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SELECTIVE INFLUENCE OF PLANT SPECIES ON MICROBIAL DIVERSITY IN THE RHIZOSPHERE

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Summary—Wheat, ryegrass, bentgrass and clover were grown for 4 wk in two different soil types. In addition, samples of these soils were amended with sucrose to mimic carbon inputs into the soil. At the end of the experiment rhizosphere microbial communities were extracted. The Biolog[R] system was used to construct sole carbon source utilisation profiles of these communities. Canonical variate analysis (CVA) was used to analyse the colour production (OD_{590}) data and to discriminate treatment effects. CVA did not differentiate between microbial communities from the two different soil types. It did, however, produce significant clustering of microbial communities from the different plant species. There was clear discrimination between the carbon sources utilised by microbial communities from the different plant rhizospheres. Carbohydrates, carboxylic acids and amino acids were the substrates mainly responsible for this discrimination suggesting plants may differ in the exudation of these compounds. Isolation and enumeration of culturable organisms from these soils confirmed the stimulatory effect of the rhizosphere on microbial growth and in particular pseudomonad proliferation. The number of pseudomonads, but not total bacterial numbers, present in the samples and inoculated into the Biolog plate was significantly correlated ($r^2 = 0.63$, $P < 0.01$) to colour formation in the wells, indicating that the Biolog system may reflect *Pseudomonas* carbon utilisation preferences. In order to further assess the significance of the technique the results need to be considered in conjunction with knowledge regarding actual carbon sources present in the rhizosphere. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Soil microorganisms play an important role in nutrient acquisition for plants. With the increasing emphasis on environmentally friendly, low input agricultural practices there is growing interest in the management of soil microbial communities to enhance plant growth. However, in order to maximise the beneficial effects of microbial activity we need a greater understanding of the factors influencing microbial diversity and activity. The abundance and activities of soil microorganisms are influenced by various environmental (e.g. soil type, nutrient status, pH, moisture) as well as plant factors (e.g. species, age). However, microbial growth in soil is carbon limited and, therefore, the presence of organic matter has the greatest influence on microbial populations (Lynch and Whipps, 1990; Wardle, 1992). *Pseudomonas*, *Flavobacterium*, *Alcaligenes* and *Agrobacterium* species have been shown to be particularly stimulated in the rhizosphere due to the release of exudates and lysates (Alexander, 1977; Curl and Truelove, 1986). The variety of organic compounds released by plants has been postulated to be a key factor influencing the diversity of microorganisms in the rhizospheres of different plant species (Rovira, 1965; Trolldenier, 1967; Bowen and Rovira, 1991; Bolton *et al.*, 1992). However, there has been no direct evidence to support this hypothesis. Many previous biodiversity studies have involved studies on cultured microorganisms, isolated using different selective media. New molecular techniques, such as 16S rRNA sequence analysis, DNA melting profiles, reassociation kinetics and DNA-to-DNA hybridisation are replacing these techniques. However, these techniques do not give any indication of functional diversity of microbial communities.

The Biolog[R] system, uses 95 different carbon sources to produce a metabolic profile of microorganisms (Biolog, 1993). It has been used to assess the metabolic diversity of microbial communities from diverse ranges of habitat (Garland and Mills, 1991), soil types (Winding, 1994), grasslands (Zak *et al.*, 1994) and tree species (Grayston and Campbell, 1996) and to determine changes in microbial metabolic functioning with heavy metal pollution (Campbell *et al.*, 1995). However, the community profile does not include fungi because of their slow growth rate (Garland and Mills, 1991). Garland (1996a) has described analytical

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approaches to community characterisation using Biolog and its use to determine temporal shifts in utilisation and to discriminate between communities from different plant species which had been grown hydroponically. Multivariate analysis has been used in all these studies to classify communities based on their metabolic profile. The utilisation of carbon, which is a key factor governing microbial growth in soil, makes the technique ecologically relevant.

Our objective was to determine the metabolic diversity of microbial communities from a range of plant species grown in different soil types. The microorganisms present in these rhizospheres were also isolated using selective plating techniques to determine quantitative differences in culturable populations. The hypothesis to be tested was that microbial communities in the rhizosphere of different plants produce characteristic metabolic profiles which override differences in soil type, and these characteristics can be used as a measure of both metabolic diversity and an indicator of carbon availability in the rhizosphere.

