3 *Gaining water and nutrients (root function)*

A radicle may be compared with a burrowing mole, which wishes to penetrate perpendicularly into the ground. By continually moving its head from side to side, or circumnutating, he will feel any stone or other obstacle, as well as any difference in the hardness of the soil, and he will turn from that side; if the earth is damper on one than the other side he will turn thitherward as a better hunting-ground. Nevertheless, after each interruption, guided by the sense of gravity, he will be able to recover his downward course and burrow to a greater depth.

(Charles Darwin, The Power of Movement in Plants, *1881)*

Seedlings of *Eucalyptus globulus* which have formed an ectomycorrhizal association with the fungus, *Hebeloma*, whose white mycelium can be seen ramifying through the soil and forming basidiomes (toadstools) above the soil. (see Colour Plate xx)

Chapter outline

Introduction

Interfaces between plants and their environment extract and exchange resources on a prodigious scale. Indeed, plants provide a chemically concentrated biomass which supports heterotrophic life. Accruing organic matter through photosynthesis is fundamental to all life but should not overshadow the ability of plants to harvest inorganic resources and especially water. Roots are the primary interface for nutrient and water acquisition.

Concentration of inorganic resources by roots is as impressive as the concentration of organil resources in shoot photosynthesis. Phosphate is concentrated by a factor of thousands during absorption. Water is sucked from seemingly dry soils to maintain biological function. At the other extreme, soils are often richer in inorganic solutes than is ideal for cell function and roots must act as a screen to prevent ingress of toxic ions such as aluminium and sodium. Few soils provide a uniformly benign substrate for root growth and function. Not only are ratios of solutes in bulk soil often incompatible with metabolism but concentrations also vary through space and time to confound extraction processes further.

Variability and scarcity of inorganic resources impose intense selective pressure on roots. Root architecture is discussed in Section 3.1 with reference to important selective pressures in our environment such as low fertility. Proteoid roots are highlighted as a special adaptation to ancient and nutrient-poor soils. The physical basis of water and nutrient flow to roots is discussed in Section 3.2, emphasising limits to water extraction. Section 3.3 describes the interface between roots and soil (rhizosphere) where roots interact with other organisms in a unique chemical and physical environment. Sections 3.4 and 3.5 cover two of the most productive biological interactions, formation of mycorrhizal roots and fixation of atmospheric nitrogen. Finally, Section 3.6 gives an overall account of how water and ions find a path to the long-distance transport system in roots. Tight regulation of developmental events in roots and gene × environment interactions recur as themes for biological success. Roots can adapt to such a variety of soil conditions that few places exclude plant life.

Figure 3.1 Root systems of young (a) wheat and (b) lupin plants. Wheat, a monocot, has a dual root system. Seminal roots emerge from the seed and nodal roots (thicker roots on the outside of the picture) emerge from the crown, a group of closely packed nodes from which tillers emerge. Lupin, a dicot, has a tap root from which lateral roots emerge and which thickens with time as continued cambial activity leads to secondary growth

3.1 Root system architecture

3.1.1 Introduction

Roots keep shoots anchored and supported. A great diversity of overall architecture among roots systems is fashioned as much by soil conditions as by genotype; hard subsoils, for example, restrict roots to surface soil layers. Root systems of monocotyledons and dicotyledons are genotypically distinct (Figure 3.1). Dicots frequently develop tap roots from a single radicle that emerges from a seed. This tap root plus primary lateral roots emerging from it form a framework on which higher-order lateral roots are formed. Such a framework strengthens due to secondary thickening as a plant matures, leading to massive roots that are often seen radiating from the base of a treetrunk (Figure 3.2a). Monocots such as grasses do not have a facility for secondary thickening and develop a fibrous root system comprised of one to several seminal roots, which emerge from the seed, plus nodal or adventitious roots, which emerge from lower stem nodes. Monocot stems are typically anchored by these nodal roots, which are much stronger and more numerous than seminal roots.

Roots do much more than anchor a plant. In addition to their obvious role in taking up water and nutrients (Section 3.6), they are also a source of hormones such as gibberellins, abscisic acid and cytokinins, which modify shoot physiology. Concentrations of some hormones respond to soil conditions, allowing roots to act as sensors of soil conditions which might affect overall plant performance. Roots also act as storage organs; examples from the Australian flora are found in the Proteaceae (*Clematis pubescens*, *Stirlingia latifolia*), Portulaceae (*Calandrinia* spp.), Juncaginaceae (*Triglochin procera*) and even the bladderwort, *Utricularia menziesii*. Also very importantly for native vegetation, roots fashion soil profiles, creating niches (biopores) which can be colonised afresh each season and

which enable roots to traverse otherwise inhospitable subsoils to gain access to water at depth. Complex physical and biological interactions between roots and soil occur in the rhizosphere (Section 3.3), where bacterial activity and root exudates stabilise biopores and modify soil chemistry.

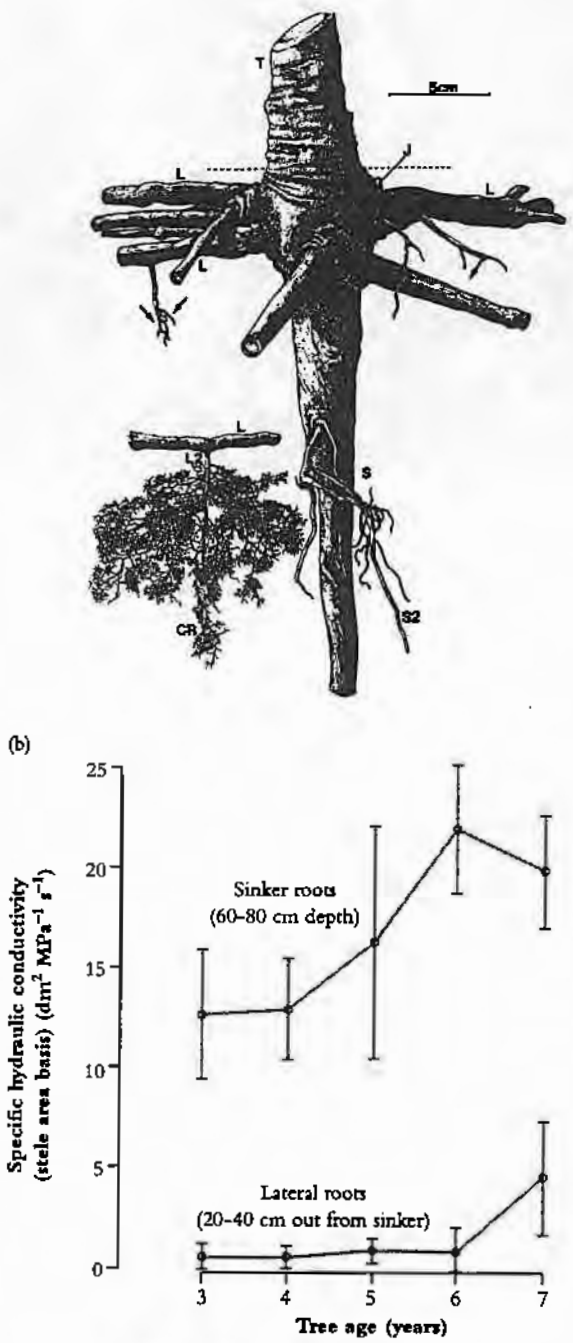

Figure 3.2 Dimorphic root system of a six-year-old *Banksia prionotes* tree growing in Yanchep, Western Australia, on a deep sand with dominant winter rainfall. The lower trunk (T) is connected through a swollen junction (J) to the root system. A system of lateral roots (L) emerge horizontally from the junction, some bearing smaller sinker roots (arrows). Other laterals give rise to ephemeral cluster roots (CR) as described in the Case study 3.1. The remainder of the root system comprises a dominant sinker root (S) which gives rise to smaller sinkers (S2). (b) Sinker roots penetrate up to 2.6 m into the sand and extract water through a low-resistance (high hydraulic conductivity) xylem pathway. Xylem in lateral roots is significantly narrower, raising axial resistance to water flow by at least one order of magnitude (Based on Jeschke and Pate 1995)

3.1.2 Root architecture and the uptake of nutrients

Total length of root per unit volume of soil (root length density, L, expressed in km m^{-3}) is often large in surface layers of the soil and typically decreases with increasing depth. Commonly, hundreds of kilometres of root per cubic metre of soil are observed near the soil surface (Table 3.1). Figure 3.3 shows L as a function of depth in a wheat crop in early spring, and under a *Eucalyptus* stand, also in spring. Dense root proliferation near the soil surface probably reflects an adaptation of plants to acquisition of nutrients such as phosphorus, potassium and cationic micronutrients such as zinc and copper). Such nutrients do not move readily in soil, hence roots branch prolifically to ensure close proximity (a few millimetres) between adsorbing surfaces and these soil-immobile ions. Roots of jarrah are also concentrated near the soil surface (Figure 3.3) but some roots penetrate very deeply to tap

Table 3.1 Densities of root systems of some annual and perennial plants expressed as root length per unit volume of soil at different depths within soil profiles. Grasses are equipped with an abundance of fine roots in surface layers that penetrate little beyond 1 m. Trees commonly have sparser root systems in surface layers than grasses but with thick axes that penetrate deeply into soil profiles. Depth of penetration is enhanced by high-density planting and can result in reduced root length densities in upper layers (see E. grandis *agroforest)*

Species	Soil depth (cm)	Root length density (km m(soil)$^{-3}$)
Pinus radiata	0–10	1.5
	10–20	3.2
	40–50	0.3
Pinus silvestris	0–15	52.5
	15–30	12.5
	45–60	3.4
	90–105	0.8
Eucalyptus grandis[1]	0–20	30 (dense planting)
(agroforest)	0–20	95 (medium density)
	0–20	110 (sparse planting)
Eucalyptus grandis[2]	0–10	81
(trial plantation)	10–20	21
	20–30	18
	30–40	14
	40–50	8
Vitis vinifera	0–20	250
Citrus sinensis[3]	0–14	30
Cereals	0–15	150
	25–50	40
Grasses	0–15	500

(Notional values from numerous sources; see Bowen and Nambiar (1984) for additional information on *Pinus*; [1]Eastham and Rose (1990); [2]R.N. Cromer, unpublished data for *E. grandis* growing as a plantation forest near Gympie (Qld) and irrigated with nutrient solution; [3]Eissenstat (1991).

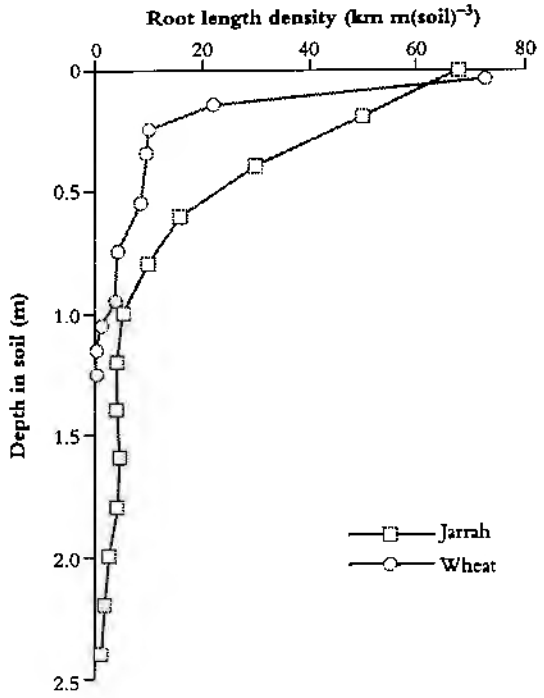

Figure 3.3 Root length density in relation to depth in the soil for a wheat crop (circles) and a jarrah (*Eucalyptus marginata* Sm.) forest (squares). Both have a dense population of roots near the surface but wheat roots barely penetrate below 1 m, whereas jarrah roots penetrate to well below the 2.5 m shown here, often to 20 m
(Derived from Carbon et al. 1980 (Jarrah); and F.X. Dunn, unpublished data (wheat))

subsoil moisture. Such water escapes the wheat roots and finishes up as ground water.

Many plants are mycorrhizal (Section 3.4). They form symbioses (mycorrhizas) with certain fungi which obtain fixed carbon from the host plant, in turn supplying the host with poorly mobile nutrients, especially phosphorus. This is achieved by proliferating their hyphae to provide a much greater surface area for nutrient uptake than could be provided by roots alone. Another adaptation, common in the Proteaceae, and also occurring in some species of lupin, are proteoid roots, clusters of tiny rootlets that greatly enlarge the available surface area for ion uptake and which are inducible by low levels of phosphorus (see case study on p. 000).

Nutrients are distributed unevenly in soil, generally being concentrated in the topsoil and also dispersed elsewhere in pockets. Surface enrichment arises from diverse sources such as dead fauna, urine patches and localised application of fertiliser. Root systems respond to enriched zones of nutrients by proliferating in their vicinity. Figure 3.4 shows an example of such a proliferation; the dense cluster of roots in the centre of the figure is a response by the row of wheat plants to application of a large pellet of nitrogen fertiliser (see arrow). Such proliferations ensure plants garner nitrogen ahead of loss to competing plants or as leachate into subsoil.

Young roots absorb nutrients more rapidly than old roots. New roots supply annual plants with abundant sites for nutrient uptake, especially during establishment. A feature of the roots of perennials is that they have a large turnover of the

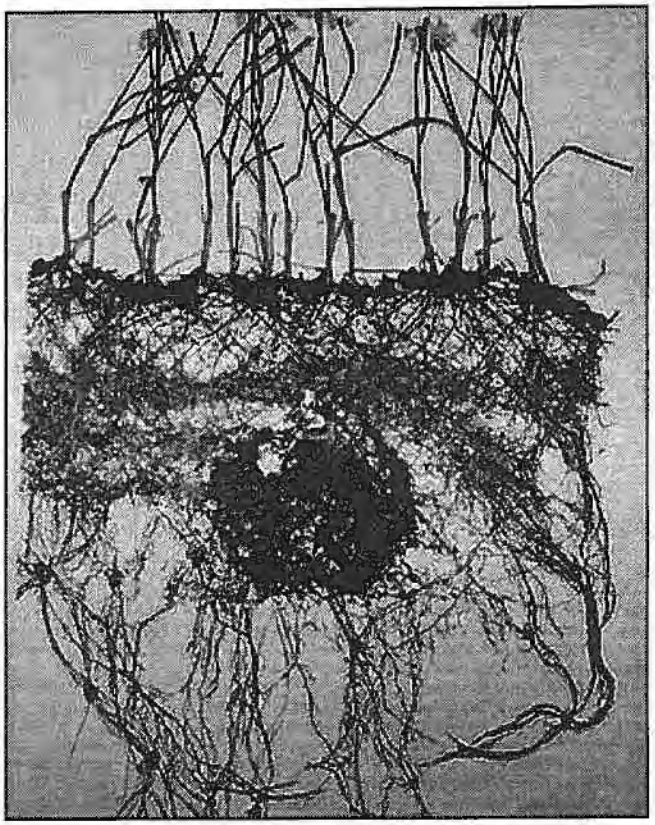

Figure 3.4 Excavated root system of wheat plants whose roots were provided with a concentrated band of ammonium sulphate fertiliser at the head of the arrow. This band is toxic at first but roots start to proliferate in its vicinity and eventually form a dense cylindrical cluster as they progressively take up the fertiliser

fine, high-order lateral roots that emerge from the secondarily thickened framework each year. This turnover drains heavily on photoassimilate, equivalent to between 40 and 90% of the standing biomass of temperate forests and half the CO_2 fixed in desert succulents (cited in Waisel *et al.* 1996). Production of fine (and often ephemeral) roots ensures uptake of nutrients over many years.

3.1.3 Root architecture and uptake of water

The dense root systems common in topsoils (Table 3.1) extract water effectively from surface soil layers. Extracting water from subsoil layers is more difficult. Australian subsoils are typically inhospitable to roots. They are dense, have a large resistance to penetration and are often sodic, that is, sodium dominates the exchange complexes on soil particles, affecting structure and water availability. Moreover, subsoils can be acutely deficient in some nutrients that are required locally by roots (e.g. zinc) or poisonously high in others (e.g. boron). Native vegetation overcomes these difficulties by forming biopores in the subsoil — highways that enable roots to create and maintain a path to water deep in the subsoil, possibly even as far as a watertable 20 m below the surface, as in the

Case study 3.1 Cluster (proteoid) roots

M. Watt

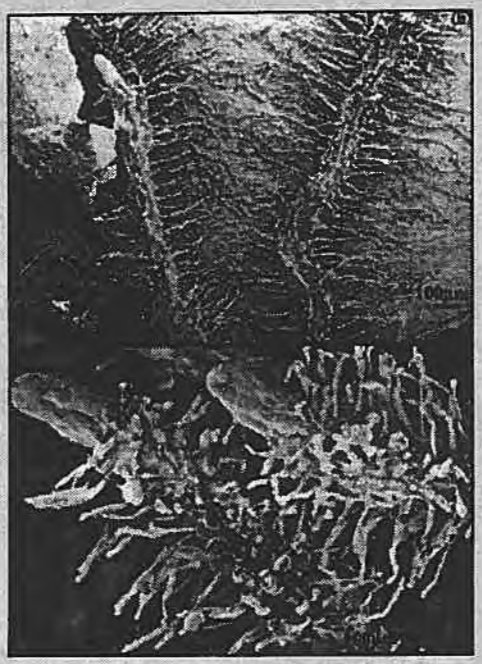

Figure 1 Cluster roots in *Banksia serrata* growing on Hawkesbury Sandstone hillslopes in the Sydney region. (a) Roots that have grown across a dead eucalypt leaf extract nutrients remaining in the decaying leaf. (b) Clusters of fine rootlets at the tips of roots increase the surface area for nutrient extraction from surrounding soil. (Scale bar ~ 1 mm)
(Original scanning electron micrograph courtesy S. Gould)

Cluster roots (Figure 1) are found worldwide in species from nutrient-deficient soils (Dinkelaker *et al.* 1995). In these soils, cluster roots enhance uptake of nutrients, especially phosphate, and help prevent soil erosion. Many species which develop cluster roots, including members of the Proteaceae where they were first described by Purnell (1960), are native to Australia. Other families such as the Casuarinaceae, Cyperaceae, Mimosaceae and Restionaceae also have heavily branched root systems (Lamont 1993); similarities in function between capillaroid roots of the Restionaceae and cluster roots of the Proteaceae might be expected as they are both major Gondwanan families. Significantly, few species with cluster roots are mycorrhizal, implying that root clusters fulfil a similar role to mycorrhizal fungi.

Australian soils generally contain low concentrations of plant -available phosphate, much of it bound with iron–aluminium silicates into insoluble forms or concentrated in the remains of decaying plant matter. Because very little of this phosphate is soluble, most roots extract it only slowly. Plants with cluster roots gain access to fixed and organic phosphate through an increase in surface area and release of phosphate-solubilising exudates (Section 3.3). Hence plants with cluster roots grow faster on phosphate-fixing soils than species without clusters, supporting claims that cluster roots are an adaptation to phosphate deficiency.

Cluster roots have a distinct morphology. Intense proliferation of closely spaced, lateral 'rootlets' occurs along part of a root axis to form the visually striking structures. Root hairs develop along each rootlet and result in a further increase in surface area compared to regions where cluster roots have not developed (e.g. 26 times in *Leucadendron laureolum*; Lamont *et al.* 1984).

Factors driving cluster root formation in relation to overall morphology of a root system remain a matter for conjecture. In the Proteaceae, clusters generally form on basal laterals so that they are abundant near the soil surface where most nutrients are found. For example, *Banksia serrata* produces a persistent, dense root mat capable of intercepting nutrients from leaf litter and binding the protecting underlying soil from erosion (Figure 1). New clusters differentiate on the surface of this mat after fires and are well placed to capture nutrients. In contrast, *Banksia prionotes* forms ephemeral clusters which export large amounts of nutrients during winter (Jeschke and Pate 1995). *Lupinus albus* has more random clusters which appear on up to 50% of roots (Figure 2).

