

Resource Exchange in the Rhizosphere: Molecular Tools and the Microbial Perspective

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Abstract

The interface between living plant roots and soils (the rhizosphere) is a central commodities exchange, where organic carbon flux from roots fuels decomposers that, in turn, can make nutrients available to roots. This ongoing exchange operates in the path of vast, transpiration-driven water flow. How the spatio-temporal patterning in resource availability around plant roots affects rhizosphere community composition, activity, and nutrient cycling remains unknown. This review considers how molecular approaches contribute to the exploration of rhizosphere resource exchange, highlighting several recently developed methods linking microbial identity with substrate uptake and gene expression. In particular, strengths and weaknesses of genetically engineered bioreporters are discussed, because currently they alone provide in situ spatio-temporal information at scales of rhizosphere organisms. The soil spatial context is an emerging frontier in ecological soils research. We conclude with parallels linking empirical investigation in the rhizosphere with the quest for understanding general rhizosphere function in Earth's diverse ecosystems.

1. INTRODUCTION

Four hundred million years ago, the first true plant roots evolved and grew downward into substrate—surrounded, from the beginning, by microbes and a soil faunal community. Today, plant roots pack or pepper the upper soil layers of diverse ecosystems over much of Earth's terrestrial surface. Because plant productivity is often limited by soil nutrient availability, the interface between living roots and soils (the rhizosphere, Lynch 1990) is a central commodities exchange, where organic carbon flux from roots fuels microbial and faunal decomposers that can, in turn, make nutrients available to those roots. Moreover, the majority of all water moving from soil to the atmosphere worldwide is transpired from leaves, and that transpired water passed first from soil into plants through the rhizospheres of innumerable roots. The ongoing exchange of carbon and nutrients in the rhizosphere thus operates in and is shaped by vast water flow toward roots. What functional commonalities underlie rhizosphere resource exchange, no matter the organismal players? And why ask such a question?

One practical reason to seek general rules is simply that, given the tremendous diversity of soil microbes, soil fauna, and plants, it is virtually impossible to investigate the intricacies of every potential rhizosphere interaction in every environmental circumstance. Yet, an understanding of controls over belowground function is becoming increasingly important as natural and agro-ecosystems around the globe are exposed to anthropogenic pressures (Drinkwater & Snapp 2006, Pregitzer et al. 2006, Wardle et al. 1998). In addition, the chemistry and development of soil present today have been strongly affected by the actions of rhizospheres over evolutionary time frames (Richter et al. 2006), and the evolution of true plant roots and their extension deep into substrate is hypothesized to have led to a revolution in planetary carbon and water cycling during the Devonian period (e.g., Algeo & Scheckler 1998, Beerling & Berner 2005). What is the biogeochemical function of the rhizosphere on Earth today? In what major ways is rhizosphere function belowground similar across terrestrial ecosystems, and in what fundamental ways can it differ?

These questions pursue common themes describing rhizosphere function, aiming to link mechanism at rhizosphere scales with pattern at ecosystem scales. Gathering empirical data belowground at both these spatial scales, however, is challenging. Key organisms in the rhizosphere, including microbes (bacteria and fungi) and their predators (e.g., protozoa, nematodes), inhabit microenvironments strongly influenced by soil structure, and they respond to conditions and resources at micrometer to millimeter scales that are difficult to characterize. Molecular methods are particularly promising for gathering empirical data at rhizosphere scales, and they are the focus of this review. The link to larger scales is challenging, not only because of inherent scaling issues and dramatic soil heterogeneity, but also because the assays of soil biogeochemistry used as estimators of belowground function at larger scales are often carried out on cored soil samples, where hyphae and roots are severed, and soil structure has been disturbed. Ultimately, the insights obtained by using molecular techniques to probe the microbial perspective of the environment and soil organismal response, at microscales, need to be coupled with improved nondestructive estimates

of soil biogeochemical process rates at larger scales, through, for example, development of soil sensors capable of continuous measures of dynamic solute pools or gases in soil pore space.

In this review we consider how molecular approaches can contribute unique empirical data to the exploration of rhizosphere function. We first briefly discuss the major drivers behind molecular rhizosphere research and then describe a common framework in which to consider rhizosphere resource exchange, noting the recent call for model systems to support hypothesis-driven inquiry. We briefly review promising molecular methods, highlighting several that have been recently developed to link microbial identity with function (substrate uptake or gene expression). In particular, we emphasize the strengths and weaknesses of genetically engineered bioreporter bacteria (microbiosensors) that are unique in providing in situ temporal and spatial information at the scale of individual microbes. This spatial context for rhizosphere function—the 3D matrix in unsaturated soil—is an emerging frontier in soils and rhizosphere research. We conclude by exploring conceptual parallels linking empirical investigation in the rhizosphere itself with the quest for understanding general rhizosphere function in the diverse array of Earth's ecosystems.

PCR: polymerase chain reaction

1.1. Drivers of the Molecular Revolution in Rhizosphere Research

The recent explosion of soils research using molecular methods reveals astonishing diversity and fascinating relationships among organisms and their soil environment (e.g., Singh et al. 2004)—far more information than can be adequately reviewed in this space. Common techniques use PCR (polymerase chain reaction) to amplify selected fragments of DNA isolated from soil organisms, and most share an emphasis on fingerprinting the microbial community present (but not necessarily active) based on that DNA (see Section 2 and **Table 1**). These techniques are destructive; soil is harvested and DNA (sometimes RNA) extracted. Small-scale spatial information is lost; functional information (beyond phylogenetic) is often limited. Nevertheless, within these limitations, several major intellectual drivers have contributed to the rapid development and successful application of molecular methods to rhizosphere ecology. Environmental engineers are searching for specific rhizosphere organisms capable of degrading/transforming soil pollutants (see, e.g., Kuiper et al. 2004 for a review). Within agricultural research, efforts are ongoing to detect and biologically control specific disease organisms around roots (see, e.g., Compant et al. 2005 and Hawes et al. 2000 for reviews). In addition, soil microbial and ecosystems ecologists have documented remarkable soil biodiversity and the potential for individual plants, plant species, soil types, and management regimes to influence rhizosphere microbial diversity (see, e.g., Garbeva et al. 2004, Kent & Triplett 2002 and Lynch et al. 2004 for reviews).

In a recent meta-analysis, Hawkes et al. (2006) summarized 16S ribosomal DNA (bacterial rDNA) data from 13 papers examining rhizospheres of two woody dicots, nine herbaceous dicots, and three grasses. At least 35 taxonomic orders of bacteria were present, including abundant Proteobacteria (often γ -Proteobacteria,

Table 1 Strengths and limitations of methods commonly used for analyzing microbial diversity^a

Strengths	Limitations
PCR-based methods: DGGE, RFLP, and related techniques (Acinas et al. 2005; Blackwood & Paul 2003; Marschner et al. 2001; Smalla et al. 1998, 2001; Spiegelman et al. 2005)	
-Amenable to high throughput analysis -Rapid and relatively easy to implement	-Subject to PCR biases -Relatively insensitive; phylotypes must be present at >1–5% in order to be reliably analyzed -A single band (phylotype) may represent more than one microbial species or type
PCR-based methods: sequencing of amplified genes (Hong et al. 2006, Thompson et al. 2005)	
-Can give a detailed picture of the diversity and contents of an amplified rhizosphere library	-Labor intensive; small numbers of samples analyzed -Subject to PCR biases
FISH (fluorescent in situ hybridization) (Spiegelman et al. 2005, Zwirgmaier 2005)	
-Can be used to visualize the location and prevalence of microbial species, or other taxonomic groups, at the single-cell level -Can be used to enumerate cells in samples	-Cells erroneously missed or labeled when probe is not universal or specific enough, respectively -Background fluorescence can make visualizing labeled cells difficult -Can be hard to detect labeled cells that constitute a small fraction of the community
Culturing (Ferrari et al. 2005, Joseph et al. 2003, Zengler et al. 2002)	
-Straightforward if methods for selective culturing of desired organisms exist	-Most rhizosphere microbes are currently not culturable (but methods development is underway)
Metagenomics (Daniel 2005, Handelsman 2004, Rondon et al. 2000)	
-Sequence information gained from large sections of environmental DNA, even whole genomes in some cases -Large cloned fragments can be used for the isolation of new enzymes or microbial products	-Time consuming and expensive -Cannot reconstruct large genomic contigs from organisms that are not highly represented in the environmental DNA -Subject to DNA isolation, cloning, and expression biases
PLFA (Bossio et al. 1998, Spiegelman et al. 2005)	
-Rapid and inexpensive	-Provides limited taxonomic information -Lipid profiles of single species can be influenced by environmental conditions

