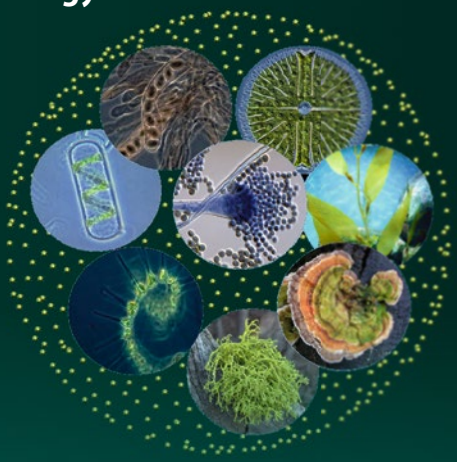
Advances in Environmental Microbiology 6



Christon J. Hurst *Editor*

Understanding Terrestrial Microbial Communities

Springer

Advances in Environmental Microbiology

Volume 6

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When I was born into this life during 1954 there was an older brother named Preston waiting for me! I am not certain about what my brother thought of the changes which my presence brought to his existence, but there I was and we became fairly inseparable. Not even his red haired Raggedy Ann, which had been my brother's 1952 Christmas present, came between my brother and I. Although, Raggedy Ann may have been a more patient listener.

Raggedy Ann, Preston and Christon in December 1954

My favorite childhood memory with my brother was when we played train ride in the basement of our house. Our parents had found some cardboard boxes and arranged the boxes as part of a circle on the basement floor. During many evenings, my brother and I would then each sit down inside of a box and pretend that we were taking an imagined journey.
A few years later, a sister named Embeth was born into our lives. Raggedy Ann had by then disappeared but Embeth came into this world with red hair, and we three Hurst children began a true life journey together.

Preston, Christon and Embeth in 1960

My little sister was a good listener, and my favorite childhood memory with my sister was when I used to read books to her and show to her the illustrations in the books as I turned the pages. Embeths favorite books for our reading time were the Mrs. Piggle-Wiggle series by Betty MacDonald.
The three of us siblings have grown older together and I happily remember journeys with them. Eventually, my brother and I took train rides on the Algoma Central Railway in Ontario, Canada, for canoeing trips with our Boy Scout troop. One time, Preston and I along

with his wife and son took a journey to Nashville, Indiana, not by train but by automobile and that was a particularly good day!

Preston and Christon Hurst in Nashville Indiana on October 2nd 2002

And many years later, I eventually took a trip to Germany with my sister during which I signed the contract to begin this book series.

Embeth at Cafe Winuwuk near Bad Harzburg Germany on July 25th 2013

I have proven to be more durable than was the Raggedy Ann doll, although perhaps I still am not as patient as a listener. And, my sister finds delight each time I show to her a new book that I have published. When this volume is printed I will read the dedication to my sister and show to her the pictures as I turn these front pages. Embeth will then smile and say to me, "Chrissy, you know that I now could read that for myself". My brothers comment will be "Chrissy, we need to find some new cardboard boxes and set up a train on the floor". And so, I lovingly dedicate my work on this book to Preston and Embeth who are my two dearest friends.

Series Preface

The light of natural philosophy illuminates many subject areas including an understanding that microorganisms represent the foundation stone of our biosphere by having been the origin of life on Earth. Microbes therefore comprise the basis of our biological legacy. Comprehending the role of microbes in this world which together all species must share, studying not only the survival of microorganisms but as well their involvement in environmental processes, and defining their role in the ecology of other species, does represent for many of us the Mount Everest of science. Research in this area of biology dates to the original discovery of microorganisms by Antonie van Leeuwenhoek, when in 1675 and 1676 he used a microscope of his own creation to view what he termed "animalcula," or the "little animals" which lived and replicated in environmental samples of rainwater, well water, seawater, and water from snow melt. van Leeuwenhoek maintained those environmental samples in his house and observed that the types and relative concentrations of organisms present in his samples changed and fluctuated with respect to time. During the intervening centuries we have expanded our collective knowledge of these subjects which we now term to be environmental microbiology, but easily still recognize that many of the individual topics we have come to better understand and characterize initially were described by van Leeuwenhoek. van Leeuwenhoek was a draper by profession and fortunately for us his academic interests as a hobbyist went far beyond his professional challenges.