Rootlets not only represent an increase in surface area but also exude protons and organic acids solubilising phosphate and making it available for uptake. Exudates from cluster roots represent up to 10–23% of the total weight of an *L. albus* plant, suggesting that they constitute a major sink for photoassimilates. However, not all this additional carbon comes from photosynthesis because approximately 30% of the carbon demand of clusters is met by dark CO_2 fixation via phosphoenolpyruvate carboxylase (Johnson *et al.* 1994). Because cluster roots form on roots of *L. albus* even when phosphate supply is adequate, growth of *L. albus* in soils with low phosphate availability is not restricted by an additional carbon 'drain' to roots. On the other hand, the great many species which produce cluster roots in response to environmental cues like phosphate deficiency (Dinkelaker *et al.* 1989) or seasons (e.g. *Banksia prionotes*) might experience a carbon penalty to support these roots.

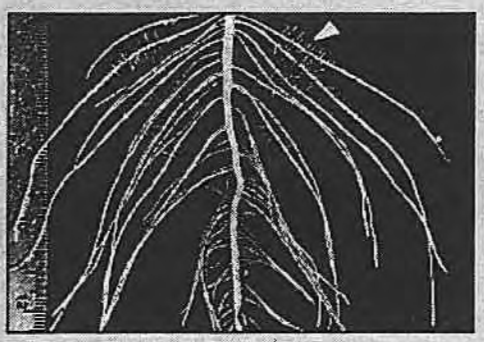

Figure 2 Basal roots of a two-week-old *Lupinus albus* plant grown in nutrient culture with 1 μM phosphate. Proteoid roots have emerged along the primary lateral roots (arrowhead) and the oldest proteoid rootlets have reached a determinate length of 5 mm. As rootlets approach their final length, they exude citrate for 2–3 days. (mm scale on left side).
(Original macrophotograph courtesy M. Natt, Research School of Biological Sciences, ANU).

Cluster roots on *L. albus* are efficient with respect to carbon consumption by generating citrate on cue. Most of the citrate exuded by clusters is released during a two to three day period when the cluster is young (Watt *et al.* 1997). A large root surface area in clusters works in concert with this burst of exudation to solubilise phosphate before it is re-fixed to clay surfaces or diffuses away (Gardner *et al.* 1981).

Form and function are thus coordinated in time and space, so that cluster roots can mine a pocket of phosphate-rich soil which would otherwise not yield its nutrients. Cluster roots are an elegant adaptation of root structure and biochemistry to nutrient-poor soils.

References

Dinkelaker, B., Römheld, V. and Marschner, H. (1989). 'Citric acid excretion and precipitation of calcium citrate in the thizospere of white lupin (*Lupinus albus* L.)', *Plant Cell and Environment*, **12**, 285–292.

Dinkelaker, B., Hengeler, C. and Marschner, H. (1995). 'Distribution and function of proteoid roots and other root clusters', *Botanica Acta*, **108**, 183–200.

Gardner, W.K., Parbery, D.G. and Barber, D.A. (1981). 'Proteoid root morphology and function in *Lupinus albus*', *Plant and Soil*, **60**, 143–147.

Jeschke, W.D. and Pate, J.S. (1995). 'Mineral nutrition and transport in xylem and phloem of *Banksia prionotes* (Proteaceae)', *Journal of Experimental Botany*, **46**, 895–905.

Johnson, J.F., Allan, D.L. and Vance, C.P. (1994). 'Phosphorus stress induced proteoid roots show altered metabolism in *Lupinus albus*', *Plant Physiology*, **104**, 657–665.

Lamont, B.B. (1993). 'Why are hairy root clusters so abundant in the most nutrient impoverished soils of Australia?', *Plant and Soil*, **156**, 269–272.

Lamont, B.B., Brown, G. and Mitchell, D.T. (1984). 'Structure, environmental effects on their formation, and function of proteoid roots in *Leucadendron laureolum* (Proteaceae)', *New Phytologist*, **97**, 381–390.

Purnell, H.M. (1960). 'Studies of the family Proteaceae 1. Anatomy and morphology of the roots of some Victorian species', *Australian Journal of Botany*, **8**, 38–50.

Watt, M., Millar, A.H., Day, D.A. and Evans, J.R. (1997). 'Organic acid efflux from the protedid roots of phosphorus limited *Lupinus albus*, *Abstracts of papers presented at the ASBMB and ASPP Annual Conferences*, Melbourne, 29 September to 2 October, P1–103, ASBMB Inc.: Kent Town, South Australia.

jarrah forests in Western Australia. Clearing native vegetation for agriculture has disconnected subsoil biopores from the topsoil, and the roots of crops may have trouble in finding them. Nevertheless it is common for roots of crops to colonise biopores; a particularly clear example is given in Figure 3.5 which shows dozens of roots clustered together in a pore about 5 mm in diameter that traverses a soil core collected from subsoil under a wheat crop.

Clearing native perennial vegetation for agriculture has generally replaced deep-rooted perennials with shallow-rooted annuals. These annuals, whether crop or pasture, do not have time to grow their deep roots into the subsoil, and consequently considerable amounts of rain may percolate beyond the reach of roots. Such water moves slowly through the deep subsoil, moving laterally as well as vertically, and in so doing often transports salt to lower parts of a landscape, discharging at the surface and leading to dryland salinity. A major challenge facing Australian agriculture is to prevent long-distance movement of this escaped water. Any long-term solution will depend on manipulating root-system architecture. Replanting agricultural landscapes with trees sometimes helps but is not a universal solution because water will not move to roots through the unsaturated zone of the soil over distances greater than about 1 m. Effects from trees are therefore quite local unless tree roots can reach a watertable where lateral movement of water is rapid.

3.2 Extracting water and nutrients from soil

Soils exhibit sharp variations in water and nutrient supply which must be accommodated by root distribution and activity. Furthermore, the supply of water and nutrients is not constant over time, with diurnal opening and closing of stomata influencing water flow to roots and affecting, in turn, mass flow of nutrients from soil to plant. Remarkably, roots can modify this heterogeneous soil solution to generate consistently large amounts of sap to support shoot activity. Plants growing in soils with adversely low or high levels of water or ions are confronted with even greater regulatory challenges.

While nutrient flow through soil and the long-distance pathways of plants is dependent on sustained water flow, other factors also exert an influence on nutrient supply to shoots. For example, ion acquisition by roots is subject to soil chemical factors such as vast variations in solubilities and mobilities of the main nutrient ions (Section 3.3). Orthophosphate, for example, diffuses through soil at least 50 times slower than potassium and 500 times slower than nitrate! Ultimately, though, any nutrient ion dissolved in the soil solution is available to diffuse along gradients in concentration or be swept to the root surface in a mass flow driven by water uptake. Once an ion arrives at a root surface, uptake process-

es begin to exert their influence, generating a sap which will enter xylem vessels and be delivered to shoots. These processes will be discussed in following sections.

Bulk flow of water, with its cargo of nutrients, is central to long-distance transport and will be the theme of this section. Water flow will be analysed in terms of hydraulic gradients and resistances through the soil–plant–atmosphere continuum. While any quantitative description of flux is dependent on the species and environmental conditions, the principles governing water and ion movement into plants are universal.

3.2.1 Where are water and nutrients found in soil?

Most soils are chemically and physically heterogeneous. Evaporation of water from the soil surface and extraction of water by roots can leave deep soil layers wetter than more superficial layers (Figure 3.6). Replenishment by rain showers first wets the surface soil, with progressively deeper layers becoming moist as water infiltrates the soil profile (Figure 3.6 — March to July). Nutrients too are often concentrated in the surface layers of the soil where biological activity is high (Figure 3.7). Deeper soil layers can have toxic levels of ions such as sodium, chloride and boron.

Models developed to describe water and nutrient extraction from soil quantitatively must take into account the uneven distribution of resources in soil, transport properties along pathways from soil to schoots and feedback signals exerted by plants to coordinate supply with internal demand. Nutrient uptake depends on water flow through the soil–root–shoot pathways (Section 3.6); some nutrients remain in the transpiration stream throughout the pathway while others interact chemically with surfaces along the route. That is resistance to long-distance transport is highly dependent on the in organic nutrient species being transported and transpirational flow rate. Indeed, the transpiration streem as a whole passes through a series of variable resistances on its route to leaf surfaces (Section 5.2). Models for water and nutrient uptake attempt to quantify these resistances in order to predict resource delivery from soil to plants.

The basic laws describing water flow through soils described below show a relationship between water status and root density. Water used by crop species has been successfully modelled using this theory of water flow but natural ecosystems have much more variable soil profiles and root distributions. Building models for nutrient acquisition depends on developing comprehensive knowledge of water flow.

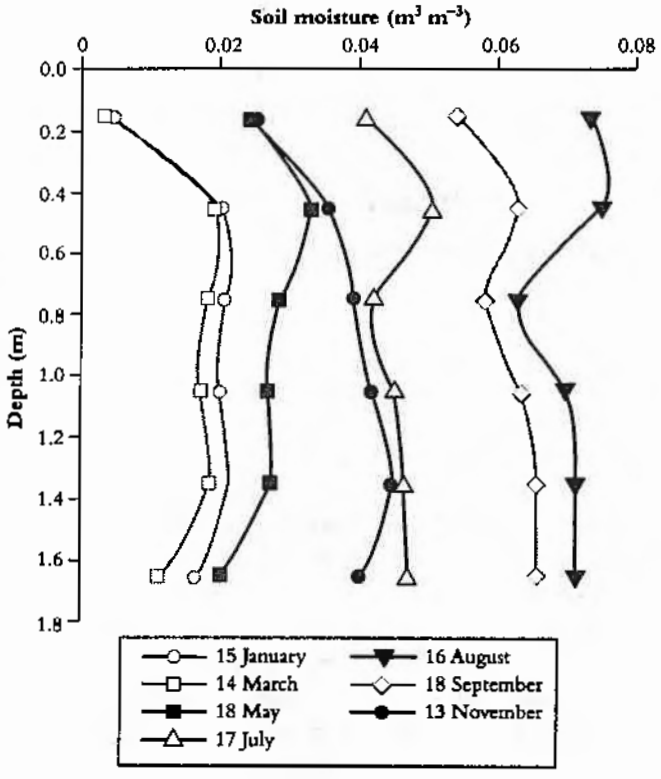

Figure 3.6 Moisture content (m^3 m^{-3}) of a podsolised sandy soil in Tasmania measured by neutron moisture monitoring to a depth of 1.65 m throughout a season. A young *Pinus radiata* stand was growing on this site. Spatial and temporal variability in water status can be observed by this technique. For example, soil became progressively wetter until late winter then began to dry in spring. In spite of the high hydraulic conductivity of this sandy soil, surface layers of the profile wetted first (March to May) then deeper soil layers became wetter towards winter (May to July). Sampling was on: 15 January; 14 March; 18 May; 17 July; 16 August; 18 September and 13 November
(Courtesy of D. Sheriff; acknowledgements to CSIRO Forestry and Forest Products and ANM Forest Management)

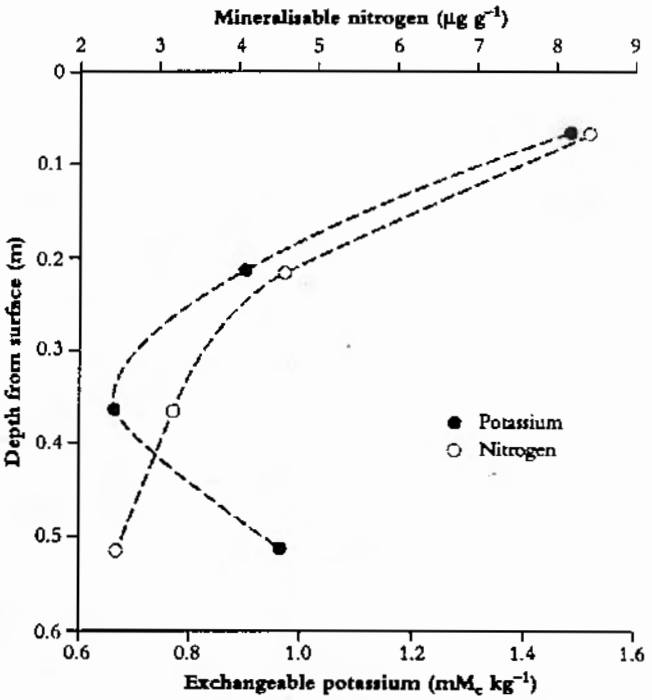

Figure 3.7 Concentrations of mineralisable nitrogen and exchangeable potassium in the top 0.6 m of the soil described in Figure 3.6 showing strong gradients in concentration of these two major nutrient resources.
(Courtesy of C. Carlyle; acknowledgements to CSIRO Forestry and Forest Products and ANM Forest Management)

3.2.2 Water flow through soil through soil

Soil is porous and holds water in its pores by capillary forces. As a soil dries, the larger pores drain and the remaining pores hold water ever more tenaciously. Water in these pores is under suction (negative hydrostatic pressure, P — Section 15.1) and this suction typically ranges from about 10 kPa to about 2 MPa in soils supplying water to plants. At suctions of less than 10 kPa, water is held in such large pores that it is likely to drain quickly away; at suctions greater than 2 MPa, most plants are at their limit for exerting sufficient suction to extract the water.

Flow of water through soil is induced by gradients in hydrostatic pressure, P. The rate of flow, F (m s^{-1}), depends on both the gradient in P and on hydraulic conductivity, K (m^2 MPa^{-1} s^{-1}), of the soil, thus:

$$F = K \frac{dP}{dx} \tag{3.1}$$

where x (m) is distance. This equation is *Darcy's Law*. Conductivity, K, varies enormously, by about a million-fold, over the range of available water content. This large range comes about because water flows much more easily in a large pore than in a small one (Poiseuille's Law — Section 5.2).

3.2.3 Calculating water depletion around roots

Water removed by transpiration results in drier soil around roots compared with bulk soil, with profound consequences for rhizosphere biology, chemistry and nutrient fluxes. As soil dries near the root surface, water flows radially from bulk soil to replenish it. Calculated distributions of water content and pore water pressure with radial distance from an absorbing root (Figure 3.8) show a pronounced increased in suction adjacent to absorbing surfaces.

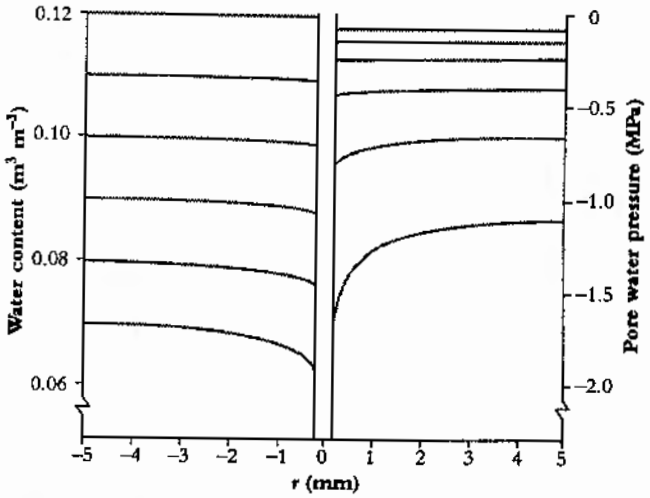

Figure 3.8 *Calculated* distributions of volumetric soil water content (left) and pressure in water-filled pores (right) as functions of distance from a model root. Pressures become more negative over time, indicating increasing suction. Horizontal lines denote water status on each of six successive days (day 1 → day 6). The steepening of curves at later times reflects how transport of water from bulk soil to the root surface becomes increasingly difficult as the soil dries. r = distance from central axis of root

Roots are cylindrical sinks for water. A radial flow of water from the bulk soil towards roots of transpiring plants is maintained by suction at the root surface. However, because K falls away with falling water content, there is a limit to how fast roots can extract water from soil. Once this limit has been reached, increasing suction by roots simply steepens the gradient in P to match the fall in K close to root surfaces so that the product of the two (Equation 3.1) remains the same.

Although soil water is driven by gradients of pressure, it is more convenient when water content is changing to describe this flow in terms of gradients in volumetric water content, θ (m^3 m^{-3}). The coefficient relating flow rate to the gradient in water content is known as diffusivity, D (m^2 s^{-1}), and the appropriate equation is formally analogous to *Fick's First Law* of diffusion:

$$F = D \frac{d\theta}{dx} \tag{3.2}$$

This equation can be elaborated to allow for cylindrical flow, and then solved to derive a simple expression to quantify the gradient in soil water content around roots as follows:

$$\Delta\theta \cong \frac{Q}{DL\pi} \tag{3.3}$$

where $\Delta\theta$ is the difference in volumetric water content between bulk soil and the root surface (m^3 m^{-3}), Q is the flow rate of water through the soil (m^3 m^{-3} s^{-1}) and L is the average length of absorbing root per unit volume of soil (root length density — m m^{-3}).

Like K, D varies with soil water content, although not so widely. Laboratory measurements of D, which are so far the only ones made with any accuracy, show a decrease of at least 50-fold as soil dries, for example in sandy loam from about 10^{-7} to about 10^{-9} m^2 s^{-1}.

The decrease in soil moisture near absorbing roots can be calculated by substituting values into this equation. In a damp (not wet) soil (water potential, ψ = −100 kPa), D might be 10^{-8} m^2 s^{-1} and L a modest 10^4 m m^{-3} (1 cm cm^{-3}). If Q, the transpiration rate, is 5×10^{-7} m^3 m^{-3} s^{-1} (about 10 mm of water lost from the surface 200 mm of soil each day), then θ at the root surface will be only 0.0015 m^3 m^{-3} less than in the bulk soil. This corresponds to less than a 0.1% decrease in water content close to the root of a transpiring plant.

Now imagine a sparser root system with undiminished transpiration; L drops to 5×10^2 m m^{-3} and D to 7.5×10^{-9} m^2 s^{-1} as soil around the roots dries. To sustain transpiration, θ would have to be 0.04 m^3 m^{-3} lower at the root surface or about 25% drier than bulk soil. Shoot water potential must then decrease as the soil around roots dries if water transport

is to be maintained. A point will be reached where resistance to water flow through soil is so great that a plant's ability to generate water potential gradients is insufficient to sustain transpiration. Drought ensues. (Chapter 15).

3.2.4 Observations of water uptake by roots

(a) Movement through bulk soil

In field soil, root length density in the topsoil is usually so high (Table 3.1, Figure 3.3) that the local rate of uptake of water is never likely to be limited by soil properties. However, in subsoil, roots become sparse and water flow through soil might limit uptake rates. Even when low root length densities are taken into account, water uptake is often much slower than simple theory would predict.

One possible reason for the discrepancy between theory and observation in water uptake by deep roots is that these roots do not ramify randomly through the soil. Subsoils are sometimes dense and difficult to penetrate so roots grow predominantly in pre-existing fissures or in continuous large pores, biopores, made by previous roots or soil fauna (Figure 3.5). A second reason is extrapolation from laboratory measurements in repacked soil of D to undisturbed soil in the field. The structure of undisturbed soil might inhibit water flow to roots. For example, soil aggregates formed naturally often result in particles of clay, usually in the form of small plates, becoming oriented parallel to the surface. Such orientation would increase greatly the path length for water flow but there are no reliable measurements of D on undisturbed subsoil to confirm this. As a consequence, water in subsoils that might be physically 'available' is not necessarily extractable by plant roots.

(b) Resistance at root surfaces

A substantial resistance to water uptake exists at the interface between soil and root, known as interfacial resistance. The interfacial boundary is only a few hundred micrometres thick and rich in organic substances secreted by the root to form a rhizosphere. Soil particles compressed by the advancing root are also embedded in this zone.

Two properties of an interfacial zone could influence water flow into roots. First, exclusion of ions at root membranes might result in a large build up of these ions outside the membranes. High osmotic pressures outside roots would impede the uptake of water (Section 17.2). This has been confirmed in a study of water uptake by lupin and radish plants (Aylmore and Hamza 1990). Exposure of roots to soil solutions containing 0–100 mol m^{-3} NaCl for only eight hours was used to analyse the impact of osmotic effects of interfacial ion build up in the absence of toxic effects. By increasing NaCl concentrations from 0 to 100 mol m^{-3}, ion concentrations at the root surface rose and water extraction from around the root declined (Figure 3.9). Hydraulic resistances for whole plants about doubled when they were grown in saline soils, presumably due to this interfacial, salt-induced resistance. Gradients are likely to become especially large as soil becomes drier because the diffusion coefficient for solutes falls by a few orders of magnitude as soil dries, so that any excluded solutes will diffuse away from the root surface only very slowly.

