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Abstract:

The soil environment contains the largest pool of carbon on Earth, with controls on soil carbon residency and flux being an emergent property of microbial metabolism. Despite the fact that microbial interactions have metabolic implications, the contribution of interactions are often overlooked regarding the carbon cycle. Here, we hypothesize that microbial interactions are intrinsically coupled to carbon cycling through eco-evolutionary principles. Interactions drive phenotypic responses that result in allocation pattern shifts and changes in carbon use efficiency. These changes promote alterations in resource availability and community structure, thereby creating selective pressures that contribute to diffuse evolutionary mechanisms. The outcomes then feed back into microbial metabolic operations with consequences for carbon turnover, continuing a feedback loop of microbial interactions, evolutionary processes, and the carbon cycle.

1.1 Introduction

Soil holds the largest store of carbon on Earth, estimated to be >2300 Pg C (Jobbágy & Jackson 2000). Flux rates of carbon from the soil exceed anthropogenic emissions by up to ten times (Chapin, Matson & Mooney, 2002). Owing to the scale of soil carbon inputs into the atmosphere, and major concerns over human disruption of the carbon cycle, it is important to understand the drivers of the soil carbon flux. In particular, microbial communities are known to influence rates of carbon cycling, yet microbial processes that govern the turnover of carbon in the soil are not fully understood (Prosser 2012).

Microbial processes have been difficult to study owing to the microscale at which they take place, the spatial and temporal fluctuation of conditions in the soil, and the incredible diversity of interacting organisms and abiotic parameters. With advancements in molecular tools, the diversity of the soil biota

and its associated carbon cycling potential have become more resolved. Yet less attention has focused on how ecological factors influence the evolution and phenotypic expression of microbial traits that affect carbon cycling in the soil environment. In this chapter, we will focus specifically on the impact of microbial interactions on traits involved with carbon cycling.

The soil environment presents challenges for microbial survival, which has driven the evolution of many extracellular metabolites. Microbes must protect themselves from predators, resist abiotic assaults, compete effectively and function under feast or famine conditions. Extracellular enzymes breakdown complex nutrients in the environment into useable forms for microbes. Toxins attack predators and competitors for nutrients. Biofilm polymeric substances protect microbes against desiccation and antibiotics, and slows diffusion of nutrients away from the producing cells. At the same time, thermodynamic limitations of cellular metabolism constrain microbes in the amount of products they are able to generate per given amount of time and unit of energy (Koch, 1985). Varying life history strategies have evolved to increase fitness according to persistent selective pressures, with these strategies constraining how a cell uses its resources.

Any pressures that shift the cellular balance away from reproduction act as selective forces specific to the microbial environment. Therefore, the higher the incurred cost to fitness and the longer it occurs, the more likely a change in allocation will lead to evolutionary changes. Presumably, costly traits such as production of extracellular goods, will be maintained if the benefit outweighs the fitness cost. Benefits to a producing cell may be direct, as is the case with enzymes that scavenge high energy resources, or indirect, such as a reduction in competition for resources. Selection also acts on multiple levels, from genes to communities, with varying outcomes (Boyle et al. 2012).

While many stressors of the soil environment have been explored in terms of their impact on carbon cycling, microbial interactions have not been widely addressed in this capacity. Here, interactions will be defined as processes driven by one microbe that have either positive or negative effects on survival and reproduction of one or more other microbes. We will focus on interactions that influence phenotypic expression and genotypic capacity of traits with consequences for carbon cycling. For example, microbial traits affect decomposition and transformation of soil organic matter and rates of soil heterotrophic respiration. Microbial interactions act as pressures that shift the cellular allocations underlying these processes by altering fitness cost:benefit ratios.

Beyond changes in allocation, microbial interactions also may influence community structure. Community structure may be important to carbon cycling if organisms show inter-taxa variation in their capacity for C cycling and if the breakdown of carbon is limited by cellular processes (Schimel & Schaeffer 2012). Indeed, there is extensive evidence that changes in microbial community structure have impacts on carbon turnover (Balser & Firestone 2005; Matulich & Martiny 2014). More broadly, changes in diversity are often linked to altered functioning (Tilman et al. 2009; Bell et al. 2005). Interactions that alter diversity, such as niche partitioning, or prevent competitive exclusion, such as non-transitive interaction networks and negative frequency-dependent selection, therefore, will have effects on community functioning.

Controls on carbon cycling are an emergent property of microbial interactions in the soil, constrained by metabolism and eco-evolutionary principles. Microbial interaction networks therefore cannot be decoupled from the soil carbon cycle. The purpose of this chapter is to explore the implications of microbial interactions in soil carbon cycling (Fig. 1). We hypothesize that changes in allocation patterns resulting from interactions will lead to both ecological and evolutionary consequences for carbon cycling. Furthermore, we hypothesize that microbial interactions have important ramifications for

community structure that feed into its associated function, defining rates of cycling and responses to peturbations. In addition, the effect of microbial interactions on soil carbon flux has relevance across multiple spatial and temporal scales, including the global scale over decades to centuries.

1.2 Allocation Patterns

Metabolic theory posits that the biochemistry and physiology that define metabolism constrains the uptake, transformation and secretion of energy and matter, as well as the rates of these processes. Metabolic control over energy and matter fluxes dictates ecological interactions among organisms and continues up to the collective nutrient cycling at ecosystem levels (Brown et al. 2004; Elser 2006). It is well established that microbial metabolism is fundamental to biogeochemical cycling (Falkowski et al. 2008).

Metabolism constrains life history strategy. Life history strategies are a set of defined traits that have evolved together or have been integrated through horizontal gene transfer, and allow persistence in specific environments. Metabolism sets limits on energetic capacity, stoichiometric demands, flux rates and allocation of resources, and the ability of a microbe to acquire, maintain, and transcribe genes for production of functional molecules that comprise a life history strategy. Though there is evidence of horizontal transfer of major metabolic pathways in microbes (Falkowski et al. 2008), the more complex pathways may be difficult to transfer because of their multi-gene nature and incongruity with preexisting pathways (Schimel & Schaeffer 2012). This has likely led to the deeply conserved nature of these metabolic units (Martiny et al. 2013).

The acquired combination of traits associated with a life history strategy frames the way that microbes can respond to environmental cues and interactions with other individuals, as well as the impact this may have on soil carbon cycling. Many of the horizontally transferred genes make up the secretome of soil microbes, and consequently have significance for soil organic matter (SOM) degradation and interactions with other microbes (Rankin et al. 2011). Synthesis of these gene products is often costly for the cell carrying these genes. As a result, though they are transient in nature, they are maintained according to the strength of conferred fitness benefits. Life history strategies may limit feasible evolutionary possibilities, however. For example, a microbe that has evolved a low carbon use efficiency (CUE) as a competitive mechanism may not survive if a is was acquired horizontally that forces constitutive production of a metabolically expensive product.

Microbial growth has been shown to drive soil SOM decomposition (Neill & Gignoux 2006). Therefore, metabolic mechanisms related to growth may have a large influence on soil carbon dynamics. The soil environment hosts a wide diversity of microbial growth strategies, beyond the simple dichotomy of copiotrophs and oligotrophs (Ernebjerg & Kishony 2012; Vieira-Silva & Rocha 2010). Growth in soil typically displays a negative relationship with yield (Lipson 2015). While this relationship is partly determined by a life history strategy corresponding to a maximum growth rate imparted by rRNA copy number, or codon usage bias (Vieira-Silva & Rocha 2010; Stevenson & Schmidt 2004; Goldfarb et al. 2011), there is phenotypic variation in CUE dependent upon maintenance costs. Maintenance costs vary with conditions, and may increase with nutrient limitation, starvation, physiological stress, allocation to storage, extracellular products, and transporters (Lipson 2015; Matsumoto et al. 2013). Maximum possible microbial CUE has been estimated at approximately 60% of acquired carbon being assimilated into biomass or ATP (Schmidt & Konopka 2008).

A genetic capacity for high metabolic versatility is often observed in microbes with a life history strategy adapted to resource limitation. This versatility has been associated with the potential for metabolic inefficiencies through uncoupling mechanisms, though carbon limitation reduces loss of metabolic efficiency by overflow metabolism. Overflow metabolism occurs when there is an excess supply of carbon so that it is only partially oxidized and then secreted from the cell. Uncoupling is a metabolic option for a cell with branched respiratory chains. Respiratory chains typically function to conserve energy, but simultaneously must remove reducing equivalents to balance the cellular redox potential. If a sudden influx of carbon occurs, the respiratory chain must uncouple, lest it produces more energy than it can consume (Teixeira De Mattos & Neijssel 1997).

Investment in acquiring resources, part of cellular maintenance costs, generally lowers the overall metabolic efficiency of the cell (Teixeira De Mattos & Neijssel 1997). The phenotypic response of microbes living in resource-limited conditions can be defined as 1) synthesis of enzymes that acquire limiting resources to maximize uptake rates, 2) synthesis of enzymes targeting alternative forms of the limiting resources, 3) decrease in anabolism to match the uptake of the limiting resources, and 4) use of storage polymers to compensate resource deficiencies (Harder & Dijkhuizen 1983; Schmidt & Konopka 2008).

Many of the effector molecules associated with maintenance costs are proteins. Protein production requires the greatest amount of energy and resources of all microbial processes (Koch 1985). Even under optimal conditions, maximum growth rate is limited by macromolecular synthesis, energy production, and transport of molecules, all processes driven by proteins. Therefore, allocation of resources towards non-growth protein synthesis represents a decrease in fitness (Chubukov et al. 2014). This burden creates a strong selective pressure for microbes to reduce nonessential protein production.

Both selective gene loss and genetic regulatory mechanisms contribute to reducing the cost incurred by a microbe in protein production. Because microbial interactions may increase cellular maintenance costs, thereby affecting allocation, they may have a large impact on ecosystem carbon cycling.

1.2.1 Interaction-Mediated Phenotypic Plasticity

Phenotypic plasticity is beneficial in highly heterogeneous environments, allowing microbes to adjust their response to a range of conditions. This has the potential to ameliorate the severity of circumstances causing negative fitness effects for the microbe on a short term scale. Phenotypic plasticity arguably carries costs with its maintenance, though. Evolutionary biologists have analyzed the costs and limits on phenotypic plasticity (DeWitt et al. 1998), as well as constraints on the evolution of plasticity. A loss of plasticity may be due to accumulation of mutations or loss of genes if it is unused (Murren et al. 2015). Generally, genomes with more regulatory and sensing elements that confer the capacity for plasticity are larger. Multiple studies have found loss of core metabolic genes in obligate symbiotic, parasitic or commensal microbes. In contrast, some free-living microbes have streamlined that maintain the core genes, but reduce the relative amount of intergenic spacer DNA and number of paralogous genes (Giovannoni et al. 2014). However, there is still a lack of evidence to indicate that large genomes in microbes carry a high enough cost to be selected against.

Interaction-induced phenotypic alterations are often initiated via direct contact, metabolic byproducts, or diffusible autoinducer molecules that interact with regulatory pathways, such as quorum signals, volatile organic compounds (VOCs), or even toxins (Effmert et al. 2012; Decho et al. 2011; Davies et al. 2006; Straight & Kolter 2009). Multiple studies have shown coordinated phenotypic responses to environmental or competitive stressors within and between populations (Challis & Hopwood 2003;

Rigali et al. 2008). When this occurs, autoinducers are considered signals. In some cases, however, phenotypic responses are induced that are not part of an effort to enact a cooperative, coordinated response. For example, it is possible that some autoinducer producers may force metabolic changes in other microbes for their own benefit, which is termed coercion. Some microbes appear to have evolved the capacity for 'crosstalk,' or the ability to eavesdrop on heterospecific autoinducers in the surrounding environment. These are known as cues. (Traxler & Kolter 2015; Netzker et al. 2015; Federle & Bassler 2003; Diggle et al. 2007).

