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 material that becomes stabilized in soil. While most root carbon is decomposed to 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 exudates released by the host plant. A plant's root exudate pool undergoes compositional changes as the plant grows, reproduces and senesces. In the well-studied annual grasses *Avena barbata* and *A. fatua*, this changing rhizosphere substrate pool, and 'bloom' of organisms that responds, is phylogenetically coherent; Acidobacteria and Actinobacteria are consistently depleted, whereas Alpha- and Beta-proteobacteria 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 effect on the colonization of nearby soil minerals, degradation of prior season's root litter, and the balance of stabilized versus lost soil carbon.

X.1 Introduction

Complex interactions among roots, soil microbes, and soil mineral surfaces play key roles in the soil carbon (C) cycle. Decades of research have illustrated that root-microbe interactions facilitate plant immune responses and acquisition of nutrients, water, and trace metals (Jones and Dangl 2006, Pii et al. 2015, Berg 2009, Columbo et al. 2014). It is less well-recognized that roots are also precursors for most soil organic matter and play a critical role in the broader soil C cycle by shaping soil microbial community assembly and dynamics. A holistic understanding of the pathways by which C moves from root tissue to the surrounding soil and is ultimately stabilized, is an essential foundation for efforts to improve plant nutrition, soil health, and to ultimately manage terrestrial C sinks.

Plant roots provide 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 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, it contributes biomass (or better put, 'necromass' –the cellular materials 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 (DOC) pool, and into the surrounding mineral soil, thus regulating how soil C is ultimately stabilized.

In this chapter, we synthesize 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 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 and root metabolites
- Role of soil moisture
- Downstream effects on soil carbon stocks and fluxes

X. 2 **Rhizosphere and Carbon Flux**

Over 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, and the fraction that remains has the potential to become stabilized in soil. Microbial communities play key roles in soil C stabilization: 1) they incorporate organic carbon into their cellular materials and products--that 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 in 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 soil organic matter 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). All this material, along with microbial cell necromass, is likely to be the molecular starting point for stabilized carbon. However, although the general importance of rhizosphere processes for the soil C cycle 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.

X.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 (e.g. maize, wheat, etc.). However, as a recent meta-analysis by Perez-Jaramillo et al (2018) 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, plant growth promotion to disease protection (Kolton et al. 2012, Yin et al. 2013, Hartman et al. 2017). Thus, to study 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.

Over the past 20 + years, our group has worked with annual grasses naturalized in California grasslands, *Avena barbata* (slender wild oat) and *Avena 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, Hawkes et al. 2006, Waldrop and Firestone 2006a, Waldrop and Firestone 2006b, DeAngelis et al. 2007, Eviner and Firestone 2007, DeAngelis et al. 2008, DeAngelis et al. 2009). Phenotypic and genotypic variability of both grasses 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 (Nuccio et al. 2016). Both communities have an approximately three-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, nitrate reductase) (Swarbreck et al. 2011a, Swarbreck et al. 2011b). Using seedstock of *Avena spp.* collected from wildland systems and combining these lineages with soils that have supported 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. 1). To carry out multi-factorial studies 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 roles 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, to associations with mineral surfaces). Using multiple labeling chambers with automated controls for $^{13}CO_2$ 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 Facility, or 'EPIC' facility at University of California, Berkeley). Combined with the rhizobox containers mentioned above, this experimental system has enabled the replicated multi-factories studies of *Avena spp*. growth (with time, elevated CO_2, or litter/soil mineral additions) that we discuss below.

X.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. 2014, Edwards et al. 2015), implying that the root microbiome's relationship with the 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 plant ages. For example, in the rhizosphere microbiomes of *A. fatua,* Shi et al. (2015) showed a significant compositional succession with time (Fig. 2); this pattern was remarkably consistent from one growing season to the next (Shi et al. 2015). Similar patterns have also been observed in *Zea mays*, *Arabidopsis thaliana*, wheat and rice (Chaparro et al. 2014, Li et al. 2014, Donn et al. 2014, 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. 2019, Shi et al. 2018, Yergeau et al. 2018, Chaparro et al. 2014). In *Avena,* we find that microbial gene transcription changes more quickly than overall community composition as roots grow (Nuccio et al. 2019). In addition, gene transcripts related to soil organic matter decomposition and carbohydrate depolymerization are differentially affected in the rhizosphere versus bulk soil as the plant matures (Nuccio et al. 2019, 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. 2019, Starr et al. 2019).