MATERIALS AND METHODS

Soil was collected from fields of permanent grassland at two sites in northeast Scotland. The first site at Craibstone (NJ 865105, Countesswells Association) was a dystic Cambisol (pH 4.65; 4.63% C; 0.34% N). The second site at Lower Affleck (NJ 864239, Tarves Association) was a eutric gleysol (pH 5.65; 2.20% C; 0.15% N). The soils were sieved to < 6 mm and kept at 4°C over night prior to use.

Samples of each soil (250 g) were placed in 7 cm pots and the moisture content adjusted to 70% field capacity. Seed (200 mg) of each of the following plant species was sown into each of three replicate pots of each soil type: *Lolium perenne*, *Agrostis capillaris*, *Triticum aestivum* and *Trifolium repens*. Three replicates of each soil type were also included, to which 10 ml of a solution of sucrose (1 mg in 10 ml) was added three times weekly, to mimic the addition of carbon from the plant roots (Newman, 1985). Sucrose was chosen because it is frequently cited as a root exudate of many plant species and carbohydrates are one of the major carbon and energy sources for microbial growth in the rhizosphere (Foster and Bowen, 1982). Three unamended replicate pots of each soil type were also included as controls. The plants were grown in the greenhouse during June 1995 for 4 wk (mean temperature 23.6 ± 3.4°C day, 14.5 ± 1.9°C night; 16 h day length). The moisture content of each pot was maintained at 70% WHC by weighing the pots three times per week and adding water as necessary.

Carbon utilisation profiles

At harvest the soil was removed from the pot. Rhizosphere soil was collected by shaking roots by hand to remove the adhering soil. The soil (10 g) was then shaken in 100 ml one-quarter strength Ringers solution (Oxoid) for 10 min to extract rhizosphere microorganisms. In the control and sucrose treated soils, the soil was thoroughly mixed before sampling. Biolog GN microplates (Biolog Inc, Hayward, CA, U.S.A.) were used to determine the metabolic diversity of the microbial communities associated with the different plant species. These 96 well microtitre plates contain 95 different carbon sources and a control well with no carbon (Biolog, 1993). In addition to nutrients in dehydrated form each well contains tetrazolium violet as a redox indicator dye (Bochner, 1989). The 10^{-4} dilution (50 ml) of each rhizosphere sample was spun at 750 *g* for 10 min to separate soil and to minimise the addition of soil or root derived carbon into the system. A 150 μl aliquot of each sample was dispersed into each well of the Biolog GN plates. Plates were incubated at 15°C and colour formation in the wells measured every 24 h, for 96 h using an optical density (OD_{590} nm) microplate reader.

Earlier studies showed correlation between inoculum cell density and the rate of colour development (Garland and Mills, 1991; Haack *et al.*, 1995). This meant samples of different cell density could appear dissimilar due to variation in rate of C utilisation irrespective of whether there were actual differences in the pattern of C utilisation. However, Garland (1996a) has demonstrated that there is still a 10–20% variation in the average well colour development (AWCD) in Biolog plates inoculated with identical inoculum densities. In order to eliminate the variation in AWCD, which may arise from different cell densities in the different samples Garland (1996a) recommended comparison of samples of equivalent AWCD. The AWCD of each plate was calculated for each 24-h period, as the mean of the optical density values for all 95 wells minus the blank well. The AWCD of different carbon substrate groups [e.g. carbohydrates, carboxylic acids, amino acids, amides, miscellaneous (aromatic and phosphorylated chemicals and alcohols) and polymers] was also calculated (Zak *et al.*, 1994) and treatment effects assessed by a three-way ANOVA using Genstat 5.3 (NAG Ltd, Oxford, U.K.). An average well colour development of 0.2 absorbance units was used as a reference point for analysis (Garland, 1996a) (i.e. 48-h plate readings from the wheat, ryegrass and clover samples; 72-h readings from the bentgrass and sucrose samples and 96-h readings from the unamended samples were compared). The data was analysed using canonical variate analysis (CVA), a form of multivariate analysis, which minimises within group (replicate) variation. CVA (Genstat 5.3, NAG Ltd, Oxford, U.K.) differ-

