Chapter 22

Do Root Exudates Exert More Influence on Rhizosphere Bacterial Community Structure Than Other Rhizodeposits?

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22.1 INTRODUCTION

In terrestrial ecosystems, plants are the key primary producers and use their complex root systems to exploit soils for resources. The soil environment influenced by roots is known as the rhizosphere and supports diverse microbial communities that are generally more densely populated than those in root-free soil (Dennis et al., 2008; Marilley and Aragno, 1999). These relatively dense communities are supported by carbon-containing materials lost by roots (rhizodeposition) and have both direct and indirect effects on plant health and nutrition (Cambell and Greves, 1990; Weller, 1988). Improved understanding of below ground plant–microbe interactions will facilitate development of improved management strategies for environment or commercial purposes.

Rhizodeposits include a wide variety of compounds (Table 22.1) derived from sloughed-off root cells and tissues, mucilages, and exudates originating from intact roots, and soluble lysates and volatile compounds released from damaged cells (Curl and Truelove, 1986; Dakora and Phillips, 2002; Uren, 2001).

Most studies that investigate rhizosphere microbial community structure do not consider the composition of all rhizodeposits and focus instead on the soluble exudates. For practical reasons, collecting root rhizodeposits is usually done in hydroponic plant culture which favors the collection of only soluble exudates. In order to exploit rhizosphere interactions for environmental or commercial benefits, however, it is essential to understand how all plant-derived substrates influence soil microorganisms. For example, we need to know the spatial distribution of substrates derived from different pools of rhizodeposits, how different components of rhizodeposits select for particular microbial taxa, and whether plant root traits can be altered by breeding or genetic modification (GM) to achieve greater control over rhizosphere microbial communities. In this chapter, we review current understanding of rhizosphere carbon and microbial community dynamics in space and time, focussing on bacteria and evaluating the

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Table 22.1 Compounds reported to be present in rhizodeposits.

Sugars	Arabinose, fructose, galactose, glucose, maltose, mannose, various mucilages, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose, desoxyribose
Amino acids	α-Alanine, β-alanine, γ-aminobutyric, α-aminoadipic, arginine, asparagine, aspartic, citrulline, cystathionine, cysteine, cystine, deoxymugineic, 3-epihydroxymugineic, glutamine, glutamic, glycine, histidine, homoserine, isoleucine, leucine, lysine, methionine, mugineic, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
Organic acids	Acetic, aconitic, ascorbic, aldonic, benzoic, butyric, caffeic, citric, *p*-coumaric, erythronic, ferulic, formic, fumaric, glutaric, glycolic, lactic, glyoxilic, malic, malonic, oxalacetic, oxalic, *p*-hydroxybenzoic, piscidic, propionic, pyruvic, succinic, syringic, tartaric, tetronic, valeric, vanillic
Fatty acids	Linoleic, linolenic, oleic, palmitic, stearic
Sterols	Campesterol, cholesterol, sitosterol, stigmasterol
Vitamins	*p*-Amino benzoic acid, biotin, choline, *N*-methyl nicotinic acid, niacin, thiamine, riboflavin, pyridoxine, pantothenate
Enzymes	Amylase, invertase, peroxidase, phenolase, acid/alkaline phosphotase, polygalacturonase, protease
Flavonones and Nucleotides	Adenine, flavonone, guanine, uridine/cytidine
Miscellaneous and Inorganic	Auxins, scopoletin, hydrocyanic acid, glucosides, unidentified ninhydrin-positive compounds, unidentified soluble proteins, reducing compounds, ethanol, glycinebetaine, inositol and myoinositol-like compounds, Al-induced polypeptides, dihydroquinone, sorgoleone, isothiocyanates, alcohols, fatty acids, alkyl sulfides, inorganic ions, and gaseous molecules (e.g., CO_2, H_2, H^+, OH^-, HCO_3^-)

Information compiled from published sources (Curl and Truelove, 1986; Dakora and Phillips, 2002; Uren, 2001).

importance of root exudates relative to other rhizosphere carbon pools.

22.2 RHIZODEPOSITION

The total allocation of photosynthetic carbon to roots is estimated to range from 20–30% for cereals to 30–50% for pasture plants (Kuzyakov and Domanski, 2000). Around 50% remains in the roots, 33% is released by root or microbial respiration (assumed to make equal contributions) within a few days, with the remainder incorporated into soil microbial biomass and organic matter (Kuzyakov, 2006; Kuzyakov and Domanski, 2000). Rhizodeposition represents approximately 11% of net fixed carbon and 27% of carbon allocated to roots (Jones et al., 2009). However, there is considerable variation between different reports, so the carbon economy of plants and the relative sizes of various pools of rhizodeposits remain controversial. Exudates appear to comprise the largest fraction of nonvolatile rhizodeposits (Meharg and Killham, 1988), with sugars and amino acids the most abundant components (Farrar et al., 2003). Total rhizodeposition is underestimated in such studies because sloughed-off cells and tissues are removed prior to analysis (Iijima et al., 2000).

