007). Decreasing bacterial diversity over time with root growth is not surprising; if certain members of an assemblage increase in dominance and a constant mass of DNA is sampled, then the traditional richness (and diversity indices) will decline (Fig. 2.4).

Overall, our research using the *Avena spp.* "wild model" system indicates that rhizosphere microbiomes change in composition, function, and responses to plant exudates as plants mature (Bird et al. 2011; Shi et al. 2015; Zhalnina et al. 2018),

with increasing microbial network complexity, altered functional potential, and shifting viral–host linkages over time (DeAngelis et al. 2008; Shi et al. 2016; Nuccio et al. 2020; Starr et al. 2019). Together, these results imply that temporal changes in rhizosphere microbial composition and function may impact not only plant–microbe interactions but also the broader soil C cycle.

## 2.5 Role of Rhizosphere Communities in the Soil Carbon Cycle

It is generally accepted that decomposition of plant litter is mediated by a succession of soil microbial populations (Sylvia et al. 2004); however, the mechanisms underlying rhizosphere community succession and assembly, and their subsequent impact on C cycling are just beginning to be explored and connected. DeAngelis et al. (2009) showed that in the presence of *Avena spp.* roots, microbial community composition and C utilization patterns are significantly different from those in bulk soil. Subsequent studies assessing the microbial capability to breakdown complex C and N sources (using chitinases and proteases) have demonstrated enhanced activity in the rhizosphere and spatial differences within root zones (DeAngelis et al. 2009; Shi et al. 2015, 2016). An analysis of homoserine lactone signals suggests that density-dependent regulation is partially responsible for the enhanced capacity of the *Avena* rhizosphere community to break down macromolecular compounds (DeAngelis et al. 2008). Proteomics analyses indicate that rhizosphere bacteria actively synthesize proteins associated with sugar transport and utilization (Pett-Ridge and Firestone 2017), while research on specific root exudates, such as oxalic acid, suggests that some exudates may promote carbon loss by liberating organic compounds from protective mineral associations (Clarholm et al. 2015; Keiluweit et al. 2015). Metatranscriptomic analyses of soil from the *A. fatua* rhizosphere and near decaying roots indicate the development of distinct carbohydrate depolymerization microbial guilds based on shared gene expression over time, and suggest that a succession of microbial functions occurs as individual roots are colonized, age, and decay (Nuccio et al. 2020). Finally, although little is known about the ecology of bacteriophages or viruses of fungi and other eukaryotes in soil, Starr et al. (2019) found significant composition differences and temporal changes in both hosts and RNA viruses in a comparison of rhizosphere, decaying root and bulk soil habitats. Since viral replication can lead to host cell death and release of soluble carbon, virus-mediated lysis of bacterial and fungal cells may play a role in the redistribution of cellular debris and the ultimate fate of root-derived C. Taken together, these studies provide evidence that plant roots alter both resource availability and the ecology of soil microbial decomposers, and shape how plant C is processed.

Several of our studies with *Avena spp.* specifically address how rhizosphere microbial communities mediate the conversion of plant root litter to either SOM or $CO_2$. Using a broad-brush community characterization approach ($^{13}C$ PLFA-phospholipid fatty acid analysis), Bird et al. (2011) followed the decomposition of intact $^{13}C$-labeled *Avena spp.* roots for two subsequent growing periods after plant senescence. The $^{13}C$ (originating as root carbon) was observed in a succession of

microbial community components, and with time, different groups of soil organisms acted as the primary decomposers of the decaying root debris. The presence of actively growing root systems stimulated the movement of $^{13}C$ into Gram-positive and Actinobacteria groups, which are known for their oxidative enzyme capacities (Waldrop and Firestone 2004).

