Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil

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Summary

- Plant root mucilages contain powerful surfactants that will alter the interaction of soil solids with water and ions, and the rates of microbial processes.
- The lipid composition of maize, lupin and wheat root mucilages was analysed by thin layer chromatography and gas chromatography-mass spectrometry. A commercially available phosphatidylcholine (lecithin), chemically similar to the phospholipid surfactants identified in the mucilages, was then used to evaluate its effects on selected soil properties.
- The lipids found in the mucilages were principally phosphatidylcholines, composed mainly of saturated fatty acids, in contrast to the lipids extracted from root tissues. In soil at low tension, lecithin reduced the water content at any particular tension by as much as 10 and 50% in soil and acid-washed sand, respectively. Lecithin decreased the amount of phosphate adsorption in soil and increased the phosphate concentration in solution by 10%. The surfactant also reduced net rates of ammonium consumption and nitrate production in soil.
- These experiments provide the first evidence we are aware of that plant-released surfactants will significantly modify the biophysical environment of the rhizosphere.

Key words: lipid analysis, phospholipid, rhizosphere, root mucilage, soil properties, surfactant.

Introduction

Root-derived mucilage is a crucial component of the rhizosphere, contributing to many fundamental plant–soil interactions, such as root penetration, soil aggregate formation, microbial dynamics and nutrient turnover (McCully, 1999). Many functions have been suggested for mucilage, but little is known about its influence on specific physical properties of the rhizosphere. Passioura (1988) noted that soil contains surface active materials of biological origin, which modify the surface tension of soil solution. He suggested that, if roots exuded similar surfactants (or stimulated their production by microorganisms), they would reduce the tension of water in the soil at a given water content. This would influence the ability of the roots to take up water, especially at higher tensions.

In a previous study, Read & Gregory (1997) measured the surface tension of mucilage collected from the roots of 3–4-d-old, axenically grown maize (Zea mays L. cv. Freya) and lupin (Lupinus angustifolius L. cv. Merrit) seedlings. The surface tension of both maize and lupin mucilage was reduced to *c.* 48 mN m^{-1} at total solute concentrations > 0.7 mg ml^{-1}, indicating the presence of powerful surfactants. Also, during collection of mucilage with Pasteur pipettes, small bubbles and planar membranes frequently formed. Stable membrane formation is a sensitive indicator of the presence of a surfactant (Ballard *et al.*, 1986). The similar reductions of surface tension measured in both maize and lupin mucilage suggested that the type of surfactant present was the same.

The major components of mucilages are sugars. Monomeric neutral sugars increase the surface tension of water and are not surface active (Shaw, 1980). During analysis of the

neutral sugar composition of maize mucilage by gas chromatography of peracetylated derivatives (Osborn *et al.*, 1999), several compounds more volatile than the observed sugars were also detected. Subsequent investigation by gas chromatography-mass spectrometry (GC-MS) indicated that some of these compounds possessed hydrocarbon chains, up to 18 carbon atoms in length. It has been suggested that polar glycolipids are synthesised as intermediates during the production of mucilage at the root tip (Green & Northcote, 1979). These glycolipids and other phospholipids associated with plant cell membranes would be expected to show marked surface activity (Ballard *et al.*, 1986).

Early studies of the root epidermis using electron microscopy reported the presence of oily droplets in mucilage of onion (*Allium cepa*) (Scott *et al.*, 1958). The droplets stained red with Sudan III, indicating the presence of saturated fatty substances. Similarly, Dawes & Bowler (1959) conducted a microscopic study of the root hair structure of radish (*Raphanus sativus*). The root hairs were covered with a mucilaginous layer and staining with Sudan III indicated the presence of fatty substances in the root hair mucilage and in the mucilage of the root epidermal cells. In the light of these observations, it seemed probable that the reductions in surface tension observed in experiments with maize and lupin mucilage were attributable to the presence of lipids.

The presence of lipid surfactants may have substantial effects on the water retention and hydraulic conductivity of the rhizosphere, on chemical adsorption, and on microbial processes. Matric potential is one of the most important components of the water potential in soil/plant systems (Campbell, 1985), governing the amount and distribution of water in the soil pore space and mediating many other soil processes, directly and indirectly (Young & Ritz, 2000). The water potential under a curved air–water interface, in idealised pore space, is given by the capillary rise equation:

$\Psi_m = -2\gamma/r\rho_w$ where r is the radius of curvature of the interface, γ is the surface tension and ρ_w is the density of water. Therefore, lowering the surface tension of the soil solution should produce a proportional increase in matric potential, allowing the plant to extract more available water from the soil.

The effect of surfactants on the hydraulic conductivity of soil has been investigated in bioremediation studies, where surfactants have been applied in bulk to soil to flush out hydrophobic organic contaminants. Saturated hydraulic conductivities and unsaturated diffusivities of loams were reduced by up to two orders of magnitude in the presence of surfactants such as sodium dodecylsulphate (Allred & Brown, 1994; Liu & Roy, 1995; Tumeo, 1997), seriously decreasing the effectiveness of surfactant-based remediation techniques. Suggested mechanisms for the effect include expansion and sodium dispersion of the clays, fine particle mobilisation and precipitation of divalent salts of the surfactants.

In addition to effects on physical properties, surfactants may also modify the chemical properties of the rhizosphere. For example, adsorption of root mucilage and polygalacturonic acid decreased subsequent P adsorption (Gaume *et al.*, 2000). However, the effect of root mucilage simply as a source of surfactants has not been previously considered. It is possible that root-produced surfactants may affect phosphate adsorption by competing directly for adsorption sites, or by altering the energetics of the interaction between the phosphate ion and the adsorption site. Root mucilage has been found to affect rates of nitrogen immobilisation and mineralisation (Mary *et al.*, 1993), but these results were considered in the context of mucilage as a carbon source for heterotrophic microbes. Because mucilage affects soil physical properties in the rhizosphere and thereby modifies the environment in which soil microbes function, there may well be consequences for microbial viability and activity.

The aims of this study were: first to detect and identify any fatty acids in root mucilage and to identify the parent lipid type; and second to evaluate the likely effects of root-released phospholipid surfactants on soil matric potential, phosphate adsorption and soil N dynamics in the rhizosphere. Lipids present in root mucilage were analysed by GC-MS and thin layer chromatography (TLC), and alterations in soil properties were assessed using a commercially available phospholipid surfactant (lecithin), which is chemically similar to the surfactants found in root mucilages.