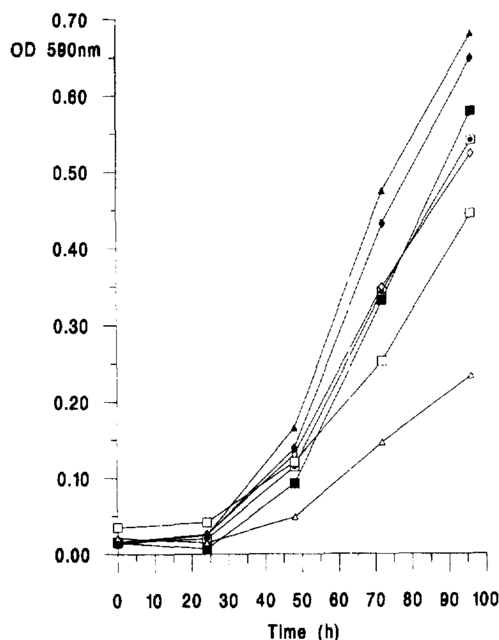

Fig. 1. Average well colour development profiles (OD_{590}) for all samples for each of the six groups of carbon substrates in the Biolog GN plate. □, All substrates; ■, carbohydrates; ◇, carboxylic acids; ◆, amino acids; △, amides; ▲, miscellaneous; □, polymers. Values are means of 36 replicate samples.

entiated samples based on their overall patterns of C utilisation and by referring to ordination coordinate loadings, identifying which carbon sources were most responsible for the discrimination.

Plate counts of microbial populations

The same rhizosphere community samples used in the carbon utilisation profiles were serially diluted and suspensions (100 μl) spread in duplicate on to the following selective media: Tryptone Soy agar (one-tenth strength, Oxoid) plus cycloheximide (50 mg l^{-1}) for enumeration of bacteria and actinomycetes; *Pseudomonas* isolation agar (Oxoid) selective for populations of pseudomonads; Rose Bengal agar (Oxoid) for enumeration of yeasts and fungi. The plates were incubated at 25°C and colonies counted after 4 d on the *Pseudomonas* and Rose Bengal agar and after 14 d on the Tryptone Soy agar. ANOVA was used to determine statistically significant treatment differences according to the *F*-test and the LSD for 95% confidence interval ($LSD_{0.05}$) was used in multiple comparisons. Morphologically different isolates from the *Pseudomonas* agar were cultured and identified using the Biolog® system (Biolog, 1993). MicroLog software (Biolog Inc, Hayward, CA, U.S.A.) was used to identify cultures based on their metabolic profiles.

RESULTS

Colour formation in the Biolog wells generally followed the same sigmoidal pattern with incubation times, but the rate varied for different groups of carbon compounds (Fig. 1). The fastest rates of utilisation over 96 h for different types of C sources was in the order: miscellaneous, amino acids, carbohydrates, carboxylic acids, polymers and finally amides. The carbon utilisation profiles of the microbial communities from the rhizosphere of the different plant species from the two soil types were similar (data not shown). The microbial communities from wheat, ryegrass and clover rhizospheres had significantly ($P < 0.05$) greater utilisation of all six groups of compounds than those from bentgrass and sucrose amended and unamended (control) soil (Fig. 2). In addition to greater total utilisation of the six groups of carbon sources by the microbial communities from the rhizospheres of wheat, clover and ryegrass, there was a much faster rate of utilisation of carbon over the 96-h period (Fig. 2). Addition of sucrose to soil resulted in higher utilisation rates of most carbon sources than in control soil. In particular the rates of utilisation of sucrose and its hydrolysis products glucose and fructose were 6, 3 and 12 times faster, respectively, in the sucrose amended Countesswells soil than in control soil (data not shown). However, in Tarves soil sucrose additions repressed fructose utilisation altogether, but increased glucose and sucrose utilisation rates 3-fold, suggesting catabolic repression (data not shown).

Multivariate analysis was performed on the OD_{590} readings from plates of equivalent AWCD (0.2 OD_{590}) (i.e. wheat, ryegrass, clover—48-h readings; bentgrass, sucrose amended—72-h readings; unamended—96-h readings). This enabled visualisation of relationships between the different samples. Principal component analysis, which is a method of multivariate analysis frequently used to examine this type of data (Garland and Mills, 1991; Garland and Mills, 1994) was found not to give good separation of samples on the first principal components (data not shown). However, CVA of the equivalent colour response data gave good differentiation of the samples from different plant species, indicating that they had different patterns of carbon utilisation (Fig. 3). Using individual treatment values (soil plus plant) there was found to be clear discrimination between samples from different plant rhizospheres but not soil type (data not shown).