For *Avena spp.,* the root microbiome is a subset of taxa which are stimulated from the background soil community; our work suggests roots stimulate or inhibit about 8% of the resident soil bacterial and archaeal community (DeAngelis et al. 2009). While many of the bacterial populations affected by *Avena spp*. roots occur within phyla (such as 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 emphasize that *Avena spp*. roots affect only a portion of

resident soil bacteria and archaea (DeAngelis et al. 2009); rhizosphere microbiome patterns 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. 3). In a study of rhizosphere microbial community development during root growth, Shi et al. (2015) showed that the relative abundance of many taxa from the Alphaproteobacteria and Betaproteobacteria responded positively to the presence of a root, while most Actinobacteria and Acidobacteria responded negatively. There are important exceptions—within the Actinobacteria, for example, some populations from the *Microbacteriaceae*, *Streptomyces*, and *Catenulispora* appeared to prefer the rhizosphere to background soil. Overall, positive and negative responses to the root are phylogenetically clustered according to 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 ≥ 1.96), indicating clustering within both deep and shallow branches of phylogenetic tree (NRI, NTI, respectively, Vamosi et al. 2009). Both the Shi et al. (2015) study and Nuccio et al. (2016) suggest that this phylogenetic coherence between net positive and net negative root response indicates 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 find 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 the plant matures (Shi et al. 2016). We see some evidence for such interactions in our earlier *Avena spp.* studies that 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 traditional richness (and diversity indices) will decline (Fig. 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 the plant matures (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. 2019, Starr et al. 2019). Together, these results imply that temporal changes in rhizosphere microbial composition and function may impact more than plant-microbe interactions, but also the broader soil C cycle.

X.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 follow-on impacts on C cycling are just beginning to be explored. In the presence of *Avena spp.* roots, DeAngelis et al. (2009) showed microbial community composition and C utilization patterns are significantly different than in bulk soil. 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, Shi et al. 2016). Analysis of homoserine lactone signals suggest 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) and 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 *Avena 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. 2019). Finally, although little is known about the ecology of bacteriophage 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 soil organic matter, or CO_2. Using a broad-brush characterization approach (^{13}C PLFA), Bird et al. (2011) followed the decomposition of intact ^{13}C labeled *Avena spp.* roots for two seasons after plant senescence and found the ^{13}C (originating as root carbon) in a succession of microbial community components over the two following seasons (Bird et al. 2011). Over time, different groups of soil organisms acted as the primary decomposers of the decaying root debris. The presence of an actively growing root system stimulated the movement of ^{13}C into Gram-positive and Actinobacteria groups, which have been associated with oxidative enzyme capacities (Waldrop and Firestone 2004).

In a more recent study, Shi and coworkers (2018) followed the decomposition of ^{13}C root litter in the presence of an active *Avena fatua* rhizosphere over two growing seasons. In this study, growing roots suppressed rates of root litter decomposition and significantly affected 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 macromolecule 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 alteration of the soil microbial functional gene profiles.

In a third study on the interaction between growing roots, decaying roots and soil microbial communities, Nuccio et al. (2019) extracted gene transcripts (metatranscriptomes) from soil near live and decaying roots in microcosms containing *Avena fatua*. Focusing on CAZyme functional domains and enzymes involved in the degradation of macromolecular plant compounds, they 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 have specialized for 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 source of carbon (C) that enters belowground food webs, it is the microbial transformation of this C that determines whether it is retained as soil organic matter (SOM) or returned back to the atmosphere.

X.6 The 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 1; and (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 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 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 the response 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 a plant grows. Our studies indicate that the *Avena spp*. rhizosphere's chemical landscape, comprising osmolytes, fatty acids, senescence hormones, amino acids, sugars, and nucleotides (Table 1), changes during succession (Fig 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 5, Estera 2017).

Recent studies have identified direct predictive links between plant exudate composition and the rhizosphere microbiome. Zhalnina and coworkers (2018) used a combination of comparative genomics and 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. Around older roots (that have ceased producing exudates and may have begun to senesce), Nuccio et al. (2019) show that distinct microbial populations (e.g. Streptomycetaceae and Catenulisporales from the 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 key determinants of soil C turnover.