It is the goal of this series to present a broadly encompassing perspective regarding the principles of environmental microbiology and general microbial ecology. I am not sure whether Antonie van Leeuwenhoek could have foreseen where his discoveries have led, to the diversity of environmental microbiology subjects that we now study and the wealth of knowledge that we have accumulated. However, just as I always have enjoyed reading his account of environmental microorganisms, I feel that he would enjoy our efforts through this series to summarize what we have learned. I wonder, too, what the microbiologists of still future centuries would think of our efforts in comparison with those now unimaginable discoveries which they will have achieved. While we study the many wonders of microbiology, we also

further our recognition that the microbes are our biological critics, and in the end they undoubtedly will have the final word regarding life on this planet.

Christon J. Hurst in Heidelberg

Indebted with gratitude, I wish to thank the numerous scientists whose collaborative efforts will be creating this series and those giants in microbiology upon whose shoulders we have stood, for we could not accomplish this goal without the advantage that those giants have afforded us. The confidence and very positive encouragement of the editorial staff at Springer DE has been appreciated tremendously and it is through their help that my colleagues and I are able to present this book series to you, our audience.

Cincinnati, OH Christon J. Hurst

Volume Preface

If the world suddenly were to be without its microbes, then none of the plants and animals which we perceive as comprising higher levels of life in our ecosystem could survive. This book presents a summary of knowledge regarding the natural terrestrial microbial processes which represent a key component of maintaining healthy life on our planet. The authors begin by explaining how microorganisms sustain the soil ecosystem through a recycling of carbon and nitrogen. That basic knowledge is followed by chapters which describe integration of soil microbiology processes into ecosystem science, usage of natural processes to achieve successful bioremediation including the accomplishment of safe and effective landfill operation, and design of composting processes which can help us to reduce the amount of wastes that we place into landfills. This book also presents an understanding of how human land usage patterns, including restoration efforts, affect soil microbial communities and how wetland microbial communities respond to anthropogenic pollutants. The book concludes with an understanding that many of the fungi which function by environmentally recycling the carbon and nitrogen of organic materials do sometimes begin their degradative action too soon, with the result being infectious diseases that are destructive of plants and can injure or even kill vertebrate species.

I am tremendously grateful to Andrea Schlitzberger, Markus Spaeth, and Isabel Ullmann at Springer DE, for their help and constant encouragement which has enabled myself and the other authors to achieve publication of this collaborative project.

Cincinnati, OH — Christon J. Hurst

Contents

Chapter 1
Carbon Cycle Implications of Soil Microbial Interactions

Kelly I. Ramin and Steven D. Allison

Abstract The soil environment contains the largest pool of organic carbon on the Earth's surface, with soil carbon residency and flux controlled by microbial metabolism. Despite the fact that microbial interactions have metabolic implications, these interactions are often overlooked in conceptual models of the soil 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 and Jackson 2000). Flux rates of carbon from the soil exceed anthropogenic emissions by up to ten times yearly (Chapin et al. 2002). Owing to the scale of soil carbon inputs into the atmosphere, and major concerns over human disruption of the global carbon cycle, it is important to understand the drivers of the soil carbon flux. Because microbes are responsible for the degradation and transformation of organic matter, soil carbon cycling is dependent upon microbial metabolism (Falkowski et al. 2008). 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

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cycling potential have become more resolved. Many stressors in the soil environment have been explored for their impact on carbon cycling. Yet less attention has focused on how microbial interactions influence the evolution and phenotypic expression of microbial traits that affect carbon cycling in the soil environment. This chapter will therefore discuss the impact of microbial interactions on traits involved with carbon cycling.