Many factors including space and time influence rhizodeposition quantitatively and qualitatively (Carvalhais et al., 2011; Darwent et al., 2003; Dennis et al., 2009; Jaeger et al., 1999; McDougal and Rovira, 1970; Vanegeraat, 1975). Rhizodeposition is increased by environmental stresses (e.g., phosphate or iron deficiency), microorganisms, and the presence of solid rooting media. Despite this, most rhizosphere carbon flow research has been undertaken in sterile solution culture, which tends to exclude sloughed-off root cells and tissues and is not a realistic substitute for plants growing in soil (Jones and Darrah, 1992, 1993a, 1993b; Muhling et al., 1993). Furthermore, many studies focus on young roots although investigation of pulse-labeled ^{14}C photosynthate partitioning to roots, rhizosphere respiration, and soil residues showed decreases with increasing plant age (28–600 days) by 43%, 28%, and 20%, respectively (Nguyen, 2003). To gain a more realistic understanding of rhizodeposition patterns and turnover of rhizodeposits, experiments in soil throughout the full life-cycle of plants are needed.

22.3 WHERE ARE THE SITES OF RHIZODEPOSITION?

The root apical region terminates with the root cap where cells synthesize and secrete polysaccharide mucilages estimated to represent 2–12% of total rhizodeposition (Darwin and Darwin, 1880; Juniper et al., 1966; Nguyen, 2003; Rougier and Chaboud, 1985; Sievers et al., 2002). Up to 10% of all carbon released by roots is found in

hotspots corresponding to lysed sloughed-off root cells (Iijima et al., 2000). The lag period between detachment and lysis of root cells is unclear and the relative influence on the plant versus the soil remains uncharacterized. These factors are predicted, however, to influence the spatial availability of rhizodeposits and consequently the distribution of microorganisms in soil. When cells separate from the root cap, they form a physical and biological interface between the root and the soil; in the majority of species they remain metabolically active and are known as border cells (Hawes, 1990, 1991; Stephenson and Hawes, 1994). Border cells are reported to influence soil microorganisms by species-specific chemoattraction and/or repulsion (Hawes, 1990, 1991) and competition for resources such as sugars (Stubbs et al., 2004). They are also implicated in stimulating sporulation (Gochnauer et al., 1990) and suppressing phytopathogens (Gunawardena and Hawes, 2002; Hawes et al., 1998, 2000; Zhao et al., 2000). The root apical meristem (RAM) is categorized into three types: closed, basic-open, and intermediate-open (Groot et al., 2004) and is thought to influence the number of border cells (Hamamoto et al., 2006; Hawes et al., 2003). The RAM of monocotyledons is generally the closed type with a discrete root cap distinct from the root proper (Sievers et al., 2002). Species with closed RAM release fewer border cells than those with open organization, for example, *Brassica napus* and *Arabidopsis thaliana* release no border cells although sheets of dead mucilage-producing "border-like" cells are sloughed-off (Bengough and McKenzie, 1997; Hamamoto et al., 2006; Hawes et al., 2003; Vicre et al., 2005).

For some compounds, root exudation is an active ATP-dependant transporter-specific process (Badri et al., 2008; Jones et al., 2004; Loyola-Vargas et al., 2007). This could provide a mechanism by which roots could exert localized regulation of microbial communities, but the majority of exudates are released by passive, basal exudation at meristematic regions immediately behind the root cap (Darwent et al., 2003; Dennis et al, 2010; Farrar et al., 2003; Jaeger et al., 1999; McDougal and Rovira, 1970; Norton et al., 1990; Vanegeraat, 1975) and illustrated in Figure 22.1.

Steep chemical gradients are generated between plant and soil in the active meristematic zones where the majority of photosynthetic carbon is allocated to support root cell division, resulting in exudation by passive diffusion (Dennis and Jones, 2006; Dennis et al., 2010; McDougal and Rovira, 1970; Norton et al., 1990; Vanegeraat, 1975).

The meristematic zone also secretes specific signal molecules such as flavonoids that attract rhizobia (Hirsch, 1999; Spaink, 1995; see Chapter 51) and strigolactones that stimulate both hyphal branching in arbuscular mycorrhizal fungi and seed germination in the root-parasitizing weed striga (Akiyama et al., 2005; see Chapters 33, 34, 34). However, plant pathogenic nematodes, fungal pathogens, and mycorrhizal fungi favor infection of the elongation zone and rhizobia infect via the root hairs or cracks where lateral roots emerge (Bird, 1996; Gunawardena and Hawes, 2002). Presumably, the apex has moved on by the time these organisms have reacted to the exudate components, assigning them to the elongation zone and beyond. In the elongation zone, c. 0.5 mm behind the RAM, rhizodermal cells expand to 10–20 times their original length facilitating root growth rates of 20–90 mm per day (Foster, 1986). This results in the apical region (root cap, meristematic and elongation zones) moving through soil at about 0.2–1.0 μm/s.

Figure 22.1 The sites of root exudation. Carbon-containing compounds labeled with ^{14}C exuded from a 6-day old *Brassica napus* root system over a 6 h period following 1 h of growth in a $^{14}CO_2$-enriched atmosphere as described (Dennis and Jones, 2006). The root exudation pattern was captured by 6 h exposure of a phosphor storage plate to a sheet of moistened filter paper placed over the root–soil profile during labeling.