In a more recent study, Shi et al. (2018) followed the decomposition of $^{13}C$ root litter in the presence of an active *A. fatua* rhizosphere over two growing seasons. In this study, growing roots suppressed the rate of root litter decomposition and significantly affected the bacterial, archaeal, and fungal community composition. Ribosomal RNA gene copy numbers of these microbes were on average 20% higher in the presence of growing roots, affecting the relative abundance of at least nine bacterial phyla. Genetic potential measurements made with GeoChip functional gene arrays (He et al. 2007) showed that microbes living near plant roots had relatively more genes coding for low molecular weight compound degradation enzymes, whereas those from unplanted soil had relatively more macromolecular degradation genes (Shi et al. 2018). To evaluate how community structure, genetic potential, and environmental variables all interacted to control root litter decomposition, Shi et al. (2018) used a Mantel analysis to test for pair-wise correlations. The resulting model suggests that the primary impact of live roots on decomposition appears to result from an alteration of soil microbial functional gene profiles.

In a third study on the interaction between growing roots, decaying roots, and soil microbial communities, Nuccio et al. (2020) extracted gene transcripts (metatranscriptomes) from soil near live and decaying roots in microcosms containing *A. fatua*. Focusing on Carbohydrate-Active Enzymes (CAZyme) functional domains and enzymes involved in the degradation of macromolecular plant compounds, Nuccio et al. used a genome-centric approach to show that carbohydrate depolymerization was carried out by a series of microbial guilds with distinct spatial and temporal response patterns in different soil habitats (rhizosphere and detritusphere). These microbial guilds appear to specialize in their use of the different substrates made available by roots of different ages and decomposition stages. While these root substrates—exudates, mucilage, root hairs, and root biomass—are the initial sources of C that enter belowground food webs, the microbial transformation of this C is what determines whether it is retained as SOM or is returned back to the atmosphere.

## 2.6 Role of Root Exudates

About 30–60% of C assimilated by plants is transferred to roots (Lynch and Whipps 1990), and up to 50% is exuded into the rhizosphere in a range of forms (Table 2.1; van Dam and Bouwmeester (2016)). Many of the interactions between roots and the surrounding microbial community are accomplished through chemical communication driven by root exudates. These interactions have been implicated in plant defense (Baetz and Martinoia 2014), nutrient acquisition (Khorassani et al. 2011), and the regulation of soil bacterial and fungal community composition (Broeckling et al. 2008; Haichar et al. 2008; Shi et al. 2011). However, the mechanisms that

**Table 2.1** Commonly detected exudates of *A. barbata* and *A. fatua* measured from hydroponically grown plants, seedlings, and rhizosphere soil

| Class | Compound | Source |
|---|---|---|
| Sugars and derivatives ($n = 24$) | α-D-glucosamine phosphate, arabinose, arbutin, cellotetraose, D-threitol, fructose, galactonic acid, galactose, glucose, inositol, lyxose, maltose, myoinositol, *N*-acetyl-D-mannosamine, neohesperidin, rhamnose, ribitol, ribose, sorbitol, sorbose, sucrose, threonic acid, xylitol, xylose[a] | E, S, Z[b] |
| Carboxylic acids and derivatives ($n = 12$) | 2-Hydroxybutyric acid, 3-hydroxy-3-methylglutaric acid, α-ketoglutaric acid, *cis*-aconitic acid, fumaric acid, lactic acid, maleic acid, malic acid, malonic acid, oxalic acid, pyruvic acid, succinic acid | E, S, Z |
| Amino acids and derivatives ($n = 30$) | 2-Aminoisobutyric acid, 5-aminovaleric acid, alanine, arginine, asparagine, aspartic acid, cysteine, gamma-amino-*n*-butyric acid, glutamic acid, glycine, histidine, homoserine, isoleucine, L-citrulline, L-homoserine, L-hydroxyproline, L-pyroglutamic acid, leucine, lysine, methionine, *N*-acetylaspartic acid, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine | E, S, Z |
| Aromatic acids and derivatives ($n = 15$) | 2,3-Dihydroxybenzoic acid, 3-dehydroshikimic acid, 4-hydroxybenzoic acid, 4-hydroxyphenylpyruvic acid, benzoic acid, caffeic acid, cinnamic acid, ferulic acid, nicotinic acid, *p*-coumaric acid, phthalic acid, quinic acid, shikimic acid, syringic acid, vanillic acid | E, S, I, Z |
| Fatty acids and derivatives ($n = 12$) | Adipic acid, arachidic acid, elaidic acid, lauric acid, lignoceric acid, linoleic acid, methylhexadecanoic acid, oleic acid, palmitic acid, palmitoleic acid, pelargonic acid, stearic acid | E, S, Z |
| Sterols | Cholesterol | S |
| Glycerol and derivatives ($n = 3$) | Glycerol, glycerol-α-phosphate, glycerol-β-phosphate | S |
| Nucleosides and nucleotides ($n = 12$) | Adenine, adenosine, cytidine, deoxyguanosine, guanine, guanosine, hypoxanthine, inosine, thymidine, uracil, uridine, xanthine | E, S, Z |
| Plant hormones ($n = 4$) | Abscisic acid, indole-3-acetic acid, jasmonic acid, salicylic acid | Z |
| Betaines ($n = 6$) | Betonicine, carnitine, choline, glycine betaine, stachydrine, trigonelline | Z |
| Miscellaneous ($n = 14$) | 1,2,4-Benzenetriol, acetol, biotin, butyrolactam, D-lyxosylamine, dehydroabietic acid, pantothenic acid, riboflavin, sinapyl alcohol, syringylaldehyde, taurine, thiamine, urea, vanillin | E, S, I, Z |