^aReferences are generally to papers that give an overview of each technique or discuss its strengths or limitations. Also included are references that highlight soil or rhizosphere biology. Spiegelman et al. (2005) provide an excellent overview.

though α - and β -Proteobacteria also were present), gram-positive members of the CFB (*Cytophaga-Flavobacterium-Bacteroides*) group, and smatterings of Actinobacteria among others. Known capabilities of these groups hint at complex rhizosphere biogeochemistry; methanotrophy, nitrification, and diazotrophy are known in Proteobacteria, and the CFB group can use complex substrates (Hawkes et al. 2006). How biodiversity affects ecosystem function is a continuing debate (e.g., Coleman & Whitman 2005, Fitter et al. 2005 for comment and review); causal links between the diversity of soil organisms present and variation in soil function are very difficult to establish, in part because rhizosphere organisms detected using 16S or 18S rDNA are not necessarily active. As techniques to purify and amplify mRNA from soils improve, identification of the active members of the rhizosphere community, and their functional gene expression, will become more tractable.

Finally, molecular approaches have also contributed to understanding of the specific signaling systems within plant-microbe-faunal and symbiotic rhizosphere interactions (e.g., Graham & Miller 2005, Griffiths et al. 2006, Harrison 2005, Thies et al. 2001), as well as root interactions with specific rhizosphere bacteria causing disease (e.g., *Agrobacterium*, *Erwinia*) or having antibiotic properties (e.g., some *Pseudomonas*) (e.g., Brenner & Winans 2005).

1.2. Components of Rhizosphere Commonalities

Our focus is on finding common themes underlying rhizosphere organismal interactions in and with soil, with the ultimate goal of offering better understanding of controls over belowground processes across varied terrestrial ecosystems. Perhaps the most basic rhizosphere commonalities are the rhizosphere components themselves: a plant root (altering soil conditions and resource availability), substrate into which the root is growing, and a surrounding symbiotic, saprotrophic, and grazing soil community. Rhizosphere resource exchange is supported and constrained by these biotic and abiotic components. Crawford et al. (2005) advocate stepping back from the evolutionary and ecological macroscale to consider that (a) the soil environment is highly spatially structured and heterogeneous, leading to controls over belowground function that are linked with physical structure and location at very small scales, and (b) horizontal gene transfer is rampant so genetic information shuffles relatively easily among microbes, potentially muddying the correspondences between microbial functions and phylogenetic lineages. With these ideas in mind, we briefly discuss roots, the soil community, and the soil itself below, incorporating references to instances where molecular data have been, or promise to be, particularly useful for probing rhizosphere resource exchange.

1.2.1. Component 1: plant roots. The rhizosphere is defined by the presence of a plant root, and all plant roots develop at the active tip with the same basic design, no matter their evolutionary lineage (Raven & Edwards 2001). Key developmental regions of roots are (**Figure 1a**) the tip zone (including root cap, meristem, and immature elongation region), the active uptake zone (where phloem and xylem are mature, and soil water and nutrients enter during transpiration), and the suberized zone (where a wax-like suberin barrier limits solute and water exchange with the surroundings). This ubiquitous developmental plan generates patterns of resource availability, soil water potential, and water flux in the rhizosphere that vary in space (e.g., around the three root zones) and in time (e.g., night versus day, **Figure 1a**). Some basic patterns, for example, rhizodeposition from the root tip during root growth, have long been described from roots growing in artificial liquid or sterilized solid media (e.g., see Whipps 1990 for a review). (Rhizodeposition, indicated in green in **Figure 1**, is the deposition of organic compounds to soil around the root, including secretion of mucilage, sloughing of root cap cells, and exudation of soluble compounds.) Exudation is likely enhanced at the tip because of basic root structure—xylem and phloem have not matured in the tissue immediately behind