Microbial interactions may act to alter the expression of various traits that have implications in carbon cycling, such as growth rate and extracellular products. The production of many exoproducts is temporally and spatially modulated through intercellular signals within and between populations (Diggle et al. 2007; Huang et al. 2013; Strickland et al. 2013), as may be differentiation and predatory behavior (Straight et al. 2006; Müller et al. 2014; Schuster et al. 2003). Autoinducers are also involved in efficiency sensing: detection of diffusion rates to optimize production amounts of extracellular products (Hense et al. 2007). The impact of autoinducers on fitness for an individual microbe in relation to its community, through both competition and cooperation, confers a level of importance that is reflected in the capacity for a wide diversity of genes for signals found in many microbes (Challis & Hopwood 2003; Krug et al. 2008; Schuster et al. 2003). Furthermore, as mediators of interactions that result in altered expression of functional traits, autoinducers are fundamental to ecosystem function (Seneviratne 2015; Zhuang et al. 2013).

Autoinducer efficacy and persistence in the soil environment is affected by the size and adsorption properties of autoinducer molecules, and may be altered by pH and the ratio of clay to organic material (Traxler & Kolter 2015; Subbiah et al. 2011; Lv et al. 2013). Mineral soil is comprised of approximately

50% air and water-filled pores, which are temporally and spatially dynamic (O'Donnell et al. 2007). This creates a high surface area within the soil matrix, on which many soil microbes form biofilms. Biofilms alter the autoinducer potential of a community through changes in diffusion rates, redox gradients and pH (Stewart 2003; Decho et al. 2011). Therefore, autoinducers have the potential to feed back on the behavior of the biofilm community. Additionally, some microbes produce degrading enzymes, agonists, and antagonists of autoinducer molecules (Wang & Leadbetter 2005; Xavier & Bassler 2005). Not only do these compounds serve to manipulate microbial interactions, but some of the degraded products may form new carbon and nutrient sources, and act as antimicrobial compounds or iron chelators (Leadbetter & Greenberg 2000; Kalia 2013).

1.2.1.1 Soil Biofilms

Biofilm formation is important to many soil microbes for survival. It offers protection against several soil environment stressors such as predation, desiccation, and toxin exposure (Matz & Kjelleberg 2005; Mah & O'Toole 2001; Roberson & Firestone 1992; Jefferson 2004). The prevalence of biofilm formation amongst bacteria, estimated to be at 99% of taxa, suggests evolutionary evidence of life in biofilm as an important adaptation. Though fungi, algae, protozoa, and yeast also grow in biofilms alongside bacteria, the primary focus in research of biofilms has been on bacteria (Jass et al. 2002; Vu et al. 2009). Regardless of taxonomic identity, biofilms create conditions for close contact between microbes by immobilizing the biofilm cells, consequently promoting interactions and coexistence of multiple microbial guilds. Because they form on SOM and localize enzymes, they facilitate efficient degradation and soil carbon turnover (Burmolle et al. 2012).

The exact composition of biofilms varies widely and has been difficult to study, but contains polysaccharides, proteins, lipids, nucleic acids, and other biopolymers such as humic substances, along with the resident microbes. While some of the matrix can be easily degraded as a nutrient source, humic substances are resistant to degradation, contributing to long-term carbon soil stocks (Flemming & Wingender 2010). The combined, 3-dimensional matrix of molecules is broadly termed 'extracellular polymeric substances,' or EPS. Each species of bacteria produces a distinct set of polysaccharides and proteins for their respective EPS, which is integrated in multispecies biofilms (Vu et al. 2009). Biofilm matrix architecture varies widely based on EPS molecular structure and environmental conditions, with the different architectures impacting important physical parameters of microbial existence, such as diffusion gradients (Flemming & Wingender 2010).

It is well established that the shift from a planktonic lifestyle to a biofilm is accomplished through changes in gene expression often engendered through intercellular autoinducers (Parsek & Greenberg 2005; Jefferson 2004). For example, Lopez *et al* (2009) found that a diverse set of natural molecules that cause potassium leakage by temporarily creating membrane pores in *Bacillus subtilis* were responsible for inducing biofilm formation. These molecules are produced by other strains as well as *B. subtilis* itself. They proposed that a membrane receptor was likely able to detect lowered intracellular concentrations of potassium and initiate a transcriptional response leading to biofilm production. Though the specific interacting molecules were not always determined, several other studies have shown either induction or an increase of biofilm formation in strains of bacteria grown together above levels when grown in monocultures (Burmolle et al. 2007; Bleich et al. 2015; Shank et al. 2011), whereas other studies have found inhibition of biofilm production (Powers et al. 2015). Monoculture biofilm formation may be a cooperative mechanism (West et al. 2007); however, induction of biofilm

production by heterospecific strains could also mean that biofilm formation is a defensive or coercion strategy.

Many of the differentially expressed genes associated with the transition from planktonic to biofilm life code for metabolic function and starvation responses (Stewart 2003; Jefferson 2004; Donlan 2002; Booth et al. 2011; Sauer & Camper 2001; Prigent-combaret et al. 1999). Expression is predominantly initiated by environmental cues. However, through the development of a biofilm, initial colonizers transform their created biofilm environment through cell autoinducers, waste products, and degradation of SOM (Stewart 2003). Because of restricted diffusion, this transformation creates microenvironments that magnify spatial and temporal heterogeneity within the biofilm, causing changes in microbial phenotype relative to available resources and interacting organisms (Stewart & Franklin 2008). SOM degradation rates may be changed by partitioning tasks, facilitating syntrophic interactions, or coordinating degradation more effectively (Jefferson 2004; Bernstein et al. 2012; Ackermann 2015; Huang et al. 2013). Additionally, there is evidence that microbes have bistable switches that respond to intercellular autoinducer that may also affect the phenotypic heterogeneity displayed within a mature biofilm (Chai et al. 2008; Dubnau & Losick 2006). This heterogeneity may lead to an increase in population-level efficiency through specialization in tasks or a reduction in the waste of resources, and may act to slow soil carbon turnover rates (Folse & Allison 2012).

Given the dramatic change in phenotype that accompanies the transition to a sedentary lifestyle within a biofilm, it is difficult to isolate the changes in cellular efficiency or changes in allocation of resources due to production of EPS. However, initial colonization is marked by high production of metabolically expensive carbon compounds and proteins, so an immediate reduction in growth might be expected. In fact, a decline in growth has been broadly observed and discussed (Burmolle et al. 2014; Mah & O'Toole

2001). Additionally, as the amount of EPS accumulates, diffusion distance of oxygen, nutrients, and waste products increases to and away from active cells, further creating conditions that might decrease growth rates through nutrient limitation, triggering of a stress response, and transition of metabolism to inherently less efficient anaerobic respiration or fermentation (Stewart 2003; Mah & O'Toole 2001; Prigent-combaret et al. 1999). Thus, it is possible that conditions generated through biofilm structural and chemical differentiation created by indirect microbial interactions lead to lower metabolic efficiency. Likewise, the stress response that has been noted in biofilms represents a shift toward allocation of resources to maintenance (Schimel et al. 2007). All of these mentioned mechanisms serve to alter soil carbon cycling by reducing yield and increasing respiration.

Alternatively, decreased diffusion associated with the EPS matrix also benefits microbes. Extracellular products, or public goods, such as enzymes, quorum molecules, and siderophores, remain closer to the producing cell, increasing its return on investment (Burmolle et al. 2014; Flemming & Wingender 2010). This may allow the producing cells to devote more of their resources towards growth, improving metabolic efficiency and biomass accumulation. Also, recycling of products from lysed cells, metabolic waste, and synergistic interactions that arise may improve resource supply, explaining an increase in growth with biofilm formation. One study showed that 63% of four-species biofilm-producing consortia synergistically increased biofilm production relative to strains grown independently in the lab (Ren et al. 2014). The highest-producing four-species consortia contained a dominant biofilm producer, *Xanthomonas retroflexus*, however, all of the interacting species in that group increased in both biofilm production and relative cell number compared to monocultures. Only two of the thirty-five combinations of 4-species consortia showed decreased biofilm production.

1.2.1.2 Growth and Dormancy

Interactions among microbes, whether positive or negative, and direct or indirect, influence cellular maintenance costs and therefore affect soil carbon cycling. The ability of microbes to maintain relatively high growth rates down to nanomolar or micromolar concentrations of substrate (Schmidt & Konopka 2008) due to the maximization of uptake represents a strong adaptation for exploitative competition, which may be further enhanced through detection of competitors. In a study by Ernebjerg and Kishonv, 2012, colonies of soil microbes were more likely to form in early growth when plated at intermediate densities, suggesting that the strains were broadly impacting each other's growth, though the mechanism was unclear (Ernebjerg & Kishony 2012).

Manipulation of the tradeoff between growth rate and yield can affect competitive interactions. By switching to a low-yield strategy, bacteria disproportionately acquire available resources (Pfeiffer et al. 2001; Lipson 2015). While this low-yield strategy might not immediately improve fitness, it functions to decrease fitness of competitors by reducing resources available for growth. This strategy has the effect of increasing carbon turnover and CO_2 flux. High rates of resource flux and cell diffusion favor a low yield strategy, wheras a spatially structured environment favors a high yield strategy, therefore, this mechanism is unlikely to be widely distributed in a soil biofilm environment.

Rapid growth is an effective competitive maneuver, though this strategy may only be successful if substrates are available (Stevenson & Schmidt 2004; Goldfarb et al. 2011; Moorhead & Sinsabaugh 2006). Growth form may also give a competitive advantage. In sand microcosms, the fast-growing, rod-shaped *Bacillus weihenstephanensis* outcompeted a slower-growing, filamentous *Streptomyces atratus* under high aqueous pore connectivity (Wolf et al. 2013). Yet, under dry conditions with low connectivity*, S. atratus* became dominant.

Pseudomonas fluorescens was shown to inhibit, but not lyse, mycelial growth in *Fusarium culmorum* (Strunnikova et al. 2007). Because the effect was eliminated when glucose or cellulose was added to the soil, the mechanism was likely caused by more rapid or effective procurement of resources. Alternatively, the bacterium *Clostridium phytofermentans* enhances its growth in the presence of fungi by producing chitin-degrading enzymes to lyse fungal hyphae. The lysed fungal biomass provides nutrients so that *C. phytofermentans* can ferment cellulose (Tolonen et al. 2014).

Competitive interactions have traditionally been broken down into the categories of interference and exploitative competition. Yet, interference competition often supports exploitation by competitors. Some microbes may respond to nutrient stress, which is associated with exploitative competition, by slowing growth and producing growth inhibitory antibiotics (Rigali et al. 2008; Cornforth & Foster 2013; Garbeva & de Boer 2009). Also, specific competitors or groups (e.g. gram positive or negative) may induce a tailored, antagonistic response through autoinducers (Tyc et al. 2015; Garbeva et al. 2011). This response hinders growth or development, or kills competitors. Other types of secondary metabolites are produced to inhibit growth or kill microbial competitors, such as toxins, biosurfactants, volatile organic compounds (VOCs), etc., but not all of these carry a discernable fitness cost (Li et al. 2013).