The data presented in Fig. 3 show that there was clear discrimination between samples from rhizosphere of wheat, which had lower coordinate values on canonical variate (CV) 1 (which explained 38.9% of the variance in the data), when compared with the other samples. On CV2 (explained 28.1% data variance) samples from the bentgrass and ryegrass rhizospheres were distinct, having lower coor-

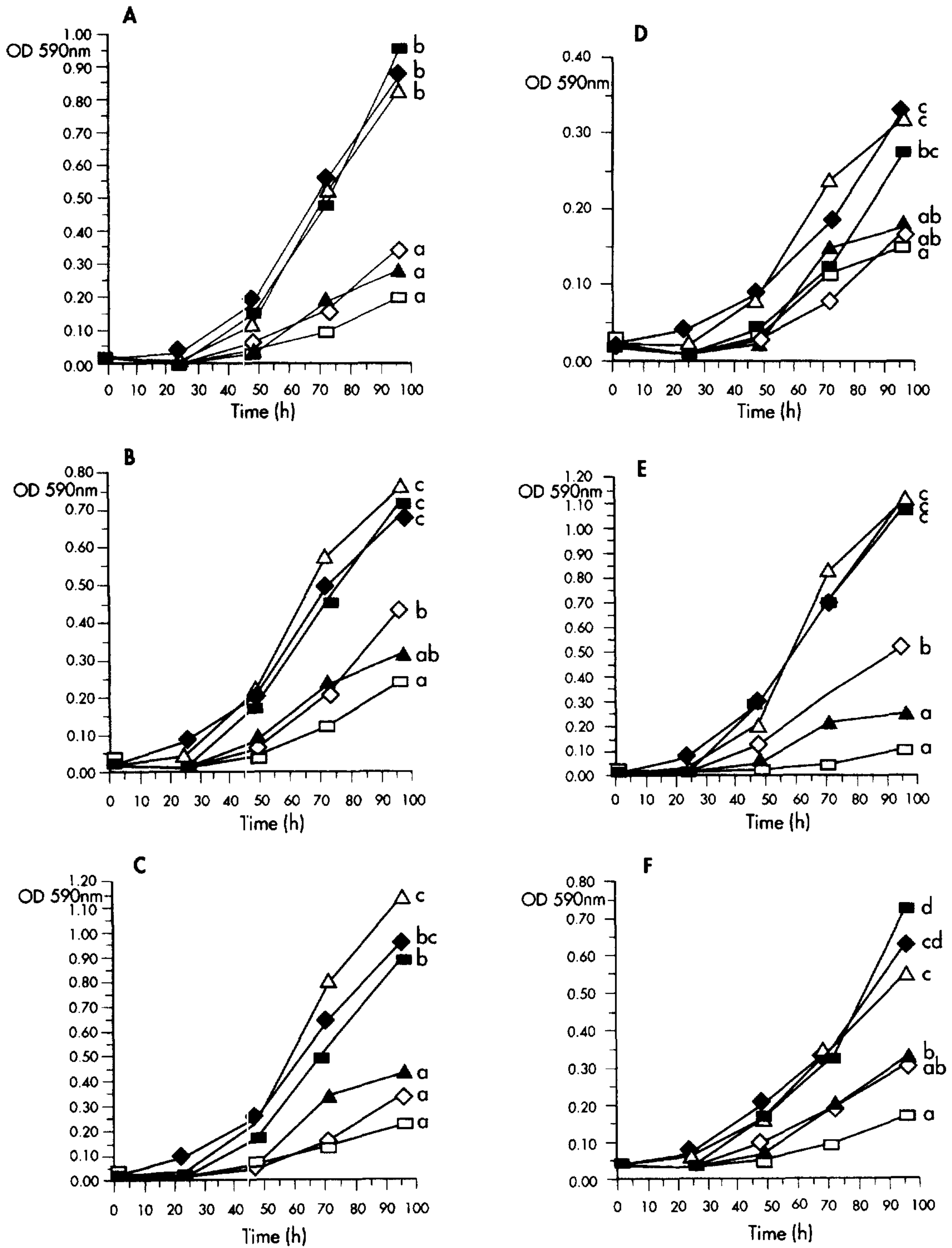

Fig. 2. Average well colour development profiles for samples from ryegrass ■, bentgrass ◇, wheat ◆, clover rhizospheres △ and sucrose amended ▲ and unamended □ soils for (A) carbohydrates, (B) carboxylic acids, (C) amino acids, (D) amides, (E) miscellaneous and (F) polymers. Values are means of six replicate samples. Values with the same letter are not significantly different $P < 0.05$).