The second possible impediment to water flow from soil to root is that physical gaps might form at the soil–root interface, either through roots growing into pores much wider

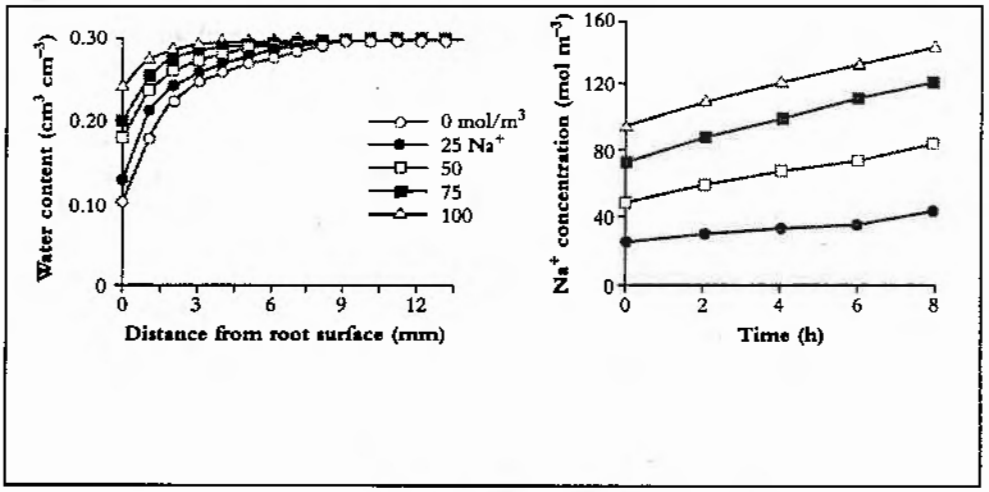

Figure 3.9 (a) Volumetric water content ($cm^3\ cm^{-3}$) in soil up to 12 mm from the surface of radish roots and (b) Na^+ concentrations at these root surfaces, monitored over an eight-hour period. Radish plants were grown for 18 days in non-saline soil at which time up to 100 mol m^{-3} NaCl was added to the soil and transpiration was elevated by fans. CAT scans were used to measure water distributions near roots and Na^+-sensitive (Na^+-LIX) microelectrodes to measure Na^+ concentrations at root surfaces. Estimates of water and ion levels were made in the top 3 cm of the soil profile. High salt treatment depressed water uptake, leaving root surfaces wetter than those in non-saline soil. Steady build up of salt around roots placed them under 'osmotic drought'

(Based on Aylmore and Hamza 1990)

than the root axis or because of roots shrinking within a pore into which they once fitted snugly. What would induce a root to shrink? A fall in water potential of roots could cause shrinkage as thin-walled cortical cells begin to collapse during water deficits. Observations made in rhizotrons (glass-walled tunnels used for observing the behaviour of roots in the field) clearly show a diurnal shrinkage in cotton roots of up to 40% where the roots are growing in large pores but we still do not know whether roots growing in intimate contact with soil particles are similarly prone to shrink. A few observations made using neutron autoradiography have shown no shrinkage in roots growing in the field (Taylor and Willatt 1983).

3.2.5 Roots responding to soil constraints

Roots respond to selection pressures imposed by temporal and spatial patterns in water and nutrient supply such as those shown in Figures 3.6 and 3.7. For example, seasonally fluctuating water tables are reflected in shifts in root growth of swamp paperbark in order to maximise extraction of non-saline water. Similarly, heterogeneous spatial distributions of water and individual nutrients call for root structures able to extract all resources required for growth. In many trees species, superficial roots deplete the enriched surface layers of soil-immobile nutrients while sinker roots tap water and soil-mobile nutrients (e.g. nitrate) which leach deeper into the soil profile. For example, dimorphic root systems of *Banksia prionotes* (Figures 3.2) are characterised by sinker roots which have almost constant resistance (high conductance) along their axes and are therefore well adapted to remove water from deep in the soil. Finer lateral roots with very low conductances appear to play a more minor role in water uptake (Figure 3.2b). *B. prionotes* also has proteoid roots which absorb much of the plant's nutrient requirements. Thus, roots have become genetically modified to accommodate the variability in resource availability that characterises each soil type and climate. Photo assimilate costs of producing such complex root systems can be high.

3.3 Soil–root interface

As a general rule, the surface area of a root system exceeds the leaf canopy it supports. Even disregarding root hairs, the interface between roots of a three-week-old lupin plant and soil is about 100 cm^2 while a four-month-old rye plant under good conditions has more than 200 m^2 of root surface (Dittmer 1937). Trees have difficult root systems to quantify but kilometres of new roots each year generate hundreds of square metres of root surface. Such a root–soil interface arises through the simultaneous activity of up to half a million root meristems in a mature tree.

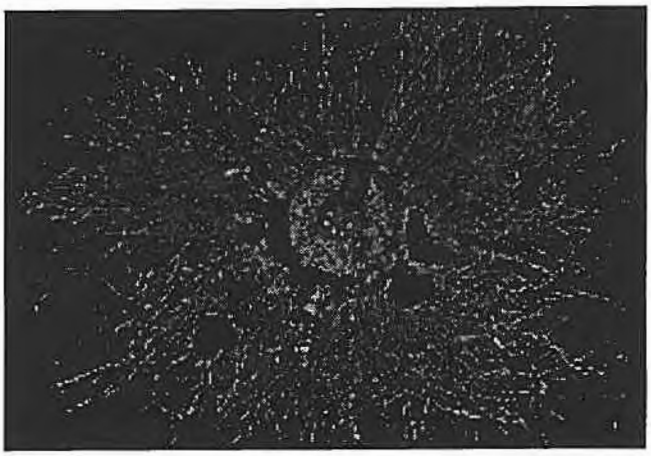

Figure 3.10 Transverse view of a young, soil-grown wheat root, sectioned by hand and stained with Toluidine Blue. Most soil in the rhizosheath was washed away during preparation, revealing many long root hairs extending from main axis (diameter 0.6 mm). Root hairs allow this root to explore 21 times more soil volume then would be possible without hairs. A lateral root can be seen extending from the pericycle which surrounds the stele (see Colour Plate xx)
(Courtesy of M. Watt)

Many roots form fine extensions to epidermal cells called root hairs, amplifying the effective surface area of the soil–root interface many times. Dittmer (1937) estimated that the surface area of root hairs in rye plants was more than that of the root axes on which they grew; similar observations have been made for trees. The aggregate *length* of root hairs in the rye plants studied by Dittmer increased 18 times faster than that of the main axes. Thus, up to 21 times more soil is explored when root hairs are present (Figure 3.10).

Anchorage and extraction of inorganic soil resources both call for a large area of contact between roots and soil. However, this vast interface is much more than a neutral interface; events within it allow resources to be extracted from the most unyielding soils. Intense chemical and biological activity in a narrow sleeve surrounding roots, particularly young axes, give rise to a *rhizosphere*.

Many root phenomena suggest a specific role for the rhizosphere. For example, roots have long been thought to find a relatively frictionless path through soils because of exudation of organic substances and cell sloughing but the chemical and physical processes that underpin this phenomenon are still quite unclear (Bengough and McKenzie 1997). Production of new roots around local zones of enrichment (Section 3.1) is made far more effective through rhizosphere activity associated with these young roots. Phosphate availability is particularly likely to be improved by the presence of a rhizosphere. Potential mechanisms will be discussed below.

Enhancement of root growth under conditions which favour high root : shoot ratios and the attendant rhizosphere surrounding those roots (rhizosheath) require a substantial input of organic carbon from shoots. Some is used in structural roles, while roots and microbes also require large amounts of carbon to sustain respiration. Even in plants growing in nutri-

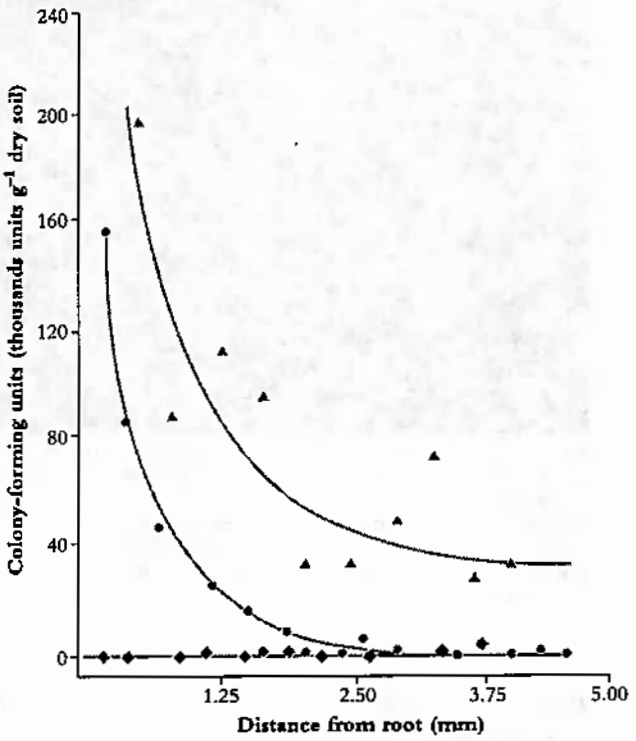

Figure 3.11 Concentration of *Enterobacter cloacae* (RP8) around wheat roots when the bacterium was introduced by inoculating seeds (circles) or soil (triangles). Uninoculated controls are shown as diamonds. Approximately 3 mm of the soil around roots supports an elevated bacterial population (Dijkstra *et al.* 1987; reproduced with permission from Marcel Dekker, Inc.)

ent-adequate, moist soils, 30–60% of net photosynthate finds its way to roots (Marschner 1995). Carbon allocation to roots can be even greater in poor soils or during drought. The rhizosphere accounts for a large amount of root carbon consumption. Barber and Martin (1976) showed that 7–13% of net photosynthate was released by wheat roots over three weeks under sterile conditions while 18–25% was released when roots were not sterile. This difference might be considered carbon made unavailable for plant growth because of demand in the rhizosphere (Section 3.3.3).

Rhizosphere chemistry and physics differ from the adjacent soil matrix and root tissues. Gradients in solutes, water and gases combine with microbial activity to produce a unique compartment through which roots perceive bulk soil. This zone of influence extends not more than 3 mm from the root axis (Figure 3.11), partly due to the low diffusion coefficients of most solutes that move through the rhizosphere (10^{-12} to 10^{-15} m^2 s^{-1} for ions such as orthophosphates). Even a relatively mobile ion such as nitrate, with a diffusion coefficient (D) of around 10^{-9} m^2 s^{-1} in soil solution, diffuses through about 1 cm of soil in a day. (Because the time required (t) for diffusion of ions is a function of the square of distance traversed (l), where $t = l2/D$ a nitrate ion would take four days to travel 2 cm, nine days to travel 3 cm and so on. Similarly, organic carbon diffuses away from roots only slowly, sustaining a microbial population as it is consumed in the rhizosphere.

Roots advancing through soil perceive a wide range of chemical and biological environments: a rhizosphere simultaneously fulfils buffering, extraction and defence roles allowing roots to exploit soils. A rhizosphere is thus a dynamic space, responding to biological and environmental conditions and often improving acquisition of soil resources. New roots develop an active rhizosphere which matures rapidly as the root axis differentiates.

3.3.1 Rhizosphere chemistry

Photoassimilate diffuses from roots into the rhizosphere where it is either respired by micro-organisms or deposited as organic carbon ('rhizodeposition'). Some of this photoassimilate loss is in the form of soluble metabolites but polymers and cells sloughed off the root cap also provide carbon substrates. Grasses undergo cortical cell death as a normal developmental process, providing further carbon substrates to support a rhizosphere microflora. Nitrogen and some other inorganic nutrients which are co-released with plant carbon are often reabsorbed by roots. Extraction of minerals from bulk soil also relies strongly on rhizosphere processes, especially near the root apices. Compounds exuded from roots interact with soil components in direct chemical reactions (e.g. adsorption reactions) and through microbially mediated events (e.g. immobilisation reactions). In addition, complex polysaccharides of microbial and root origin give rise to a gelatinous mucilage which associates with soil particles to form a rhizosheath (Section 3.3.3). Rhizosheaths have physical and chemical implications for root function. Hydraulic continuing between soil and roots is, for example, thought to be enhanced by the hydrated mucigel. Negatively charged groups on side-chains of mucilagenous polysaccharides attract cations like Ca^{2+}, providing exchange sites from which roots might absorb nutrients.

Such a diversity of chemical reactions in the rhizosphere is partly an outcome of the array of root-derived compounds. For example, phenolic compounds can be released by root cells in large amounts (Marschner 1995), both as a result of degradation of cell walls and from intracellular compartments. Release of organic acids (principally citric, fumaric and malic acids) solubilises phosphate from surfaces to which they are adsorbed in many species, including those of the family Proteaceae. A modest release of organic acids accounting for about 0.1% of the root mass each week is sufficient to enhance phosphate acquisition in a selection of annual legumes (Ohwaki and Hirata 1992). In more extreme cases, up to a quarter of the dry weight of *Lupinus albus* plants is released from cluster roots, mostly as citrate (see case study 3.1 Roots). Even the fungal hyphae of mycorrhizal eucalypt and pine roots can secrete photoassimilates, in the form of oxalic acid, causing phosphorus to be solubilised from insoluble calcium apatite (Malajczuk and Cromack 1982).

The main families of low molecular weight compounds which react with inorganic ions are phenolics, amino acids

and organic acids. Heavy metals such as aluminium, cadmium and lead are complexed by phenolics, affecting the mobility and fate of these ions in contaminated soils. Manganese is complexed by organic acids, as are ferric ions, which also interact chemically with phenolic compounds and amino acids. For example, highly specialised amino acids (phytosiderophores) can complex ferric ions and enhance uptake from soils by rendering iron soluble. Low iron status actually stimulates release of phytosiderophores into the rhizosphere (Marschner 1995). Other metals such as zinc and copper might also be made more available to the plant through the chelating action of phytosiderophores. Chemical processing by chelating agents is dependent on plant perception of nutrient deficiencies, leading to an ordered change in rhizosphere chemistry. A significant demand on photoassimilates is required to sustain chelation of nutrient ions.

Enzymes are also released from roots, particularly phosphatases, which cleave inorganic phosphate from organic sources. The low mobility of orthophosphates means that phosphatases can be an important agent in phosphorus acquisition, especially in heathland soils where the native phosphorus levels are low relative to the phosphorus-rich remnants of decaying plant material.

pH is another important rhizosphere property. Roots can acidify the rhizosphere by up to two pH units compared to the surrounding bulk soil through release of protons, bicarbonate, organic acids and CO_2. In contrast, the rhizosphere of roots fed predominantly with nitrate was more alkaline than bulk soil. A distinct rhizospheric pH arises because of the thin layer of intense biological activity close to roots, especially young roots. In addition to proton fluxes, release of CO_2 by respiring roots and microbes is likely to cause stronger acidification of the rhizosphere near root apices where respiration is most rapid.

Rhizosphere acidification affects nutrient acquisition by liberating cations from negative adsorption sites on clay surfaces and solubilising phosphate from phosphate-fixing soils. Furthermore, micronutrients present as hydroxides can be released at low pH, conferring alkalinity tolerance on those species with more acidic rhizospheres. So, the rhizosphere is a space which ensheathes particularly the youngest, most active parts of a root in a chemical milieu of the root's making. In this way, acquisition of soil resources is strongly controlled by processes within roots. Local variations within soil are buffered by rhizosphere chemistry, enabling roots to exploit heterogeneous soils effectively.

3.3.2 Rhizosphere biology

Microbial activity, sustained by photoassimilates secreted from roots, contributes substantially to rhizosphere properties. The level of microbial activity is also influenced by availability of nitrogen as a substrate for microbial growth. Soils with high

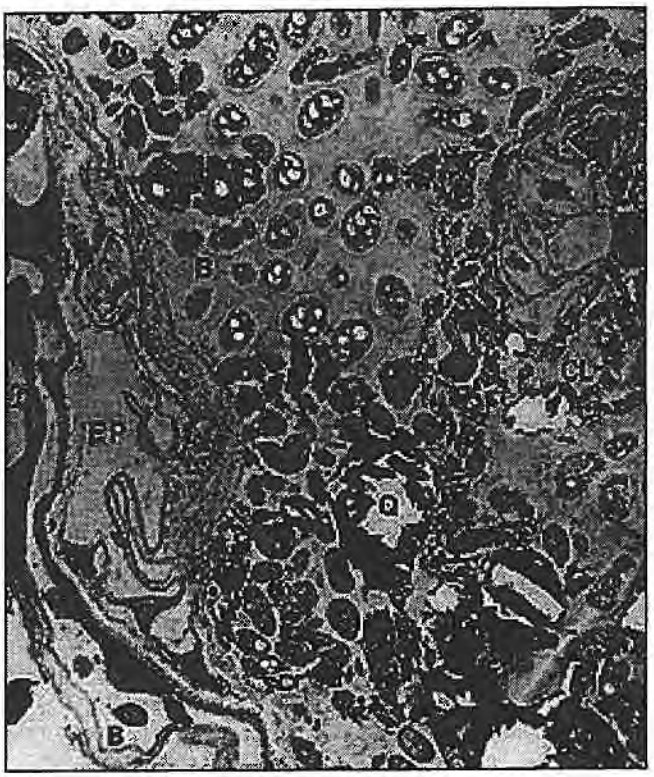

Figure 3.12 Mature rhizosphere from roots of clover (*Trifolium subterraneum* L.). The outer cortex has been crushed and epidermal cells (EP) have become distorted, leading leakage of substrates into the rhizosphere. The rhizosphere is rich in micro-organisms with bacteria (B) clearly visible. Soil (Q) and clay (CL) particles are held together in the inner rhizosphere by a mucilage of polysaccharides. Sustained losses of carbon required to maintain this microflora are thought to come from exudation and senescence of root cells. (× 10 000)
(Foster *et al.* 1983; reproduced with permission from the American Phytopathological Society)

fertility and biological activity have microbial densities 5–50 times greater in the rhizosphere then in bulk soil. The diversity of rhizosphere microflora is spectacular (Figure 3.12) and still incompletely described. Early studies in South Australia showed that *Pseudomonas* and *Arthrobacter* species are present on roots in ratios which reflect the rapid doubling-times and competitive dominance of *Pseudomonas* (Bowen and Rovira 1976). Within genera, species of *Pseudomonas* have frequencies varying up to 500-fold. In general, gram-negative bacteria, fungi and ascomycetes (Section 3.4) are most abundant.

Rhizosphere microorganisms are not uniformly distributed along roots. Apices are almost free of microbes but densities can increase dramatically in subapical zones. Very mature root axes with lateral branches are sparsely populated with microbes. In maize roots, the frequency of microorganisms is only 4% nearest the root apex, rising to 20% in subapical zones (Schönwitz and Ziegler 1986). Even within these zones, there are large variations in distribution, with radial epidermal walls of roots secreting exudates which can support huge microbial populations, up to 2×10^{11} microbes cm^{-3}.

Roots do of course influence adjacent soil throughout their length by setting up gradients of water, gases and ions. For example, in waterlogged soils leakage of O_2 from

aerenchymatous roots leads to oxidation of metal ions and local build up of aerobic microflora around roots of agricultural plants (Chapter 18). In general, however, the most active microbial populations and rates of chemical transformation in the rhizosphere occur in the subapical zones of the root. In supporting these processes, root-associated microbes metabolise inorganic nitrogen, depositing protein nitrogen in the process of immobilisation. Microbial activity also produces plant growth regulators such as auxin, cytokinins and gibberellins, possibly in amounts sufficient to influence root morphogenesis. Ethylene can also be produced by rhizospheric fungi, potentially inducing root morphological changes such as lateral root initiation.

3.3.3 Costs and benefits of a rhizosphere

Root function and overall plant performance can benefit conspicuously from processes in the rhizosphere. Infection by rhizobia (Section 3.5) and mycorrhizal fungi (Section 3.4) improve the nutritional status of many species and rhizobial strains have even been used to manipulate rhizosphere biology. Under natural conditions, intense microbial competition occurs in the rhizosphere, as seen by the success of *Pseudomonas* spp. discussed in Section 3.3.2, but the variability of soils, plant species and environmental conditions make it impossible to predict rhizosphere composition.