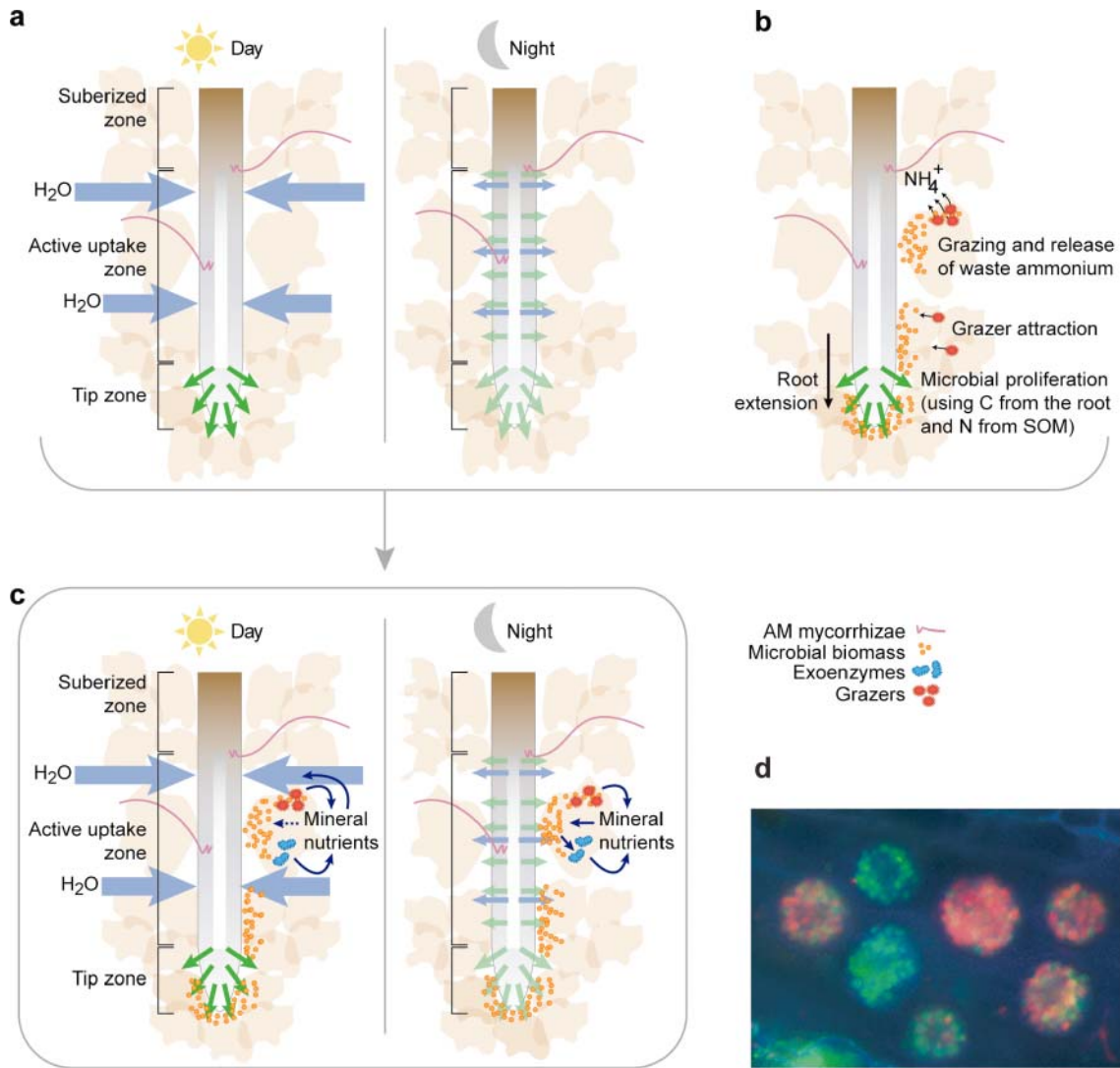


Figure 1

Organisms and interactions in the rhizosphere. (a) Root-driven water fluxes and rhizodeposition (including efflux of solution from roots during hydraulic redistribution at night). (b) Clarholm's (1985) model of tri-trophic resource exchange in the rhizosphere food web. (c) Combined influences of water flux, rhizodeposition, and community interactions in the rhizosphere. (d) Protozoa imaged in the rhizosphere of an alfalfa root. *Sinorhizobium meliloti* bioreporters that were genetically engineered to express red fluorescent and green fluorescent protein (Bringhurst et al. 2001) are visible in vacuoles after having been engulfed by protozoa.

the meristem, so some sugars and other cellular building blocks move from mature phloem to the meristem via an apoplastic route (through cell walls), where membranes and transporters are less able to control diffusion into soil (e.g., Bretharte & Silk 1994). How far various rhizodeposits can spread into nonsterile rhizosphere soil, and the complexities of their distributions, has remained unknown.

The recent development of living bioreporter bacteria (microbiosensors, **Table 2**, Section 2) that can be deployed in nonsterile soil allows empirical investigation of such patterns. For example, Jaeger et al. (1999) genetically engineered *Erwinia herbicola* 299R to report (by producing ice nucleation protein) amounts of available sucrose and tryptophan in nonsterile soil around roots of *Avena barbata*. As expected, sucrose availability was maximal near the root tip and decreased dramatically with distance along the root axis, but, intriguingly, at 12–16 cm from the tip, sucrose report was still significantly above bulk soil levels. In contrast, tryptophan availability was minimal at the root tip back to 8 cm along the axis, then increased greatly until 12–16 cm from the tip (the most basipetal sampling point). Casavant et al. (2002) used an *Enterobacter cloacae* bioreporter to detect arabinose around barley seeds and the root/seed junction, but arabinose was not found at root tips. Further, Bringham et al. (2001) showed, using microbiosensors engineered from *Sinorhizobium meliloti*, that galactosides (common sugars in phloem sap) were present in nonsterile, rhizosphere soil around root hairs of a variety of grasses and legumes, and around locations where lateral roots emerged from the main root, but not at root tips. The microbiosensors' green fluorescent protein (GFP) report was detected by confocal microscopy up to 200 μm from the root surface. Such molecular data promise to provide a useful empirical envelope in which to examine mathematical predictions of solute distribution patterns around roots (e.g., Darrah 1991, Scott et al. 1995, and reviewed in Tinker & Nye 2000), particularly when the empirical data and modeling are developed in parallel.

Other microscale patterns in conditions and resource availability that result from the development and function of roots have not yet been as well explored. For example, microbiosensors indicate that common soluble organic compounds are not confined to soil around the root tip; transpiration should influence movements of solutes in soil around the root active uptake zone, with soil solution flow toward roots opposing diffusion of solutes away from the root (**Figure 1a**). At night, when transpiration stops, diffusion of any substances away from the active uptake zone could occur unimpeded, and if hydraulic redistribution is occurring, a flux of organic molecules out could even be aided by water flow out of the root (**Figure 1a**). [Hydraulic redistribution is the movement of water from regions of higher to regions of lower soil water potential via plant roots, and it is widespread (e.g., Caldwell et al. 1998).] Can this hypothesized diel rhythm in root-derived substrate availability around the active uptake zone (higher at night, lower during the day) be detected by rhizosphere microbiosensors? Moreover, because transpiration-driven water flux does not occur at the tip, is there an opposite rhythm driven by photosynthesis (higher exudate availability during the day, lower at night) in rhizosphere soil around the root tip? The existence and effects of such rhythms in single roots, and root systems of varying architecture, remain to be tested in situ in nonsterile soil.

GFP: green fluorescent protein

Root water potential, too, varies in space and time in different root zones. Simple single-root models predict how far into rhizosphere soils of different textures and water contents the variation in root water potential propagates, and, e.g., Hillel (1998) calculates gradients in water potential developing out to more than several millimeters from the root over days. [See Tinker & Nye (2000) for more extensive discussion.] However, thermocouple psychrometers, time domain reflectometry, and tensiometers assay soil moisture at much larger scales. Recently, genetically engineered microbes have been developed that report total soil water potential at millimeter and micrometer scales in their surroundings, producing more GFP per cell as soil dries (Cardon & Herron 2005; P.M. Herron, D.J. Gage, Z.G. Cardon, submitted, building upon Axtell & Beattie 2002). This cellular-scale report can be viewed *in situ* using an epifluorescence microscope, without destructive harvest of rhizosphere soil. Expected transpiration-induced water potential gradients have been observed using these biosensors—rhizosphere water potential is least negative around the root tip, where xylem is not mature and transpiration-driven water uptake is minimal, and it becomes more negative around the active uptake zone where metaxylem has matured (P.M. Herron, D.J. Gage, Z.G. Cardon, submitted).

Water potentials and velocities of water movement toward roots in the active uptake zone have important implications for rhizosphere function, including organismal movements and resource exchanges (e.g., Scott et al. 1995). Protozoal grazers, for example, move more easily when water potentials are high (that is, when the soil is wet). Also, at the scales in which rhizosphere organisms operate, velocities of water movement strongly influence advective (bulk flow-linked) versus diffusion-linked delivery of nutrients through the rhizosphere, but in many current models the advective delivery to roots is ignored for most nutrients (Darrah et al. 2006). The behavior of roots as leaky pipes (Landsberg & Fowkes 1978) and the changes in axial and radial conductivity of roots during development behind the root tip (Doussan et al. 1998, Zwieniecki et al. 2002) suggest there should be dramatic variation in the radial velocity of transpiration-driven soil solution movement toward the root axis, increasing from zero at the tip to a maximum (but not constant) velocity in the active uptake zone (**Figure 1a,c**, *blue arrows* of different lengths). For example, Doussan et al.'s modeling, developed using Varney and Canny's dye-based data (cited in Doussan et al. 1998), finds a 30% increase in maximum radial water flow into roots between approximately 2–3 cm and 7–10 cm from the main root tip in corn, along with further variation possibly associated with maturation of early and late metaxylem elements and development of the endodermis and hypodermis, etc. (Doussan et al. 1998).