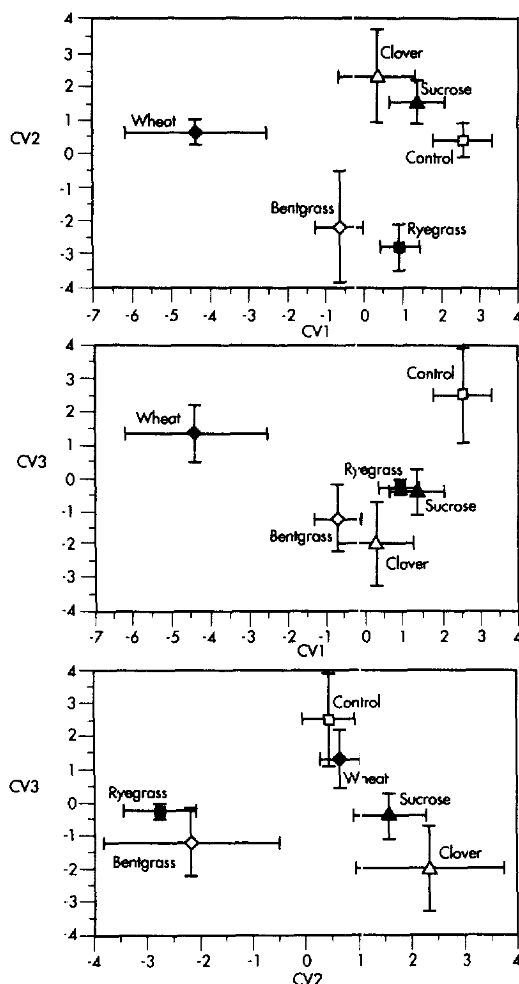

Fig. 3. Canonical variate scores for the first three ordination axes of rhizosphere samples of ryegrass, bentgrass, wheat, clover, sucrose amended and unamended soil. Values are means of six replicate samples plus SD for each crop type.

dinate values on this axis. Samples from the unamended (control) soils were distinct on CV3 (variance 18.7%) having higher coordinate values on this axis than other samples. Correlation and analysis of the loadings of the original carbon sources on CV1 indicated that the microbial communities from the rhizosphere of wheat had higher utilisation of a number of carbohydrates (L-rhamnose, α-D-glucose), amino acids (D-serine and L-asparagine) and glycogen, than the communities from the other rhizosphere soils (carbon sources negatively correlated to the first ordination axes) (Table 1). The carbon sources positively correlated with the first ordination axes were utilised to a lesser degree by communities from the wheat rhizosphere (Table 1). On CV2 the distinction of the microbial communities from the bentgrass and ryegrass rhizospheres was due to greater utilisation of glucose-1-phosphate and D-psicose relative to other rhizosphere and non-rhizosphere communities (negative values) (Table 1). Communities from these two grass rhizospheres had lower utilisation of the same compounds compared with the wheat rhizosphere (positive score CV2, negative score CV1), in addition to lower utilisation of other carboxylic acids, L-serine and D-trehalose (Table 1). Microbial communities from the unamended (control) soils had greater utilisation of L-leucine (positive values on CV3) than communities from other soils (Table 1).

Microbial populations

Plate counts of the culturable populations of bacteria and fungi from each of the rhizosphere samples showed that the wheat rhizosphere contained significantly ($P < 0.05$) higher populations of bacteria than all other samples, except for the clover rhizosphere (Fig. 4). In addition, the wheat rhizosphere samples contained the highest fungal populations (Fig. 4). There were significantly higher populations of bacteria in the clover rhizosphere than in unamended soil (Fig. 4). All four plant rhizosphere soils contained significantly ($P < 0.05$) greater populations of pseudomonads than either sucrose-amended or unamended soils. The sucrose-amended soils also contained significantly higher populations of pseudomonads than the unamended soils (Fig. 4). There was no significant effect of soil type on the populations of culturable organisms associated with each plant species (data not shown).

The AWCD of the Biolog plates was found to be significantly positively correlated ($r^2 = 0.63$, $P < 0.01$) with the numbers of pseudomonads present in the sample (Fig. 5). The correlation of pseudomonad numbers to AWCD was greater in the Countesswells soil ($r^2 = 0.76$, $P < 0.001$) than the Tarves soil ($r^2 = 0.50$, $P < 0.05$) (data not shown). The poorer correlation in Tarves soil was due to the combination of high pseudomonad numbers, but low carbon utilisation in the bentgrass rhizosphere samples. The correlation of AWCD to total bacterial numbers ($r^2 = 0.43$) and fungal numbers ($r^2 = 0.13$) was not significant.

The bacterial species isolated on *Pseudomonas* agar were identified using Biolog GN plates. Table 2 summarises the results of the identifications indicating the dominating pseudomonads associated with the different plant species. *P. fluorescens* Type B. and *P. corrugata* were found in all treatments. *P. marginalis* was found in all rhizosphere and sucrose amended soils. *Acinetobacter calcoaceticus* was found in the rhizosphere of ryegrass and clover. The rhizosphere of clover appeared to support the greatest diversity of pseudomonads, with seven different species identified (Table 2).