A significant proportion of photoassimilate is used to support a rhizosphere, reflecting the high cost of microbial activity and polymer exudation. This pattern is repeated in many species with up to 20% of plant carbon consistently lost by roots. Relative rates of microbial and root respiration are almost impossible to estimate in roots growing in undisturbed soils because of the intimacy of roots and microbes. In addition to consuming large amounts of plant carbon, microbes produce phytotoxins which can impose further restrictions on root function.

Mechanisms describing how a rhizosphere *benefits* its host are even more elusive because of the diversity of reactions in such a small space. Chelation is identified as a major influence on nutrient acquisition and might also help ameliorate ion toxicities. Physical properties of the rhizosphere are even less well understood, with questions such as root lubrication, root–mucilage shrinkage and interfacial water transport not yet resolved. Limited data do not support earlier notions of mucilage as a water-holding matrix and while secretions might help roots advance through soil, most friction is thought to be between root axes and cap cells (Bengough and McKenzie 1997). Physical properties of mucilage do not suggest it is an ideal lubricant. Whether the dynamic properties of a rhizosphere bring constant benefits to a plant or simply passively coexist with growing roots, remains a critical question.

3.4 Mycorrhizal associations

Around 90% of higher plants are infected by fungi and form mycorrhizal associations. Fungal symbiosis with a plant host is based on the provision of carbon from plant to fungus and inorganic nutrients from fungus to plant. Benefits to the host are considerable. Without mycorrhizal associations many higher plants would be unable to complete their life cycles. Both modern agriculture and natural ecosystems such as forests and grasslands rely strongly on mycorrhizal associations. Competitive advantages which come from efficient nutrient extraction from low-fertility soils have ensured that mycorrhizal symbioses have spread widely. Curiously, though, some genera have no mycorrhizas but have adapted to nutrient-poor sites in other ways (e.g. *Lupinus* and *Banksia*).

3.4.1 Main types of mycorrhizas

Mycorrhizal associations are classified according to the way in which fungi interact with a host plant root, in particular the nature of the interface that forms between host plant and fungus. This classification leads to a number of distinct types of mycorrhizal association; however, only three of these are widely distributed in the plant kingdom: arbuscular mycorrhizas, ectomycorrhizas and ericoid mycorrhizas. Mycorrhizal types generally form with a characteristic group of plant species but there are occasional examples of overlap such as eucalypts which have both arbuscular mycorrhizal and ectomycorrhizal types. Arbuscular mycorrhizas occur in a vast array of herbaceous genera (in fact, some 80% of all plant species), while ectomycorrhizas are most common in tree species (including the families Betulaceae, Pinaceae, Fagaceae, Dipterocarpaceae, Leguminaceae and Myrtaceae). Ericoid mycorrhizas are confined to genera within the Ericales, including Ericaceae and Vacciniodeae in the northern hemisphere and Epacridaceae in the southern hemisphere.

Not only do the three types differ in host preference and in the structures they form during association with the host root, they also differ in the ways by which they enhance host plant growth. Indeed, each type appears to have evolved to suit a particular soil habitat, with arbuscular mycorrhizal infection most common in vegetation that is native to regions of relatively high mean annual temperatures and rates of evapotranspiration, where mineralisation of soil organic matter to inorganic nitrogen is rapid. By contrast, ectomycorrhizal associations are largely confined to trees in habitats where temperatures and evapotranspiration are lower, leading to slower rates of decomposition and accumulation of plant litter in soil. In heathlands of polar and alpine regions, where temperatures and rates of evapotranspiration are further reduced (and in some Mediterranean-type heaths such as those found in Australia), ericoid mycorrhizas predominate.

In gross terms, key nutritional differences between these three environments are as follows. In upland soils dominated by ericoid mycorrhizas, the processes of ammonification and nitrification (conversion of organic nitrogen to NH_4^+ and NO_3^-, respectively) are severely inhibited, leading to an accumulation of organic nitrogen and rendering nitrogen the major growth-limiting nutrient. In the context of Australian heaths, nitrogen may be equally limiting in the sandy heathland soils, where its availability will rely on the (often moisture-limited) decomposition of plant debris. In forests where ectomycorrhizas dominate, ammonification occurs more readily, but nitrification remains slow and nitrogen availability is the major nutritional limitation to plant growth. By contrast, nitrification generally occurs freely in soils that favour arbuscular mycorrhizal associations. The mobility of NO_3^- in soil means that nitrogen is available relatively freely to plant roots so that phosphorus availability often becomes limiting to growth.

Each type of mycorrhizal association has thus evolved distinctive symbiotic forms to enhance host plant growth and survival (Read 1991). While such strategies endow largely nutritional benefits upon the host, mycorrhizal infection is also known to enhance plant water status, confer protection against root pathogens, contribute to soil structure via hyphal binding of soil particles and render plants less susceptible to toxic elements in some circumstances. This section will, however, focus solely upon nutritional benefits to the host plant.

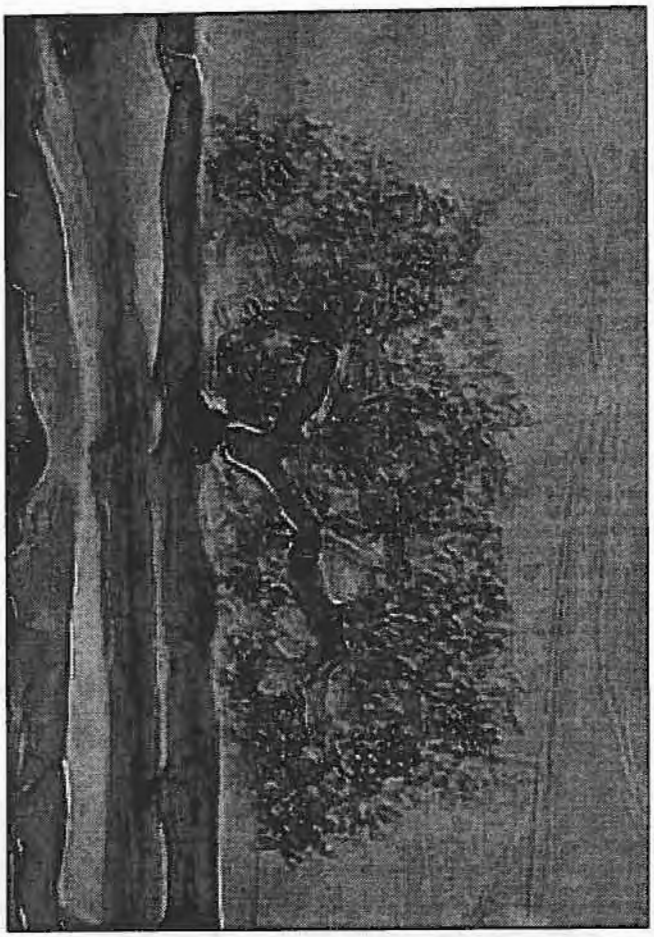

Figure 3.13 Mature arbuscule of *Glomus mosseae* which has formed in a root cortical cell of leek (*Allium porrum*). A large surface area between fungus and host cell enables exchange of organic solutes and mineral nutrients across the interface formed by the two membrane systems. Roots were cleared, stained with Chlorazol black and viewed with interference contrast microscopy (see Colour Plate xx)
(Courtesy of M. Brundett; reproduced with permission of ACIAR and CSIRO Forestry and Forest Products)

3.4.2 Fungus–root interfaces

Arbuscular mycorrhizal associations are formed by fungi from the family Glomales (Zygomycetes). During the infection process, fungal hyphae penetrate the epidermal cell layer before forming highly branched structures called arbuscules within individual cells of the cortex (Figure 3.13). Hyphae do not penetrate the endodermis or enter the stele. Arbuscules represent the major interface across which nutrient and carbon exchange takes place in arbuscular mycorrhizal symbioses. Although the plant cell wall is penetrated during infection, the host plasma membrane remains intact and apparently functional, proliferating to surround the arbuscular branches as they develop. The interface between fungus and plant thus comprises membranes of both partners separated by a region of apoplasm, a feature common to all types of mycorrhizal association (Smith *et al.* 1994). The highly branched nature of arbuscules is thought to increase the surface area to volume ratio of the host plant by up to 20-fold, providing an extensive interface across which nutrient exchange can take place. Ericoia mycorrhizal fungi (largely ascomycetes) form a similar interface between cell walls and membranes in the fine hair roots of the Ericales. Fungi penetrate the epidermal cells, forming dense hyphal coils which are surrounded by host plasma membrane, although the extent to which this increases the surface area of host plasma membrane has not yet been determined (Figure 3.14).

Ectomycorrhizal symbioses are formed largely by higher fungi in the Basidiomycotina and Ascomycotina, which form mycorrhizas with the short lateral roots of trees. Unlike arbuscular mycorrhizas and ericoid mycorrhizas, ectomycorrhizal fungi do not normally penetrate host cell walls. Rather, they form an entirely intercellular interface, with extensively branched hyphae penetrating between epidermal and cortical cells, forming a network known as the Hartig net (Figure 3.15). Depending upon the tree species involved, the Hartig net may extend as far as the endodermis, the highly branched hyphae providing a potentially vast surface area for nutrient exchange between the partners. Ectomycorrhizas are further differentiated from the other two main mycorrhizal types by the fact that the fungus forms a dense hyphal mantle around each short lateral root, sealing the normal absorptive surface of lateral roots from the soil environment to a greater or lesser extent. In such cases, the short lateral root will become entirely dependent upon the fungal symbiont for its nutrient supply.

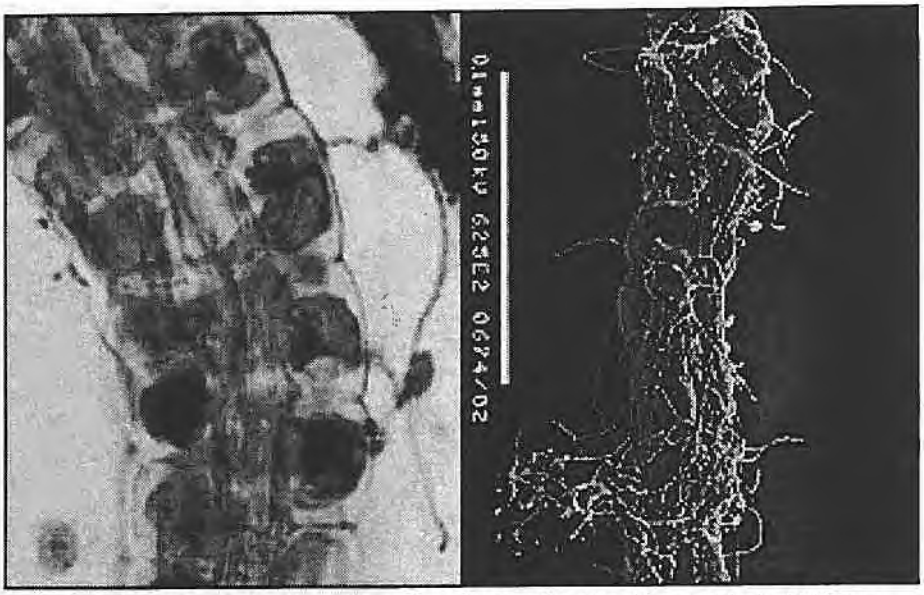

Figure 3.14 (a) Hair root of *Lysinema ciliatum*, from the Australian Epacridaceae, stained with Trypan blue showing enlarged epidermal cells containing intracellular hyphal coils. Hyphae only penetrate single epidermal cells (see Colour Plate xx). (b) Scanning electron micrograph of *L. ciliatum* showing enlarged 'balloon-like' epidermal cells and loose hyphal weft (see Colour Plate xx)
(Courtesy of K. Dixon)

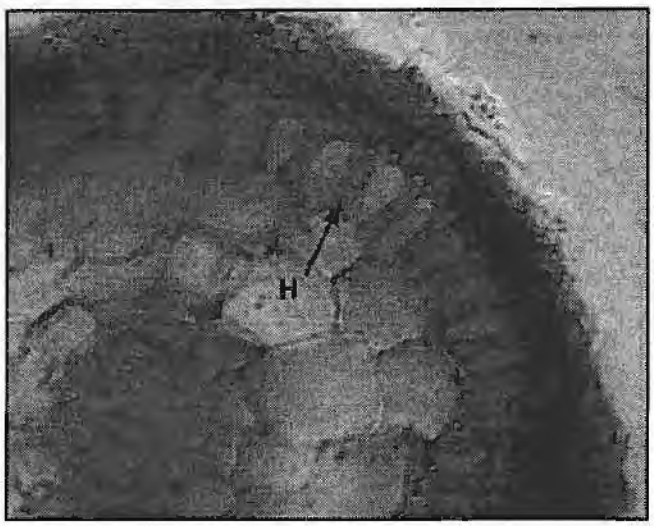

Figure 3.15 Transverse section of an ectomycorrhiza showing a Hartig net (H) in roots of *Eucalyptus globulus*. Fungal hyphae are structurally modified, making intimate contact with root epidermal cells (E) and enabling exchange of resources through the interface between fungus and host. Magnification × 300 (see Colour Plate xx)
(Courtesy of I. Tommerup and M. Brundett; reproduced with permission from CSIRO Forestry and Forest Products)

3.4.3 Functional aspects of mycorrhizas

The association between fungus and host plant delivers nutrients via: (1) mobilisation and absorption by fungal mycelia; (2) translocation to the fungus–root interface and (3) transfer across the fungus–root interface (Cairney and Burke 1996).

(a) Mobilisation and absorption of nutrients

In addition to hyphae in direct contact with the root surface, all mycorrhizal fungi produce mycelium (extramatrical mycelium) which grows from the infected root surface into surrounding soil. Both arbuscular mycorrhizal and ectomycorrhizal fungi produce copious extramatrical mycelium, with arbuscular mycorrhizal mycelia extending for several centimetres from the infected root surface and ectomycorrhizal mycelium potentially spreading for up to several metres. In either case, the mycelium extends well beyond the nutrient depletion zone for immobile nutrients around individual roots and displays a complex architecture that renders it an efficient nutrient-collecting network. Extramatrical mycelium is the component of mycorrhiza which efficiently mines bulk soil for scarce nutrients and translocates absorbed nutrients to the fungus–root interface where transfer to the host plant is effected.

Extramatrical mycelium of many ectomycorrhizal fungi spread as a diffuse mat of individual hyphae where the leading edge progressively differentiates by hyphal aggregation behind the growing front to form complex linear multihyphal structures known as rhizomorphs (Figure 3.16). Hyphae up to 35 mm in diameter at the core of rhizomorphs are devoid of cell walls and can therefore play a role in transport of unorganic nutrients or photoassimilates. In arbuscular mycorrhizas, diffuse hyphae (diameter 1–5 μm) at the growing front provide a vast surface area for nutrient absorption, while larger diameter hyphae (up to 10 μm) constitute an excellent translocatory infrastructure for efficiently moving solutes from bulk soil through the rhizosphere to the root surface (Read 1992).

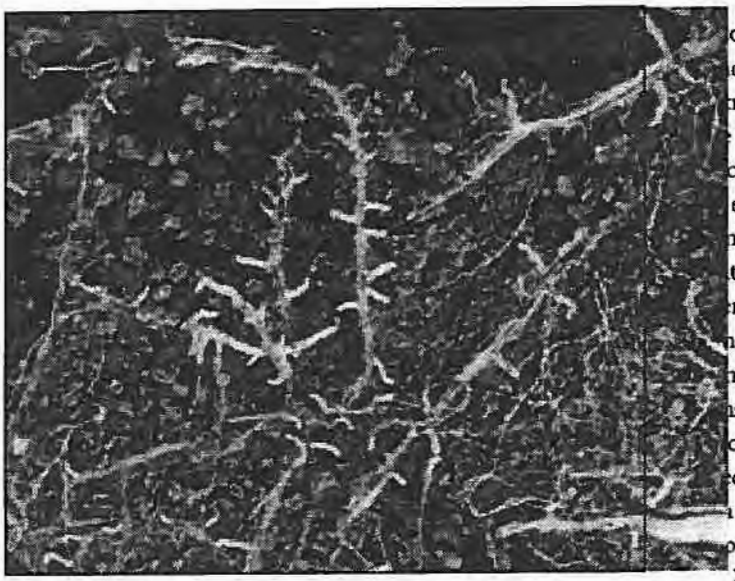

Figure 3.16 Abundant mycelium (M) of *Scleroderma* ramifies through soil forming a sheath (S) around roots of Eucalypt (E). Resultant ectomycorrhizas (arrow) benefit the host through enhanced nutrient uptake (especially phosphorus) from surrounding soil. (? see Colour Plate xx)
(Courtesy of I. Tommerup; reproduced with permission from CSIRO Forestry and Forest Products)

Many experiments have demonstrated a relationship between arbuscular mycorrhizal infection and improved plant phosphorus status. Arbuscular mycorrhizal fungi do not appear to have access to sources of soil phosphorus that are otherwise unavailable to non-mycorrhizal roots. However, extramatrical mycelium provides a large surface area for orthophosphate absorption from bulk soil through production of up to 250 m of mycelium cm^{-1} of colonised root. Increased plant absorption of nitrogen and other macronutrients such as calcium and sulphur and micronutrients including zinc and copper also appear simply to reflect the increased absorptive surface of the extramatrical mycelium. Some arbuscular mycorrhizal fungi might still be shown to extract phosphate from organic forms in soil through the action of extracellular phosphatases. Absorption of orthophosphate is maximised by the action of a high-affinity transporter which is expressed only in extramatrical mycelium of arbuscular mycorrhizal fungi during symbiosis with the plant (Harrison and van Buuren 1995).

The extramatrical mycelium of ectomycorrhizal fungi increases the absorptive area of a root system several-fold. This increase is undoubtedly important in extending the volume of soil explored by the host plant and consequently the quantity of minerals available. Ectomycorrhizal fungi, however, use additional strategies to enhance nutrient acquisition. Many secrete extracellular proteinases and peptidases that effectively hydrolyse organic nitrogen sources to liberate amino acids which can be absorbed by the fungi. Like northern hemisphere forest soils, where nitrogen mineralisation through the action of these enzymes is well established, Australian forest soils have considerable organic nitrogen which can be mineralised. Even though the rate of mineralisation by ectomycorrhizal fungi is unquantified in southern hemisphere forests, omycorrhizal-derived enzymes are likely to be of impor- ce in tree nutrition. Ectomycorrhizal fungi also produce racellular phosphomonoesterates and phosphodiesterases, latter mediating access to phosphorus sequestered within cleic acids. Some ectomycorrhizal fungi produce hydrolyt- enzymes within the cellulase, hemicellulase and lignase ilies that may facilitate hyphal entry to moribund plant terial in soil and access to mineral nutrients sequestered rein. In these ways ectomycorrhizal fungi shortcircuit con- ntional nutrient cycles, releasing nutrients from soil organ- matter with little or no involvement of saprotrophic organ- s. Ectomycorrhizal fungi also release siderophores capable complexing iron and oxalate to improve potassium uptake. ducing agents released by ectomycorrhizal fungi enhance uptake from stable oxides (e.g. MnO_2), further con- outing to host plant nutrition.

In contrast, ericoid mycorrhizal fungi produce little extramatrical mycelium and infection does not significantly increase the absorptive surface of the host root system (Figure 3.14). Hair root systems of plants in the Ericales form extremely dense mats with a potentially large absorptive area in heathland soils, reducing the need for extensive extramatrical mycelium. A major contributor to nutrient acquisition in the Ericales is production of a complex array of extracellular enzymes that can release nitrogen and, to a lesser extent, phosphorus from simple organic compounds and plant litter. Ericoid mycorrhizal fungi are important sources of these enzymes. This is of particular importance in high-rainfall, low-temperature environments where the activities of decomposer organisms are extremely limited and organic matter accumulation is large.

(b) Movement of carbon and nutrients across the fungus–root interface

Regardless of the mycorrhizal type, nutrients arrive at the fungus–root interface within the symplasm of the fungus (Figure 3.17). Transfer to the host plant involves efflux across the fungal plasma membrane and subsequent absorption from the apoplasm of the interface across the plasma membrane of the host root cells. Escape of substrates from the interface is minimised by elaborate fungal structures. Impermeable extracellular material deposited between hyphae of the mantle in some ectomycorrhizas and at the points of hyphal entry into cells in arbuscular mycorrhizas and ectomycorrhizas create a defined apoplasmic compartment. Not only does this prevent leakage from the interface apoplasm but it also means that local chemical and physical conditions can be controlled by the activities of both partners.