In most terrestrial plants, abundant root hairs may develop when rhizodermal cells are fully extended. Their tips secrete mucilage, binding soil particles and improving root–soil contact (Curl and Truelove, 1986; Scott et al., 1958). Lectins that bind specific sugar molecules

are present in legumes at the tips of root hairs. They have a role in the early stages of nodulation by binding compatible rhizobia and may be involved in other recognition processes (Hirsch, 1999); glycoproteins present on root hairs of some species are also implicated in signaling (Ridge et al., 1998; Ridge and Rolfe, 1986; Samaj et al., 1999).

Rhizoplane-colonizing bacteria commonly inhabit the network of depressions at the junctions between rhizodermal cells and are often covered in a thin transparent coating of mucilage (Chin-A-Woeng et al., 1997). Such biofilms arise from interactions between root and microorganism cells, although mucilage layers are also found on axenically grown plant roots (Foster, 1986; Rudrappa et al., 2008). When rhizodermal and cortical cells age and lyse, they become impregnated with suberin and colonized by microorganisms (Esau, 1977; Watteau et al., 2002).

Figure 22.2 summarizes the origins of the various pools of rhizodeposits. At the root apices these pools arise from mucilages, exudates, and sloughed-off tissues and root cells that may or may not lyse their contents in the vicinity. Where the root is fully developed, rhizodeposits originate from the lysis of rhizodermal cells; mucilaginous sheets in the depressions between cells; and cells or tissues and remnants of soluble exudates that were released at the apices. Although root exudate quality and quantity responds rapidly to subtle changes (Dilkes et al., 2004), it is not known if similar processes regulate the production of mucilages and the sloughing-off of root cells and tissues. Root mucilages can increase the water holding capacity of rhizosphere soil (Young, 1995) and their production can depend on the water status of the plant (Carminati et al., 2011).

22.4 WHAT HAPPENS TO RHIZODEPOSITS IN SOIL?

The extent of the rhizosphere of each plant species will vary with both the quantity and composition of soluble rhizodeposits, and the local soil properties that influence mobility of compounds (Jones et al., 2004). There is rapid breakdown or mineralization of most rhizodeposits, largely due to microorganisms (Kuzyakov et al., 2001; Nguyen et al., 1999): mucilages reported to have a half-life of approximately 3 days (Jones et al., 2009); amino acids, sugars, and organic acids 30–120 min (Ryan et al., 2001). The latter was estimated from adding ecologically realistic concentrations to a spatially constrained root mat formed at the base of the plant container, so may not be truly representative of root systems in soil but may be considered "average" for the whole rhizosphere because bacterial density and the consequent turnover of rhizodeposits is generally greater at basal when compared with apical root regions (Chin-A-Woeng et al., 1997; Dennis et al., 2008).

22.5 WHAT TRAITS DO RHIZOSPHERE BACTERIA NEED?

The bacterial genes and traits that lead to rhizosphere colonization including utilization of rhizodeposits, effective colonization of root or rhizosphere soil surfaces, and competition with other organisms have been extensively studied over recent years. Despite contradictory reports on the role of bacterial motility in rhizosphere colonization (de Weger et al., 1987; Howie et al., 1987; Simons et al., 1997), it is reduced if either flagella or ATP production is disrupted (Dekkers et al., 1998a, 1998b). Growth rate, like motility, is energy dependent and subject to the ability of microorganisms to obtain essential nutrients. A relatively fast growth rate is important for effective root colonization and is linked to genes for nutrient uptake (de Weert et al., 2006), amino acids and vitamin B1 synthesis (Simons et al., 1997), and organic acid utilization (Bloemberg and Lugtenberg, 2001). The O-antigen of lipopolysaccharide (LPS) is implicated in regulating growth rates and also in root colonization (Dekkers et al., 1998a, 1998b). Thus, for bacteria, chemotaxis toward roots and rapid growth in response to carbon-containing compounds are important traits determining rhizosphere competiveness.

The rhizosphere environment is reported to be oligotrophic (nutrient poor), with carbon concentrations ranging from 10 to 100 μg C/g dry soil (Darrah, 1991), further depleted by microorganisms (Morita, 1988). The root-free bulk soil is usually carbon-poor relative to the rhizosphere with most soil bacteria considered oligotrophs rather than copiotrophs that grow relatively fast and prosper in nutrient-replete conditions. However, evidence suggests that rhizospheres select for an increased proportion of copiotrophic bacteria (2–7%) compared to less than 1% in to bulk soil (Zelenev et al., 2005b). In the lettuce rhizosphere, the ratio of copiotrophic to oligotrophic bacteria was highest at the root apex where most exudates are released, declining toward the root base although the opposite was observed in a different plant species, tomato (Maloney et al., 1997). Simulation of the rhizosphere by adding glucose to bulk soil stimulated the proportion of active cells to 5% of the total (Christensen et al., 1999) and root-derived mucilage increased bulk soil biomass C by 23% and the number of cultivable bacteria by 450% (Benizri et al., 2007). A wide range of growth rates for different types of bacteria that utilize root exudates and/or mucilages as sole carbon sources is reported in the literature (Baudoin et al., 2003; Benizri et al., 2002; Knee et al., 2001), providing a mechanism by which plant species could influence rhizosphere microbial