For the purposes of this chapter, interactions will be defined as processes driven by one microbe that have either positive or negative effects on survival or 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. We propose that microbial interactions act as pressures that result in changing the cellular allocation of resources underlying these processes. These pressures alter fitness cost/benefit ratios and ultimately impact carbon cycling.

This chapter also aims to address how microbial interactions influence community structure. Community structure may be important to carbon cycling if organisms show inter-taxa variation in their capacity for carbon cycling and if the breakdown of carbon is limited by cellular processes (Schimel and Schaeffer 2012). There is extensive evidence that changes in microbial community structure have impacts on carbon turnover (Balser and Firestone 2005; Matulich and Martiny 2014). More broadly, changes in diversity are often linked to altered functioning (Tilman et al. 2001; Bell et al. 2005). Interactions that alter diversity at the microsite, such as niche partitioning, or prevention of competitive exclusion, such as non-transitive interaction networks and negative frequency-dependent selection, therefore, will likely have effects on community carbon cycling (Cordero and Datta 2016).

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. 1.1). 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 associated community functioning. While these metabolic constraints on carbon transformation and shifts in allocation that change the fate of carbon may take place at the microsite, evidence suggests that microbial metabolic processes collectively scale up and contribute to carbon cycling at the ecosystem level (Brown et al. 2004; Elser 2006; Sinsabaugh et al. 2015). Therefore, the effect of microbial interactions on soil carbon flux potentially has relevance across multiple spatial and temporal scales, including the global scale over decades to centuries.

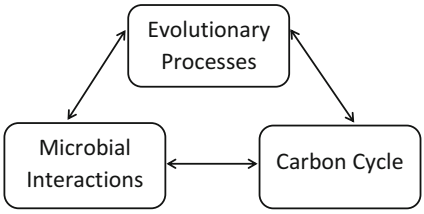

Fig. 1.1 A conceptual diagram of the feedback between microbial interactions, evolutionary processes, and the carbon cycle

1.2 Allocation Patterns

Microbial growth has been shown to drive soil organic matter (SOM) decomposition, indicating that metabolic mechanisms that impact growth rate have a large influence on soil carbon dynamics (Neill and Gignoux 2006). While growth rate is partly determined by rRNA copy number, or codon usage bias (Vieira-Silva and Rocha 2010; Stevenson and Schmidt 2004; Goldfarb et al. 2011), carbon use efficiency (CUE) is phenotypically variable and depends upon maintenance costs. As a metric, CUE defines the amount of growth achieved per unit of acquired carbon and may be an important control on carbon sequestration in soil (Allison et al. 2010; Bradford and Crowther 2013). Maximum possible microbial CUE has been estimated at approximately 60% of acquired carbon being assimilated into biomass or ATP but declines with growing maintenance costs (Schmidt and Konopka 2009). Maintenance costs vary with conditions and may increase with temperature, nutrient limitation, starvation, physiological stress, allocation to storage, extracellular products, and transporters (Lipson 2015; Matsumoto et al. 2013).

Microbes often face competition for limited resources in the soil environment. The investment in acquiring resources, part of cellular maintenance costs, generally lowers the overall metabolic efficiency of the cell (Teixeira De Mattos and Neijssel 1997). The phenotypic response of microbes living in resource-limited conditions includes synthesis of enzymes that acquire limiting resources to maximize uptake rates, synthesis of enzymes targeting alternative forms of the limiting resources, a decrease in anabolism to match the uptake of the limiting resources, and use of storage polymers to compensate resource deficiencies (Harder and Dijkhuizen 1983; Schmidt and Konopka 2009).