Table 1 Commonly detected exudates of *Avena barbata* and *Avena 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, myo-inositol, N-acetyl-D-mannosamine, neohesperidin, rhamnose, ribitol, ribose, sorbitol, sorbose, sucrose, threonic acid, xylitol, xylose*	E, S, Z**
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,	E, S, Z

	lysine, methionine, N-acetylaspartic acid, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine	
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

*Exudates were measured by gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS) and/or high-performance liquid chromatography (HPLC)

**E – Estera 2017; S - Shi, unpublished; I - Iannucci et al. 2012; Z - Zhalnina et al. 2018

X. 7 Effects of Elevated CO_2 and Root Exudates

Elevated CO_2 (eCO_2) can promote higher rates of photosynthesis and increased allocation of C to roots and various soil C pools (Table 2). In *Avena spp*., elevated CO_2 changes exudate patterns and temporal patterns of exudation over time (Fig 6). Hence eCO_2 studies provide a unique opportunity to assess 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. Elevated CO_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 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 also 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 impacts of eCO_2 on plant–microbe interactions (Drigo et al. 2010, 2013) remain poorly understood. These interactions may influence plant growth and subsequently 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 predictions of rhizosphere-driven C cycling.

In our research with *Avena spp.*, we have found that plants grown under elevated (700 ppm) CO_2 increased both C allocated belowground and the amount of root-derived ^{13}C in the mineral-associated fraction (Table 2). The increase in C associated with the soil mineral fraction ('heavy fraction'), suggests the 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. 6). Since eCO_2 both increased and decreased specific exudate components (Fig. 6), additional work is needed to parse how these changes affect the long-term fate of plant-derived exudate C.

Table 2 Root biomass and plant-derived soil carbon pools after growing *Avena spp*. for one season under elevated CO_2 (eCO_2) and ambient CO_2 (aCO_2) conditions in $^{13}CO_2$ growth chambers. 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 established methods (Golchin et al. 1994, Bird et al. 2011). *P* values shown in bold indicate significant changes between ambient and eCO_2 treatments ($P < 0.05$). Data are presented as means ± standard errors (n =8).

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**

X. 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, Hu et al. 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 mediated 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 cycle 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, where soil moisture was relatively high, but as soil moisture levels declined, the plants suppressed decomposition rates of soil litter. In our group's studies with *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. 2019) and this affects rates 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, which are two common bacterial osmotic stress genes (He et al. 2007).

Altered bacterial community composition and bacterial and fungal functional gene profiles also accompany reduced water in rhizosphere soil (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, and that bacteria tend to be substantially more sensitive and responsive to soil moisture than fungi (Barnard et al. 2013). Taken together, these results suggest bacterial communities in the rhizosphere may be

differentially affected by the water stresses common in Mediterranean climate grassland, likely impairing their metabolic activities and leading to downstream impacts on decomposition rates and rhizosphere C cycling.

X.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 in the extent of colonization of nearby soil minerals, decomposition of a prior season's root litter, and the balance of stabilized vs 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 both the quantity and composition of mineral-associated SOM was largely a factor of mineralogy and influence of nearby roots (Whitman et al. 2018). 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.

X.10 Conclusions

Interactions between plants and soil microorganisms are of primary importance to terrestrial ecosystem functions and particularly the cycling of C. Drawing heavily on 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 critically important aspects of root-microbial interactions that have been commonly underappreciated and provide rough outlines of a mechanistic roadmap for how plant root C enters microbial and mineral soil pools. Most root C entering soil returns to the atmosphere as CO_2, but a small portion becomes stabilized as longer-lived soil organic matter. 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 show 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 soil when interpreted in the physical-chemical soil-root environment. However, creating a predictive roadmap for the pathways plant C takes as it enters soil continues to provide a long-term challenge for soil scientists.

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Figure Captions:

Fig. 1 Plant eCO_2/isotope chambers at the Environmental Plant Isotope Chamber Facility (EPIC) at University of California, Berkeley. The sixteen replicate plant growth chambers pictured here are used for full-factorial experiments with controlled light, moisture, isotope, and atmospheric CO_2 concentrations.

Fig. 2 Rhizosphere communities undergo compositional succession as roots grows, senesce, and die. 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. (Shi et al. 2015)). Microbial communities were assessed at 3, 6, 9, and 12 weeks during the lifespan of *Avena fatua*. B. Bacterial DNA copies per gram soil measured by qPCR.

Fig. 3 The rhizosphere-responsive communities of a wild annual grass are phylogenetically coherent. (A) Maximum likelihood tree depicting bacteria that are significantly enriched (blue) or depleted (red) in the *Avena fatua* rhizosphere over time (outer rings: 3-12 weeks). The inner ring signifies the phylogenetic affiliation of the OTUs by phylum. B) 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 over one 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 (Nuccio et al. 2016). The tree was visualized using ITOL (Letunic and Bork 2011). 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).

