MINIREVIEW

Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities?

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Abstract

This review evaluates the importance of root exudates in determining rhizosphere bacterial community structure. We present evidence that indicates that: (1) the direct influence of root exudates on rhizosphere bacterial communities is limited to small spatiotemporal windows related to root apices; (2) upon rapid assimilation by microorganisms, root exudates are modified, independent of plant influences, before rerelease into the rhizosphere by the microorganisms themselves – thus, at short distances from root apices, rhizosphere carbon pools are unlikely to be dominated by root exudates; and (3) many of the major compounds found in root exudates are ubiquitous in the rhizosphere as they are found in other pools of rhizodeposits and in microbial exudates. Following this argument, we suggest that the importance of root exudates in structuring rhizosphere bacterial communities needs to be considered in the context of the wider contribution of other rhizosphere carbon pools. Finally, we discuss the implications of rhizosphere bacterial distribution trends for the development of effective strategies to manage beneficial plant–microorganism interactions.

Introduction

Plants are the key primary producers in most terrestrial ecosystems and generally exploit soils for resources using complex root systems. The soil environment that is influenced by the presence and activities of roots, the rhizosphere, generally supports bacterial communities that are less diverse (Marilley *et al.*, 1998; Marilley & Aragno, 1999) and of greater size (Lynch & Whipps, 1990; Semenov *et al.*, 1999) than those associated with root-free soil. As carbon availability often limits microbial growth in soil, this 'rhizosphere effect' is thought to result primarily from the release of carbon-containing compounds from roots, known collectively as rhizodeposits. Rhizosphere bacteria have both direct and indirect effects on plant health and nutrition (Weller, 1988; Cambell & Greves, 1990), and an improved understanding of their ecology should facilitate the development of rhizosphere management strategies for environmental and/or commercial purposes.

Rhizodeposits include a wide variety of substances (Table 1) that originate from sloughed-off root cells and tissues, mucilages, volatiles, and soluble lysates and exudates that are released from damaged and intact cells, respectively (Curl & Truelove, 1986; Uren, 2001; Dakora & Phillips, 2002). Despite the fact that similar rhizodeposits can originate from different sources, researchers rarely consider this when discussing their results and often refer to root exudates as if they represent all root-derived carbon. Root exudates are implicated as a key determinant of rhizosphere microbial community structure. However, most studies do not characterize rhizodeposits, and those that demonstrate a link between specific root-derived products and rhizosphere microbial community responses fail to confirm that the products originate from root exudates rather than lysates or any other source of rhizodeposits. Therefore, despite an abundance of literature demonstrating that rhizodeposits influence microbial community structure, evidence supporting claims that root exudates are the key determinant is not available.

It is clear that an improved understanding of the relationship between rhizosphere microbial ecology and rhizodeposition may facilitate the development of rhizosphere

Table 1. Organic compounds release by plant roots

Sugars:
Arabinose, fructose, galactose, glucose, maltose, mannose, mucilages of various compositions, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose, deoxyribose
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, praline, 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
Growth factors and vitamins:
p-Amino benzoic acid, biotin, choline, *N*-methyl nicotinic acid, niacin, pathothenic, thiamine, riboflavin, pyridoxine, pantothenate,
Enzymes:
Amylase, invertase, peroxidase, phenolase, acid/alkaline phosphatase, polygalacturonase, protease
Flavonones and purines/nucleotides:
Adenine, flavonone, guanine, uridine/cytidine
Miscellaneous:
Auxins, scopoletin, hydrocyanic acid, glucosides, unidentified ninhydrin-positive compounds, unidentifiable soluble proteins, reducing compounds, ethanol, glycinebetaine, inositol, and myo-inositol-like compounds, Al-induced polypeptides, dihydroquinone, sorgoleone, isothiocyanates, inorganic ions and gaseous molecules (e.g. CO_2, H_2, H^+, OH^-, HCO_3^-), some alcohols, fatty acids, and alkyl sulphides.

Adapted from Uren (2001), Curl & Truelove (1986), and Dakora & Phillips (2002).

management strategies. However, to alter the structure of rhizosphere microbial communities via modified rhizodeposition profiles, it is critical that we determine which pools of rhizodeposits exert the greatest influence on rhizosphere microorganisms. By doing so, we will know whether it is more effective to modify the expression level of certain transporters, manipulate the contents of root cells, or alter the rate at which root cells and tissues are released and/or lyse. This review aims to synthesize current understanding of rhizosphere carbon and bacterial community dynamics in space and time to evaluate the importance of root exudates relative to other pools of rhizosphere carbon in shaping microbial community structure. Our focus is on bacterial communities, but we recognize the importance of other rhizosphere biota and include a discussion of these organisms throughout the text, albeit to a lesser extent. We begin by summarizing what is known about the spatial and temporal dynamics of rhizodeposition and microbial community structure. We then present a range of studies that claim that root exudates structure rhizosphere microbial communities and argue that they provide insufficient evidence to demonstrate that rhizodeposits, and indeed rhizosphere carbon, originating from other sources, contribute less than root exudates to the observed microbial responses. Finally, we discuss the implications of microbial distributions for realizing effective rhizosphere management strategies and present a range of mechanisms by which these distribution trends may be influenced.

Rhizodeposition

Estimates for the total allocation of photosynthates to roots range between 30% and 50% for pasture plants and 20% and 30% for cereals such as wheat and barley (Kuzyakov & Domanski, 2000). For cereals, roughly half of this carbon remains in the roots, approximately one-third is released from the rhizosphere by root or microbial respiration within a few days, and the remaining fraction is incorporated into rhizosphere microbial biomass and soil organic matter (SOM) (Kuzyakov & Domanski, 2000). Assuming that roots and microorganisms contribute equally to respiration in the rhizosphere (Kuzyakov *et al.*, 2001; Kuzyakov, 2006) rhizodeposition represents approximately 11% of net fixed carbon and 27% of carbon allocated to roots (Jones *et al.*, 2009). However, estimates of carbon economies within plants are controversial and vary considerably between different workers. Likewise, estimates for the relative sizes of various pools of rhizodeposits are uncertain. Root exudates are reported to comprise the largest fraction of nonvolatile rhizodeposits (Meharg & Killham, 1988), and of these, sugars and amino acids are thought to be released in the greatest quantities (Farrar *et al.*, 2003). However, before analysis, rhizosphere solutions are often filtered, thereby removing sloughed-off cells and tissues despite their potential significance in the total rhizodeposition budget (Iijima *et al.*, 2000).