Ectomycorrhizal fungi derive carbon for growth and metabolism from host roots, largely as photoassimilate. Sucrose is thought to be inverted in root cell walls and glucose is then absorbed by hyphae from the interface apoplasm.

Identifying control steps in phosphate transport across a fungus–root interface has proved difficult because fungi store phosphate as polyphosphates, making it difficult to estimate

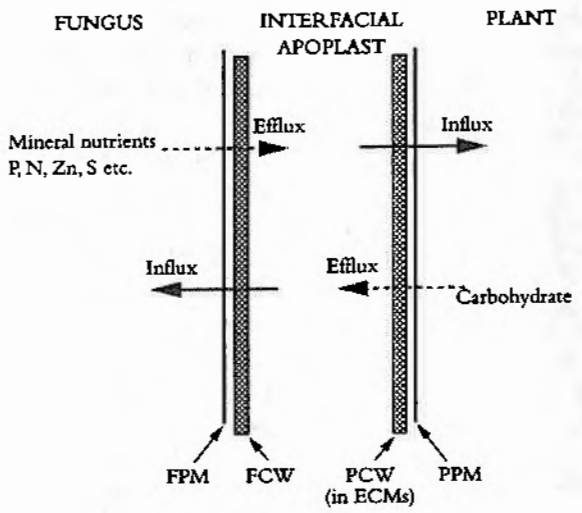

Figure 3.17 A simplified view of the symbiotic interface showing fungal (FPM) and plant (PPM) plasma membranes through which fluxes of solutes occurs. Carbohydrates efflux into the interfacial apoplasm where they become available for influx to the fungus. Mineral nutrients extracted from soil by the fungus are effluxed to the same space, making them available for the host plant. Fungal cell wall (FCW) occupies the apoplasm in all mycorrhizal associations but plant cell wall (PCW) is only present in the interface of ECMs, where fungal hyphae are intercellular. In AM and ERM associations, hyphae penetrate PCW and therefore only FCW occupies the interface.
(Based on Smith and Read 1997; reproduced with permission from Academic Press)

the concentration gradient of free orthophosphate across the fungal plasma membrane. Indeed, rates of phosphate release from these polyphosphate reserves might determine phosphate efflux rate to the apoplasm. Transport proteins in fungal and host plasma membranes must also play a central role in phosphate uptake by mycorrhizal roots and considerable effort is being made to discover the combination of phosphate transporters and channel proteins coordinating this flux. Cloning of a high-affinity transporter from arbuscular mycorrhizal fungi is a start in this search. Absorption of phosphate across the host plasma membrane is believed to be mediated by a $2H^+$/orthophosphate symporter energised by an H^+-ATPase. A quantitative picture of how mycorrhizal associations transport phosphate will require more knowledge of transport kinetics, fungal phosphate metabolism, channel gating factors (Section 4.1) and interaction between fungal and host genomes.

3.5 Symbiotic nitrogen fixation

3.5.1 Acquiring atmospheric nitrogen

Plant growth is frequently limited by nitrogen. Plants generally obtain nitrogen from soil reserves of nitrate or ammonium (so-called minetal nitrogen) but these reserves are often scarce.

Natural ecosystems can 'run down' with respect to nitrogen through soil leaching and fire. Relative abundance of nitrogen-fixing species will then increase. For example, a walk from east to west across Fraser Island, Queensland, will take you across progressively older and more nitrogen-deficient sand dunes, and from rainforest to heathland.

In agriculture, harvest of saleable commodities (animal or plant) involves a removal of site nitrogen that might be replaced by further mineralisation of soil nitrogen, import of mineral nitrogen (fertiliser) or fixation of atmospheric dinitrogen (N_2).

The earth's atmosphere is rich in N_2 (about 78% N_2) but it is very unreactive. Hydrogen will react with N_2 at high temperatures and pressures on a catalyst (Haber process, Equation 3.4). Large quantities of ammonia (NH_3) are produced by this method for industrial and agricultural use. Amazingly, some prokaryotes have the ability to catalyse this reaction, with the enzyme nitrogenase donating at least four pairs of electrons to every N_2 molecule to effect reduction to two NH_4^+ and at least one H_2 (Equation 3.5). Efforts are continuing to duplicate biological N_2 fixation in a 'test tube', as a cheaper alternative to the Haber process.

$$N_2 + 3H_2 \rightarrow 2NH_3 \qquad (3.4)$$

(reaction at 100–1000 atm, 400–550°C, catalysed by Fe)

$$N_2 + 16ATP + 8e^- + 10H^+ \rightarrow 2NH_4^+ + H_2 + 16ADP + 6P_i \qquad (3.5)$$

(reaction at ambient conditions, catalysed by the Fe–Mo-containing enzyme, nitrogenase)

Biological N_2 fixation is energetically expensive even though it occurs at ambient conditions — estimates fall between 3 and 7 g carbon respired g^{-1} nitrogen fixed (Layzell 1992). Photoassimilate consumed to support N_2 fixation is unavailable for other processes such as growth. Consider a crop fertilised with 140 kg N ha^{-1}. An N_2 fixer could replace this fertiliser, but only at a cost of at least 420 kg C ha^{-1}. As most plant dry matter contains 40% carbon, this is equivalent to a loss of one tonne of dry matter per hectare!

In non-agricultural systems, high rates of N_2 fixation can give a species a nutritional advantage over competitors, but again only by drawing on photoassimilates. Legumes tend thus to be colonisers of environments low in mineralised nitrogen, where they hold a competitive advantage.

3.5.2 A range of N_2-fixing associations

Nitrogen-fixing bacteria can be free-living in water or on solid substrates like soiil or rocks. Sandstone buildings discolour black, for example, because of the presence of an N_2-fixing cyanobacterium. Nitrogen released by decay of such organisms can then be used by plants.

Table 3.2 Symbioses between N_2-fixing prokaryotes and vascular plants

Prokaryotic symbiont		Vascular symbiont		Location
Class	*Genus*	*Division*	*Family/Genus*	
Cyanobacteriales	*Anabaena*	Pteridophyla	*Azolla*	Extracellular pocket
	Nostoc	Cycadophyta	All genera?	Intercellular in nodules
	Nostoc	Anthophyta	*Gunnera*	Intracellular, in glands
Actinomycetales	*Frankia*	Anthopyta	Betulaceae, Casuarinaceae, Coriariaceae, Datiscaseae, Elegnaceae, Myricaceae, Rhamnaceae, Rosaceae	Intracellular, in nodules
Eubacteriales	*Acetobacter*	Anothypta	?Saccharum	Rhizosphere, intercellular
	Azospirillum	Anthophyta	?	Rhizosphere
	Azobacter	Anthophyta	?	Rhizosphere
	Rhizobium	Anthophyta	*Parasponia*	Intracellular, in nodules
	Rhizobium		Leguminosae	Intracellular, in nodules

(Adapted from Sprent 1984)

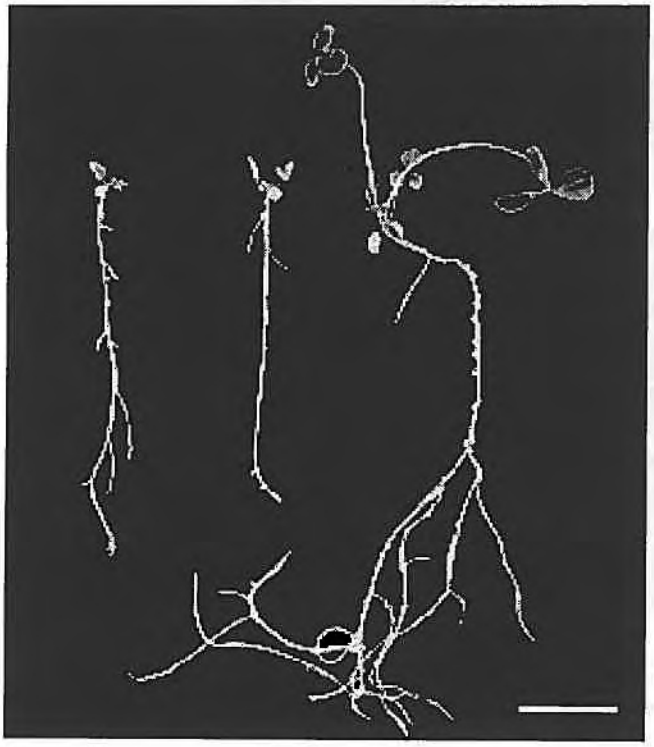

Figure 3.18 An effective symbiosis between white clover (*Trifolium repens*) and nitrogen-fixing bacteria (*Rhizobium leguminosarum* bv. *trifolii*) supports vigorous growth in host plants on a nitrogen-free growing medium (right side of illustration). In that case, a positive interaction has occurred between host and bacterial genes for both nodule formation (*Nod*$^+$) and nitrogen fixation (*Fix*$^+$). By contrast, a genetic mutant *Nod*$^+$*Fix*$^-$ (centre specimen) results in formation of a few rudimentary nodules that lack nitrogen-fixing capacity, while host plants show no nodulation response to *Nod*$^-$ bacteria (left side of illustration). (Scale bar = 1 cm)
(Original photomicrograph courtesy Barry Rolfe)

However, some plants have evolved a tighter relationship with N_2-fixing bacteria, involving an exchange of carbon and nitrogen between plant host and partner. Several different types of these symbioses have evolved (Table 3.2). Roots or leaves of some plants form a loose association with N_2-fixing bacteria, with plant exudates used as a carbon source by the bacteria. In *Azolla*, the cyanobacterium *Anabaena* is located in cavities on the underside of modified leaves, with a secretory trichome delivering sugars and absorbing fixed nitrogen. In other plants, the N_2 fixer is located in intercellular spaces of the host plant, as reported for sugar cane. These less intimate associations supply host plants with substantial amounts of nitrogen.

In more highly developed associations, plants localise the symbiotic association within a modified root or 'nodule' (Figure 3.18). In cycads, the microsymbiont *Anabaena* is located in intercellular spaces of the mid-cortex of short, highly branched, modified roots (Figure 3.19A and B). In another class of symbioses, the actinorhizal plants, the microsymbiont *Frankia* (an actinomycete, or filamentous bacterium) is located within the cortical cells of a modified root. This group includes the genus *Casuarina*. *Parasponia* is the only non-legume known to form an association with the rod-shaped bacterium *Rhizobium*. Unlike legumes, the *Parasponia* nodule has a central vascular bundle and the microsymbiont is always encapsulated within cellulosic material (termed a 'persistent infection thread').

3.5.3 Rhizobial associations

Nodules formed by members of the family Leguminoseae have a central zone of infected cells, surrounded by a cortex of uninfected cells (Figure 3.19C). A root vascular strand branches within the cortex of the nodule. This structure is quite distinct from nodules of the cycads or actinorhizal plants, which have a central vascular bundle and an infected cortex (a typical root vascular anatomy). Different legume species display various nodule growth patterns, but they can be roughly classified as either of indeterminate growth (i.e. with an apical meristem and consequent elongated shape) or determinate growth (i.e. a spherical meristem which ceases activity at nodule maturity).

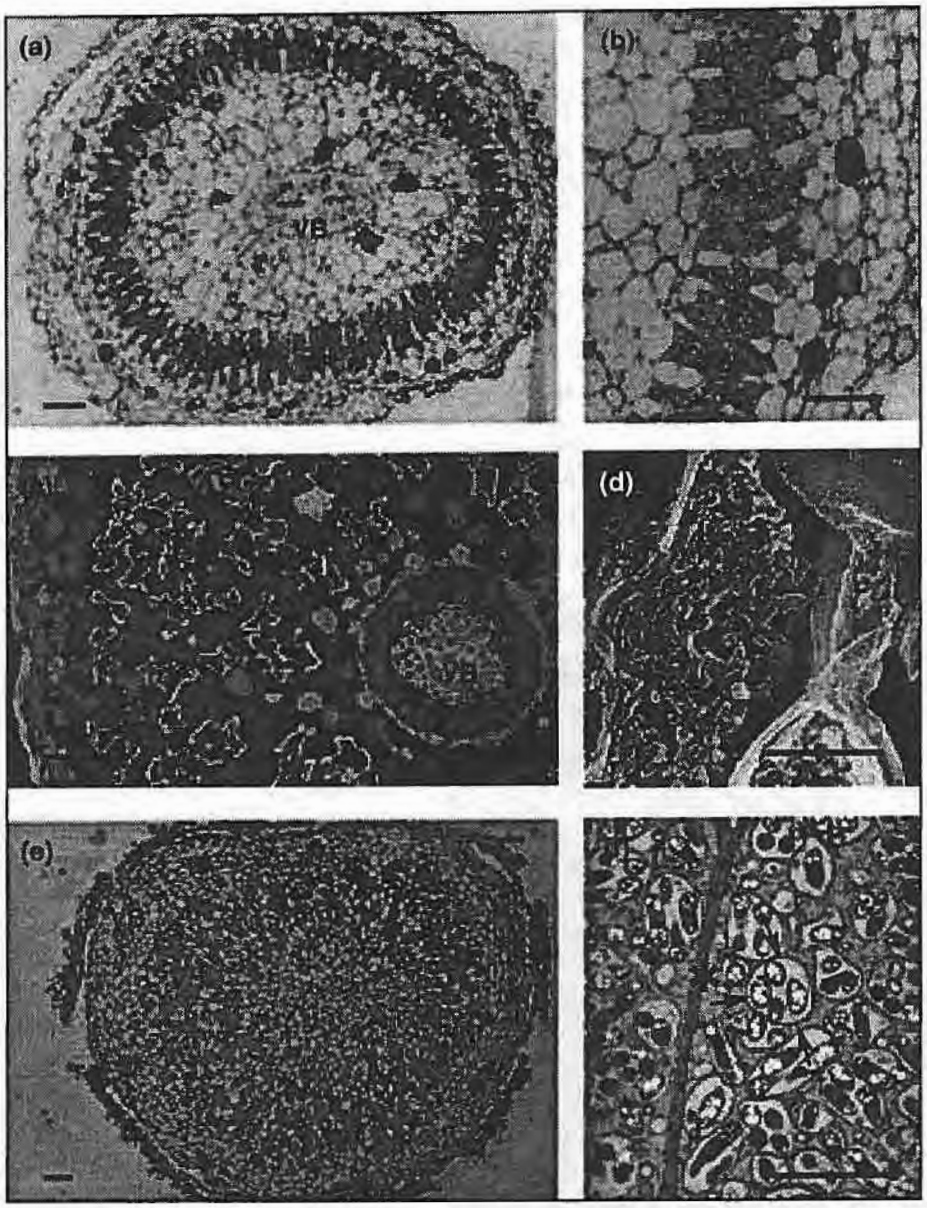

Figure 3.19 Nodule anatomy showing (a) a cycad (*Macrozamia miquellii*) nodule consisting of a central vascular strand (VB) and an infected cortical region (stars); (B) a cyanobacterium, a microsymbiont, is located in the intercellular spaces of this infected cortex; (C) a nodule of the river-oak (*Casuarina cunninghamii*) consisting of a central vascular bundle with infected cells in the cortex (arrows) identified by suberisation and lignification of their walls (section stained with berberine sulphate and viewed under epi-fluorescence optics); (D) scanning electron micrograph of an actinomycete microsymbiont (a filamentous bacterium) encapsulated within threads (arrows) throughout the plant cytoplasm; (E) a legume (*Macroptilium lathyroides*) nodule consisting of a central infected region with scattered infected cells (arrows) enclosed in a cortex. Vascular strands (VB) are present in the cortex; (F) transmission electron micrograph of a soybean (*Glycine max* L.) nodule containing a microsymbiont enveloped by plasma membrane to form 'symbiosomes' — packets of bacteria within the cell cytoplasm (arrows). Bar represents 100 mm in A, B, C and E; 5 μm in D and F.

The association between rhizobia and legumes is a controlled infection. Typically, the bacterial partner infects the plant through root hairs, and is then encapsulated by polysaccharide material produced by the host plant, forming infection threads. Infection threads then grow into the root cortex, while bacteria multiply within each thread. Finally, bacteria are released from the infection threads and engulfed by plant cells in a form of phagocytosis. This process results in a bacterium (sometimes several) encapsulated by a plant cell membrane (Figure 3.19D). Encapsulating membranes control the delivery of photoassimilate to bacteria, thus ensuring a symbiotic rather than a parasitic relationship. These units are termed 'symbiosomes' (Figure 3.19F).

Evolution of this partnership might be similar to that of other endosymbionts such as mitochondria and chloroplasts. Perhaps a future step in the evolution of a legume–rhizobial symbiosis will be retention of bacteria within plant cells to create a new organelle! If this were to happen, the legume would no longer be dependent on the presence of a microsymbiont for infection. Cells could maintain a low resident

population of the new organelle, like plastids in non-photosynthetic tissue, and allow proliferation under set conditions within nodule structures.

In some legume symbioses, bacteria are not released from infection threads. This character is one of several that distinguish each of the three legume subfamilies Caesalpinoideae, Mimosoideae and Papilionoideae (e.g. cassia, acacia and soybean, respectively). The Caesalpinoideae are largely trees or shrubs, and the few species which nodulate have little nodule mass proportional to plant biomass (Sprent and Raven 1985). In most of the caesalpinoid species that do nodulate, the microsymbiont remains encapsulated in an infection thread throughout the life of a nodule. In some species the infection threads are thin walled, while in others bacteria are released into the cytoplasm (Figure 3.19E). Naisbitt *et al.* (1992) suggested that this variation represents an evolutionary sequence within extant species. The Papilionoideae is considered the most advanced of the legume subfamilies.

Biological interactions between host plant and bacterium are subtle. Just as legumes vary genetically, so do the rod-shaped bacteria (rhizobia) that infect various legumes. Not all rhizobia are equally infective (able to infect and form nodules) or effective (able to fix N_2) on all legumes. An appropriate bacterial partner must therefore be matched genetically with each legume for optimal N_2 fixation. The *nod* genes of rhizobia encode proteins which catalyse synthesis of *nod* factors, specific compounds responsible for recognition of the bacterium by a host legume. Pure cultures of rhizobia are produced commercially, generally in a peat-moss-based medium, for inoculating legume seed prior to planting.

3.5.4 Linking functions with structures

(a) Protecting nitrogenase from O_2

A basic conflict arises in sociological N_2 fixation: nitrogenase is destroyed by O_2, yet aerobic respiration is essential to sustain the high energy demand of N_2 fixation. Nitrogen-fixing bacteria must be protected from O_2, while a level of aerobic respiration occurs in the host cell cytoplasm. In cycads, cyanobacteria provide their own O_2 protection. Nitrogenase is located in specialised cells (heterocysts) which have an O_2-impermeable lining of glycolipid. An analogous structure (a vesicle) affords protection to nitrogenase in the microsymbiont *Frankia* within most actinorhizal nodules (see Feature 3.1). In *Parasponia* and most caesalpinoid nodules the persistent infection threads provide O_2 protection to nitrogenase (Sprent and Raven 1985).

There is one major problem with structures of 'fixed' resistance. As respiration rate varies (i.e. O_2 flux), O_2 concentration inside the structure must also vary: following Fick's Law of diffusion, O_2 *flux* into the nodule will change in proportion to the O_2 *concentration* gradient at constant resistance. Free water bathing the nodule is equilibrated with the atmosphere (20.8% O_2), therefore containing approximately 360 μM O_2 in solution, while nitrogenase is destroyed by submicromolar concentrations of dissolved O_2. For practical purposes then, an O_2 gradient of 360 (outside) to 0 (inside) μM must be maintained. If respiration rate was halved without a change in resistance, would the O_2 concentration gradient also halve from 360 to 180 mM. An O_2 concentration of 180 μM O_2 would destroy nitrogenase. So, resistance must vary too.

A legume-nodule cortex copes with variations in respiration rate by providing a variable level of O_2 protection (Layzell and Hunt 1990). According to their model, a layer of cells adjacent to the infected zone either lacks radial intercellular spaces (preventing inflow of O_2) or has intercellular spaces filled with water. The thickness of this layer could vary under osmotic control to set nodule permeability. Diffusion of O_2 through water is about 10 000 times slower than through air, so flooding of radial air spaces in the nodule cortex would be an effective way of decreasing O_2 diffusion into infected tissue.