Slowed growth may accompany allocation shifts to cellular maintenance costs of antibiotic production, but it has also been proposed that slowed growth is a protective mechanism against antibiotic attacks (Mah & O'Toole 2001). The reason why slowed growth imparts protection is unclear, however, because resistance to antibiotics may carry a fitness cost, it could be associated with a shift in allocation away from growth (Andersson & Levin 1999; Andersson & Hughes 2010; Dykes & Hastings 1998). Garbeva *et*

al, (2011) found differential regulation of ribosomal protein and stress response genes along with induction of antibiotic production, suggesting that slowed growth is partly due to a cellular stress response. Also, slowed growth may be caused by mechanisms to suppress antibiotic production in neighboring cells, or to increase antibiotic production in the defending cell (Tyc et al. 2014; Abrudan et al. 2015; Galet et al. 2014). Oddly, given the fitness cost of production of some growth inhibitory molecules, it is surprising that 33% of soil bacteria constitutively produce antibiotics (Tyc et al. 2014). This finding may support the notion that antibiotics serve as autoinducer molecules.

Another tradeoff occurs between growth rate and lag time, which are negatively correlated (Ernebjerg & Kishony 2012; Geisel et al. 2011). Microbes with a life history strategy evolved for stressful environments generally have longer lag times, and slower growth rates. Dormancy or a reduced metabolic state have indirect fitness consequences for a population by freeing up the resources that cells would have consumed for their kin (Ratcliff et al. 2013). These microbes may be the persister cells noted in biofilms, that are more inclined to switch into a dormant or reduced metabolic state (Stewart & Franklin 2008). In the soil environment, approximately 80% of all bacteria are in a dormant state (Lennon & Jones 2011). Though the reduced metabolic state is energetically prudent, the cost of going into this state is not zero. Multiple metabolic processes must first prepare to shut down, including machinery to go into and out of dormancy, as well as resting structures (Lennon & Jones 2011). In a model, Geisel *et al* (2011) analyzed the fitness advantages related to dormancy, and found that given the fitness cost of delayed response to new resources, environments with long stress periods are the most beneficial for microbes that rapidly transition to dormancy.

1.2.2 Social Adaptation

Natural selection acts on genetic variation, often a single, specific locus in microbes (Mitri & Foster 2013). Evolution plays a strong role in microbial survival with the strong selective pressures imposed through interactions, constant fluctuations within the soil environment, and the relatively short generation times of microbes. Of major concern for fitness is how microbes compete for and effectively acquire resources. Some disproportionately exploit resources before their competitors can fully gain access to them, while others produce toxins to kill or slow down their competitors. The selective pressures introduced as a result of these actions may further engender evolutionary changes that impact the rate of resource turnover and fate. At the very least, competitive interactions can change resource allocation within the bounds of a microbe's life history strategy.

Studies of social evolution are often performed in microbes due to their relative simplicity. However, there is debate over whether the interactions in laboratory studies are truly social in nature. The argument is that for a trait to be social, it must have evolved specifically in response to pressures by neighboring cells, and is relevant only to a specified set of environmental parameters (Rainey et al. 2014). Generally, the effects of resources and environmental conditions are easier to decipher in studies compared to the determination of social evolution caused by interactions with a specific partner (Haloin & Strauss 2008). Social behaviors have fitness effects for both the actor and the recipient. Cooperative behaviors can be mutually beneficial, in which the actor and recipient receive positive fitness results, or altruistic, in which the actor does not. Likewise, competitive behaviors can be broken down into selfish, with the actor receiving a fitness benefit while the recipient is harmed, or spiteful, with both being harmed (Hamilton 1964). Even though laboratory experiments often cannot specifically prove that the evolutionary response to selective pressures in the experiment is solely due to the interaction and therefore social, these experiments inform potential mechanisms that may occur

through interactions, and as such, are important to begin understanding how evolution impacts carbon cycling.

1.2.2.1 Horizontal gene transfer

The accessory genome- those genes contained within a microbe that are horizontally transferred via mobile genetic elements (MGE) such as transposons, bacteriophages, and plasmids- predominantly codes for secreted proteins (Nogueira et al. 2012), but can also encode metabolic traits (Falkowski et al. 2008; Ochman et al. 2000). Many of these MGEs encode functions that benefit the host and impact neighboring cells, such as antibiotic resistance and enzymes. The genes encoding the secretome, molecules secreted outside of the cell or integrated into the cellular membrane or wall, evolve faster than the rest of the genome, indicating strong selective pressure on these traits. Also, because these traits are horizontally transferred they are key players in bacterial adaptation (Nogueira et al. 2012). Indeed, because many of these traits are involved in interactions with other microbes, they are subject to an evolutionary arms race.

The Core Genome Hypothesis postulates that despite the genetic fluidity of prokaryotes, there is a set of core genes that can be used to reasonably identify distinct species (Riley & Lizotte-waniewski 2009). However, many of the genes responsible for microbial interactions are part of the accessory genome, which constitutes upwards of 90% of a bacteria's pan-genome (Touchon et al. 2009; Haq et al. 2014), blurring species lines (Goldenfeld & Woese 2007). Within a spatially explicit environment, a communal gene pool may form (Norman et al. 2009), with the spread of MGEs increasing relatedness of interacting individuals. Gene sharing will alter the costs and benefits of cellular interaction mechanisms, and influence the evolution of public goods (McGinty et al. 2013).

Transmission of MGEs increases at higher cellular densities (Sorensen et al. 2005). For example, the mycosphere, or the area around fungal hyphae, has been postulated to be an area with concentrated horizontal gene transfer (HGT), including between bacteria and fungi (Zhang et al. 2014). Biofilms promote HGT by creating a matrix for microbes to interact closely for conjugation, maintaining the naked DNA of lysed cells in proximity to the biofilm's residents for transformation, and even potentially facilitating viral infection for transduction (Donlan 2002; Flemming & Wingender 2010; Hausner & Wuertz 1999; Burmolle et al. 2014; Sorensen et al. 2005; Molin & Tolker-Nielsen 2003). Because of this, it is likely that plasmids and bacteriophages have incorporated genes that facilitate biofilm formation to ensure their own propagation (Jefferson 2004; Madsen et al. 2012). Furthermore, the biofilm acts as a reservoir of genetic information, allowing rapid adaptation to fluctuating conditions, and redefinition of an ecological niche (Haq et al. 2014).

The cost of the transferred genes depends on the mechanisms of transfer and the genes that they carry (Rankin et al. 2011). For example, because bacteriophages cause cellular lysis, they incur the greatest fitness cost. Also, loss of important functions when genes are integrated into the core genome and disrupt a gene, replication of the MGE, intergenomic competition with other genetic elements, and creation of transmission structures such as pili, are all additional costs that may be associated with horizontal gene transfer (Rankin et al. 2011). Due to these costs, microbes often shed MGEs that are not useful. Therefore, MGEs have evolved mechanisms that either encourage or force their own maintenance within the host cell. For example, addiction complexes contain a toxin-antitoxin complex, with the antitoxin degrading more rapidly than the toxin (Zhang et al. 2012). This parasitic mechanism ensures maintenance of the plasmid by lowering fitness to zero upon loss. Similarly, greenbeard genes contain toxin-antitoxin complexes, but are associated with MGEs that cause cell lysis (Biernaskie et al.

2013). When the cell dies, its intracellular contents will also kill surrounding microbes that do not contain the same MGE.

In response to opposing fitness interests between the host microbe and MGEs, MGEs have likely evolved to carry traits that are beneficial to the host. Additionally, the biosynthetic cost of the secreted and outer membrane proteins is lower than those for purposes elsewhere in the cell, indicating that these MGEs had evolved to be more efficient for the host cell, improving the likelihood of maintenance within the cell (Nogueira et al. 2009; Smith & Chapman 2010). This is likely not only due to the intergenomic conflict of the core and accessory genome, but also because of the imposed costs of social interactions.

1.2.2.2 Cheaters

Because production of extracellular products bears a cost, and other members of a population are likely producing the same products, it is evolutionarily expedient for an individual microbe to evolve a loss of production. The loss of function represents an increase in fitness, having a positive competitive effect against surrounding producers. Still, the degree of fitness gain is affected by diffusion rates, spatial structure, available resources, and surrounding biotic interactions.

Regulatory mechanisms may be a better evolutionary bet over loss of function in highly variable environments. However, regulatory mechanisms are more evolutionarily complicated to acquire than perhaps a point mutation that results in loss of function, or ejection of a MGE. Despite that, larger bacterial genomes, as are common in the soil environment, are positively associated with regulatory elements (Giovannoni et al. 2014), though constitutive production may be forced through mafia tactics coded on MGEs, such as addiction complexes and greenbeards (Travisano & Velicer 2004; Rankin et al. 2012).

Evidence shows that autoinducers can regulate public good production (Hense et al. 2007; Darch et al. 2012; Strickland et al. 2013; Barnard & Salmond 2007). Darch *et al* (2012) proposed that quorum sensing is an effective manager of public good production because public goods are more efficient at higher cell densities, that decreased the collective burden of production.

Autoinducers are often the mechanism by which cheating can arise due to efficiency sensing and coordinated population responses (Stephen P. Diggle et al. 2007). When microbes evolve to be deaf to signals, they are no longer induced to generate public goods. Cheating in autoinducers gives only a small fitness benefit due to eliminated production costs for the signals themselves (Diggle et al. 2007; Hense et al. 2007). The greatest benefit is due to reduced production of the public good tied to the autoinduction. Also, cheaters can overproduce signals, coercing their neighbors to produce more public goods. Evidence shows that high relatedness, or kin selection, act as buffers to the evolution of cheating in public good-producing populations (Diggle et al. 2007; Popat et al. 2015).

An example of public goods commonly involved in cheating is siderophores. Because soil is an aerobic environment, bioavailability of iron is often limited. Siderophores chelate iron, an important element involved in many metabolic pathways. Many species of bacteria and fungi produce multiple types of siderophores and their receptors, some that are more metabolically costly than others (Dumas et al. 2013). It is common for cheaters to arise that pirate xenosiderophores, those produced by other species. Cheating is done by expressing siderophore receptors, that are likely acquired through HGT (Cornelis & Bodilis 2009). There is often a concomitant reduction in the production of endogenous siderophores, and an increase in fitness (Galet et al. 2015; Miethke et al. 2013). Traxler *et al* (2012) found that siderophore piracy can be used exploitatively, as well. In their experiment, *Amycolatopsis sp.* AA4 arrested development of *Streptomyces coelicolor* through manipulation of iron availability via siderophore production, then through piracy of *S. coelicolor* siderophores.

Cheating in biofilm formation is not generally based public goods production, such as the EPS, but rather competition for limited resources and oxygen (Xavier & Foster 2007). Cells at the surface of the biofilm experience higher nutrient and oxygen levels. There is evidence that mutants arise with increased ability to produce biofilm compounds, effectively pushing themselves to the surface of the biofilm (Kim et al 2014), suffocating the wild type strain. This allocation to biofilm polymers, however, comes at the expense of reproduction as indicated by lower density of mucoid variant cells (high biofilm-producing) compared to wild-type cells. In fact, mucoid variants from the original population evolved repeatedly (parallel evolution) and outcompeted their predecessors. Genomic analysis confirms that increased competitive ability was not achieved through faster growth, but through increased biofilm polymer production (Kim et al. 2014).

Enzymes are especially important for carbon cycling, as they are the proximate agents by which microbes can access SOM and begin degradation and subsequent mineralization of carbon. Extracellular enzymes can also contribute to production of recalcitrant SOM in the soil (Burns et al. 2013), affecting the amount of carbon that is sequestered. Because enzymes are proteins, their production costs are relatively high, potentially resulting in reduced fitness through allocation of carbon and energy towards enzymes, as well as lowered metabolic efficiency. Between 1-5% of assimilated carbon and nitrogen has been estimated to support extracellular protein secretion (Frankena et al. 1988). This cost trades off with the increase in fitness due to resource acquisition from enzyme activity (Allison et al. 2011).