Fig. 4 The diversity of bulk soil and rhizosphere microbial community associated with *Avena fatua* as indicated by a). OTU richness and b). phylogenetic diversity (a measure of biodiversity which incorporates phylogenetic difference between species) in rhizosphere and bulk soils across plant growth stages. Data are present as means ± standard errors (n=16). ANOVA P probability values are shown in each figure. Data were generated based on the large phylogenetic tree (Fig. 3) used to calculate phylogenetic diversity (Faith's PD) was created 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.

Fig. 5 Plot of a partial least squares discriminate analysis (PLS-DA) components 1 and 2 for elevated and ambient metabolite samples collected over 9 weeks from a sterile plant growth experiment. Sterilized *Avena 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 Hoaglands solution once per week. SPCs were sampled at weeks one, two, three, four, six, and nine for root exudate profiles and analyzed via GC-MS. Metabolite abundances of identified peaks from GC-MS were then normalized and analyzed via PLS-DA and ANOVA. Data was normalized from root exudate samples from weeks one, two, three, four, six and nine. There was a significant difference in the metabolic profiles over time, as the plant grew, regardless of CO_2 treatment. Colors represent different timepoints collected and circles represent 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).

Fig. 6 Heat maps of with metabolite cluster trees from the sterile plant growth experiment where *A. barbata* was grown in sterile plant chambers (SPCs). **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 discriminate analysis (PLS-DA VIP). Warm colors reflect a larger abundance of a metabolite and cooler colors reflect a decreased abundance. Heat map and cluster tree constructed using a Euclidean distance measure and ward clustering algorithm. Heat map summarizes the root exudate changes in each SPC sample over time. Specifically, root exudates produced in weeks one, two and three are in less abundance in weeks six and nine. Conversely, root exudates produced in weeks six and nine are not produced in the

earlier weeks of one, two, and three. **B.** Heat map with metabolite cluster tree showing auto scaled abundances for root exudates that have a significant difference detected between elevated and ambient CO_2 treatments as analyzed by a 2-way ANOVA and Tukey's HSD with a $p < 0.05$. Out of 125 different metabolites detected from root exudate samples, only seven metabolites were found to be significantly different between the two CO_2 treatments. Tree shows degree of similarity among metabolites based on Euclidean distance and metabolites are clustered to minimize sum of squares.