Any O_2 leaking through this cortex can diffuse freely in the intercellular air spaces of infected tissue and dissolve in the cytoplasm of infected cells. O_2 gradients which might be expected within infected cells because of rapid bacterial respiration are largely avoided by the presence of leghaemoglobin (Lb) (a molecule similar to the haemoglobin in mammalian blood). O_2 diffuses to Lb molecules where it is bound to form high concentrations of oxygenated Lb (estimated at 0.7 mol m^{-3} by Bergersen (1982). Effective nodules are pink because of oxygenated Lb; indeed this colour change can be used to estimate free O_2 concentrations. Soybean nodules seem to regulate the free O_2 in infected cells at between 5–60 nM (e.g. Layzell and Hunt 1990). Finally, residual O_2 diffuses through the symbiosome to bacteria, supporting a level of aerobic respiration.

'Conventional' chemistry may not be appropriate when describing O_2 movement in cells because O_2 molecules in cellular compartments are so scarce. A sphere of 1 μm radius — roughly the size of a mitochondrion or bacterium — containing a solution with 10 nM O_2 will contain only 24 molecules of O_2.

(b) Carbon supply and nitrogen export

Nodules are metabolically highly active. A typical maximum rate of nitrogenase activity in soybean nodules, as measured by gas exchange (discussed below) is 300 μmol electron pairs g^{-1} (nodule) h^{-1}. This value is useful to bear in mind when reading the literature about N_2 fixation, with low values possibly indicating unhealthy or disturbed plants. As nitrogenase is at best 75% efficient, with respect to N_2 fixation (Equation 3.5), this rate is equivalent to the fixation of some 150 μmol N g^{-1}(dry weight) h^{-1}. Reduced nitrogen is exported from nodules to the host plant while carbon is imported into the nodule, supporting energy needs of fixation (through respiration) and providing carbon skeletons for packaging nitrogen as an organic molecule.

Photoassimilate (host to nodule) and nitrogen-based resources (nodule to host) must pass through the endodermis (Figure 3.18C) of nodule vascular bundles. Radial walls of this endodermis have Casparian bands and tangential walls have relatively few plasmodesmata, so this cell layer restricts apoplasmic and symplasmic flow of carbon into nodules and nitrogen out of nodules (Brown *et al.* 1995). Transport pathways in nodules are still being elucidated but sucrose probably moves via the symplasm from host plant phloem to infected cells. Assimilated nitrogen is present in the apoplasm, and probably moves in both apoplasm and symplasm from infected cells to vascular bundles before moving symplasmically through the endodermis. Membrane unloading into the vascular apoplasm is aided by either several layers of pericycle cells or a single layer with extensive wall ingrowths that serve to increase membrane surface area ('transfer cells'; Chapter 5). The concentration of nitrogenous solutes in the xylem apoplasm causes a hydrostatic pressure to develop, and this results in a mass flow of nodule xylem sap to adjacent roots. The water that accompanies sucrose entering the nodule as phloem sap is re-exported with assimilated nitrogen in the xylem. Nodules are thus analogous to 'glands' that secrete nitrogenous compounds.

FEATURE ESSAY 3.1 Protecting nitrogenase from oxygen

W.B. Silvester

While dinitrogen (N_2) fixation occurs in many free-living bacteria in soil, it is only when bacteria such as rhizobia and *Frankia* enter symbiotic relationships with a plant that really large quantities of nitrogen are fixed. Crop, livestock and wool production in Australia and New Zealand are highly dependent on plant protein derived from N_2 fixation in nodules of legumes (e.g. lupins, lucerne and clovers). In these systems, solar energy drives both photosynthesis and biological reduction of N_2 thereby providing a basis for low-energy, efficient production.

Biochemical conversion of N_2 to ammonia (NH_3) by bacteria is energetically expensive and extremely sensitive to O_2 (Section 3.5). Both proteins that make up the nitrogenase complex are irreversibly denatured by even the slightest 'sniff' of O_2 and all systems that support nitrogenase have evolved mechanisms of creating a low O_2 environment at the site of N_2 fixation. Nodule respiration exerts a major influence on O_2 partial pressure (pO_2) in nodules because it consumes O_2; an interesting affirmation of the central role of O_2 is provided by the problem of *cold lability* of nodules.

It is common practice in physiological work to harvest tissues onto ice as a way of slowing down metabolism, preventing loss of substrates and retaining tissue integrity. However, during the late 1960s, Esam Moustafa, working at Palmerston North in New Zealand showed that chilled legume nodules lost most of their activity after warming up, compared to control nodules which were kept at air temperature during harvest. This phenomenon of cold lability defied explanation until the work of Fraser Bergerson in Canberra and others demonstrated the pivotal role of O_2 and respiratory O_2 uptake in modulating nodule function. When nodules are chilled, respiration slows down (Q_{10} about 1.8) but O2 continues diffusing into the nodule (Q_{10} about 1.1), so that lower temperatures disproportionately affect respiration. The build up of O_2 due to lowered respiration and higher solubility of O_2 at lower temperatures conspire to destroy nitrogenase enzymes in chilled nodules.

Meanwhile, we were working with a group of non-leguminous nodulated plants which were physiologically and geographically distinct from legumes. These actinorhizal plants are found in temperate or cool temperate areas and have representatives in a wide variety of angiosperm families. They include *Casuarina* (sheoke) in Australia; *Coriaria* (tutu) in New Zealand and *Alnus* (alder). They have major roles as N_2 fixers and while the nodules look superficially like those of legumes, they form through a symbiosis with a different bacterium, *Frankia*.

Frankia was first isolated in 1978 and shown by John Tjepkema soon after to fix N_2 in air which contains 21% O_2. He also showed that actinorhizal nodules are highly aerated structures. This is in strong contrast to legume nodules which maintain a very low pO_2 within the infected zone and to *Rhizobium* which in pure culture will only fix N_2 at very low pO_2.

We became interested in the mechanisms whereby *Frankia* and actinorhizal nodules are able to cope with atmosphere levels of O_2. *Frankia* is a rather complex bacterium, which produces both sporangia and a highly specialised cell called a vesicle (Figure 1). We imagined the vesicle might be the site of N_2 fixation and therefore provide a clue to O_2 protection in this bacterium. Working with two graduate students, Richard Parsons and Sharon Harris, we probed the possibility of modifying the ability of *Frankia* to cope with O_2.

When we grew *Frankia* in stirred cultures gassed with various pO_2, we found that the bacteria were very sensitive to transfer from one O_2 level to another. Abrupt O_2 shocks killed them. If cultures were not shocked, it was possible to grow *Frankia* at almost any O_2 level. Thus, when cultures are grown at low O_2 (e.g. 5 kPa), optimum pO_2 for nitrogenase is 5 kPa; likewise it is possible to grow cultures at 70 kPa O_2

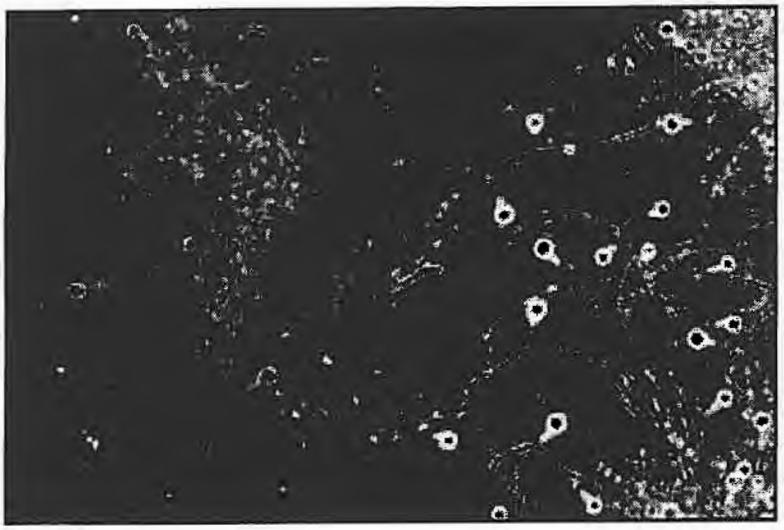

Figure 1 Dark-field microscopy showing *Frankia* (strain Ccl3) grown at (a) 3 kPa O_2 and (b) 60 kPa O_2. *Frankia* consists of fine hyphae (*c.* 1 μm diameter) and when induced to fix N_2 it produces rounded terminal vesicles 2–3 μm in diameter. Vesicles respond dramatically to O_2 level by producing a thickened envelope (see Colour Plates xx and xx)

(over three times atmospheric levels) and nitrogenase activity optimises at around 70 kPa O_2.

This mechanism for exquisite adaptation to extreme O_2 levels, and ability to express nitrogenase at very high pO_2 only became clear by using some interesting optical techniques. John Torrey and colleagues had shown that the vesicle is surrounded by a thick envelope of multilayered lipid which is assumed to be an O_2 diffusion barrier, and we proposed that this envelope must adapt to the O_2 level at which *Frankia* is growing. We hit upon dark-field microscopy as a way to visualise the vesicle walls. This technique relies on light from a steep angle outside the field of view in a microscope being refracted off surfaces such as cell walls. Lipid material is highly refractive and shows up particularly well under dark-field. Vesicles from *Frankia* cultures grown at 3 kPa O_2 are very thin walled, but at high pO_2 dark-field images show a massive wall around the vesicles (Figure 1).

Lipids are difficult to visualise under most light and electron microscopes and the only way in which the envelope could be seen was to use freeze-fracture electron microscopy. We collaborated with Stan Bullivant at Auckland University to obtain freeze-fracture views of the envelope and confirmed that at high pO_2 the vesicle may have over 100 layers of lipid tightly packed into a thick envelope (Figure 2).

While the vesicle envelope obviously responds to O_2 levels in free-living *Frankia*, what happens in root nodules? Is the nodule an essential part of the O_2 protection mechanism as it is in legumes? We gassed root systems of a variety of nodulated actinorhizal plants with various pO_2 and showed that *Frankia* nodules could likewise adjust to ambient pO_2. However, unlike legume nodules, there seem to be several different mechanisms of O_2 protection. In *Alnus* nodules, for example, vesicle envelopes are the important site of adjustment, while in some other nodules, vesicles do not form and we postulate that the host cell wall is an important O_2 barrier. Just to make matters more complex, some *Frankia* nodules, particularly *Casuarina*, also have

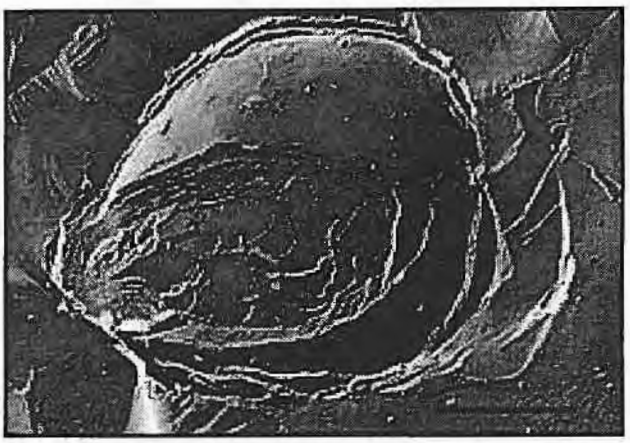

Figure 2 Freeze-fracture electron micrograph of a vesicle of *Frankia* grown at 40 kPa O_2. Note multiple lipid layers in the vesicle envelope. (Scale bar = 1 μm)

leghaemoglobin and function more like legume nodules. We now accept that actinorhizal nodules run the full range of physiologies: *Casuarina* resembles legumes, relying on low internal pO_2 and oxygenated leghaemoglobin, whereas *Alnus* has very prominent vesicles which apparently provide much of the O_2 protection.

No symbiotic mechanism for O_2 protection of nitrogenase has evolved as the most efficient. If, in an *Alnus* symbiosis, a vesicle is proven to be the prime site of O_2 protection, this has some implications for developing novel symbioses. Protection against O_2 in legume nodules is largely host provided but a bacterially derived O_2 protection mechanism as in *Frankia* might one day be transferable to legumes. A much-simplified nodule could then be envisaged to sustain biological N_2 fixation.

Further reading

Schwintzer, C.R. and Tjepkema, J.D. (1990). *The Biology of* Frankia *and Actinorhizal Plants*, Academic Press: New York.

Benson, D.R. and Silvester, W.B. (1993). 'Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants', *Microbiology Reviews*, 57, 293–319.

3.5.5 Measuring N_2 fixation

Rates of N_2 fixation can be measured by a number of techniques to address questions of nodule efficiency and nitrogen cycling in agricultural and natural plant systems. Nitrogenase is pivotal for initial reduction of N_2 but this same enzyme will also reduce acetylene (C_2H_2) to ethylene (C_2H_4). Acetylene is an effective competitor with N_2 for nitrogenase so the rate of C_2H_4 synthesis is proportional to nitrogenase activity. Acetylene reduction gives an instantaneous estimate of the N_2 fixation rate. Another instantaneous technique requires flushing nodulated roots with an argon : oxygen gas mixture (79 : 21) to displace all N_2. All electron flux through nitrogenase is then diverted to the reduction of protons to H_2 rather than N_2 to NH_4^+ (Equation 3.5). The rate of H_2 evolution by roots can thus be used to estimate nitrogenase activity.

Alternative approaches to 'instantaneous' estimates of N_2 fixation provide an integrated rate of fixation over periods of hours or days. The proportions of inorganic and organic nitrogen compounds in xylem sap are affected by the ratio of inorganic nitrogen taken up to symbiotic N_2 fixation; this can be exploited in genera of legumes in which amides and ureides are major products of N_2 fixation. Soybean, for example, exports less than 10% of nitrogen to shoots in the form of ureides when supplied nitrate but more than 80% when all nitrogen is biologically fixed. Thus, relative ureide levels in sap give an estimate of N_2 fixation.

Many experiments now rely on ^{15}N-based techniques to obtain an integral of fixation over the life of a plant. These techniques rely on a difference in ratio of the stable isotopes of nitrogen (^{15}N and ^{14}N) in soil and atmosphere (Figure 3.20). The soil must be enriched in ^{15}N relative to the atmosphere — either naturally (the process of denitrification causes a fractionation of the two isotopes, leaving the soil enriched in ^{15}N) or by artificial ^{15}N addition. The N_2-fixing plant of interest is sampled, together with an adjacent non-N_2-fixing plant (e.g. grass) whose ^{15}N enrichment represents that of soil nitrogen. ^{15}N enrichment in digested plant material and soil is analysed isotopically in a mass spectrometer and contribution of biological N_2 fixation calculated.

A typical 'good' rate of fixation for a (non-irrigated) field of subtropical legumes in northern Australia is *c.* 60–100 kg N ha^{-1} $year^{-1}$. About the same amount of nitrogen is harvested as seed from a crop of cowpea, soybean or chickpea, so growing these legumes does not add net nitrogen to the soil; it does, however, spare nitrogen which would otherwise be removed at harvest. Irrigated legume-based pastures in temperate Australia or New Zealand fix 250–300 kg N ha^{-1} $year^{-1}$ and make a substantial contribution to the low energy costs of agriculture in these regions. Selection of appropriate

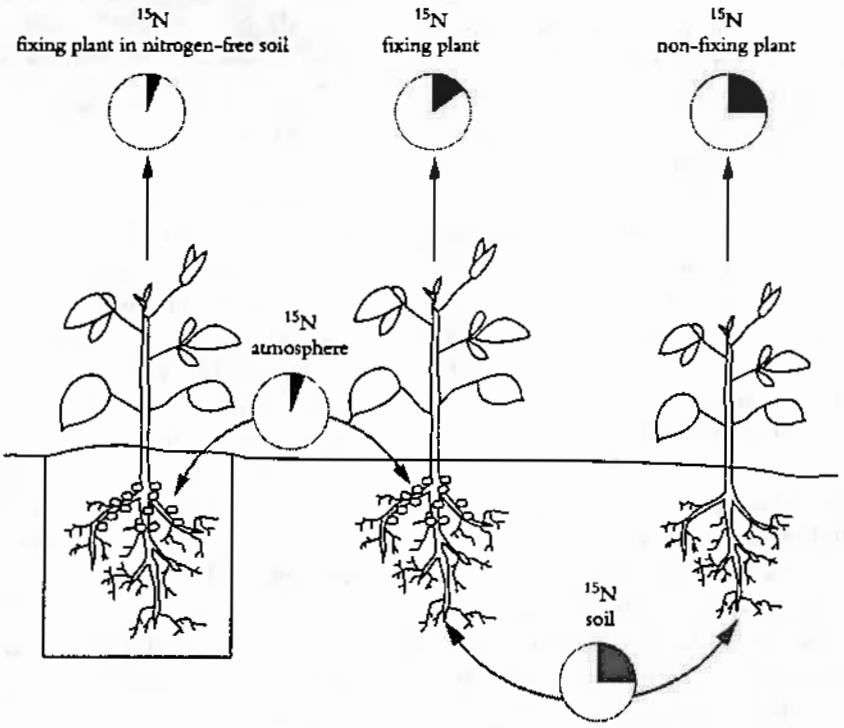

Figure 3.20 Basis of the natural abundance method for assessing the contribution of N_2 fixation to legume nutrition. This method entails measuring plant $^{15}N/^{14}N$ ratio by mass spectroscopy. Natural differences in $^{15}N/^{14}N$ ratio between soil and atmospheric nitrogen are exploited. Legumes to the left and right of the figure each have a unique source of nitrogen, while a test plant in the middle relies on both fixed nitrogen and soil inorganic nitrogen. Plants (left) denied a source of inorganic nitrogen (e.g. nitrate) fix atmospheric nitrogen and therefore have low $^{15}N/^{14}N$ ratios. Plants without nodules (right) take up only soil-derived nitrogen and are enriched with ^{15}N (high $^{15}N/^{14}N$ ratios). ^{15}N 'signatures' of these two sets of plants can be used to estimate the relative contributions of soil and atmospheric nitrogen as nitrogen sources in the test plant, and therefore to assess the significance of N_2 fixation.

biological N_2 fixers could greatly improve N_2 fixation in tropical legume crops.

3.6 Absorption of water and nutrients by roots

In terrestrial plants, water and solutes must move from the bulk soil through a rhizosphere before entering roots. Within a root, radial transport carries resources to the central stele where they are released into xylem vessels and made available for long-distance (axial) transport. Uptake is achieved via this tortuous route through different matrices with a high degree of control and responsiveness to plant requirements. Inorganic nutrients are delivered to cells along the transpiration pathway and a proportion is subsequently transferred to phloem vessels for use in a wide variety of synthetic events throughout the plant.

For example, inorganic ions (e.g. potassium and orthophosphate) are delivered to growing root tips through the incoming phloem sap and therefore root tips are largely reliant on ion and water uptake elsewhere in roots. Because growing root tips do not absorb most nutrients locally, they are somewhat independent of fluctuations in external nutrient concentrations as roots elongate through soil. Ion uptake and phloem retranslocation therefore both contribute to establishment of new roots by delivering an appropriate nutrient mix to the cytoplasm of immature root apical cells. In soils contaminated with heavy metals, relatively unvacuolated apical cells can be protected from toxic ions because these ions are sequestered in vacuoles of more mature root cells. The entire process of acquisition of inorganic resources is dependent on the initial entry of ions into roots, the subject of Section 3.6.

3.6.1 Radial uptake: a dynamic component of resource acquisition

Water plants are bathed in a solution buffered against sudden variation but land plants must extract water and ions from soil, a much more stochastic environment. Sharp variations in soil composition occur through time and space. Soil water content can, for example, swing from dryness to saturation over minutes while sharp gradients in nutrient concentration occur over less than a centimetre. Integrating water and nutrient extraction from such a heterogeneous source is achieved through a high degree of plasticity in root structure and function. Structural modifications might include local root branching to deplete nutrient-rich zones (Figure 3.4) or pockets of moisture. Function can alter even more quickly in response to variation, seen, for example, in the rapid up-regulation of K^+ and orthophosphate absorption when roots are deprived of these ions (Drew *et al.* 1984).