[a]Exudates were measured by GC–MS, LC–MS, and/or high-performance liquid chromatography (HPLC)

[b]E—Estera (2017); S—Shi (unpublished); I—Iannucci et al. (2012); Z—Zhalnina et al. (2018)

underlie how root exudates influence microbe-mediated C cycling are complicated and difficult to study within an intact soil matrix. For example, the increased concentration of labile soil C near roots has been shown to both stimulate and repress soil organic carbon mineralization (Kuzyakov et al. 2000; Fontaine et al. 2007), and some studies suggest that exudates are just as likely to persist within soil as root tissue carbon (Sokol et al. 2018). One specific complication is the highly complex nature of root exudate compounds, which vary with plant genotype, root maturity, and in response to environmental stimulations (Jones 1998). Another difficulty is accurate characterization of exudate chemical composition because of the large background signal contributed by soil and microbial components (Kuzyakov and Domanski 2000).

Advances in sequencing approaches and high-resolution metabolite analysis have recently made it possible to measure direct links between specific exudate compounds and responses of specific microbial populations. It seems likely that the increased microbial activity and growth in the rhizosphere is fueled by root exudation patterns, which change in composition and abundance as plants grow. Our studies indicate that the chemical landscape of the *Avena spp*. rhizosphere, comprising osmolytes, fatty acids, senescence hormones, amino acids, sugars, and nucleotides (Table 2.1), changes during plant growth in a successional pattern (Fig. 2.5). Indeed, as community composition, richness, and microbe–microbe interactions are changing during the growth of an *Avena* plant, plant exudation profiles also shift in a remarkably similar manner (Fig. 2.5, Estera 2017).

Recent studies have identified direct predictive links between plant exudate composition and rhizosphere microbiome. Zhalnina et al. (2018) used a combination of comparative genomics and liquid chromatography–mass spectrometry (LC–MS)/MS exometabolite profiling of *Avena* root exudate consumption by sequenced bacterial isolates to show that developmental processes in *A. barbata* generated consistent patterns in root exudate composition. They showed that the chemical succession of *Avena* root exudates interacted with microbial metabolite substrate preferences (specifically for amino acids, osmolytes, and aromatics) that were predictable from the microbe's genome sequences. They hypothesized that the combination of plant exudation traits and microbial substrate uptake traits interacted to yield the patterns of microbial community assembly observed in the rhizosphere of this annual grass. Nuccio et al. (2020) show that, around older roots (that have ceased producing exudates and may have begun to senesce), distinct microbial populations (e.g., Streptomycetaceae and Catenulisporales from Actinobacteria) begin to have high d-CAZy gene transcription, expressing many enzymes involved in cellulose and xylose breakdown. Thus, it appears that temporal changes in root exudates over time and space may be directly linked to the successional changes in the rhizosphere microbial community identified by Shi et al. (2015) and may be the key determinants of soil C turnover.