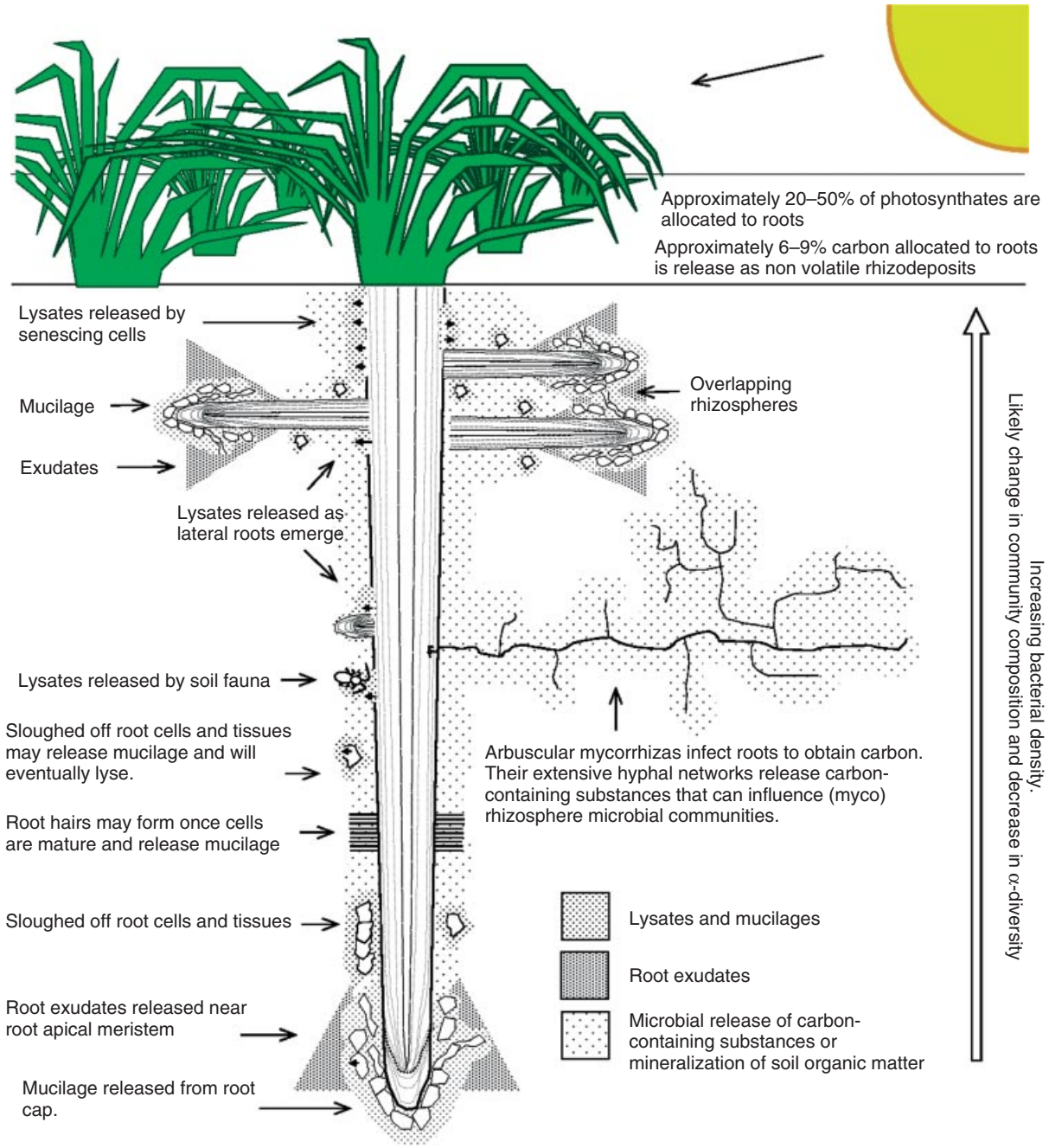

Figure 22.2 Origins of the various rhizodeposit pools. Rhizodeposits at root apices originate from mucilages, exudates, and sloughed-off root cells/tissues that may lyse rapidly or more slowly away from the vicinity of the apex. Root exudate availability is predicted to decrease rapidly with increasing distance from apices due to microbial mineralization. In regions surrounding root zones where cells are fully developed, rhizodeposits originate from, rhizodermal cell lysis, mucilaginous sheets found in the depressions between cells, and nonmineralized compounds, cells and tissues released at root apices. Additionally, microorganisms metabolize rhizodeposits and rerelease carbon in different compounds. In regions dominated by microbe-derived carbon, especially in the mycorhizosphere, the plant may exert little direct control over microbial communities.

community structure. However, this does not prove that root exudates alone are responsible: most components are present in other rhizodeposit pools and can also arise from microbial activity. To justify claims that root exudates are the key determinant of microbial community structure, we believe that stronger evidence is needed.