Materials and Methods

Analysis of surfactants in root mucilages

Germination of seeds and collection of mucilage Seeds of maize (Zea mays L. cv. Freya), lupin (Lupinus angustifolius L. cv. Merrit) and wheat (Triticum aestivum L. cv. Charger) were surface-sterilised in sodium hypochlorite solution (2% for 10 min), then rinsed thoroughly in sterile deionised water. The seeds were then germinated on moist filter papers in Petri dishes, in the dark at 26°C. After 4–5 d, mucilage was collected from the tips of the germinating roots using a drawn glass Pasteur pipette, in a laminar flow cabinet. The mucilage was centrifuged at 12 000 r.p.m. for 30 min, decanted and filtered (0.2 μm nylon syringe filter) to remove all the insoluble plant material. The harvested mucilages were freeze-dried immediately (10^{-1} mbar at −40°C).

For comparison, the root tissues of each species were also processed to extract lipids, using the method described by Christie (1989). Excised roots from the seedlings were homogenised in isopropanol, filtered and the residue re-extracted with fresh isopropanol. The filtrates were combined and the solvent was removed on a rotary evaporator. The residue was dissolved in 2 : 1 chloroform/methanol and washed with 0.88% aqueous potassium chloride solution in a modified 'Folch' procedure (Ways & Hanahan, 1964; Christie, 1989). The resulting organic layer containing the purified lipid was separated, filtered and the solvent removed on a rotary evaporator.

Analysis of fatty acids

Derivatisation Lipids were transesterified with sulphuric acid in methanol according to the method described by Christie (1989). This produces methyl ester derivatives of the component fatty acids. The freeze-dried mucilage was dissolved in toluene to which a 1% solution of sulphuric acid in methanol was added. The mixture was left for 18 h at 50°C in a stoppered tube. Water containing 5% sodium chloride was then added and the esters were extracted twice with hexane. The hexane layer was washed with 2% aqueous potassium bicarbonate solution and dried over anhydrous sodium sulphate. The solution was filtered and the solvent volume reduced in a stream of nitrogen, prior to analysis. The purified lipid extracts from root tissues were dissolved in toluene and transesterified in the same manner.

Analysis by GC-MS Analyses were carried out on a Hewlett Packard 5890 GC fitted with a Restek Rtx-50 column (15 m long, 0.25 mm i.d., 0.1 μm coating) with helium as the carrier gas at an inlet pressure of 5 psi, coupled to a Hewlett Packard 5970 Mass Selective Detector. The initial temperature of the column was 50°C for 3 min, then increasing at 15°C min^{-1} to 250°C, held for 5 min.

The fatty acid methyl esters were identified from their mass spectra (Christie, 1989) and by comparison of retention times to standards. Results are reported using standard fatty acid nomenclature. The number in front of the colon indicates the number of carbon atoms in the fatty acid; the number after the colon indicates the number of double bonds in the carbon chain. Where the configuration/position of the double bond is known, 'c' (*cis*) or 't' (*trans*) is shown followed by a number representing the position relative to the carboxyl end of the molecule.

Analysis of parent lipids The freeze-dried sample was transferred to a test tube with acidified brine. Methanol was added to the tube and vortex mixed, then twice the volume of chloroform was added and also vortex mixed. The lower layer was separated and the solvent removed. The residue was taken up in chloroform and spotted onto a TLC plate that was developed with 65 : 25 : 4 chloroform/methanol/water. The plate was dried then sprayed with Phospray reagent which stains phosphorus-containing spots blue. Relative retentions were compared with phospholipid standards with phosphatidylethanolamine migrating ahead of phosphatidylcholine.

Measurement of surface tension Surface tension was measured by the capillary rise method (Nelkon & Ogborn, 1978) using small precision-bore capillaries (radius = 0.315 mm), thoroughly cleaned in sodium hydroxide solution. Capillary rise (typically ranging between 25 and 45 mm) was measured at 20°C with a travelling microscope using the equilibrium position of the receding meniscus (Read & Gregory, 1997). Individual measurements are accurate to ± 1 mN m^{-1}.

Effect of phospholipid on soil properties

Selection of lipid The use of root-derived mucilage as a source of surfactant was impractical for these experiments because large quantities were required, and because it would have been impossible to separate effects of the surfactant from those of other components of the mucilage. Therefore a commercially available phosphatidylcholine surfactant (soybean lecithin (Sigma Chemical Co., St. Louis, MO, USA) was used. Although lecithin probably contains a higher proportion of unsaturated fatty acids than the surfactant lipids in mucilages, measurements showed that the surface tension of a 500-mg l^{-1} solution was about 50 mN m^{-1}, which is comparable with the surface tension observed for root mucilages.

Selection of soils Bullionfield soil, collected from Scottish Crop Research Institute, Dundee, was used throughout the experiments. This is a dark brown, sandy loam formed over sandstone and is slightly acidic. For comparison, when it was appropriate, Sonning soil and acid-washed sand were also used in the experiments. Sonning soil was collected from Lamyard field at The University of Reading Farm, Sonning, Berkshire, UK. This soil is a freely draining, sandy loam, formed on fluvial valley gravels and is neutral to slightly acidic.

Water release properties The water release properties of Bullionfield soil and acid-washed sand were measured on tension tables and pressure plates. Half the soil and sand samples were treated with deionised water and half with lecithin solution at a concentration of 500 mg l^{-1}. The soil was air-dried and sieved to < 2 mm, the sand was dry sieved to < 250 μm. The soil was packed into Perspex rings (40 mm internal diameter, 10 mm deep, sealed at the base with 20 μm nylon mesh) to give a dry bulk density of 1.1 Mg m^{-3}. Acid-washed sand cores were packed in the same way, but to a dry bulk density of 1.7 Mg m^{-3}. The samples were saturated from the bottom with deionised water or lecithin solution (500 mg l^{-1}), then placed on tension tables held at tensions ranging from 0.5 to 17 kPa or pressure plates at tensions of 250, 900 and 1500 kPa. Three replicates were used for each treatment, at each tension. Samples were weighed periodically until equilibrium water content had been achieved, which was usually within 24 h on the tension tables, although, for the pressure plates, longer equilibration times were required.

Phosphate adsorption Phosphate adsorption/desorption was measured using the method described by Rowell (1994). Three replicate soil samples (2.5 g air-dry, < 2 mm) were shaken for 24 h with six standard phosphate solutions in 10 mM calcium chloride. Bullionfield soil is a strong phosphate adsorber, so standard solutions of higher concentration than usual (containing 0, 5, 10, 20, 30 and 50 μg P ml^{-1}) were required. The experiment was repeated with Sonning soil, which is more weakly phosphate-adsorbing, for comparison.

Half the soil samples were treated normally, while half were shaken with standard solutions also containing 500 mg l^{-1} lecithin. At the end of the shaking period, the suspensions were filtered through Whatman no. 41 filter papers and P concentration was determined by the phosphomolybdate method, measuring the absorbance at 880 nm using a Perkin Elmer Lambda 2 UV/Visible spectrometer. The absorbance measurements ranged between 0.003 and 0.699 and the standard error within each set of three replicates was never greater than ±0.003. The presence of lecithin had no effect on the development of the phosphomolybdate complex or the calibration of the method.