Furthermore, the quantity and composition of rhizodeposits is influenced by many factors (Jones *et al.*, 2004) and varies in time and space with respect to the position on the root (McDougal & Rovira, 1970; Vanegeraat, 1975; Jaeger *et al.*, 1999; Darwent *et al.*, 2003). Despite this, most rhizosphere carbon-flow research has been undertaken in sterile solution culture, which is a problem because rhizodeposition is increased by the presence of solid rooting media, by environmental stresses (e.g. phosphate or iron deficiency), and the presence of microorganisms (Jones & Darrah, 1992, 1993a, b; Muhling *et al.*, 1993). Our understanding of rhizodeposits is based, therefore, on analyses of samples that may exclude sloughed-off root cells and tissues, and poorly represent the conditions that prevail in the rhizospheres of

soil grown plants. These knowledge gaps limit our understanding of rhizosphere microbial ecology. Therefore, the pattern of rhizodeposition and turnover in soil requires better characterization. In particular, trends in rhizodeposition throughout the full life cycle of plants are poorly understood as most studies have focused on young roots. Nguyen (2003) reported that the partitioning of pulse-labelled ^{14}C photosynthates to roots, rhizosphere respiration, and soil residues decreased with increasing plant age (28–600 days) by 43%, 28%, and 20%, respectively. However, there is a lack of studies with which to compare these observations.

Where are rhizodeposits released?

The terminal structure of the root apical region is the root cap; cells towards its periphery are involved in the production and secretion of polysaccharide mucilages that may represent 2–12% of the total rhizodeposition (Darwin & Darwin, 1880; Juniper *et al.*, 1966; Rougier & Caboud, 1985; Sievers *et al.*, 1999; Nguyen, 2003). Ultimately, these cells separate from the cap (Hawes & Lin, 1990; Stephenson & Hawes, 1994). In most plant species, these detached cells are metabolically active and are known as border cells (Hawes, 1991), forming a physical and biological interface between the root and the soil. They are thought to influence bacterial communities in a variety of ways including stimulation of sporulation (Gochnauer *et al.*, 1990), suppression of phytopathogens (Hawes *et al.*, 1998, 2000; Zhao *et al.*, 2000; Gunawardena & Hawes, 2002), species-specific chemoattraction and/or repulsion (Hawes & Lin, 1990; Hawes *et al.*, 2000), and competition for resources such as sugars (Stubbs *et al.*, 2004). The number of border cells varies between different plant species and this variability appears to relate to the organization of the root apical meristem (RAM) in dicotyledons (Hawes *et al.*, 2003; Hamamoto *et al.*, 2006) and is categorized into three types: closed, basic-open, and intermediate-open (Groot *et al.*, 2004). The RAM of monocotyledons is generally of a closed-type construction, with the root cap distinct from the root proper (Sievers *et al.*, 1999). Species with a closed RAM organization release fewer border cells than those with an open organization, for example *Brassica napus* and *Arabidopsis thaliana* release no border cells (Hawes *et al.*, 2003; Hamamoto *et al.*, 2006), although sheets of dead mucilage-producing 'border-like' cells are sloughed off (Bengough & McKenzie, 1997; Hawes *et al.*, 2003; Vicre *et al.*, 2005). Lysis of sloughed-off root cap cells creates substrate hotspots in the rhizosphere that may represent 10% of all the carbon released by roots (Iijima *et al.*, 2000). The influence of plant and soil factors on the size of this pool is not clear and the lag period between detachment from the root cap and cell lysis has not been characterized; however, these factors will influence the spatial availability of rhizodeposits and therefore the potential distribution of rhizosphere bacteria and other organisms.

The exudation of some compounds is an active process (Jones *et al.*, 2004; Loyola-Vargas *et al.*, 2007) and mediated by highly specific transporters requiring ATP (Badri *et al.*, 2008). This is important because it indicates a mechanism for the root to specifically control the local exudation of substances that could directly regulate microbial communities. However, the vast majority of root exudates are thought to be released passively (basal exudation) at meristematic root regions immediately behind the root cap (Fig. 1; McDougal & Rovira, 1970; Vanegeraat, 1975; Norton *et al.*, 1990; Jaeger *et al.*, 1999; Darwent *et al.*, 2003; Farrar *et al.*, 2003). To our knowledge, evidence indicating that root exudates are released in significant quantities from older root regions is not available. In the meristematic zone, cells that constitute the root proper originate from the divisions of initial cells in the RAM. This intense activity results in the cells associated with this region receiving the majority of carbon allocated below ground, with consequential steep electrochemical gradients that lead to exudation by passive