1.2.2. Component 2: rhizosphere communities. Spatial and temporal heterogeneity in rhizosphere conditions and resources thus emerges from general root structure and function, and that heterogeneity is hypothesized to be important for community activity and resource exchange (e.g., Clarholm 1985). The rhizosphere microbial and soil faunal community has very diverse membership, including symbiotic and saprotrophic bacteria and fungi, grazing protozoa (e.g., heteroflagellates, ciliates, and amoebae), nematodes, mites, enchytraeid worms, and a host of other soil organisms (Griffiths et al. 2006, Hawkes et al. 2006, Johnson & Gehring 2006, Moore

et al. 2006). In 1985, Clarholm provided a now-classic, simple, function-oriented framework for exploring resource exchange among roots, microbes, and grazers in the rhizosphere. Very briefly, labile (easily used) carbon (*green arrows* in **Figure 1b**) is lost from the root tip as it extends through soil, and soil microbial activity increases as a result of the root carbon input. Building new microbial biomass (*yellow dots* in **Figure 1b**) requires not only carbon but also a source of nitrogen and other essential nutrients. (Use of the term nutrients varies between microbial and ecosystems literatures; here we use the word nutrients to represent essential, noncarbon elements such as nitrogen, phosphorus, and sulfur.) Focusing on nitrogen, if concentrations of mineral nitrogen in soil solution are not high, microbes can use exoenzymes to attack soil organic matter (SOM) for nitrogen to make their biomass, thus moving it into an actively cycling pool. Eventually, grazers (*red furry blobs*, **Figure 1b**; real protozoa in soil, **Figure 1d**) are attracted to and consume the microbial biomass. Meanwhile, the root tip continues to extend through soil, away from soil particles populated with now-thriving microbiota, and the root axis matures. Grazers consuming microbial biomass release waste ammonium, because of stoichiometric differences in carbon and nitrogen between biomasses of the two organism types or because of inefficiencies of assimilation, or both. If that mineral-nutrient pool is near the active uptake zone of the root (**Figure 1b**), ammonium can be taken up by the plant. In sum, through a multitrophic interaction, the root has exchanged carbon (which fed microbes initially, Cheng & Gershenson 2006) for nitrogen (released by grazers).

Clarholm's (1985) trophic interactions (**Figure 1b**) and the water fluxes into and out of roots (**Figure 1a**) interact (**Figure 1c**), but how the resulting spatio-temporal patterning in resource availability affects rhizosphere community biomass, gene expression, composition, trophic interactions, and nutrient cycling remains unknown. The balance of mineralization (release) of nutrients from SOM by microbes and immobilization (uptake) of nutrients into microbial biomass is key for nutrient availability to plants, because neither nutrients in SOM nor mineral nutrients immobilized in microbial biomass are immediately available for plant root uptake. Factors influencing the balance of mineralization and immobilization include stoichiometric constraints (C:N of soil community predators, prey, and chemical substrates), efficiencies of resource use, energy and nutrient status of microbes and, thus, likelihood of their producing exoenzymes to attack SOM, mineral nutrient availability in soil solution, and availability of SOM (e.g., Chen & Stark 2000, Clarholm 1985, Moore et al. 2004, Robinson et al. 1989, Schimel & Bennett 2004, Schimel & Weintraub 2003).

Of these factors, the energy and nutrient status of rhizosphere microbes clearly could be directly influenced by roots via rhizodeposition and transpiration-driven variation in water flow. Our combined model in **Figure 1c** shows, for example, that a diel rhythm of resource availability around the active uptake zones of roots (described in Section 1.2.1 and **Figure 1a**) could expose rhizosphere microbes to higher solute flux from roots at night, potentially providing carbon to rhizosphere microbes and increasing microbial growth demand for nutrients. As a result, depletion of the dissolved mineral nutrient pool in rhizosphere soil solution by microbes likely becomes more severe. This period of relative nutrient-limitation at night (caused by

depletion of solutes from soil solution and potentially exacerbated by movement of organic compounds from the root into soil) could spur microbial production of long-lived exoenzymes (*blue blobs* in **Figure 1c**) that release mineral nutrients from SOM. Once transpiration starts again, relative carbon limitation of microbial growth could develop around the active uptake zone as transpiration again opposes diffusion of substances from the root surface. But, exoenzymes in soil, outside microbes, will still actively attack SOM and release nutrients that could be poached by the plant because the carbon-limited microbes are not growing (immobilizing nutrients) rapidly (**Figure 1c**, Day). Overall, average rhizodeposition from the entire root system (much of it at root tips) may be maximal during the day and lower at night (e.g., Murray et al. 2004), but organisms in the rhizosphere around the root's active uptake zone may experience exactly the opposite fluctuation. Over the course of days, the rhizosphere community around the active uptake zone may thus be pushed back and forth from carbon (daytime) to nutrient (nighttime) limitation on a diel cycle. Are the effects of such zone-specific rhythms on microbial gene expression detectable using functional gene arrays? Though the implications of such a hypothetical rhizosphere pump are not yet clear, preliminary mathematical modeling (Cardon & Rastetter 2004) suggests that persistence of a long-lived exoenzyme pool, releasing nutrients from SOM and replenished by periodic pushing of the rhizosphere microbial community into a state of moderate night-time nutrient limitation, enhances plant root access to nutrients in the long term.

Of course, interactions among organisms in the rhizosphere are governed by more than resources. Molecular and signal coordination among diverse members of the rhizosphere community extends beyond the well-known symbioses and is thought to be mediated through common active signaling compounds such as plant phenolics, phytohormones, and quorum-sensing signals (e.g., Bonkowski 2004, Hirsch et al. 2003, Loh et al. 2002). These signals can influence species-specific rhizosphere interactions and potentially can act much more broadly. For example, Bonkowski & Brandt (2002) found lateral root proliferation in *Lepidium sativum* seedlings was associated with the presence of protozoal grazers, perhaps because protozoa promote increased numbers of bacteria capable of producing auxin. The generality of such effects remains to be determined (Bonkowski 2004, Bonkowski & Brandt 2002, Clarholm 2005).

1.2.2.1. Perspectives on control of rhizosphere food webs. To conceptually organize such a dizzying array of potential signal molecules and interactions of rhizosphere organisms concentrated at the root surface, Phillips et al. (2003) proposed a framework founded on the idea of rhizosphere control points. These control points are defined as regulatory elements within organisms (e.g., genes coding for receptors binding rhizosphere signals) that can be acted upon by selective pressures in the rhizosphere, leading to altered fitness of those organisms and resulting in broader effects within the rhizosphere food web. Given this definition, there likely is a very large number of control points even within a very simple community. The strength of their effects on rhizosphere food web function can be examined using molecular techniques such as targeted mutation to knock out function. The framework is intriguing because it seeks to link molecular investigation of specific signals, receptors, and environmental

responses within particular organisms to rhizosphere population and community ecology. The genetic nature of Phillips et al.'s control points leads them to suggest that "genes lie at the heart of rhizosphere food web regulation."

In contrast, Moore et al. (2003) argue that control points within rhizosphere food webs are embedded within the whole web structure itself, through which energy and resources flow in various paths (channels), in characteristic ways. For example, energy from plants can flow directly via a root channel to symbionts (e.g., mycorrhizae) and up the food chain that is using mycorrhizal hyphae as its base. Additionally, energy and resources can move from roots to upper trophic levels of rhizosphere food webs through bacterial (fast) or fungal (slow) saprotrophic channels with distinctly different dynamics. The relative importance of fast bacterial and slow fungal channels varies from ecosystem to ecosystem; the fungal channel dominates when more detritus, or more resistant detritus, is present (e.g., no-till agriculture, mor forest humus, Couteaux & Bottner 1994), and the bacterial channel dominates with soil disturbance and/or the prevalence of easily metabolized organic compounds (e.g., tilled agriculture, mull forest humus). Overall food web stability is enhanced by this flexible, multichannel structure (e.g., Moore et al. 2006). This view of control has the appeal of generality because rhizosphere stability and energy flow control are determined by community structural patterns, not particular species per se. Molecular techniques targeted toward describing community structure and capturing population dynamics could contribute to empirical tests of these ideas.