In both soils, the amount of P initially present was small compared with the amount in the standard solutions. Also, because the lecithin was added at the same time as the phosphate in the standard solutions, it was possible that any effects were due to the lecithin adsorbing to the soil surface before the phosphate, that is blocking P-adsorption, rather than desorbing P from soil particle surfaces. To test this, a further experiment was conducted with Bullionfield soil. Soil samples (2.5 g) were shaken for 24 h with 10 ml of 100 µg P ml^{-1} solution (in 10 mM calcium chloride). Then, 15 ml of 10 mM calcium chloride solution was added to half the samples and 15 ml of lecithin solution (1000 mg l^{-1}, in 10 mM calcium chloride) was added to the rest. The samples were shaken again for 24 h, filtered, and solution P concentration determined by the phosphomolybdate method.

Soil N dynamics The effect of lecithin on net N mineralisation rate in Bullionfield soil was investigated using a ^{15}N pool dilution technique (Gibbs & Barraclough, 1998). In this technique, a small quantity of $^{15}NH_4^+$ is added to the soil. Provided the ^{15}N label mixes with the indigenous soil NH_4^+, then the decline over time in ^{15}N abundance in the NH_4^+ pool is a direct indicator of the rate at which mineralisation introduces unlabelled NH_4^+ into the pool. The soil was sieved < 6 mm while moist. Incubations were carried out using 40 g samples of wet soil (water content 0.24 g g^{-1} dry soil), packed into 20 mm high plastic rings, 63 mm diameter, covered at the base with 280 µm nylon mesh. The rings were packed to a density of 1.1 Mg dry soil m^{-3} and sealed with parafilm. Four pin holes allowed gaseous exchange, but restricted water loss. The soil samples were incubated at 20°C for 14 d prior to treatment, periodically watered back to their original weight and re-sealed with parafilm. Three replicates were used for each treatment. After 14 d equilibration, the soil was amended with 10 µg N g^{-1} dry soil as $(^{15}NH_4)_2SO_4$, containing 5 atom% ^{15}N, in 1 ml of deionised water. Lecithin was added to the ammonium sulphate solution at three concentrations 0, 250 and 500 mg l^{-1}, so the surfactant was added to the soil simultaneously with the labelled ammonium. The ^{15}N-labelled solution was added dropwise from a pipette over the whole surface area of each ring. The samples were then covered and returned to a constant temperature room at 20°C. Half the samples were extracted by shaking the soil with 200 ml of 1 M KCl for 1 h, 1 d after amendment (T_1); with the rest extracted 3 d later (T_4). NH_4^+ and NO_3^- concentrations were determined colorimetrically using a Tecator FIAstar 5010 flow injection auto analyser (Foss Tecator AB, Höganäs, Sweden). Then, NH_4^+ and NO_3^- were concentrated by diffusion (in the presence of magnesium oxide for NH_4^+ and magnesium oxide and Devarda's alloy for NO_3^-) onto separate Whatman GF/D glassfibre discs acidified with 10 µl 2.5 M $KHSO_4$. ^{15}N: ^{14}N ratios of the discs were determined using a VG 622 mass spectrometer coupled to a Europa Scientific Roboprep combustion analyser (Europa Scientifia, Crewe, UK).

Results

Analysis of surfactants in root mucilages

Fig. 1 shows the fatty acid methyl ester profile of lipids extracted from the root tissues of each plant species. The chromatograms are dominated by three fatty acids: 16 : 0 (11.46 min), 18 : 2 c9, c12 (12.78 min) and 18 : 3 c9, c12, c15 (12.94 min). The most intense peak was different for each of the three species: maize, 18 : 2; lupin, 18 : 3; wheat 16 : 0. Peaks assigned to 18 : 0 (12.70 min) and 18 : 1 c9 (12.67 min) were easily detected, overlapping on the chromatograms slightly, but both were relatively minor components. Very small peaks due to 14 : 0 (10.09 min) and 12 : 0 (8.58 min) could sometimes be detected above the baseline noise. The relative amounts of the major fatty acid components of root tissues and mucilages, estimated from GC peak areas, are summarised in Table 1.

In contrast with root tissues, the filtered mucilages consisted almost entirely of saturated fatty acids, especially 16 : 0

Table 1 Fatty acid methyl ester analysis of the root tissues and mucilage of maize, lupin and wheat (concentrations in mol%, based on relative GC peak areas from Figs 1 and 2)

	Maize:			Lupin:		Wheat:	
Fatty acid	Root tissue	Unfiltered mucilage	Filtered mucilage	Root tissue	Filtered mucilage	Root tissue	Filtered mucilage
16 : 0	33.2	42.9	72.6	29.5	58.2	45.2	87.6
18 : 1	2.6	0	0	5.5	0	2.1	0
18 : 0	2.0	9.7	21.5	1.2	27.1	3.7	11.4
18 : 2	55.3	47.4	5.9	18.9	14.7	36.4	1.0
18 : 3	6.9	0	0	44.9	0	12.6	0

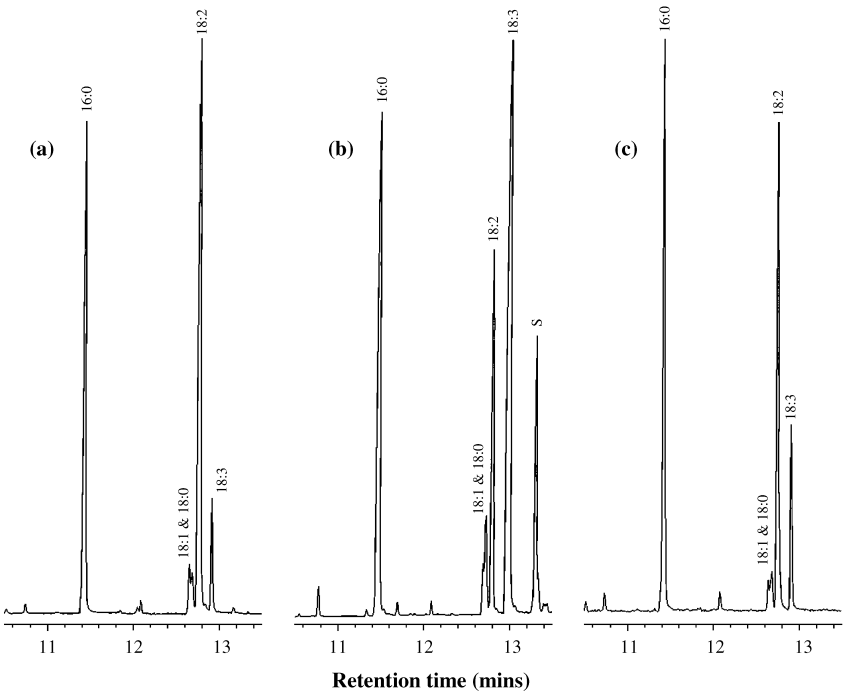

Fig. 1 Gas chromatograms showing the fatty acid composition of the root tissues of: (a) maize (b) lupin and (c) wheat, following extraction, hydrolysis and methyl esterification. Five fatty acids were typically present: saturated (16 : 0 and 18 : 0) and unsaturated (18 : 1, 18 : 2 and 18 : 3). The peak labelled 'S' is due to a 19 : 0 standard which was occasionally added prior to derivatisation.