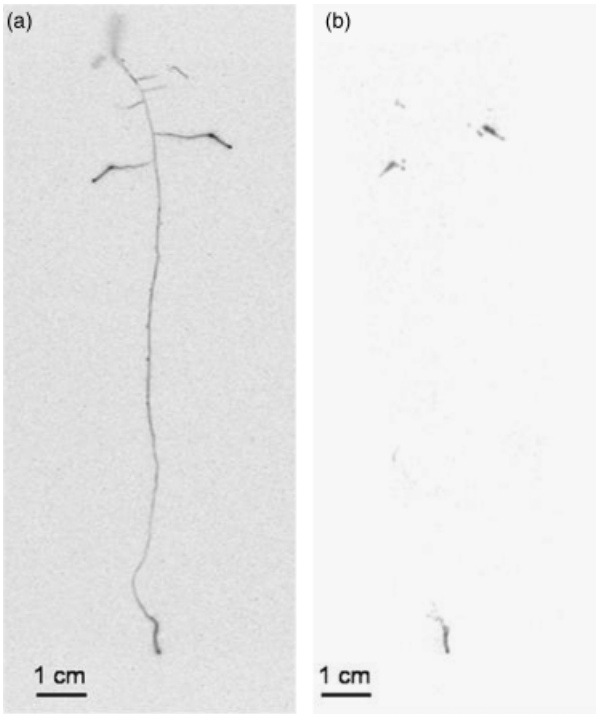

Fig. 1. Allocation of carbon-containing compounds (a) within and (b) exuded from a 6-day-old *Brassica napus* root system 1–6 h after a 1-h period of growth in a $^{14}CO_2$-enriched atmosphere. The allocation of ^{14}C-labelled compounds within the root was captured using the method of Dennis & Jones (2006). The pattern of root exudation was captured by exposing a phosphor storage plate (6 h) to a sheet of moistened filter paper that was placed over the root–soil profile during labelling.

diffusion (McDougal & Rovira, 1970; Vanegeraat, 1975; Norton *et al.*, 1990; Dennis & Jones, 2006).

The meristematic zone graduates into the zone of elongation, where, approximately 0.5 mm behind the meristematic zone, rhizodermal cells expand to 10–20 times their original length. The process of elongation yields root growth rates that are typically in the range of 20–90 mm day^{-1} (Foster, 1986), which means that cells in the elongation and meristematic zones as well as those associated with the root cap move through soil at an approximate rate of 0.2–1.0 μm s^{-1}. The meristematic zone appears to be a favoured site of infection by plant pests and pathogens and is where symbiotic rhizobia are attracted by specific flavonoid signal molecules (Spaink, 1995; Hirsch *et al.*, 2003). It is also the location to which strigolactones attract arbuscular mycorrhizal fungi and induce hyphal branching before symbiosis (Akiyama *et al.*, 2005). Presumably, the apex has moved on by the time bacterial and fungal cells have reacted to these exudate components, assigning them to the elongation zone.

When a rhizodermal cell has fully extended, root hairs may develop in huge abundance, secreting mucilage at their tips, which helps to bind soil particles, thereby improving the root–soil contact (Scott *et al.*, 1958; Curl & Truelove, 1986). In legumes, lectins (which bind specific sugar molecules) are present at the tips of root hairs and are involved in the recognition of rhizobia in the early stages of nodulation (Hirsch, 1999); however, their role in other plant–microorganism interactions is not clear. At the surface of root hairs of legumes and some *Brassicaceae*, sugars that bind to specific lectins have been reported (Ridge & Rolfe, 1986; Ridge *et al.*, 1998), and it is suggested that glycoproteins found at the surface of the root hairs of various species including maize are also involved in plant–microorganism signalling (Samaj *et al.*, 1999). The external face of each rhizodermal cells bulges out into the rhizosphere, creating a network of depressions at the junctions between cells. Rhizoplane bacteria are commonly observed to inhabit these depressions, and are often covered in a thin transparent mucilage coating (Chin-A-Woeng *et al.*, 1997). Similar mucilage layers have been observed on the roots of axenically grown plants (Foster, 1986); however, roots and microorganisms generally interact to produce these biofilms (Rudrappa *et al.*, 2008). As rhizodermal and cortical cells age, they lyse, become impregnated with suberin, and are colonized by microorganisms (Esau, 1977; Watteau *et al.*, 2002).

The origins of various pools of rhizodeposits are summarized in Fig. 2. Briefly, rhizodeposits at root apices originate from mucilages, exudates, and sloughed-off root cells/tissues that may or may not lyse their contents in the vicinity. Rhizodeposits surrounding areas of the root where the cells are fully developed originate from (1) rhizodermal cell lysis, (2) mucilagenous sheets found in the depressions between cells, and (3) nonmineralized compounds, cells, and tissues released at root apices. Very subtle changes within the rhizosphere can induce rapid shifts in the quality and quantity of root exudates (Dilkes *et al.*, 2004), but the responsiveness of mucilages and sloughed-off root cells/tissues to such changes is unknown.

The fate of rhizodeposits in soil

Plant species are likely to vary in the radial extent of their rhizosphere, determined by the amount and composition of their soluble rhizodeposits, which may exhibit relative differences in mobility in soil (Jones *et al.*, 2004). Generally, the mineralization of rhizodeposits is thought to be rapid (Nguyen *et al.*, 1999; Kuzyakov & Cheng, 2001). For example, mucilages are reported to have a half-life of approximately 3 days (Jones *et al.*, 2009), and Ryan *et al.* (2001) reported that most amino acids, sugars, and organic acids are mineralized with a half-life of 30–120 min when added to the rhizosphere at ecologically realistic concentrations. However, these latter estimates were arrived at by adding the compounds to a root mat (a dense population of roots formed at the base of a container in which a plant is grown as a consequence of spatial constraint). While it is possible to find root mats in nature (e.g. between rock cracks), their form is not representative of most root systems. Exudate turnover rates based on root mats may be considered 'averages' for the entire rhizosphere because bacterial density is generally greater at basal when compared with apical root regions of plants grown in soil (Chin-A-Woeng *et al.*, 1997; Dennis *et al.*, 2008); therefore, root exudate turnover rates are likely to be greater at the base when compared with the apices.