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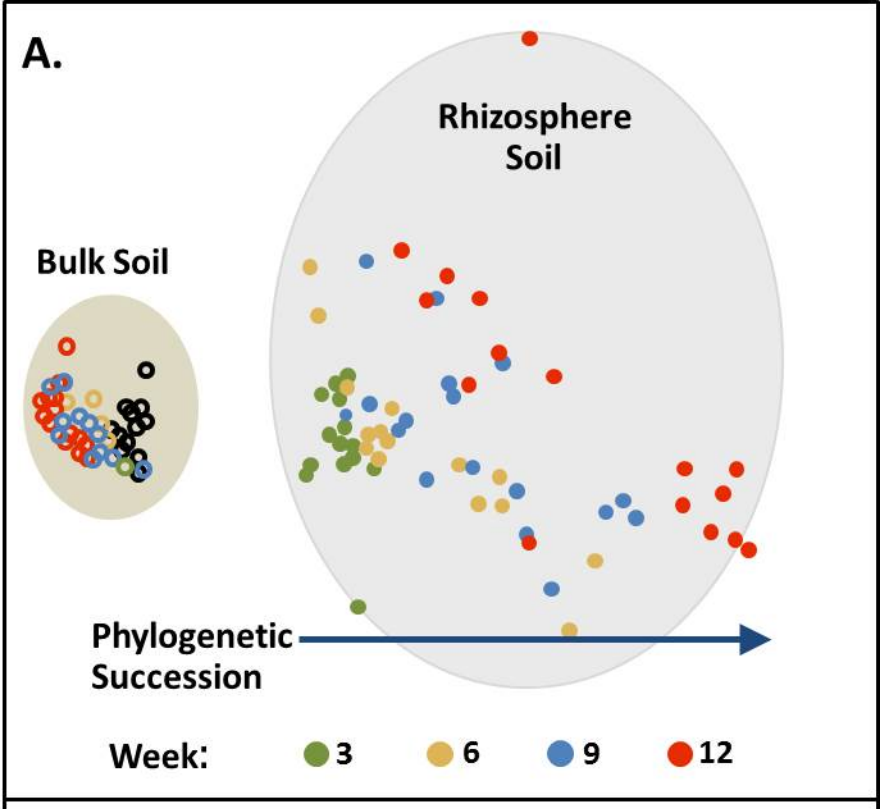
