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Rhizodeposition and Its Impact on Microbial Community Structure and Function in Trees

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S u m m a r y

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Rhizodeposition is a key process influencing nutrient availability through its stimulation of microbial activity in the rhizosphere. Microbial community structure and function in the rhizosphere has been hypothesised to be a consequence of variation in the quantity and quality of rhizodeposition. This paper discusses the character of carbon loss from trees and describes the use of community level physiological profiles (CLPP) to characterise microbial communities from the rhizosphere and rhizoplane of hybrid larch (*Larix eurolepis*), Sitka spruce (*Picea sitchensis*), sycamore (*Acer pseudoplatanus*) and non- rhizosphere (bulk) samples from a farm woodland site. Canonical variate analysis (CVA) was used to analyse the carbon utilisation data and discriminate treatment effects. CVA discriminated the rhizosphere and rhizoplane microbial communities from the bulk soil. The carbon profiles of the rhizosphere microbial communities from the three tree species were similar, but there was significant discrimination of the communities from the three tree rhizoplanes. These differences were mainly attributable to differences in carboxylic acid utilisation. Isolation and enumeration of culturable microorganisms from these soils confirmed the stimulatory effect of the rhizosphere on microbial growth, and in particular on pseudomonad proliferation, and that there are qualitative and quantitative differences in microbial communities associated with these tree species. The possible relationship between microbial diversity and carbon availability in the rhizosphere of different tree species is discussed.

I n t r o d u c t i o n

Trees are increasingly being grown in a wide range of land use systems. However, the sustainable production of timber from these systems will require a greater understanding of factors influencing nutrient availability and uptake in trees (MAHENDRAPPA & al. 1986, MILLARD 1996). Soil microorganisms have a key role

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in regulating the availability of plant nutrients through the mineralisation of soil organic matter and the solubilisation of soil minerals (LEE & PANKHURST 1992). Microbial growth in soil is carbon limited; carbon enters the soil from plants as litter material, dead roots and rhizodeposition. Litter and dead roots, are most important for the organic matter supplied to the soil and its turnover. Rhizodeposition describes the carbon flux entering the soil from plant roots and is composed of several groups, distinguished by their mode of release, exudates, secretions, lysates, gases and mucilage. However, it is the presence of readily available carbon substrates present in rhizodeposition as root exudates, which are considered to have the greatest stimulatory effect on microbial growth and activity. Readily degradable compounds such as amino acids and monosaccharides found in root exudates have been postulated to be part of a fast pool of carbon which is rapidly utilised and cycled through the microbial cells, grazers and plants 8-10 times a year (COLEMAN & al. 1983). More recalcitrant compounds, the bulk of soil organic matter, including humic substances, has been estimated to be cycled through the microbial-plant pathway once every 10-100 years (COLEMAN & al. 1983). The active micro- and mesofauna operate in the fast organic pool and it is the cycling of nutrients within this pool, which is most relevant to plant growth (LEE & PANKHURST 1992).

Estimates of the carbon assimilated by plants, which is then released into the soil through root turnover and rhizodeposition vary considerably, with values of up to 40% being frequently quoted (VAN VEEN & al. 1991). Perennials, like trees, release more of their fixed carbon than annuals with as much as 73% of the C fixed by *Pseudotsuga menziesii* being released belowground (FOGEL & HUNT 1983). The amount of carbon lost as root exudates has been reported to be a small percentage of the total C released by some workers (1.7 %, LAMBERS 1987), but a considerable proportion by others (10 %, JOHANSSON 1992, JONES & DARRAH 1995). The variation in estimates has resulted because of the different methodologies used to measure root exudation and the bias in the interpretation of the results. A wide variety of compounds have been shown to be present in root exudates, the dominant components being sugars, amino acids and organic acids (KRAFFCZYK & al. 1984, SUNDIN & al. 1990). However, the numerous studies on herbaceous root exudation contrast with paucity of data on tree root exudates. A recent review of the data in this area showed that the compounds present in tree root exudates include carbohydrates, amino acids, organic acids, fatty acids, phenolic acids, vitamins, volatiles and growth factors (GRAYSTON & al. 1996).