Rhizosphere bacterial communities

To proliferate and establish in the rhizosphere, bacteria must be able to utilize rhizodeposits, effectively colonize root or rhizosphere soil surfaces, and be able to compete with other organisms. Over the past 20 years, considerable progress has been made in understanding the bacterial traits and genes that facilitate effective rhizosphere colonization. Motility appears to be an important trait for rhizosphere colonization (De Weger *et al.*, 1987; Simons *et al.*, 1997), although there are a few reports to the contrary (Howie *et al.*, 1987). Motility is energy-dependent; therefore, colonization ability can be decreased if either flagella or ATP production is disrupted (Dekkers *et al.*, 1998a, b). Another trait that is important in effective root colonization by bacteria is their growth rate, which is partly dependent on their ability to obtain components that are essential for growth and/or maintenance. Growth rate has been linked to genes involved in nutrient uptake (De Weert *et al.*, 2006), the ability to synthesize amino acids and vitamin B1 (Simons *et al.*, 1997),

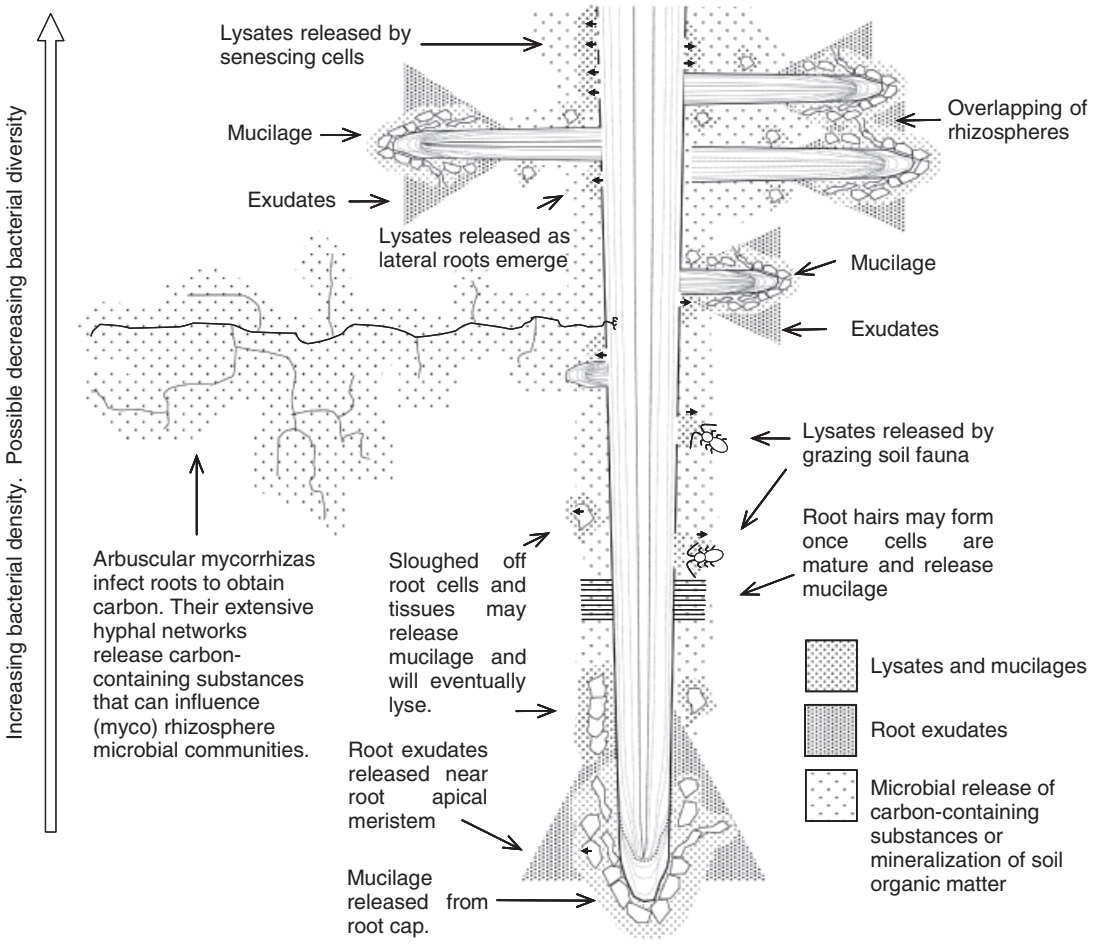

Fig. 2. Origins of various pools of rhizodeposits. At root apices, rhizodeposits originate from mucilages, exudates, and sloughed-off root cells/tissues that may or may not lyse their contents in the vicinity. The availability of root exudates is likely to decrease rapidly with increasing distance from root apices due to microbial mineralization. Surrounding areas of the root where cells are fully developed rhizodeposits originate from rhizodermal cell lysis, mucilagenous sheets found in the depressions between cells, and nonmineralized compounds, cells, and tissues released at root apices. Microbial communities will also release root-derived carbon in an altered form. Plant may exert little control over microbial communities associated with regions that are dominated by microorganism-derived carbon. This may be particularly apparent in mycorhizospheres.

and to utilize organic acids (A.H.M. Wijfjes *et al.*, unpublished data, cited in Bloemberg & Lugtenberg, 2001). The O-antigen of lipopolysaccharide plays a role in root colonization and may be implicated in regulating growth rates (Dekkers *et al.*, 1998b). In summary, the abilities of bacterial cells to move towards roots in response to carbon-containing compounds (chemotaxis) and grow rapidly are important traits that enable a bacterial species to be competitive in the rhizosphere.

Bulk soil is generally carbon poor relative to the rhizosphere; therefore, the majority of soil bacteria are thought to be oligotrophs (Zelenev *et al.*, 2005). Rhizosphere environments have also been reported to be oligotrophic, with carbon concentrations ranging from 10 to 100 $\mu g\,C\,g^{-1}$ of dry soil (Darrah, 1991), which may be further depleted by microorganisms (Morita, 1988). However, evidence suggests that rhizospheres may select for an increased proportion of copiotrophic bacteria relative to bulk soil environments. For example, Zelenev *et al.* (2005) reported that the proportion of cultivable bacteria increased from $< 1\%$ in bulk soil to 2–7% in the rhizosphere (Zelenev *et al.*, 2005), and in the rhizosphere of lettuce, Maloney *et al.* (1997) found that the ratio of copiotrophic to oligotrophic bacteria was high at the root apex (the main site of root exudate release) and declined towards the root base. In tomato plants, however, they found the opposite pattern, possibly reflecting differences in the quantity and quality of rhizodeposits. Furthermore, Christensen *et al.* (1999) reported that addition of