22.6 BACTERIAL COMMUNITY STRUCTURE IN THE RHIZOSPHERE

As well as containing more copiotrophs, rhizosphere bacterial populations are more numerous than those of bulk soil (Chin-A-Woeng et al., 1997; Gamalero et al., 2004; Rovira et al., 1974; Tesarova and Repova, 1984; Watt et al., 2006), with bacterial density following the trend: basal region > bulk soil > apical region as shown in Figure 22.3 and reported in the literature (Chin-A-Woeng et al., 1997; Dennis et al., 2008; Duineveld and Van Veen, 1999; Jones and Darrah, 1992; Liljeroth et al., 1991; Olsson et al., 1987; Parke et al., 1986). This counters the view that exudates determine the overall structure of rhizosphere bacterial communities, since they are released at root apices and rapidly mineralized. An exception is the Libyan Desert grass, *Aristida coerulescens*, where bacterial densities at the root apex are reported to be greater than those at the base, possibly a result of the extreme environmental conditions in the upper horizons of a hot desert soil (Naim, 1965). Another report that the bacterial population density peaks at both the base and the apex of wheat roots may reflect two sources of rhizodeposits: lysates attributed to the loss of cell integrity more prevalent around the root base; and exudates released predominately at the root tip (Vanvuurde and Schippers, 1980).

The number of bacterial species and their relative abundance (α-diversity) in the rhizosphere differs from bulk soil (Marilley and Aragno, 1999; Marilley et al., 1998). The community structure also varies between root zones (Dennis et al., 2008; Duineveld and Van Veen, 1999; Marschner et al., 2002; Semenov et al., 1999), due to the heterogeneous abundance and distribution of rhizosphere microorganisms with respect to position along longitudinal root axes (Chin-A-Woeng et al., 1997; Dennis et al., 2008). Bacterial communities occur in clusters occupying a relatively small proportion of the available surfaces at root apices but almost cover the root base (Chin-A-Woeng et al., 1997). Since indices of α-diversity are affected by differences in community size, and it is not possible to make concomitant measurements of community size and composition using most current methods, the sampling effort required to generate representative estimates of bacterial diversity within the apical region is likely to be much greater than within the basal region. However, because the root will have had little time to influence the local microbial community at the root apex, we hypothesize that bacterial α-diversity of the apical zone will be similar to that of bulk soil communities. By the same reasoning, microbial communities associated with older root regions will be less diverse than in bulk soil due to the longer period of time that they have been exposed to, and influenced by the root. An indication that bacterial diversity follows the general trend: bulk soil > apical region > basal region was obtained using a new microsampling technique on the roots of *B. napus* and is illustrated in Figure 22.3 (Dennis et al., 2008).

22.7 ARE ROOT EXUDATES KEY DETERMINANTS OF RHIZOSPHERE MICROBIAL COMMUNITY STRUCTURE?

Rhizosphere microbial communities can be either stimulated or inhibited by components of root exudates (Hartmann et al., 2009) and different microbial species

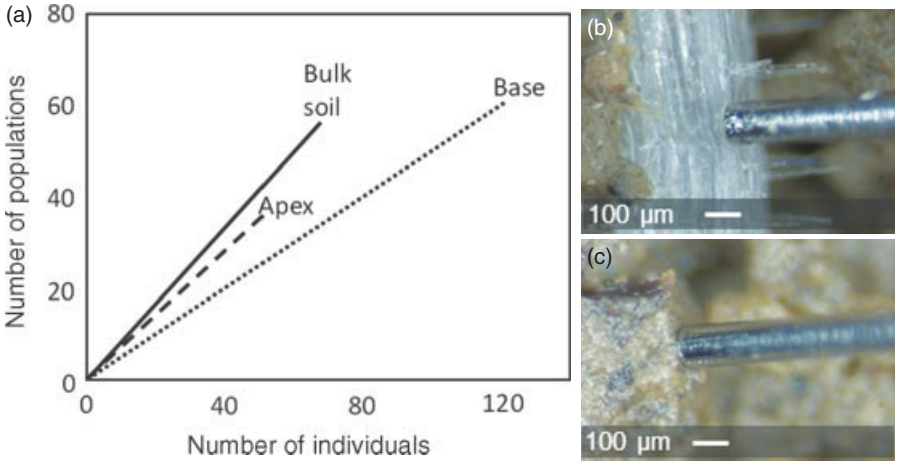

Figure 22.3 Comparison of bacterial diversity in different root zones. Rarefaction of bacterial communities associated with bulk soil and the root apex and base of 6-day old *B. napus* plant grown in soil (a). Individuals represent colonies isolated from each environment using a novel microsampling technique, categorized using ERIC-PCR fingerprinting (Dennis et al., 2008). Panels (b) and (c) show microsampling tips sampling root and soil surfaces, respectively.

vary in their ability to utilize and compete for substrates. Carbohydrates, organic acids, and amino acids are components of root exudates that stimulate positive chemotactic responses in bacteria (Somers et al., 2004). Some plants show extreme variation, for example, the cluster or proteoid roots formed by species of the Proteaceae, Casuarinaceae, Mimosaceae, Fabaceae, Myricaceae, and Moraceae (Dinkelaker et al., 1995) in response to phosphorus (also to some extent, iron) deficiency. Young cluster roots (2–3 days) of *Lupinus albus* are characterized by high malate exudation but citrate dominates in mature roots, increasing once growth has ceased for a few days and decreasing as the cluster root senesces (Neumann et al., 2000). Rhizosphere microbial communities of different-age *L. albus* cluster and noncluster roots with differing exudation profiles were investigated using denaturing gradient gel electrophoresis (DGGE) analysis of 16S and 18S rRNA gene amplicons (Marschner et al., 2002). Bacterial and fungal communities were associated with different root classes and with specific root exudate components: citric acid was the major influence on fungi whereas *cis*-aconitic, citric, and malic acid all influenced bacteria. Other studies have shown up to 1000-fold more opine-utilizing bacteria in the rhizopspheres of genetically modified plants that exuded opines (amino acid–sugar conjugates), compared with non-opine producing controls (Mansouri et al., 2002; Oger et al., 1997; see Chapter 110).