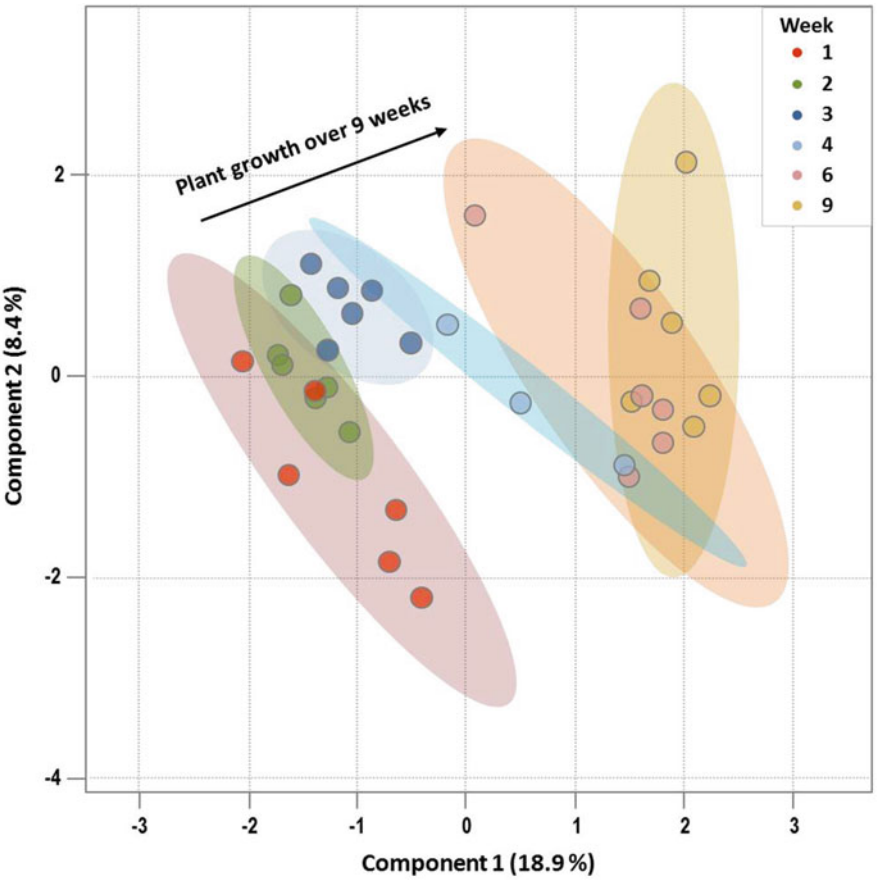


**Fig. 2.5** Plot of partial least squares discriminant analysis (PLS-DA) components 1 and 2 for metabolite samples collected over 9 weeks from a sterile plant growth experiment. Sterilized *A. barbata* seedlings were planted in sterile plant chambers (SPCs) with sterilized sand, and grown in either 400 ppm (ambient) or 700 ppm (elevated) $CO_2$ conditions. The pore space of the SPCs were fully drained and refreshed with diluted Hoagland solution once a week. SPCs were sampled at weeks 1, 2, 3, 4, 6, and 9 for root exudate profiles analyzed via gas chromatography–mass spectrometry (GC–MS). Metabolite abundances of identified GC-MS peaks were then normalized and analyzed via PLS-DA and ANOVA. Data were normalized from root exudate samples from weeks 1, 2, 3, 4, 6, and 9. There was a significant difference in the metabolic profiles over time, as plants grew, regardless of $CO_2$ treatment. Colors represent the different time points at which the samples were collected and circles represent the individual samples collected. Components 1 and 2 account for 27.3% of the variance in the dataset and are significant predictors of time. Ellipses indicate the 95% confidence interval for each sample grouping (#1–#9)

