6
Mineral Nutrition

1. Introduction

Next to water, nutrients are the environmental factor that most strongly constrains terrestrial productivity. The productivity of virtually all natural ecosystems, even arid ecosystems, responds to addition of one or more nutrients, indicating widespread nutrient limitation. Species differ widely in their capacity to acquire nutrients from soil. Some plants can take up Fe, P, or other ions from a calcareous soil from which others cannot extract enough nutrients to persist. In other soils, the concentrations of aluminum, heavy metals, or sodium chloride may reach toxic levels, whereas some species have genetic adaptations that enable them to survive in such environments. This does not mean that metallophytes *need* high concentrations of heavy metals or that halophytes *require* high salt concentrations to survive. These species perform well in the absence of these adverse conditions. Their distribution is restricted to these extreme habitats because, on one hand, these plants resist the adverse conditions, whereas most other plants do not. On the other hand, metallophytes and halophytes generally perform less well than most other plants in habitats without toxic levels of minerals or salts. Terms like metallophytes, halophytes, and others that we will encounter later in this chapter therefore refer to the **ecological amplitude** of the species rather than to their physiological requirements (Fig. 2 in Chapter 1 on assumptions and approaches).

This chapter deals with the acquisition and the use of nutrients by plants, focusing on terrestrial plants that absorb nutrients predominantly via their roots from soil. Leaves are also capable of acquiring nutrients. For example, volatile nitrogenous and sulfurous compounds, which may occur either naturally or as air pollutants in the atmosphere, can be taken up through the stomata. Nutrients in the water on wet leaves are also available for absorption by leaves. This may be of special importance for aquatic and epiphytic plants as well as for mosses and even *Sequoia sempervirens* (coast redwood) (Burgess & Dawson 2004). Other mechanisms to acquire nutrients include those found in carnivorous plants, which acquire nutrients from their prey, symbiotic associations with microorganisms, and parasitic associations with host plants. These will be treated in separate chapters.

2. Acquisition of Nutrients

Most terrestrial plants absorb the inorganic nutrients required for growth via their roots from soil. For the uptake into the root cells, transport proteins ("carriers", "channels", and "transporters") are used (Sect. 2.2.1). Before describing mechanisms associated with transport across the plasma membrane, we discuss the movement of nutrients in soil.

2.1 Nutrients in the Soil

2.1.1 Nutrient Availability as Dependent on Soil Age

In relatively young landscapes, following recent volcanic activity or glaciation, phosphorus (P) availability is relatively high, and nitrogen (N) tends to

H. Lambers et al., *Plant Physiological Ecology*, Second edition, DOI: 10.1007/978-0-387-78341-3_6,

be the key nutrient that limits plant productivity. In ancient, highly weathered soils that characterize much of Australia and the Cape region in South Africa, P is the key-limiting nutrient. **Chronosequences** (gradients of soil age) over various geological time scales up to 4 million years constitute natural experiments that allow the study of causes of variation in availability and forms of N and P (Walker & Syers 1976, Vitousek 2004) (Fig. 1A) and of plant strategies for accessing different forms of nutrients (Lambers et al. 2008). These strategies broaden the options for uptake of resources from soils that differ in chemical composition. Individual strategies such as mycorrhizas, N_2-fixing symbioses

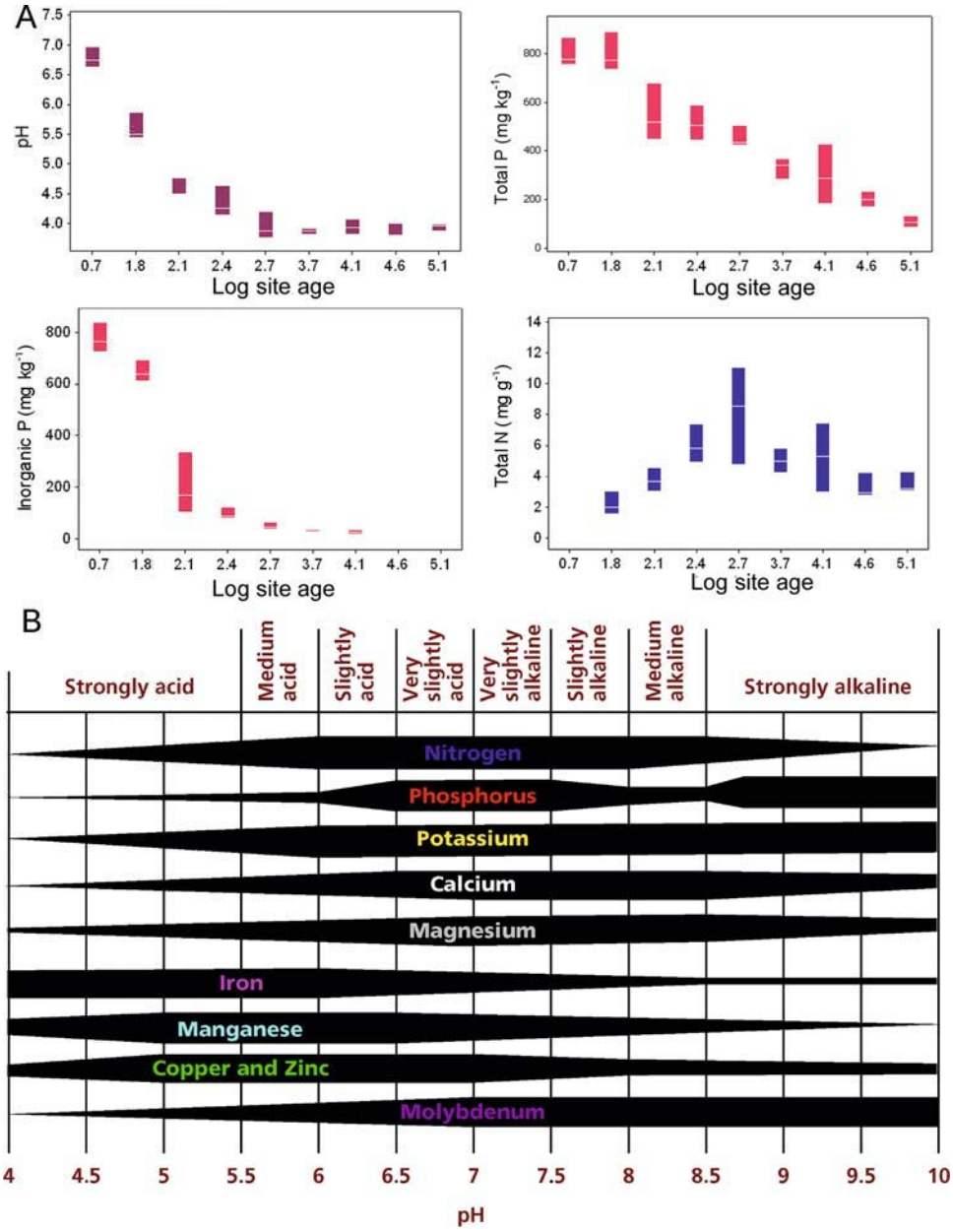


FIGURE 1. (A) Summary of mineral soil properties along the Franz Josef soil chronosequence. Box plot symbols: *horizontal lines* are the median; *shaded bars* give 25 and 75% percentiles, based on Richardson et al. (2004). (B) The availability of a number of essential nutrients in the soil as dependent on soil pH.

(Chapter 9A), and P-absorbing cluster roots (Sect. 2.2.5) may augment each other's activities. Together, these strategies allow plants to grow and compete under a wide range of conditions, including extremely nutrient-impoverished soils, such as those in ancient landscapes.

2.1.2 Nutrient Supply Rate

Nutrient supply rates in the **soil** ultimately govern the rates of nutrient acquisition by plants. **Parent material**, the rocks or sediments that give rise to soil, determines the proportions of minerals that are potentially available to plants. For example, granite is resistant to weathering and generally has lower concentrations of P and cations required by plants than does limestone. Other parent materials such as serpentine rock have high concentrations of heavy metals that are either not required by plants or are required in such low concentrations that their high concentrations in serpentine soils can cause toxic accumulations in plants. Various ecological factors (climate, vegetation, topography, and surface age) strongly influence weathering rates and rates of leaching loss and, therefore, the relationship between parent material and nutrient availability (Jenny 1980).

The **atmosphere** is the major source of N, through both biotic N_2 fixation (Sect. 2 of Chapter 9A on symbiotic associations) and deposition of nitrate and ammonium in precipitation. Atmospheric deposition of P is considerably less but can be important in extremely P-impoverished biomes, such as ocean basins downwind from deserts (Brown et al. 1984, Soderberg & Compton 2007). There is also substantial input from wet and dry deposition. Some cations [e.g., sodium (Na)] may come primarily from sea salt, particularly in coastal regions, but other nutrients [calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K)] come predominantly from dust (from deserts, agricultural areas, and unpaved roads) and from industrial pollution. These atmospheric inputs can be substantial. For example, atmospheric inputs of Ca are equivalent to 62, 42, and 154% of uptake by forests in the eastern United States, Sweden, and the Netherlands, respectively (Hedin et al. 1994), which is considerably higher than annual inputs by weathering. In ecosystems receiving aeolian dust, atmospheric deposition may contribute a substantial proportion of the P requirement of natural vegetation (Gressel & McColl 1997), especially in nutrient-impoverished landscapes (Soderberg & Compton 2007). Thus, atmospheric inputs may determine external mineral supply to ecosystems much more than generally appreciated.

Soil pH is a major factor in determining the **availability** of nutrients in soils. High concentrations of hydrogen ions (low pH) cause modest increases in nutrient input by increasing weathering rate (Johnson et al. 1972), but even greater loss of base cations by leaching. Acid rain is a recent source of soil acidity caused by atmospheric deposition of nitric and sulfuric acid in precipitation. Protons first displace cations from the exchange complex on clay minerals and soil organic matter. Sulfate anions can then leach below the root zone, carrying with them mobile mineral cations (e.g., K, Ca, and Mg) and leaving behind a predominance of hydrogen and Al ions (Fig. 1B) (Driscoll et al. 2001). The availability of other ions is strongly affected by pH because this affects their oxidation state and solubility (e.g., P, S, and Al)or the biological processes that control production and consumption (e.g., N) (Fig. 1B).

In the short term, recycling of nutrients from dead organic matter is the major direct source of soluble nutrients to soils (Table 1). Soluble cations like K and Ca are leached from dead organic matter, whereas organically bound nutrients like N and P must be released by **decomposition**. Plants can only take up inorganic phosphate (P_i), predominantly as $H_2PO_4^-$, which is released by plant or microbial enzymes that release P_i from organic P forms (**phosphatases**). N is released from dead organic matter yielding soluble organic N, which may be further decomposed to NH_4^+ (N **mineralization**). NH_4^+ may then be oxidized, via NO_2^-, to NO_3^- (**nitrification**), and NO_3^- may be converted to gaseous N_2 or N_2O (**denitrification**) (Fig. 2A). The rates of these steps depend on temperature and soil conditions (e.g., pH and redox potential); however, nitrification may also be affected by inhibitors released from

Table 1. Major sources of available nutrients that enter the soil.

	Source of nutrient (% of total)		
Nutrient	**Atmosphere**	**Weathering**	**Recycling**
Temperate forest			
N	**7**	**0**	**93**
P	**1**	**<10?**	**>89**
K	**2**	**10**	**88**
Ca	**4**	**31**	**65**
Arctic tundra			
N	**4**	**0**	**96**
P	**4**	**<1**	**96**

***Source*: Chapin 1991.**

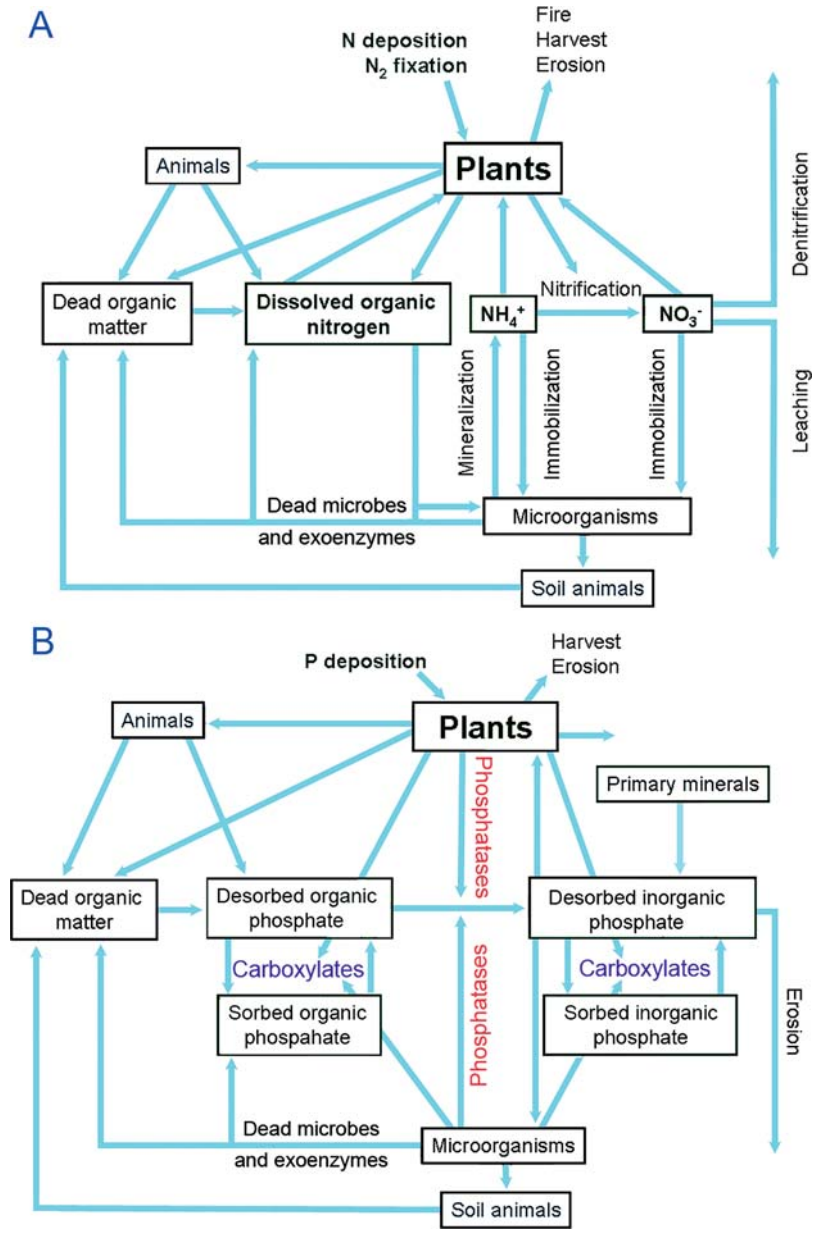


FIGURE 2. (A) A simplified view of the terrestrial N cycle. All N pools (*boxes*) and transformations (*arrows*) are affected by both plants and microorganisms. Dead plants, animals, and microorganisms are decomposed, releasing dead organic matter and then dissolved organic N (e.g., amino acids, urea). Some of the dissolved organic N in soils originate from living organisms. Both plants and microorganisms are capable of using dissolved organic N. Microorganisms use the dissolved organic N as a carbon source, releasing N that is in excess of their requirement as NH_4^+. Both plants and microorganisms can use NH_4^+ as a source of N. Incorporation of NH_4^+ into soil microorganisms leads to N-immobilization; the reverse transformation is called mineralization. Immobilization predominates at high availability of a carbon source, whereas mineralization is favored by a shortage of a source of carbon for microorganisms. Under aerobic conditions, some NH_4^+ is transformed into NO_3^-, in a process called nitrification. In alkaline soil, nitrification predominantly results from autotrophic microorganisms, whereas in acid soil heterotrophic microorganisms are probably most important. NO_3^- is available for both plants and microorganisms; as with NH_4^+, some of the NO_3^- may be immobilized, or lost from the system through leaching or denitrification; denitrification can be inhibited by

roots (Lata et al. 2004), as is further discussed in Chapter 9E on interactions among plants. At each step, plants or soil microorganisms can take up soluble N, or N can be leached from the system, reducing the substrate available for the next N transformation. Therefore, the supply rates of the different forms of "available N" to plants and microbes must follow this same sequence: dissolved organic $N \geq NH_4^+ \geq NO_3^-$ (Eviner & Chapin 1997). If N supply rate always follows the same sequence in all soils, why do the quantities and relative concentrations of these soluble forms of N differ among ecosystems?

First, microbes generally release P_i or NH_4^+ to the soil solution when their growth is more strongly limited by carbon than by nutrients (Schimel & Bennett 2004). On the other hand, they **immobilize** nutrients when decomposing plant litter with low nutrient concentrations and/or high concentrations of labile carbon (e.g., inputs of straw). Second, environmental conditions further modify rates of specific N transformations. For example, cold anaerobic soils in arctic Alaska limit N mineralization and nitrification (an aerobic process), so amino acid N concentrations are relatively high and NO_3^- concentrations low (Kielland 1994). On the other hand, in many arid and agricultural soils, high temperatures promote rapid mineralization and nitrification, and denitrification (an anaerobic process) occurs slowly, so NO_3^- is the most abundant form of soluble N. Finally, N-uptake rates by plants and microorganisms modify availability of each N form to other organisms. For example, low concentrations of NO_3^- in acidic conifer forest soils may be caused by rapid microbial NO_3^- uptake (Stark & Hart 1997), and not only by slow nitrification rates (Lodhi & Killingbeck 1980). Plant species in a N-limited, arctic tundra community are differentiated in timing, depth, and chemical form of N uptake, and species dominance is strongly correlated with uptake of the most available soil N forms (Jones et al. 2005, McKane et al. 2002).

The activity of **phosphatases** that release P_i from organic P sources (Sect. 2.2.5.1) implies that organic phosphate is hydrolyzed independently of the utilization of organic matter by microorganisms (Fig. 2B). In addition, root exudates can greatly enhance weathering of primary minerals and mobilize phosphate **sorbed** to soil particles (Sect. 2.2.5.2); "sorption" refers to both adsorption (precipitation) onto soil particles and absorption inside such particles (Barrow 1984). When compared with the N cycle (Fig. 2A), the **P cycle** is, therefore, considerably less dependent on microbial decomposition of organic matter than the **N cycle**, on both biological and geological time scales (Fig. 2B; Gressel & McColl 1997, Johnson et al. 2003).

In summary, each nutrient is returned from dead organic matter to plant-available forms through distinct processes that occur at different rates in response to quite different environmental controls. Consequently, nutrients in the soil are seldom available in the proportions required by plants.

2.1.3 Nutrient Movement to the Root Surface

As roots grow through the soil, they **intercept** some nutrients. This amount, however, is often less than the amount contained in the growing root, and therefore cannot serve as a net source of nutrients to the rest of the plant. That is, roots do not move toward the nutrients; rather the nutrients must move to the roots by mass flow or diffusion (Table 2).

Rapid transpiration in plants may result in substantial nutrient transport from the bulk soil to the root surface via **mass flow**. The extent to which mass flow is responsible for ion transport to the roots depends on the concentration of the different ions in the bulk solution relative to the requirement for plant growth (Table 2; Prenzel 1979).

If less nutrients arrive at the root surface than are required to sustain plant growth, the concentration at the root surface drops, due to absorption by the roots. This creates a concentration gradient that drives ion **diffusion** toward the root (e.g., for P_i and K^+). Other ions are delivered more rapidly by mass flow than they are required by the roots (e.g., Ca^{2+}), which causes precipitation on the root surface (often as $CaSO_4$) (Barber & Ozanne 1970). Diffusion from the bulk soil to the root surface depends both on the **concentration gradient** and on the **diffusion coefficient**. This coefficient, which varies among soil types, differs by three orders of

FIGURE 2. (continued) specific compounds released from living roots or litter. (B) A simplified representation of the major processes and components of the terrestrial P cycle in plant–soil systems. Several processes explained for the N cycle (e.g., mineralization, immobilization) play a similar role in the P cycle; however, leaching of P tends to be negligible, due to the low mobility of P in soil. Note that plants have considerably greater control over the P cycle than over the N cycle, e.g., via the release of phosphatases and carboxylates.

Table 2. The significance of root interception, mass flow, and diffusion in supplying *Zea mays* (corn) and a sedge tundra ecosystem with nutrients.*

Nutrient	Amount taken up by the crop	Approximate amounts supplied by		
		Root interception	Mass flow	Diffusion
Zea mays				
Nitrogen	190	2	150	38
Phosphorus	40	1	2	37
Potassium	195	4	35	156
Calcium*	40	60	165	0
Magnesium*	45	15	110	0
Sulfur	22	1	21	0
Copper*	0.1	–	0.4	–
Zinc	0.3	–	0.1	–
Boron*	0.2	–	0.7	–
Iron	1.9	–	1.0	–
Manganese*	0.3	–	0.4	–
Molybdenum*	0.01	–	0.02	–
Sedge tundra ecosystem				
Nitrogen	22	–	0.1	21.9
Phosphorus	1.4	–	0.01	1.4
Potassium	9.7	–	0.6	9.1
Calcium	20.9	–	52	0
Magnesium	47.1	–	39.1	8.0

***Source*: Clarkson 1981, Barber 1995, Jungk 1991; tundra data calculated from Shaver & Chapin 1991 and Chapin, unpublished.**

*** All data in kg ha^{-1}. The corn data pertain to a typical fertile silt loam and a crop yield of 9500 kg ha^{-1} and the tundra data a wet sedge meadow with a low-nutrient peat soil. The amount supplied by mass flow was calculated from the concentration of the nutrients in the bulk soil solution and the rate of transpiration. The amount supplied by diffusion is calculated by difference; other forms of transport to the root (e.g., mycorrhizas) may also be important but are not included in these estimates. The elements marked * are potentially supplied in excess by mass flow; they may accumulate at the soil/root interface and diffuse back into the bulk soil.**

magnitude among common ions. It is large for NO_3^-, which therefore moves quickly to the root surface in moist soils, even when there is little water uptake. The diffusion coefficient is also fairly large for K^+ so that most plants can acquire sufficient K to sustain growth. Diffusion coefficients are very low for zinc (Zn^{2+}) and P_i (Table 3), due to specific interactions with the clay minerals of the soil cation-exchange complex. Hence, variation in soil clay content is one of the factors that affect the diffusion coefficient. N and P, which are the two macronutrients that most frequently limit plant growth, are seldom supplied in sufficient quantities by mass flow to meet the plant requirement; therefore, diffusion generally limits their supply to the plant, particularly in natural ecosystems. When soil solution concentrations are much higher, as they are in agricultural soils, mass flow delivers a major fraction of all N required for pant growth (Table 2; Yanai et al. 1998).

Most estimates of the importance of mass flow consider only water movement associated with transpiration. Bulk movement of soil solution, however, also occurs as a "wetting front" after rain. The wetting front carries ions with it and replenishes "diffusion shells" where plant uptake has reduced

Table 3. Typical values for diffusion coefficients for ions in moist soil.*

Ion	Diffusion coefficient ($m^2\ s^{-1}$)
Cl^-	$2–9 \times 10^{-10}$
NO_3^-	1×10^{-10}
SO_4^{2-}	$1–2 \times 10^{-10}$
$H_2PO_4^-$	$0.3–3.3 \times 10^{-13}$
K^+	$1–28 \times 10^{-12}$

***Source*: Clarkson 1981.**

*** The range of values represents values for different soil types.**

nutrient concentrations around individual roots. In arctic tundra, where permafrost causes substantial lateral movement of water, bulk water flow accounts for 90% of the nutrient delivery to deep-rooted species (Chapin et al. 1988). Bulk water movement may have a large (but currently unknown) influence on nutrient supply in other wet ecosystems. Soil heterogeneity may influence the importance of bulk water flow for nutrient supply to roots. Roots and rainwater both move preferentially through soil cracks created by small animals or soil drying. These effects of soil heterogeneity may increase the importance of bulk water movement as a mechanism of nutrient supply more than is currently appreciated.