Metabolic theory posits that thermodynamics define absolute constraints on the uptake, transformation, and secretion of energy and matter, as well as the rates of these processes (Brown et al. 2004). These controls over energy and matter fluxes also dictate ecological interactions among organisms by defining a bacterium's ability to grow, produce molecules that impact surrounding bacteria, respond to declining resources, or counter chemical attacks. The cellular response to interactions may lead to a shift in allocation of resources that impacts the rate of carbon turnover and its ultimate fate in the soil environment. These metabolic interactions influence what percentage of acquired carbon is transformed and immediately released into the atmosphere, converted to biomass or extracellular products, or stored as recalcitrant compounds in the soil.

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 toward nongrowth 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.

In addition to the increase in resource-acquiring mechanisms, microbes in the soil alter their growth rates and production levels of other potentially costly molecules in response to interactions. Toxins attack predators and competitors for nutrients. Defense systems respond to interspecific assaults. Biofilm polymeric substances protect microbes against desiccation and antibiotics while slowing diffusion of nutrients away from the producing cells. Siderophores chelate iron to make it bioavailable. Production of these may also represent a decrease in fitness for a microbe.

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 their products are unused or being produced by other community members (Murren et al. 2015). Multiple studies have found loss of core metabolic genes in obligate symbiotic, parasitic, or commensal microbes. In contrast, some free-living microbes have streamlined their genomes by maintaining core functional genes while reducing the relative amount of intergenic spacer DNA and number of paralogous genes (Giovannoni et al. 2014; Solden et al. 2016). Microbes must balance their capacity for plasticity with the burden of DNA replication, immediate ecological and environmental pressures, and availability of genetic material through horizontal gene transfer (HGT).

1.2.1.1 Interaction Agents in the Soil Environment

Interaction-induced phenotypic alterations are often initiated via direct contact, metabolic by-products, 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 and Kolter 2009). Multiple studies have shown coordinated phenotypic responses to environmental or competitive stressors within and between populations (Challis and 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 “cross talk” or the ability to eavesdrop on heterospecific

autoinducers in the surrounding environment. These autoinducers are known as cues (Traxler and Kolter 2015; Netzker et al. 2015; Federle and Bassler 2003; Diggle et al. 2007a, b).

Microbial interactions may act to alter the expression of various traits that have implications in carbon cycling, such as growth rate and production of extracellular products. The production of many exoproducts is temporally and spatially modulated through intercellular signals within and between populations (Diggle et al. 2007a, b; 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 and 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 are affected by the size and adsorption properties of the autoinducer molecules and may be altered by pH and the ratio of clay to organic material (Traxler and 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). Additionally, some microbes produce degrading enzymes, agonists, and antagonists of autoinducer molecules (Wang and Leadbetter 2005; Xavier and 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 and Greenberg 2000; Kalia 2013).

Another direct mechanism that may force interspecific changes in microbial phenotype, and hence shifts in resource allocation, is contact-dependent inhibition (CDI) (Ruhe et al. 2013; Blanchard et al. 2014). This not only causes shifts in resource allocation and a decrease in growth for the CDI-producing cell but also decreases in growth or death of the recipient. This mechanism requires close proximity for action, conditions that arise in soil microbial biofilms.

Finally, microbes may cause changes to neighboring cells' phenotypes through indirect agents. Metabolic by-products can change the local abiotic conditions, such as pH, creating stressful conditions and altering metabolic efficiency of neighbors. Likewise, metabolic by-products can alter efficiency as newly available resources that benefit neighbors through cross-feeding.

1.2.1.2 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 and Kjelleberg 2005; Mah and O'Toole 2001; Roberson and Firestone 1992; Jefferson 2004). The prevalence of biofilm formation among bacteria, estimated to be at 99% of taxa, supplies 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 establish conditions that alter contact between microbes by immobilizing the biofilm cells next to each other, forming barriers to inhibit interactions, or altering diffusion rates of extracellular molecules.

The exact composition of biofilms varies widely 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 soil carbon stocks (Flemming and Wingender 2010). The combined, three-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 into 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 and Wingender 2010). The dramatic change in phenotype that accompanies the transition to a sedentary lifestyle within a biofilm makes it 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 observed in some cases (Burmolle et al. 2014; Mah and O'Toole 2001).