The pseudomonads isolated from the various rhizosphere and control soils, and identified using Biolog, utilised all the carbon sources identified by multivariate analysis (Table 1) as being important

Table 1. Correlation of C source utilisation variables to ordination axes derived from canonical variate analysis (CVA) of plant rhizosphere samples showing carbon compounds responsible for discrimination of the samples taken from wheat rhizosphere soils (CV1), ryegrass and bentgrass rhizosphere soils (CV2) and unamended soils (CV3)

CV1		CV2		CV3	
Carbon source	Loading	Carbon source	Loading	Carbon source	Loading
α Ketobutyric acid	10.730	Glycyl-L-aspartic acid	9.759	L-leucine	8.156
Succinic acid	9.134	α-D-glucose	9.575		
2,3 Butane diol	9.081	Glycogen	9.393		
m-Inositol	7.231	D-trehalose	8.850		
α-D-lactose	6.916	L-asparagine	8.545		
Monomethyl succinate	6.469	D-serine	7.659		
		Quinic acid	7.436		
		α-Hydroxy butyric acid	7.274		
		Malonic acid	7.200		
		L-serine	7.154		
		L-rhamnose	6.976		
Glycogen	−10.980	Glucose-1-phosphate	−7.498	D,L-lactic acid	−7.103
L-rhamnose	−8.769	D-psicose	−6.923	Alaninamide	−7.060
α-D-glucose	−8.435			D-trehalose	−6.600
D-serine	−7.612				
L-asparagine	−6.766				

in discrimination between the communities (data not shown). However, there were differences in utilisation between the pseudomonad species. For example, *P. corrugata* and *P. fluorescens* Type G did not utilise α-D-lactose in contrast to the other Pseudomonads. *Burkholderia* (prev. *Pseudomonas*) *cepacia* utilised glycogen and L-rhamnose, which were not utilised by the other *Pseudomonas* species. Similarly, *P. marginalis* did not utilise D-psicose, but had much greater utilisation of α-hydroxybutyric acid than the other pseudomonads.

DISCUSSION

We have shown that microbial communities from the rhizospheres of different plant species produce characteristic C source utilisation patterns. Comparison of samples with equivalent AWCD ensured that discrimination between samples could

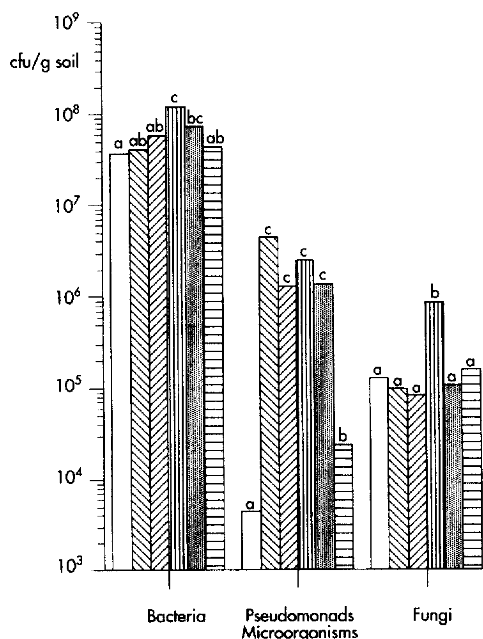

Fig. 4. Populations of microorganisms in unamended soil □ and in the rhizosphere of ryegrass ▧, bentgrass ▨, wheat ▥, clover ▦ and sucrose amended ▤ soil. Values are means of six replicate samples. Bars with the same letter are not significantly different $P < 0.05$.

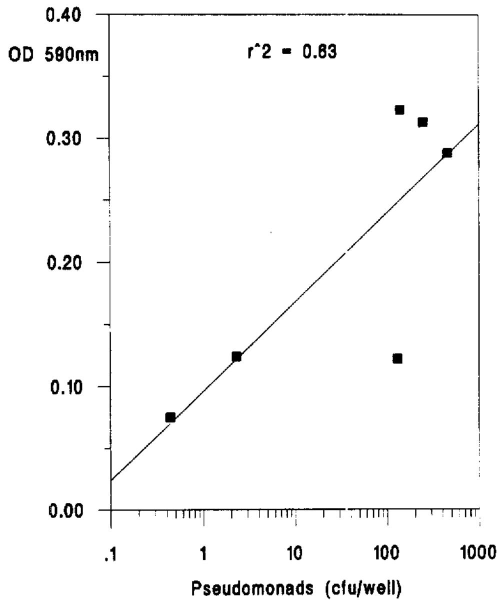

Fig. 5. Correlation between AWCD and inoculum density of culturable pseudomonads.

be attributed to definite qualitative differences in microbial communities rather than quantitative (density) effects. These patterns of C utilisation were similar for communities growing under the same plant species in both soil types when grown under controlled environmental conditions.