Mass flow and diffusion cannot always account for the nutrient transport to the root surface. Mass flow delivers very little P_i to the roots, and the diffusion coefficient for P_i in soil is too low to allow much P_i to move by diffusion (Table 3). Some organic phosphate molecules may diffuse more rapidly and become available for the roots, but generally diffusion of organic phosphate is also slow (Sect. 2.2.5.1). If plants do not have access to this source of P, then special adaptations or acclimations are required to acquire P_i when its concentration in the soil solution is low (Sect. 2.2.2). Mycorrhizas are an additional important mechanism of nutrient transport to the root (Sect. 2 of Chapter 9A on symbiotic associations).

Because NO_3^- moves more readily to the roots' surface, it would appear to be available in larger quantities than NH_4^+. Is NO_3^- really the predominant source of N for any plant? That depends to a large extent on environmental conditions. Where both NO_3^- and NH_4^+ are present, NH_4^+ is the preferred source (Garnett & Smethurst 1999, Kronzucker et al. 1999a). When amino acids are available, these can also represent a major source of N (Kielland 1994, Warren 2006). When the soil pH is low, the rate of nitrification, i.e., the oxidation of NH_4^+ to NO_2^- and then to NO_3^- by NH_4^+-oxidizing and NO_2^--oxidizing autotrophic bacteria, respectively, tends to be slow (Lodhi & Killingbeck 1980). Under these conditions NO_3^- will not be a major source of N. The same is true for anaerobic soils, since nitrification is an aerobic process. When soils are cold, such as in arctic Alaska, mineralization is slow, very little NH_4^+ is made available, and a large fraction of the total pool of soil N is present as amino acids (Kielland 1994, Lipson & Näsholm 2001). Under such conditions, amino acids tend to be a major source of N (Henry & Jefferies 2003), but arctic plants will also absorb NO_3^- or NH_4^+ and assimilate it, if supplied in sufficient amounts. Most plants from acid soils, similarly, appear to be capable of absorbing and assimilating NO_3^- and very few species appear to be incapable of using NO_3^- as a source of N (Atkin 1996, Min et al. 1999). The potential to utilize amino acids as N sources is, however, common in most plant communities, regardless of soil fertility (Schmidt & Stewart 1999, Kielland et al. 2006).

Low water availability reduces diffusion rates below values in moist soils, because air replaces water in pores of dry soil, greatly lengthening the path from the bulk soil to the root surface (increased "tortuosity"). Ion mobility in soil can decrease by two orders of magnitude between a soil water potential of –0.01 and –1.0 MPa, which is a range that does not strongly restrict water uptake by most plants (Fig. 3). Because diffusion is the rate-limiting step in uptake of the most strongly limiting nutrients (Table 2), reduction in water availability can greatly reduce plant growth. Two lines of evidence suggest that this may be a major causal mechanism by which low water supply restricts plant growth (Chapin 1991):

(1) Tissue concentrations of growth-limiting nutrients often decline with water stress (Fig. 4), whereas one would expect tissue concentrations to increase if water restricted growth more than nutrient uptake.
(2) Nutrient addition enhances growth of some desert annuals more than does water addition (Gutierrez & Whitford 1987).

The implication of this is that, with current predictions of climate change, plant growth in Mediterranean regions will become more limited by P (Sardans et al. 2007).

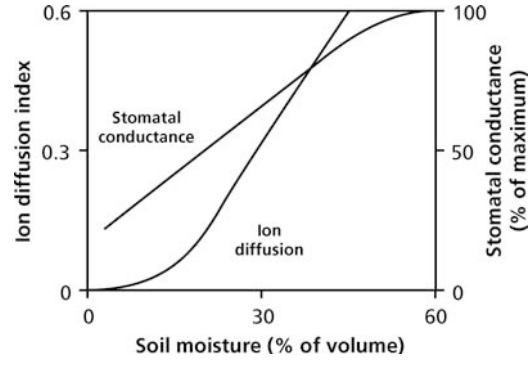


Figure 3. The rate of ion diffusion (deduced from the diffusion impedance factor for Cl^-) and leaf conductance to water vapor as dependent on soil moisture for *Nerium oleander* (oleander) grown in a sandy loam (after Chapin 1991).

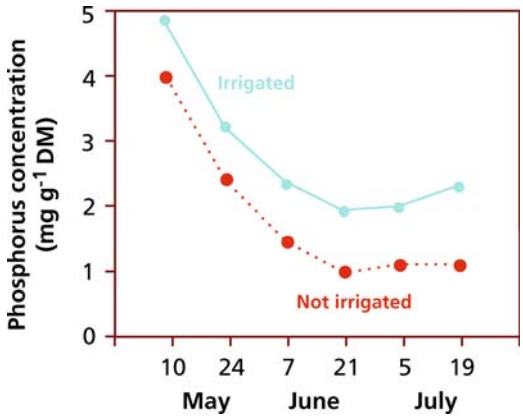


FIGURE 4. P concentration in the shoots of *Hordeum vulgare* (barley) grown with or without irrigation (after Chapin 1991).

For soil-mobile ions, such as NO_3^-, tissue concentrations vary with soil moisture availability in exactly the opposite manner as found for immobile ions. That is, in plants of Australian semi-arid mulga woodlands, the NO_3^- concentration in the tissue tends to be high and the rate of NO_3^- assimilation tends to be low, when the availability of soil moisture is low. After a shower, the NO_3^- concentration in the soil rises rapidly, and the rate of NO_3^- assimilation in the tissue increases, whereas the concentration of NO_3^- in the tissue declines (Erskine et al. 1996).

2.2 Root Traits That Determine Nutrient Acquisition

Rates of nutrient uptake depend on the quantity of root surface area and the uptake properties of this surface. Once nutrients arrive at the root surface, they must pass the plasma membrane of the root cells. As with carbon uptake by photosynthesis (Sect. 2.2 of Chapter 2A on photosynthesis), the rate of nutrient uptake depends on both the concentration in the environment and the **demand** by the plant as well as on the inherent capacity of a plant to take up certain nutrients. The plant's demand is determined by its growth rate and the concentration of the nutrient in the tissues. At a high internal concentration, the capacity for uptake of that nutrient tends to be **down-regulated** so as to avoid nutrient toxicity. Despite this feedback mechanism, plants may show **luxury consumption** of specific nutrients (i.e., absorption at a higher rate than required to sustain growth), leading to the accumulation of that nutrient. Many species from N-rich sites [e.g., *Urtica dioica* (stinging nettle), *Spinacia oleracea* (spinach), and *Lactuca sativa* (lettuce)] show luxury consumption of NO_3^- and accumulate NO_3^- in their vacuoles (Martinoia et al. 1981). Some species from severely P-impoverished habitats [e.g., *Hakea prostrata*(harsh hakea), *Banksia grandis*(bull banksia), and *Protea compacta* (bot river sugarbush)] exhibit **P toxicity** when exposed to slightly higher P levels than that occurring in their natural habitat, because they fail to sufficiently down-regulate P uptake as internal P concentration increases (Lambers et al. 2008).

2.2.1 Increasing the Roots' Absorptive Surface

Because diffusion is the major process that delivers growth-limiting nutrients to plant roots (Table 2), the major way in which plants can augment nutrient acquisition is by increasing the size of the root system. The relative size, expressed as the **root mass ratio** (root mass as a fraction of total plant mass), is enhanced by growth at a low nutrient supply (**acclimation**) (Brouwer 1962). Similarly, plants **adapted** to low nutrient supply typically have a high root mass ratio. Increased root allocation is particularly important for those ions that diffuse slowly in soil (e.g., P_i). In a heterogeneous soil, roots tend to proliferate in those zones with highest availability of N or P, rather than in depleted zones, thus maximizing the effectiveness of each unit of root production (but see Sect. 2.2.5).

The effective absorbing root surface can be enlarged by **root hairs** (Table 4). These root hairs vary in length from 0.2 to 2 mm, depending on species. Root hair length may increase from 0.1 to 0.8 mm, due to reduced supply of NO_3^- or P_i (Bates & Lynch 1996). The diameter of most roots involved in ion uptake is between 0.15 and 1.0 mm, so the presence of root hairs allows a considerably larger cylinder of the soil to be exploited by the root than could be achieved by a root without root hairs. Root hairs have greatest effect on absorption of those ions that diffuse slowly into soil; they are probably not important for the uptake of Si, since mutants of *Oryza sativa* (rice) that lack root hairs take up Si at the same rates as the wild type, and Si transporters involved in Si uptake are not expressed in root hairs (J.F. Ma et al. 2001b, 2006). In low-P soils root hairs may be responsible for as much as 90% of total P_i uptake (Föhse et al. 1991). Total root hair length in cereals may be 20–50 m m^{-1} (of roots from which they emerge); the higher values are

TABLE 4. Phosphorus uptake of seven plant species in relation to morphological root properties (root radius and root hairs).

Species	P_i uptake (10^{-12} mol m^{-1} s^{-1})	Root radius (μm)	Root hairs		
			Number per mm	Average length (mm)	Surface area of root hairs (m^2 m^{-2})
Allium cepa	84	2290	1	0.05	6.5×10^{-3}
Lolium perenne	69	660	45	0.34	1.2
Triticum aestivum	91	770	46	0.33	1.2
Brassica napus	320	730	44	0.31	1.3
Solanum lycopersicum	186	1000	58	0.17	0.6
Spinacia oleracea	485	1070	71	0.62	1.9
Phaseolus vulgaris	60	1450	49	0.20	0.4

Source: Föhse et al. 1991.

typical for P-efficient cultivars (Gahoonia & Nielsen 2004). Species with a high frequency of long root hairs yield relatively more when P is limiting, in comparison with those with less frequent or shorter root hairs which need a high P_i supply for good growth. Increasing the root mass ratio or production of root hairs must incur costs, in terms of investment of carbon, N, and other resources. To achieve a 200% expansion of the root surface by root hairs incurs less than 2% of the costs associated with a similar increase realized by a greater investment in roots (Clarkson 1996). **Mycorrhizal associations** are even more effective in terms of enlarging the P_i-absorbing surface per unit cost, even if we consider that the fungus requires additional plant-derived carbohydrates for its functioning (Sect. 2.6 of Chapter 9A on symbiotic associations).

2.2.2 Transport Proteins: Ion Channels and Carriers

Roots transport nutrients across their plasma membrane either by **diffusion** *down* an electrochemical potential gradient or by **active transport** *against* an electrochemical potential gradient. The electrochemical potential gradient is caused by the extrusion of protons by a **proton-pumping ATPase** that pumps H^+ from the cytosol across the plasma membrane. This creates an electrical potential difference of approximately 80–150 mV (negative inside) across the plasma membrane (Fig. 5A); however, values outside this range have also been measured (Cheeseman & Hanson 1979, Szczerba et al. 2006a). The proton pump functions like the ATPase in the thylakoid membrane of the chloroplast (Sect. 2.1.3 of Chapter 2A on photosynthesis) and the inner membrane of mitochondria (Sect. 2.5.1 of Chapter 2B on plant respiration); however, here the ATPase acts in reverse: it uses ATP and extrudes protons. Cations tend to move inward and anions outward along this electrochemical potential gradient. The **Nernst equation** allows us to calculate that monovalent cations are at electrochemical equilibrium (no driving force for movement) if the concentration of the cation is 40- to 150-fold lower outside than inside the cell. For monovalent anions, the reverse can be calculated: the concentration of an anion at electrochemical equilibrium is 40- to 150-fold lower inside than outside the cell. When concentration gradients are less than this, ions may move in the direction predicted by the electrochemical gradient; when the concentration gradients exceed these values, ions may move in the opposite direction (Fig. 5B).

For most ions, diffusion across the lipid bilayer of the plasma membranes is a very slow process, unless facilitated by special transport proteins. Such transport proteins include **ion-specific channels** (i.e., "pores" in the membrane through which ions can move single file) (Roberts 2006). These channels function is a similar way as the water-channel proteins discussed in Sect. 5.2 of Chapter 3 on plant water relations. The ion channels are either open or closed, depending on the membrane potential or the concentration of specific effectors (Fig. 5A). Ion channels have the advantage that they allow massive transport, albeit only down an electrochemical potential gradient. If such a gradient does not exist or when the gradient is in the opposite direction, channels cannot be used for net transport. In that case, transport may require, first, the extrusion of protons via a H^+-pumping ATPase (Fig. 5A). The proton gradient can then be used for uptake of ions, in a proton-cotransport mechanism via **carrier proteins** (Fig. 5A). Such carriers are like enzymes: they bind their substrates, followed

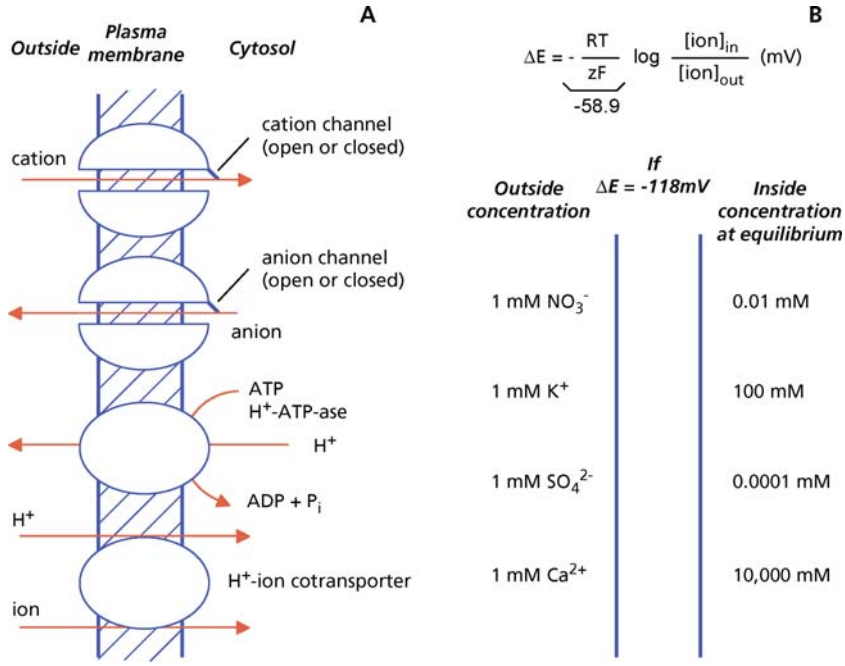


FIGURE 5. (A) Ion transport across the plasma membrane. The membrane potential is negative (i.e., there is a negative charge inside and a positive charge outside). Cations can enter via a cation channel, down an electrochemical potential gradient. Anions (e.g., NO_3^-) can only leave the cytosol via an anion channel, down an electrochemical potential gradient. An H^+-ATPase ("proton pump") extrudes protons from the cytosol, thus creating a proton-motive force. Protons can be used to drive ion uptake against an electrochemical potential gradient. For further explanation, see the text. (B) Schematic representation of the concentration of monovalent and divalent anions and cations that is expected if the plasma membrane is perfectly permeable for these ions in the absence of energy-requiring mechanisms at a membrane potential of 118 mV. The Nernst equation gives the relationship between the membrane potential ΔE and the outside and inside ion concentrations. R is the gas constant; T is the absolute temperature; z is the valency of the ion for which the equilibrium concentration is calculated; and F is Faraday's number. For further explanation, see the text.

by a specific reaction (release of the substrate at the other side of a membrane), and may be allosterically regulated. Carriers tend to have a much lower transport capacity than channels. Both types of proteins are subject to turnover so that continuous protein synthesis is required to maintain ion transport.

Although ions can move via a channel down an electrochemical potential gradient across the plasma membrane, it should be noted that also ion transport via channels is eventually an **active process**, because charge balance must be accomplished, by the H^+-pumping ATPase, at the expense of ATP; otherwise, membranes subjected to, say, NH_4^+ or Na^+ uniport would electrically supercharge and "combust" very quickly (Gerendás & Schurr 1999, Britto & Kronzucker 2006).

Both channels and carriers are, in principle, ion specific, but other ions with similar structure might occasionally enter the cell via these transport proteins. This may account for the entry of some Na^+, heavy metals, and Al in plant roots. Transport proteins are involved in the **influx** of nutrients from the rhizosphere, as well as in the transport of some of the acquired nutrients into the **vacuoles** and the release into the **xylem vessels** (De Boer & Wegner 1997). Channels and carriers are also involved in ion **efflux**, sometimes spectacularly so, as during stomatal movements (Sect. 5.4.2 of Chapter 3 on plant water relations), or they may be responsible for efflux of nutrients, which may occur simultaneously with nutrient influx. Uptake of Na^+ ions from a saline soil occurs down an electrochemical potential gradient, in which case the ions may be extruded with an energy-dependent carrier mechanism (Sect. 3.4.1; Davenport & Tester 2000). Silicon (as silicic) acid is transported into the root cells by a passive channel, but out of the cells by an active transporter (Ma et al. 2006, 2007).

Transport from the rhizosphere across the plasma membrane into the cytosol (influx) is mostly

against an electrochemical potential gradient for all anions and sometimes also for some cations. Such transport must involve an active component (i.e., it requires **metabolic energy)**; however, transport mediated by channels also requires metabolic energy, although indirectly to generate the electrochemical potential gradient. This requires respiratory energy: ATP is used to extrude protons, catalyzed by an H^+-ATPase, so that a membrane potential is created (inside negative). **Efflux** of ions, from the cytosol to the rhizosphere, is mostly down an electrochemical potential gradient for anions; the efflux of NO_3^- may be very low in some circumstances, but it may also be of similar magnitude as the influx (Kronzucker et al. 1999b), especially in slow-growing plants grown with a high nutrient supply (Scheurwater et al. 1999). Like the NO_3^--uptake system, the NO_3^--efflux system is NO_3^- inducible, and it strongly increases with increasing internal NO_3^- concentrations. The efflux system requires both RNA and protein synthesis, but has a much lower turnover rate than the uptake system (Aslam et al. 1996). NO_3^- efflux may contribute significantly to the respiratory costs associated with nutrient acquisition (Sect. 5.2.3 of Chapter 2B on plant respiration). NO_3^- efflux may reflect a fine control of net uptake, compared with the coarse control of gene expression. Ion efflux from roots is not restricted to Na^+ and NO_3^-, but is quite common for a range of other cations and anions (Demidchik et al. 2002, Roberts 2006).

2.2.3 Acclimation and Adaptation of Uptake Kinetics

2.2.3.1 Response to Nutrient Supply

Nutrient uptake by roots increases in response to increasing nutrient supply up to some maximum uptake rate, where a plateau is reached (Fig. 6A) which is very similar to the CO_2 or light-response curves of photosynthesis (Sect. 2.2 of Chapter 2A on photosynthesis; Epstein & Hagen 1952). If nutrient uptake is not limited by diffusion of the nutrient to the root surface, then the shape of this curve is also similar to that obtained with enzymes in solution (Michaelis–Menten kinetics). This leads to the suggestion that the **maximum inflow rate** (I_{max}) may be determined largely by the abundance or specific activity of transport proteins in the plasma membrane; the K_m describes the **affinity** of the transport protein for its ion. This analogy may not be entirely accurate, however, because the access of ions to carriers and ion channels in plasma membranes of a structurally complex cortex is probably quite different from the access of substrates to an enzyme in a stirred solution. Nonetheless, I_{max} is a useful description of the capacity of the root for ion uptake, and K_m describes the capability of the root to utilize low concentrations of substrate (low K_m confers high affinity). Affinities and transporter abundance may be reasonably inferred, provided influx is properly measured, and this can be difficult at high nutrient concentrations (Szczerba et al. 2006b). C_{min} is the minimum ion concentration at which net uptake occurs (analogous to the light- and CO_2-compensation points of photosynthesis). C_{min}, the minimum ion concentration at which net uptake occurs (Fig. 6A), is determined by the balance of influx by ion-transport proteins and efflux along an electrochemical potential gradient. The experimental determination of C_{min} is difficult. For instance, in nonsterile conditions much of the nutrient remaining in solution is in microorganisms. If these are filtered out, then the C_{min} is often spectacularly lower than is usually determined in this critical experiment.

For many nutrients, roots have both a **high-affinity uptake system**, which functions well at low external concentration but has a low I_{max}, and a **low-affinity system**, which is slow at low external concentrations but has a high I_{max} (Forde 2002, Bucher 2007). The high-affinity system is most probably carrier mediated, whereas the low-affinity system may reflect the activity of a channel, at least for K^+. However, there are also "**dual-affinity transporters**", e.g., for NO_3^- (Liu & Tsay 2003). Switching between the two modes of action is regulated by **phosphorylation**; when phosphorylated, the transporter functions as a high-affinity NO_3^- transporter, whereas, it functions as a low-affinity NO_3^- transporter when dephosphorylated. This regulatory mechanism allows plants to change rapidly between high- and low-affinity NO_3^- uptakes. The ecophysiological significance of low-affinity systems for NO_3^-, which only allow significant uptake at NO_3^- concentrations well above that in most natural soils, still remains to be demonstrated (Sect. 2.2.3.2).

When nutrients are in **short supply**, plants tend to show a **compensatory** response in that the I_{max} is increased and a high-affinity transport system is sometimes induced. For example, plants exhibit a high capacity (i.e., high I_{max}) to absorb P_i when grown at a very low supply of P_i, a high potential to absorb NO_3^- and NH_4^+ under conditions when N is in short supply, a high potential to absorb K^+ or SO_4^{2-} when K or S are limiting (Table 5). Information about other nutrients is sparse, but it suggests that there is little stimulation of the inflow of Ca, Mg, and Mn (Robinson 1996). The compensatory increase in

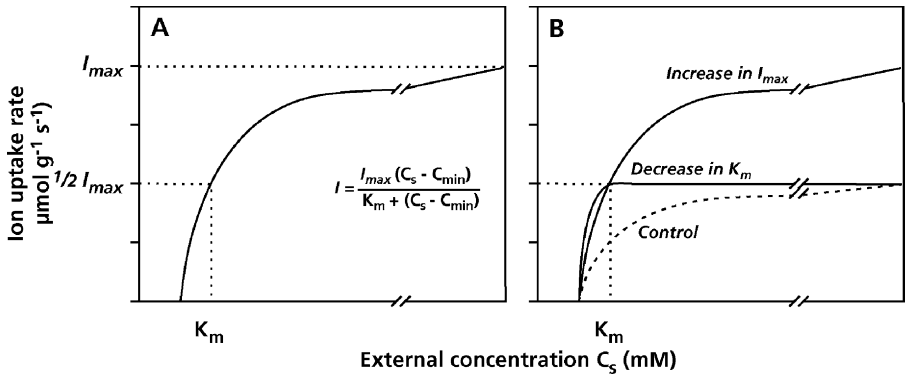


FIGURE 6. (A) The relationships between uptake rates (net inflow = *I*) of ions and their external concentrations (*C*). At C_{min} the net uptake is zero (influx = efflux). (B) Uptake kinetics in control plants and in plants grown with a shortage of nutrients. Note that both induction of a different high-affinity system and up-regulation of the same low-affinity system enhance the capacity for nutrient uptake at low external concentration.

I_{max} for P, N, and K in response to a shortage of these nutrients occurs over a 2- to 15-day period, but can be as fast as hours (Siddiqi et al. 1990). It is specific to the nutrient that limits growth: N limitation increases the capacity to absorb both NH_4^+ and NO_3^-, but it decreases the capacity to absorb other nonlimiting nutrients (Table 5). The appearance of a high-affinity system (low K_m) is especially strong for K, and happens within an hour (Smart et al. 1996).

TABLE 5. Effect of a shortage of one nutrient or of water and exposure to a low irradiance on the maximum rate of nutrient uptake (I_{max}).*

Limiting factor	Ion absorbed	Uptake rate by stressed plants (% of control)
Nitrogen	Ammonium	209
	Nitrate	206
	Phosphate	56
	Sulfate	56
Phosphorus	Phosphate	400
	Nitrate	35
	Sulfate	70
Sulfur	Sulfate	895
	Nitrate	69
	Phosphate	32
Water	Phosphate	13
Light	Nitrate	73

Source: Chapin 1991.
*Values for *Hordeum vulgare* (barley), except for water stress [*Solanum lycopersicum* (tomato)]. Stress is due to low availability of the resource listed in the left-hand column.