Root exudation can be influenced by many factors both environmental (culture conditions, nutrient and water availability, pH, temperature, light, CO_2 concentration and presence of microorganisms) and plant (species and age) derived and these have been reviewed by GRAYSTON & al. 1996. It has been hypothesised that variation in root exudate patterns may select for a specific microbial community in the rhizosphere. Soil microorganisms vary in their functional potential (e.g. N and P transformations, pathogen suppression) and therefore the community composition will have important consequences for tree growth (GRAYSTON & al. 1996). However, there is little knowledge on the diversity of the microbial community in tree rhizospheres (LINDERMAN 1988, GRAYSTON & al. 1996).

Community level physiological profiles (CLPP), constructed using Biolog microplates, have shown their potential as an ecologically relevant method to characterise microbial communities from the rhizosphere of different plant species (GARLAND 1996, GRAYSTON & al. 1998) and a range of habitats, including forests (GRAYSTON & CAMPBELL 1996, PRIHA & al. 1999). The technique measures utilisation of a variety of carbon compounds and is therefore a meaningful assay of communities because carbon is a major factor governing microbial growth in soil (WARDLE 1992).

The aim of this study was to determine microbial community structure and function in the rhizosphere and on the rhizoplane of three tree species, using selective plating techniques and CLPP. The hypothesis to be tested was that microbial communities from the rhizoplane and rhizosphere of the three tree species will be different, as a consequence of variation in rhizodeposition between the different zones and tree species, and this will be reflected in a difference in CLPP between these communities.

Materials and Methods

Site

Soil and root samples were collected from three replicate stands of hybrid larch (*Larix eurolepis*), Sitka spruce (*Picea sitchensis*) and sycamore (*Acer pseudoplatanus*) at a farm woodland site at Lower Affleck in north-eastern Scotland (57°18'20"N/2°13'33"W) The trees were six years old and had been planted three years previously at a density of 4,000 stems ha^{-1}. Triplicate cores (5 cm diam.) were taken to a depth of 15 cm from each of three tree species within each stand and bulked for each tree. In addition, bulk, non-rhizosphere soil samples were also taken from unplanted areas of the woodland.

Microbial analysis

Rhizosphere microbial communities were extracted by shaking 10 g of rhizosphere soil (soil adhering to roots) in 100 cm^3 of ¼ strength Ringers solution (Oxoid) for 10 min, on a wrist action shaker. Rhizoplane communities were then extracted by shaking 5g of the washed roots in 50 cm^3 of Ringers solution containing 20 g glass beads for 10 min. After ten-fold serial dilution in Ringers, suspensions (0.1 cm^3) were spread in duplicate on the following media. Tryptone Soy agar (1/10 strength, Oxoid) plus cycloheximide (50 mg dm^{-3}) to enumerate bacteria, *Pseudomonas* isolation agar (Oxoid) selective for pseudomonads and Czapek-Dox agar (Oxoid) plus ampicillin (10 mg dm^{-3}), streptomycin and tetracycline (50 mg dm^{-3}) for enumeration of fungi. Plates were incubated at 25 °C and colonies counted weekly until no new growth appeared. An ANOVA was used to determine statistically significant treatment differences after log transformation of the count data. Isolates from the *Pseudomonas* isolation agar were identified using the Biolog system (BIOLOG 1993). Microlog software Biolog Inc.) was used to identify cultures based on their metabolic profiles.

Biolog GN microplates (Biolog Inc., Hayward, CA, USA), which contain 95 different carbon sources, a control well with no carbon source and a redox indicator dye (tetrazolium violet) (BIOLOG 1993) were used to construct community level physiological profiles (CLPP) of the rhizosphere and rhizoplane microbial communities. Direct microscopic counts of microorganisms in the samples were made using epifluorescence microscopy after staining with acridine orange. A 50 cm^3 aliquot of each rhizosphere and rhizoplane sample diluted appropriately in Ringers solution to give an inoculum density of 10^4 organisms cm^{-3} was centrifuged at 2000 rpm for 10 min to separate soil and minimise addition of soil or root derived carbon into the system. A 0.15 cm^3 aliquot of each sample was dispensed into each well of the GN plate and the plates incubated at 15°C for 5 days and

colour development (carbon utilisation) measured as absorbance at 590 nm every 24 h using a microplate reader (Vmax, Molecular Devices, Oxford, UK). The data was analysed by canonical variate analysis (CVA) (Genstat 5.3, NAG Ltd., Oxford, UK) to differentiate samples based on their overall patterns of carbon utilisation and to identify which carbon sources were most responsible for the discrimination.