glucose to bulk soil to simulate rhizosphere conditions increased the proportion of active cells detected by direct staining to 5% of the total (Christensen *et al.*, 1999), and addition of root-derived mucilage was demonstrated to increase bulk soil biomass C by 23% and the number of cultivable bacteria by 450% relative to a nonamended bulk soil control (Benizri *et al.*, 2007). Numerous studies demonstrate a wide range of growth rates for different types of bacteria utilizing root exudates and/or mucilages as the sole carbon sources (Knee *et al.*, 2001; Benizri *et al.*, 2002; Baudoin *et al.*, 2003), indicating that the relative capacity for bacteria to metabolize and compete for carbon sources influences the structure of microbial communities. It is sensible, therefore, to assume that major components of rhizodeposits could influence the structure of rhizosphere bacterial communities. However, given that most of the components found in root exudates are also present in other pools of rhizodeposits and may also derive from microorganisms and their activities, we believe that stronger evidence is needed to justify claims that root exudates are the key determinant of microbial community structure.

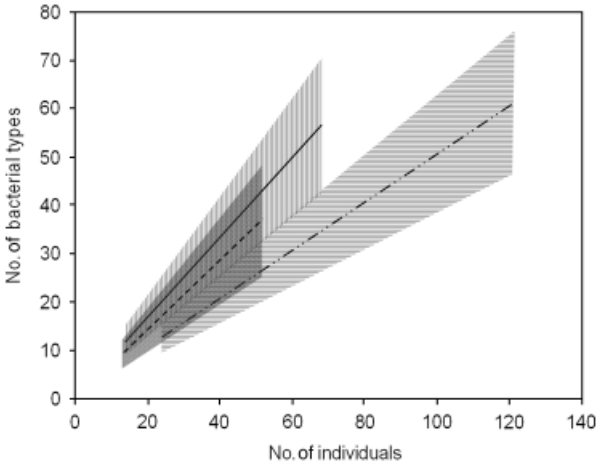

Fig. 3. Individual-based rarefaction curves (lines) with their associated 95% confidence limits (shaded areas), representing bacterial diversity in bulk soil (solid line, vertical shading), at the root apex (dashed line, dark shading), and at the root base (dotted and dashed line, horizontal shading) of 6-day-old *Brassica napus* roots. Individuals represent the colonies isolated from each environment. ERIC-PCR fingerprinting was used to categorize each individual into bacterial types (see Dennis *et al.*, 2008).

Rhizosphere bacterial community structure

Generally, the abundance of bacteria in the rhizosphere is reported to be greater than that in root-free soil (Rovira *et al.*, 1974; Tesařová & Řepová, 1984; Chin-A-Woeng *et al.*, 1997; Gamalero *et al.*, 2004; Watt *et al.*, 2006), and rhizosphere bacterial density follows the trend: basal region > bulk soil > apical region (Fig. 3; Parke *et al.*, 1986; Olsson *et al.*, 1987; Liljeroth *et al.*, 1991; Chin-A-Woeng *et al.*, 1997; Duineveld & van Veen, 1999; Dennis *et al.*, 2008). Given that root exudates are predominantly released at root apices and are rapidly mineralized, this trend is not consistent with the view that root exudates determine the overall structure of rhizosphere bacterial communities. There are a few examples, such as the Libyan desert grass *Aristida coerulescens*, where bacterial densities at the root apex are reported to be greater than those at the base (Naim, 1965), but environmental conditions in the upper horizons of a hot desert soil are likely to be extreme. Peak bacterial population density has also been reported at the base and the apex of wheat roots (Van Vuurde & Schippers, 1980); however, the authors suggested that this observation may reflect two sources of rhizodeposits: exudates, released predominantly at the root tip, and lysates associated with a loss of cell integrity that is more prevalent around the root base.

Numerous studies have demonstrated differences in the composition of bulk soil relative to rhizosphere bacterial communities (Marilley *et al.*, 1998; Marilley & Aragno, 1999), as well as differences between root zones (β diversity; Semenov *et al.*, 1999; Duineveld *et al.*, 2001; Marschner *et al.*, 2002). However, the number of species and their relative abundance within specific root locations (α diversity) are a particularly poorly understood aspect of rhizosphere microbial ecology. This is largely related to the fact that the abundance and distribution of rhizosphere microorganisms are heterogeneous and vary considerably with respect to position along longitudinal root axes (Chin-A-Woeng *et al.*, 1997; Dennis *et al.*, 2008). At the root base, bacterial communities have been observed to more or less cover the rhizoplane, but at root apices, they are present as clusters that occupy a relatively small proportion of the available root surface (Chin-A-Woeng *et al.*, 1997). Therefore, given that α diversity indices are known to be sensitive to differences in community size, and most methods do not facilitate concomitant measurements of community size and composition, the amount of sampling effort required to generate representative 'within apical region' estimates of bacterial diversity is likely to be much greater than within basal root regions. We hypothesize that the α diversity of bacterial communities at root apices will be similar to that of bulk soil communities because the root will have had little time to influence the local microbial community. Following this argument, the diversity of microbial communities associated with older root regions will be lower than that in bulk soil due to the long period of time that they have been exposed to, and influenced by the root relative to younger regions. This relationship was investigated on the roots of *B. napus* using a new microsampling technique (Dennis *et al.*, 2008), and although the results were not conclusive,

there was some indication that bacterial diversity follows the general trend: bulk soil > apical region > basal region (Fig. 3).