Compensatory changes in I_{max} involve synthesis of additional transport proteins for the growth-limiting nutrient, and an up-regulation of mRNA levels coding for a high-affinity uptake system (Sect. 2.2.3.2). A decrease in K_m could be due to induction of a high-affinity system, or to allosteric effects on or phosphorylation of existing transporters (Smart et al. 1996, Liu & Tsay 2003). Both an increase in capacity (I_{max}) of a low-affinity system and induction of a high-affinity system may enhance the uptake capacity at a low nutrient supply (Fig. 6B).

An increase in I_{max} or a decrease in K_m is functionally important if processes at the root surface limit nutrient uptake, as would be the case for NO_3^-. The significance of the up-regulation of the uptake system for the plant is that the concentration of the limiting nutrient at the root surface is decreased which increases the concentration gradient and the diffusion of the limiting nutrient from the bulk soil to the root surface. The significance of such up-regulation for plants growing in soil is relatively small for immobile ions such as P_i, when compared with that for mobile ions such as NO_3^-. For immobile ions, it is the mobility in the soil, rather than the I_{max} of the roots, that determines the rate at which roots can acquire this nutrient from the rhizosphere (Sects. 2.1.2 and 2.3). Rather than considering an *up-regulation* of I_{max} for P uptake at a *low* P availability uptake as being functionally important, a *down-regulation* at *higher* P supply is probably important in avoiding **P toxicity** (Shane et al. 2004a, b).

2.2.3.2 Response to Nutrient Demand

Any factor that increases plant **demand** for a specific nutrient appears to cause an increase in I_{max} for that nutrient. Up-regulation of the system for NO_3^- uptake upon an increased demand involves the NO_3^- concentration in the root itself as well as **systemic signals** from the shoot, imported via the phloem (King et al. 1993). The signals that arrive via the phloem probably include a low concentration of amino acids and/or an increased concentration of organic acids (Touraine et al. 1994). In experiments on effects of the demand for P_i, K^+, or SO_4^{2-}, effects of demand can be simulated by a period of starvation, as discussed in 2.2.3.1. For example, in *Arabidopsis thaliana* (thale cress) the expression of genes that encode a P_i transporter and the capacity to take up P_i increases with decreasing internal P concentration (Dong et al. 1999). The same happens with genes that encode NH_4^+ transporters and the capacity to take up NH_4^+ when the external NH_4^+ supply increases (Rawat et al. 1999). The influence of demand and starvation on NO_3^- transport, however, is more complex (Fig. 7).

For NO_3^-, as is the case for many other ions, there are two inducible uptake systems: a **high-affinity transport system** (HATS) and a **low-affinity transport system** (LATS); other genes encoding NO_3^--uptake systems are constitutively expressed (Miller & Cramer 2005). In the complete absence of external NO_3^- (rather than low external concentrations as described in Table 5), the uptake capacity is very low. In *Hordeum vulgare* (barley) and *Lotus japonicum* (birdsfoot-trefoil), the mRNA for the HATS is almost absent after 72 hours of NO_3^- deprivation. Upon re-exposure of the roots to NO_3^-, this is first taken up by the constitutive HATS. After 30 minutes, there is a huge rise in mRNA encoding the HATS, and after 2–4 hours the inducible HATS is reassembled in the plasma membrane (Trueman et al. 1996a), and the rate of NO_3^- uptake increases (Siddiqi et al. 1990). The general experience, however, is that plants receiving NO_3^-, but in amounts inadequate for supporting maximum growth, de-repress their NO_3^--transport activity (Table 5), so net NO_3^- uptake increases in experimental conditions where the plants are given a sudden dose of NO_3^- (Fig. 7).

The significance of the low-affinity uptake systems, which only function at external NO_3^- concentrations well above that normally found in soil, is puzzling. Concentrations in the range of 5–20 mM, however, do occur in the rhizosphere of crop plants and ruderals (i.e., species that occupy disturbed sites where nitrification rates are generally high) (Wolt 1994). In *Arabidopsis thaliana* (thale cress), the gene that encodes the inducible low-affinity system is expressed in epidermal cells close to the root tip, and in cells beyond the epidermis and even the endodermis further away from the tip; but it is never expressed in the vascular cylinder (Huang et al. 1996). The low-affinity NO_3^--uptake systems cannot be passive, because transport occurs against an electrochemical potential gradient even at an external NO_3^- concentration of 1 mM (Siddiqi et al. 1991). The constitutive system may serve as a **NO_3^--sensing system**, because it is associated with a plasma membrane-bound nitrate reductase. The

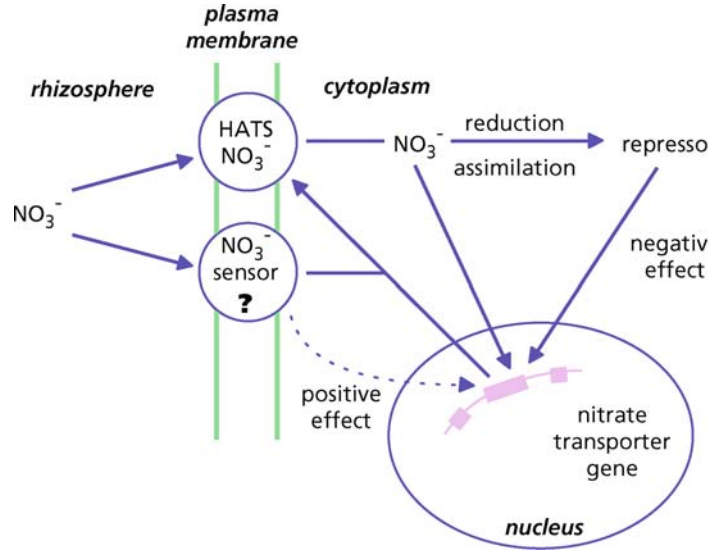


FIGURE 7. Regulation of the inducible high-affinity NO_3^- uptake system (HATS) by NO_3^-. The HATS is affected both by external NO_3^- supply and by internal demand. Situation 1: If *no nitrate* is present in the rhizosphere, there is no positive effector. The gene encoding the NO_3^- transporter is repressed and the system cannot respond immediately to the addition of NO_3^-. Situation 2: If there is an *inadequate nitrate* concentration in the rhizosphere, NO_3^- is sensed (probably by the constitutive HATS) and the gene encoding the NO_3^- transporter is transcribed and the system responds to the addition of NO_3^-. Products of the reduction and assimilation of NO_3^- (amino acids, organic acids) have a negative effect on the transcription of the gene encoding the HATS.

concerted action of the constitutive system and its associated nitrate reductase may lead to the production of intermediates that induce both the inducible high-affinity system and cytosolic nitrate reductase. Both the constitutive and the inducible systems are carrier-mediated proton-cotransport systems, requiring the entry of two protons for every NO_3^- taken up (Mistrik & Ullrich 1996, Trueman et al. 1996b).

C_{min} for a given ion decreases in minutes to hours in response to decreases in supply of that ion, due to decreases in its cytoplasmic concentration, which reduce leakage across the plasma membrane and therefore efflux rates (Kronzucker et al. 1997). The increase in I_{max} when plants acclimate to low availability of a given nutrient increases the plant's capacity to absorb nutrients from solutions of low concentration (Fig. 8). This compensation, however, is always less than 100%, so tissue concentrations increase under conditions of high nutrient supply (**luxury consumption**) and decrease under conditions of low nutrient supply (high nutrient-use efficiency) (Sect. 4). A low capacity to down-regulate I_{max} for P_i uptake is typically associated with species occurring on severely P-impoverished soils (Fig. 8).

A plant's response to nutrient stress, e.g., a short supply of P, requires a capacity to sense the internal nutrient status, e.g., leaf [P]. Recent studies have demonstrated the novel functions of **micro-RNAs** (miRNAs) in regulating adaptive responses to nutrient stresses. Plant miRNAs usually down-regulate the abundance of their target mRNAs by post-transcriptional cleavage of the targeted mRNA. For example, miR399 is up-regulated during Pi deficiency which results in down-regulation of *UBC24*,a gene involved in **targeted protein degradation**. Plants over-expressing miR399 or defective in the gene involved in targeted protein degradation (*UBC24*)display P toxicity because of increased P uptake, enhanced root-to-shoot translocation, and retention of P in their old leaves. This suggests that the miR399-mediated regulation of *UBC24* expression is critical in **P homeostasis**. Similar results have been found for plants that are deprived of S. The existence and conservation of miRNAs and their target genes involved in P and S uptake among many plant species point to the evolutionary importance of these miRNA-mediated nutrient-stress responses (Chiou 2007).

The nature of genetic adaptation to infertile soils differs among ions. Plants adapted to infertile soils typically have a low capacity to absorb immobile ions like P which follows from their relatively low growth rate and hence a low demand for nutrients.

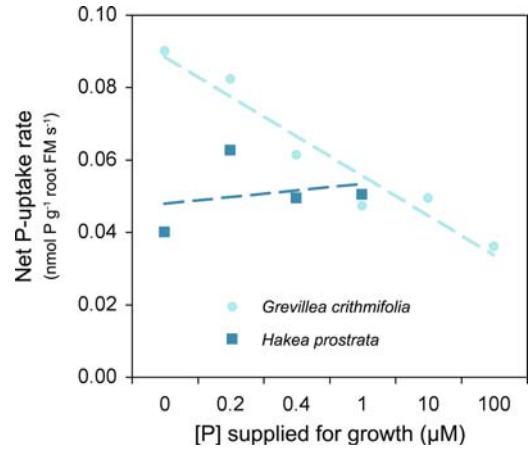


FIGURE 8. Net P_i-uptake rates for intact whole root systems, calculated from P_i-depletion curves. The nutrient solution for the uptake studies contained 5 μM P. Uptake rates are plotted against the external P concentration during plant growth, for *Grevillea crithmifolia* and *Hakea prostrata* (harsh hakea). Note down-regulation of net P-uptake rates (the common response) in *Grevillea crithmifolia*, and a lack of down-regulation of net P-uptake rates in *Hakea prostrata* (which accounts for this species showing signs of P toxicity upon fertilization with P). After Shane et al. (2004b) and Shane & Lambers (2006).

2.2.3.3 Response to Other Environmental and Biotic Factors

The responses of nutrient-uptake kinetics to changes in water, light, and other factors are readily predicted from changes in plant demand for nutrients. **Water stress** may reduce the capacity of roots to absorb nutrients, if it reduces growth, and therefore plant demand for nutrients (Table 5, Fig. 9A). Similarly, plants adapted to dry environments typically have low relative growth rates (Chapter 7 on growth and allocation), and, consequently, low capacities to absorb nutrients. The effect of **irradiance** on nutrient-uptake kinetics depends on nutrient supply. With adequate nutrition, low light availability reduces nutrient uptake (Table 5, Fig. 9B). By contrast, nutrient uptake by nutrient-limited plants is not strongly affected by light availability.

Low temperature directly reduces nutrient uptake by plants, as expected for any physiological process that is dependent on respiratory energy (Fig. 10A; Macduff et al. 1987). Plants compensate through both acclimation and adaptation for this temperature inhibition of uptake by increasing their capacity for nutrient uptake (Fig. 10B,C). In contrast to plants from dry and infertile environments, arctic and alpine plants often grow quite rapidly and so

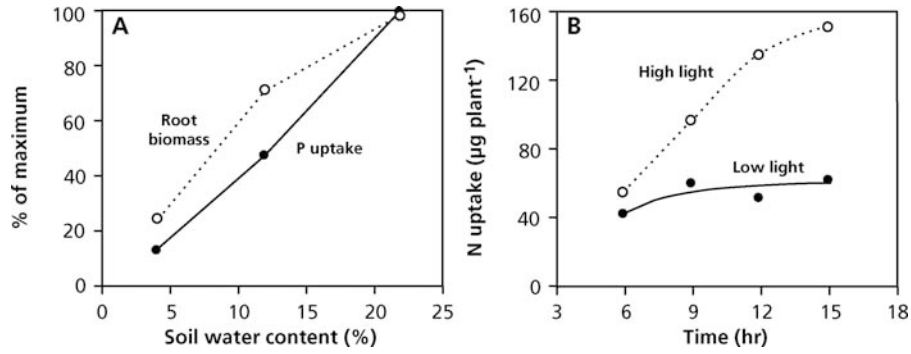


FIGURE 9. Effect of soil water content on root biomass and P_i uptake per unit root biomass in *Solanum lycopersicum* (tomato) (A) and of growth irradiance on ammonium uptake per plant in *Oryza sativa* (rice) (B) (after Chapin 1991).

exploit the short growing season; therefore, they have a substantial demand for nutrients.

When plants are grown with an adequate nutrient supply and store nutrients, **grazing** of leaves reduces plant nutrient demand and therefore reduces nutrient-uptake capacity (Clement et al. 1978). By contrast, grazing of nutrient-stressed plants can deplete plant nutrient stores, so that plants respond by increasing nutrient-uptake capacity (Chapin & Slack 1979). Plants that are adapted to frequent grazing, such as grasses from the Serengeti Plains of Africa, similarly increase their capacity to absorb P_i when clipped to simulate grazing (McNaughton & Chapin 1985).

2.2.4 Acquisition of Nitrogen

N can be absorbed by plants in three distinct forms: **NO_3^-**, **NH_4^+**, and **amino acids**. N assimilation (i.e., the conversion of inorganic to organic N) has a substantial carbon cost: NO_3^- must first be reduced to NH_4^+, which must then be attached to a carbon skeleton before it can be used in biosynthesis. Thus, the carbon cost of assimilation which is generally large is NO_3^- >> NH_4^+ > amino acids (Zerihun et al. 1998). Depending on the species, NO_3^- is reduced either in the roots or transported to the leaves, where it is reduced in the light. The first step in the reduction is catalyzed by **nitrate reductase**, which is an inducible enzyme; the gene encoding nitrate reductase is transcribed in response to NO_3^- application (Campbell 1996). The protein is rather short lived, being degraded with a half-time of a few hours (Miller & Cramer 2005). In addition, the activity of the enzyme is controlled by phosphorylation. In the leaf, the enzyme is turned off at night by **phosphorylation**, which allows inactivation of nitrate reductase by an inhibitor protein.

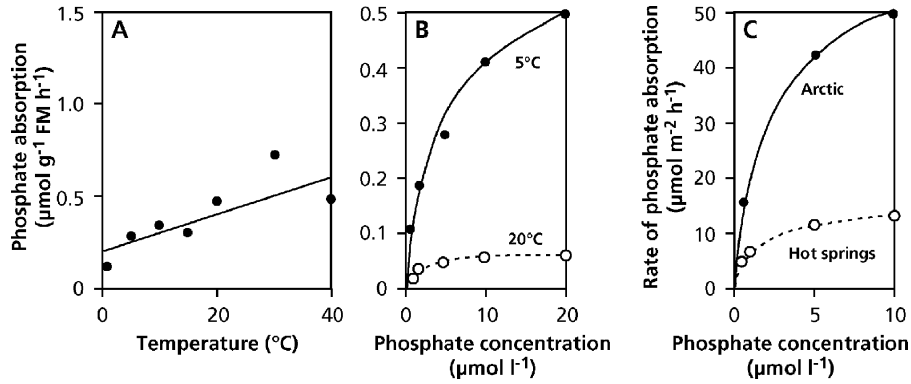


FIGURE 10. Response of P_i uptake by *Carex aquatilis* (a tundra sedge) to temperature at different time scales: (A) immediate response, (B) response following acclimation, and (C) response of adapted genotypes (measured at 5°C) (after Chapin 1974; copyright Ecological Society of America, and Chapin & Bloom 1976).

A protein phosphatase reactivates the enzyme when irradiance increases (Kaiser & Huber 2001). NO_3^- assimilation is energetically expensive because of the costs of NO_3^- reduction. NH_4^+ is toxic to plant cells, and therefore must be assimilated rapidly to amino acids. NO_3^- reduction to NH_4^+ requires approximately 15% of plant-available energy when it occurs in the roots (2% in plants that reduce NO_3^- in leaves) with an additional 25% of available energy for NH_4^+ assimilation (Bloom et al. 1992). One might think that the lower costs when NH_4^+, rather than NO_3^-, is used as the source of N by the plant would allow for a higher growth rate. This does not always occur, however, because of either adjustments in leaf area ratio (Sect. 2.1.1 of Chapter 7 on growth and allocation) or possibly a lower efficiency of root respiration (Sect. 2.6 of Chapter 2B on plant respiration).

The distribution of nitrate reductase activity and the presence/absence of NO_3^- in xylem sap suggest the following ecological patterns (Andrews 1986):

1. All species increase the proportion of NO_3^- reduced in the shoot as NO_3^- supply increases which suggests a limited capacity for NO_3^- reduction in the root system.
2. Temperate perennials and annual legumes reduce most NO_3^- in the roots under low NO_3^- supply.
3. Temperate nonlegume annuals vary considerably among species in the proportion of NO_3^- reduced in roots under low NO_3^- supply.
4. Tropical and subtropical species, both annuals and perennials, reduce a substantial proportion of their NO_3^- in the shoot, even when growing at a low NO_3^- supply.

Despite these general patterns, some NO_3^- reduction occurs in leaves of most plants, particularly in ruderals. Leaf nitrate reductase activity is typically highest at midday in association with high light intensities. Some plants, particularly those in the Ericaceae, show low levels of nitrate reductase (Smirnoff et al. 1984), presumably because NO_3^- availability is generally low in habitats occupied by these species. Leaves of most Gymnospermae and Proteaceae reduce NO_3^- only after induction by feeding leaves with NO_3^- which suggests that these species also reduce most NO_3^- in the roots (Smirnoff et al. 1984).

Plant species differ in their preferred forms of N absorbed, depending on the forms available in the soil. For example, arctic plants, which experience high amino acid concentrations in soil, preferentially absorb and grow on amino acids, whereas *Hordeum vulgare* (barley) preferentially absorbs inorganic N (Chapin et al. 1993); *Picea glauca* (white spruce) preferentially absorbs NH_4^+ (Kronzucker et al. 1997). Much of the early work on NO_3^- and NH_4^+ preference is difficult to interpret because of inadequate pH control (Sect. 2.2.6) or low light intensity. Species from habitats with high NO_3^- availability (e.g., calcareous grasslands), however, often show preference for NO_3^- and have higher nitrate reductase activities than do species from low-NO_3^- habitats. Most plants are capable of absorbing any form of soluble N, however, especially if acclimated to its presence (Atkin 1996).

Plants can also acquire N from the air. This is an important avenue of N uptake by N-limited forests exposed to rain that has high NO_3^- due to fossil fuel combustion or high NH_4^+ due to volatilization from agricultural lands and stockyards (Clarkson et al. 1986). Natural and agricultural vegetation acts as a major "sink" for atmospheric pollutants in terrestrial ecosystems. When the needles of *Picea abies* (Norway spruce) are exposed to NO_2, they rapidly induce nitrate reductase and assimilate the N (Von Ballmoos et al. 1998). It has been estimated that the total emissions of NO_x (i.e., a combination of NO and NO_2) are around 150 million tons per year, and that more than half of this is from a natural origin. In metropolitan areas, however, 75% of the NO_x may be due to road traffic. The capacity to assimilate NO_2 from the air varies greatly among species. Some species [e.g., *Magnolia kobus* (kobus magnolia), *Eucalyptus viminalis* (manna gum), and *Nicotiana tabacum* (tobacco)] may derive more than 10% of their N from NO_2. Information about the species that can assimilate a lot of NO_x may be useful in choosing street trees in polluted areas (Morikawa et al. 1998).

2.2.5 Acquisition of Phosphorus

There are numerous traits involved in acquiring sufficient quantities of P_i from soil. Some of these traits are specific for P_i (e.g., root phosphatases); other traits (e.g., root hairs and root mass ratio) promote uptake of all ions, but are most critical for P_i because of the low diffusion coefficient of P_i in soil (Table 3) and therefore the small volume of soil that each root can exploit. The specialized association with a mycorrhizal fungus will be discussed in Sect. 2.3 of Chapter 9A on symbiotic associations.

2.2.5.1 Plants Can Also Use Some Organic Phosphate Compounds

In agricultural soils, 30–70% of all P is present in an **organic** form; in nutrient-poor grasslands, peat soils, and forest soils this may be as much as

80–95% (Macklon et al. 1994, Turner 2006), or 99% in organic tundra soils (Kielland 1994). A major form of soil P is **inositol phosphate**, which consists of esters containing four, five, or six P molecules, or stereo-isomers thereof (Turner & Richardson 2004). Many species [e.g., *Lupinus albus* (white lupin) (Adams & Pate 1992), *Carex acutiformis* (pond sedge) (Pérez Corona et al. 1996), *Trifolium subterraneam* (subclover) (Hayes et al. 2000), *Triticum aestivum* (wheat) (Richardson et al. 2000)] can use nucleic acids, phospholipids, glucose 1-phosphate, and glycerophosphate (all present in the soil), in addition to P_i, due to the activity of **phosphatases** in the soil. Production of phosphatases by the roots provides an additional source of P_i; these enzymes hydrolyze organic P-containing compounds, releasing P_i that is absorbed by roots (Richardson et al. 2007). Phosphatase production is enhanced by a low P_i supply to the plants. Phosphatases cannot hydrolyze **phytate** (the calcium salt of *myo*-inositol hexakisphosphate), the major form of organic P in seeds; **phytase** is required to release P_i from this source. Some plants may release phytase into the rhizosphere, but for many plants phytate is a poor source of P (Hayes et al. 2000, Richardson et al. 2000). Roots may, however, exude organic substances that act as substrates for microorganisms, which produce enzymes that hydrolyze organic phosphate, including phytate (Richardson 1994). Whatever the exact mechanism by which organic P is hydrolyzed, the concentration of organic P near the root surface may decrease by as much as 65% in *Trifolium alexandrinum* (berseem clover) and 86% in *Triticum aestivum* (wheat) (Tarafdar & Jungk 1987). This shows that these roots do have access to organic forms of P in the soil (Fig. 11).

The capacity to use organic P varies among species and also depends on soil conditions. It may range from almost none to a capacity similar to that of the rate of P_i uptake (Hübel & Beck 1993).

2.2.5.2 Excretion of Phosphate-Solubilizing Compounds

Some plants that are adapted to low-P soils excrete **acidifying** and/or **chelating** compounds (e.g., citric acid and malic acid). Acidification enhances the solubility of P_i in alkaline soils; however, in acid soils, when phosphate is bound to Al or Fe, phosphate solubility is not enhanced by a pH decrease in the rhizosphere (Fig. 1B). Chelating compounds, including citrate and malate, occupy sites that bind phosphate (ligand exchange), and thus solubilize phosphate **sorbed** to soil particles. Both acidification (in alkaline soils) and chelation (all soils) processes enhance the concentration gradient for P_i between the bulk soil and the root surface (Lambers et al. 2006). Crop species vary widely in their capacity to access sparingly available P (Pearse et al. 2006), and such variation offers potential for improving crops for specific soils, intercropping, and crop rotations (Kamh et al. 1999, 2002, Nuruzzaman et al. 2005, Li et al. 2007).