Enzyme cheaters evolved repeatedly out of producer populations, indicating the strong selective pressure for this adaptation (Allison et al. 2014). Cheaters have been shown to benefit from high diffusion rates, especially with increased cost of enzyme production (Allison 2005; Allison et al. 2014; Folse & Allison 2012). Keeping enzymes attached to the cell surface is an effective strategy to keep the products of degradation close to the enzyme-producing cell, and eliminate the loss of the enzyme itself through diffusion. Yet surface attachment may limit access to substrates that are further from the cell.

In biofilms, where the majority of microbes grow in the soil, loss of enzymes through diffusion will be partially mitigated by the EPS matrix.

Limiting diffusion with structured environments decreased overall cheater success, allowing either coexistence or dominance by producers (Allison 2005; Allison et al. 2014). Structured environments localize enzymes and maintain highly related individuals together in patches excluding cheaters. Likewise, low nutrient concentrations allowed producers to form insulated patches against cheaters (Mitri et al. 2011; Nadell et al. 2010). The fitness of cheaters is also frequency-dependent (West et al. 2007; Ross-Gillespie et al. 2007). With higher percentages of cheats, there is less product supply causing a decline in both producers and cheaters. This may lead to boom and crash cycles in microbial populations with intrinsically high growth rates under high resources, such as in newly forming biofilms. However, in nutrient-limited environments that impose slow growth rates, such as mature biofilms, the relative number of cheaters in populations might be more likely to stabilize (Morris et al. 2012).

1.2.2.3 Black Queen, Cross-feeding, and Syntrophy

Under specific conditions, cheating strategies may lead to long term stabilization of mutualisms (Morris et al. 2012). The Black Queen Hypothesis stipulates that production of public goods will lead to cheaters in the community that do not produce these goods. The products must be expensive to produce, and only made by a fraction of the community, but vital to the community. These conditions establish the producer as a helper, making it essential to community survival. However, Morris (2015) suggests that, over time, cheating may arise in the helper for other public good traits, creating a mutual interdependency. Microbes may partially maintain their produced public goods inside of the cell, which impacts the probability of evolution towards mutual interdependency (Estrela et al. 2015). The stabilization of mutual dependency was shown in an experiment in which strains were grown together that had null mutations for different amino acid production pathways (Pande et al. 2014). The cross-

feeding mechanism created a division-of-labor fitness advantage over the ancestral strain that was stabilized through negative frequency-dependent selection for the pair of metabolic dependents. The fitness advantage was likely incurred by eliminating metabolic machinery required for the null mutation, and instead allocating the saved resources towards growth.

In the case of enzymes, decreasing production, either through cheating or cooperative auxotrophies, may slow carbon turnover in the soil by decreasing the relative amount of active enzymes to break down carbon substrates relative to the number of individuals in the community (Folse & Allison 2012; Oliveira et al. 2014). Alternatively, cheating may facilitate a transition to a more efficient community metabolism based on a mutual auxotrophy. The increased metabolic efficiency may allow faster growth, increasing total carbon consumption in the community and the rate of carbon turnover (Pande et al. 2014).

Evolution of cross-feeding interactions may arise through optimization of metabolic pathway length (Pfeiffer & Bonhoeffer 2004; Costa et al. 2006). Cross-feeding is pervasive in microbial communities, and often a plastic trait (Ponomarova & Patil 2015). Production of ATP in metabolic pathways involves multiple enzymes that carry out each step. These enzymes are energetically expensive and resource intensive. By eliminating some of the ATP-generating steps, the resource is only partially degraded and secreted from the cell. This increases the ATP production rate, though at the expense of yield (Pfeiffer & Bonhoeffer 2004). The partially degraded compound is then used by another microbial community member.

More complex cross-feeding patterns have been found in nature. These syntrophies, or "obligate mutualistic metabolisms," are broadly described as a service mutualism, in which one species provides a chemical resource in exchange for a benefit from its interacting partner (Harcombe 2010; Bull & Harcombe 2009). Syntrophies are largely anaerobic processes that are beneficial to the participants

because they shift the metabolic reactions toward thermodynamic favorability (Morris et al. 2013; McInerney et al. 2008). Biofilms can function to keep interacting partners in close proximity (Little et al. 2008), ultimately increasing carbon turnover in mature biofilms with anoxic regions.

1.3 Community Structure

Diversity-function relationships generally show a positive, asymptotically saturating relationship between species richness and ecosystem function (Tilman et al. 2009; Bell et al. 2005; Tiunov & Scheu 2005; Langenheder et al. 2010). Conversely, there are contrasting results of a 'negative complementarity effect.' This decreasing function with increasing diversity, has been hypothesized to be caused by competitive interactions (Becker et al. 2012; Jousset et al. 2011; Van der Wal et al. 2013; Szczepaniak et al. 2015). These conflicting patterns with increasing species diversity indicates that a community's composition may have an impact on its overall function.

Indeed, microbial community composition has been shown to affect ecosystem function in multiple studies (Tiunov & Scheu 2005; Bell et al. 2005; Langenheder et al. 2010; Reed & Martiny 2007). A recent literature synthesis that investigated the relationship between altered community composition and related function found that 75% of the papers that explicitly tested for a link between community structure and processes found a statistically significant link (Bier et al. 2015).

The remaining 25% that showed a decoupled composition and function may have been due to redundancy of function among community members (Allison & Martiny 2008). There are, however, exceptions to the link between community and function. The techniques used to evaluate who is present in the community often do not take into account the metabolic states of the individual members of the community (Baldrian et al. 2012; Lennon & Jones 2011). Additionally, the presence of genes or gene transcripts is not necessarily representative of the activity level of the present community members (Schimel & Schaeffer 2012). Mouillot *et al* (2011) showed that functional diversity, rather than

taxonomic diversity was more predictive of ecosystem multifunctionality, with a few specialist species contributing disproportionately to primary production and degradation. This finding supports the notion that HGT of functional traits rapidly redefines an ecological niche (Ochman et al. 2000).

Community composition only indirectly controls the turnover of soil carbon by altering the genetic potential of the community and the context within which microbes operate. Ultimately, it is microbial physiology that directly controls carbon turnover (Allison 2012). Schimel and Schaeffer (2012) propose that for the community composition to impact soil carbon turnover rates, 1) the organisms must vary in the functional traits that they possess, and 2) that the biological reactions they facilitate must be either the rate-limiting or the fate-controlling step in carbon breakdown. The authors argue that the rate-limiting step is more likely due to abiotic soil constraints, and therefore, it is the fate-controlling step that confers the importance of microbial composition onto community function.

1.3.1 Community composition is determined by microbial interactions

The composition of microbial communities, along with available resources and conditions, determine microbial interactions. The resource competition theory posits that the species with lower resource requirements will outcompete other species with higher requirements when they are both limited by the same resource. However, species can coexist if they are either limited by different resources, or if they have nearly identical resource requirements (Tilman 1981). Soils are spatially and temporally heterogeneous, though. Microsite variation alters the outcome of many competitive interactions beyond the resource competition theory, as do the additional competitive mechansims (Hibbing et al. 2010). A strategy that works in one location may not be as effective in the neighboring location. For example, in environments with low nutrients or low diffusion rates, the competitive ability conferred by a rapid growth rate is diminished (Dechesne et al. 2008). Rapid depletion of resources upon initial

colonization of a substrate surface and increase of EPS will likely create that scenario, increasing coexistence.

1.3.1.1 Spatially defined interactions

The proximity of microbes to each other is relevant to interactions and microbial processes. HGT shapes communities and their function, and increases with microbial density and activity (van Elsas & Bailey 2002). However, in a soil simulation parameterized using photos taken at the microscale in soil, Raynaud & Nunan (2014) determined that the average distance between microbes in the soil is 12.46 µm, with distances decreasing and aggregation increasing closer to the surface of the soil. In lower density bulk soil, the average number of interacting species was 11±4 within 20 µm, whereas in the higher-density rhizosphere, it was closer to 284±30 species (Raynaud & Nunan 2014).

Results from modeled two-species interactions show that spatial separation may result from microbial interactions, with antagonism leading to self-segregation and mutualism to homogenization (Blanchard & Lu 2015). Separation allows microbes to coexist that might normally compete (Ettema & Wardle 2002; Dechesne et al. 2008). So, while competitive exclusion may occur on a very small scale, diversity is maintained through the larger soil ecosystem, with stability of some communities dependent upon spatial structure (Kim et al. 2008). Furthermore, as was previously discussed with biofilms, each microsite is changed through the interactions it hosts, contributing to temporal heterogeneity. For example, metabolic byproducts may change the local pH, which may impact diffusible signals, changing microbial interactions (Decho et al. 2011).

Non transitive interaction networks have been studied to determine how diversity can be maintained despite antagonistic interactions with rock-paper-scissor dynamics. Spatial structure allows sensitive strains to survive close to toxin-producing strains through shielding by strains resistant to the toxin (Kerr et al. 2002; Narisawa et al. 2008). This dynamic functions in communities with one toxin producing

strain, or in communities with diverse toxin producers, with diverse toxin production leading to increased ecological stability (Biernaskie et al. 2013; Prasad et al. 2011; Kelsic et al. 2015). These models, however, do not account for the effects of antagonism strength on microbial interactions. For example, synthesis of communities with varying strength of bacteriocin action suggests that potent bacteriocins led the producers to extinction by stimulating heightened attack responses from their opponents, whereas weak bacteriocins supported coexistence through mild responses (Majeed et al. 2013).

1.3.1.2 Inhibition and reduction of niche overlap

Similar to the effects of physical separation with the previous examples, non-transitive interaction networks are applicable to modulation of antagonism through multispecies interactions. Neighboring cells can decrease antibiotic production of a focal species' antagonist, eliminating negative fitness impacts on the focal species and allowing all three to coexist (Tyc et al. 2015). Thus the identity of interacting species plays a strong role in ecological processes. Abrudan *et al.* (2015) demonstrated that inhibitory interactions were reduced by induction of antibiotic production combined with suppression of antibiotic production in competing species, which allowed maintenance of diversity. Moreover, they found that interactions were environmentally mediated.

Species with high niche overlap are predicted to be more competitive with each other (Freilich et al. 2011). Often, this means that phylogenetically related species engage in stronger competition than more phylogenetically distant species (Jousset et al. 2011). Sympatric *Streptomycete* species showed higher degrees of antibiotic inhibition and reciprocated production than with allopatric *Streptomycete* species, with niche overlap being positively correlated with antiobiotic inhibition (Kinkel et al. 2014; Vetsigian et al. 2011). This result supports the hypothesis that antibiotics function to mediate community interactions in attempts to reduce niche overlap. Indeed, sub-lethal levels of antibiotics

altered independent growth rates of several *Streptomyces* strains on distinct substrates, as well as their range of substrate use (Jauri et al. 2013). Niche overlap declined in 56% of the isolate-isolate-antibiotic combinations, suggesting that sub-lethal antibiotics acted as an "escape from competition" mechanism. Consequently, antibiotic production may by an instrument to initiate niche differentiation, leading to speciation. Even monoculture biofilms undergo adaptive diversification to eliminate intraspecific competition and form synergistic communities through spatial partitioning and cross-feeding, which leads to higher productivity (Poltak & Cooper 2011).