Results

All three rhizosphere samples contained significantly higher populations of bacteria, including pseudomonads, than the bulk soil (Fig. 1). Quantitatively, there were no significant differences in the bacteria or fungi found in the rhizosphere or on the rhizoplane of the different tree species, but the larch and sycamore rhizosphere samples contained higher numbers of bacteria and fungi than the Sitka spruce rhizosphere. In addition, the larch rhizosphere and rhizoplane samples contained more pseudomonads than both the sycamore and Sitka spruce rhizosphere and rhizoplanes (Fig. 1). However, identification of the bacterial species isolated on the *Pseudomonas* isolation agar, using Biolog GN plates, indicated that there were fewer species of pseudomonads associated with larch than with the other tree species and approximately 70% were non-fluorescent species (e.g. *Burkholdaria cepacia*) (Fig. 2). The pseudomonads associated with sycamore and Sitka spruce were an equal mixture of fluorescent (e.g. *P. fluorescens C, P. fluorescens G, P. putida, P. corrugata*) and non-fluorescent species. Of the total prokaryote population, actinomycetes dominated in all soils except sycamore (55-60 % total prokaryotes). However, in the rhizoplane samples bacteria were more dominant, accounting for 53-86% of the total prokaryote population.

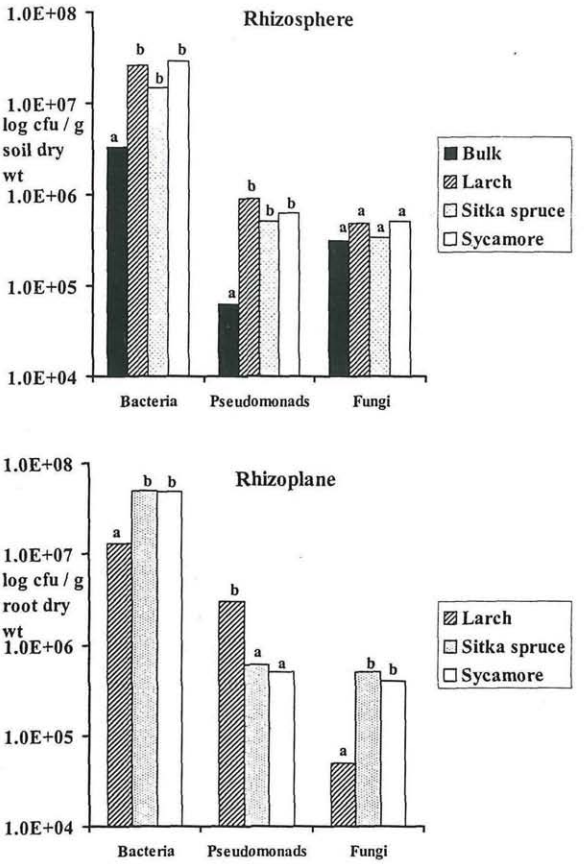

Fig. 1. Populations of bacteria, pseudomonads and fungi in the rhizosphere and on the rhizoplane of hybrid larch, Sitka spruce and sycamore. (Bars with the same letter are not significantly different $P < 0.05$).

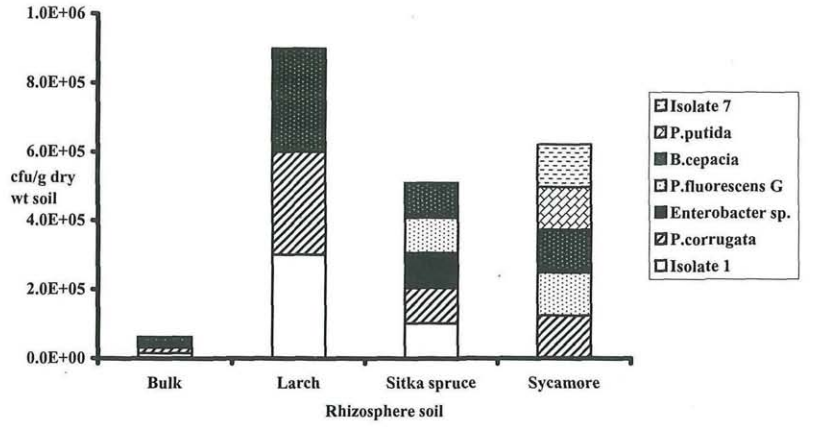

Fig. 2. Species of pseudomonads found in the rhizosphere of hybrid larch, Sitka spruce, sycamore and bulk soil taken from a farm woodland.