The capacity to excrete carboxylates is very pronounced in members of the Proteaceae, which do not form a mycorrhizal association, but have **proteoid roots** (Fig. 12). The term "proteoid roots" was given because the structures were first discovered in the family of the Proteaceae (Purnell 1960). Similar structures have since been found in many other families, and now the term **cluster roots** is used more commonly. Proteoid cluster roots consist of clusters of longitudinal rows of extremely hairy

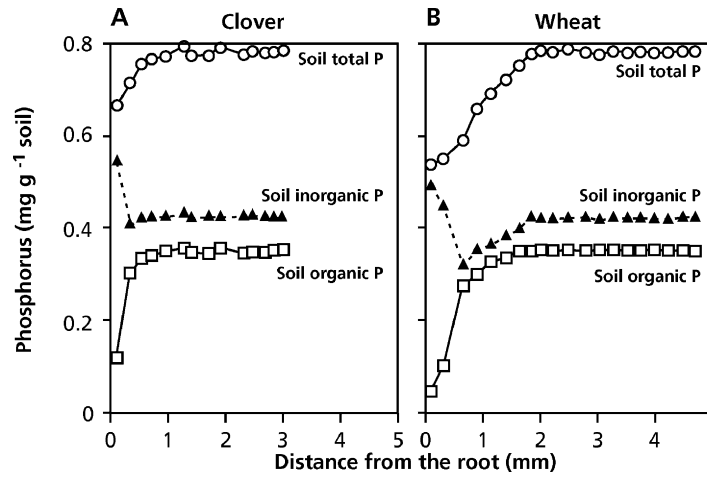


FIGURE 11. Distribution of total, inorganic and organic P in the rhizosphere of *Trifolium alexandrinum* (clover, 10 days old) and *Triticum aestivum* (wheat, 15 days old) grown in a silt loam (Tarafdar & Jungk 1987).

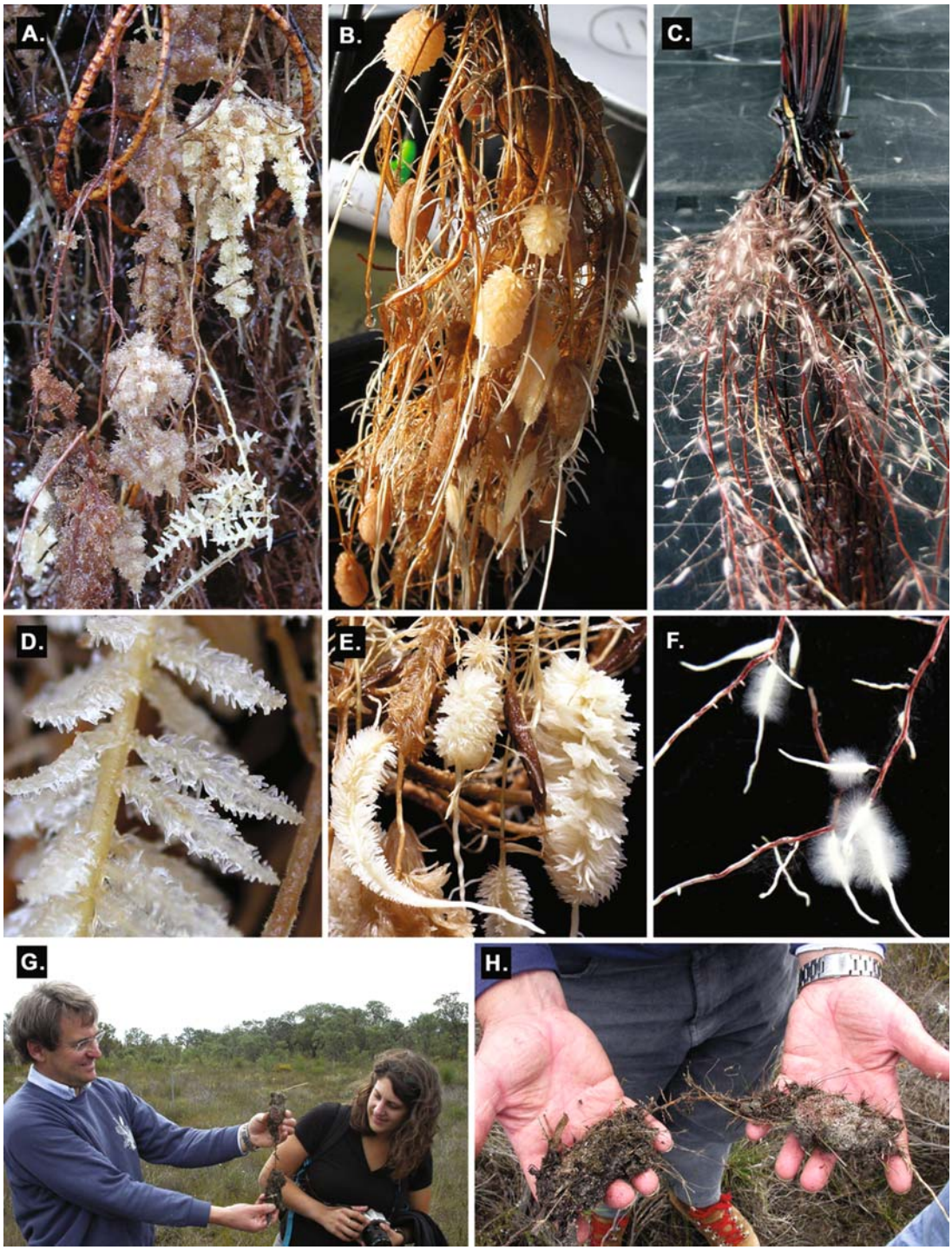


FIGURE 12. Root-cluster morphology of Proteaceae and Cyperaceae species. In A–F plants were grown hydroponically at very low P supply ($\leq$ 1 μM). (A) *Dryandra sessilis* (parrot bush) root system with "compound" "proteoid" root clusters; bar is 20 mm. (B) *Hakea prostrata* (harsh hakea) root system with "simple" proteoid-root clusters; bar is 30 mm. (C) *Tetraria* (sedge) species root system with "dauciform" root clusters; bar is 20 mm. (D) Young, compound proteoid-root cluster of *Banksia grandis* (bull banksia) terminates with third-order determinate, branch rootlets; bar is 3 mm. (E) Simple proteoid-root clusters of *Hakea sericea* (silky

rootlets, which originate during root development, 1–3 cm from the root tip. One lateral branch may contain one, two, or several clusters, centimeters apart from each other. Clusters may consist of unbranched rootlets [simple cluster roots, as in *Hakea* species (Proteaceae) and *Lupinus albus* (white lupin, Fabaceae) (Fig. 12B,E)],or they may have branched rootlets [compound cluster roots, as in *Banksia* species (Proteaceae) (Fig. 12A,D) (Shane & Lambers 2005)]. The cluster roots excrete carboxylates, phenolics, and phosphatases (Lambers et al. 2006), but this process takes place during only a few days after their formation (Neumann et al. 2000). Many sedges (Cyperaceae) produce **dauciform roots**, carrot-shaped roots with long root hairs (Fig. 12C,F), which are physiologically similar to the cluster roots in Proteaceae and Fabaceae (Shane et al. 2005). A third type of root clusters, **capillaroid roots, is** restricted to some species in the Restionaceae (Lambers et al. 2006).

Root clusters are almost universal in the Proteaceae; they also occur in species belonging to the Betulaceae, Casuarinaceae, Cucurbitaceae, Cyperaceae, Elaeagnaceae, Fabaceae, Moraceae, Myricaceae, and Restionaceae. Many species that form cluster roots are nonmycorrhizal or weakly mycorrhizal (e.g., Cyperaceae, some Fabaceae, Proteaceae, Restionaceae), but this is not universal (e.g., Betulaceae, Casuarinaceae, Elaeagnaceae, some Fabaceae) (Lambers et al. 2006).

In Australia and South Africa, nonmycorrhizal cluster-bearing species of the Proteaceae occur on the most heavily leached and P-impoverished soils. Mycorrhizal species of the Myrtaceae, on the other hand, are found on soil with higher P levels. Species of the Casuarinaceae, which are both mycorrhizal and cluster bearing, occupy an intermediate position (Lambers et al. 2006). This distribution pattern is explained by the fact that cluster roots are very effective at acquiring P from soils in which phosphate is largely **sorbed** to soil particles; they effectively "**mine**" the soil for P_i. Arbuscular mycorrhizal associations, on the other hand, act as "**scavengers**" for P_i (Sect. 2.2 of Chapter 9A); they are more effective when the P_i concentration in solution is somewhat higher than that in soils where Proteaceae are more abundant (Fig. 13A).

The development of root clusters is suppressed by an increased supply of P_i (Fig. 9A.9 in Sect. 2.3.2 of Chapter 9A on symbiotic associations; Reddell et al. 1997, Keerthisinghe et al. 1998, Shane & Lambers 2006). Because the formation of cluster roots is suppressed by foliar application of P_i, the induction must be controlled systemically by the internal P concentration, rather than by that in the soil (Gilbert et al. 1998).

Proteoid roots of *Lupinus albus* release 40, 20, and 5 times more citric, malic, and succinic acid, respectively, than lupin roots in which the development of proteoid roots is suppressed by P. The mechanism that allows the massive and rapid release of carboxylates is not yet known, but we know that it is mediated by anion channels (Zhang et al. 2004). Although the excretion of citrate is highest close to the root tip, the capacity to absorb P from the medium is equally high close to, and further away from, the tip. In situ, however, most of the P_i in the soil will be depleted by root cells close to the tip, leaving little to be absorbed by the older zones. The mechanism by which citrate and other chelating substances enhance P uptake is by **solubilizing P_i** that is **sorbed** to soil particles; both inorganic and organic P compounds are solubilized, the latter then becoming available for hydrolysis by **phosphatases** (Fig. 13B).

The capacity to excrete acidifying and/or chelating compounds is not restricted to species with morphological structures such as cluster roots. Species in the Brassicaceae also excrete citric acid, thus enhancing the capacity to solubilize rock phosphate (Hoffland et al. 1989). Some species induce a dissolution of poorly soluble phosphate at a faster rate than that of P_i uptake, leading to accumulation in the rhizosphere (Hinsinger 1998). Neighboring plants may profit from the capacity to release inorganic P from sparingly soluble sources. Intercropping, i.e., growing at least two crop species on the same plot of land at the same time, can enhance plant productivity. *Zea mays* (corn) yields 43% more and *Vicia faba*(faba bean) yields 26% more when the species are intercropped on a low-P soil, instead of grown as a monoculture on the same soil (Table 6). Using permeable and

FIGURE 12. (continued) hakea) at various stages of development terminate with second-order determinate branch rootlets (white root clusters are young-mature, whereas brown ones are senescent or dead). (F) Higher magnification of dauciform-root clusters of *Tetraria* species in C. Root hair density is extremely high on individual dauciform roots; bar is 10 mm. (F) *Tetraria* species. In G and E, simple proteoid-root clusters of *Hakea ceratophylla* that tightly bind the sand excavated at the University of Western Australia's Alyson Baird Reserve at Yule Brook (Western Australia) (courtesy M.W. Shane, School of Plant Biology, the University of Western Australia, Perth, Australia). A-F: copyright Elsevier Science, Ltd.

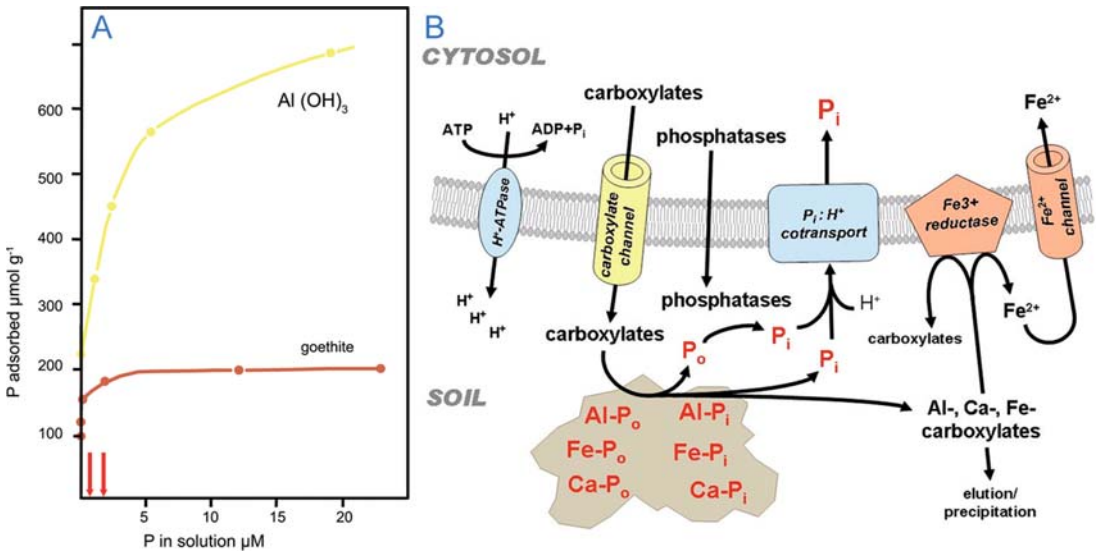


FIGURE 13. (A) P_i-sorption isotherms on goethite (at pH 6.3) and $Al(OH)_3$ (at pH 5.8), using $Ca(H_2PO_4)_2$. Goethite is a common, Fe-containing compound in soil. $Al(OH)_3$ was used for the sake of comparison, since no reduction of the metal was possible. Note that P_i is "not readily available" for *Lolium perenne* (perennial ryegrass) until about 40% of all the goethite is "covered". P availability then increases, reaching a maximum at 2 µM in solution, when 75% of the goethite is covered by sorbed P_i. Mycorrhizas increase the availability for ryegrass in the range 0.5–2 µM, marked by the arrows, when 60–70% of the goethite surface is "covered by sorbed P_i. Modified after Parfitt (1979). (B) Effects of carboxylates (and other exudates) on inorganic (P_i) and organic P (P_o) mobilization in soil. Carboxylates are released via an anion channel. The exact way in which phosphatases are released is not known. Carboxylates mobilize both inorganic and organic P, which both sorb to soil particles. Phosphatases hydrolyze organic P compounds, once these have been mobilized by carboxylates. Carboxylates will also mobilize some of the cations that bind P. Some of these cations (especially Fe) move to the root surface for uptake by the roots. Others move down the soil profile. Modified after Lambers et al. (2006).

TABLE 6. Average biomass and grain yield of *Zea mays* (corn) and *Vicia faba* (faba bean) grown in continuous monoculture, in a continuous intercropping system, or in a continuous rotational system for 4 years in a low-P, high-N soil in China.

	Crop	Cropping system	Average for 2003–2006 kg ha^{-1}	% Increase
Grain yield	Corn	Continuous monoculture	12810	–
		Intercropped with faba bean	18910	49
		Rotated with faba bean	17360	37
	Faba bean	Monoculture	4290	–
		Intercropped with corn	5240	22
		Rotated with corm	5720	29
Above-ground biomass	Corn	Monoculture	26920	–
		Intercropped with faba bean	39990	49
		Rotated with faba bean	36990	38
	Faba bean	Monoculture	10380	–
		Intercropped with corn	12660	22
		Rotated with corn	13000	21

Source: Li et al. 2007.

impermeable root barriers, the positive effects on corn can be ascribed to rhizosphere acidification by faba bean. The positive effect on faba bean is due to exploration of a different rooting depth (Li et al. 2007). P-solubilizing effects may also benefit the following crop (Table 6). When *Triticum aestivum* (wheat) is grown in rotation with *Lupinus albus*(white lupin) on a low-P soil in northern Nigeria, wheat benefits from the P-solubilizing activity of white lupin as the preceding crop (Kamh et al. 2002).

2.2.6 Changing the Chemistry in the Rhizosphere

The availability of several **micronutrients** in the rhizosphere is greatly affected by physiological processes of the roots (Table 7). For example, **proton extrusion** by roots may reduce rhizosphere pH by more than 2 units from that in the bulk soil (Hinsinger et al. 2003); the capacity to affect the pH is strongest at a soil pH of 5–6. Roots also have the capacity to **reduce** compounds in the rhizosphere or at the plasma membrane which is particularly important for the acquisition of Fe, when available in its less mobile oxidized state in soil. On the other hand, roots in flooded soils can **oxidize** compounds in the rhizosphere, largely by the release of oxygen (Sect. 3.5). This can reduce the solubility of potentially toxic ions like aluminum and sulfide. Roots often excrete exudates that **mobilize** sparingly soluble micronutrients, or stimulate the activity of rhizosphere microorganisms and therefore the mineralization of N and P.

Table 7. The availability of a number of micronutrients, aluminum, and toxic heavy metals for plants when the pH decreases, and the reason for the change in availability.

Microelement	Effect of decreased pH on availability of the microelement	Cause of the effect
Aluminum	Increase	Increased solubility
Boron	Increase	Desorption
Cadmium	Increase	Cadmium-organic ligand complexation
Copper	No effect	
Iron	Increase	Reduction, increased solubility
Manganese	Increase	Desorption, reduction
Molybdenum	Decrease	Adsorption
Zinc	Increase	Desorption

Source: Marschner & Römheld 1996, Krishnamurti et al. 1997.

2.2.6.1 Changing the Rhizosphere pH

The pH in the rhizosphere is greatly affected by the **source of N** used by the plant, because N is the nutrient required in largest quantities by plants and can be absorbed as either a cation (NH_4^+) or an anion (NO_3^-). Roots must remain electrically neutral, so when plants absorb more cations than anions, as when $\mathbf{NH_4^+}$ is the major N source, more **protons** must be extruded (reducing rhizosphere pH) than when $\mathbf{NO_3^-}$ is the major N source, in which case the pH tends to rise slightly. An additional cause of the decline in rhizosphere pH when NH_4^+ is the source of N is that, for each N that is incorporated into amino acids, one H^+ is produced. Because NH_4^+ is assimilated exclusively in the roots, whereas NO_3^- is assimilated partly in the roots and partly in the leaves, the production of H^+ is greatest with NH_4^+. A somewhat smaller decrease in pH also occurs when atmospheric N_2 is the sole source of N for legumes or other **N_2-fixing** systems (Sect. 3 of Chapter 9A on symbiotic associations). The drop in pH with NH_4^+ as N source is due to exchange of NH_4^+ for H^+ (or uptake of NH_3, leaving H^+ behind). The rise in pH with NO_3^- as the source of N is thought to be associated with the generation of hydroxyl ions during its reduction according to the overall equation: $NO_3^- + 8\ e^- + 1.5\ H_2O \rightarrow NH_3 + 3\ OH^-$. A more comprehensive analysis, however, which also accounts for primary transport at the plasma membrane and N metabolism subsequent to NO_3^- reduction shows that NO_3^- entry does not raise the pH intracellularly (Britto & Kronzucker 2005). To compensate for an increase in pH associated with NO_3^- accumulation, protons are taken up; some hydroxyl ions are neutralized by the formation of organic acids (mainly malic acid) from neutral sugars. As a result, plants grown with NO_3^- contain more organic acids (mainly malate) than those using NH_4^+ or N_2.

Application of ammonium or urea as fertilizers can create major agricultural problems, since both the pH in the rhizosphere and that of the bulk soil will decline in the longer term. This may mobilize potentially toxic ions, including Al and Mn, and reduce the availability of required nutrients (Fig. 1 and Sect. 3.1).

Rhizosphere pH affects the availability of both soil micronutrients and potentially toxic elements that are not essential for plant growth (Al)

(Table 7). The solubility of iron (Fe) decreases a 1000-fold for each unit increase in soil pH in the range 4–9; that of manganese (Mn), copper (Cu), and zinc (Zn) decreases a 100-fold. Mn and Fe also become more available when they are reduced (to Mn^{2+} and Fe^{2+}, respectively). Although Fe is abundant in the Earth's crust, it predominates as insoluble Fe^{3+} precipitates, which are largely unavailable to plants, especially at neutral or alkaline pH. **Fe-deficiency** symptoms in calcareous soils can be prevented by supplying NH_4^+, which acidifies the rhizosphere, rather than NO_3^-, which tends to further increase the pH around the roots; however, it is only effective in the presence of nitrification inhibitors that prevent the microbial transformation of NH_4^+ to NO_3^- (Marschner 1991). Net nitrification is often favored by a high pH, which increases nitrification more than NO_3^- immobilization by soil microbes. In practice, supplying Fe in a chelated or reduced form is more effective (Table 8).

The availability of molybdenum (Mo) decreases with a decreasing pH, and that of Cu, which tends to be complexed in the soil, is unaffected by pH. As a result, when grown in soil with $(NH_4)_2SO_4$, the concentrations of Fe, Mn, Zn, and B are higher in plant biomass than those in plants given $Ca(NO_3)_2$ (Table 8).

Plants can strongly reduce rhizosphere pH by excreting organic acids (Sect. 2.2.6) or by excreting protons which occur when the uptake of major cations (e.g., K^+) exceeds that of anions (Hinsinger et al. 2003). In calcareous soils, this acid excretion occurs to an extent that bulk soil pH is lowered.

Some nutrient deficiencies cause plants to reduce **rhizosphere pH**. When the Fe supply is insufficient, *Helianthus annuus* (sunflower) plants lower the pH of the root solution from approximately 7 to 4. Similar responses have been found for *Zea mays* (corn) and *Glycine max* (soybean) genotypes with a low susceptibility to Fe deficiency ("lime-induced chlorosis"). Fe deficiency-induced acidification of the rhizosphere is mediated by the proton-pumping ATPase at the plasma membrane, with cations being exchanged for H^+ (Fig. 14). Zn deficiency can also cause a lowering of the rhizosphere pH (Römheld 1987). Organic acid-mediated dissolution of Fe plays a significant role in elevating the concentration of Fe complexes in the rhizosphere, especially when Fe occurs as $Fe(OH)_3$, but less so when it is present as Fe oxides (Fe_2O_3 and Fe_3O_4) (Jones et al. 1996a).

Lowering the pH in response to Fe deficiency may coincide with an increased capacity to reduce Fe at the root surface, due to the activity of a specific **Fe reductase** in the plasma membrane (Schmidt 2003). Reducing and chelating compounds (phenolics) may be excreted, solubilizing and reducing Fe^{3+} (Deiana et al. 1992). This is the typical response of Fe-efficient dicots and monocots other than grasses ("strategy I" in Fig. 15). Excretion of reducing and chelating compounds also enhances the availability and uptake of Mn. In calcareous soils with a low concentration of Fe and a high concentration of Mn, this strategy may lead to Mn toxicity. When the buffering capacity of the soil is large and the pH is fairly high, "strategy I" is not very effective.

2.2.6.2 Excretion of Organic Chelates

Grasses exude very effective chelating compounds, particularly when Fe or Zn are in short supply

Table 8. The effect of the form of nitrogen applied to a sandy loam [*Triticum aestivum* (wheat) and *Brassica oleracea* var. *botrytis* (cauliflower)] or a calcareous soil [*Arachis hypogaea* (peanut)] on concentrations of micronutrients or chlorophyll.*

	Micronutrient concentration (mg kg^{-1} DM)				
N-source	Fe	Mn	Zn	B	Chlorophyll concentration [mg (g^{-1} FM^{-1})]
Nitrate	55	23	18	3.5	0.89
Ammonium	68	45	24	12.9	0.85
Ammonium + nitrification inhibitor					1.76
Nitrate + FeEDDHA					2.96

Source: Marschner 1991.

* Chlorophyll concentration is a good indicator for the availability of Fe in the rhizosphere. To inhibit the transformation of ammonium into NO_3^- by nitrifying bacteria, a nitrification inhibitor (nitrapyrin) was added. FeEDDHA is a chelated form of Fe, which is readily available to the plant. Concentrations were measured in mature leaves (B), young leaves (chlorophyll), or the entire shoot (other micronutrients).