Grayston and Campbell (1996) found the major influences on the C utilisation profiles of microbial communities taken from the rhizosphere of hybrid larch and Sitka spruce, were soil type and previous crop history. There were differences in the metabolic profiles of the communities from the two tree species at all sites, due to variation in carboxylic acid utilisation, but this was overshadowed by the site differences. The two soils used in our experiment were of different types, but had similar crop histories. In addition, the same plant species were grown in the two soil types for 4 wk before sampling. This suggests that under controlled conditions plant species have a major selective influence on microbial community structure, a view shared by others (Rovira, 1965; Atkinson *et al.*, 1975; Miller *et al.*, 1989). It also suggests that the total functional diversity of microorganisms in different soils may be similar and that a combination of environmental and plant factors influence which organisms are active, become culturable (Colwell *et al.*, 1985) and proliferate under different conditions. However, the proof of this hypothesis must await the determination of total genetic microbial diversity (rRNA sequence analysis) from a range of soils to assess whether all soils possess similar microbial genetic pools.

The differences in metabolic profiles of microbial communities from the different crop types and the unplanted soils, suggest differences in community structure between crops. Differential utilisation of C sources between crop types suggests a differential availability of these C sources in the various crop rhizospheres, which may have influenced the proliferation of particular communities of microorganisms. In addition the absence of a plant or smaller plant biomass could have led to a decreased or less active microbial community in the unplanted and bentgrass rhizosphere soils, respectively, resulting in the slower overall rate of utilisation of C sources by these communities. The C source, which was utilised to a greater degree by the microbial communities from the non-rhizosphere soils (L-leucine), may have been present in the soils as a result of exudation, root cell senescence, mineralisation of organic matter or dead bacterial and fungal cells or hyphae. This compound may be less readily utilised by the majority of the microbial community and could, therefore, have remained present in the soils for a longer period. The stimulation of organisms capable of degrading L-rhamnose, α-D glucose, D-serine, L-asparagine and glycogen in the wheat rhizosphere and the lower utilisation of these same compounds by communities from bentgrass and ryegrass rhizospheres suggests that wheat may exude such compounds to a greater extent than other species. With the exception of glycogen, all these compounds have been reported as root exudate constituents (Campbell *et al.*, 1997). However, it must be emphasised that the Biolog technique

Table 2. Bacterial species isolated on *Pseudomonas* agar from the rhizospheres of ryegrass, bentgrass, wheat and clover grown in Countesswells and Tarves soils and in sucrose amended and unamended soils

Bacterial species	Plant species					
	Ryegrass	Bentgrass	Wheat	Clover	Sucrose	None
Pseudomonas fluorescens Type A				✓		
P. fluorescens Type B	✓	✓	✓	✓	✓	✓
P. fluorescens Type F	✓				✓	✓
P. fluorescens Type G	✓	✓	✓	✓		
P. corrugata	✓	✓	✓	✓	✓	✓
P. marginalis	✓	✓	✓	✓	✓	
P. tolassi				✓		
Burkholderia cepacia				✓		
Acinetobacter calcoaceticus/gen 13	✓					
A. calcoaceticus/*baumannii*/gen 2				✓		