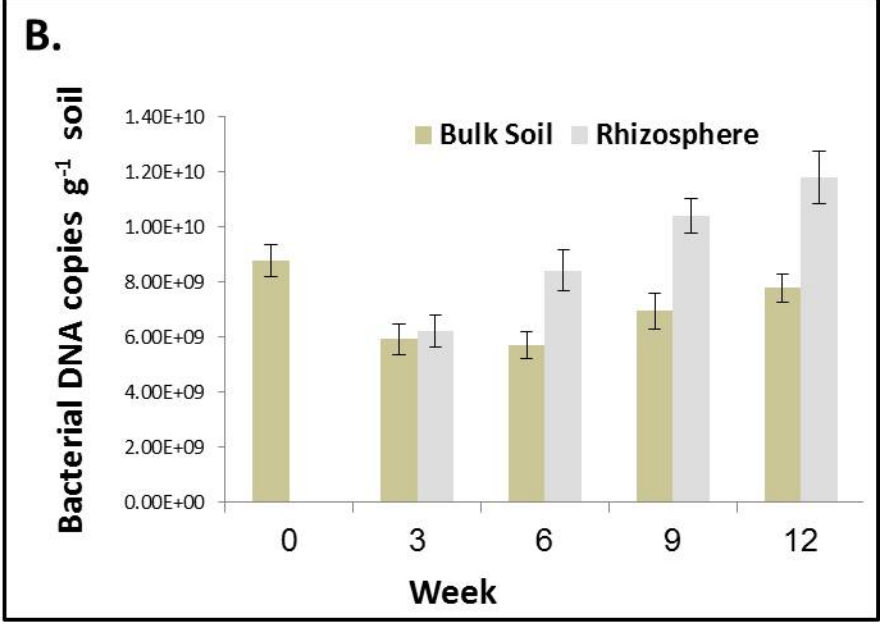
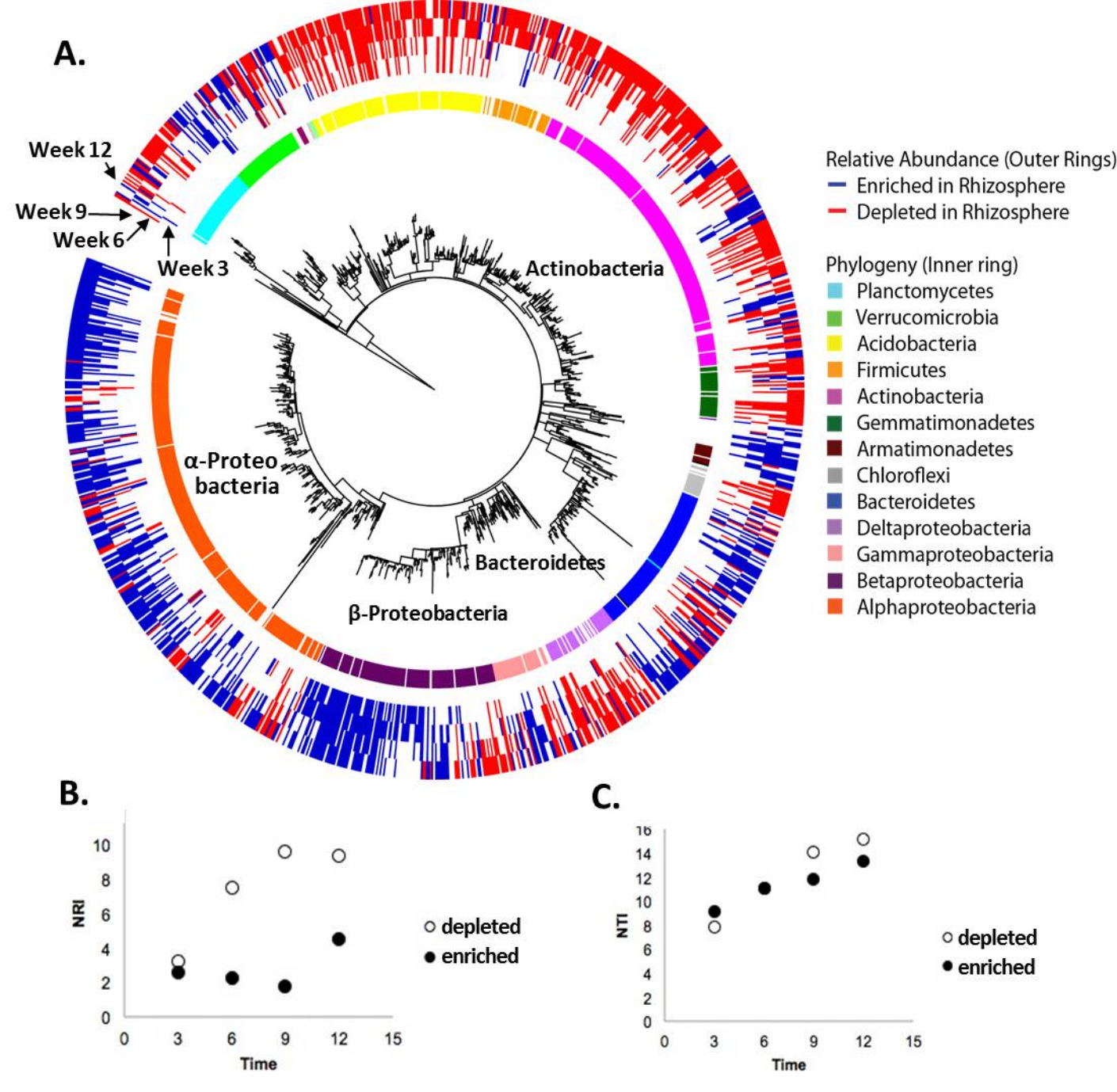
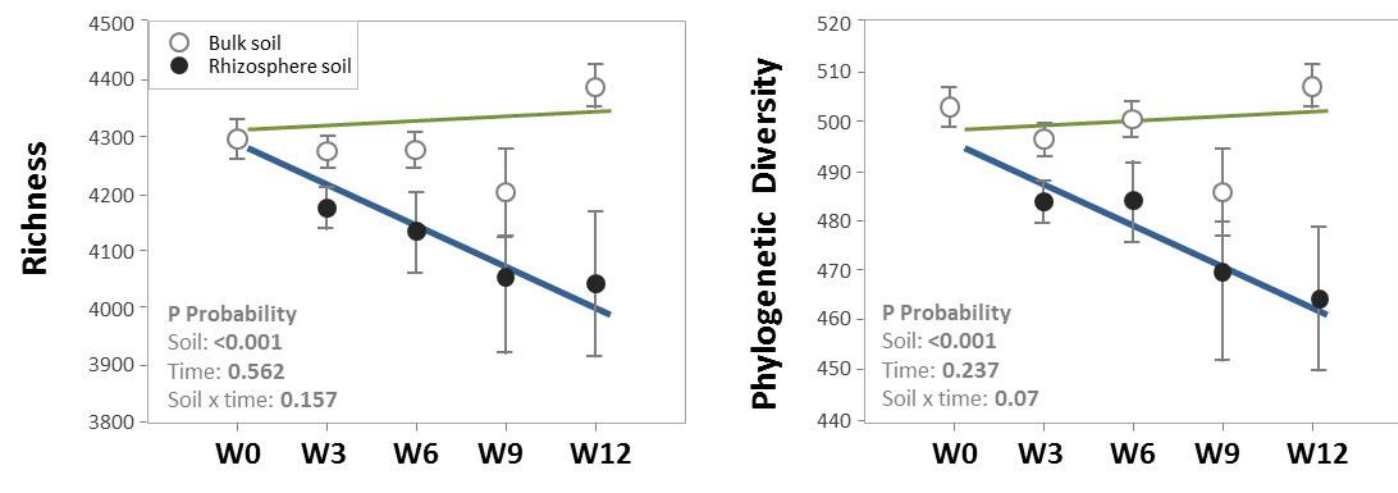