Finally, Young & Ritz (1998) advance a third perspective, that the soil habitat itself exerts significant control over function of rhizosphere food webs. Its heterogeneous three-dimensional soil structure and dynamic water content dramatically affect delivery paths for resources and mobility of organisms interacting in food webs, serving as a physical heart of rhizosphere function (see Section 1.2.3 below).

1.2.2.2. *The mycorrhizal menagerie.* Plant roots have likely been involved in mycorrhizal symbioses and surrounded by saprotrophic microbes for 400 My (Harrison 2005). Today, the majority of plants are mycorrhizal, and for those plants, rhizosphere resource exchange occurs in two complementary domains: at the symbiotic physical interface between root and fungal tissues and in rhizosphere soil between plant roots and saprotrophic microbes. The various mycorrhizal types have differing capacities to attack SOM in order to release nutrients, with arbuscular mycorrhizal (AM) fungi (common, e.g., in grasslands) the least capable, and ericoid (ERM) and ectomycorrhizae (ECM; common in cold northern latitudes) very capable of saprotrophic activity (Chapman et al. 2006, Johnson & Gehring 2006, Read & Perez-Moreno 2003). Interestingly, it remains unclear whether, or to what extent, mycorrhizal hyphae can facilitate saprotrophic microbial attack on SOM in the rhizosphere (e.g., Fitter 2005, Jones et al. 2004), though Wamberg et al. (2003), using the molecular fingerprinting technique denaturing gradient gel electrophoresis (DGGE), detected that mycorrhizal hyphae can have significant effects on membership and perhaps activity in the soil bacterial community. Biogeographical patterns in mycorrhizal symbiosis and saprotrophic capability likely contribute to major differences

DGGE: denaturing
gradient gel electrophoresis

in root-mycorrhizal-saprotrophic resource exchange in various terrestrial ecosystems. We return to these contrasts later.

The specific signals and responses involved in establishment of mycorrhizal symbioses have been the subject of intense molecular investigation (see Harrison 2005 and Graham & Miller 2005 for reviews), and the challenge is to weave this information into a better understanding of larger-scale phenomena. Graham & Miller (2005) note, for example, that an in-depth characterization of the mycorrhizal transporter responsible for uptake of a soil nutrient may not help with scaling up to understanding nutrient uptake in an ecosystem if release of that nutrient by saprotrophic microbiota is limiting. An ecosystem-level view of controls should help prioritize areas for molecular research.

1.2.3. Component 3: the soil structural stage for rhizosphere function. The organisms driving essential ecosystem functions in soil through their gene expression, resulting physiology, and trophic interactions are operating at a scale that is extremely difficult to envision let alone examine empirically. Just as Earth's surface is heterogeneous at a scale we can perceive, soil particles' surfaces are heterogeneous for microbes, with water films of different thicknesses, and "mountains" and "valleys" of various resource availability. This soil structure strongly influences general rhizosphere resource exchange (e.g., Hillel 1998, Tinker & Nye 2000). The three-dimensional matrix is highly heterogeneous, with various-sized organic or inorganic particles piled together with space in between. If soils are saturated, that space is filled with water, constraining diffusion of gases relative to the unsaturated state, but facilitating diffusion of dissolved nutrients or carbon, and movements of swimming protozoal predators. As soil drains and dries, a number of changes take place. Spaces between particles become filled with gas, largest first and smallest last; water becomes confined to films on particles. Movements of swimming organisms are severely constrained; the tortuosity of the path for water movement in water films increases dramatically, as does the length of paths along which dissolved substances diffuse. Soil hydraulic conductivity plummets, and soil water potential drops, following characteristic trajectories of water potential as a function of soil moisture content that depend strongly on soil texture. Within this structural background, the chemical nature of soil also influences the potential for resource exchange; for example, clays and organic matter can provide cation exchange capacity that holds cations such as ammonium tightly in place. Crawford et al. (2005) suggest that a major current challenge within soil ecology is the need to monitor biological activity *in situ* within this physical structure, continuously, at multiple scales.

1.3. Model Systems for Function-Focused Rhizosphere Research?

Independently, both Phillips et al. (2003) and Crawford et al. (2005) call for development of model microcosms to facilitate hypothesis-driven, function-focused rhizosphere research. Crawford et al. champion the need for targeted investigation of soil structural and physical controls over soil ecology, and Phillips et al. emphasize the need for simplified soil communities of tractable size and genetic manipulability.

Such well-controlled biological systems would lend themselves well to integration of empirical results with mathematical modeling (e.g., see Darrah et al. 2006, Tinker & Nye 2000, Toal et al. 2000 and Wu et al. 2005 for reviews). They would also link beautifully to emerging function-focused molecular methods, including functional DNA arrays detecting expression of known genes among community members, stable isotope probing revealing use of ^{13}C -labeled rhizodeposits by identifiable microbes, and development of microbiosensors producing visual reports of rhizosphere conditions, resources, or microbial activity that can be assayed continuously (**Table 2**).

Ideally, both the expression of particular genes and a metric of numbers of organisms and/or groups of organisms (population and community biology, at the microscale) should be monitored, because both gene expression and trophic interactions contribute to biogeochemical cycling. Eventually, in more natural soil systems, rDNA genes from broad groups of soil prokaryotes, fungi, and predators might be sequenced and used to track changes in populations, but there are limitations still to such an approach, not least of which is the paucity of sequence information for soil organisms. Again, this effort would be greatly simplified at first in model microcosms, though even harvest design would be challenging, given the vast differences in sizes and numbers of such key soil organisms as nematodes and bacteria. To begin exploring genetic, trophic, and soil physical controls over rhizosphere resource exchange, not simply function in one type of microcosm, organisms should be included in various combinations, forming trophic webs of various structures and sizes (e.g., Ingham et al. 1985) in multiple soils with varied physical properties. The ultimate goal is to use simplified microcosms to test mechanistic hypotheses, and, from information gleaned, develop hypotheses testable in natural ecosystems.

2. MOLECULAR METHODS—COMMON AND CUTTING-EDGE

2.1. Diversity

Over the past 25 years, the development of molecular techniques has allowed microbial ecologists to explore microbial diversity far beyond that exposed by analysis of culturable microbes. There are 243,909 aligned and annotated bacterial 16S rRNA sequences alone in the current ribosome database at Michigan State University (Coleman & Whitman 2005), and, though large, this database represents a miniscule fraction of the total prokaryotic diversity estimated to exist worldwide (Curtis et al. 2002, Gans et al. 2005, Schloss & Handelsman 2004). The most powerful and convenient methods for investigating microbial diversity have been thoroughly reviewed elsewhere (**Table 1**); generally, they require the isolation of microbial DNA from the environment, followed by amplification of conserved regions (i.e., DNA regions with very similar or identical sequence) by PCR, and sequencing or other analyses [for example, DGGE separating fragments on gels, or T-RFLP (terminal-restriction fragment length polymorphism)] designed to characterize the diversity of the amplified fragments (Garbeva et al. 2004, Kent & Triplett 2002, Spiegelman et al. 2005). Resulting phylotypes from DGGE and T-RFLP are “operational taxonomic units”

RFLP: restriction fragment length polymorphism

SIP: stable isotope probing

FISH: fluorescent *in situ* hybridization

based on DNA fragment patterns; they do not necessarily correspond directly to single species. PCR-based methods ultimately underestimate diversity because of several inherent limitations. First, amplification of DNA in proportions matching those in complex environments is unlikely because microbes differ in their susceptibility to lysis and in the extractability of their nucleic acids. Second, primers often do not amplify DNA from all target organisms (Baker et al. 2003, Smalla et al. 1998). Third, genes are amplified at different efficiencies and their representation in the final pool of amplified products may not represent their environmental abundance. Finally, it is becoming clear that sampling of single or a small number of genes can vastly underestimate genome diversity at the species level (Thompson et al. 2005). Whether such genomic diversity within species confers functional diversity or contributes to differential selection is not always clear.