The rationale behind claims that root exudates are the key determinant of rhizosphere microbial community structure

Root exudates are known to contain compounds that can exert stimulatory and inhibitory influences on rhizosphere microbial community structure and composition (Hartmann *et al.*, 2009). Components of root exudates such as carbohydrates, organic acids, and amino acids have been demonstrated to stimulate positive chemotactic responses in bacteria (Somers *et al.*, 2004), and the capacity for different microbial species to utilize and compete for substrates is known to differ. In response to phosphorus, and to some extent iron deficiency, special root formations known as cluster or proteoid roots are formed by a range of plant species, such as the *Proteaceae*, *Casuarinaceae*, *Mimosaceae*, *Fabaceae*, *Myricaceae*, and *Moraceae* (Dinkelaker *et al.*, 1995). In *Lupinus albus*, young cluster roots (2–3 days) are characterized by high malate exudation (Neumann *et al.*, 1999). Once growth has ceased, cluster roots show an increase in the exudation of citrate for a few days, but then exudation decreases as the root feature senesces (Neumann *et al.*, 2000). Marschner *et al.* (2002) investigated the effect of differing exudation profiles on microbial communities associated with noncluster and cluster roots of differing ages in the rhizosphere of *L. albus* using DGGE analysis of 16S and 18S rRNA gene fragments. They observed strong differentiation between bacterial and fungal communities associated with different root classes and related these effects to specific root exudate components. Differences in – fungal community structure were attributed to citric acid and differences in – bacterial community structure were attributed to *cis*-aconitic, citric, and malic acid. In another study, Oger *et al.* (1997) demonstrated that genetically modified plants that produce opines had 80% more opine-utilizing bacteria in their rhizospheres when compared with non-opine-producing controls. They concluded that this was related to an increased rate of opine exudation from the roots of the modified plants.

In addition to these stimulatory substances, roots also exude a range of secondary metabolites that inhibit the growth of fungal and bacterial pathogens in response to inducers such as salicylic acids, jasmonic acids, and chitosans, which stimulate a defence response (Walker *et al.*, 2003). In their natural environment, it is likely that plant defence responses are constantly stimulated and differences in the resulting secondary metabolites such as saponins, glucosinilates, and hydroxamic acids may account for the resistance or the susceptibility of particular plant species/cultivars to root pathogens. Fungal pathogens that infect oats, tomato, and potato roots are resistant to the saponins (avenacin A-1, α-tomatine, and α-chaconine, respectively) that these species exude (Morrissey & Osbourn, 1999), but there is no evidence for the differential selection of rhizosphere bacteria. Similarly, soil bacteria are not affected by glucosinilates that degrade to toxic (iso)thiocyanates produced by Crucifer and Brassica species, which may deter herbivorous pests and inhibit some fungal root pathogens (Morrissey & Osbourn, 1999), although this may be controversial (Rumberger & Marschner, 2004). The bacterial root pathogen *Erwinia* remains unaffected by 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 (Niemeyer, 1988). Roots also secrete some antibacterial compounds such as rosemarinic acid and naphthoquinones (Walker *et al.*, 2003), and some plant species have been shown to produce compounds (unidentified) that interfere with bacterial quorum sensing: this is a prerequisite for the expression of many bacterial genes involved in both pathogenic and beneficial plant–microorganism interactions (Hentzer *et al.*, 2002; Bauer & Mathesius, 2004; Bais *et al.*, 2006; Degrassi *et al.*, 2007). In summary, there is strong evidence to indicate that components of root exudates exert a selective influence on rhizosphere microbial communities. However, most of these compounds can be found in other rhizosphere carbon pools, including those that derive from microorganisms.

Do root exudates exert a stronger influence than other rhizosphere carbon pools?

Assuming the mineralization rates of Ryan *et al.* (2001) for sugars and amino/organic acids, and an average root growth rate of 0.2–1.0 $\mu m\, s^{-1}$ (Foster, 1986), the concentration of exudates surrounding the root would be halved at a distance of just 0.4–7.2 mm behind the meristem, and at a distance of 2–47 mm, the concentration would decline to approximately 1% of that originally released. Within the area, where root exudates are relatively abundant, microbial community structure is likely to shift in favour of those species that compete most effectively for the available resources. However, as the quantity and composition of root exudates respond rapidly to very subtle environmental changes (Dilkes *et al.*, 2004), the selective pressure exerted on microbial communities is heterogeneous in space and time.

Once root-derived or other carbon substrates are assimilated by microorganisms, some of it is rapidly respired, a significant fraction is used for growth and/or maintenance, and some may be lost as exudates and polysaccharide mucilages from the microorganisms themselves. Very little is known about the quantity and composition of

rhizosphere microbial exudates; however, they have been implicated in many functions including quorum sensing, microbial attraction and suppression, and modulation of nutrient availability, root activity, and root architecture (Bais *et al.*, 2005). Any remaining carbon is likely to return to the rhizosphere following cell lysis and may fuel a subsequent wave of colonists. This boom and bust carbon economy may manifest itself as waves of bacteria initiated by exudates released by passing root apices (Zelenev *et al.*, 2005). However, as it is cycled through successive microbial communities, the composition of the available carbon compounds will change, making it increasingly unlikely that root exudates determine the structure of microbial communities in older root regions (Fig. 2). This is important because the majority of bacteria are associated with basal root regions. Inhibition or promotion of growth of microorganisms at the root base may be mediated by root lysates and mucilages and/or compounds released by microorganisms. However, root exudates could also influence microbial communities associated with older root regions upon their release from the apices of emerging lateral roots.

Another likely source of carbon substrates and other nutrients is SOM. De Nobili *et al.* (2001) demonstrated that when exposed to the types and concentrations of carbon compounds commonly found in rhizospheres, microorganisms accelerate SOM decomposition. However, when exposed to root exudates and more recalcitrant SOM, rhizosphere microorganisms preferentially utilize the relatively simple components of root exudates (Kuzyakov, 2002). Therefore, given that many of the components found in root exudates are also present in other rhizosphere carbon pools, SOM decomposition may be significant only in areas where other more labile substrates are depleted. Interestingly, Cheng *et al.* (1996) found that nitrogen, not carbon, limits rhizosphere bacterial respiration, which indicates that SOM mineralization could strongly influence rhizosphere microbial community structure (Helal & Sauerbeck, 1986; Cheng *et al.*, 1996).