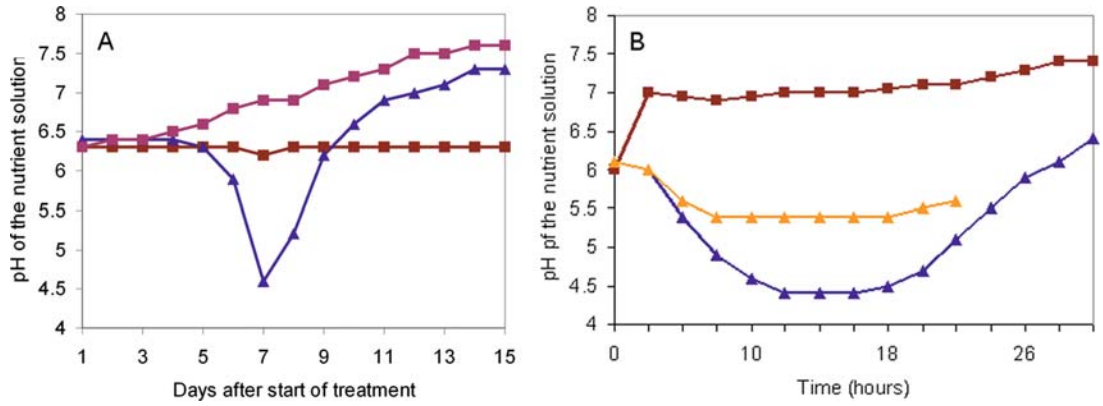


FIGURE 14. Changes in pH in the root environment of *Cicer arietinum* (chickpea) as affected by Fe supply. (A) Effects of Fe supply in the absence and presence of an organic buffer (MES, 4-morpholineethanesulfonic acid) on the acidification of the nutrient solution. (B) Effects of an inhibitor of the plasma membrane ATPase (vanadate) on the acidification of the nutrient solution (Ohwaki & Sugahara 1997).

(Fig. 15). These chelators are called **phytosiderophores**, because of their role in the acquisition of Fe. However, these chelators are also important for the uptake of metals like Zn, when these are in short supply, and hence the term phytometallophore is also used (Cakmak et al. 1996). Phytosiderophores are probably released through an anion channel; concomitant K^+ uptake ensures charge balance (Sakaguchi et al. 1999). Fe diffuses in the form of an Fe-phytosiderophore chelate to the root surface, and is absorbed as such by root cells ("strategy II"; Fig. 17). The system responsible for uptake of the Fe chelate is induced by **Fe deficiency**. In strategy II, Fe reduction takes place after uptake into the root cells, rather than prior to uptake as in strategy I. The capacity of a genotype to release phytosiderophores is inversely related to its sensitivity to Fe or Zn deficiency. For example, *Hordeum vulgare* (barley) is less sensitive to Fe deficiency and excretes more phytosiderophores than sorghum (*Sorghum bicolor*) and corn (*Zea mays*) (Marschner & Römheld 1996). Genotypes of wheat [*Triticum aestivum* (bread wheat) and *Triticum durum* (durum wheat)] that are more resistant to Zn deficiency exude more phytosiderophores than do more sensitive genotypes (Cakmak et al. 1996, Rengel & Römheld 2000).

Phytosiderophores are similar to and sometimes derived from nicotinamine (Fig. 16). Nicotinamine itself is also an effective chelator and probably plays a role in chelating Fe inside the cell, in both strategies I and II (Scholz et al. 1992). Phytosiderophores are specific for each species and are more effective in chelating Fe than are many synthetic chelators used in nutrient solutions. They also form

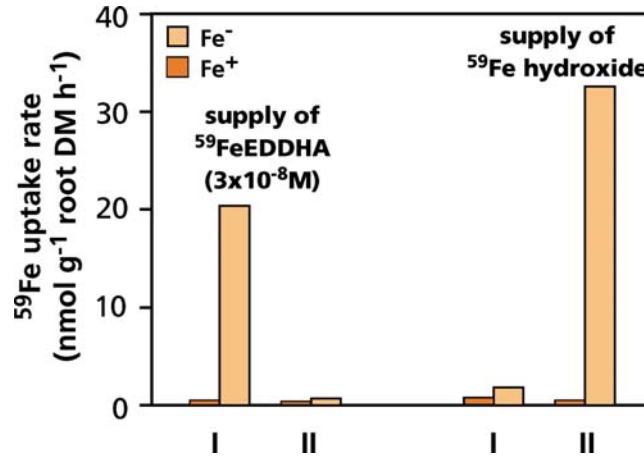


FIGURE 15. The response to Fe deficiency of species following two contrasting "strategies". Strategy II is restricted to grasses. Strategy I is found in monocots, with the exception of grasses, and in dicots. Plants are grown with or without Fe and then supplied with ^{59}FeEDDHA or ^{59}Fe hydroxide (Römheld 1987). Copyright Physiologia Plantarum.

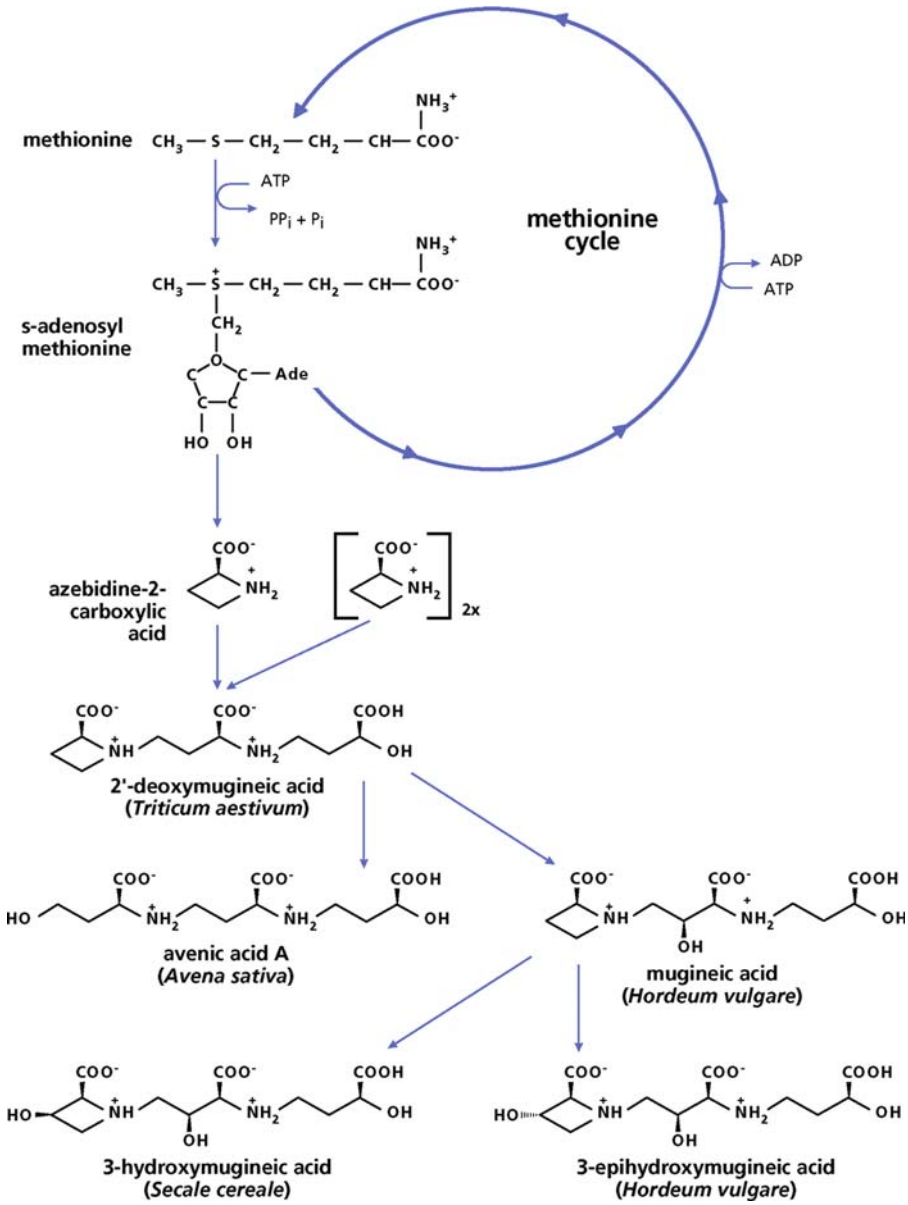


FIGURE 16. Scheme for the biosynthesis of phytosiderophores, which are hydroxy- and amino-substituted imino-carboxylic acids exuded by graminaceous monocotyledonous plants (Ueno et al. 2007). Copyright Trustees of The New Phytologist.

stable chelates with Cu, Zn, and, to a lesser extent, Mn and enhance the availability of these nutrients in calcareous soils. Fe-efficient species belonging to strategy I or II show an enhanced capacity to absorb Fe upon withdrawal of Fe from the nutrient solution (Fig. 17).

Phytosiderophores that are excreted by Fe-efficient grasses can also enhance the Fe status of some Fe-inefficient dicotyledonous neighboring plants, both in nutrient solution and in pot experiments. This mechanism offers an explanation for the success of **intercropping** *Arachis hypogaea* (peanut) with *Zea mays* (corn) in northern China (Zuo et al. 2000) and for the re-greening of fruit trees when grown in combination with *Festuca rubra*(red fescue) (Ma et al. 2003).

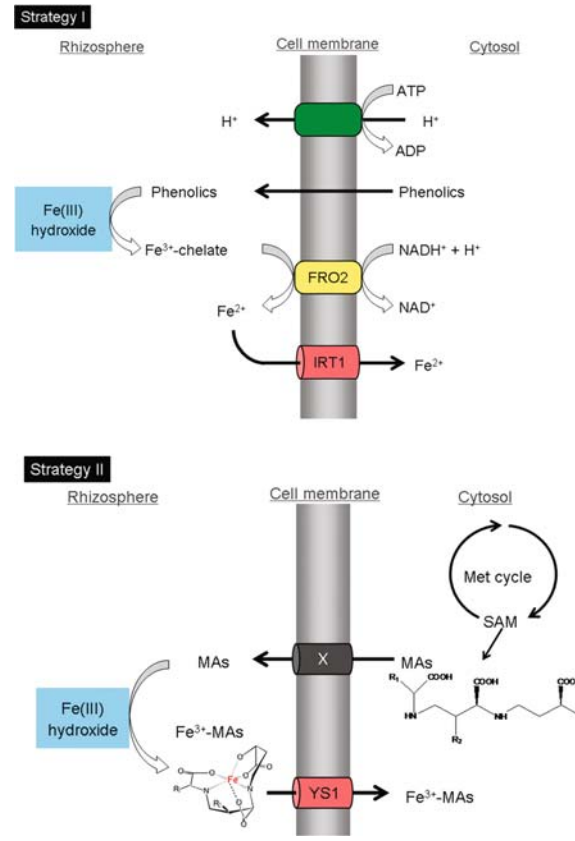


FIGURE 17. Induction of the capacity to absorb Fe as affected by Fe deficiency in dicotyledonous and non-graminaceous dicotyledonous species (strategy I) and in graminaceous species (strategy II) (Ma 2005).

Carboxylates are a common component of root exudates. They are excreted in response to a shortage of P, Fe, K, and some other cations (Jones 1998, Neumann & Römheld 1999). Depending on the dissociation properties and number of carboxylic groups, carboxylates can carry varying negative charges, thereby allowing the complexation of metal cations in solution and the displacement of anions from the soil matrix. For this reason, carboxylates play a role in many soil processes, including the mobilization and acquisition of nutrients by plants (e.g., P_i and Fe) and the detoxification of metals (e.g., Al, Pb), microbial proliferation in the rhizosphere, and the dissolution of soil minerals, leading to **pedogenesis** (e.g., laterite formation and podzolization) (Pate et al. 2001). Organic acids transform high-molecular-mass humus compounds into smaller ones (molecular mass less than 10000). Upon transformation of the humus complex, Ca, Mg, Fe, and Zn are released from the humus complex. Organic acids are far more effective than their K^+-salts or inorganic acids; their action is likely a combination of acidification and chelation (Albuzzio & Ferrari 1989).

2.2.7 Rhizosphere Mineralization

Root exudation of organic acids, carbohydrates, and amino acids, and the sloughing of polysaccharides from growing root tips, usually accounts for less than 5% of total carbon assimilation, but it may increase substantially when P availability is low (Table 2 in Chapter 2B on respiration). Root exudates have major effects on microbial processes in soils which are often carbon limited (Chapter 10A on decomposition). The densities and activity of microorganisms, especially bacteria, and microbial predators are much greater in the rhizosphere than they are in bulk soil, and they are enhanced by factors, such as elevated atmospheric CO_2 concentrations, that increase root exudation (Cheng & Johnson 1998). The effects of root exudates depend on soil fertility (Sect. 3.2 of Chapter 10A on decomposition). In infertile soils, stimulation of root exudation by elevated CO_2 concentrations tends to increase N immobilization by rhizosphere microbes and reduces plant uptake (Diaz et al. 1993). By contrast, in more fertile soils, where microbes are more carbon limited, the stimulation of root exudation by

elevated [CO_2] increases N mineralization and plant uptake (Zak et al. 1993). Annual N uptake by vegetation is often twice the N mineralization estimated from incubation of soils in the absence of roots (Chapin et al. 1988). Much of this discrepancy could involve the more rapid nutrient cycling that occurs in the rhizosphere, as fueled by root exudation.

2.2.8 Root Proliferation in Nutrient-Rich Patches: Is It Adaptive?

When N, K, or P are limiting for plant growth and only available in localized root zones, roots tend to **proliferate** in these zones more than they do in microsites with low nutrient availability. Roots experiencing nutrient-rich patches can also enhance their physiological ion-uptake capacities compared with roots of the same plant outside the patch zone (Hodge 2004). Local proliferation, however, is found only if the elongating tip of the axis from which the laterals emerge has experienced these favorable local conditions while elongating. If it has not, or if the plant as a whole does not experience nutrient deficiency, then no laterals emerge in favorable zones (Drew et al. 1973; Drew 1975). Local root proliferation occurs similarly in species from nutrient-rich [*Holcus lanatus* (common velvetgrass), *Lolium perenne* (perennial ryegrass)] and nutrient-poor habitats [*Anthoxanthum odoratum* (sweet vernalgrass), *Festuca rubra*(red fescue)] (Franken et al. 1999). Recent discoveries on molecular aspects of plant responses to N-rich patches are discussed in Box 6.1.

It would seem that the proliferation of roots in response to a localized nutrient supply is functional, but is it really? When *Triticum aestivum* (wheat)

Box 6.1
Molecular Control of Local Root Proliferation

Local root proliferation in response to patches enriched in N, P, or K is well documented (Sect. 2.2.8; Zhang & Forde 1998). In roots of *Arabidopsis thaliana* (thale cress), NO_3^- induces a gene (*ANR1*) that codes for a NO_3^--specific transcription factor; this gene is not affected by K or P (Zhang & Forde 1998, Forde 2002). Transgenics in which expression of this key gene is repressed no longer respond to NO_3^--rich zones by lateral-root proliferation. When NO_3^- is supplied to the entire root system, lateral-root growth is unaffected by NO_3^- in the range of 0.01–1 mM, whereas it is inhibited in the transgenic plants. A mutant that has only 0.5% of the nitrate reductase activity of the wild type exhibits a response that is similar to that of the wild type. This shows that NO_3^- itself, rather than an assimilation product, is responsible for the effects on localized root proliferation.

Root proliferation corresponds with an increased rate of cell production in the lateral-root meristem (Sect. 2.2.5 in Chapter 7 on growth and allocation). An auxin-resistant mutant does not respond to the NO_3^- signal, suggesting involvement of the phytohormone auxin (Box 7.1 in Chapter 7) in the NO_3^--stimulated lateral-root expansion (Zhang et al. 1999). Lateral-root primordia originate from pericycle founder cells. Sophisticated mass-spectroscopy-based techniques have been used to determine the exact map of the sites of biosynthesis of auxin and its distribution in *Arabidopsis thaliana*. This has highlighted the importance of the phytohormone during lateral-root initiation and emergence (Casimiro et al. 2003).

The systemic inhibitory effect (i.e., the suppression of root proliferation when a high NO_3^- concentration is supplied to the entire root system) acts by suppressing the development of lateral-root primordia at a stage just after emergence through the epidermis. Mature laterals are insensitive to NO_3^-, and the stunted lateral roots that are produced in 50 mM NO_3^- grow out as normal after plants have been transferred to 1 mM NO_3^-. Therefore, the post-emergence stage is specifically susceptible to the systemic, inhibitory signal (Zhang et al. 1999).

To explain the manner in which down-regulation of *ANR1* leads to suppression of the growth of lateral roots in well-fed plants and to the absence of a response to a local NO_3^- supply, thefollowing model has been suggested. First, there is the localized stimulatory effect that requires the presence of NO_3^- at the lateral-root tip and *ANR1* expression. Second, there is a systemic inhibitory effect that results from the influence of NO_3^- supply on

continued

Box 6.1. *Continued*

the N status of the shoot; this effect does not depend on expression of *ANR1* and might involve cytokinins (Box 7.1 and Sect. 5.4.4 in Chapter 7 on growth and allocation). This model is consistent with a response of the lateral roots of wild-type plants in NO_3^--rich patches, and with the lack of a response in the transgenics. It is also consistent with the inhibition of lateral-root growth of the transgenics when NO_3^- is supplied to the entire root system; the positive effect of *ANR1* is blocked, and there is only the inhibitory effect of the N status of the entire plant (Fig. 1).

The localized stimulatory effect begins with perception of the NO_3^- signal by a NO_3^- sensor, involving a specific NO_3^- transporter, NRT1.1 (Fig. 7; Remans et al. 2006). The signal is then transduced through a pathway that involves the products of *ANR1*. The transcription factor encoded by this gene activates a set of genes that modulate meristematic activity in the lateral-root tip. An auxin-sensitivity gene (*AXR4*) interacts with *ANR1*, but it is not yet clear how the two genes interact.

The systemic inhibitory effect requires the uptake of NO_3^-; the more NO_3^- is taken up, the stronger the inhibition. Evidence from experiments with a mutant deficient in nitrate reductase suggests that the plant senses the internal NO_3^- pool. The nature of the inhibitory signal is unknown, but it probably originates in the shoot, because applying 50 mM KNO_3 to one half of a split-root system leads to the suppression of lateral-root development in both halves. The inhibitory signal appears to be sensed specifically during a critical phase of lateral-root development after emergence from the parent root and just prior to the point at which the cells of the newly differentiated lateral-root meristem become activated and elongation of the mature lateral root begins.

The two opposing effects of NO_3^- provide a regulatory system that enables root branching to respond to both the plant's N status and the local availability of NO_3^-. In this way, the intensity of the response to a localized NO_3^- source (i.e., the foraging response) can be adjusted according to the plant's demand for N, so that resource allocation within the plant as a whole can be optimized (Zhang & Forde 2002).

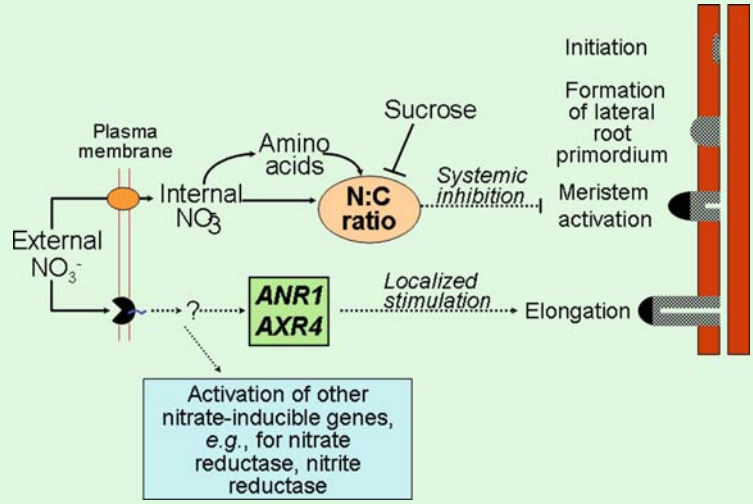


FIGURE 1. Dual-pathway model for regulation of lateral-root growth and development by NO_3^- in *Arabidopsis thaliana*(thale cress). *Broken lines* indicate signaling steps; *solid arrows* indicate transport or metabolic steps. The localized stimulatory effect depends on the external NO_3^- concentration and acts on the mature lateral-root tip to increase meristematic activity. The systemic inhibitory effect depends on the internal N status of the plant and acts on a critical stage of lateral-root development prior to activation of the lateral-root meristem. Both effects are specific to the lateral roots, and growth of the primary root is largely insensitive to the supply of NO_3^- (Forde 2000). With permission from Oxford University Press.

plants are grown with a localized ^{15}N-labeled organic residue in soil, rates of N uptake per unit root length greatly increase during growth through the localized source of N. Plants obtain only 8% of the N that they ultimately absorb during the first 5 days of exploitation of the localized source. Only after this initial absorption do the roots proliferate in the residue; over the next 7 days they absorb 63% of the total N obtained from the local source. After that time, massive proliferation occurs in the residue, but relatively little further N is captured (Fig. 18). This suggests that local proliferation is of only limited importance for the capture of the N released from locally decomposing organic matter. When plants are competing for nutrients, however, local proliferation is advantageous. For example, when *Lolium perenne* (perennial ryegrass) grows together with *Poa pratensis* (smooth meadow-grass), *L perenne* produces greater root densities in the patch than does *Poa pratensis*, and it also captures more N from the patch (Hodge et al. 1999). Proliferation, triggered by the local source of N, might also be advantageous in the longer term to take up nutrients other than N.

The extent of the response to a localized supply depends on the overall nutrient status of the plant. Thus, if one half of the roots receives no nutrients at all, then the response is considerably stronger than if that half is supplied with a moderate amount (Table 9; Robinson 1994). The development of an individual root obviously depends both on the nutrient availability in its own environment and on other roots of the same plant.

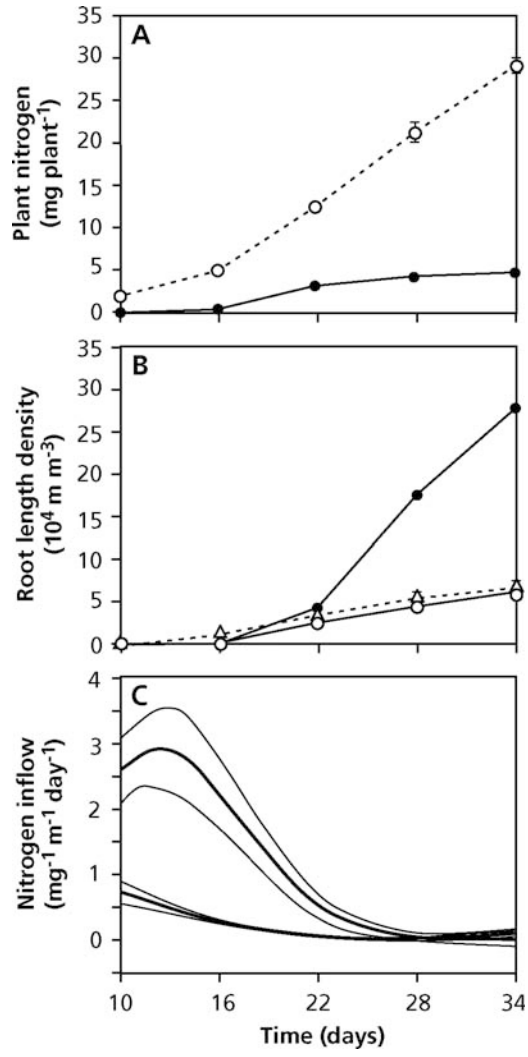


FIGURE 18. The response of *Triticum aestivum* to a localized organic residue, enriched with ^{15}N. (A) Total N in the plant (*open symbols*) and N in the plant derived from the organic residue. (B) Total root length density in the residue (*filled symbols*) and in the soil above (*triangles*) and below (*triangles*) the residue; (C) N uptake for the whole root system (*lower curve*) and for the part of the roots that proliferated in the localized residue (*upper curve*) (Van Vuuren et al. 1996).

2.3 Sensitivity Analysis of Parameters Involved in Phosphate Acquisition

The contribution of different parameters involved in the uptake of P_i can be assessed using **simulation models**. Such models are increasingly used to analyze ecophysiological problems.

Nye and co-workers (Bhat & Nye 1973, Nye & Tinker 1977, Tinker & Nye 2000) analyzed the significance of root hairs using an experimental and a mathematical approach. They measured the (labeled) P_i concentration at the root surface of an oil seed root with dense root hairs. In addition, they simulated the P_i concentration under one of the following two assumptions: (1) root hairs are not involved in P_i uptake, and (2) root hairs effectively increase the cylinder intercepted by the root. There was good agreement between the simulated and the experimental data, only when they assumed that **root hairs** are effective (Fig. 19). This work corroborated earlier ideas based on the significance of root hairs for the acquisition of immobile ions, including P_i (Table 4).