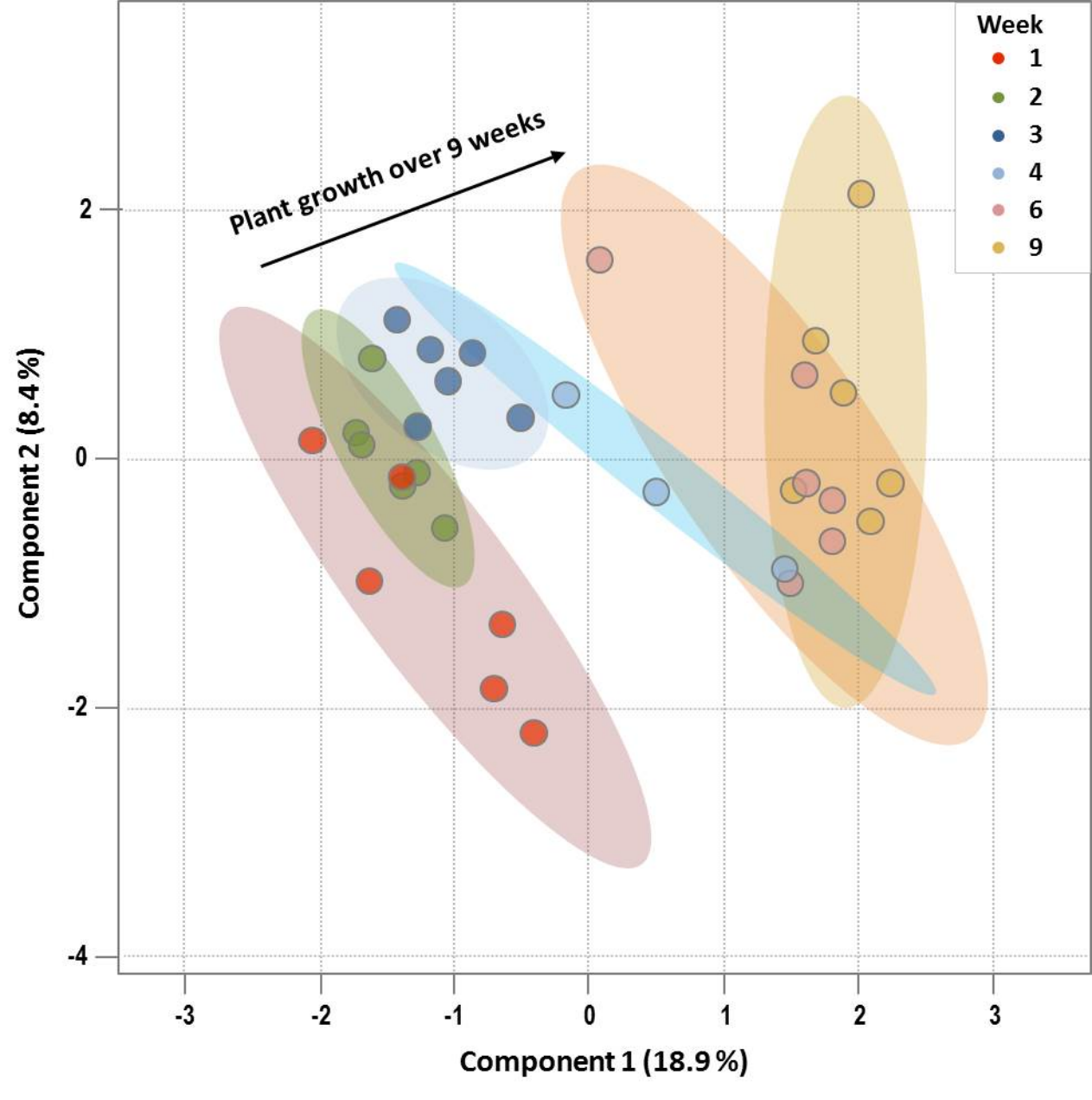
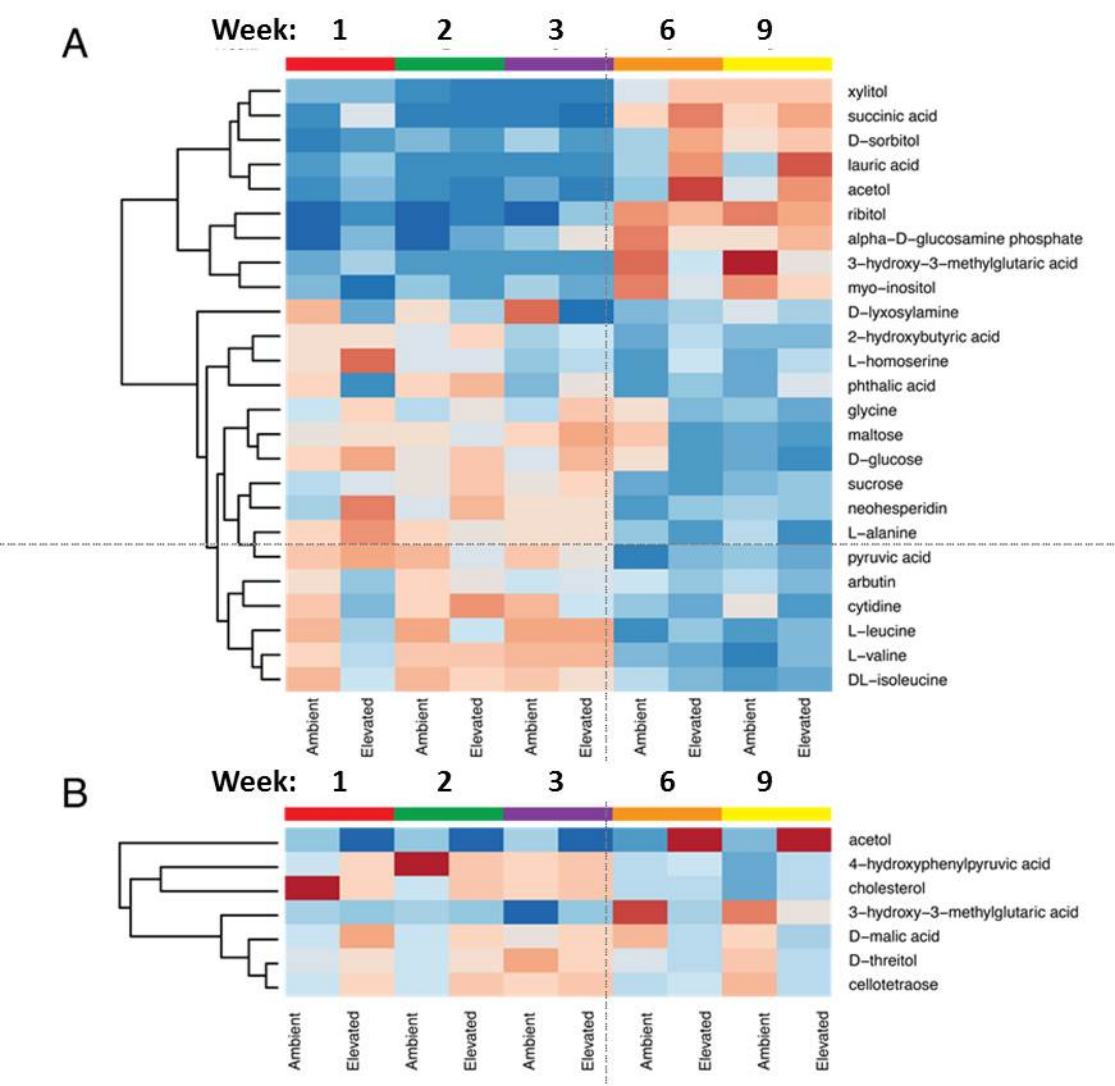