It is not our intention to suggest that root exudates do not influence the structure of rhizosphere microbial communities. However, we believe that insufficient evidence is available to support claims that root exudates are the key determinant of rhizosphere microbial community structure. In light of evidence that strongly suggests that the availability of root exudates is limited to small spatiotemporal windows surrounding root apices, we hypothesize that rhizosphere microbial communities respond to other rhizosphere carbon pools for the majority of their coexistence with their plant host. This does not mean that the plant is not able to influence the inhabitants of its rhizosphere, but simply that other pools of rhizodeposits may have a similar, if not greater, overall influence than root exudates. Nonetheless, root exudates are likely to be of great importance in initiating the rhizosphere effect in very young seedlings and on emerging lateral roots. It is important to note that the studies that claim to have demonstrated the importance of root exudates in structuring rhizosphere microbial communities fail to consider the potential influence of other pools of rhizodeposits. For example, the study by Oger *et al.* (1997) demonstrated more opine-utilizing bacteria in the rhizospheres of plants engineered to produce more opines. We assume that the production of these opines was not isolated to apical root regions; therefore, it is likely that opines were also released by cell lysis in regions of the root that were not associated with rhizosphere carbon pools dominated by root exudates. However, the influence of other pools of rhizodeposits was not considered. We believe that a more accurate interpretation of such studies would be to suggest that observed microbial community responses are linked to differences in rhizodeposition rather than just root exudates. While we recognize the difficulties associated with differentiating between different pools of rhizodeposits, we think that this is an important distinction. In order to design effective rhizosphere management strategies, it is necessary to consider where microbial communities associated with different root regions obtain the majority of their resources, as many applications will require modifications to rhizosphere microbial community structure and composition at specific root locations.

Implications of and potential mechanisms to adjust microbial distributions for rhizosphere management

A key goal of rhizosphere microbial ecology is to facilitate the development of agricultural systems that deliver high levels of food security while reducing the environmental impacts associated with current food production systems. In part, realization of this technology is likely to be achieved by encouraging the proliferation of indigenous or introduced microorganisms that increase plant nutrient uptake, promote plant growth directly, or suppress plant pathogens. Irrespective of the strategy used to do so, consideration of the spatial and temporal aspects of rhizosphere processes such as nutrient uptake and pathogen infection is likely to be critical to the success of the management systems used. For example, in order to control a plant pathogen that infects at root apices, it is likely that the organisms that suppress the invasion will need to be present at the same location. Likewise, in order to maximize root uptake of a specific nutrient or heavy metal ion, mobilized by rhizosphere microorganisms, the relevant organisms should be associated with the area of the root that is most active in the uptake of that ion. Information regarding spatial trends in plant nutrient uptake is disparate, and so given the emphasis of this discussion, we briefly summarize the current

knowledge on this topic. Uptake of magnesium (Grunes *et al*., 1993), copper (Papeschi *et al*., 2000), cadmium (Pineros *et al*., 1998), and particularly calcium (Ryan *et al*., 1990) is characterized by maximal influx in the elongation zone, and the root apex appears to be the principal site of iron uptake (Clarkson & Sanderson, 1978). Evidence suggests that magnesium and calcium ions are taken up by an apoplastic route; therefore, their uptake is impeded in older root regions by the formation of suberin in the rhizodermal and endodermal cell layers, which effectively blocks apoplastic flow (Robards *et al*., 1973; Ferguson & Clarkson, 1975; Ferguson & Clarkson, 1976). For iron, however, greater uptake is thought to occur at root apices because greater quantities of compounds that are associated with its uptake are released there (Clarkson & Sanderson, 1978). In young roots (< 7 cm long), phosphate (Rubio *et al*., 2004), potassium (Marschner, 1995), and nitrate and ammonium (Henriksen *et al*., 1992; Colmer & Bloom, 1998; Taylor & Bloom, 1998) are reported to be taken up more uniformly along the root. Therefore, microbial mineralization of SOM in the rhizosphere is likely to benefit the plant by modulating the availability of these nutrients in regions that have been depleted by passing root apices.

Strategies that may facilitate improved control of the biomass and distribution of indigenous and introduced microorganisms can be broadly grouped into those that involve rhizodeposits and those that do not. Approaches that fall into the latter category include attempts to select for specific microbial groups by applying chemical amendments to soils (Devliegher *et al*., 1995), modifications to proteins in rhizodermal cell membranes involved in plant–microorganism signalling (Samaj *et al*., 1999), and changes to root growth and architecture. These strategies are largely beyond the scope of this review (see Ryan *et al*., 2009 for detailed information); however, some may be very effective. Dekkers *et al*. (2000), for example, demonstrated that introduction of an effective root tip colonizing bacterium, *Pseudomonas fluorescens* WCS365, into the rhizosphere of tomato suppressed the plant pathogenic fungus *Fusarium oxysporum* f.sp. *radicis-lycopersici*, which often infects at root apices. The biocontrol ability of WCS365 is thought to be partly related to *sss* (sodium solute transporter superfamily) genes that appear to be involved in its ability to effectively colonize root tips – the introduction of this gene into other *Pseudomonas* strains that were less efficient colonizers of root apices increased their abundance at these root regions by 8–40-fold (Dekkers *et al*., 2000).