1.3.1.3 Fungal interactions

Fungi act as ecosystem engineers, creating pores that form new habitats and mining new resources for other microbes. Soil pore structure has been observed to be non-random in nature, with a highly structured bacterial distribution (Young & Crawford 2004). Using this evidence, in an experimental manipulation, Crawford *et al* (2012) found that at scales below 53 um, fungal hyphae were highly correlated with soil pore organization. Additionally, increasing the fungal:bacterial ratio increased soil aggregate formation, indicating that soil community structure plays a role in aggregate stabilization and pore formation in soil. The formed pores are speculated to improve local conditions for the engineering species by opening up channels for oxygen exchange and increasing water flow potential. This increases nutrient exchange and bacterial colonization through increased connectivity. When varying hydration conditions were modeled as a function of the pore matrix potential, microbial dispersal increased dramatically (Kim & Or, 2015). In addition, fungi facilitate bacterial movement in conditions with low water potential by providing a highway bacterial biofilm formation and motility (Pion et al. 2013). Highly mobile bacteria species stimulated migration by less-mobile species along fungal hyphae, with no obvious fitness decline (Warmink et al. 2011; Warmink & Van Elsas 2009).

Fungi dominated the litter horizon in a forest ecosystem, with the fungal: bacterial ratio evening out in the organic horizon (Baldrian et al. 2012). Bacteria often benefit from fungi due to the fungal release of extracellular enzymes that create nutrient "hotspots" or metabolic intermediates from degrading recalcitrant carbon sources (Van der Wal et al. 2013). Increases in bacterial biomass are correlated with increasing fungal biomass in soil microcosms (Šnajdr et al. 2011). Bacteria have lower yield than do fungi, though, so changes in the fungal:bacterial ratio have implications for ecosystem CO_2 flux; for instance, changes in this ratio coincided with changes in respiration rate (Lipson et al. 2009).

The impact of increasing fungal community diversity on function is dependent upon whether the interactions are predominantly positive or negative (Van der Wal et al. 2013). Abundance of fungal species is not always positively correlated with functional contribution. For example, in a forest ecosystem, it was found that many of the less abundant species contributed disproportionately to cellobiohydrolase production in the litter horizon (Baldrian et al. 2012). Additionally, the species within the active and dormant fractions of the community were inconsistent, though the relative diversity was the same.

1.3.2 Evolutionary feedbacks on carbon cycling

Though multiple pairwise evolution experiments have been performed in the laboratory, the relevance of these experiments for communities of interacting microbes is unclear (Turcotte et al. 2012; Johnson & Stinchcombe 2007). Various pressures imposed by interactions with multiple species simultaneously may result in microevolution of a population that cannot be accounted for in simple two-species experiments (Johnson & Stinchcombe 2007). Furthermore, the geographic mosaic theory of coevolution posits that biotic and abiotic factors impact evolutionary results across geographic ranges (Thompson 2005). Even though it is possible to extrapolate the fundamental niche of an organism, ecological interactions alter the niche, resulting in an altered range of conditions that permit survival.

Diffuse evolution refers to evolution that is caused by one species' effect on the evolving species, but depends upon multiple other species within the environment. Diffuse coevolution occurs when the selection is reciprocal (Strauss et al. 2005). Research on the interplay between ecological interactions and evolutionary mechanisms is still in its early stages (Johnson & Stinchcombe 2007). By impacting evolutionary rate and direction, diffuse coevolution alters interactions between members of a community of microbes, which then feeds back on function, further affecting community interactions (Lawrence et al. 2012; Fussmann et al. 2007; Schoener 2011). The limited studies on eco-evolutionary dynamics indicate that evolution can take place on ecological timescales (Schoener 2011).

The community context of diffuse evolution may indicate that whole communities evolve together through direct or indirect mechanisms (Little et al. 2008; Barraclough 2015). Generally, it has been seen that over time, antagonistic communities evolve to be less competitive, with this effect increasing with increasing diversity (Fiegna, Moreno-Letelier, et al. 2015) Competition caused a decrease in resource use diversity, which was associated with a decrease in relative growth rate and yield compared to the ancestral strain, though this effect saturated at higher species richness (Fiegna, Scheuerl, et al. 2015). The decrease in growth rate and yield mirrors microbial adaptation to resource limited conditions, with increased uptake machinery and enzyme production for resource acquisition (Schmidt & Konopka 2008).

Further eco-evolutionary dynamics were highlighted in an experiment performed by Lawrence *et al.* (2012). Here, a community of four strains of bacteria was evolved over several generations. The researchers found that the strains grown in a community evolved faster than those in monoculture. Additionally, the interacting species evolved resource use divergence, and cross-feeding on metabolic waste products, indicative of character displacement and positive interactions. Three of the four community-evolved species did more poorly than their ancestors when grown in monoculture, revealing some degree of coadaptation. When compared to the community of ancestors, the evolved community had smaller population sizes, but higher CO_2 flux rate, suggesting a shift in community CUE. This

experiment demonstrated that community interactions can increase evolutionary rates above selection caused by abiotic pressures alone. It also reveals that adaptations caused by community interactions may function to transform environmental conditions, strengthening selective pressures, and altering carbon cycling through evolution-induced metabolic shifts.

1.4 Conclusion

Through effects on physiology, public goods, and social evolution, microbial interactions play a large role insoil carbon cycling. The rate of microbial metabolism controls uptake, transformation, and allocation of carbon (Brown et al. 2004). Because microbial interactions change phenotypic allocation of carbon and drive selection that alters metabolic traits, these interactions are tied to the carbon cycle. Further, the dynamics of populations and communities are largely determined by the rate of metabolism and the metabolic products of the member organisms. Waste products, biofilm formation, and growth rate affect a microbe's neighboring cells. Finally, ecosystem processes of energy flux and biomass production are also determined by metabolism. Sinsabaugh *et al* (2015) showed an allometric relationship between extracellular enzyme-substrate and production-biomass reactions, indicating that these are linked metabolic processes with relevance at the ecosystem scale.

Though some techniques have been developed to study microbes in the soil environment (O'Donnell et al. 2007), the ability to determine ecological and evolutionary processes at the microscale is still limited. The determinants of soil community structure and all of its associated network interactions are yet uncertain, as is the ability to deduce its functional capacity relative to spatial and temporal conditions (Prosser 2012).

Models provide an alternative approach to extrapolate microbial metabolic processes, interactions with the abiotic and biotic environment, and effects on soil carbon cycling and storage (Kim & Or 2015; Allison 2012; Liang et al. 2011; Allison et al. 2010). Metabolic reconstructions are able to predict cellular

and community processes such as biomass yields, formed consortia, evolution, stress adaptations, and the impact of specific phylotypes (Oberhardt et al. 2009; Khandelwal et al. 2013; Carlson & Taffs 2010). Furthermore, metabolic models can be used to explore ecosystem-level processes (Klitgord & Segre 2011). Combining models with new microscale experiments could rapidly advance a predictive understanding of microbial interactions in soil. Such efforts are critical given the myriad mechanisms by which microbial interactions potentially influence the carbon cycle.

References:

Abrudan, M.I. et al., 2015. Socially mediated induction and suppression of antibiosis during bacterial coexistence. *Proceedings of the National Academy of Sciences*, pp.1–6.

Ackermann, M., 2015. A functional perspective on phenotypic heterogeneity in microorganisms. *Nature Reviews Microbiology*, 13(8), pp.497–508.

Allison, S.D., 2012. A trait-based approach for modelling microbial litter decomposition. *Ecology letters*, 15(9), pp.1058–70.

Allison, S.D., 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecology Letters*, 8(6), pp.626–635.

Allison, S.D. et al., 2011. Evolutionary-Economic Principles as Regulators of Soil Enzyme Production and Ecosystem Function. In G. Shukla & A. Varma, eds. Soil Biology. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 229–243.

Allison, S.D. et al., 2014. Extracellular enzyme production and cheating in Pseudomonas fluorescens depend on diffusion rates. *Frontiers in microbiology*, 5(April), p.169.

Allison, S.D. & Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences of the United States of America*, 105 Suppl(Supplement_1), pp.11512–9.

Allison, S.D., Wallenstein, M.D. & Bradford, M.A., 2010. Response to warming dependent on microbial physiology. *Nature Geoscience*, 3(April).

Andersson, D.I. & Hughes, D., 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature reviews. Microbiology*, 8(4), pp.260–71.

Andersson, D.I. & Levin, B.R., 1999. The biological cost of antibiotic resistance. *Current opinion in microbiology*, 2(5), pp.489–93.

Baldrian, P. et al., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME Journal*, 6(2), pp.248–258.

Balser, T.C. & Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry*, 73(2), pp.395–415.

Barnard, A.M.L. & Salmond, G.P.C., 2007. Quorum sensing in Erwinia species. *Analytical and Bioanalytical Chemistry*, 387(2), pp.415–423.

Barraclough, T.G., 2015. How Do Species Interactions Affect Evolutionary Dynamics Across Whole Communities? *Annu. Rev. Ecol. Evol. Syst*, 46, pp.25–48. Available at: www.annualreviews.org.

Becker, J. et al., 2012. Increasing antagonistic interactions cause bacterial communities to collapse at high diversity. *Ecology letters*, 15(5), pp.468–74.

Bell, T. et al., 2005. The contribution of species richness and composition to bacterial services. *Nature*, 436(7054), pp.1157–60.

Bernstein, H.C., Paulson, S.D. & Carlson, R.P., 2012. Synthetic Escherichia coli consortia engineered for syntrophy demonstrate enhanced biomass productivity. *Journal of Biotechnology*, 157(1), pp.159–166.

Bier, R.L. et al., 2015. Linking microbial community structure and microbial processes: an empirical and conceptual overview. *FEMS microbiology ecology*, (May), p.fiv113.

Biernaskie, J.M., Gardner, A. & West, S. a, 2013. Multicoloured greenbeards, bacteriocin diversity and the rock-paper-scissors game. *Journal of evolutionary biology*, 26(10), pp.2081–94.

Blanchard, A.E. & Lu, T., 2015. Bacterial social interactions drive the emergence of differential spatial colony structures. *BMC systems biology*, 9, p.59.

Bleich, R. et al., 2015. Thiopeptide antibiotics stimulate biofilm formation in Bacillus subtilis. *Proceedings of the National Academy of Sciences of the United States of America*, 112(10), pp.3086–91.

Booth, S.C. et al., 2011. Differences in Metabolism between the Biofilm and Planktonic Response to Metal Stress. *Journal of Proteome Research*, pp.3190–3199.

Boyle, R.A., Williams, H.T.P. & Lenton, T.M., 2012. Natural selection for costly nutrient recycling in simulated microbial metacommunities. *Journal of Theoretical Biology*, 312, pp.1–12.

Brown, J.H. et al., 2004. Toward a metabolic theory of ecology. *Ecology*, 85(7), pp.1771–1789.

Bull, J.J. & Harcombe, W.R., 2009. Population dynamics constrain the cooperative evolution of cross-feeding. *PLoS ONE*, 4(1).

Burmolle, M. et al., 2014. Interactions in multispecies biofilms: Do they actually matter? *Trends in Microbiology*, 22(2), pp.84–91.

Burmolle, M., Hansen, L.H. & Sorensen, S.J., 2007. Establishment and early succession of a multispecies

biofilm composed of soil bacteria. *Microbial Ecology*, 54(2), pp.352–362.

Burmølle, M, Kjøller, AH & Sørensen, SJ 2012, An invisible workforce: biofilms in the soil. in G Lear & GD Lewis (eds), *Microbial biofilms: current research and applications.*Caister Academic Press, pp. 61-71.

Burns, R.G. et al., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology and Biochemistry*, 58, pp.216–234.

Carlson, R.P. & Taffs, R.L., 2010. Molecular-level tradeoffs and metabolic adaptation to simultaneous stressors. *Current Opinion in Biotechnology*, 21(5), pp.670–676.