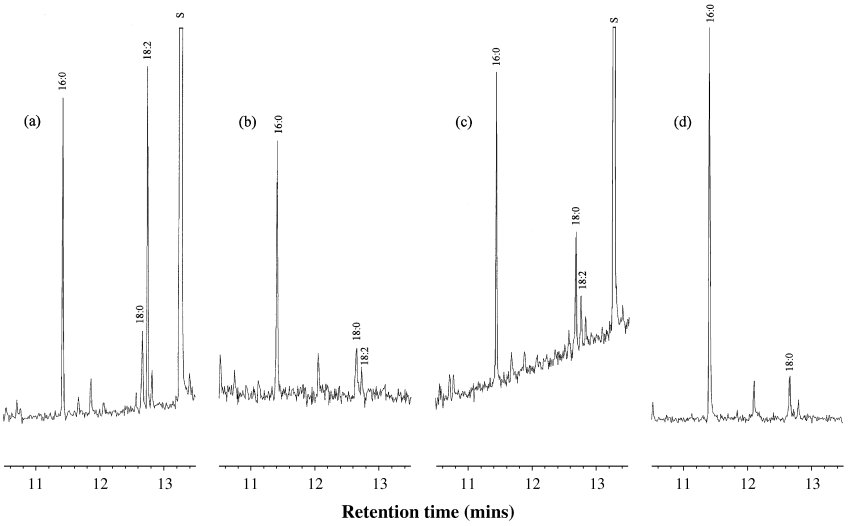

Fig. 2 Gas chromatograms showing the fatty acid composition of: (a) unfiltered maize mucilage; (b) maize mucilage after filtration through a 0.45-μm syringe filter to remove all insoluble plant material; (c) filtered lupin mucilage; (d) filtered wheat mucilage; following extraction, hydrolysis and methyl esterification. In the filtered mucilages, the two saturated fatty acids (16 : 0 and 18 : 0) predominate. The peak labelled 'S' is due to a 19 : 0 standard which was occasionally added prior to derivatisation.

with some 18 : 0, along with some unsaturated 18 : 2 (Fig. 2; Table 1). The intensity of these chromatograms was very low, particularly with lupin mucilage. All the chromatograms shown in the figures were obtained using the mass spectrometer in total ion detection mode. Selected ion detection, monitoring just five or six major fatty acid fragment ions, simplified the analysis and was useful for confirmation purposes. However, the selected ion mode distorts relative peak areas, depending on the choice of ions used, and therefore was less useful for quantitative analysis.

Analysis of unfiltered maize mucilage also produced a chromatogram of low intensity. The resulting fatty acid profile was intermediate between those of maize root tissue (Fig. 1) and filtered maize mucilage (Fig. 2). The unsaturated 18 : 2 fatty acid was relatively more abundant, with the saturated acids, especially 16 : 0, accounting for the rest (Table 1).

TLC of the filtered mucilages showed that phosphatidylcholine was the main phospholipid class present, although traces of phosphatidylethanolamine were also detected.

Fig. 3(a) shows the surface tension of filtered wheat mucilage over a range of total solute concentrations. The wheat mucilage was harvested at a solute concentration (determined by evaporation to constant weight) of *c.* 2.5 mg ml^{-1} and subsequently diluted for the surface tension measurements.

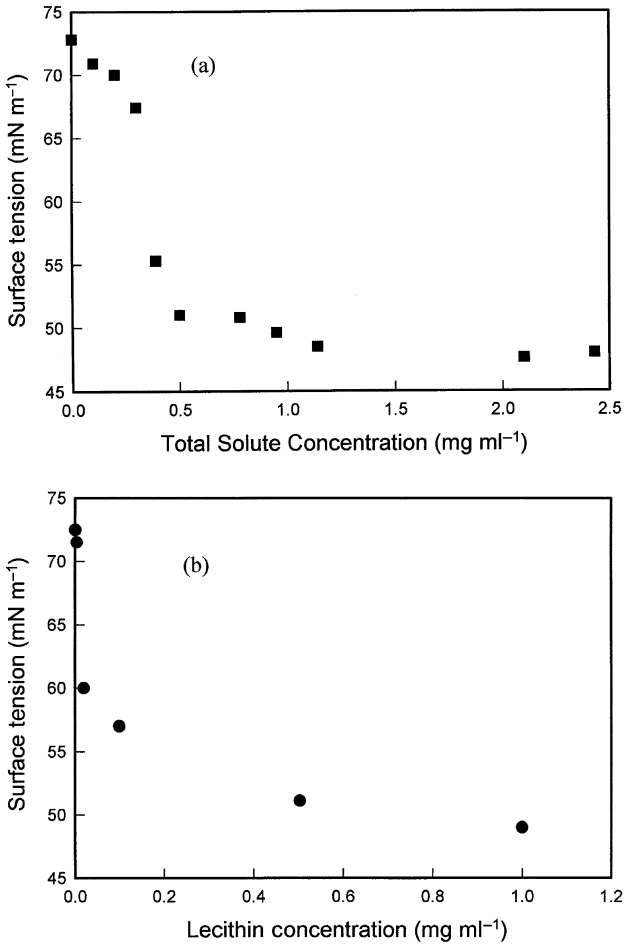

Fig. 3 Variation of surface tension (at 20°C) of (a) filtered wheat mucilage, and (b) soybean lecithin (a phosphatidylcholine), in aqueous solution.