The transition from a planktonic lifestyle to a biofilm is accomplished through multiple changes in gene expression. 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 and Camper 2001; Prigent-combaret et al. 1999). These changes in gene expression can be initiated by environmental cues but have also been observed to be engendered through intercellular autoinducers (Parsek and 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 versus 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 coercive strategy.

Through the progressive stages of development of a biofilm, colonizers transform their created biofilm environment through cell autoinducers, waste products, and degradation of soil organic matter (SOM) (Stewart 2003). This transformation creates microenvironments that magnify spatial and temporal heterogeneity within the biofilm due to restricted diffusion, leading to changes in microbial phenotype relative to available resources and interacting organisms (Stewart and Franklin 2008). Some microbial processes also have bistable switches that respond to intercellular autoinducers that may affect the phenotypic heterogeneity displayed within a mature biofilm (Chai et al. 2008; Dubnau and Losick 2006). These mechanisms that increase heterogeneity may lead to an increase in community- or population-level efficiency through specialization in tasks and reduction of the unicellular burden of enzyme production, or a reduction in the waste of resources through cross-feeding, and may act to alter soil carbon turnover rates (Folse and Allison 2012; Jefferson 2004; Bernstein et al. 2012; Ackermann 2015; Huang et al. 2013).

The physical structure of EPS in the soil affects microbial processes and interactions by affecting diffusion rates. As the amount of EPS accumulates, diffusion rates of oxygen, nutrients, and waste products decrease, 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 and 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).

Alternatively, decreased diffusion associated with the EPS matrix may benefit microbes. Extracellular products that are available to and benefit all members of a community—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 and Wingender 2010). Because restricted diffusion effectively lowers the productive need of these molecules, it may allow the producing cells to devote more of their resources toward growth, improving metabolic efficiency and biomass accumulation. 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 monoculture biofilms. Only 2 of the 35 combinations of 4-species consortia showed decreased biofilm production relative to monocultures.

1.2.1.3 Growth and Dormancy

Interactions among microbes, whether positive or negative and direct or indirect, have the potential to affect growth and soil carbon cycling. Exploitation competition between microbes is indirect and involves depletion of a common limiting resource, with the winner having a higher capacity for resource acquisition. Higher resource acquisition increases growth rate, effectively starving the loser of resources. An evolutionary focus on this strategy may only be successful when resources are available (Stevenson and Schmidt 2004; Goldfarb et al. 2011; Moorhead and Sinsabaugh 2006). Given the highly variable availability of resources, it is unsurprising that the soil environment hosts a wide diversity of microbial growth strategies, beyond the simple dichotomy of copiotrophs and oligotrophs (Ernebjerg and Kishony 2012; Vieira-Silva and Rocha 2010). Yet the ability of microbes to maintain relatively high growth rates down to nanomolar or micromolar concentrations of substrate due to the maximization of uptake suggests a strong selective advantage for exploitative competition (Schmidt and Konopka 2009).

Indeed, some bacteria may have evolved measures to manipulate their growth rate as a competitive measure. By switching to a high-growth rate low-yield strategy, bacteria disproportionately acquire available resources even though their metabolic efficiency declines (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 their growth. This strategy has the effect of increasing carbon turnover and flux but is only beneficial under conditions with high rates of resource diffusion (Lipson 2015). Therefore, this mechanism would likely only occur at the surface of biofilms where high diffusion rates of oxygen and resources take place.