Chai, Y. et al., 2008. Bistability and Biofilm Formation in Bacillus subtilis. *Molecular Microbiology*, 67(2), pp.254–263.

Challis, G.L. & Hopwood, D. a, 2003. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by Streptomyces species. *Proceedings of the National Academy of Sciences of the United States of America*, 100 Suppl , pp.14555–14561.

Chubukov, V. et al., 2014. Coordination of microbial metabolism. *Nature Reviews Microbiology*, 12(5), pp.327–340.

Cornelis, P. & Bodilis, J., 2009. A survey of TonB-dependent receptors in fluorescent pseudomonads. *Environmental Microbiology Reports*, 1(4), pp.256–262.

Cornforth, D.M. & Foster, K.R., 2013. Competition sensing: the social side of bacterial stress responses. *Nature reviews. Microbiology*, 11(4), pp.285–93.

Costa, E., Pérez, J. & Kreft, J.U., 2006. Why is metabolic labour divided in nitrification? *Trends in Microbiology*, 14(5), pp.213–219.

Crawford, J.W. et al., 2012. Microbial diversity affects self-organization of the soil-microbe system with consequences for function. *Journal of The Royal Society Interface*, 9(71), pp.1302–1310.

Darch, S.E. et al., 2012. Density-dependent fitness benefits in quorum-sensing bacterial populations. *Proceedings of the National Academy of Sciences of the United States of America*, 109(21), pp.8259–8263.

Davies, J., Spiegelman, G.B. & Yim, G., 2006. The world of subinhibitory antibiotic concentrations. *Current Opinion in Microbiology*, 9(5), pp.445–453.

Dechesne, A., Or, D. & Smets, B.F., 2008. Limited diffusive fluxes of substrate facilitate coexistence of two competing bacterial strains. *FEMS Microbiology Ecology*, 64(1), pp.1–8.

Decho, A.W., Frey, R.L. & Ferry, J.L., 2011. Chemical challenges to bacterial AHL signaling in the environment. *Chemical Reviews*, 111(1), pp.86–99.

DeWitt, T.J., Sih, A. & Wilson, D.S., 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, 13(2), pp.77–81. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0169534797012743.

Diggle, S.P. et al., 2007. Cooperation and conflict in quorum-sensing bacterial populations. *Nature*, 450(7168), pp.411–414. Available at: http://www.nature.com/doifinder/10.1038/nature06279.

Diggle, S.P. et al., 2007. Evolutionary theory of bacterial quorum sensing: when is a signal not a signal? *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 362(1483), pp.1241–9.

Donlan, R.M., 2002. Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases*, 8(9), pp.881–890.

Dubnau, D. & Losick, R., 2006. Bistability in bacteria. *Molecular Microbiology*, 61(3), pp.564–572.

Dumas, Z., Ross-Gillespie, A. & Kummerli, R., 2013. Switching between apparently redundant iron-uptake mechanisms benefits bacteria in changeable environments. *Proc Biol Sci*, 280(1764), p.20131055.

Dykes, G.A. & Hastings, J.W., 1998. Fitness costs associated with class IIa bacteriocin resistance in Listeria monocytogenes B73. *Letters in Applied Microbiology*, 26(1), pp.5–8.

Effmert, U. et al., 2012. Volatile Mediated Interactions Between Bacteria and Fungi in the Soil. *Journal of Chemical Ecology*, 38(6), pp.665–703.

van Elsas, J.D. & Bailey, M.J., 2002. The ecolgy of transfer of mobile genetic elements. *FEMS Microb. Ecol.*, 42, pp.187–197.

Elser, J., 2006. Biological stoichiometry: a chemical bridge between ecosystem ecology and evolutionary biology. *The American naturalist*, 168 Suppl , pp.S25–35.

Ernebjerg, M. & Kishony, R., 2012. Distinct growth strategies of soil bacteria as revealed by large-scale colony tracking. *Applied and Environmental Microbiology*, 78(5), pp.1345–1352.

Estrela, S., Morris, J.J. & Kerr, B., 2015. Private benefits and metabolic conflicts shape the emergence of microbial interdependencies. *Environmental Microbiology*, 18, pp.1415–1427.

Ettema, C.H. & Wardle, D. a., 2002. Spatial soil ecology. *Trends in Ecology and Evolution*, 17(4), pp.177–183.

Falkowski, P.G., Fenchel, T. & Delong, E.F., 2008. The microbial engines that drive Earth's biogeochemical cycles. *Science (New York, N.Y.)*, 320(5879), pp.1034–9.

Federle, M.J. & Bassler, B.L., 2003. Interspecies communication in bacteria. *Journal of Clinical Investigation*, 112(9), pp.1291–1299.

Fiegna, F., Moreno-Letelier, A., et al., 2015. Evolution of species interactions determines microbial community productivity in new environments. *The ISME Journal*, 9(5), pp.1235–1245.

Fiegna, F., Scheuerl, T., et al., 2015. Saturating effects of species diversity on life-history evolution in bacteria. *Proceedings. Biological sciences / The Royal Society*, 282(1815), p.20151794–.

Flemming, H. & Wingender, J., 2010. The biofilm matrix. *Nature reviews. Microbiology*, 8(9), pp.623–33.

Folse, H.J. & Allison, S.D., 2012. Cooperation, competition, and coalitions in enzyme-producing microbes: social evolution and nutrient depolymerization rates. *Frontiers in microbiology*, 3(September), p.338.

Frankena, J., Van Verseveld, H.W. & Stouthamer, a H., 1988. Substrate and energy costs of the production of exocellular enzymes by Bacillus licheniformis. *Biotechnology and bioengineering*, 32(6), pp.803–812.

Freilich, S. et al., 2011. Competitive and cooperative metabolic interactions in bacterial communities. *Nature Communications*, 2, p.589.

Fussmann, G.F., Loreau, M. & Abrams, P.A., 2007. Eco-evolutionary dynamics of communities and ecosystems. *Functional Ecology*, 21(3), pp.465–477.

Galet, J. et al., 2014. Gluconic acid-producing Pseudomonas sp. prevent γ-actinorhodin biosynthesis by Streptomyces coelicolor A3(2). *Archives of Microbiology*, 3, pp.619–627.

Galet, J. et al., 2015. Pseudomonas fluorescens Pirates both Ferrioxamine and Ferricoelichelin Siderophores from Streptomyces ambofaciens. *Applied and Environmental Microbiology*, 81(9), pp.3132–3141.

Garbeva, P. et al., 2011. Transcriptional and antagonistic responses of Pseudomonas fluorescens Pf0-1

to phylogenetically different bacterial competitors. *The ISME journal*, 5(6), pp.973–985.

Garbeva, P. & de Boer, W., 2009. Inter-specific interactions between carbon-limited soil bacteria affect behavior and gene expression. *Microbial Ecology*, 58(1), pp.36–46.

Geisel, N., Vilar, J.M.G. & Rubi, J.M., 2011. Optimal resting-growth strategies of microbial populations in fluctuating environments. *PLoS ONE*, 6(4).

Giovannoni, S.J., Cameron Thrash, J. & Temperton, B., 2014. Implications of streamlining theory for microbial ecology. *The ISME journal*, 8(8), pp.1553–1565..

Goldenfeld, N. & Woese, C., 2007. Biology's next revolution. *Nature*, 445(January), p.369.

Goldfarb, K.C. et al., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Frontiers in microbiology*, 2(May), p.94..

Haloin, J.R. & Strauss, S.Y., 2008. Interplay between ecological communities and evolution: review of feedbacks from microevolutionary to macroevolutionary scales. *Annals of the New York Academy of Sciences*, 1133, pp.87–125..

Hamilton, W.D., 1964. The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, 7(1), pp.1–16.

Haq, I.U. et al., 2014. The Genome of the Fungal-Interactive Soil Bacterium Burkholderia terrae BS001-A Plethora of Outstanding Interactive Capabilities Unveiled. *Genome biology and evolution*, 6(7), pp.1652–68.

Harcombe, W., 2010. Novel cooperation experimentally evolved between species. *Evolution*, 64(7), pp.2166–2172.

Harder, W. & Dijkhuizen, L., 1983. Physiological Responses to Nutrient Limitation. *Annual review of*

microbiology, 37, pp.1–23.

Hausner, M. & Wuertz, S., 1999. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Applied and environmental microbiology*, 65(8), pp.3710–3.

Hense, B. a et al., 2007. Does efficiency sensing unify diffusion and quorum sensing? *Nature reviews. Microbiology*, 5(3), pp.230–239.

Hibbing, M.E. et al., 2010. Bacterial competition: surviving and thriving in the microbial jungle. *Nature reviews. Microbiology*, 8(1), pp.15–25.

Huang, Y. et al., 2013. In silico and experimental methods revealed highly diverse bacteria with quorum sensing and aromatics biodegradation systems - A potential broad application on bioremediation. *Bioresource Technology*, 148, pp.311–316..

Jass, J., Roberts, S.K. & Lappin-Scott, H.M., 2002. Microbes and enzymes in biofilms. In R. G. Burns & R. D. Dick, eds. *Enzymes in the Environment: Activity, Ecology, and Applications*. New York, NY: Marcel Dekker, Inc., pp. 307–326.

Jauri, P.V. et al., 2013. Subinhibitory antibiotic concentrations mediate nutrient use and competition among soil Streptomyces. *PLoS ONE*, 8(12).

Jefferson, K.K., 2004. What drives bacteria to produce a biofilm? *FEMS Microbiology Letters*, 236(2), pp.163–173.

Jobbágy, E.G. & Jackson, R.B., 2000. the Vertical Distribution of Soil Organic Carbon and Its. *Ecological Applications*, 10(2), pp.423–436..

Johnson, M.T.J. & Stinchcombe, J.R., 2007. An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology and Evolution*, 22(5), pp.250–257.

Jousset, a et al., 2011. Genotypic richness and dissimilarity opposingly affect ecosystem functioning. *Ecology letters*, 14(6), pp.537–45.

Kalia, V.C., 2013. Quorum sensing inhibitors: An overview. *Biotechnology Advances*, 31(2), pp.224–245.

Kelsic, E.D. et al., 2015. Counteraction of antibiotic production and degradation stabilizes microbial communities. *Nature*, 521(7553), pp.516–519. Available at:

Kerr, B. et al., 2002. Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature*, 418(6894), pp.171–174.

Khandelwal, R.A. et al., 2013. Community Flux Balance Analysis for Microbial Consortia at Balanced Growth. *PLoS ONE*, 8(5).

Kim, H.J. et al., 2008. Defined spatial structure stabilizes a synthetic multispecies bacterial community. *Proceedings of the National Academy of Sciences of the United States of America*, 105(47), pp.18188–18193.

Kim, M. & Or, D., 2015. Individual-based model of microbial life on hydrated rough soil surfaces. *PLoS ONE*, pp.1–32.

Kim, W. et al., 2014. Importance of positioning for microbial evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 111(16), pp.E1639–47.

Kinkel, L.L. et al., 2014. Sympatric inhibition and niche differentiation suggest alternative coevolutionary trajectories among Streptomycetes. *The ISME journal*, 8(2), pp.249–56.

Klitgord, N. & Segre, D., 2011. Ecosystems biology of microbial metabolism. *Current Opinion in Biotechnology*, 22(4), pp.541–546.

Koch, A.L., 1985. The macroeconomics of bacterial growth. In M. Fletcher & G. D. Floodgate, eds.

Bacteria in their Natural Environments. Academic Press, pp. 1–42.