A.
Rhizosphere Soil
Bulk Soil
Phylogenetic Succession
Week:
3
6
9
12


B.
Bacterial DNA copies g-1 soil
1.40E+10
1.20E+10
1.00E+10
8.00E+09
6.00E+09
4.00E+09
2.00E+09
0.00E+00
Bulk Soil
Rhizosphere
0
3
6
9
12
Week


A.
Week 12
Week 9
Week 6
Week 3
Actinobacteria
α-Proteo bacteria
Bacteroidetes
β-Proteobacteria
Relative Abundance (Outer Rings)
Enriched in Rhizosphere
Depleted in Rhizosphere
Phylogeny (Inner ring)
Planctomycetes
Verrucomicrobia
Acidobacteria
Firmicutes
Actinobacteria
Gemmatimonadetes
Armatimonadetes
Chloroflexi
Bacteroidetes
Deltaproteobacteria
Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
B.
NRI
Time
depleted
enriched
C.
NTI
Time
depleted
enriched


Richness
Bulk soil
Rhizosphere soil
4500
4400
4300
4200
4100
4000
3900
3800
P Probability
Soil: <0.001
Time: 0.562
Soil x time: 0.157
W0
W3
W6
W9
W12
Phylogenetic Diversity
520
510
500
490
480
470
460
450
440
P Probability
Soil: <0.001
Time: 0.237
Soil x time: 0.07
W0
W3
W6
W9
W12


Week
1
2
3
4
6
9
Plant growth over 9 weeks
Component 2 (8.4 %)
Component 1 (18.9 %)
2
0
-2
-4
-3
-2
-1
0
1
2
3


A
Week: 1 2 3 6 9
xylitol
succinic acid
D−sorbitol
lauric acid
acetol
ribitol
alpha−D−glucosamine phosphate
3−hydroxy−3−methylglutaric acid
myo−inositol
D−lyxosylamine
2−hydroxybutyric acid
L−homoserine
phthalic acid
glycine
maltose
D−glucose
sucrose
neohesperidin
L−alanine
pyruvic acid
arbutin
cytidine
L−leucine
L−valine
DL−isoleucine
Ambient
Elevated
Ambient
Elevated
Ambient
Elevated
Ambient
Elevated
Ambient
Elevated
B
Week: 1 2 3 6 9
acetol
4−hydroxyphenylpyruvic acid
cholesterol
3−hydroxy−3−methylglutaric acid
D−malic acid
D−threitol
cellotetraose
Ambient
Elevated
Ambient
Elevated
Ambient
Elevated
Ambient
Elevated
Ambient
Elevated