Barber and co-workers (Silberbush & Barber 1983, Barber 1995) analyzed the sensitivity of P_i uptake by pot-grown soybean plants to various soil and root factors (Fig. 20). The simulated

TABLE 9. Root development of *Pisum sativum* (garden pea) in a split-root design, in which root halves were grown in different pots and supplied with different nutrient concentrations from the time they were 24 mm long.*

Nutrient strength pot 1–pot 2	Root dry mass (mg)			Ratio pot 1/pot 2	Shoot dry mass (mg)
	Pot 1	Pot 2	Total		
0–50	51	450	501	0.11	806
1–50	60	427	487	0.14	847
10–50	142	370	512	0.38	874
25–50	194	269	463	0.72	935
50–50	300	283	582	1.05	1032
10–0	225	61	286	3.77	463
25–0	343	52	394	6.76	670

Source: Gersani & Sachs 1992.
* Plants were harvested when they were 3 weeks old.

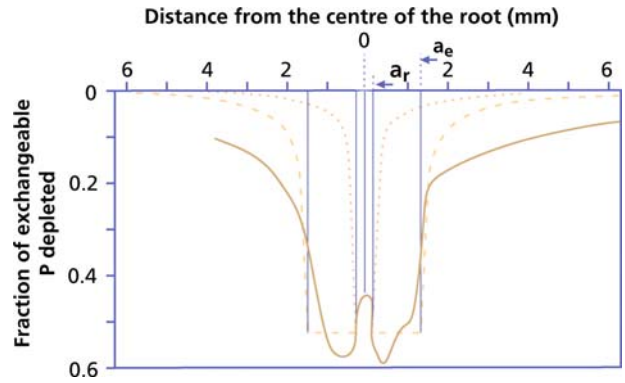


FIGURE 19. Calculated and measured P_i concentration profiles around a *Brassica napus* (oil seed) root. P_i profiles are calculated under the assumption that root hairs do (ii, *outer broken lines*) or do not (i, *inner broken lines*) play a role in P_i uptake. The *solid line* gives the experimentally determined profile. The radii given are the radius of the root axis only (a_r) and that of the root plus root hairs (a_e) (Bhat & Nye 1973 and Nye & Tinker 1977).

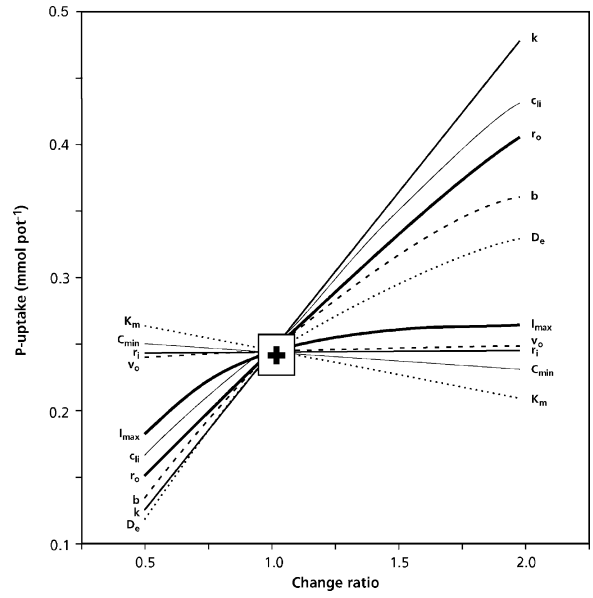


FIGURE 20. Effects of changing parameter values (from 0.5 to 2.0 times the standard value) on simulated P_i uptake by roots of *Glycine max* (soybean). k is the rate of root elongation, C_{li} is the initial P_i concentration in solution, r_o is the root diameter, b is the buffer power of the soil, D_e is the diffusion coefficient of P_i in the soil, I_{max} is the maximum P_i inflow rate, v_o is the rate of transpiration, r_i is the spacing between individual roots, C_{min} is the lowest concentration at which P_i uptake is possible, and K_m is the P_i concentration at which the rate of P_i uptake is half of that of I_{max} (Silberbush & Barber 1983). With kind permission, from the Annual Review of Plant Physiology, Vol. 36, copyright 1985, by Annual Reviews Inc.

uptake agreed well with their experimental results. Their results demonstrated that P_i uptake is much more responsive to changes in the rate of **root elongation** (*k*in Fig. 20) and **root diameter** (r_o) than to changes in **kinetic properties** of the uptake system: K_m, I_{max}, and C_{min}. Soil factors such as **diffusion coefficient** (D_e) and **buffer power** (b) have greater effects if their values are decreased than if they are increased. **Transpiration** (v_o) has no effect at all on the rate of P_i uptake. The spacing between roots (r_i) was such that there was no inter-root competition; hence, changes in the value for this parameter had no effect. It is clear that, for a relatively immobile nutrient such as P, kinetic parameters are considerably less important than are root traits such as the rate of elongation and root diameter. This is consistent with the generalization that diffusion to the root surface rather than uptake kinetics is the major factor determining P_i acquisition. For more mobile ions, such as NO_3^-, kinetic properties play a somewhat more important role (Kirk & Kronzucker 2005).

This example shows how simulation models can be helpful to explore our intuitive ideas elegantly, if they are used in combination with experimental approaches.

3. Nutrient Acquisition from "Toxic" or "Extreme" Soils

The term *toxic* or *extreme* soil is clearly anthropomorphic. For example, a soil of a rather high or low pH may be toxic for some species, but a favorable habitat for others. Similarly, the presence of high concentrations of "heavy metals" may prevent the establishment of one species, but allow completion of the life cycle of another. As pointed out in Sect. 1, the occurrence of species in sites that we tend to call "toxic" does not necessarily mean that adapted plants grow better in such sites. We use terms like **halophytes** and **calcifuges** to refer to the **ecological amplitude** of the species. The **physiological amplitude** of a species is usually much broader than its ecological amplitude (Sect. 3 of Chapter 1 on assumptions and approaches). The restriction of a species to extreme soils might indicate that adapted plants are the only ones that can survive in these soils, due to their specialized mechanisms and that they are outcompeted on soils that we consider less extreme.

The following sections discuss specialized plant traits associated with phenotypic **acclimation** and genotypic **adaptation** to extreme soils and their consequences for species distribution.

3.1 Acid Soils

Soils naturally tend to become acid with age (Fig. 1), as a result of several processes (Bolan et al. 1991):

1. **Decomposition of minerals** by weathering, followed by leaching of cations, such as K^+, Ca^{2+}, and Mg^{2+} by rain. This is particularly important in humid regions.
2. **Production of acids** in soils (e.g., due to hydration and dissociation of CO_2, formation of organic acids, oxidation of sulfide to sulfuric acid and nitrification of ammonia).
3. Plant-induced production of acidity, when an **excess of cations** over anions is taken up (e.g., when N_2 or NH_4^+, rather than NO_3^-, is used as N source for plant growth).

Soils may also acidify due to human activities such as input of nitric and sulfuric acids from "acid rain"; the addition of acidic fertilizer, such as ammonium sulfate or urea, or the exposure of acidic mine tailings.

Soil acidity modifies the availability of many mineral nutrients (Fig. 1) as well as the solubility of Al. Although a **low soil pH** per se may limit the growth of plants, **Al toxicity** is considered a major yield-limiting factor in many acid soils, especially in the tropics and subtropics (Kochian et al. 2005). In acid soils, concentrations of Mn may also increase to toxic levels, generally at a somewhat higher pH than that which causes Al toxicity. P, Ca, Mg, K, and Mo may decline to an extent that deficiency symptoms arise (Table 7 in Sect. 2.2.6).

3.1.1 Aluminum Toxicity

Aluminum is of the most abundant metal in the Earth's crust and the third most abundant element. Like all trivalent cations, it is toxic to plants. Aluminum hydrolyzes in solution, such that the trivalent cation dominates at **low pH** (Fig. 21). In addition, at low pH aluminum is released from chelating compounds (J.F. Ma et al. 2001a). Many species have a distinct preference for a soil with a particular pH. **Calcifuge** ("chalk-escaping"; also called "acidophilous", acid-loving) species resist higher levels of soluble Al^{3+} in the root environment. There are several potential sites for injury due to Al (Fig. 22):

1. the cell wall
2. the plasma membrane

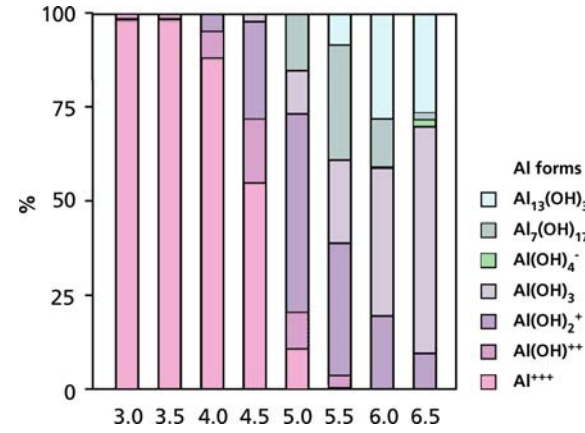


FIGURE 21. Calculated distribution of total inorganic Al concentration over various monomeric and polymeric forms as a function of pH. Calculations are based on parameters given by Nair & Prenzel (1978).

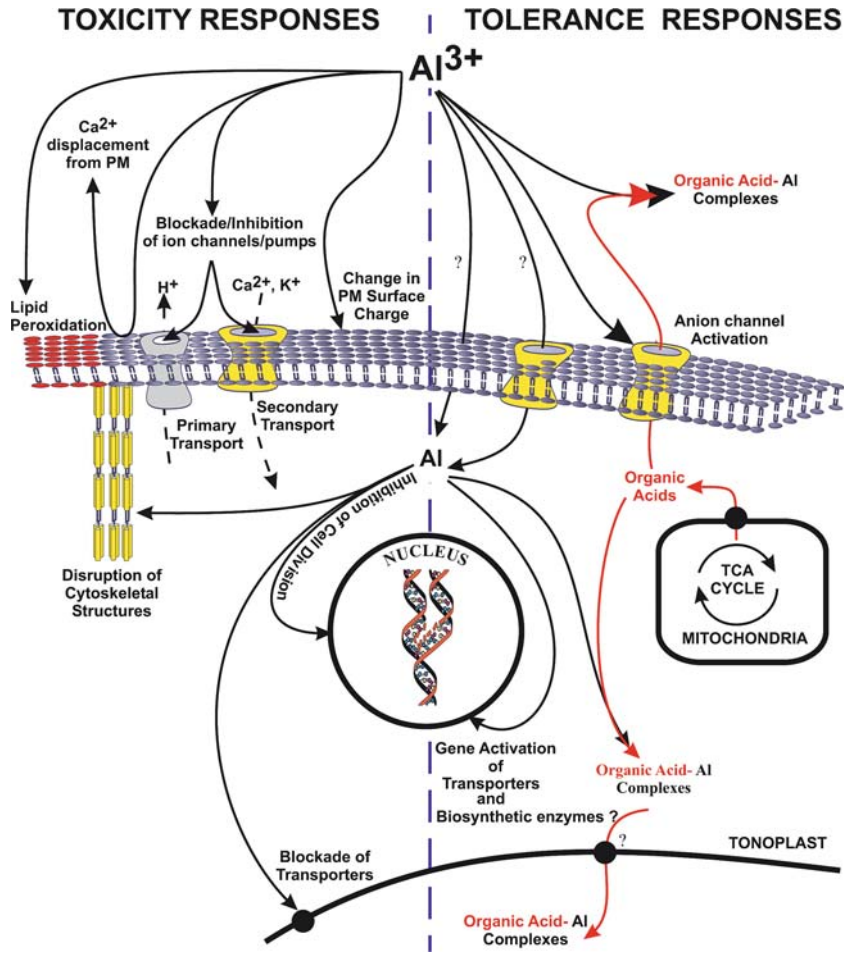


FIGURE 22. Possible mechanisms of Al toxicity and Al resistance in plants. Al toxicity targets are illustrated on the left side of the diagram. For clarity, the interactions of Al with the cell wall are not shown. On the right side, Al-resistance mechanisms (Al exclusion and internal Al detoxification) are based on the formation of Al complexes with carboxylates. The Al-exclusion mechanism involves the release of carboxylate anions via an Al-gated anion channel at the plasma membrane. The internal Al-detoxification mechanism involves chelation of cytosolic Al by carboxylate anions with the subsequent sequestration into the vacuole via unknown transporters (Kochian et al. 2005).

3. signal-transduction pathways
4. the root cytoskeleton
5. DNA/nuclei

The **root apex** appears to be the most sensitive region for Al toxicity. When most of the roots are exposed to Al, but root tips are in a solution without Al, plant growth is not affected. On the other hand, when only the root tips are exposed to Al, toxicity symptoms are readily visible (Kochian 1995). Inhibition of root elongation is the primary Al-toxicity symptom (Ryan et al. 1994). Inhibition of root elongation in the root tip is due to interference with the formation of **cell walls**, decreasing cell-wall elasticity by cross-linking with pectin (Kochian et al. 2005, Ma et al. 2005). Root cells become shorter and wider. As a consequence, root elongation is impaired and the roots have a "stubby" appearance (Fig. 23) and a low specific root length, when grown in the presence of Al (Table 10; Delhaize & Ryan 1995).

Important toxic effects of Al occur at the **plasma membrane**. These are partly due to the inhibition of the uptake of Ca and Mg (Table 11), due to blockage of ion channels in the plasma membrane (Kochian et al. 2005). Some of the symptoms of Al toxicity are very similar to those of a deficiency of other ions. This may be due to competition for the same site in the cell walls (some cations), precipitation of Al complexes (with P_i), or inhibition of root elongation, which reduce the absorption capacity (Kochian et al. 2005). Inhibition by Al of the uptake of Ca and Mg decreases the concentration of these cations in the cell, causing Ca- and/or Mg-deficiency symptoms. Ca is required during cell division for spindle formation and to initiate metaphase/anaphase transition. Hence, the presence of Al prevents cell division and root development (Kochian et al. 2005). Interference with Mg uptake causes Mg deficiency symptoms (i.e., chlorotic leaves with brown spots), and stubby discolored roots (Kochian et al. 2005). Al toxicity also resembles boron deficiency, but the reason for this is not clear (LeNoble et al. 1996a).

Some Al is rapidly taken up in the symplast as well, possibly via carriers whose function is to take up Mg or Fe, or via endocytosis (Kochian 1995). In

Table 10. The effects of aluminum concentration on various root parameters of *Mucuna pruriens* (velvet bean).*

$[Al^{3+}]$ (mg L^{-1})	DM (g)	FM (g)	D (mm)	L (m)	SRL (m g^{-1})
0	6.4	126	0.37	1160	175
0.1	6.6	155	0.44	1100	166
0.2	6.6	126	0.46	931	141
0.4	3.3	55	0.51	253	76

***Source*: Hairiah et al. 1990.**

*** DM, dry mass; FM, fresh mass; D, diameter; L, root length per plant; SRL, specific root length (per gram dry mass of roots).**

***Note*: The increase in root dry mass was not statistically significant.**

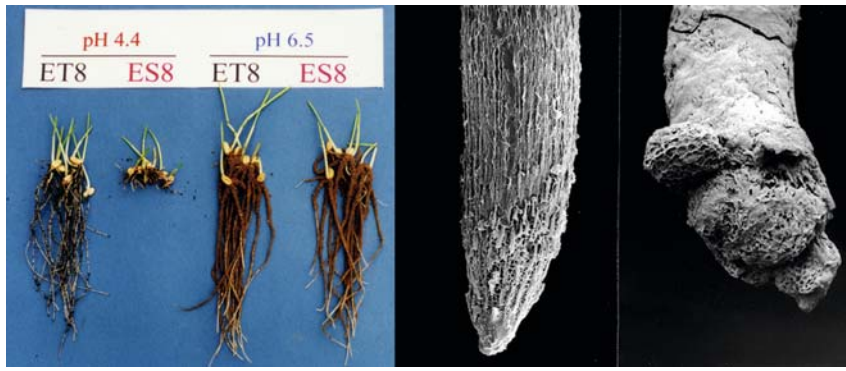


Figure 23. (*Left*) Seedlings of an Al-sensitive (ES8, *right*) and a near-isogenic Al-resistant (ET8, *left*) line of *Triticum aestivum* (wheat) grown in soil at pH 6.5, where Al is harmless for roots, and at pH 4.4, where aluminum is toxic if not chelated (courtesy J.F. Ma, Plant Stress Physiology Group, Research Institute for Bioresources, Okayama University, Kurashiki, Japan; Ma 2000, by permission of Oxford University Press). (*Right*) Scanning electron micrograph of the root tips of the two near-isogenic lines shown in the top panel; the photo on the right shows a root tip of the Al-sensitive line, and the one on the left a root tip of the Al-resistant line. The seedlings were grown for 4 days in a solution containing 5 mM $AlCl_3$ in 200 mM $CaCl_2$ at pH 4.3 (courtesy E. Delhaize, CSIRO, Canberra, Australia; Delhaize & Ryan 1995). Copyright American Society of Plant Biologists.

TABLE 11. Aluminum, phosphorus, calcium, and magnesium concentration [mmol (kg dry mass)$^{-1}$] in roots and shoot of *Sorghum bicolor* (sorghum), grown for 35 days at three levels of Al (zero, low: 0.4 mg L^{-1}, high 1.6 mg L^{-1}) and P [low, medium, and high: 285, 570, and 1140 mmol plant^{-1} (35 days)$^{-1}$].*

		Shoot				Root			
P level	Al level	Al	P	Ca	Mg	Al	P	Ca	Mg
Low	Zero	–	26	171	69	–	29	28	22
Medium	Zero	–	30	151	63	–	34	21	20
High	Zero	–	38	139	63	–	39	19	23
Low	Medium	1	27	127	36	7(29)	30	20	16
Medium	Medium	1	29	108	37	5(40)	34	18	16
High	Medium	1	40	85	36	5(40)	46	20	19
Low	High	1	93	61	23	11(36)	70	16	14
Medium	High	1	108	51	21	13(31)	76	15	15
High	High	1	335	65	25	131(45)	263	16	16

Source: Tan & Keltjens 1990.
* Values in brackets indicate the percentage removable with 0.05 M H_2SO_4 (i.e., the fraction in the apoplast).

the cytosol, with a neutral pH, it is no longer soluble, and the Al^{3+} concentration is less than 10^{-10} M, due to the formation of nontoxic forms of Al, e.g., $Al(OH)_3$. Al may also displace Ca and/or Mg from sites where they have a vital function in activation of enzymes; interference with calmodulin (a major component of **signal-transduction pathways** in plants) and the **cytoskeleton** may be particularly harmful.

Because of the very high affinity of Al for proteins and P-containing compounds, including ATP, phospholipids, and **DNA**, these very low Al concentrations are potentially phytotoxic (Ma et al. 1998). Most of these effects occur *after* the very rapid (1–2 hours) inhibition of root elongation. They are, therefore, not the primary cause of inhibition of plant growth (Kochian 1995). Cell division is also inhibited; mitosis appears to be arrested in the S-phase of DNA replication.

Leaf disorders (e.g., Fe-deficiency symptoms) occur several days after exposure to Al. In *Triticum aestivum* (wheat),which exhibits Fe uptake according to strategy II (Sect. 2.2.6), Fe deficiency is due to inhibition of the biosynthesis and release of phytosiderophores (Chang et al. 1998).

3.1.2 Alleviation of the Toxicity Symptoms by Soil Amendment

Al toxicity symptoms can be diminished by addition of extra **magnesium** or **calcium**. **Phosphate** addition also has a positive effect, because it precipitates Al, either outside or in the roots. There is some evidence that the toxicity symptoms can be alleviated by Mg (especially in **monocotyledons**) and Ca (especially in **dicotyledons**) (Keltjens & Tan 1993, Silva et al. 2001a). This pattern is consistent with the higher requirement for Ca in dicots (Sect. 4). Cation amelioration of Al toxicity is probably caused by a reduction of Al accumulation (Ryan et al. 1997). In *Glycine max* (soybean), adding 50 μM Mg to a nutrient solution containing toxic levels of Al increases exudation of citrate (which chelates Al) by the tap root tips several fold. This suggests that alleviation of Al toxicity by Mg is due to increased production and exudation of citrate (Silva et al. 2001b).

The ability of high-molecular-mass organic acids, such as **humic acid** and **fulvic acid**, to bind Al is well documented. These substances form much more stable complexes with Al than do citrate, malate, and oxalate, which are excreted by roots of Al-resistant plants (Sect. 3.1.3). Fulvic acid and humic acid are constituents of humus, peat, and leaf litter, which can be added to alleviate toxic effects of Al (Harper et al. 1995).

Some of the symptoms of Al toxicity [e.g., inhibition of root elongation of *Cucurbita pepo* (squash) growing in nutrient solution] are relieved by the addition of boron (LeNoble et al. 1996a). Incorporation of boron in an acidic high-Al subsoil promotes the depth of rooting and total root growth in *Medicago sativa* (alfalfa) (LeNoble et al. 1996b).

3.1.3 Aluminum Resistance

Recent progress in several laboratories has set the stage for identification and characterization of the genes and associated physiological mechanisms

that contribute to Al resistance in important crop species grown on acid soils. This provides the necessary molecular tools to address a major, worldwide agronomic problem (Kochian et al. 2005). Different mechanisms can be discerned to account for a plant's resistance to potentially toxic levels of Al:

1. Al exclusion from the root apex (avoidance)
2. Al tolerance

There is clear evidence for both **exclusion** mechanisms (Kochian 1995) that confer Al resistance and for **internal detoxification** in species that accumulate Al, such as *Hydrangea macrophylla* (hydrangea), *Camellia sinensis* (tea), *Richeria grandis* (a tropical cloud-forest tree) (Ma et al. 1997), and *Fagopyrum esculentum* (buckwheat) (Zheng et al. 1998). Internal detoxification of Al in Al-accumulating species is probably based on binding of Al to **citrate** or **oxalate** in leaf cells (Ma et al. 1997, Zheng et al. 1998).

Work on the aluminum-resistant species *Fagopyrum esculentum*(buckwheat) and comparisons of resistant and sensitive genotypes of *Phaseolus vulgaris* (common bean), *Triticum aestivum* (wheat), *Zea mays* (corn), and *Arabidopsis thaliana* (thale cress) highlight the importance of **carboxylates** (**citrate**, **malate**, and **oxalate**) release by roots, especially by root tips (Figs. 23 and 25; Zheng et al. 1998). Some species, e.g., *Lupinus albus* (white lupin) release carboxylates in response to both Al supply and P deficiency, but the response differs in the exact part of the root system from which the carboxylates are released (Wang et al. 2007).

In resistant wheat genotypes, Al activates a channel that allows the exudation of carboxylates. Transporters responsible for Al-activated release of carboxylates have been identified in several species (Delhaize et al. 2007, Furukawa et al. 2007, Magalhaes et al. 2007). Higher rates of exudation reflect higher rates of carboxylate synthesis, rather than higher concentrations in the root tips. The excretion of carboxylates is accompanied by K^+ efflux, so the positive effect of the chelator is not negated by lowering the pH. Mucilage exuded by the root cap may allow the malate concentration to remain sufficiently high over extended periods to protect the root tip (Delhaize & Ryan 1995). Wheat genotypes that excrete both malate and **phosphate** at the root tip show a threefold greater resistance to Al. Contrary to the inducible release of malate, the release of phosphate is constitutive (Pellet et al. 1996). Microbial degradation of the malate released by the roots of Al-resistant plants could potentially limit the effectiveness of these compounds in sequestering Al because the half-life of the released organic acids is less than 2 hours. For rapidly growing roots (> 15 mm day^{-1}), however, the residence time of the malate-releasing root tips in any zone of soil is around 5 hours, because root tips and their carbon release to the rhizosphere move quickly enough, so that the size of the microbial biomass in the rhizosphere of the root tip does not change much from the time the tip enters a zone. Electron microscopy and physiological studies confirm that there is little microbial proliferation at the root apex. Carboxylate release protects the root tip from the toxic effects of Al, despite some microbial breakdown of malate in the rhizosphere (Jones et al. 1996b).