Additionally, interference competition, in which competitors directly and aggressively fight over resources, often supports exploitative efforts. 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 and Foster 2013; Garbeva and de Boer 2009). This slowed growth may accompany an allocation toward cellular maintenance costs of antibiotic production, but it has also been proposed that the slowed growth is a preemptive protective measure against antibiotic attacks (Mah and O'Toole 2001). The reason why slowed growth imparts protection is unclear. However, because resistance to antibiotics may also carry a fitness cost, the slowed growth could be associated with this shift in allocation away from growth and toward resistance (Andersson and Levin 1999; Andersson and Hughes 2010; Dykes and 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. Slowed growth may also be caused by production of coercive molecules to suppress antibiotic production in a neighboring cell or to trigger antibiotic production in a third cell that is forced into the role of bodyguard (Tyc et al. 2014; Abrudan et al. 2015; Galet et al. 2014). Given the fitness cost of production of some growth inhibitory molecules, it is surprising that one study found 33% of soil bacteria constitutively produce antibiotics, lending credence to the hypothesis that antibiotics may also serve as autoinducers (Tyc et al. 2014).

Dormancy or a reduced metabolic state will have indirect fitness consequences for a population by freeing up for their kin the resources that microbes otherwise would have consumed for themselves (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 and Franklin 2008). In the soil environment, approximately 80% of all bacteria are in a dormant state (Lennon and 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 for cellular shutdown, including production of machinery to go into and out of dormancy, as well as resting structures (Lennon and Jones 2011). Ultimately, microbial interactions affect the rate at which neighboring microbes transition into a dormant state, either through exploitation or kin selection, thus altering soil carbon turnover rates.

1.3 Evolution of Traits with Carbon Cycling Consequences

Studies of social evolution are often performed using microbes due to their relative simplicity. 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 behaviors, these experiments inform about potential mechanisms that may occur through interactions and, as such, are important to begin understanding how evolution impacts carbon cycling (Rainey et al. 2014). Social behaviors have fitness effects for both the actor and the recipient. Cooperative behaviors can be mutually beneficial, in which both the actor and recipient receive positive fitness results, or altruistic, in which the actor does not. Likewise, competitive behaviors are broken down into selfish, with the actor receiving a fitness benefit while the recipient is harmed, or spiteful, with both being harmed (Hamilton 1964). Natural selection acts on genetic variation, often a single, specific locus in microbes (Mitri and Foster 2013). For many social evolutionary mechanisms, relatedness is determined at one specific gene, such as for a public good or toxin (Table 1.1).

Pressures that shift the cellular balance away from reproduction, such as those that occur through microbial interactions, act as selective forces that may have implications for carbon cycling. 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

Table 1.1 Potential effects of microbial interactions on soil carbon cycling

Interaction type	Effect upon 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 increase 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

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.

Conversely, costly traits may be maintained if the cost of loss increases, as occurs with enforcement tactics carried on mobile genetic elements (MGEs).

1.3.1 Horizontal Gene Transfer

Many of the genes responsible for microbial interactions and carbon cycling are part of the accessory genome, which constitutes upwards of 90% of a bacterial taxon's pan-genome (Touchon et al. 2009; Haq et al. 2014; Rankin et al. 2011). The accessory genome—those genes contained within a microbe that are shared through

HGT via mobile genetic elements (MGEs) such as transposons, bacteriophages, and plasmids—predominantly codes for secreted proteins but can also encode metabolic traits and pathways (Falkowski et al. 2008; Ochman et al. 2000; Nogueira et al. 2012). The more complex pathways may be difficult to transfer, however, because of their multigene nature and incongruity with preexisting pathways (Schimel and Schaeffer 2012). This has likely led to the deeply conserved nature of these large metabolic units (Martiny et al. 2015).

Transmission of MGEs increases at higher cellular densities (Sorensen et al. 2005; McGinty et al. 2013). 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 and Wingender 2010; Hausner and Wuertz 1999; Burmolle et al. 2014; Sorensen et al. 2005; Molin and 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). Therefore, 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; Norman et al. 2009).