Roots respond to signals that stimulate defense responses (salicylic acids, jasmonic acids, chitosans) by exuding a range of secondary metabolites that inhibit fungal and bacterial pathogen growth (Walker et al., 2003). Plant defense responses are constantly stimulated by diverse soil microorganisms to produce a range of secondary metabolites (saponins, glucosinilates, hydroxamic acids), which in part account for the resistance or susceptibility of particular plant species/cultivars to root pathogens. Fungal pathogens of oat, tomato, and potato roots are resistant to the saponins (avenacin A-1, α-tomatine, and α-chaconine, respectively) exuded by these species; in crucifers and brassicas, glucosinilates that degrade to toxic (iso)thiocyanates deter herbivorous pests and fungal root pathogens (Morrissey and Osbourn, 1999). There is little evidence that these compounds affect bacteria although some differences in rhizosphere community structure related to glucosinilates have been reported (Rumberger and Marschner, 2004). The antifungal hydroxamic acids DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) produced by maize roots do not appear to affect the bacterial root pathogen *Erwinia* (Niemeyer, 1988). However, roots do also secrete antibacterial compounds including rosemarinic acid and, naphthoquinones (Walker et al., 2003). Some plant species produce as-yet unidentified compounds that interfere with bacterial quorum sensing, essential for expression of genes involved in many plant–microbe interactions, both pathogenic and beneficial (Bais et al., 2006; Bauer and Mathesius, 2004; Degrassi et al., 2007; Hentzer et al., 2002). Thus, some components of root exudates exert a strong selective influence on rhizosphere microbial communities, but many of these compounds also occur in other rhizosphere carbon pools including those that derive from lysed cells and microorganisms.

22.8 DO ROOT EXUDATES EXERT A STRONGER INFLUENCE THAN OTHER CARBON POOLS IN THE RHIZOSPHERE?

Assuming a root growth rate of 1.0 μm/s (Foster, 1986) and the half-life sugars and amino/organic acids in soil to be 30 min (Ryan et al., 2001), then 1 mm behind the meristem, the concentration of exudates surrounding the root would be halved and at a distance of 10 mm the concentration would fall to less than 1% of that originally released (Dennis et al., 2010). These estimates are based on the maximum rates in the cited references: the minimum rates indicate a halving of exudate 4 mm behind the meristem and less than 1% at 30 mm, illustrating the relatively small window where exudate-derived substrate is abundant and favors the most competitive copiotrophic microorganisms. However, in reality, selective pressures will vary spatially and temporally as exudation will respond rapidly to even small changes in the environment (Dilkes et al., 2004).

Once carbon substrates are assimilated by microorganisms, some are lost rapidly by respiration and some are secreted as soluble metabolites, exopolysaccharides, and other compounds. The remainder are incorporated into cellular material to be released on cell death and lysis. Little is known about the quantity and composition of rhizosphere microbial secretions but many are implicated in functions including quorum sensing, attracting or antagonizing other microorganisms, and modulating root activity and architecture with consequences for nutrient availability (Bais et al., 2005). Release of microbial cell lysates may fuel a subsequent wave of colonists manifested as waves of bacterial abundance initiated by exudates released by passing root apices (Zelenev et al., 2005a). As the root-derived C is cycled through successive microbial communities, compositional changes make it increasingly unlikely that root exudates determine the structure of microbial communities in older root regions (Figure 22.2). As lateral roots emerge, they will influence communities associated with older root regions but at basal root regions, where the largest numbers of bacteria are found, lysates and mucilages and microbial

metabolites derived from these are likely to play a more important role.

The soil organic matter (SOM) provides a universal microbial substrate and when exposed to carbon compounds typical of root exudates, SOM decomposition is accelerated (De Nobili et al., 2001). However, rhizosphere microorganisms use the relatively simple components of root exudate in preference to the more recalcitrant SOM (Kuzyakov, 2002); thus, SOM decomposition may be significant only where more labile substrates are depleted. However, since carbon availability alone does not appear to limit bacterial respiration in the rhizosphere (Cheng et al., 1996), it is possible that SOM mineralization has a major influence on rhizosphere microbial community structure (Cheng et al., 1996; Helal and Sauerbeck, 1986).