Al resistance may be based not only on the release of carboxylates and phosphate, but also on an Al-induced root-mediated elevation of the pH in the rhizosphere adjacent to the root tip (Degenhardt et al. 1998, Larsen et al. 1998). Because the solubility of Al is pH dependent, increases in rhizosphere pH reduce the concentration of Al^{3+} (Fig. 21).

At a high pH, calcifuge species may show **Fe-deficiency** symptoms. This is probably associated with their Al-resistance mechanism, which may immobilize other ions as well, including Fe. Root growth of calcifuge species may be stimulated by low Al concentrations. This growth-enhancing effect of Al is most pronounced at low pH (high H^+ concentration). It is probably associated with the alleviation of the toxic effects of a low pH which is a general effect of cations; trivalent cations have the strongest effect, followed by divalent and then monovalent ones (Kinraide 1993). The growth of **calcicole** ("chalk-loving"; also called acidifuge, "acid-escaping") species, which naturally occur on soils with a high pH (Sect. 3.2), may also be stimulated by Al, but the optimum Al concentration for such species is about 5 μM, as opposed to 20–30 μM for calcifuge species, such as *Nardus stricta* (matgrass) and *Ulex europaeus* (gorse).

3.2 Calcareous Soils

Calcifuge ("chalk-escaping") species have a distinct preference for a soil with a low pH. They tend to have a very low ability to solubilize the $\mathbf{P_i}$, **Fe**, and **Zn** and in limestone, but resist higher levels of soluble Al^{3+} in the root environment (Sect. 3.1); NO_3^- availability will be low, and NH_4^+ will be a more important source of N (Sect. 2.1.2). Carbonate-rich soils may contain high levels of Fe and this may arrive at the root surface, but calcifuge species are unable to acquire sufficient Fe to sustain

rapid growth. The lack of a high capacity to utilize the forms of Fe, Zn, and other trace elements that prevail in alkaline soils (Sect. 2.2.6) leads to "**lime chlorosis"** and may be the cause of failure of establishment of calcifuge species in such soils. Some calcifuge plants [e.g., *Carex pilulifera* (pill sedge)] are unable to translocate sufficient Fe to their leaves when grown in calcareous soil; Fe may accumulate in or precipitate on their roots. Others [e.g., *Veronica officinalis* (heath speedwell)] may increase the amount of Fe that is transported to their leaves, but accumulate this Fe in a form that is not metabolically active (Table 12; Zohlen & Tyler 1997, 2000). In addition, calcifuges tend to lack the capacity to access the prevalent poorly soluble P sources in alkaline substrates (Sect. 2.2.5). **Calcifuges** have very low leaf **P** concentrations to support physiological functions and consequently low biomass production, when grown in calcareous soil (Zohlen & Tyler 2004).

Calcicole species are associated with soils of high pH. Their growth may be stimulated by high Ca concentrations, which are saturating for calcifuge species; however, this is not the major factor explaining their distribution. More importantly, calcicole species do not resist **Al** in their root environment (Sec. 3.1).

The solubilization of P_i and Fe by carboxylate exudation in calcicoles inevitably also enhances the concentration of Ca. Indeed, high Ca concentrations may be found in the xylem sap of calcicole species. Because Ca is an important "**second messenger**" (e.g., in the regulation of stomatal conductance) (Sect. 5.4.2 of Chapter 3 on plant water relations), how does a calcicole plant avoid being poisoned by Ca? Calcicoles store excess Ca as crystals, sometimes in leaf trichomes (De Silva et al. 1996). Calcifuge herbs are unable to avoid excessive uptake of **calcium** from calcareous soil (Zohlen & Tyler 2004).

3.3 Soils with High Levels of Heavy Metals

Heavy metals are characterized by their high density, which is greater than 5 g mL^{-1}. Their biological activity, however, is due to **ligand properties**. Moreover, some of the "heavy metals" (e.g., arsenate) do not quite fit the above definition, and hence the term "heavy metal" is a bit of a misnomer (Duffus 2002); it will be used in this chapter, as it is a term that is common in the ecophysiological literature. Some heavy metals [cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn)] are essential micronutrients for plants but become toxic at elevated concentrations. Their role as an essential micronutrient may be as a cofactor or activator of specific enzymes or to stabilize organic molecules. Other heavy metals [e.g., cadmium (Cd), lead (Pb), chromium (Cr), mercury (Hg), silver (Ag), uranium (U), and gold (Au)] are not essential for plant functioning.

3.3.1 Why Are the Concentrations of Heavy Metals in Soil High?

High levels of heavy metals in soils may have a geological or anthropogenic origin. In 1865 the first reference to heavy metal **hyperaccumulation** in plants was made when *Thlaspi caerulescens* (alpine pennycress) growing on Zn-rich soils near the German–Belgium border was reported to contain 17% of Zn in its ash. However, it was the discovery in 1948 by Minguzzi and Vergnano of extreme Ni accumulation in *Alyssum bertolonii* from serpentine hills in Italy, reaching 10 mg Ni g^{-1} dry mass, that marks the beginning of an increasing interest in this subject (Assunção et al. 2003). Brooks et al. (1977) first coined the term **hyperaccumulator** to define plants with Ni concentrations higher than 1000 μg g^{-1} dry mass. There is increasing

Table 12. Total, "metabolically active", and "HCl-soluble" Fe in freshly sampled leaf tissue of two calcifuge species, grown in acid silicate soil, calcareous soil, and calcareous soil amended with calcium phosphate.*

Species	Soil	Total	Metabolically active	HCl soluble
Carex pilulifera	Acid	781	283	691
	Calcareous	491	163	272
	Calcareous + P	360	115	202
Veronica officinalis	Acid	1148	689	818
	Calcareous	1588	399	654
	Calcareous + P	1311	480	593

Source:Zohlen & Tyler 1997.

* Expressed as nmol g^{-1} dry mass.

Note: The "metabolically active" fraction was extracted with 1,10-phenantroline, an Fe-complexing reagent considered to extract mainly Fe^{2+}; the HCl-soluble fraction is considered the fraction that is important in chlorophyll synthesis.

evidence that hyperaccumulation confers protection against herbivores and microbial pathogens (Chapters 9A and 9B; Poschenrieder et al. 2006).

Serpentine soils have naturally high levels of nickel (Ni), chromium (Cr), cobalt (Co), and magnesium (Mg), but low concentrations of Ca, N, and P. The flora associated with these soils is rich in specially adapted **endemic species** (Arianoutsou et al. 1993). It has been known in Europe for centuries that rock formations containing high levels of certain metals (e.g., Cu) are characterized by certain plant species associated with these sites (**metallophytes**). This is also true for southern Africa, where only certain herbaceous species [e.g., *Senecio coronatus* (woolly grassland senecio)] establish in metal-rich sites (Przybylowicz et al. 1995). Such metal-**hyperaccumulating** plants may contain very high levels of heavy metals. Hyperaccumulators of Co, Cr, Cu, Pb, or Ni have concentrations >1 mg g^{-1} dry mass, and hyperaccumulators of Mn or Zn contain up to 10 mg g^{-1} dry mass. Recently a fern, *Pteris vittata*(Chinese brake fern) was found to hyperaccumulate arsenate (As). In As-spiked soils, it accumulates 23 mg g^{-1} dry mass of As in its above-ground biomass (fronds) (L. Ma et al. 2001c). Metal-resistant species can be used as indicators to identify potential mining sites (e.g., *Hybanthus floribundus*, from the Eastern Goldfields area of Western Australia, to find Ni) (Brooks 1998).

In sites close to mines, where the remains of the mining activity have enriched the soil with heavy metals, or under electricity pylons, which cause zinc contamination due to corrosion of their galvanized surfaces, metal-resistant genotypes emerge [e.g., of *Agrostis capillaris* (colonial bentgrass), *Agrostis stolonifera* (creeping bentgrss), *Anthoxanthum odoratum* (sweet vernalgrass), *Deschampsia caespitosa* (tufted hair-grass), and *Festuca ovina* (sheep's fescue) (Al-Hiyaly et al. 1990)]. The shoots of such plants may contain as much as 1.5 mg g^{-1} Zn on a dry mass basis, a level that is highly toxic to other plants (Brown & Brinkmann 1992). Along roadsides, which are often enriched in lead (Pb) from automobile exhaust, Pb-resistant genotypes occur. Some *Agrostis tenuis* (common bentgrass) genotypes grow even better in soils that contain as much as 10 mg g^{-1} Pb than in unpolluted control soil (McNeilly 1968). Resistant genotypes are usually resistant only to one metal, unless more than one heavy metal is present in high level at such a site.

Cadmium (Cd) pollution has increased drastically in recent decades as a result of combustion of fossil fuel, disposal of pigments and stabilizers for plastics, application of sewage sludge, and the use of phosphate fertilizers. This has led to concern about possible health and ecosystem effects. A comparison of several cultivars of two *Lupinus* species [*Lupinus albus* (white lupin), and *Lupinus angustifolius* (narrow-leaved lupin)] with *Lolium multiflorum* (Italian ryegrass) showed much greater uptake by the grass. Because the lupins release considerably more **carboxylates**(citrate, malate, and succinate) into the rhizosphere than the grass does, cadmium is possibly chelated by root exudates which would reduce its availability for uptake (Römer et al. 2000).

3.3.2 Using Plants to Clean or Extract Polluted Water and Soil: Phytoremediation and Phytomining

Some metal-accumulating species have been used to remove heavy metals from polluted water [e.g., *Eichhornia crassipes* (water hyacinth)]. Terrestrial metallophytic species are also potentially useful to remove heavy metals from polluted sites, a process termed **phytoremediation** (Chaney et al. 1997, Krämer 2005). It requires plants that show both a high biomass production and metal accumulation to such high levels that extraction is economically viable. For example, *Brassica juncea* (Indian mustard) accumulates high levels of Cd, even when the Cd level in solution is as low as 0.1 mg L^{-1} (Salt et al. 1995), *Thlaspi montanum* (Fendler's pennycress) and *Thlaspi goesingense* (tiny wild mustard) accumulate high levels of Ni (Krämer et al. 1997, Boyd & Martens 1998), and *Thlaspi caerulescens* (alpine pennycress) accumulates Zn and Cd (Robinson et al. 1998, Frey et al. 2000). The combination of high biomass production and hyperaccumulation is often found in Brassicaceae (cabbage family). After accumulation of heavy metals from the polluted soil, the plants have to be removed and destroyed, taking care that the toxic metal is removed from the environment. Phytoremediation technologies are currently available for only a small subset of pollution problems, such as As. As removal employs naturally selected hyperaccumulator plants [e.g., *Pteris vittata* (brake fern)], which accumulate very high concentrations of arsenic specifically in above-ground tissues (Krämer 2005).

Metal-accumulating plants can also be used as a "green" alternative to environmentally destructive opencast mining practices. Such production of a crop of high-biomass plants that accumulate high metal concentrations is termed **phytomining** (Brooks et al. 1998, Robinson et al. 1999). Phytomining also offers the potential to exploit ore bodies that are uneconomic to mine by conventional methods. Promising results have been obtained using a

number of hyperaccumulating species to extract gold; **ammonium thiocyanate** is added to soil, because this is commonly used for making gold soluble in mining operations. In the presence of this compound, *Brassica juncea* (Indian mustard) accumulates nearly 60 μg g^{-1} dry mass, whereas these plants typically contain only 1 μg g^{-1} plant (Anderson et al. 1998).

3.3.3 Why Are Heavy Metals So Toxic to Plants?

The biochemical basis of metal toxicity is not always clear. Heavy metals are "Lewis acids", which can accept a pair of electrons from a coordinate covalent bond; that is, they react with naturally occurring "Lewis bases" in the cell, such as $-S^-$ groups, $-OH^-$ groups, amino groups, and carboxylic acid termini. Cd, Pb, and Hg, which are nonessential, affect **sulfhydryl groups** and **N atoms** in proteins and thus inactivate these. For a redox-active metal, an excess supply may result in uncontrolled redox reactions, giving rise to the formation of toxic **free radicals**. For example, $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$, followed by $Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\cdot + H^+$. Free radicals may lead to **lipid peroxidation** and membrane leakage (Clemens 2001). Other heavy metals may inactivate major enzymes by **replacing** the **activating cation**. For example, Zn may replace Mg in Rubisco, reducing the activity of this enzyme and hence the photosynthetic capacity (Clijsters & Van Assche 1985). Like Zn, Cd also affects photosynthesis. Fluorescence measurements indicate that the Calvin cycle is the primary process affected, and this subsequently leads to a "down-regulation" of photosystem II (Krupa et al. 1993). Cd affects the mineral composition even in Cd-resistant species such as *Brassica juncea* (Indian mustard). It reduces the concentration of Mn, Cu, and chlorophyll in the leaves, even at a concentration in solution that has no effect on biomass production (Salt et al. 1995).

Most primary effects of heavy metals occur in the **roots**, which show reduced elongation upon exposure. Metal resistance can be quantitatively assessed by determining the effect of the metal on root elongation (Table 13). The increment in root dry mass tends to be affected less than that in root length, leading to "stubby" roots (Brune et al. 1994). Zn inhibition of water uptake may be due to binding of the metal to water-channel proteins (Sect. 5.2 of Chapter 3 on plant water relations). Mn toxicity leads to interveinal chlorosis and reduced photosynthesis (Macfie & Taylor 1992).

Table 13. The effect of zinc on root elongation of a Zn-sensitive and a Zn-resistant ecotype of *Deschampsia caespitosa*.

Zn sensitive		Zn resistant	
Zn concentration (μM)	Root elongation rate (%)	Zn concentration (μM)	Root elongation rate (%)
1	100	1	100
25	82	250	82
50	78	500	64
100	62	1000	53

***Source*: Godbold et al. 1983.**
***Note*: The plants were exposed to different Zn concentrations in solution for 10 hours.**

3.3.4 Heavy-Metal-Resistant Plants

Resistance in higher plants has been demonstrated for the following heavy metals: Cd, Cu, Fe, Hg, Mn, Ni, Pb, and Zn. **Metal resistance** is sometimes partly based on **tolerance**. For example, damage by Pb outside the plasma membrane can be prevented by modification of extracellular enzymes so that they are no longer affected by Pb. This has been shown for extracellular phosphatases in Pb-resistant genotypes of *Agrostis tenuis* (common bentgrass). **Avoidance** mechanisms generally account for resistance in a range of species. At a cellular level, these mechanisms include (Fig. 24) the following:

1. **Exclusion** of the metal:
   a. binding by mycorrhizal fungi;
   b. binding to root cell walls;
   c. chelation by root exudates;
   d. reduced net uptake: decreased influx or increased efflux.
2. Uptake followed by storage, typically occurring in **hyperaccumulators**:
   a. chelation of metals in the cytosol;
   b. repair of metal-damaged proteins;
   c. compartmentation of metals in specific compartments, e.g., vacuoles or trichomes.

In addition, mechanisms that are expressed at the level of whole plants play a role. These include differences in the proportion of absorbed metals that are either retained in the roots or loaded in the xylem for export to the shoot (Assunção et al. 2003).

Some mycorrhizal fungi (predominantly ectomycorrhizal fungi; Sect. 2 of Chapter 9A on symbiotic associations) can retain Zn and thus reduce the Zn

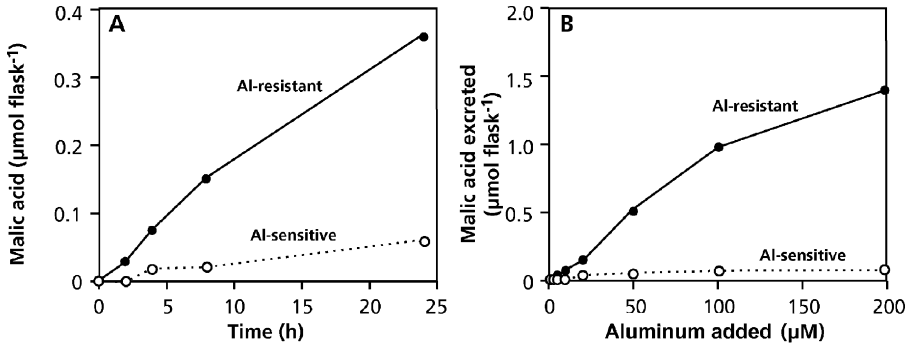


FIGURE 24. (A) Malate release from the roots of seedlings of an Al-resistant and an Al-sensitive genotype of *Triticum aestivum* (wheat) incubated in nutrient solution containing 50 mM Al. (B) Effect of Al concentration in the nutrient solution on malate release of the same genotypes as shown in (A) (after Delhaize et al. 1993). Copyright American Society of Plant Biologists.

content of their host, *Pinus sylvestris*(Scots pine). Although the root cell walls are is in direct contact with heavy metals in the soil solution, adsorption onto these must be of limited capacity and be of little consequence for resistance.

As with Al (Sect. 3.1.3), heavy metals can be chelated by exudates released from roots of resistant plants. For example, Ni-resistant plants of *Thlaspi arvense*(field pennycress) exude histidine and citrate, which chelate Ni and thus reduce its uptake by roots (Nian et al. 2002, Salt et al. 2000). Pb-resistant varieties of *Oryza sativa*(rice) release oxalate into the rhizosphere to detoxify Pb (Yang et al. 2000). Cu induces release of malate and citrate from roots of *Triticum aestivum*(wheat). Therefore, although not as widely explored as carboxylate release as a mechanism to reduce uptake of Al, a similar mechanism does appear to play a role in preventing entry of heavy metals (Fig. 25).

The clearest example of reduced uptake as a resistance mechanism is for As, first discovered in *Holcus lanatus*(common velvetgrass). Arsenate, which is structurally similar to phosphate, is taken up by the same transport system as P_i, and the As-resistant plants exhibit an absence of the high-affinity P_i-uptake system (Meharg & Macnair 1992). Enhanced efflux plays a role in bacteria and fungi, but has not yet been found in higher plants (Sharples et al. 2000, Hall 2002).

Chelation of heavy metals following their uptake involves several SH-containing compounds. **Cd resistance** is associated with the presence of SH-containing **phytochelatins** (PCs) (Fig. 26A,B). PCs are poly(γ-glutamyl-cysteinyl)-glycines, which bind metals. Unlike other peptides, with an α-carboxyl peptide bond, they are not made on ribosomes, but via a specific pathway from **glutathione** (which can also bind metals on its own). Upon exposure of tobacco [*Nicotiana rustica* (Aztech tobacco)] plants to Cd in the root environment, Cd-binding peptides,[γ-$(Glu\text{-}Cys)_3$-Gly and $\gamma(Glu\text{-}Cys)_4Gly$] are produced. Inhibition of PC synthesis leads to loss of the cadmium-detoxification mechanism. Together with Cd, some of the PCs are almost exclusively located in the **vacuole** (Vögeli-Lange & Wagner 1990), and an ATP-dependent mechanism transporting the Cd-PC complex has been identified in tonoplasts of *Avena sativa* (oat) (Salt & Rauser 1995). The formation of PCs, followed by uptake of the Cd-PC complex in the vacuole, probably plays a crucial role in Cd resistance. **Cu resistance** in *Arabidopsis thaliana* (thale cress) correlates with the level of expression of genes that encode **metallothioneins**, a group of cysteine-rich metal-binding proteins. Metallothioneins also have a high affinity for Cd and Zn. They were first discovered as the substances that are responsible for Cd accumulation in mammalian kidney. Like other proteins, but unlike phytochelatins, metallothioneins are synthesized on ribosomes (Robinson et al. 1993, Murphy & Taiz 1995).

Evidence for protection against heavy-metal-induced damage comes from enhanced expression of **heat-shock proteins (HSPs).**These proteins characteristically show increased expression in response to exposure of plants to stress, including heavy metals as well as high temperature (Sect. 3.2 of Chapter 4B on effects of radiation and temperature).

Compartmentation of accumulated metals may occur in the vacuole or in the apoplastic space (e.g., for Zn, Cd, Ni, and Cu) (Krämer et al. 2000). Epidermal cells, with the exception of stomatal cells, may also be used for storage of the metals (Brune et al. 1994, Frey et al. 2000). Cd, Mn, Zn, and Pb are

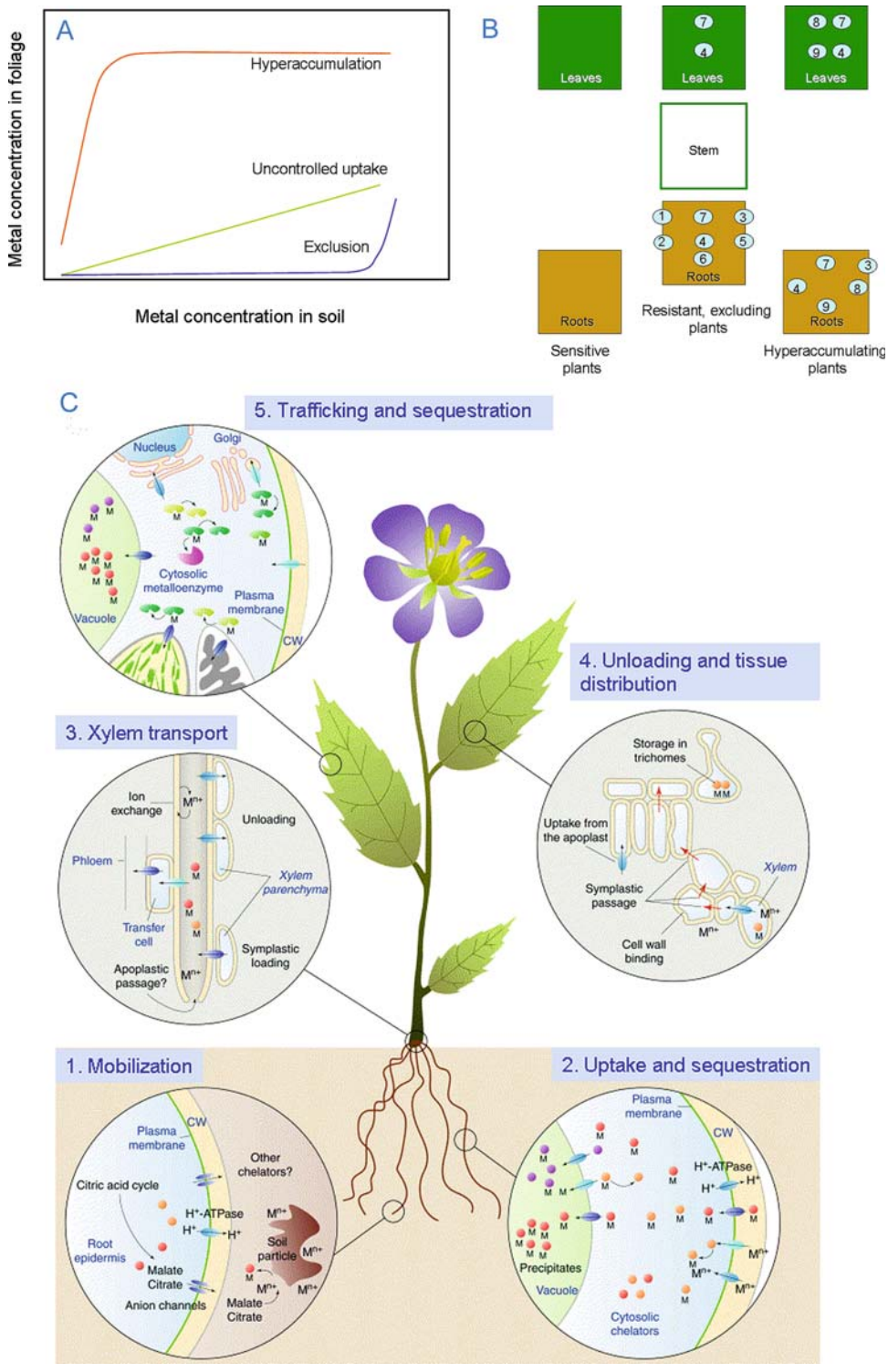


FIGURE 25. Summary of potential responses of higher plants to heavy metals. (A) Typical responses of sensitive plants, plants that exclude heavy metals from their foliage, and hyperaccumulating plants. For further explanation, see text (Callahan et al. 2005). (B) Mechanisms of metal toxicity and resistance in higher plants.