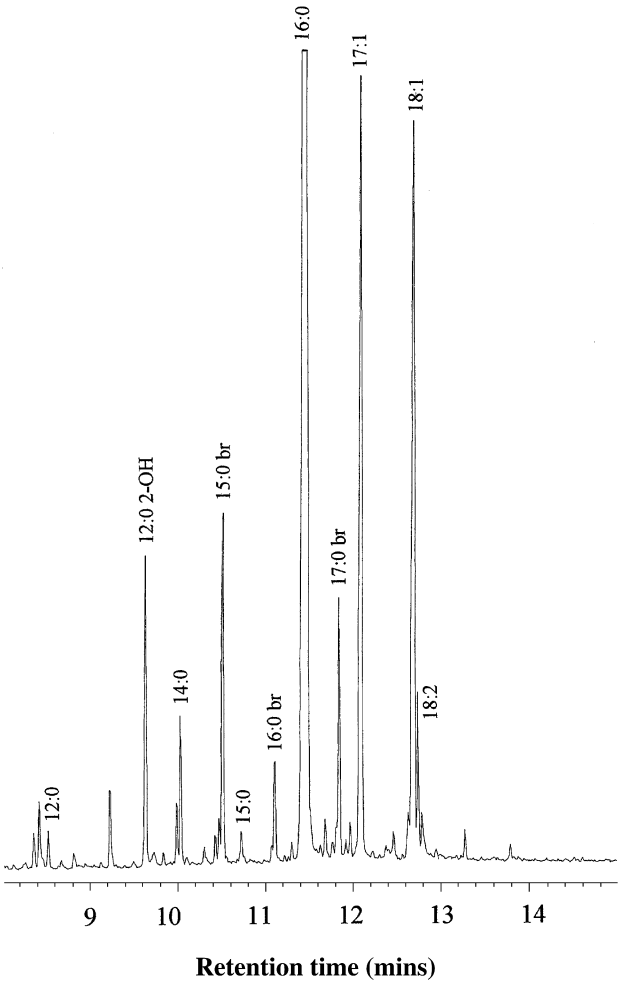

Fig. 4 Fatty acid analysis of a wheat mucilage sample showing clear signs of bacterial contamination. The profile is intense relative to the mucilage chromatograms (i.e. the quantity of fatty acids in the sample is much greater) and consists of fatty acids with odd-numbered carbon chain lengths, branched chains ('br') and occasionally hydroxyl-substitution (12 : 0 2-OH), in addition to the even-numbered, unbranched fatty acids characteristic of plants.

As concentration increased, the surface tension decreased to < 50 mN m^{-1} at *c.* 0.5 mg ml^{-1}, after which it remained about 48 mN m^{-1} to concentrations up to 2.4 mg ml^{-1}. For comparison, the surface tension of soybean-derived lecithin (Sigma Chemical Co. 99%), a typical phosphatidylcholine, was also measured. Surface tension decreased in a similar manner to the wheat mucilage, reaching a value of *c.* 49 mN m^{-1} at a concentration of 1000 mg l^{-1} (Fig. 3b). Although surface tension was still decreasing slightly, this concentration was close to the solubility limit of the soybean lecithin used.

Despite sterilisation, some batches of seeds developed signs of bacterial contamination. Fatty acid analysis of these mucilage samples produced a different profile (Fig. 4), consisting of odd-numbered carbon chain lengths and branched chains, in addition to the even-numbered, unbranched fatty acids characteristic of plants. Hydroxyl-substituted fatty acids were also present (e.g. 2-hydroxydodecanoic acid, 12 : 0 2-OH). The amount of fatty acids in the contaminated samples was much greater, producing relatively intense gas chromatograms. Similarly, TLC showed much higher lipid concentrations where bacterial contamination was present, and the predominant lipid was phosphatidylethanolamine instead of phosphatidylcholine.

Effect of phospholipid on soil properties

Water release properties Fig. 5 shows the moisture release of Bullionfield soil at a density of 1.1 Mg m^{-3}, with and without lecithin. The presence of the surfactant always reduced the equilibrium water content at any given tension. The effect was greatest at low tension where water content was reduced by as much as 10%, at the same potential, by the lecithin. The surface tension of the lecithin solution is about 65% that of pure water so it was possible to calculate the expected moisture release curve for comparison with the measured values (Fig. 5). At low tension, the agreement between

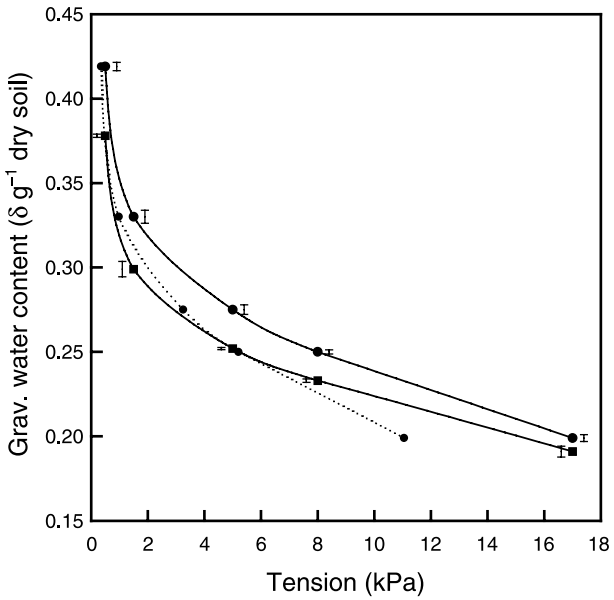

Fig. 5 Water release curves for Bullionfield soil at a density of 1.1 Mg m^{-3} with pure water (circles, solid line) and with lecithin solution at a concentration of 500 mg l^{-1} (squares, solid line), measured on tension tables. Error bars, offset from the data points for clarity, represent one standard error. For comparison, a moisture release curve calculated from the water content of Bullionfield soil with pure water, but plotted at 65% of the tension, is also shown (circles, dotted line).

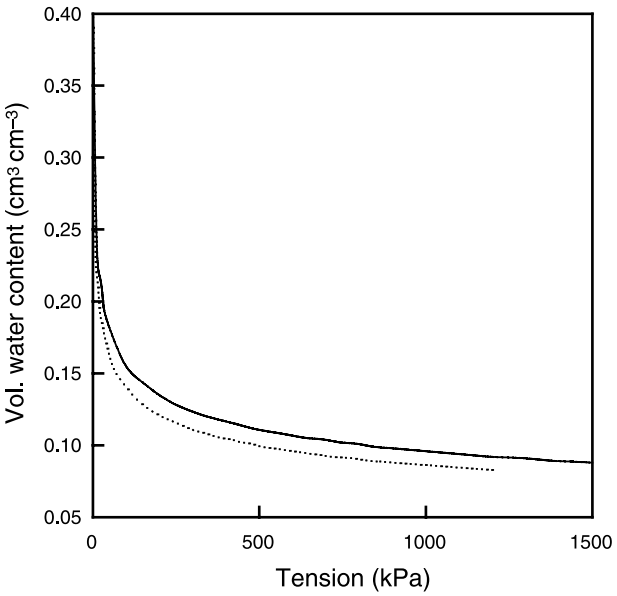

Fig. 6 Moisture release curve for Bullionfield soil with pure water, calculated using van Genuchten's (1980) equation fitted to the experimental data obtained from tension table and pressure plate experiments (solid line). The moisture release curve calculated from the same water contents plotted at 65% of the suction is also shown (dotted line).

this calculated line and the measured data was good, but at higher tensions (> 4 kPa) the lines diverge and the effect of the surfactant was smaller than predicted.