While we agree that root exudates have some influence on rhizosphere microbial community structures, we believe that current evidence is not sufficient to support claims that root exudates are the key determinant. Rather, evidence suggests that the direct influence of root exudates is limited to small spatiotemporal windows surrounding root apices, and we propose that other rhizosphere carbon pools will have a similar, if not greater, influence in selecting rhizosphere microbial communities for the majority of their coexistence with their plant host. Nevertheless, root exudates probably exert the major rhizosphere effect in very young seedlings and on emerging lateral roots. However, many reports claiming to demonstrate the importance of root exudates in structuring rhizosphere microbial communities overlook the potential influence of other rhizodeposits pools. An example is the observation of increased numbers of opine-utilizing bacteria in the rhizospheres of genetically modified plants that synthesized opines (Oger et al., 1997; see Chapter 110) where it is likely that cell lysis released opines in root regions distant from the root exudate-dominated apices. A more accurate interpretation of such studies is that responses observed in microbial communities are likely linked to differences in rhizodeposition rather than root exudates alone, a distinction we think is important despite the difficulties associated with differentiating between different pools of rhizodeposits. It is important to consider where microbial communities associated with different root regions get the majority of their resources, in order to develop effective strategies for rhizosphere management. The root physical environment offers relatively abundant water and nutrients for soil microbes. Together with the powerful influence of a general source of carbon supplied by rhizodeposition, these factors must be the main drivers for rhizosphere colonization by microorganisms. Root exudates are likely to have a much localized influence relative to the generalized effect of rhizodeposition.

22.9 MODIFYING MICROBIAL DISTRIBUTION IN THE RHIZOSPHERE

Designing agricultural systems with high levels of food security while reducing the environmental impacts currently associated with agriculture is a key goal of rhizosphere microbial ecology. One strategy is to encourage the proliferation of beneficial indigenous or introduced microorganisms that promote plant growth directly, increase nutrient uptake, or suppress pests and pathogens. Understanding spatial and temporal aspects of these rhizosphere processes is critical for their successful exploitation: to control a plant pathogen that infects at root apices, organisms that suppress the invasion must be present at the same location. Likewise, rhizosphere microorganisms that mobilize specific nutrients need to be associated with the root zone most active in their uptake.

There is conflicting information regarding spatial trends in plant nutrient uptake. Briefly, there is maximal influx of magnesium (Grunes et al., 1993), copper (Papeschi et al., 2000), cadmium (Pineros et al., 1998), and particularly calcium (Ryan et al., 1990) in the elongation zone, whereas root apices are the principal sites of iron reductase and phytosiderophore production and consequently where most iron is taken up although Fe^{3+} phytosiderophore uptake can occur along the length of the root (Romheld and Marschner, 1986). Uptake of magnesium and calcium ions, thought to be apoplastic, is impeded in older root regions due to suberin formation in the rhizodermal and endodermal cell layers effectively blocking apoplastic flow (Ferguson and Clarkson, 1975, 1976; Robards et al., 1973). Young roots (<7 cm long) are reported to exhibit uniform uptake of phosphate (Rubio et al., 2004), potassium (Marschner, 1995), nitrate, and ammonium (Colmer and Bloom, 1998; Henriksen et al., 1992; Taylor and Bloom, 1998). When these ions are released from SOM during mineralization by rhizosphere microorganisms, they will become available to the plant in regions previously depleted by passing root apices.

There are several ways in which the distribution of indigenous and introduced microorganisms could be managed, for example, the microbial biomass is increased by additions of SOM (e.g., composts) and specific groups are encouraged by applying chemical amendments to soils (Devliegher et al., 1995). Other approaches have attempted to modify root growth and architecture and proteins in rhizodermal cell membranes involved in plant–microbe signaling (Samaj et al., 1999). These methods are not strictly relevant to rhizodeposition and more details can be found in other reviews (Ryan et al., 2009). Modification of rhizosphere bacteria can be very effective, for example, introduction of an effective

root tip–colonizing bacterium, *Pseudomonas fluorescens* WCS365, into the tomato rhizosphere suppressed the plant pathogenic fungus *Fusarium oxysporum* f.sp. *radicis-lycopersici*, which often infects at root apices (Dekkers et al., 2000). The ability to colonize root tips was partly related to *sss* (sodium solute transporter superfamily) genes in WCS365 and when introduced into other *Pseudomonas* strains less efficient at colonizing apices, the genes appeared to increase their abundance at these root regions by 8- to 40-fold (Dekkers et al., 2000).

Other tactics could involve modifying the response of microbial inoculants to rhizodeposits, the supply of rhizodeposits to microorganisms, or a combination of both. These approaches need not necessarily involve GM of either microorganism or plants: effective root-tip-colonizing bacteria can be selected by repeatedly inoculating plants with microorganisms isolated from their root apices (de Weert et al., 2004; Kuiper et al., 2001). Effective root tip-colonizers can subsequently be screened for traits beneficial to plant growth and reintroduced as inoculants. Further desirable traits can be introduced by GM if appropriate. Such organisms are likely to contain genes enabling proliferation in response to exudates and resistance to any antimicrobial components it contains (Rumberger and Marschner, 2004). Interactions are complex: release of antimicrobial compounds from Arabidopsis roots was blocked by one pathogenic *Pseudomonas syringae* strain using a mechanism dependent on its type III secretory system (Bais et al., 2005). In comparison to research to increase the production of antimicrobial compounds, relatively little attention has been given to strategies involving the introduction of genes that would increase the capacity for microorganisms to resist antimicrobial rhizodeposits or utilize xenobiotic compounds released by roots (Ryan et al., 2009). Plants modified to contain genes for opine synthesis select organisms capable of utilizing the opines produced, and a variant on this theme has been proposed to generate rhizopine-producing plants (see Chapter 110). Rhizopines (scyllo-inosamine or 3-*O*-methyl-scyllo-inosamine), produced and also specifically degraded by some rhizobia (strains of *Sinorhizobium meliloti*) in the alfalfa root nodules they induce and occupy, are toxic to many other microorganisms (Murphy et al., 1987; Saint et al., 1993). Despite the potential of this system to facilitate selection of plant-beneficial nitrogen-fixing organisms, attempts to clone and express the *S. meliloti* rhizopine synthesis genes in plants and the degradative genes in other bacteria have not been successful to date (Gardener and de Bruijn, 1998; Ryan et al., 2009; see Chapter 110).