2.2. Function

Recent technical advances, described in more detail below, have moved microbial ecology beyond simply analyzing community diversity toward uncovering ecological activities of community members. Stable isotope probing (SIP) and mRNA-fluorescent *in situ* hybridization (mRNA-FISH) are newer nucleic acid-based methods that tag cells actively metabolizing an isotopically labeled compound or expressing certain genes, respectively. DNA arrays detect expression of selected functional genes in the environment; microbial bioreporters (**Table 2**) report their perception of, or response to, their environments. SIP and DNA arrays are just beginning to be applied to soil and rhizosphere environments, and mRNA-FISH to marine sediments and groundwater. Bioreporters have been used in rhizosphere studies to a limited extent for about 10 years (**Table 2**).

2.2.1. Stable isotope probing. For SIP experiments, microbial communities are exposed to ^{13}C -labeled substrate, resulting in the biosynthesis of heavy, ^{13}C -labeled macromolecules by organisms using the substrate for growth. DNA with a high enough fraction of ^{13}C can be physically separated from unlabeled DNA by equilibrium density centrifugation (Meselson & Stahl 1958); this ^{13}C -labeled subpool provides genes to identify organisms that used the labeled substrate (Dumont & Murrell 2005; Lu & Conrad 2005; Radajewski et al. 2000, 2003; Saleh-Lakha et al. 2005; Singh et al. 2004; Wellington et al. 2003). Separation of ^{13}C -labeled nucleic acids from high G/C nucleic acids requires that at least 20% of the nucleic acid carbon be ^{13}C (Manefield et al. 2002). This requirement poses challenges for rhizosphere research in which photosynthetic fixation of $^{13}\text{CO}_2$ provides the label; ^{13}C labeling of the DNA of rhizosphere organisms using rhizodeposits can be low (Ostle et al. 2003, Singh et al. 2004). Further challenges in interpretation of labeling patterns stem from cross feeding of organisms, either through conversion of labeled compounds by primary consumers or trophic interactions (Ostle et al. 2003, Radajewski et al. 2003).

RNA, in comparison with DNA, is more efficiently labeled because many RNA molecules are rapidly and continually synthesized in growing organisms. They are single stranded and short relative to the genomic DNA fragments, and thus are likely

Table 2 Representative bioreporter studies in the rhizosphere, grouped by report type**GFP Report**

Induced by PCBs (Boldt et al. 2004) *Pseudomonas fluorescens* bioreporters in microcolonies were induced by PCBs in contaminated alfalfa rhizosphere soil. Comparison with growth rate bioreporters suggested that the PCB-degrading cells were not growing at high rates.

Induced by galactosides (Bringhurst et al. 2001) *Sinorhizobium meliloti* bioreporters were induced by galactosides in nonsterile rhizosphere soils around grasses and legumes. Protozoal grazing was detectable because engulfed bioreporter bacteria remained fluorescent.

Induced by arabinose (Casavant et al. 2002) An *Enterobacter cloacae* life history bioreporter (passing induction to all descendents) detected arabinose around barley seeds and the root/seed junction, but not at root tips, perhaps because arabinose is only cleaved from cell walls after elongation and differentiation of plant cells is complete.

Induced by toluene (Casavant et al. 2003) A *Pseudomonas fluorescens* life history bioreporter was induced by vapor-phase toluene, even at low or transient concentrations. In the absence of toluene, compounds in barley rhizosphere activated the bioreporter in localized areas.

Induced by nitrate (DeAngelis et al. 2005) An *Enterobacter cloacae* bioreporter detected lower levels of nitrate in the rhizosphere of wild oat than in bulk soil, suggesting detection of competition for nitrate between microbes and plant roots. (Ice nucleation report also used.)

Induced by low water potential (Cardon & Herron 2005; P.M. Herron, D.J. Gage & Z.G. Cardon, submitted) *Pseudomonas putida* and *Pantoea agglomerans* biosensors reported total soil water potential (Ψ); *P. agglomerans* reported more negative rhizosphere Ψ with increased axial distance from corn root tips, confirming the predicted gradient based on corn root anatomy.

Induced by high bioreporter growth rates (Ramos et al. 2000) *Pseudomonas putida* bioreporters host a growth rate-regulated rRNA promoter fused to unstable *gfp*. Bioreporters grew slowly in nonsterile barley rhizosphere, but rapidly around sloughing root sheath cells in sterile soils.

Induced by quorum sensing (Steidle et al. 2001) *Pseudomonas putida* bioreporters were induced by homoserine lactones, illustrating that quorum sensing-based gene expression occurs in nonsterile rhizosphere soil around tomato roots.

Lux Report

Constitutively expressed (Beauchamp & Kloepper 2003) Root colonization by bioluminescent, plant growth-promoting *Pseudomonas putida* inoculated onto soybean seeds was tracked in nonsterile soil; numbers were much lower on root sections near root tips.

Induced by root exudates (Darwent et al. 2003) *Pseudomonas fluorescens* bioreporters indicated nitrate limitation did not increase specific barley root exudation rates in sand microcosms, but did increase total root system length and thus overall root system exudation.

Induced by P. starvation (Kragelund et al. 1997) Luminescence from *Pseudomonas fluorescens* bioreporters was detected in the rhizosphere of barley plants in sterile, but not nonsterile, soils.

Constitutively expressed (Ratray et al. 1995) *Enterobacter cloacae* bioreporters luminesced throughout rhizospheres of wheat in sterile soils, but luminescence was restricted to shoot/root junctions in nonsterile wheat rhizospheres, perhaps owing to competition with native bacteria.

Constitutively expressed (Roberts et al. 1999) Bioluminescent *Enterobacter cloacae* migrated from cucumber seed to roots during root growth in nonsterile soil, but colonization was not even, and numbers of bioreporters were much lower near root tips.

Constitutively expressed (Sakai et al. 1997) *Pseudomonas fluorescens* bioreporters luminesced in sterile rhizosphere soil around spinach, but luminescence decreased in nonsterile soils.

Induced by copper (Tom-Petersen et al. 2001) Copper available to a *Pseudomonas fluorescens* bioreporter in soil amended with complex organic material was less than total copper content, suggesting that copper complexation with organic matter reduces bioavailability.

Ice Nucleation Protein Report

Induced by tryptophan and by sucrose (Jaeger et al. 1999) Two *Erwinia herbicola* biosensors reported increasing tryptophan and decreasing sucrose availabilities in nonsterile rhizosphere soil as a function of axial distance from root tips of *Avena barbata*.

to be labeled to higher specific activities. Using a short (6 hour) $^{13}\text{CO}_2$ pulse delivered to grassland plants in limed and unlimed field plots at the Sourhope Field Experimental Site, Rangel-Castro et al. (2005) successfully enriched RNA from rhizosphere fungi and bacteria that had consumed ^{13}C -labeled rhizodeposits. DGGE analysis of heavy and light RNA fractions identified distinct phylotypes that had preferentially used either carbon from the $^{13}\text{CO}_2$ -labeled rhizodeposits or from other unlabeled (^{12}C) soil sources, respectively. Overall, the microbial community using plant rhizodeposits in more fertile, limed soils was more complex than that in unlimed soils. In another example, Lu & Conrad (2005) pulse-labeled rice growing in microcosms and found preferentially labeled RNA from rice cluster I archaea in rhizosphere soil, suggesting these methanogens may be particularly important in transforming root-derived carbon to methane. SIP can similarly be combined with phospholipid fatty acid analysis (PLFA; **Table 1**; e.g., Butler et al. 2003, Lu et al. 2004, Treonis et al. 2004). Lu et al. (2004) found rapid differential labeling of straight-chain and branched fatty acids extracted from rhizosphere microorganisms after pulse-labeling rice. Treonis et al. (2004) found preferential ^{13}C labeling of fatty acids indicative of fungi and gram-negative bacteria in the rhizosphere of pulse-labeled grasses; interestingly, labeling of PLFAs was similar in limed and unlimed treatments at Sourhope, perhaps because PLFA is less specific than DGGE analysis of extracted RNA.