## 2.7 Effect of $eCO_2$ and Root Exudates

Elevated $CO_2$ can promote higher rates of photosynthesis and increased allocation of C to roots and various soil C pools (Table 2.2). In *Avena spp*., $eCO_2$ changes exudate composition and temporal patterns of exudation over time (Fig. 2.6). Hence $eCO_2$ studies provide a unique opportunity to assess the effects of altered root exudation patterns on microbial community succession and function, and in turn, how these population dynamics influence C transformations and stabilization processes. $eCO_2$ concentrations stimulate many plant responses and lead to higher rates of photosynthesis, increased belowground biomass production, and soil deposition of labile C (Hungate 1999; Liu et al. 2009; Phillips et al. 2011) as well as lower transpiration rates and potentially increased soil water content due to reduced stomatal conductance (Hungate 1999). Previous studies suggest that $eCO_2$ disproportionately affects root-associated microbial communities compared to those in the surrounding bulk soil (Drigo et al. 2008, 2009, 2010), and appears to consistently increase fungal populations in rhizosphere soil (Carney et al. 2007; Cheng et al. 2012; Drigo et al. 2013). In one study, $eCO_2$ increased both rhizosphere fungal populations and the activities of carbon decomposition enzymes, resulting in an overall loss of soil carbon (Carney et al. 2007).

However, the effect of $eCO_2$ on the temporal variation in soil and rhizosphere microbial communities, and the impact of $eCO_2$ on plant–microbe interactions (Drigo et al. 2010, 2013) remain poorly understood. These interactions may influence plant growth and net primary productivity by altering beneficial microbial colonization and/or pathogen infection. Therefore, it is important to examine the effect of $eCO_2$ on the abundance, composition, and function of rhizosphere microbial communities over time; the integration of such information could greatly improve the predictions of rhizosphere-driven C cycling.

From our research on *Avena spp.*, we have found that plants grown under elevated (700 ppm) $CO_2$ increased both C allocated belowground and the amount of root-

**Table 2.2** Root biomass and plant-derived soil carbon pools after growing *Avena spp*. for one season under $eCO_2$ and ambient $CO_2$ ($aCO_2$) conditions in $^{13}CO_2$ growth chambers

| Treatment | $aCO_2$-Planted | $eCO_2$-Planted | *P*-value |
|---|---|---|---|
| Root biomass (g) | 0.57 ± 0.03 | 0.88 ± 0.10 | **0.039** |
| Total belowground $^{13}C$ | 225.6 ± 20.0 | 266.5 ± 22.9 | **0.050** |
| $^{13}C$ soil excluding roots | 101.1 ± 12.9 | 153.1 ± 18.1 | **0.035** |
| $^{13}C$-fLF (μg C/g soil) | 79.9 ± 9.2 | 103.5 ± 12.9 | 0.275 |
| $^{13}C$-oLF (μg C/g soil) | 4.9 ± 1.0 | 7.5 ± 1.6 | 0.192 |
| $^{13}C$-HF (μg C/g soil) | 68.2 ± 8.6 | 112.5 ± 12.7 | **0.001** |

Total belowground $^{13}C$ is in μg $^{13}C$/g soil + roots. $^{13}C$ soil excluding roots is in μg $^{13}C$/g soil. $^{13}C$ associated with different soil fractions was measured by isotope ratio mass spectrometry (IRMS) following separation of soil into three fractions: free light fraction (fLF), occluded light fraction (oLF), and heavy fraction (HF), according to the established methods (Golchin et al. 1994; Bird et al. 2011). *P* values shown in bold indicate significant changes between $aCO_2$ and $eCO_2$ treatments ($P < 0.05$). Data are presented as mean ± standard errors ($n = 8$)