No significant effects due to the surfactant were apparent in samples equilibrated on pressure plates. To determine whether or not a measurable effect should have been observed, moisture release data for Bullionfield soil with pure water were fitted to van Genuchten's (1980) equation, over a wide range of potential (Fig. 6). A moisture release curve was then calculated for the same water contents at 65% of the tension of the fitted van Genuchten line. Over the range 200–700 kPa, the difference in moisture content at a given tension corresponds to about 0.012 cm^3 cm^{-3} or 0.011 g g^{-1}.

Fig. 7 shows the moisture release curve of acid-washed sand at a density of 1.7 Mg m^{-3}, with and without lecithin. Over the range 0–18 kPa tension, the sand lost about 70% of the water held at saturation. The effect of the surfactant on the moisture release curve was large compared with that observed with Bullionfield soil, with a 50% reduction in water content occurring at 4 kPa tension. Once again, experimental data were compared with a moisture release curve calculated at 65% of the tension with pure water (Fig. 7) and showed a larger decrease in water content than predicted at small tensions (0–7 kPa) with close agreement at tensions from 7 to 12 kPa.

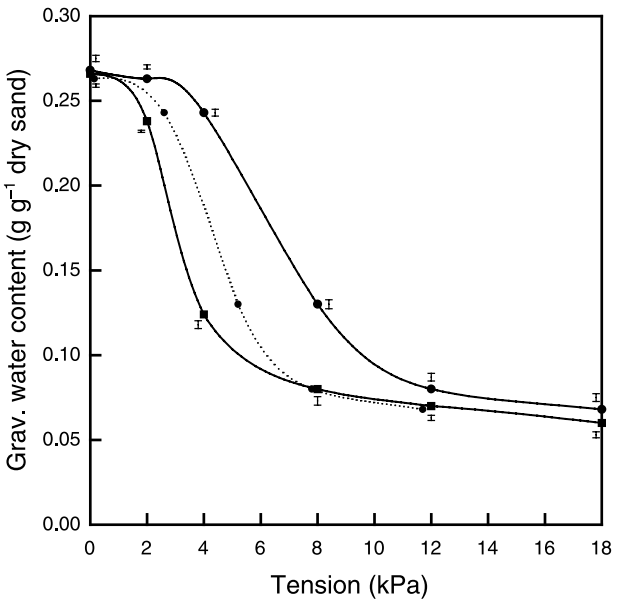

Fig. 7 Water release curves for acid-washed sand at a density of 1.7 Mg m^{-3} with pure water (circles, solid line) and with lecithin solution at a concentration of 500 mg l^{-1} (squares, solid line), measured on tension tables. Error bars, offset from the data points for clarity, represent one standard error. For comparison, a moisture release curve calculated from the water content of Bullionfield soil with pure water, but plotted at 65% of the tension, is also shown (circles, dotted line).

Phosphate adsorption Fig. 8 shows the adsorption isotherms of Bullionfield and Sonning soils, with and without the presence of lecithin in the standard solutions. Even at the highest P concentration used (50 µg P ml^{-1}), very little desorption occurred with the 0 µg ml^{-1} standard. The Sonning soil was less strongly adsorbing, approaching saturation at

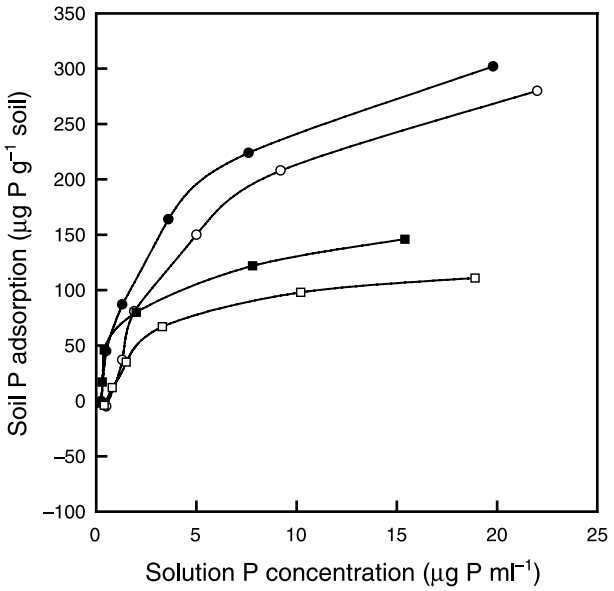

Fig. 8 The effect of surfactant on the phosphate adsorption isotherms of Bullionfield and Sonning soils: Bullionfield soil with 10 mM calcium chloride solution (closed circles); Bullionfield soil with 10 mM calcium chloride solution also containing 500 mg l^{-1} lecithin (open circles); Sonning soil with 10 mM calcium chloride solution (closed squares); Sonning soil with 10 mM calcium chloride solution also containing 500 mg l^{-1} lecithin (open squares).

the highest P concentration, but also only giving a small desorption at 0 µg P ml^{-1}. With both soils, the presence of lecithin decreased soil adsorption and increased the amount of P in solution. The effect was greater in the Sonning soil.

In a subsequent experiment, phosphate was preadsorbed onto Bullionfield soil prior to the addition of lecithin, in order to test the ability of the surfactant to re-mobilise the phosphate. In the initial phase, 336 µg P g^{-1} was preadsorbed onto the soil. After shaking with lecithin solution, 15 µg P ml^{-1} was left in solution and 250 µg P g^{-1} soil was adsorbed; a similar ratio to that obtained when phosphate and lecithin were added simultaneously. The calcium chloride solution alone was much less effective in removing the adsorbed P; after shaking, only 9 µg P ml^{-1} were in solution and 310 µg P g^{-1} soil were still adsorbed.