Selection of desirable modifications to rhizodeposition have been selected for inadvertently by repeated crossing of high performance crop lines, such as wheat varieties that release malate to detoxify Al^{3+}, which otherwise can be a problem in acid soils (de Sousa, 1998). Accordingly, either conventional or GM breeding could be used to develop rhizosphere management strategies such as modifying rhizodeposition patterns to give plant roots a greater capacity to synthesize and release certain compounds such as citrate (de la Fuente et al., 1997; Koyama et al., 2000), malate (Yang et al., 2007), and opines (Oger et al., 1997). Another approach focuses on the release of rhizodeposits, involving the Al^{3+}-activated malate transporter (ALMT), and the multidrug and toxic compound extrusion (MATE) gene families (Ryan et al., 2009). The most promising gene within the ALMT family encodes a channel for malate transport that, unlike others within the family, is not dependent on Al^{3+} (Kovermann et al., 2007). In a wide range of organisms, transport of a range of compounds is controlled by MATE genes: these include citrate (Yokosho et al., 2009), flavonoids (Debeaujon et al., 2001), and alkaloids and antibiotics (Li et al., 2002). There are 59 MATE orthologs in *A. thaliana* (Yazaki, 2005) and at least 40 in the rice (*Oryza sativa*) genome (Yokosho et al., 2009), but few have been studied in any detail. Rhizodeposition rates may also be controlled by modifying genes involved in programed cell death (Dickman et al., 2001) and/or increasing numbers of border or border-like cells released by roots (Knox et al., 2007). Genes inducing apoptosis (Chao and Korsmeyer, 1998) could be modified to increase rhizodeposition. When genes from various origins inhibiting apoptosis have been overexpressed in a range of plant species, the resultant phenotypes are more resistant to abiotic and biotic stress (Awada et al., 2003; Chu et al., 2008; Dickman et al., 2001; Qiao et al., 2002; Shabala et al., 2007).

From these examples, it is clear that GM technologies can provide a range of opportunities for modification of the supply of rhizodeposits to microorganisms. Increasing intracellular synthesis of specific compounds will not only raise concentrations in root exudates but they are also likely to be distributed throughout the rhizosphere in lysates from damaged or senescing cells. This provides a potential mechanism to deliver compounds via rhizodeposits that inhibit wound- or root-infecting pathogens, or to select for specific microorganisms in rhizosphere zones that are depleted of root exudates. It is reasonable to assume that elevated transporter activity will increase exudation at root apices, but it is less easy to predict changes, if any, on the spatial distribution of exudation sites. Furthermore, if exudation remains limited to root apices, microbial communities associated with basal root regions will not be affected. Nevertheless, it is possible that one or a combination of techniques (e.g., mutagenesis, selective breeding, and GM) to alter plant and microorganism traits will lead to novel and exciting crop

management strategies in the future. For example, the expression of apoptosis-inducing genes in border/border-like cells in response to specific microorganisms would provide them with lysates preferentially. Similarly, the ability of plants to determine their rhizosphere inhabitants would be enhanced by manipulating root cells in zones with low rhizodeposition to synthesize and exude appropriate compounds.

22.10 CONCLUSION

In this review we present evidence that the direct influence of root exudates on rhizosphere microbial communities is probably limited to small spatiotemporal windows that surround root apices. Root-derived carbon is rapidly assimilated by microorganisms and is likely to be further modified, independent of plant influences, prior to rerelease into the rhizosphere, meaning that rhizosphere carbon pools are unlikely to be dominated by root exudates at short distances from root apices. Also, many of the major components of root exudates are also found in other pools of rhizodeposits (including rereleased microbial metabolites), so will be present throughout the rhizosphere. In the light of this, we suggest that the contribution of root exudates in structuring rhizosphere bacterial communities must be considered in the context of other rhizosphere carbon pools. It will be particularly important to determine the growth-limiting source of nutrients in each root zone in order to design more effective rhizosphere management strategies involving microbial communities associated with different root regions.

In this review we have considered a wide range of approaches offering the potential to modify both the spatial and the temporal distribution rhizodeposits, and the responses of microorganisms to these substances. We present novel and exciting opportunities that could engineer the rhizosphere to overcome the vagaries of the natural soil environment by exploiting the selective influence of plants on the composition and structure of their associated microbial communities.

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