Water and nutrient acquisition are also influenced by shoot factors. Water status in leaves helps determine hydraulic gradients through the soil–plant–atmosphere continuum and thereby influences water uptake. Shoots are also essential for maintenance of ion uptake activity in roots because they supply photoassimilates for energy production in roots.

So, radial flow of water and ions across roots should be viewed as one important part of the entire pathway for resources entering a plant. Experiments on radial flow of water and ions reveal some of the critical sites of control for uptake of these soil-borne resources.

3.6.2 Extracting water and nutrients via the rhizosphere

Ions taken up into the main axis of a root, a lateral branch, or a fine unicellular root hair must cross a sleeve of rhizosphere up to a few millimetres thick. Even in solution cultures, where turbulence is maximised by air bubbles, unstirred layers of solution exist within 10–200 mm of root surfaces. Young, active roots in soil must draw large quantities of water and nutrients through this complex space in order to generate xlem sap. Balancing solute and water flow into roots is therefore critical, with small variations in flux having a marked effect on solute concentrations around roots. Some outcomes of such an imbalance in uptake rates are described below.

Ions have characteristic rates of release from bulk soil into the transpiration stream, depending on adsorption isotherms and solubilities. Moreover, rates of influx of individual ions into roots vary widely. Hence concentrations of ions in the rhizosphere also vary widely. Ions which are readily released from soil into the transpiration stream, swept to the root surface but taken up more slowly than water, tend to accumulate at the interfacial zone. Figure 3.9 describes this phenomenon when roots were exposed to NaCl, showing that water deficits can result. Roots supplied with essential nutrients rather than NaCl also exhibit solute build up during rapid transpiration. Most striking are plants in calcareous soils where deposits of calcium in the rhizosphere form an encrusted sleeve around roots.

By contrast, plants not experiencing extreme transpiration rates and growing in soils of low to moderate fertility have sustained uptake of ions such as orthophosphate and K^+, leading to lower concentrations around roots. This is especially so when low ion diffusivities prevent replenishment of these ions from the bulk soil (Figure 3.21). Local fluctuations in solute concentrations are significant for roots because ion uptake systems respond directly to local concentrations (Chapter 4). Adequate nutrient concentrations in bulk soil might therefore mask local deficiencies at the root surface.

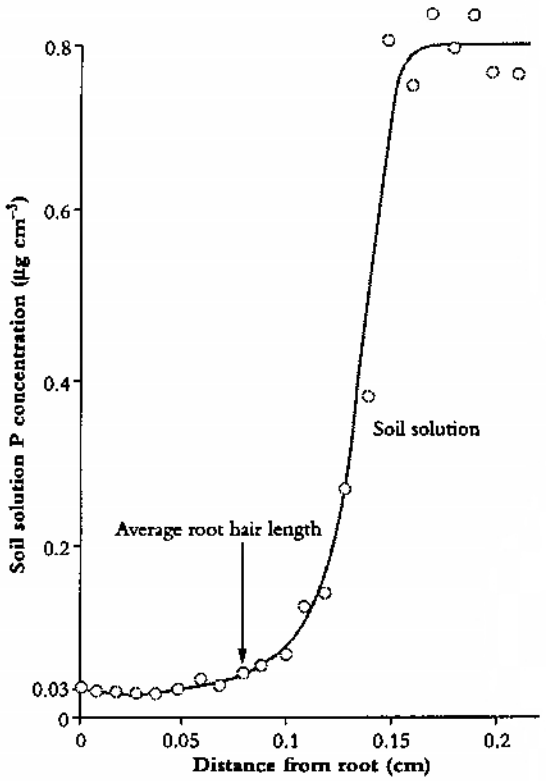

Figure 3.21 Depletion of soil solution phosphate around maize roots over a three-day period in a sandy soil. Note that within the zone explored by root hairs (about 0.8 mm from the root axis) all phosphate is depleted
(Based on Hendriks *et al.* 1981)

3.6.3 Pathways and fluxes

Nutrient concentrations are optimised for plant growth in hydroponic solution cultures, favourably modified in fertilised agricultural soils where phosphorus and nitrogen status and pH might be adjusted, and totally unmodified in natural ecosystems. Most essential nutrients for plant growth are present in soil solution at concentrations well below those found in plant tissues (e.g. phosphorus, potassium and nitrogen) while other ions can be in excess around roots (e.g. baron, aluminium and sodinm). Plants nevertheless colonise all these environments and produce biomass at impressive rates by controlling water and ion influx. The sensitivity with which roots recognise ions in soil solution is critical to plant survival. For example, exclusion of undesirable ionic species by root membranes will leave them relatively harmlessly in the rhizosphere whereas passage of these ions to shoots will have more dire outcomes such as leaf abscission and necrosis. Equally, membranes allow root cells to absorb essential ions selectively, even when they are chemically similar to deleterious ions (Section 17.2).

Water and ions move through root tissues along either a symplasmic pathway (intracellular), an apoplasmic pathway (extracellular) or a combination of both (transcellular pathway) (Figures 3.22a and b). While each route is explicitly

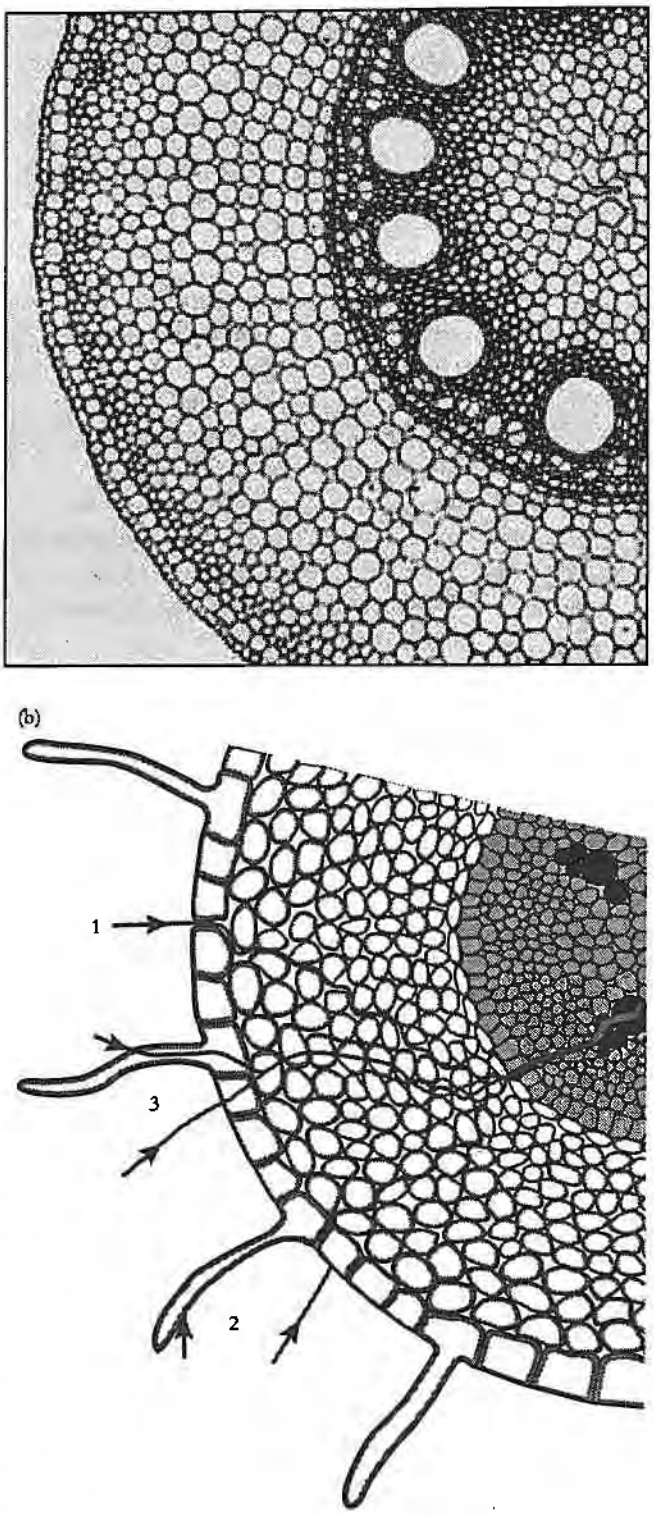

Figure 3.22 Transverse section of a maize root. Note an exodermal layer underlying the epidermis, and distinctive endodermis bounding the stele. Pink Toluidine Blue staining characterises suberised cells of these major barriers to radial flow. Late metaxylem vessels with large diameters are the dominant conduit for axial transport of sap. Smaller xylem elements, xylem parenchyma and phloem bundles lie within tissues adjacent to late metaxylem vessels. Magnification Vertical scale bar = 1 mm. (? see Colour Plate xx) (b) Sketch of a transverse root section showing three proposed pathways of ion and water flow across roots: (1) apoplasmic, (2) symplasmic and (3) transcellular (transvacuolar). Note that ions are predominantly forced to enter the symplasm at the endodermis and no further discrimination in pathway is evident.
((a) Courtesy of A.W.R. Robards)

defined, it is, in practice, technically difficult to determine flow rates along each pathway.

By definition, non-apoplasmic flow requires transport across membranes but the intracellular distance traversed and number of membranes crossed when ions travel through cells is variable. Water and ions can move through a series of plasmodesmal connections, thereby remaining in the cytoplasm until reaching the stele. Conductivity in this case is largely regulated by plasmodesmal resistance. Alternatively, water and some ions enter vacuoles and are therefore subject to transport properties of the tonoplast ('transcellular flow'). Ultimately, most water and ions enter the apoplasm when released into mature xylem vessels, either from xylem parenchyma cells or after rupture of immature xylem elements.

Alternatively, flow across the cortex might be largely apoplasmic as water and ions are drawn through intercellular spaces and cell walls up to the endodermis, where they generally enter the symplasm. Concentrations of ions in the rhizosphere, transpiration rates, ionic species and membrane transport properties all have an effect on the proportion of flow through each pathway. Cells deep within the cortex might have a lower capacity for active uptake of ions into the symplasm than outer cell layers but can none the less absorb K^+ when concentrations are high (Clarkson 1996). Entry of anions to deep layers of the cortex is likely to be limited by charge repulsion from dissociated, negative carboxyl groups in cell walls (Donnan Free Space). In general, cations also pass through cell walls more slowly than through solutions, particularly if many of the carboxyl groups in cell walls are not occupied by Ca^{2+} ions. None the less, apoplasmic flow of water through roots can sustain large ion fluxes during periods of high transpiration.

Estimates of net flux of water and ions do not reveal the absolute rates of influx and efflux: there is evidence for leakage of many ions (e.g. nitrogen and phosphorus) out of root cells and water can also cross membranes bidirectionally when water potential gradients favour water loss from roots in very dry soil. The case for efflux of orthophosphate, nitrate and sulphate has been made particularly convincingly (see Case study 4.1; Marschner 1995) with evidence that minimum ion concentrations extracted by roots are largely determined by efflux rates. Downregulating efflux of an ion allows roots to extract that ion to a lower concentration. Electrochemical gradients are not the only factors in ion efflux: ion-specific channels and carrier proteins in membranes can confer genetic control on efflux rates. Outwardly directed K^+ channels and Na^+ efflux pumps are two membrane transport proteins likely to play an important role in efflux.

3.6.4 Barriers to apoplasmic flow

Ionic composition of soil solution is not strongly modified while passing through cell walls. Weak charge fields around wall polymers adsorb some divalent cations but monovalent cations and anions pass through largely free of interactions. However, hydrophobic layers in specialised cell walls force ions to cross membranes and provide important sites for selectivity. Ions either follow electrochemical gradients into cells via channels in membranes or are pumped via energy-carriers located in membranes (Section 4.2). Rapid ion uptake is possible through channels when electrochemical gradients strongly favour influx (e.g. calcium) whereas influx of cations such as potassium (at low concentrations) and anions such as nitrate and orthophosphate is achieved by energy-dependent transporters (Chapter 4).

(a) Endodermis

An endodermal cell layer constitutes the prime barrier to apoplasmic flow in roots (Figure 3.23a). State I endodermis forms within a few millimetres of the root apex when a single layer of cells around the stele lay down hydrophobic polymers of suberin and lignin in transverse and radial walls, leaving longitudinal walls unchanged (Figure 3.23b). Phenolics in these deposits can be stained to reveal the Casparian bands. Even in this early phase, Casparian bands begin to lower the permeability of cell walls to water and solutes, especially helped by tight adherence of cytoplasm to the suberised walls.

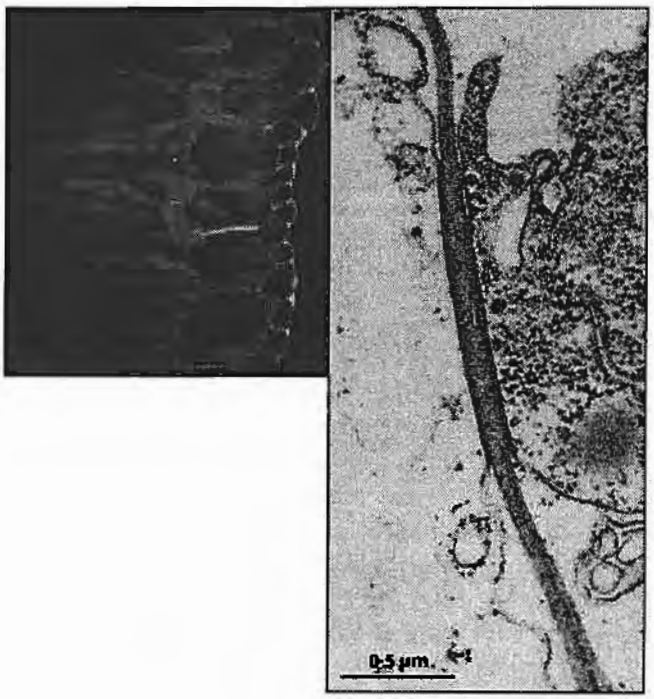

Figure 3.23 **(a) Scanning electron micrograph of an isolated endodermis (large cells) and pericycle (small cells) prepared from a barley root. Radial walls of the endodermal cells are tightly appressed because of the apoplasmic discontinuity created by suberisation of these walls. Magnification Vertical scale bar = 20 µm. (b) Transverse sections of endodermal cells of barley roots taken 4 cm from the root apex. Roots were plasmolysed to reveal the attachment of plasma membrane to Casparian bands (CB) which is typical of State I endodermis. Apoplasmic discontinuity is achieved by the hydrophobic barrier Casparian bands impose in radial walls. Roots were fixed in glutaraldebyde then osmium tetroxide, dehydrated and embedded in epoxy resin. Bar represents 0.5 µm.**
(Both figures courtesy of A.W.R. Robards; (b) reproduced with permission from Academic Press).

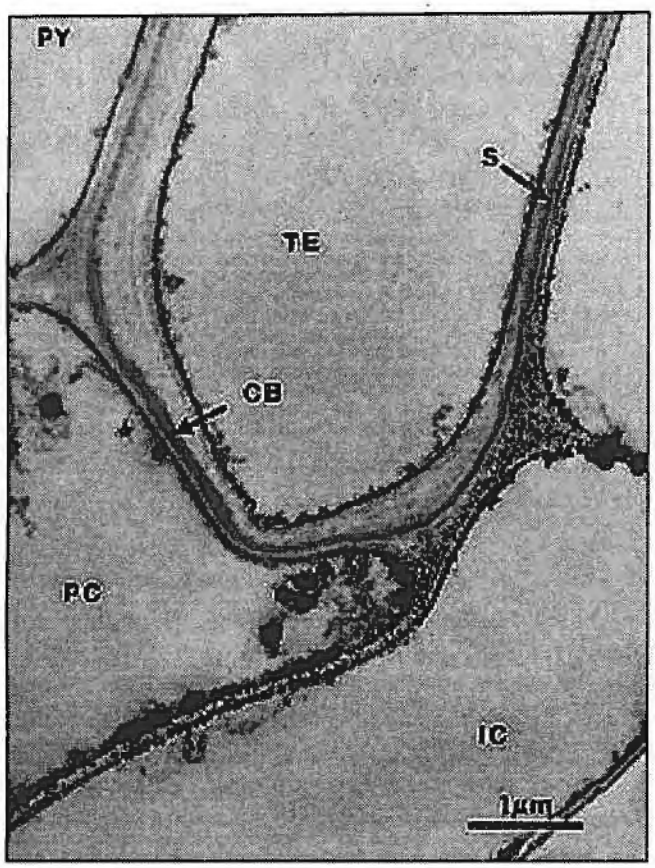

Figure 3.24 Electron micrograph of a transverse section of *Puccinellia peisonis* root showing a mature endodermal cell (TE) and adjacent passage cell (PC). Chloride ions supplied to roots while they were intact were subsequently precipitated using silver ions (Ag^+), leaving electron-dense AgCl at the barrier imposed by suberin (S). Chloride ions arriving from the inner cortex (IC) must travel either symplasmically to the stele or cross through a relatively scarce passage cell (PC). This root was grown in a non-aerated solution containing 200 mol m^{-3} NaCl then sectioned 1 cm from the apex. The root was fixed in buffer containing osmium tetroxide and embedded in uranyl acetate. Bar represents 1 μm.
(Courtesy of R. Stelzer)

Further differentiation of Casparian bands occurs within centimetres from the apex, where suberin lamellae tighten the apoplasmic seal (State II endodermis). Ultimately State III endodermis forms by deposition of cellulose around endodermal cells. In grasses, outer tangential walls have less cellulose than other walls but often all endodermal walls are thickened leaving only pits to preserve plasmodesmal continuity between cortex and stele. The effectiveness of this seal is shown when Ag^+ is used to precipitate Cl^- ions supplied to roots of *Puccinellia peisonis* (Figure 3.24). Suberised regions of the endodermal cell wall prevent further progress of Cl^- towards the stele. The endodermis minimises both passive leakage of ions out of the stele (when concentrations exceed those in the cortex) and unrestricted apoplasmic flow of ions into the stele.

The importance of root structure and the endodermis, in particular, for ion uptake was recognised early this century and led Crafts and Broyer to propose in 1938 that ion transport entailed a passive leakage of ions into xylem vessels after an initial concentration step in the symplasm. Ion leakage was attributed to O_2 deficiency in the stele. This model recognised

Table 3.3 Per cent inhibition of Cl^- uptake and transport in maize roots exposed to a range of O_2 concentrations. In a hypoxic medium, roots had a predominantly anaerobic stele within an aerobic cortex so stele-located *energy-dependent ion flux would be preferentially inhibited. Chloride uptake and transport were totally inhibited in anaerobic conditions, demonstrating that at least one energy-dependent step lies in the radial pathway for Cl^-. However, roots exposed to 0.4 mol m^{-3} Cl^- for 24 h in the presence of 0.015 and 0.05 mol m^{-3} O_2 (hypoxia) showed more severe inhibition of Cl^-* transport *from the medium to xylem vessels than Cl^-* uptake *into root tissues. This is indicative of energy-dependent Cl^- movement in the stele, possibly an active unloading step*

O_2 concentration (mol m^{-3})	Net Cl^- uptake by roots	Cl^- transport to xylem	Sap flow
<0.003	98.8	100	89
0.015	50	71	42
0.05	21	39	20

(Data provided by H. Greenway)

structural features of roots and specifically coupled the endodermis as a barrier to ion movement with its capacity to impede O_2 supply to respiring stelar cells. This mechanism has never been verified experimentally but it has attractive features such as an 'anaerobic core' which has since been identified in roots growing at diminished O_2 levels (Section 18.2). The concept of an anaerobic core has since been exploited to show that Cl^- influx into roots exposed to hypoxia was suppressed much less than Cl^- transport to xylem vessels, providing good evidence that an energy-dependent step was involved in Cl^- transport across the stele to xylem vessels (Table 3.3). This does not support passive leakage and is potent evidence for energy-dependent unloading of ions into xylem.