Krug, D. et al., 2008. Discovering the hidden secondary metabolome of Myxococcus xanthus: A study of intraspecific diversity. *Applied and Environmental Microbiology*, 74(10), pp.3058–3068.

Langenheder, S. et al., 2010. Bacterial biodiversity-ecosystem functioning relations are modified by environmental complexity. *PloS one*, 5(5), p.e10834.

Lawrence, D. et al., 2012. Species interactions alter evolutionary responses to a novel environment. *PLoS Biology*, 10(5).

Leadbetter, J.R. & Greenberg, E.P., 2000. Metabolism of acyl-homoserine lactone quorum-sensing signals by Variovorax paradoxus. *Journal of Bacteriology*, 182(24), pp.6921–6926.

Lennon, J.T. & Jones, S.E., 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature reviews. Microbiology*, 9(2), pp.119–130.

Li, P.F. et al., 2013. Co-cultivation of Sorangium cellulosum strains affects cellular growth and biosynthesis of secondary metabolite epothilones. *FEMS Microbiology Ecology*, 85(2), pp.358–368.

Liang, C. et al., 2011. An Absorbing Markov Chain approach to understanding the microbial role in soil carbon stabilization. *Biogeochemistry*, 106(3), pp.303–309.

Lipson, D. a., 2015. The complex relationship between microbial growth rate and yield and its implications for ecosystem processes. *Frontiers in Microbiology*, 6(June), pp.1–5.

Lipson, D. a. et al., 2009. The trade-off between growth rate and yield in microbial communities and the consequences for under-snow soil respiration in a high elevation coniferous forest. *Biogeochemistry*, 95(1), pp.23–35.

Little, A.E. et al., 2008. Rules of Engagement: Interspecies Interactions that Regulate Microbial

Communities. *Annu Rev Microbiol*, 62, pp.375–401.

Lopez, D. et al., 2009. Structurally diverse natural products that cause potassium leakage trigger multicellularity in Bacillus subtilis. *Proceedings of the National Academy of Sciences*, 106(1), pp.280–285.

Lv, G. et al., 2013. Influence of montmorillonite on antimicrobial activity of tetracycline and ciprofloxacin. *Journal of Asian Earth Sciences*, 77, pp.281–286.

Madsen, J.S. et al., 2012. The interconnection between biofilm formation and horizontal gene transfer. *FEMS immunology and medical microbiology*, 65(2), pp.183–95.

Mah, T.F.C. & O'Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. *Trends in Microbiology*, 9(1), pp.34–39.

Majeed, H. et al., 2013. The weak shall inherit: bacteriocin-mediated interactions in bacterial populations. *PloS one*, 8(5), p.e63837.

Martiny, A.C., Treseder, K. & Pusch, G., 2013. Phylogenetic conservatism of functional traits in microorganisms. *The ISME Journal*, 7(4), pp.830–8.

Matsumoto, Y. et al., 2013. Growth rate-coordinated transcriptome reorganization in bacteria. *BMC genomics*, 14, p.808.

Matulich, K.L. & Martiny, J.B.H., 2014. Microbial composition alters the response of litter decomposition to environmental change. *Ecology*, 96(1), p.140620135402001.

Matz, C. & Kjelleberg, S., 2005. Off the hook - How bacteria survive protozoan grazing. *Trends in Microbiology*, 13(7), pp.302–307.

McGinty, S.É.M. et al., 2013. The interplay between relatedness and horizontal gene transfer drives the

evolution of plasmid-carried public goods. *International Journal of Molecular Sciences*, 14, pp. 22246-22257.

McInerney, M.J. et al., 2008. Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. *Annals of the New York Academy of Sciences*, 1125, pp.58–72.

Miethke, M., Kraushaar, T. & Marahiel, M. a., 2013. Uptake of xenosiderophores in Bacillus subtilis occurs with high affinity and enhances the folding stabilities of substrate binding proteins. *FEBS Letters*, 587(2), pp.206–213.

Mitri, S. & Foster, K.R., 2013. The genotypic view of social interactions in microbial communities. *Annual review of genetics*, 47, pp.247–73.

Mitri, S., Xavier, J.B. & Foster, K.R., 2011. Social evolution in multispecies biofilms. *Proceedings of the National Academy of Sciences of the United States of America*, 108 Suppl , pp.10839–10846.

Molin, S. & Tolker-Nielsen, T., 2003. Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. *Current Opinion in Biotechnology*, 14(3), pp.255–261.

Moorhead, D.L. & Sinsabaugh, R.L., 2006. A Theoretical Model of Litter Decay and Microbial Interaction. *Ecological Monographs*, 76(2), pp.151–174.

Morris, B.E.L. et al., 2013. Microbial syntrophy: Interaction for the common good. *FEMS Microbiology Reviews*, 37(3), pp.384–406.

Morris, J.J., 2015. Black Queen evolution: the role of leakiness in structuring microbial communities. *Trends in Genetics*, 31(8), pp.475–482.

Morris, J.J. et al., 2012. The Black Queen Hypothesis : Evolution of Dependencies through Adaptive Gene

Loss. mBio 3(2), pp. 1-7.

Mouillot, D. et al., 2011. Functional structure of biological communities predicts ecosystem multifunctionality. *PloS one*, 6(3), p.e17476.

Müller, S. et al., 2014. Bacillaene and sporulation protect Bacillus subtilis from predation by Myxococcus xanthus. *Applied and environmental microbiology*, 80(18), pp.5603–5610.

Murren, C.J. et al., 2015. Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity*, 115(November 2014), pp.293–301.

Nadell, C.D., Foster, K.R. & Xavier, J.B., 2010. Emergence of spatial structure in cell groups and the evolution of cooperation. *PLoS Computational Biology*, 6(3).

Narisawa, N. et al., 2008. Coexistence of antibiotic-producing and antibiotic-sensitive bacteria in biofilms is mediated by resistant bacteria. *Applied and environmental microbiology*, 74(12), pp.3887–94.

Neill, C. & Gignoux, J., 2006. Soil organic matter decomposition driven by microbial growth: A simple model for a complex network of interactions. *Soil Biology and Biochemistry*, 38(4), pp.803–811.

Netzker, T. et al., 2015. Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. *Frontiers in Microbiology*, 6(April), pp.1–14.

Nogueira, T. et al., 2009. Horizontal gene transfer of the secretome drives the evolution of bacterial cooperation and virulence. *Current biology : CB*, 19(20), pp.1683–91.

Nogueira, T., Touchon, M. & Rocha, E.P.C., 2012. Rapid evolution of the sequences and gene repertoires of secreted proteins in bacteria. *PloS one*, 7(11), p.e49403.

Norman, A., Hansen, L.H. & Sørensen, S.J., 2009. Conjugative plasmids: vessels of the communal gene pool. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*,

364(1527), pp.2275–2289.

O'Donnell, A.G. et al., 2007. Visualization, modelling and prediction in soil microbiology. *Nature Reviews Microbiology*, 5(9), pp.689–699.

Oberhardt, M. a, Palsson, B.Ø. & Papin, J. a, 2009. Applications of genome-scale metabolic reconstructions. *Molecular systems biology*, 5(320), p.320.

Ochman, H., Lawrence, J.G. & Groisman, E. a, 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature*, 405(6784), pp.299–304.

Oliveira, N.M., Niehus, R. & Foster, K.R., 2014. Evolutionary limits to cooperation in microbial communities. *Proceedings of the National Academy of Sciences*, 111(50), p.201412673.

Pande, S. et al., 2014. Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria. *The ISME journal*, 8(5), pp.953–62.

Parsek, M.R. & Greenberg, E.P., 2005. Sociomicrobiology: The connections between quorum sensing and biofilms. *Trends in Microbiology*, 13(1), pp.27–33.

Pfeiffer, T. & Bonhoeffer, S., 2004. Evolution of cross-feeding in microbial populations. *The American naturalist*, 163(6), pp.E126–35.

Pfeiffer, T., Schuster, S. & Bonhoeffer, S., 2001. Cooperation and competition in the evolution of ATP-producing pathways. *Science (New York, N.Y.)*, 292(5516), pp.504–507.

Pion, M. et al., 2013. Gains of bacterial flagellar motility in a fungal world. *Applied and Environmental Microbiology*, 79(22), pp.6862–6867.

Poltak, S.R. & Cooper, V.S., 2011. Ecological succession in long-term experimentally evolved biofilms produces synergistic communities. *The ISME journal*, 5(3), pp.369–78.

Ponomarova, O. & Patil, K.R., 2015. Metabolic interactions in microbial communities: untangling the Gordian knot. *Current Opinion in Microbiology*, 27, pp.37–44.

Popat, R. et al., 2015. Conflict of interest and signal interference lead to the breakdown of honest signaling. *Evolution*, 69(9), pp.2371–2383.

Powers, M.J. et al., 2015. Inhibition of Cell Differentiation in Bacillus subtilis by Pseudomonas protegens. *Journal of Bacteriology*, 197(13), pp.JB.02535–14.

Prasad, S. et al., 2011. Antagonistic interaction networks among bacteria from a cold soil environment. *FEMS Microbiology Ecology*, 78(2), pp.376–385.

Prigent-combaret, C. et al., 1999. Abiotic Surface Sensing and Biofilm-Dependent Regulation of Gene Expression in Escherichia coli. *Journal of Bacteriology*, 181(19), pp.5993–6002.

Prosser, J.I., 2012. Ecosystem processes and interactions in a morass of diversity. *FEMS Microbiology Ecology*, 81(3), pp.507–519.

Rainey, P.B. et al., 2014. Microbes are not bound by sociobiology: Response to Kümmerli and Ross-Gillespie (2013). *Evolution*, 68(11), pp.3344–3355.

Rankin, D.J. et al., 2012. The coevolution of toxin and antitoxin genes drives the dynamics of bacterial addiction complexes and intragenomic conflict. *Proceedings. Biological sciences / The Royal Society*, 279(1743), pp.3706–15.

Rankin, D.J., Rocha, E.P.C. & Brown, S.P., 2011. What traits are carried on mobile genetic elements, and why? *Heredity*, 106(1), pp.1–10.

Ratcliff, W.C. et al., 2013. Disentangling direct and indirect fitness effects of microbial dormancy. *The American naturalist*, 182(2), pp.147–56.

Raynaud, X. & Nunan, N., 2014. Spatial ecology of bacteria at the microscale in soil. *PLoS ONE*, 9(1).

Reed, H.E. & Martiny, J.B.H., 2007. Testing the functional significance of microbial composition in natural communities. *FEMS Microbiology Ecology*, 62, pp.161–170.

Ren, D. et al., 2014. High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation. *The ISME journal*, pp.1–9.

Rigali, S. et al., 2008. Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by Streptomyces. *EMBO reports*, 9(7), pp.670–675.

Riley, M.A. & Lizotte-waniewski, M., 2009. Population Genomics and the Bacterial Species Concept M. B. Gogarten, J. P. Gogarten, & L. C. Olendzenski, eds. , 532(16), pp.1–10.

Roberson, E.B. & Firestone, M.K., 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. *Applied and Environmental Microbiology*, 58(4), pp.1284–1291.

Ross-Gillespie, A. et al., 2007. Frequency dependence and cooperation: theory and a test with bacteria. *The American naturalist*, 170(3), pp.331–42.

Sauer, K. & Camper, A.K., 2001. Characterization of Phenotypic Changes in Pseudomonas putida in Response to Surface-Associated Growth Characterization of Phenotypic Changes in Pseudomonas putida in Response to Surface-Associated Growth. *Society*, 183(22), pp.6579–6589.

Schimel, J., Balser, T.C. & Wallenstein, M., 2007. MICROBIAL STRESS-RESPONSE PHYSIOLOGY AND ITS IMPLICATIONS for Ecosystem Function. , 88(6), pp.1386–1394.