measures potential utilisation and so some caution must be used when extrapolating the data to actual carbon source availability in the rhizosphere (Garland, 1996b). Nevertheless, the fact that sucrose additions to both soils resulted in higher utilisation rates of sucrose and one of its hydrolysis products, glucose, suggests that the Biolog system is detecting functional changes in microbial communities, resulting from differing carbon source availability in the soil. Although sucrose addition to the soils also increased utilisation of many of the other carbon sources in the GN plate, which suggests non-selective increase in microbial growth in amended soils, sucrose amendment would be expected to stimulate the induction of enzymes necessary for degradation of other carbon compounds and not only those responsible for sucrose utilisation. Similarly, Vahjen *et al.* (1995) showed that soil amendment with aprotinin (protease inhibiting peptide) resulted in the utilisation of additional substrates, mainly carbohydrates, by soil microbial communities. Intriguingly, the fact that fructose utilisation, the other sucrose hydrolysis product was repressed in sucrose-amended Tarves soil, may suggest that sucrose and glucose utilisers had increased in this soil at the expense of other organisms using the full spectrum of carbon compounds available in the GN plate, such that the overall AWCD was reduced. There have been numerous studies characterising plant root exudates, the majority involving important arable crops (Hale *et al.*, 1978; Bolton *et al.*, 1992). However, the quality and quantity of root exudates have been shown to be influenced by both environmental and plant factors (Rovira, 1959; Martin, 1977; Vancura *et al.*, 1977; Kraffcyzk *et al.*, 1984). Therefore, no direct conclusions can be made regarding differences in exudates between plant species because there have been few studies carried out at the same time and using identical conditions. However, the similarity in C utilisation patterns of the microbial communities from the ryegrass and bentgrass rhizospheres suggests that these grass species may exude a similar spectrum of root exudates. The ryegrass and bentgrass used in this experiment are both C3 perennial grass species with a lower relative growth rate than wheat (Cousens, 1996) and clover (Woledge *et al.*, 1992). Therefore, it may be postulated that less carbon will be released into the rhizosphere of these grass species. Chantigny *et al.* (1996) found a correlation between the water-soluble organic C and microbial biomass C found under various annual and perennial species, suggesting differences in C deposition from the plant species. Martin (1971) showed that clover had a greater exudation rate than wheat, which was higher than ryegrass. Legumes also have a lower C-to-N ratio, <20 compared with >30, than grasses (Steele and Vallis, 1988). There is evidence that legumes exude greater concentrations of amino nitrogen than wheat (Ayers and Thornton, 1968; Vancura and Hanzlikova, 1972) and this could result in the selection of different organisms in these rhizospheres.

There have been a number of studies assessing the diversity of microbial populations in the rhizosphere of different plant species, including wheat (Neal *et al.*, 1970; Neal *et al.*, 1973; Kleeberger *et al.*, 1983; Miller *et al.*, 1989) clover and ryegrass (Sperber and Rovira, 1959; Darbyshire and Greaves, 1967) and grasses (Christie *et al.*, 1978; Lawley *et al.*, 1983). Sperber and Rovira (1959) found a greater diversity of microorganisms in the rhizosphere of clover (21 genera) than ryegrass (16 genera). They found that *Arthrobacter* species were dominant in both these rhizospheres. Similarly, Miller *et al.* (1989) found actinomycetes contributed a high percentage of the microbial community in the rhizosphere of wheat, maize and grass and pseudomonads, though a low percentage, were stimulated in the rhizosphere. The selective stimulation of *Pseudomonas* species in the rhizosphere, which has been reported previously (Martin, 1971; Alexander, 1977) was well demonstrated in this study. Pseudomonads are nutritionally very diverse (Bowen, 1980) and have a higher growth rate in soil than other bacteria. In our experiment the proportion of *Pseudomonas* species in the total bacterial population increased from 0.01% in non-rhizosphere soil to 12.5% in the rhizosphere soil. The positive correlation we found between AWCD and the number of *Pseudomonas* species in the samples agrees with the data of Heuer *et al.* (1995) who used 16 S rRNA probes to demonstrate that similarly fast growing Gram-negative *Enterobacter* species were mainly responsible for the colour development within the Biolog plates inoculated with a soil extract. The significant correlation of pseudomonad numbers to AWCD in the Biolog wells and the evidence of the selective stimulation of these organisms in the rhizosphere, coupled with the isolation of different *Pseudomonas* species from various plant rhizospheres and their differing utilisation of the range of carbon sources responsible for community discrimination, suggests *Pseudomonas* species may be responsible for the differences in the C utilisation profiles between the rhizosphere samples.

Our study demonstrates the potential of the Biolog system to rapidly determine the metabolic diversity of rhizosphere communities. However, in order to fully assess the value of the technique in determining the potential functional diversity of microbial communities the identity of the microorganisms responsible for producing the profile in the Biolog wells needs to be elucidated. Our findings suggest that it may be the activity of *Pseudomonas* species in the samples which predominate in the

Biolog wells. The differences in utilisation of carbon sources by the microbial communities from the plant rhizospheres can only have been due to the presence of different microbial species. This diversity of microorganisms associated with the different plants may have arisen due to the variation in carbon compounds exuded by the plants. Evidence from the literature suggests these plants may produce different exudate spectra. However, in order to link utilisation rates with carbon availability in the rhizosphere further concurrent studies on root exudation by these plant species are needed. In addition, the identification of other rhizosphere carbon sources will enable development of plant or ecosystem-specific plates (Campbell *et al.*, 1997), improving the definition and relevance of the technique to studies of the rhizosphere.

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