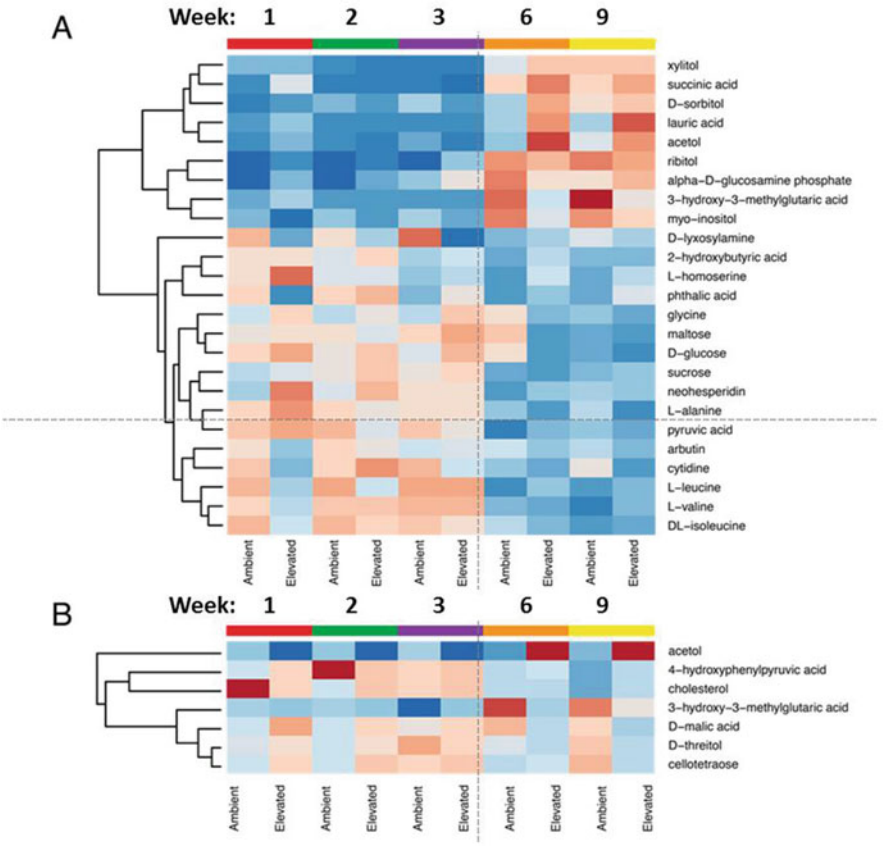


**Fig. 2.6** Heat maps and cluster trees of metabolites from a plant growth experiment where *A. barbata* was grown in sterile plant chambers (SPC). (**a**) Heat map of root exudate profiles using the top 25 metabolites that were most important in the projection of the plot from a partial least squares discriminant analysis (PLS-DA). Warm colors reflect a larger abundance of metabolites and cooler colors a decreased abundance. Heat maps and cluster trees were constructed using a Euclidean distance measure and ward clustering algorithm, respectively. Heat maps summarize the root exudate changes in each SPC sample over time. Specifically, root exudates produced during weeks 1, 2, and 3 have lower abundance that those produced during weeks 6 and 9. Conversely, some root exudates produced during weeks 6 and 9 are not produced during the earlier weeks of 1, 2, and 3. (**b**) Metabolite heat map and cluster tree showing autoscaled abundances for root exudates that are significantly different between $eCO_2$ and $aCO_2$ treatments as analyzed by a two-way ANOVA and Tukey's HSD (honestly significant difference) with $p < 0.05$. Out of 125 different metabolites detected from root exudate samples, only 7 were significantly different between the two $CO_2$ treatments. Trees show the degree of similarity among metabolites based on Euclidean distance, and metabolites are clustered to minimize the sum of squares

derived $^{13}C$ in the mineral-associated fraction of soil (Table 2.2). The increase in C associated with the soil mineral fraction ("heavy fraction") suggests a potential for increased stabilization of root C under $eCO_2$. In addition, metabolites produced in

early weeks of plant growth under $eCO_2$ conditions clustered distinctly from later produced metabolites (Fig. 2.6). Since we observed that $eCO_2$ both increased and decreased specific exudate components (Fig. 2.6), additional studies are needed to parse how these changes affect the long-term fate of plant-derived exudate C.