Soil N dynamics The results of the ^{15}N pool dilution experiment are summarised in Table 2. The Bullionfield soil did not contain a significant concentration of ammonium at the start of the experiment. A labelled ammonium pool was created but this pool was rapidly consumed. It was therefore impossible to derive reliable mineralisation rates from the experiment. However, there were significant treatment effects on rates of ammonium consumption and nitrate production. The addition of lecithin reduced the net consumption of ammonium and net production of nitrate. Where lecithin was absent, 45% of the added NH_4-N was consumed within 24 h and nearly 100% in 4 d. This rate was significantly reduced by

Table 2 The effect of lecithin on total NH_4-N and NO_3-N pools and ^{15}N enrichments in Bullionfield soil, 1 d (T_1) and 4 d (T_4) after the addition of labelled ammonium sulphate (10 µg N g^{-1} dry soil, containing 5 atom% ^{15}N)

Treatment	Total NH_4-N (µg N g^{-1} dry soil)		NH_4–^{15}N enrichment (atom%)		Total NO_3-N (µg N g^{-1} dry soil)		NO_3–^{15}N enrichment (atom%)		Net NO_3-N production (µg N g^{-1} dry soil d^{-1})
	T1	T_4	T_1	T_4	T_1	T_4	T_1	T_4	
Water	5.49 ± 0.30	0.01 ± 0.01	4.334 ± 0.041	0.661 ± 0.066	81.0 ± 1.4	88.9 ± 1.6	0.528 ± 0.007	0.784 ± 0.012	2.62
Lecithin 250 mg l^{-1}	7.84 ± 0.21	2.32 ± 0.07	4.441 ± 0.001	3.192 ± 0.030	78.4 ± 1.2	83.3 ± 1.2	0.425 ± 0.003	0.622 ± 0.009	1.63
Lecithin 500 mg l^{-1}	7.94 ± 0.11	1.76 ± 0.10	4.441 ± 0.005	2.810 ± 0.059	77.3 ± 1.2	81.8 ± 1.5	0.412 ± 0.003	0.597 ± 0.004	1.51

lecithin addition at both concentrations, with *c.* 20% of NH_4-N consumed within 24 h and *c.* 80% consumed at day 4. Similarly, between day 1 and day 4, net NO_3-N production was reduced from 2.62 μg N g^{-1} soil d^{-1} to less than 1.63 μg N g^{-1} soil d^{-1} in the presence of lecithin. Significant ^{15}N enrichment occurred in the NO_3^- pool (from 0.523 atom% at day 1 to 0.784 atom% at day 4 in the water treatment, and from *c.* 0.42 atom% at day 1 to *c.* 0.6 atom% at day 4 in the two lecithin treatments) confirming that ^{15}N was transferred to the NO_3^- pool. At the same time, ^{15}N depletion took place in the NH_4^+ pool, so pool dilution, and hence mineralisation, was occurring, but at a slower rate than NH_4^+ consumption.

Discussion

Analysis of surfactants in root mucilages

The fatty acid and lipid composition of plant tissues has been studied extensively (e.g. Body, 1974; Jarvis & Duncan, 1974; Sukhija *et al.*, 1976). The root tissues of maize, lupin and wheat analysed in this study produced characteristic plant fatty acid profiles – essentially 16 and 18-carbon fatty acids, saturated and unsaturated, but principally 16 : 0, 18 : 2 and 18 : 3. The maize analysis is in agreement with Barta (1991), who found that the most prevalent fatty acid in the neutral lipids, galactolipids and phospholipids of maize root tissues was 18 : 2. Although the predominant fatty acid is different in the various species, that is 18 : 3 in lupin, 16 : 0 in wheat (18 : 2 in white clover and 16 : 0 in rape (unpublished results)), these three fatty acids (16 : 0, 18 : 2 and 18 : 3) dominate.

In spite of the very low concentration of lipids in the root mucilages, it was possible to extract, derivatise and analyse the fatty acids from these lipids. Unexpectedly, the fatty acid profiles found in filtered root mucilages were quite different to those of the parent plant root tissues. In mucilage, saturated fatty acids predominated, especially 16 : 0; 18 : 2 was a minor component and 18 : 3 was never detected. The presence of saturated fatty acids in mucilage is in accordance with the microscope studies of Scott *et al.* (1958) and Dawes & Bowler (1959), who observed oily droplets which stained red with Sudan III.

The chromatogram obtained from unfiltered maize mucilage represents not only the lipids present in the mucilage, but also those extracted from detached root cap cells and other insoluble plant material (indicated by the predominance of 18 : 2). The relative absence of any unsaturated fatty acids in the filtered samples indicates that these chromatograms are representative of the dissolved lipid component in the mucilages, with little interference from root tissues.

In plant tissues, the most common phospholipids are phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine (Christie, 1989). The TLC results showed that most of the plant-produced lipid in mucilage is phosphatidylcholine. When bacterial contamination of the samples occurred (Fig. 4), much higher quantities of lipids were present, mostly in the form of phosphatidylethanolamine. Phosphatidylethanolamine is generally the major phospholipid in Gram-negative bacteria and is a major component of some Gram-positive genera. In contrast with higher organisms, phosphatidylcholine is rarely a major lipid in bacteria (Christie, 1989). Many microorganisms are known to produce lipid-based biosurfactants (Banat, 1995) and the majority of soil microorganisms exist in the rhizosphere. In this nonsterile environment, microbial surfactants may be as important as plant-released surfactants in modifying the physical properties of the rhizosphere. In any case, whatever their source, lipid surfactants will produce a significant reduction in surface tension close to the root.

The surface tension of maize and lupin mucilage has been reported previously (Read & Gregory, 1997). The surface tension of wheat mucilage over a range of different solute concentrations (Fig. 3a) is essentially the same as for maize and lupin. The value of 48 mN m^{-1} is very close to the surface tension of soybean lecithin solutions observed in this study, at higher concentrations (Fig. 3b). However, the commercial phosphatidylcholine will probably contain a higher proportion of unsaturated fatty acid components relative to the mucilages, which may affect the relative surface activities. Ballard *et al.* (1986) found that egg lecithin exhibited a surface tension of 44–44.9 mN m^{-1} in a variety of polar organic solvents. The lipid was assumed to be orientated with the choline head towards the solvent and hydrocarbon chains extending perpendicular to the surface, as it would be in water. In addition, the egg lecithin formed complete monolayers over organic solution surfaces at very low concentration (*c.* 10^{-4} M). This is particularly important because the total solute concentrations in mucilage, of which the lipid is a minor component, are low.

Taken together, these results indicate that the surfactants present in maize, lupin and wheat mucilage are phosphatidylcholines, composed mainly of saturated fatty acids.

From our experiments, it is unclear whether surfactant is actively secreted by the root or is present simply as a result of leakage from damaged cell membranes. However, the different fatty acid composition observed in the mucilage, compared with root tissues, suggests that some form of selection, passive or controlled, has occurred during release into the mucilage.