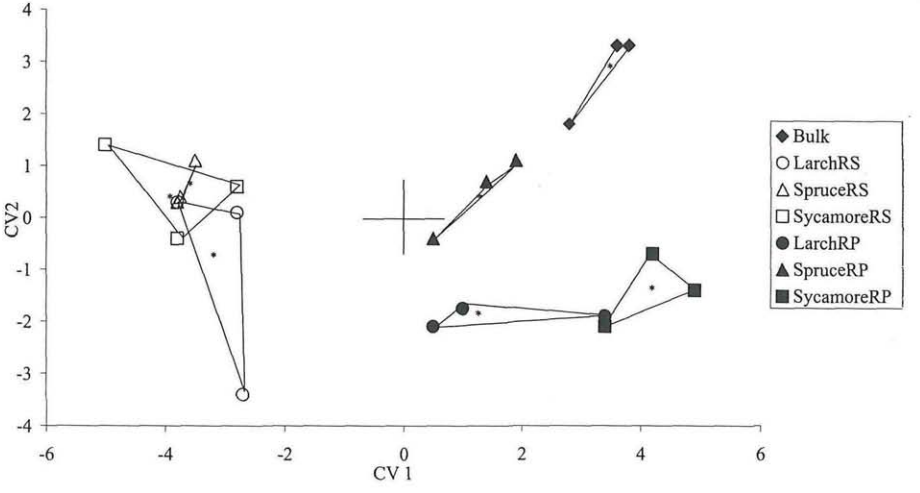

Fig. 3. Canonical variate scores for the first two ordination axes for microbial communities from the rhizosphere (RS) and rhizoplane (RP) of hybrid larch, Sitka spruce and sycamore and bulk soil from a farm woodland. Symbols represent different samples. * indicates mean score for each treatment.

Canonical variate analysis (CVA) was performed on the CLPP data from each incubation time to identify patterns of response among samples. The results presented are those after 72 h of incubation because this gave the best discrimination amongst treatments. CVA clearly differentiated the rhizosphere samples from the bulk soil on canonical variate (CV) 1 and the bulk soil from the rhizoplane samples on CV2 (Fig. 3). Differences between the microbial communities from the different tree species were observed within the rhizoplane samples, of which Sitka

spruce was the most distinct (Fig. 3). Analysis of the loadings of the carbon sources on these canonical variates indicated that the differences between the rhizosphere, bulk soil and the rhizoplane samples were mainly attributed to different utilisation of carboxylic acids. Utilisation of amino acids and sugars were very similar across all samples. No significant differences between the microbial communities from the three tree species were apparent within the rhizosphere samples (Fig. 3).

Discussion

The results form a preliminary study of the microorganisms associated with different tree species. They show clearly that the rhizosphere exerts a significant stimulatory effect on the populations of bacteria in soil and particularly the pseudomonads. Pseudomonads are a nutritionally diverse group of bacteria (BOWEN 1980) and have a higher growth rate in soil than other species and there is increasing evidence of their selective stimulation in the rhizosphere of a range of plant species (ALEXANDER 1977, GRAYSTON & al. 1998), including trees (GRAYSTON & CAMPBELL 1996, PRIHA & al. 1999). The results also suggest that the different tree species select for, and stimulate the growth of different microbial species. As well as quantitative differences in the microbial populations associated with the three tree species there were qualitative differences, in particular there were fewer species and fewer fluorescent pseudomonads found in the rhizosphere and on the rhizoplane of hybrid larch, than Sitka spruce and sycamore. There have been relatively few studies on microbial communities in the rhizosphere of trees (FITTER & GARBAYE 1994). GRAYSTON & CAMPBELL 1996, in a comparison of hybrid larch and Sitka spruce at three forest sites, showed that the rhizosphere of hybrid larch contained fewer fluorescent pseudomonads than Sitka spruce at all sites. MALAJCZUK & MCCOMB 1979 found that the populations of rhizosphere microflora and in particular fluorescent pseudomonads were more numerous in the rhizosphere of *Eucalyptus marginata* than *E. callophylla*. They postulated that the differences in both numbers and types of organisms were due to differences in root exudates produced by the two species, in particular malonic acid was produced by *E. marginata*, but not by *E. callophylla* (MALAJCZUK & MCCOMB 1977). The results from this study suggest that hybrid larch, Sitka spruce and sycamore do produce different exudates because the carbon substrates used for growth by the microorganisms from the different tree rhizoplanes varied. However, as this study was conducted at only one time point, further research will be needed to confirm the differences are consistent over time, and how they vary over the season. Changes in CLPP were mainly due to differences in carboxylic acid usage suggesting that the trees may differ in the carboxylic acids they produce which then selects for microorganisms capable of utilising these substrates. At present nothing is known about the exudates produced by these tree species. However, of the limited amount of data on tree root exudates it is known that the quantity and quality of carbon released varies considerably between species (GRAYSTON & al. 1996). Organic acids have been shown to be quantitatively the most dominant compounds released in