## 2.8 Role of Soil Moisture

Previous studies have reported a significant interaction between $eCO_2$ and gravimetric soil moisture (as well as N and P availability), possibly due to enhanced plant growth (Hu et al. 1999, 2001). Such $eCO_2$- and soil moisture-induced changes in C sources and soil microenvironments are likely to have a substantial influence on the composition and function of soil microbiota and consequently in mediating the ecosystem processes (e.g., C, N cycling) (Hungate et al. 1997; Cheng and Johnson 1998; Luo et al. 2006; Carney et al. 2007; Phillips et al. 2012).

Actively transpiring roots can impact soil C cycling processes by altering nearby soil water content. Castanha et al. (2018) report that *Avena spp.* caused increased decomposition of soil root detritus early in the growing season, when soil moisture was relatively high; however, as soil moisture levels declined, the plants suppressed decomposition rates of soil litter. In studies of *Avena spp*. we have found (not surprisingly) that rhizosphere soils have consistently lower soil moisture than unplanted soils (Shi et al. 2018; Nuccio et al. 2020) and this affects the rate of litter decomposition in the root zone versus the surrounding soil. The presence of plant roots also significantly increased the abundance of *pro*V and *pro*W, two common bacterial osmotic stress genes (He et al. 2007; Shi et al. 2018).

Altered bacterial community composition and bacterial and fungal functional gene profiles also accompany reduced water in rhizosphere soils (Webb et al. 2002). In CA annual grassland soils where *Avena spp.* grow, we have found that bacteria and fungi are differentially sensitive to soil moisture; bacteria tend to be substantially more sensitive and responsive to soil moisture than fungi (Barnard et al. 2013). These results suggest that bacterial communities in the rhizosphere may be differentially affected by the water stresses common in Mediterranean climate grasslands, likely impairing their metabolic activities and leading to downstream impacts on decomposition rates and rhizosphere C cycling.

## 2.9 Downstream Effects on Soil Carbon Stocks and Fluxes

Root-microbial dynamics have significant "downstream" effects on the soil C cycle, altering the amount and types of organic matter that become associated with mineral surfaces (Shi et al. 2018; Whitman et al. 2018), which may persist for long timescales. These effects can be measured by the extent of colonization of nearby soil minerals, decomposition of a prior season's root litter, and the balance of stabilized versus lost soil carbon. In a study where we incubated fresh minerals

(quartz, ferrihydrite, kaolinite) in the presence of an active *Avena spp*. rhizosphere, we found that both the quantity and composition of mineral-associated SOM were largely a factor of mineralogy and the influences of nearby roots (Whitman et al. 2018; Neurath, unpublished data). We also found significant differences in microbial community composition (16S rRNA and ITS) on different mineral types (Whitman et al. 2018). Because different microbial populations have different inherent ecophysiological traits (cell wall biochemistry, carbon use efficiency, growth rate) that can affect soil C persistence, the colonization patterns and habitat preferences of individual microbial populations may be foundational to the persistence of C entering soil via plant roots.

## 2.10 Conclusions

Interactions between plants and soil microorganisms are of primary importance to terrestrial ecosystem functions and particularly C cycling. Drawing heavily on the results from a "wild model" system, the common grass *Avena spp.* (wild oat) grown in CA annual grassland soils where it is ubiquitous, we summarize the important aspects of root–microbial interactions that have been commonly underappreciated, and provide the rough outlines of a mechanistic roadmap for how plant root C enters microbial and mineralized soil pools. Most of the root C entering soils returns to the atmosphere as $CO_2$, but a small portion becomes stabilized as longer-lived SOM. The actual path taken by each photosynthetically fixed plant C atom is a result of its consumption and use by bacteria, archaea, fungi, and viruses that make up the rhizosphere microbiome. Our results suggest that the sum of soil microbial ecophysiological traits (shaped by their phylogeny and defined by their genomes and gene expression) predict the fate of root C in soils when interpreted in the physicochemical soil–root environment. However, creating a predictive roadmap for the pathways taken by plant C as it enters the soil continues to be a long-term challenge for soil scientists.

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