Because many of the genes carried on MGEs code for public goods that are secreted from the cell, the potential loss of public goods by diffusion implicitly increases the cost of production to the cell and likelihood of gene ejection. As is the case with whole organisms, MGE success depends upon propagation. To resolve a potential conflict of survival between the host and the MGE, an evolutionary compromise has been observed in which the biosynthetic cost of secreted and outer membrane proteins is often lower than those for purposes elsewhere in the cell, improving the likelihood of the MGE maintenance within the cell (Nogueira et al. 2009; Smith and Chapman 2010).

It is important to consider that MGEs have also evolved mechanisms of forced maintenance. These mechanisms impact interactions between microbes as well as metabolic efficiency through shifts in allocation of resources toward fabrication of MGE products. For example, addiction complexes contain a toxin-antitoxin complex, with the antitoxin degrading more rapidly than the toxin (Zhang et al. 2012). Because the toxin remains in effect for a longer period than the antitoxin, the cell loses immunity upon loss of the MGE, and fitness lowers to zero.

Through MGEs a picture emerges of how function and interactions feed into one another. Microbes create biofilms that favor HGT, and MGEs contain traits that impact neighboring cells. The toxin-antitoxin complexes force production of their products while killing local cells that have not acquired the same complex. Depending on what other genes might be carried with these complexes, this may also have a large impact on the production of public goods that are involved in carbon cycling or sequestration. Even without a toxin-antitoxin complex, the associated increase of relatedness involved with HGT creates a dynamic of kin selection that promotes production of public goods encoded on MGEs (McGinty et al. 2013). Despite this immediate and localized increase in relatedness, HGT is thought to

contribute to the larger process of speciation (Boto 2010). In fact, genes associated with secreted proteins have been found to evolve at a relatively high rate (Nogueira et al. 2012), which may have more downstream effects on carbon cycling as discussed in the continuing sections.

1.3.2 Cheaters

Cheating is an evolutionary strategy that either eliminates the cost of production of a public good for the cheater while using the goods produced by others or disproportionately increases access to a limiting resource for the cheating microbe. The success of any cheating strategy is density dependent, as a competitive strategy only has benefits insomuch that it is distinct among its competitors (West et al. 2007; Ross-Gillespie et al. 2007). It also depends upon diffusion rates, spatial structure, and available resources. Despite the population-level benefit of cooperative public good production, cheating is a strategy that commonly arises (Allison et al. 2014; Darch et al. 2012; Kim et al. 2014). Multiple mechanisms exist to buffer populations against cheaters, including those associated with MGE maintenance, but the rapid generation time of microbes combined with the relatively high evolvability of genes for secreted products suggests that cheating mutations may occur often (Travisano and Velicer 2004; Diggle et al. 2007a, b; Popat et al. 2015).

Cheating with EPS production in biofilms alters allocation of resources, metabolic efficiency, and growth through an increase in production of EPS. Cells at the surface of a biofilm experience higher resource and oxygen levels. Cheaters have arisen with an increased ability to produce biofilm compounds, effectively pushing themselves to the surface of the biofilm to acquire more of these resources while suffocating the wild-type strain (Xavier and Foster 2007; Kim et al. 2014). This allocation to biofilm polymers, however, comes at the expense of reproduction as indicated by lower density of cheater cells compared to wild-type cells. Genetic analysis confirms that increased competitive ability was not achieved through faster growth but through increased biofilm polymer production (Kim et al. 2014).

Because microbial growth is positively correlated with SOM degradation, this competitive interaction, resulting in a decreased growth rate, may represent slowed carbon turnover and lower relative biomass. Depending upon the molecular composition of the produced EPS, more resistant forms of soil carbon may be formed. However, the increased allocation of resources toward production of EPS may be associated with decreased metabolic efficiency and consequently a greater proportion of acquired carbon being respired.

When members of a population are producing the same public goods, it is evolutionarily expedient for an individual microbe to evolve a loss of production. Because the cheater is still being provided with communal public goods, the loss of function represents an increase in fitness and has a positive competitive effect against surrounding producers. An example of public goods commonly involved in cheating is siderophores. Because soil is often an aerobic environment, bioavailability of iron