Effect of phospholipid on soil properties

Effects on water release and hydraulic conductivity According to the capillary rise equation, reducing the surface tension of soil solution should produce a proportional decrease in tension at a given water content. In our experiments, at low tension (0–10 kPa; saturation to below field capacity), this was indeed found to be the case. The surface tension of 500 mg l^{-1} lecithin solution is *c.* 35% less than that of pure water and, as expected, the addition of lecithin produced an

equivalent decrease in tension at the same water content. At tensions greater than 10 kPa, the effect of the surfactant was much less. Calculations with a simulated moisture release curve based on the van Genuchten equation, fitted to the experimental data for Bullionfield soil, show that, even at high tension (> 1 MPa), moisture content differences of > 0.01 g g^{-1} should exist. Differences of this magnitude should have been measurable in the pressure plate experiments – standard errors within replicates were typically ± 0.002 g g^{-1} – but no such differences were observed. The most likely explanation for this is that in dry soil the surfactant was not present in sufficient concentration to form a monolayer at the air–water interfaces.

In a soil at saturation, there is no air–water interface present. As soil water content decreases, the area of the soil–water interface increases and at high tensions water becomes smeared in films across particle surfaces rather than being held in pores. In air-dry soil, the air–water interface approaches the soil surface area (e.g. *c.* 5 m^2 g^{-1} for a sandy loam, up to 800 m^2 g^{-1} for a swelling clay). The area occupied by a lecithin molecule at the surface of a polar solvent is *c.* 7.5×10^{-19} m^2 (Ballard *et al.*, 1987). Therefore, in 1 ml of lecithin solution (500 mg l^{-1}, M_r *c.* 800), even assuming that all the surfactant molecules are at the solution surface, there is only sufficient lecithin to produce a monolayer covering 0.279 m^2. As soil water tension increases, the surface area upon which the lecithin must act becomes too large for complete surfactant coverage and the surface tension of the soil solution will effectively start to increase. Indeed, with the pressure plate samples, there was no discernible difference in water content caused by the addition of surfactant. The results are therefore consistent with the hypothesis that the surfactant becomes increasingly diluted per unit area of water meniscus, and consequently has a smaller effect on surface tension as the soil dries beyond 10 kPa tension.

A second possible explanation for the decreased efficacy of surfactant in dry soils is that the amount of lecithin in the samples had decreased during the experiments. In microbiologically active soils, lipids are readily decomposed (Amblès *et al.*, 1989). The drier soil samples required longer equilibration times, providing greater opportunity for microbial degradation to occur. Similarly, both the tension table and pressure plate experiments started with saturated samples, which then drained to an equilibrium moisture content. As water was removed from the samples, some surfactant will have been removed too, reducing the amount of lecithin present, especially at high tensions. This source of uncertainty is more difficult to quantify but, in reality, roots always grow in a nonsterile environment where fluxes of water are present intermittently. A root tip growing through soil produces mucilage continuously, and releases surfactant over a prolonged period. Therefore, these experiments (which involved a single application of the surfactant) are likely to have underestimated the influence that root-released surfactants may have, at least locally, in soils at high tension.

Other studies have found that saturated hydraulic conductivities and unsaturated diffusivities can be reduced by up to two orders of magnitude with the addition of surfactants such as sodium dodecylsulphate (Allred & Brown, 1994; Liu & Roy, 1995; Tumeo, 1997). Preliminary experiments to measure saturated hydraulic conductivity in re-packed cores of Bullionfield soil encountered various problems. After establishing the initial infiltration rate with pure water, subsequent flushing of cores with lecithin solution produced a significant reduction in infiltration rate. Re-flushing with pure water lead to a small recovery in the infiltration rate but not to the original value. The infiltration rate continued to decrease further during repeated cycling between water and lecithin solution. The structure of the soil core was apparently disrupted by the continuous infiltration, especially by the flow of surfactant solution through the matrix, leading to a gradual clogging of the larger transmission pores.

In summary, if plant roots can maintain sufficient levels of surfactant in the rhizosphere, then they may be able to draw water and therefore nutrients from smaller soil pores than would otherwise be accessible to them. The production of surfactants at the root surface will lead to gradients of matric potential and hydraulic conductivity extending from the root into the bulk soil. It is possible that, whilst surfactants enable more water to be extracted from the rhizosphere, the decrease in hydraulic conductivity in this zone may slow water extraction from the bulk soil.

Effects on phosphate adsorption Plant roots have evolved a range of mechanisms for increasing the availability of phosphorus, including the increased exudation of organic acids, the release of enzymes (especially acid phosphatases) and the production of root clusters (proteoid roots), allowing plants to survive in phosphorus-deficient soils (Marschner, 1995). In addition to these mechanisms, the effect of phospholipid surfactant on P adsorption is clearly to increase the amount of P in solution and therefore available to the plant. The ability of the surfactant to remove P which is already adsorbed on soil would be very important to an extending root seeking more nutrient and may offer an advantage over other organisms competing for phosphate. Clearly, lecithin is very effective at desorbing the preadsorbed P, although in this study, the initial adsorption occurred only 24 h before the subsequent desorption. The increase in phosphate desorption may be related to the apparent changes in soil structure observed in hydraulic conductivity experiments. Disaggregation by surfactants in the rhizosphere may also increase the availability of other nutrients in the region of root uptake.

Effects on soil N dynamics Although it was impossible to derive reliable mineralisation rates, the addition of lecithin to Bullionfield soil consistently reduced the rate of nitrogen conversion processes. Clearly, the presence of the surfactant

will have affected the moisture release and chemical adsorption properties of the soil microbial environment, and this may have had a detrimental effect on the microorganisms involved in nitrogen turnover. It is also possible that the lipid had a more direct effect on the microbial population. Amblès *et al.* (1989) reported that some lipids are toxic to soil microflora, although their definition of a lipid was broad and included simple hydrocarbons. The phosphatidylcholine surfactant used in this study is very similar to the lipids universally found in plant, bacterial and fungal membranes and therefore, is unlikely to be particularly toxic to soil organisms. Of course, lipid surfactants are relatively minor components of root mucilages and it may be that, in terms of microbial function, the detrimental effects of the surfactant are far outweighed by the carbon source that mucilage provides.

These experiments provide the first evidence we are aware of that plant-released surfactants will significantly affect the chemical and physical environment of soil solution in the rhizosphere, modifying soil water release characteristics and hydraulic conductivity, and also nutrient adsorption, availability and microbial turnover. The amount of lipid found in the mucilages was small. However, phospholipids are such powerful surfactants that, even at these low concentrations, considerable reductions in surface tension were observed. In soil, where the growing root tip is subject to abrasion and mechanical impedance, production of mucilage will increase (Groleau-Renaud *et al.*, 1998) and presumably, so will the production of surfactant. The root tip is continuously releasing surfactant into the surrounding soil and, whether or not soil microbes are present and producing their own surfactants, root-derived lipids will profoundly influence the properties of the rhizosphere. Furthermore, the existence of microbially produced phospholipids will reinforce the effects of decreased surface tension in the rhizosphere.

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