Carrier molecules and channels are central to ion transport and help explain ion release into xylem vessels described by Pitman (1972). Much early experimental evidence for energy dependence of ion transport came from the use of respiratory inhibitors. Inhibitors, however, reveal nothing about *sites* of active ion uptake because they perfuse the whole root and alter metabolism generally by ATP deprivation. Membrane damage and associated ion leakage from cells are a common outcome of inhibitor treatment. Other ways to study the net influx of ions into roots have been through radiotracers such as ^{32}P, ^{36}Cl and ^{86}Rb (a potassium analogue) but these experiments have limitations in that penetration of the isotopes deep into root tissues is too slow to allow firm conclusions on sites or rates of ion uptake in steady-state conditions. Discriminating short-term influx from longer-term net flux (influx minus efflux) is often fraught because optimal labelling times are tissue specific and therefore highly empirical. Approaches in which carrier and channel proteins are immunolocalised might reveal *sites* of uptake but tell little about ion fluxes at those sites in intact roots. In view of the specialised role of stelar cells in ion efflux, it might be possible to immunolocalise proteins involved in ion *unloading* separately from those which *load* ions into the cortex. A similar approach has led to immunolocalisation of ATPases involved specifically in phloem unloading in bean seed coats.

(b) Exodermis

The exodermis is a second layer of root cells which imposes a barrier to radial transport processes in most species studied (Perumalla and Peterson 1985). As in an endodermis, Casparian bands restrict radial apoplasmic movement of ions but the exodermis forms in a layer of cortical cells beneath the epidermis (Figure 3.22). Exodermal layers become functionally mature 20–120 mm from the apex, where lateral roots are initiated, and therefore only constitute a barrier to apoplasmic ion flow in root zones where an endodermis is already present. In a similar way to the endodermis, maturation of an exodermis involves further deposition of cellulosic wall material, further impeding flow of solution through walls.

Individual passage cells (Figure 3.24) in both endodermal and exodermal layers allow apoplasmic passage of ions and therefore provide points of low radial resistance (Clarkson 1996). How and why passage cells form is unknown. Some families (e.g. irises) have large numbers of passage cells while others have very few.

Studies cited here describe roots with only primary tissues. Secondary thickening dramatically alters rates of water and nutrient influx because endodermal and cortical tissues of dicotyledonous plants are replaced by secondary phloem and a cork-like layer covered with bark. Permeability changes are discussed in Section 3.6.6. Woody roots might not even take up water or nutrients, or may only do so when supply to younger roots is severely limited or gradients into the root are very steep. Monocotyledonous roots have no secondary thickening but none the less form a thorough seal from soil by maturation of an endodermis and degradation of cortical cells.

3.6.5 Transport of water and solutes

Water and nutrients are acquired by roots in a mutually dependent fashion because bulk flow of soil solution carries with it a cargo of nutrients to root surfaces. Furthermore, most essential macronutrients become more concentrated during absorption, creating water potential gradients and inducing water flow into roots ('root pressure'). Indeed, if roots fail to take up nutrients as fast as water, water uptake is gradually restricted (Section 3.6.2).

Inflow of water is driven by two processes, transpirational pull and osmotically derived 'root pressure'. Gradients in water potential generated by transpiration from shoots (suction) are sufficient to draw soil solution to the roots (Section 3.2). For example, phosphate and water uptake coincide in time when followed over several days in sugar cane plants (Figure 3.25). The degree to which this ionic mixture is modified before entering the xylem will be determined partly by ionic interactions in cell walls (Donnan Free Space) and to a much greater extent by membrane transport properties as ions enter the symplasm. Imbalance between water and ion uptake can generate apoplasmic solute levels high enough to drought a plant growing in fertile, damp soils under sunny conditions (Section 3.6.3). Reserves of ions in root cell vacuoles can help to buffer deficits of ions in the uptake stream: for example, ions of potassium, nitrogen and phosphorus stored in vacuoles can represent up to 90% of the cell's reserves. None the less, plants which are transpiring rapidly generally have a nutrient-depleted (low osmotic pressure) xylem stream compared to slowly transpiring plants (Figure 3.26).

At the other extreme, plants which have had shoots removed so xylem exudates can be sampled from cut stumps have very concentrated xylem fluid which is released under hydrostatic pressure from the roots ('root pressure'). This flow is osmotically driven (water follows ions into the roots) and apart from the droplets seen on the margins of guttating leaves in early morning, it is an inconspicuous contributor to sap flow. The contribution of 'root pressure' to sap flow in rainforest species may be significant but in the brighter environments of more open canopies, transpirational pull is almost totally responsible.

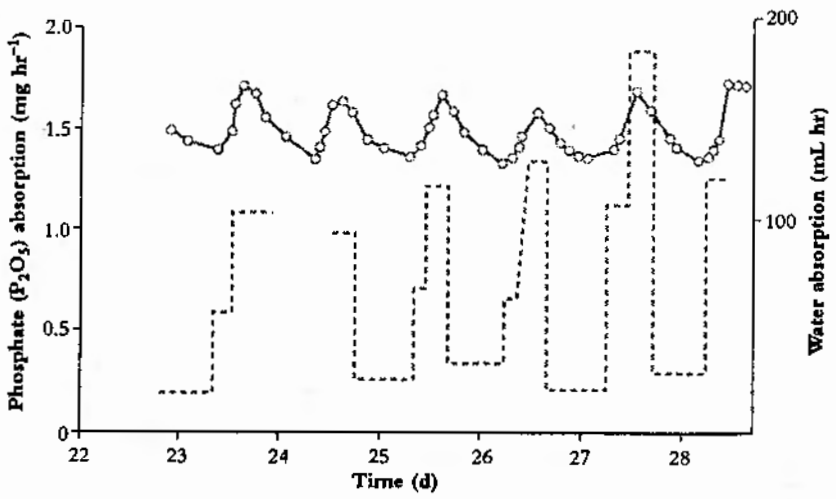

Figure 3.25 Transpiration (—) and phosphate (P) absorption (circles) by sugar cane plants over a five-day period showing diurnal fluctuations in transpiration and much smaller oscillations in phosphate absorption. Tight regulation of phosphate inflow removes the extremes of phosphate supply to shoots which would follow if uptake were passive
(Based on van den Honert 1933; reproduced with permission of Pergamon Press)

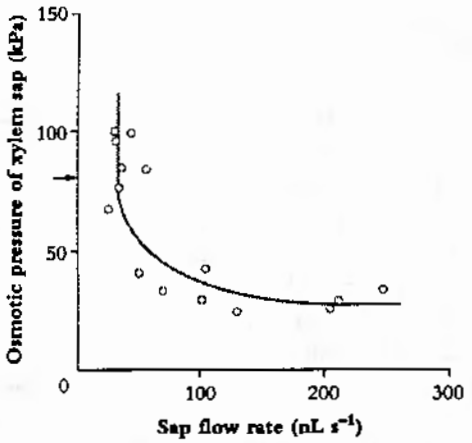

Figure 3.26 Osmotic pressure (reflecting total solute concentration) of xylem sap of young barley plants at a range of transpiration rates. Transpiration rates were imposed by varying vapour pressure deficit around the shoots and xylem sap was sampled by applying sufficient pressure to roots to cause a cut leaf tip to bleed
(Based on Munns and Passioura 1984)

3.6.6 Testing root function

Roots display marked structural complexity *along* their axes (dividing, expanding and differentiating tissues), paralleled by gradients in functional capacity. Evidence for longitudinal gradients in ion uptake capacity comes from experiments where separate root zones are supplied individually with nutrient solutions and local ion uptake monitored (e.g. Harrison-Murray and Clarkson 1973). Ions such as K^+ and orthophosphate are sometimes taken up more rapidly in the terminal few centimetres of root axes, supplementing phloem in satisfying the large nutrient demand by young, differentiating root cells. Demand for ions by shoots can also be substantial, leading to high rates of ion uptake in mature root tissues and increasing allocation of ions from these tissues to the translocation stream (Figure 3.27). Among the major nutrient ions, uptake of Ca^{2+} is most consistently localised in young root axes, furnishing these cells with Ca^{2+} which cannot be delivered in the phloem.

Water uptake, when measured locally, shows similar gradients (Figure 3.28; Sanderson 1983) although mature root axes with lateral roots contribute significantly to water inflow. Significant osmotically driven water flow ('root pressure') occurs in young root tissues during rapid ion influx.

One approach to understanding the significance of these zones for both water and nutrient transport is to place whole roots or segments of root into a root pressure probe (Steudle 1994). Using this method, build up of hydrostatic pressure in xylem vessels ('root pressure') caused by energy-dependent solute loading can be measured at the cut end of a root. Also, water potential gradients can be applied across roots, either osmotically or by hydrostatic pressure, to induce water flow and give estimates of radial hydraulic conductivity (Figure 3.29).

Resistance to radial transport of ions and water are differently distributed. Root pressure probe experiments demonstrate that minor damage to the endodermis, such as pinpricks, does not substantially alter water flow but does lower root pressure to less than half its original value within less than two minutes. Therefore, the barrier to ion uptake appears to be principally at the endodermis whereas that to water inflow is probably more generally distributed over the root membranes. Longer-term observations show that root pressures begin to recover 0.5–1 hour after damage, demonstrating repair of damaged endodermal cells. Xylem vessel walls contribute less than one-third of the radial resistance to water flow, reflecting their high degree of leakiness (Section 5.1).

Distinct differences in radial conductivity at various stages of root development can be shown with a root pressure probe. About 20 mm from a root apex, where an endodermis is present but too immature to impose a tight barrier to transport, water and solutes flow to the stele along a low resistance, largely apoplasmic pathway (Frensch *et al.* 1996). Longitudinal transport meets considerable resistance in these root zones because late metaxylem vessels are immature (Section 3.6.7). Similarly, in mature root axes where secondary laterals emerge, apoplasmic transport sometimes increases, probably because of emerging lateral roots rupturing the endodermal cell layer. Apoplasmic flow past emerging lateral roots of

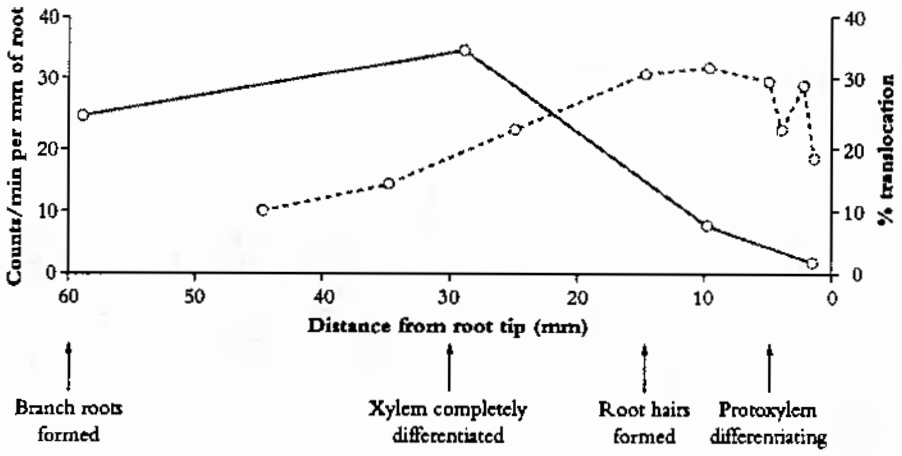

Figure 3.27 Phosphate uptake and translocation from different positions along roots of young barley plants. Roots were supplied with ^{32}P and radioactivity was monitored in root zones to assess the proportion of phosphate which remained in a zone (dashed lines) versus the proportion translocated axially (solid lines) to other plant parts (principally shoots). A peak in untranslocated ^{32}P in the zone of root hair formation reflects the efficacy of hairs in phosphate uptake. Mature root zones were effective at translocating ^{32}P, probably reflecting smaller demand for mineral nutrients in mature cells and maturity of the long-distance transport system in basal root zones
(Based on van den Honert *et al.* 1955)

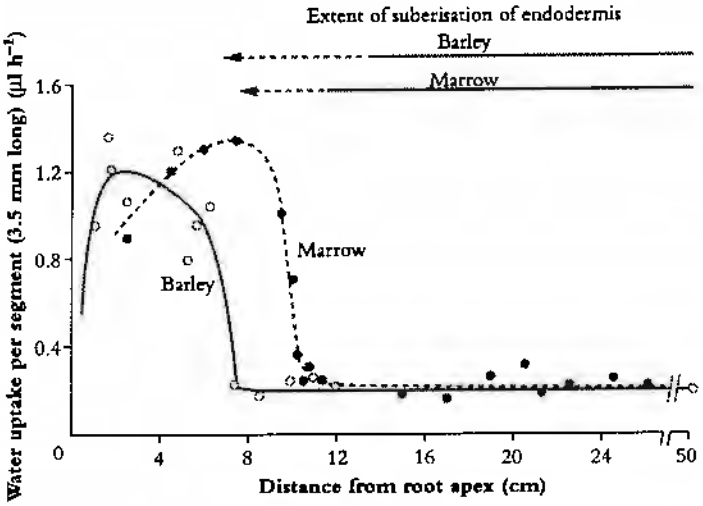

Figure 3.28 Local rates of water uptake in 3.5 mm segments of barley (*Hordeum vulgare*) and marrow (*Cucurbita pepo*) roots. Water uptake was measured using micropotometers applied to the roots of plants in normal transpiring conditions. The extent of endodermal development was also assessed for these roots and appears as horizontal lines on the figure. Water uptake is fastest in apical, non-endodermal zones under these transpirational conditions
(Graham *et al.* 1974; reproduced with permission from Academic Press)

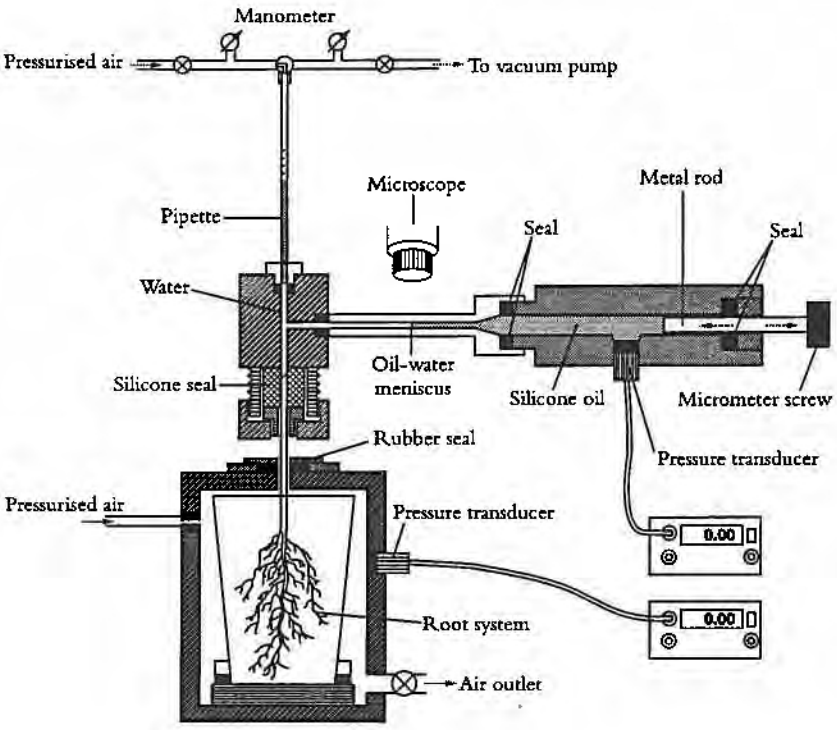

Figure 3.29 Root pressure probe for measuring water and solute relations of roots. The living root system is enclosed in a chamber which can be pressurised. After excising the shoot, a water-filled capillary is sealed to the cut stump with silicone; xylem sap can therefore flow directly into this capillary. The capillary connects to a pipette (top) and an oil-filled chamber (right) in which pressure can be monitored by following the oil–water meniscus. Roots in this apparatus remain alive and physiological for long periods, allowing water flow through the root system to be measured in a number of ways. (1) When the pipette is open to the atmosphere, roots under pressure exude sap providing a pressure–flow relationship from which root hydraulic conductivity (L_p) is derived. Unpressurised roots exude at a rate determined by solute uptake (giving 'root pressure'). (2) Suction can be applied to the cut stump to mimic 'transpirational pull', so providing another measure of L_p. (3) The cut stump can even be pressurised, forcing water out through root tissues and generating another pressure–flow relationship. This direction of flow is rare but not unknown in nature. If pressure is applied fairly briefly, osmotic pressures in xylem sap are constant and L_p in (1) to (3) agrees closely. Finally, transient flows can be induced with the aid of the probe to estimate pressure relaxations. In this case, the pipette has to be removed and the system closed. The apparatus also gives information on solutes, in particular reflection coefficients (Equation 4.4) in whole root systems for a range of solutes
(Courtesy of E. Steudle)

monocotyledons growing in the field is restricted by a lignified adhesive layer between the new epidermis and the cortex of the parent axis. This sealing phenomenon around lateral roots might be widespread but has not been extensively investigated. Overall permeability of mature roots is, however, very small because of increasing suberisation and secondary thickening of endodermal and exodermal cell layers. So, in root pressure probe experiments, the area of 'apoplasmic bypass' did not exceed 0.05% of the total cross-sectional area of the endodermis. This might still be significant because the low hydraulic resistance of an apoplasmic pathway (orders of magnitude less than a symplasmic route) coupled with the lack of ion selectivity when membranes are bypassed mean that apoplasmic influx might be important for roots exposed to toxic solutes.

Most knowledge on transport processes in roots comes from herbaceous species which lack the secondary thickening and cell senescence characteristic of mature roots in soil. Solute and water transport in oak and spruce roots show that root pressure contributes little to water flow, reflecting low overall demand for nutrients by mature plants. Transpirational water flow is dominant. When water transport was induced hydraulically, large radial resistances were measured, suggesting that impermeable endodermal and exodermal cell layers and secondary root thickening make roots very impermeable to water.

Sites of water uptake can be visualised by bathing roots in a solution containing membrane-impermeable dyes. Dye accumulates where water enters membranes (Section 5.2), for example at the endodermis and exodermis of roots. These lay-

ers are therefore thought to be major points of entry for water into the symplasm. Whether most water enters the symplasm at these cell layers in intact plants in soil is less clear because we know that dyes also accumulate around root hairs, which might therefore be the main site of water uptake into the root symplasm. The exodermis is only a barrier to water transport in mature roots where deposition of hydrophobic substances in the Casparian bands has been extensive. Young root apices do not accumulate dye, suggesting that they are not major sites for water uptake. Mature (non-cytoplasmic) late metaxylem vessels found in older root zones are necessary for full transport capacity to be engaged.

3.6.7 Axial versus radial flow

Analysis of barriers to water and nutrient flowing across roots gradually builds a picture of the radial resistances to transport. Mechanisms within roots that optimise delivery of water and ions to shoots are incompletely understood and will probably be shown to entail subtle controls on internal resistances. Some points in the pathway are certainly more critical than others and should be targeted as sites of control for resistance. Loss of cortical cells, for example, has a small effect on ion uptake, showing that the cortex is not a key site of control of radial resistance to ion flow.

In young roots, radial and axial resistances to water and ion uptake are substantial; with only protoxylem and early metaxylem vessels conducting sap towards shoots, axial resistance can limit flow. Axial resistance decreases up to three orders of magnitude away from root apices as wide late metaxylem vessels mature by death of their cell contents. As barriers to radial flow develop in these mature axes (endodermis, exodermis and secondary thickening), radial transport of water and ions assumes greater importance (Steudle 1994). Flow through symplasmic pathways still occurs but low permeabilities often restrict water and ion delivery to xylem vessels. Xylem sap is therefore a composite of the activities of all these tissue types; utilisation of water and ions gathered by this vast structural complex is discussed in Chapter 4.

Further reading

Bowen, G.D. and Rovira, A.D. (1991). 'The rhizospere, the hidden half of the hidden half', in *The Plant Roots, the Hidden Half*', eds Y. Waisel, A. Eshel and U. Kafkafi, 641–669, Marcel Dekker, Inc.: New York.

Clarkson, D.T. (1996). 'Root structure and sites of ion uptake', in *Plant Roots, The Hidden Half*, 2nd edn, eds Y. Waisel, A. Eshel and U. Kafkafi, 483–510, Marcel Dekker, Inc.: New York.

Marschner, H. (1995). *Mineral Nutrition of Higher Plants*, 2nd edn, Academic Press: London.

Robson, A. D., Abbott, L. K. and Malajczuk, N. (eds) (1994). *Management of Mycorrhizas in Agriculture, Horticulture and Forestry*, Kluwer Academic Publishers: Dordrecht, The Netherlands.

Smith, S. E. and Read, D. J. (1997). *Mycorrhizal Symbiosis*, 2nd edn, Academic Press: London.