Other divisions can be made between management strategies that aim to affect the supply of rhizodeposits to microorganisms directly and those that alter the response of microbial populations to these substances. Importantly, these approaches may or may not involve genetic engineering, which will currently influence the feasibility of their application in many parts of the world due to environmental legislation. Bacterial species that are effective at colonizing root tips can be selected for by repeatedly inoculating plants with microorganisms isolated from their root apices (Kuiper *et al*., 2001; De Weert *et al*., 2004). These 'root tip colonizers' can then be screened for traits that benefit plant growth and be reintroduced as they are, or with genetic modifications, into rhizospheres as plant growth-promoting rhizobacterial inoculants. These organisms are likely to contain genes that allow them to proliferate in response to exudates and resist the antimicrobial components that are often detected in exudates (Rumberger & Marschner, 2004). Interestingly, some bacteria have been demonstrated to block the release of antimicrobial compounds from roots using a mechanism based on the type III secretory system (Bais *et al*., 2005). However, in comparison with those involved in the production of antimicrobial compounds (Ryan *et al*., 2009), relatively little attention has been paid to potential strategies involving the introduction of genes that would increase the capacity for microorganisms to resist antimicrobial rhizodeposits or utilize xenobiotic compounds released by roots. In addition to the opine system that can be used to select for specific plant–microorganism interactions, another system has been proposed based on rhizopine (3-*O*-methyl-scyllo-inosamine)-producing plants. Rhizopine, which is found in nitrogen-fixing nodules and is toxic to many microorganisms (Murphy *et al*., 1987), can be degraded by *Sinorhizobium meliloti* (Saint *et al*., 1993). However, attempts to isolate and express rhizopine-degradation genes from *S. meliloti* in plants have failed to produce enhanced levels of rhizopine (McSpadden-Gardener & de Bruijn, 1998). This system is worth pursuing as it may facilitate the selection of plant-beneficial nitrogen-fixing organisms (Ryan *et al*., 2009).

From a plant perspective, favourable modifications to rhizodeposition are often selected for inadvertently by repeated crossing of high-performance crop lines. For example, crop breeders have unintentionally selected for wheat varieties that release malate to detoxify Al^{3+}, which is important in many acid soils (de Sousa, 1998). Conventional breeding techniques could therefore be used to develop rhizosphere management strategies, although perhaps a more direct approach involves genetic engineering. A promising strategy to modify rhizodeposition patterns is to produce plants with a greater capacity to *synthesize* certain compounds. Successful examples include increases in the release of citrate (de la Fuente *et al*., 1997; Koyama *et al*., 2000), malate (Yang *et al*., 2007), and opines (Oger *et al*., 1997) from roots. Another approach is to engineer the *release* of rhizodeposits. Currently, research on this topic is focused on 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 detected within the ALMT family encodes a protein that, unlike others within the family, is not dependent on Al^{3+} for the transport of malate (Kovermann *et al.*, 2007). MATE genes are found in a diverse range of organisms and are implicated in the transport of many compounds including citrate (Yokosho *et al.*, 2009), flavonoids (Debeaujon *et al.*, 2001), alkaloids, and antibiotics (Li *et al.*, 2002). There are 59 MATE orthologues in *A. thaliana* (Yazaki, 2005) and at least 40 in the rice (*Oryza sativa*) genome (Yokosho *et al.*, 2009), but only a fraction of these have been studied in any detail. There is also potential to increase the number of border/border-like cells released by roots (Knox *et al.*, 2007) and to alter the rate at which cells lyse by modifying genes involved in programmed cell death (Dickman *et al.*, 2001). Antiapoptosis genes of various origins have been successfully overexpressed in a range of plant species and generally result in phenotypes that exhibit greater resistance to abiotic and biotic stress (Dickman *et al.*, 2001; Qiao *et al.*, 2002; Awada *et al.*, 2003; Shabala *et al.*, 2007; Chu *et al.*, 2008). A range of apoptosis-inducing genes are also known (Chao & Korsmeyer, 1998) and may be expressed in plants.

It is clear that genetic engineering offers a range of opportunities to affect the supply of rhizodeposits to microorganisms. While increased synthesis of specific compounds within plants is likely to enhance their exudation from roots, their presence in lysates from damaged or senescing cells is likely to give them a more or less ubiquitous distribution in the rhizosphere. Increased synthesis of compounds may be useful, therefore, to select for specific microbial populations in regions of the rhizosphere that are depleted of root exudates or to deliver rhizodeposits (lysates) that are deleterious to microorganisms that infect wounds or natural openings on the rhizoplane. The influence of increasing transporter activity on the spatial distribution of root exudates is currently unknown. Presumably, increased exudation would be observed at root apices; however, the possibility of exudation occurring in other root regions should be investigated. If exudation is limited to root apices, then this strategy may not be effective in influencing microbial communities associated with basal root regions. Many exciting opportunities may exist to develop sophisticated management strategies that combine different genetic mechanisms. For example, if linked to a mechanism that recognized specific microorganisms, the expression of apoptosis-inducing genes in border/border-like cells could facilitate the preferential delivery of lysates to targeted organisms. Similarly, if linked to genes that are initiated in root cells associated with parts of the rhizosphere that are low in available rhizodeposits, coupled increases in the synthesis and exudation of certain compounds could increase the capacity for plants to determine their rhizosphere inhabitants.

Concluding remarks

The evidence presented in this review highlights that the direct influence of root exudates on rhizosphere microbial communities is likely to be limited to small spatiotemporal windows that surround root apices. Once rapidly assimilated by microorganisms, root-derived carbon is likely to be further modified, independent of plant influences, before rerelease into the rhizosphere by microorganisms themselves. Thus, at short distances from root apices, rhizosphere carbon pools are unlikely to be dominated by root exudates. However, many of the major compounds found in root exudates are likely to be present throughout the rhizosphere as they are found in other pools of rhizodeposits and in microbial exudates. Following this argument, we suggest that the importance of root exudates in structuring rhizosphere bacterial communities needs to be considered in the context of the wider contribution of other rhizosphere carbon pools. This is important because in order to design more effective rhizosphere management strategies, it is necessary to determine where microbial communities associated with different root regions obtain the majority of their resources, as many applications will require modifications to rhizosphere microbial community structure and composition at specific root locations. In this review, we have presented a wide range of approaches that offer exciting opportunities to modify the spatial and temporal distribution of rhizodeposits as well as the response of microorganisms to these substances. These strategies may facilitate the development of engineered rhizospheres in which the selective influence of plants on the composition and structure of their associated microbial communities is always dominant over influences beyond their control.

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