tree root exudates (SMITH 1976) and a wide spectrum of these compounds are produced by different trees (GRAYSTON & al. 1996). For example, pines produce aconitic and malic acid, which are not produced by sugar maple and the latter produce malonic acid, which tends not to be found in root exudates from evergreens (GRAYSTON & al. 1996). It is likely that these three tree species will vary in the quality and quantity of carbon released in their exudates due to the fact that sycamore is a deciduous broadleaf, larch, a deciduous conifer and Sitka spruce, an evergreen conifer. LEYVAL & BERTHELIN 1993 showed that pine and beech trees differed, both qualitatively and quantitatively, in the carboxylic acids they produced and these acids were not detected in the exudates after dual inoculation of the trees with a mycorrhizal fungus and an *Agrobacterium* species. This suggests that the fungus and/or bacterium may have been using these carboxylic acids as carbon sources.

In this study the rhizoplane communities had different carbon profiles, and more distinct profiles between tree species than the rhizosphere communities. Differences in rhizosphere and rhizoplane carbon sources are a result of the rhizosphere containing microbial and plant debris e.g. starch, cellulose (ROVIRA & al. 1979) and exudates modified by other microorganisms, whereas the rhizoplane would be more likely to contain unmodified exudates. A higher proportion of the prokaryote community consisted of actinomycetes in the rhizosphere than in the rhizoplane samples. Actinomycetes are slower growing than bacteria and tend to grow on polymers such as lignocellulose, starch and chitin in soil, whereas bacteria prefer simple, readily utilisable carbon sources.

Another factor that could have influenced exudation patterns and hence the microbial communities associated with the different tree species is the presence of different mycorrhizal symbionts (MOLINA & al. 1992). For example, deciduous broadleaf's such as sycamore have endomycorrhizal as well as ectomycorrhizal associates (JANOS 1980). Species of the ectomycorrhizal fungus *Suillus* are host specific and associate with larch but not spruce (MOLINA & TRAPPE 1982). Mycorrhizae differ in their carbon storage patterns and their conversion of plant sugars to metabolic intermediates (FINLAY & SODERSTROM 1992) and these differences could result in changes in the character and quantity of carbon released from tree roots.

This preliminary study has demonstrated the potential of the use of CLPP to rapidly discriminate microbial communities from different tree species based on their metabolic profiles. Further temporal sampling is required to verify that the differences in soil microbial communities between tree species are consistent over time and the degree of seasonal variation. The differences in utilisation of carbon sources by the microbial communities from the different tree species and zones can only have been due to the presence of different microbial species, as indicated using plate counts. The diversity of microorganisms associated with the different tree species may have arisen due to the variation in carbon compounds exuded by the trees and their modification by mycorrhizal symbionts. Evidence from the literature suggests that these trees may produce different exudation patterns. However, in order to link utilisation rates with carbon availability in the rhizosphere further

studies on root exudation by these tree species are needed. In addition, the identification and inclusion of other rhizosphere carbon sources in these CLPP will enable development of forest ecosystem specific plates, improving the discrimination and relevance of the technique. The beneficial effects of rhizosphere microbial communities on tree growth highlight the need for greater understanding of microbial community structure and function in the rhizosphere.

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