Schimel, J.P. & Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. *frontiers in microbiology*, 3, pp.1–11.

Schmidt, T.M. & Konopka, A.E., 2009. Physiological and Ecological Adaptations of Slow-Growing , Heterotrophic Microbes and Consequences for Cultivation. In S. S. Epstein, ed. *Uncultivated Microorganisms*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 257–276.

Schoener, T.W., 2011. The Newest Synthesis : Understanding Ecological Dynamics. *Science*, 331(January), pp.426–429.

Schuster, M. et al., 2003. Identification, Timing, and Signal Specificity of Pseudomonas aeruginosa Quorum-Controlled Genes: A Transcriptome Analysis. *Society*, 185(7), pp.2066–2079.

Seneviratne, G., 2015. Signal transduction in edaphic ecosystems governs sustainability. *Agriculture, Ecosystems & Environment*, 210, pp.47–49.

Shank, E. a. et al., 2011. Interspecies interactions that result in Bacillus subtilis forming biofilms are mediated mainly by members of its own genus. *Proceedings of the National Academy of Sciences*, 108(48), pp.E1236–E1243.

Sinsabaugh, R.L. et al., 2015. Scaling microbial biomass, metabolism and resource supply. *Biogeochemistry*, 122(2-3), pp.175–190.

Smith, D.R. & Chapman, M.R., 2010. Economical evolution: Microbes reduce the synthetic cost of extracellular proteins. *mBio*, 1(3), pp.28–32.

Šnajdr, J. et al., 2011. Saprotrophic basidiomycete mycelia and their interspecific interactions affect the spatial distribution of extracellular enzymes in soil. *FEMS Microbiology Ecology*, 78(1), pp.80–90.

Sorensen, S.J. et al., 2005. Studying plasmid horizontal transfer in situ: a critical review. *Nature reviews. Microbiology*, 3(9), pp.700–10.

Stevenson, B.S. & Schmidt, T.M., 2004. Life History Implications of rRNA Gene Copy Number in

Escherichia coli. *Society*, 70(11), pp.6670–6677.

Stewart, P.S., 2003. Diffusion in Biofilms. *Journal of Bacteriology*, 185(5), pp.1485–1491.

Stewart, P.S. & Franklin, M.J., 2008. Physiological heterogeneity in biofilms. *Nature Reviews Microbiology*, 6(3), pp.199–210.

Straight, P.D. & Kolter, R., 2009. Interspecies chemical communication in bacterial development. *Annual review of microbiology*, 63, pp.99–118.

Straight, P.D., Willey, J.M. & Kolter, R., 2006. Interactions between Streptomyces coelicolor and Bacillus subtilis: Role of surfactants in raising aerial structures. *Journal of Bacteriology*, 188(13), pp.4918–4925.

Strauss, S.Y., Sahli, H. & Conner, J.K., 2005. Toward a more trait-centered approach to diffuse (co)evolution. *New Phytologist*, 165(1), pp.81–90.

Strickland, M.S., McCulley, R.L. & Bradford, M.A., 2013. The effect of a quorum-quenching enzyme on leaf litter decomposition. *Soil Biology and Biochemistry*, 64, pp.65–67.

Strunnikova, O.K. et al., 2007. Development and relations of Fusarium culmorum and Pseudomonas fluorescens in soil. *Mikrobiologiia*, 76(5), pp.675–681.

Subbiah, M. et al., 2011. Beta-Lactams and Florfenicol Antibiotics Remain Bioactive in Soils While Ciprofloxacin, Neomycin, and Tetracycline Are Neutralized. *Applied and Environmental Microbiology*, 77(20), pp.7255–7260.

Szczepaniak, Z. et al., 2015. Antibacterial effect of the Trichoderma viride fungi on soil microbiome during PAH's biodegradation. *International Biodeterioration & Biodegradation*, 104, pp.170–177.

Teixeira De Mattos, M.J. & Neijssel, O.M., 1997. Bioenergetic consequences of microbial adaptation to

low-nutrient environments. *Journal of Biotechnology*, 59(1-2), pp.117–126.

Thompson, J.N., 2005. Coevolution: The Geographic Mosaic of Coevolutionary Arms Races. *Current Biology*, 15(24), pp.992–994.

Tilman, D. et al., 2009. Diversity and Productivity in a Long-Term Grassland Experimen ... Diversity and Productivity in a Long-Term Grassland Experiment Diversity and Productivity in a Long-Term Grassland Experimen ... *October*, 294(2008), pp.9–11.

Tilman, D., 1981. Tests of resource competition theory using four species of Lake Michigan algae. *Ecology*, 62(3), pp.802–815.

Tiunov, A. V. & Scheu, S., 2005. Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. *Ecology Letters*, 8(6), pp.618–625.

Tolonen, A.C. et al., 2014. Fungal lysis by a soil bacterium fermenting cellulose. *Environmental microbiology*, 17, pp.2618–2627.

Touchon, M. et al., 2009. Organised genome dynamics in the Escherichia coli species results in highly diverse adaptive paths. *PLoS Genetics*, 5(1).

Travisano, M. & Velicer, G.J., 2004. Strategies of microbial cheater control. *Trends in microbiology*, 12(2), pp.72–8.

Traxler, M.F. et al., 2012. Interspecies modulation of bacterial development through iron competition and siderophore piracy. *Molecular Microbiology*, 86(3), pp.628–644.

Traxler, M.F. & Kolter, R., 2015. Natural products in soil microbe interactions and evolution. *Nat. Prod. Rep.*, 32, pp.956–970.

Turcotte, M.M., Corrin, M.S.C. & Johnson, M.T.J., 2012. Adaptive Evolution in Ecological Communities.

PLoS Biology, 10(5), p.e1001332.

Tyc, O. et al., 2014. Impact of interspecific interactions on antimicrobial activity among soil bacteria. *Frontiers in Microbiology*, 5(October), pp.1–10.

Tyc, O., Wolf, A.B. & Garbeva, P., 2015. The Effect of Phylogenetically Different Bacteria on the Fitness of Pseudomonas fluorescens in Sand Microcosms. *Plos One*, 10(3), p.e0119838.

Vetsigian, K., Jajoo, R. & Kishony, R., 2011. Structure and evolution of Streptomyces interaction networks in soil and in silico. *PLoS biology*, 9(10), p.e1001184.

Vieira-Silva, S. & Rocha, E.P.C., 2010. The systemic imprint of growth and its uses in ecological (meta)genomics. *PLoS Genetics*, 6(1).

Vu, B. et al., 2009. Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules*, 14(7), pp.2535–2554.

Van der Wal, A. et al., 2013. A thready affair: Linking Fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews*, 37(4), pp.477–494.

Wang, Y. & Leadbetter, J.R., 2005. Rapid Acyl-Homoserine Lactone Quorum Signal Biodegradation in Diverse Soils †. , 71(3), pp.1291–1299.

Warmink, J. a. et al., 2011. Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. *Soil Biology and Biochemistry*, 43(4), pp.760–765.

Warmink, J.A. & Van Elsas, J.D., 2009. Migratory response of soil bacteria to Lyophyllum sp. strain Karsten In soil microcosms. *Applied and Environmental Microbiology*, 75(9), pp.2820–2830.

West, S. a. et al., 2007. The Social Lives of Microbes. *Annual Review of Ecology, Evolution, and Systematics*, 38(1), pp.53–77.

Wolf, A.B. et al., 2013. Impact of matric potential and pore size distribution on growth dynamics of filamentous and non-filamentous soil bacteria. *PLoS ONE*, 8(12).

Xavier, J.B. & Foster, K.R., 2007. Cooperation and conflict in microbial biofilms. *Proceedings of the National Academy of Sciences of the United States of America*, 104(3), pp.876–81.

Xavier, K.B. & Bassler, B.L., 2005. Interference with AI-2-mediated bacterial cell–cell communication. *Nature*, 437(7059), pp.750–753.

Young, I.M. & Crawford, J.W., 2004. Interactions and self-organization in the soil-microbe complex. *Science*, 304(5677), pp.1634–7.

Zhang, D. et al., 2012. Polymorphic toxin systems: Comprehensive characterization of trafficking modes, processing, mechanisms of action, immunity and ecology using comparative genomics. *Biology Direct*, 7(1), p.18.

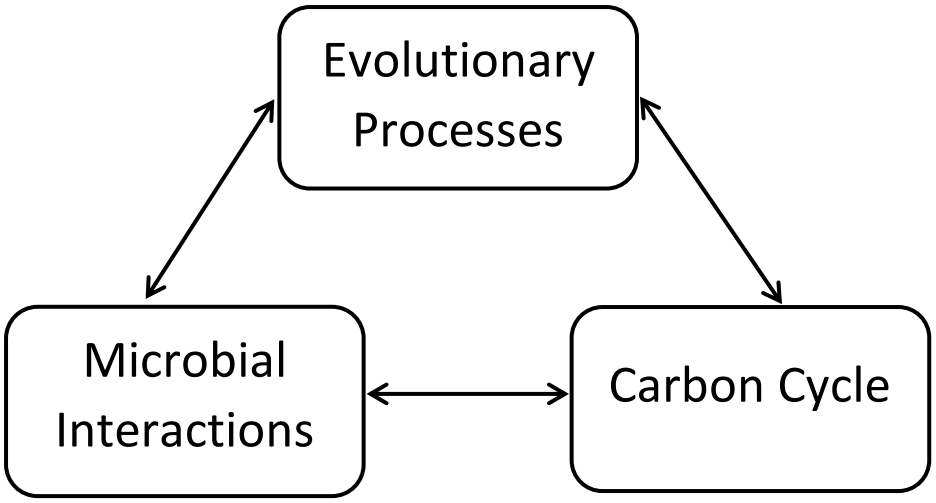
Zhang, M. et al., 2014. The mycosphere constitutes an arena for horizontal gene transfer with strong evolutionary implications for bacterial-fungal interactions. *FEMS Microbiology Ecology*.

Zhuang, X. et al., 2013. Bioactive molecules in soil ecosystems: Masters of the underground. *International Journal of Molecular Sciences*, 14(5), pp.8841–8868.

Figure legends:

Figure 1. A conceptual diagram of the feed back between microbial interactions, evolutionary processes, and the carbon cycle.

Table 1. Potential effects of microbial interactions on soil carbon cycling.



Interaction type	Soil carbon storage	Potential mechanisms
Exploitation	-	Rate of SOM degradation increases with increasing growth of exploiting population
Decrease in CUE	-	Reduction in biomass accumulation and increasing amount of carbon released as CO_2
Toxin production	+/-	Metabolic production costs may decrease carbon storage but growth inhibition might increase it. Reduction in niche overlap may contribute to increased SOM degradation.
Signal degradation	+	The targeted population will be unable to function cohesively in SOM degradation
Coercion	+/-	Effects are dependent upon what action is being coerced
Dormancy	+	Reduces total SOM degradation if dormancy caused by stressors other than nutrient limitation
Cross-feeding	-	Rate of SOM degradation increases, but yield may decrease
Syntrophy	-	Streamlines metabolic processes and facilitates SOM degradation in anoxic environments
Siderophore cheating	+/-	May increase or decrease SOM degradation depending on the relative metabolic costs of siderophore production and growth rates of the cheater and producer
Enzyme cheating and Black Queens	+	Reduction of degradation of SOM by lowering total enzyme production
Biofilm cheating	+/-	Increased carbon allocation to EPS and humic substances increases storage though associated production costs may cause greater CO_2 flux
Soil pore formation	-	Facilitates access to SOM, oxygen, and water resulting in increased degradation