2.2.2. DNA arrays. DNA microarrays are most often used to identify genes transcribed in a known organism grown in well-defined media. Arrays typically consist of thousands of different amplified gene fragments (or oligonucleotides) from a single species spotted onto a solid substrate such as a microscope slide (Schena et al. 1995). mRNA isolated from the target species is converted into fluorescently labeled cDNA and hybridized to the array, producing fluorescent spots that indicate genes that were transcriptionally active above a threshold level (DeRisi et al. 1997). For example, Mark et al. (2005) used DNA microarrays to identify unique *Pseudomonas aeruginosa* genes expressed during growth in artificial medium containing sugarbeet root exudates from two beet cultivars.

Functional gene arrays are similar to DNA arrays except they contain gene fragments or oligonucleotides from genes of functional interest (e.g., genes for enzymes important in nitrogen or carbon cycling), from a variety of organisms. RNA can be harvested from soil, converted to labeled cDNA, and hybridized to the array to identify which of the genes spotted on the array were transcriptionally active in soil (Saleh-Lakha et al. 2005, Sessitsch et al. 2002). Hybridization of harvested and labeled DNA from soil to the array identifies genes on the chip that were present in soil. Array design and fabrication are challenging. Genes of interest must be identified, and probes on the array must be designed to be specific enough to identify genes from particular organisms (or groups of organisms) among the diverse, often uncharacterized, soil community.

Schadt et al. (2005) recently described the design of an array targeting genes of soil bacteria involved in metal resistance, biodegradation, and carbon, nitrogen, and sulfur cycling. This array contained spots consisting of oligonucleotides of about 50 bases in length. Labeled genes would hybridize to spots if they and the spots were more

than 86% identical, and given that the sequence identity between the same functional genes in different species is often less than 85%, the researchers suggest that the array should be able to provide species-level information about those genes represented on the array (Rhee et al. 2004, Schadt et al. 2005). Other functional gene arrays have also been described (Cho & Tiedje 2002, Dennis et al. 2003, Taroncher-Oldenburg et al. 2002). Using similar approaches, but with probes diagnostic for microbial species and higher taxonomic groups, DNA arrays are being developed to study microbial diversity in complex environments including soil (Bodrossy & Sessitsch 2004, Loy et al. 2002, Sanguin et al. 2006, Small et al. 2001).

2.2.3. mRNA-fluorescent *in situ* hybridization. FISH is a standard technique in microbial ecology, often used to identify the phylogenetic affiliation of soil bacteria (Delong & Wickham 1989, Kobabe et al. 2004). Typically, fluorescently labeled RNA or DNA probes are designed to bind to the 16S ribosomal RNA of selected taxonomic groups. Following the fixation and permeabilization of target cells, probe is added and it hybridizes to rRNA within cells from those selected groups. Cells containing this specifically bound fluorescent probe can be visualized by epifluorescence microscopy or enumerated by flow cytometry. (*In situ* indicates that labeling of the rRNA occurs within cells, but those cells may or may not be *in situ* in their native environment.)

Recent advances have made it possible to visualize gene expression (mRNA-FISH) in individual cells, or to identify metabolically active cells, while simultaneously determining their taxonomic affiliations (Zwirgmaier 2005). To reliably detect gene expression, signal from bound probe needs to be amplified because target mRNA molecules are often present at only a few copies per cell, and they can turn over rapidly. Visualizing mRNAs by FISH has been achieved by tagging FISH probes with digoxigenin, and hybridizing the probe under conditions that optimize signal to noise. The bound probe is visualized using antibodies that are conjugated to horseradish peroxidase, which converts soluble substrates to insoluble fluorescent compounds. Using this method, marine sediment bacteria expressing methane monooxygenase, and cells in contaminated groundwater samples expressing a naphthalene dioxygenase gene, have been visualized (Bakermans & Madsen 2002, Pernthaler & Amann 2004). Combining FISH with microautoradiography, or with immunodetection of bromodioxymidine, will further allow for the visualization and simultaneous taxonomic identification of individual cells that are capable of degrading particular growth substrates or that are in the process of actively synthesizing new DNA (Gray et al. 2000, Nielsen et al. 2003, Pernthaler et al. 2002, Zwirgmaier 2005).

2.2.4. Microbial bioreporters. The function-focused methods described above require a destructive harvest of soil for assay. Bioreporters offer an alternative, often nondestructive method for investigating microbial distribution and function *in situ* in the rhizosphere (Table 2). A bioreporter is made by fusing DNA coding for an easily assayed reporter gene with a promoter of interest and then inserting the fusion into a living host bacterial strain. Common reporter genes include *lacZ*, *pboA*, and *inaZ* (all requiring destructive harvest for assay of gene products), *gfp* (coding for GFP), and genes that result in bacterial bioluminescence (*lux*). The visual reports from *gfp*

and *lux* can be assayed nondestructively, without supplying external cofactors or substrates to cells. GFP can be used to monitor gene expression in single cells using an epifluorescence microscope, providing information on micrometer to millimeter spatial scales in soil. Light from expression of *lux* genes is detectable using sensitive cameras, so a resident engineered population can report on conditions across entire imaged root systems (see **Table 2**). Use of bioreporters in complex environments is a relatively recent development (Larrainzar et al. 2005, Leveau & Lindow 2002, Prosser et al. 1996, van der Meer et al. 2004). Modeling and statistical treatment of data from bioreporters have added to the sophistication with which these tools can be used (Leveau & Lindow 2001, 2002; van der Meer et al. 2004).

Several studies have used bioreporters to explore rhizosphere biology (**Table 2**). In some cases the reporter gene was driven by a promoter induced by specific compounds in the soil (**Table 2**). In other cases, bioreporters were designed with promoters that fired without needing to be induced, thus constitutively expressing the report, and enabling detection of where the tagged bacteria were active. Such tagging is useful, for example, in testing whether growth-promoting bacteria introduced into soil on seeds can colonize the complete plant root system. However, bioreporters are living organisms, not electronic sensors, so the biology of the host organisms (e.g., substrate preferences, growth rates, promoter strengths) may influence the reporter response (e.g., Wright & Beattie 2004). Interpretation of reports must therefore be conservative. Even with this limitation, the information gained at microbial scales never before assayed is powerful. Challenges associated with interpretation of bioreports include the following:

1. In instances where production of report is to be assayed in individual bacteria, the strength of the report should be proportional to the strength, or concentration, of the signal or compound that activated the promoter driving the reporter gene (Axtell & Beattie 2002, Lee & Keasling 2005). However, in some cases the promoter is either switched on or off at some stimulus threshold in any particular cell (e.g., in the native *E. coli araC* and *lacZ* promoters; Novick & Weiner 1957, Siegle & Hu 1997), and the fraction of cells in which the promoter is on is proportional to the strength or concentration of the inducing signal. Bioreporter design and assay must take into account such differences.
2. As Leveau & Lindow's (2001) simple mathematical model of bioreports shows, factors beyond promoter activity can affect the amount of report (e.g., GFP molecules) per cell. For example, though all promoters in a population of cells may be firing at a constant rate and driving production of a constant stream of report molecules as a function of a stimulus, if various reporter cells are growing at different rates, the report molecules per cell are being divided more often into the daughter cells of bioreporters dividing quickly. Report molecules per cell could thus be lower in rapidly growing cells, even though promoter firing rates are the same in rapidly and slowly growing cells. Similarly, differential rates of degradation of report protein, or maturation of report protein, could also affect the concentration of report in cells (Leveau & Lindow 2001). Such patterns do not necessarily develop in all bioreporters; as Leveau & Lindow (2001)

point out, for example, promoter activity itself may be affected by growth rate, independent of the stimulus detected by the bioreporter. It is essential to test as much as possible for such effects under well-controlled conditions before microbiosensors are inoculated into complex environments. For example, P.M. Herron, D.J. Gage & Z.G. Cardon (submitted) developed water potential-sensing bacteria using *Pseudomonas putida*, and found that GFP per cell is a reliable report of water potential across a range of growth rates caused by varied carbon sources and salt types. Further, complex environments harbor zones of conditions and resources that may or may not be conducive to microbial activity. Kragelund et al. (1997) note, for example, that a high-level expression in a bioreporter cannot be expected from starved host cells, even if an inducing signal is present. Controls should be designed to reveal such resource limitations.

3. Finally, promoters in bioreporters may be induced by many compounds or conditions, some of which may not have been experimentally identified (e.g., Casavant et al. 2003; **Table 2**). For example, Bringhurst et al.'s (2001) galactoside bioreporter consisted of the *S. meliloti melA* promoter, normally induced by many α -galactosides, fused to *gfp*. But, the *melA* promoter has also been shown to be induced by galactose and some β -galactosides (Bringhurst & Gage 2000, 2002; Gage & Long 1998). Furthermore, carbon sources induced the *melA* promoter with varying efficiency, meaning bright bioreporter cells could have resulted from low levels of an efficient inducer (e.g., melibiose), or from high levels of an inefficient inducer (e.g., stachyose). For this reason, the inclination to view bioreporter results as absolutely quantifying levels of inducer should be avoided.

Good experimental design can diminish effects of these limitations. Bioreporters should be well-characterized in liquid medium before being deployed in complex environments. The influences of carbon source and growth rate should be investigated. Control strains that constitutively express a gene very similar to the reporter gene can be deployed in the environment under study to determine whether variations in environmental conditions (other than the resource or condition being assayed by the bioreporter) can cause variations in report levels independent of variation in promoter firing. For example, if the bioreporter gene is *gfp*, then red fluorescent protein (*rfp*) would be a good constitutively expressed control reporter (e.g., DeAngelis et al. 2005). Such control bacteria can also confirm that interesting spatial expression patterns are not due to uneven distribution or uneven growth of inoculated bioreporter cells (e.g., Bringhurst et al. 2001). If the constitutively expressed gene and the inducible bioreporter gene can be differentiated from each other, they can be engineered into the same cells (DeAngelis et al. 2005).

3. SEEKING BRIDGES ACROSS SCALES

Linking rhizosphere mechanisms to ecosystem function is a challenging goal, but already in the literature a variety of mathematical and conceptual models established at both scales have promisingly parallel components that recommend integration. At rhizosphere scales, a common functional framework should include spatially explicit, water-linked mixing of rhizosphere resources in soil hosting a community

whose activity is constrained by trophic interactions, stoichiometry, and soil structure. There are recognized gross shifts in this framework ecosystem to ecosystem, including, for example, differences in saprotrophic abilities of mycorrhizae (and their biogeographical distribution), shifts in dominance of bacterial versus fungal saprotrophs, variation in soil structure and chemistry, seasonal extents of plant control over water fluxes, etc. These known, larger ecosystem-scale patterns can be woven into the developing conceptual rhizosphere framework and their consequences considered. As a simple example, shifts in dominance of fungi and bacteria among microbial decomposers have been well-documented across ecosystems and management regimes, and the implications of the simple morphological distinction between fungi and bacteria within the soil matrix are intriguing. The three-dimensional fungal hyphal structure can support integration of resources by a single organism from a much larger volume of rhizosphere soil, potentially diminishing the effect of spatial zonation of resource availability on microbes around tip, active uptake, and suberized root zones when fungi are dominant decomposers in ecosystems.

Models can be linked within as well as across scales. For example, Clarholm's (1985) model could be considered an extremely simple form of Moore et al.'s (2003) bacterial channel of energy flow in the rhizosphere food web, in which root-derived, labile carbon fuels bacterial growth and activity, leading to increased grazing by protozoa on that biomass (and concomitant nutrient mineralization). Recognizing mycorrhizae of various saprotrophic capabilities as part of the rhizosphere community, Clarholm's model is also paralleled by Chapman et al.'s (2006) conceptual ecosystems model describing open, leaky nutrient cycles. Such cycles operate in ecosystems where AM mycorrhizae (incapable of saprotrophic activities) are dominant, including grasslands (where almost all of the upper soil layer is rhizosphere soil) or rhizospheres of AM-infected trees. In this open ecosystem cycling scheme, availability of nutrient-rich, easily decomposed plant litter and rhizodeposits supports decomposition dominated by bacteria (as in Clarholm's model), release of mineral nutrients from that bacterial biomass (e.g., by grazing mortality, though that is not specified), and ultimate availability of those mineral nutrients to plants (taken up by roots directly from soil or delivered by the AM fungi). The cycle is open or leaky in the sense that nutrients are quite available in litter and are not being strongly conserved or passed tightly from organism to organism; the nutrients can pass through abiotic soil pools, where ammonium might be nitrified to nitrate, which could be lost by leaching, denitrification, or retained in the system through immobilization. AM hyphae operate in essence as extensions of the root system in this context, providing an enhanced surface area over which nutrients can be absorbed from soil. However, as noted previously, it remains unclear whether the AM fungal hyphae themselves can stimulate the activities of saprotrophs through either signaling or provision of resources, thus indirectly influencing decomposition rates. Nondestructive molecular approaches examining activity and gene expression in saprotrophic bacteria around intact AM fungal hyphae would be particularly interesting in this context.

In contrast to ecosystems dominated by AM mycorrhizae and bacterial saprotrophs, Chapman et al.'s (2006) conceptual model of closed or conservative ecosystem cycling characterizes ecosystems dominated by slow decomposition rates and

ECM-symbioses (e.g., northern boreal ecosystems). The closed ecosystem cycling scheme emphasizes the saprotrophic capability of the ECM fungi themselves in attacking litter that is naturally less rich, as well as the ECM fungal delivery of nutrients directly to the plant roots that directly supplied them carbon. This closed, tight cycling can be viewed as a variation on the rhizosphere fungal/slow and mycorrhizal channels described by Moore et al. (2006), in which the saprotrophic fungal community and the symbiotic fungal community are one and the same. The evolution of saprotrophic ECM symbionts greatly simplified the interorganismal rhizosphere transfers otherwise required to move carbon to decomposers and nutrients from litter or SOM to roots. In cold climates where microbial activity is limited and litter is recalcitrant, Chapman et al. (2006) suggest this streamlining represents an “uncorking of the microbial bottleneck” that otherwise could restrict nutrient availability to plants.

Discussion of open (leaky) and closed (conservative) recycling schemes and their shifts over time has a long tradition within ecosystems ecology, ranging from Odum’s landmark paper in 1969 hypothesizing shifts from open to closed nutrient cycling during succession (see Vitousek & Reiners 1975 for contrast), to Bardgett et al.’s (2005) discussion of successional shifts under climate change. The rhizosphere is the microscopic stage in which these interactions are playing out, an empirical realm where molecular tools emphasizing function are invaluable for testing and inspiring understanding of belowground function in the diverse array of Earth’s ecosystems.

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