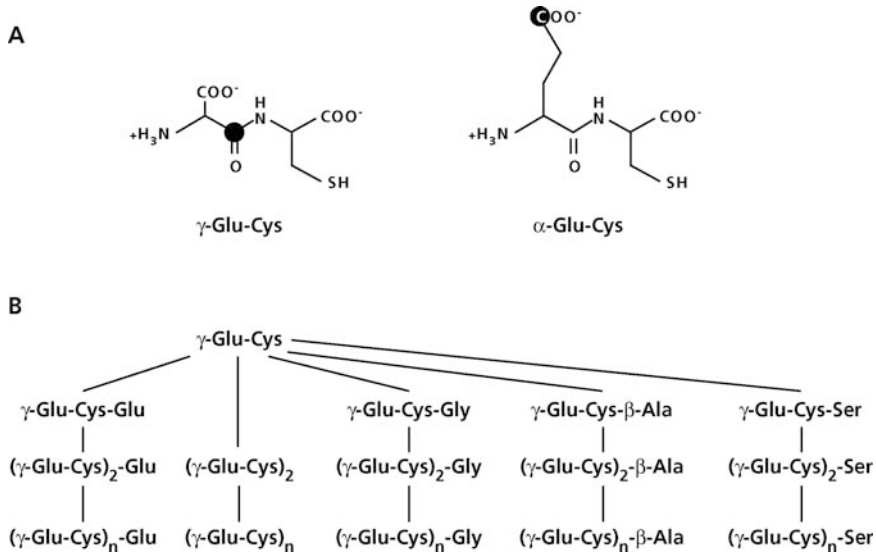


FIGURE 26. (A) The structure of γ(Glu-Cys) peptides. The γ-carboxyl-C of Glu is highlighted to indicate the difference between α- and γ-carboxyamide linkages. (B) A model summarizing the five families of γ(Glu-Cys) peptides involved in metal immobilization in plants and yeasts; the *lines* indicate family relationships and do not necessarily specify biosynthetic sequences (Rauser 1995).

preferentially accumulated in leaf trichomes [e.g., in *Brassica juncea* (Indian mustard) and *Arabidopsis halleri*(meadow rock-cress)] (Salt et al. 1995, Zhao et al. 2000).

Cu resistance in *Silene cucubalus* (bladder campion) is based on **exclusion**, whichis at least partly based on ATP-dependent Cu efflux (Van Hoof et al. 2001). Upon exposure to Cu, both resistant and sensitive *Silene vulgaris* plants accumulate **phytochelatins** (Fig. 27). When compared at tissue Cu concentrations that give a similar physiological effect, the phytochelatin concentrations in sensitive and resistant genotypes are fairly similar (Table 14). Phytochelatin synthesis is likely to be essential to bind the toxic Cu, but because phytochelatins are produced in both Cu-resistant and sensitive plants, it is apparently not the basis for Cu resistance in *Silene vulgaris*.

FIGURE 25. (continued) 1. Restriction of metal movement to roots by mycorrhizal fungi. 2. Binding to cell walls. 3. Chelation by root exudates. 4. Reduced influx across the plasma membrane. 5. Efflux into the apoplast. 6. Chelation in the cytosol by various ligands, including organic acids, phytochelatins (PC), and metallothioneins (MT). 7. Repair and protection of plasma membranes, e.g., by heat-shock proteins (HSP) and metallothioneins. 8. Transport of PC-Cd complex into the vacuole. 9. Transport and accumulation of metals in the vacuole (Hall 2002). (C) Molecular mechanisms involved in heavy metal hyperaccumulation. (1) Metal ions are mobilized by secretion of chelators and acidification of the rhizosphere. (2) Uptake of hydrated metal ions or metal-chelate complexes is mediated by various uptake systems in the plasma membrane. Inside the cell, metals are chelated, and excess metals are sequestered by transport into the vacuole. (3) From the roots, transition metals are transported to the shoot via the xylem. Presumably, the larger portion reaches the xylem via the root symplast. Apoplastic passage might occur at the root tip. Inside the xylem, metals are present as hydrated ions or as metal-chelate complexes. (4) After reaching the apoplast of the leaf, metals are differentially captured by different leaf cell types and move cell to cell through plasmodesmata. Storage appears to occur preferentially in trichomes. (5) Uptake into the leaf cells again is catalyzed by various transporters [not depicted in (5)]. Intracellular distribution of essential heavy metals (= trafficking) is mediated by specific metallochaperones and transporters localized in endomembranes (note that these processes function in every cell). Abbreviations and symbols: CW, cell wall; M, metal; filled circles, chelators; filled ovals, transporters; bean-shaped structures, metallo-chaperones (Clemens et al.2002); copyright Elsevier Science, Ltd.

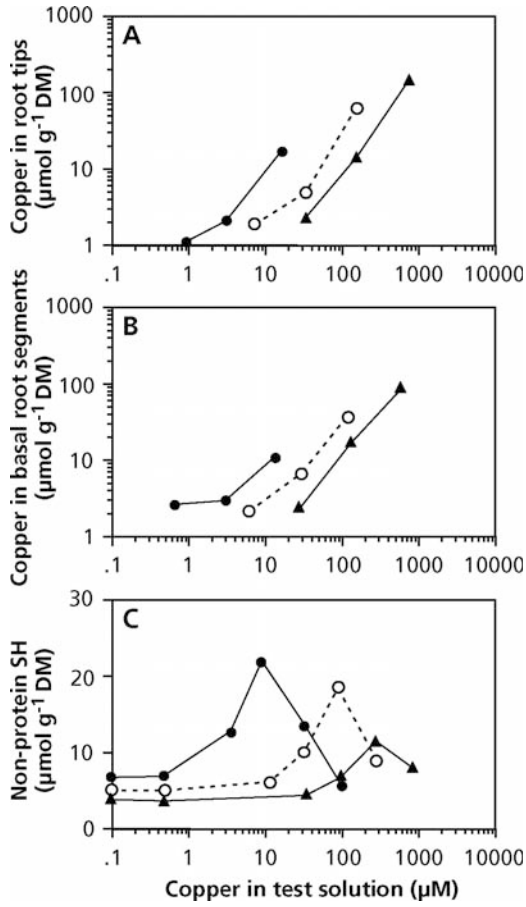


Figure 27. Copper (A, B) and phytochelatin sulfhydryl concentration (lowest panel) in the roots of one Cu-sensitive (*filled circles*) and two Cu-resistant (*open circles* and *filled triangles*) ecotypes of *Silene cucubalus*(bladder campion). Cu was measured in the apical 10 mm (A) and the adjacent 10 mm (B). Phytochelatin was measured for the entire roots (after Schat & Kalff 1992).

When compared at the same external Zn concentration (100 µM), a **Zn-resistant** ecotype of *Deschampsia caespitosa* (tufted hair-grass) accumulates less Zn in the apical parts of its roots (especially the 0–10 mm zone, but also in the 10–50 mm zone), but more in the basal parts (further than 50 mm from the apex) (Godbold et al. 1983). At the same external Zn concentration, whole roots of both ecotypes of *Deschampsia flexuosa* absorb Zn at the same rate. When compared at an external Zn concentration that has a similar effect on root growth (Table 13), the resistant ecotype accumulates more Zn than does the sensitive one (Fig. 28). As found for other Zn-resistant genotypes, it also binds a greater

Table 14. Phytochelatin sulfhydryl concentration [µmol (g dry mass)$^{-1}$] and molar ratio of phytochelatin to Cu in the roots of a Cu-sensitive and a Cu-resistant ecotype of *Silene cucubalus*(bladder campion).

	Phytochelatin concentration		Phytochelatin/Cu ratio	
Cu exposure level	Sensitive	Resistant	Sensitive	Resistant
Highest concentration without any effect	3.7	2.9	3.7	1.6
Concentration giving 50% inhibition of root growth	7.6	7.5	3.7	1.7
Concentration giving 100% inhibition of root growth	19.0	16.0	1.2	0.3

***Source*: Schat & Kalff 1992.**
***Note*: The same data were used as given in Figure 27 for the apical 10 mm.**

fraction of the Zn to its cell walls than does the sensitive one. Inside the cell, Zn is probably stored in the vacuole (as a complex with oxalate or citrate). There is very little transport of Zn to the shoot, especially in the resistant ecotypes. Zn-resistant ecotypes of *Silene vulgaris* (bladder campion) also accumulate more Zn than sensitive ones. Zn accumulates in vacuoles because of a greater capacity to transport Zn across the tonoplast (Chardonnens et al. 1999). As with Al (Sect. 3.1.1), many heavy metals are largely complexed or precipitated at cytosolic pH.

Typical Zn-hyperaccumulating species [e.g., *Thlaspi caerulescens* (alpine pennycress)] accumulate and tolerate up to 40 mg Zn g^{-1} dry mass in their shoots. When exposed to Zn levels that are toxic for most plants, *Thlaspi caerulescens* shows both enhanced Zn influx into the roots and increased transport to the shoots which makes it a promising species to be used for **phytoremediation** (Lasat et al. 1996).

After the discovery of extreme Ni hyperaccumulation in *Alyssum bertolonii* (Brassicaceae) from Italian serpentine soil in 1948, nearly 200 species have been identified as Ni hyperaccumulators. **Ni resistance** in *Alyssum lesbiacum* is associated with the presence of high concentrations of the amino acid **histidine**. Histidine plays a role in the detoxification of absorbed Ni and transport of a Ni-

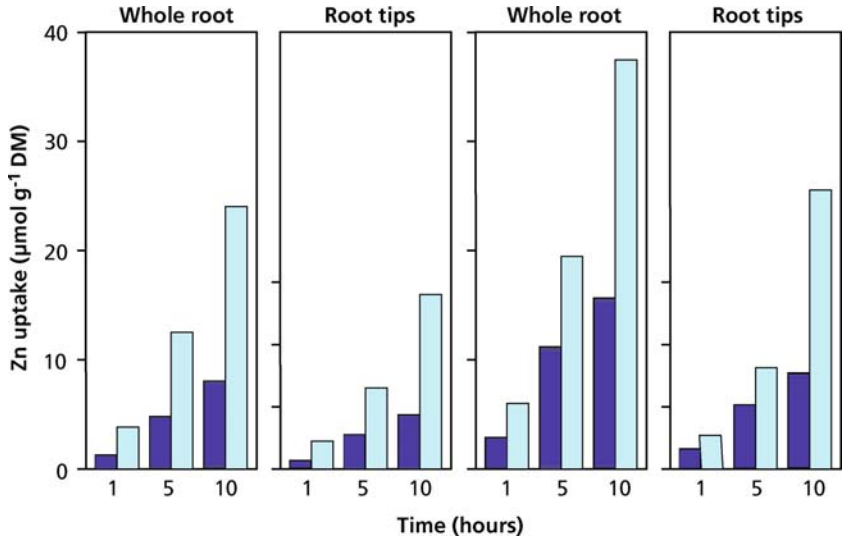


FIGURE 28. Uptake of ^{65}Zn by roots of a Zn-sensitive (*filled bars*) and a Zn-resistant (*open bars*) ecotype of *Deschampsia caespitosa*. The plants are compared at low and high external Zn concentrations, which give the same effect on root elongation (Table 14). The low Zn concentrations (panels at left) are 25 and 250 mM, and the high Zn concentrations (panels at right) are 100 and 500 mM for the sensitive and resistant ecotype, respectively. At the end of the experiment, desorption into a nonlabeled Zn solution was allowed for 30 minutes. The data therefore show uptake into the root cells only, rather than a combination of uptake and binding of labeled Zn to the cell walls (after Godbold et al. 1983).

histidine complex in the xylem to the leaves. In some *Alyssum* species Ni may accumulate to 30 mg g^{-1} leaf dry mass (Krämer et al. 1996).

3.3.5 Biomass Production of Sensitive and Resistant Plants

The biomass production of metal-resistant ecotypes tends to be less than that of sensitive ones, even when compared at a concentration of the heavy metal that is optimal for the plants (i.e., a higher concentration for the resistant plants) (Table 15). This might be due to the **costs** associated with the resistance mechanism. Alternatively, the low productivity of the resistant ecotypes may be associated with the typically low nutrient supply in their natural environment, which selects for inherently slow-growing species (Sect. 3 of Chapter 7 on growth and allocation).

When grown in nontoxic soil, Cu-resistant and Cu-sensitive ecotypes of *Agrostis tenuis* (common bentgrass) have a similar yield in monoculture. In mixtures, the yield of the resistant ecotype is reduced (McNeilly 1968). This explains why resistant ecotypes are exclusively found in environments containing high levels of heavy metals. If resistance were based on reduced uptake capacity, as for As-resistant ecotypes that are characterized by an absence of the high-affinity P_i-uptake system (Sect. 3.3.4), this would offer an explanation for this observation.

TABLE 15. Dry mass (mg per two plants) of roots and shoot of a Cu-sensitive and a Cu-resistant ecotype of *Silene cucubalus* (= *S. vulgaris*, bladder campion), after growth in nutrient solution with two Cu concentrations.

Ecotype		0.5 µM	40.5 µM
Sensitive	Roots	64	8
	Shoot	523	169
	Total	587	173
Resistant	Roots	22	33
	Shoot	146	237
	Total	168	270

***Source*: Lolkema et al. 1986.**
***Note*: The different ecotypes were grown separately.**

3.4 Saline Soils: An Ever-Increasing Problem in Agriculture

The presence of high concentrations of Na^+, Cl^-, Mg^{2+}, and SO_4^{2-} ions in saline soils inhibits growth

of many plants. On a global scale, production is severely restricted by salinity on about 380 million hectares that is potentially usable for agriculture. These areas occur predominantly in regions where evaporation exceeds precipitation (as in southern Australia) and in low-lying areas (such as the Mekong delta and many coastal stretches) where infiltration of seawater is common. The problem of saline soils is ever increasing, due to poor irrigation and drainage practices, expansion of irrigated agriculture into arid zones with high evapotranspiration rates, or clearing land which leads to rising saline water tables ("dryland salinity") (Munns 2002, 2005).

3.4.1 Glycophytes and Halophytes

Most crop species are relatively salt sensitive (**glycophytes**). A notable exception is sugar beet (*Beta vulgaris*). In saline areas, such as salt marshes, species occur with a high resistance to salt in their root environment (**halophytes**). The problems associated with high salinity are threefold:

1. A high salinity is associated with a low soil **water potential**, giving rise to symptoms similar to those of water stress;
2. Specific ions, especially Na^+ and Cl^-, may be **toxic**;
3. High levels of NaCl may give rise to an **ion imbalance** (predominantly Ca) and lead to deficiency symptoms.

Plant adaptation and acclimation to salinity involve all these aspects; we discussed acclimation associated with the low water potential in Sect. 3 of Chapter 3 on plant water relations.

Toxicity effects may include competition of Na^+ with K^+ in biochemical processes and inhibition of NO_3^- uptake by Cl^-, possibly because both anions are transported across the plasma membrane by the same carrier. The toxic effect of Na^+ far exceeds that of Cl^- (Tester & Davenport 2003). High Na^+ may replace Ca^{2+} on root cell membranes, which may give rise to leakage of K^+ from the root cells. It may also reduce the influx and enhance the efflux of Ca^{2+}. The decreased influx of Ca probably results from competition for binding sites in the cell wall which decreases the concentration at the protein in the plasma membrane responsible for Ca^{2+} influx. The toxicity of specific ions may subsequently lead to an ion imbalance and ion deficiency, especially Ca deficiency (Munns 2002). On the other hand, Ca^{2+} reduces the influx of Na^+, due to the inhibition by Ca^{2+} of a voltage-insensitive monovalent channel that allows Na^+ entry into roots (White 1999). However, confirmation of this effect using intact plants is necessary to firmly establish that Ca^{2+} does, indeed, affect influx. The addition of Ca^{2+} has often been proposed as a strategy to reduce Na^+ toxicity to crops.

At a moderate NaCl concentration in the root environment, Na^+ uptake occurs down an electrochemical potential gradient and higher Na^+ concentrations are expected inside than outside (Table 16). Roots of some **glycophytes**, however, maintain a low Na^+ concentration in the presence of 1 mM Na^+ in their medium. This indicates that either their plasma membranes are highly impermeable for this ion or Na^+ is actively excreted from these roots.

3.4.2 Energy-Dependent Salt Exclusion from Roots

The low Na^+ concentration inside the cells of glycophytes is mostly due to energy-dependent transport. At an external NaCl concentration of 1 mM,

Table 16. Experimentally determined concentrations of Na^+ and K^+ ions in *Avena sativa* (oat) and *Pisum sativum* (pea) roots, compared with values predicted on the basis of the Nernst equation.*

	Oat		Pea	
Ion	Predicted	Experimentally determined	Predicted	Experimentally determined
K^+	27	66	73	75
Na^+	27	3	73	8

***Source*: Higginbotham et al. 1967.**

***The latter values assume that no metabolic energy-dependent mechanism is involved in the transport of these cations. The membrane potential of oat and pea was –84 and –110 mV, respectively.**

Table 17. Net uptake of labeled Na^+ in a glycophyte, *Plantago media* (hoary plantain), and a halophyte, *Plantago maritima* (sea plantain), in the presence and absence of DES (diethylstilboestrol, an inhibitor of the plasma membrane ATPase).*

	Plantago media		*Plantago maritima*	
NaCl (mM)	–DES	+DES	–DES	+DES
1	**0.5**	**2.8**	**5.9**	**2.7**
10	**6**	**27.7**	**21.6**	**25.5**
50	**37.3**	**121.1**	**68.1**	**82.5**

***Source*: De Boer 1985.**

*** The uptake was measured at three levels of NaCl in the nutrient solution and are expressed as (μmol (g root dry mass)$^{-1}$) hour^{-1}). Values printed in bold are significantly different from those to their immediate left.**

inhibition of the plasma membrane H^+-ATPase increases net Na^+ uptake in the glycophyte *Plantago media* (hoary plantain), but decreases it in the halophyte *Plantago maritima* (sea plantain). This illustrates that both **ATP-dependent Na^+ excretion** and **uptake** occur in these *Plantago* species (Table 17). At higher (10, 50 mM) NaCl concentrations, the roots of the glycophyte continue to excrete Na^+, but not to the extent that accumulation in the plant is avoided. At 10 mM, there is no evidence for ATPase-mediated uptake in the halophyte, and at 50 mM there is excretion (Table 17).

3.4.3 Energy-Dependent Salt Exclusion from the Xylem

At 10 and 50 mM NaCl, when an inhibitor of the plasma membrane ATPase has no positive effect on the Na^+ concentration in the roots, the inhibitor enhances the Na^+ concentration in the leaves of *Plantago* (Fig. 29). This indicates ATP-dependent exclusion from the xylem in both the glycophyte [*Plantago media* (hoary plantain)] and the halophyte [*Plantago maritima* (sea plantain)]. Glycophytes, therefore, maintain a lower Na^+ concentration in their leaves, partly due to excretion by their roots as well as because of energy-dependent **exclusion from the xylem** (Cheeseman 1988). In *Arabidopsis thaliana* (thale cress), such exclusion is based on reabsorption of Na^+ from the xylem by surrounding xylem-parenchyma cells involving a specific Na^+ transporter (Sunarpi et al. 2005, Davenport et al. 2007).

Using labeled Na^+, it has been shown for *Glycine max* (soybean) that salt that leaks into the xylem can be reabsorbed and excreted back into the root environment; however, the extent to which this happens

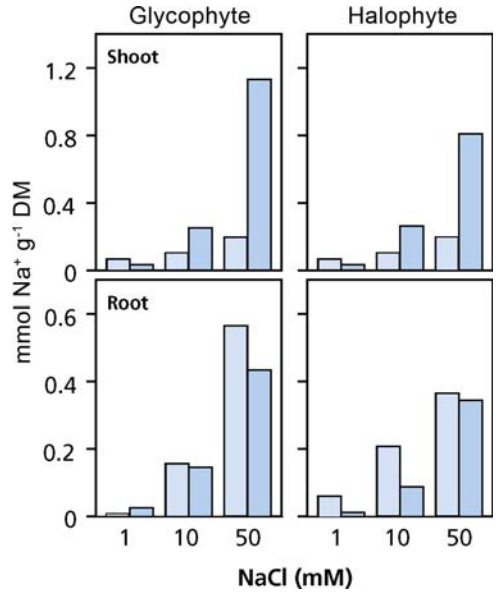


Figure 29. The effect of an inhibitor of the plasma membrane ATPase (DES, diethylstilboestrol; *open bars*) on the accumulation of labeled Na^+ in roots and shoots of a glycophyte (*Plantago media*) and a halophyte (*P. maritima*). Results for control plants are shown with *filled bars* (De Boer 1985). Reproduced with the author's permission.

in this glycophyte is rather small (Lacan & Durand 1994).

3.4.4 Transport of Na^+ from the Leaves to the Roots and Excretion via Salt Glands

Salt transported to the shoot via the transpiration stream may be exported again, via the phloem, to the roots. Using ^{22}NaCl, this was shown for *Capsicum annuum* (sweet pepper) (Blom-Zandstra et al. 1998). For another glycophyte [*Lupinus albus* (white lupin)] this was determined by analyzing phloem sap, which exudes spontaneously from white lupin stems upon cutting (Sect. 5 in Chapter 2C on long-distance transport). Export of Na^+ to the roots may be followed by excretion, as shown for *Plantago media* (hoary plantain) (Table 17).

True halophytes may have **salt glands**, which **excrete** salt from their leaves. These may remove a major part of the salt arriving in the shoot via the transpiration stream, as shown for *Cynodon*(bermudagrass) turf cultivars (Fig. 30). **Salt exclusion** in the roots can be estimated from the difference in net Cl^- uptake and the product of the transpiration rate and

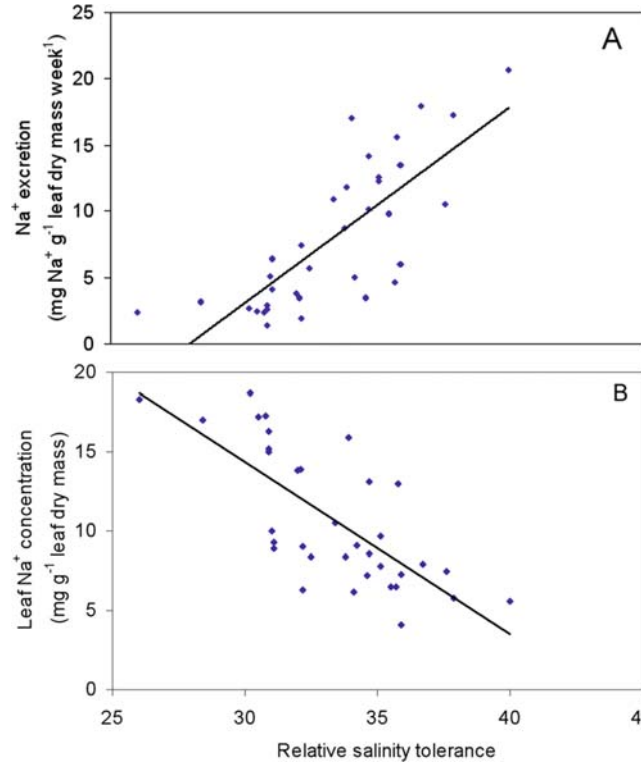
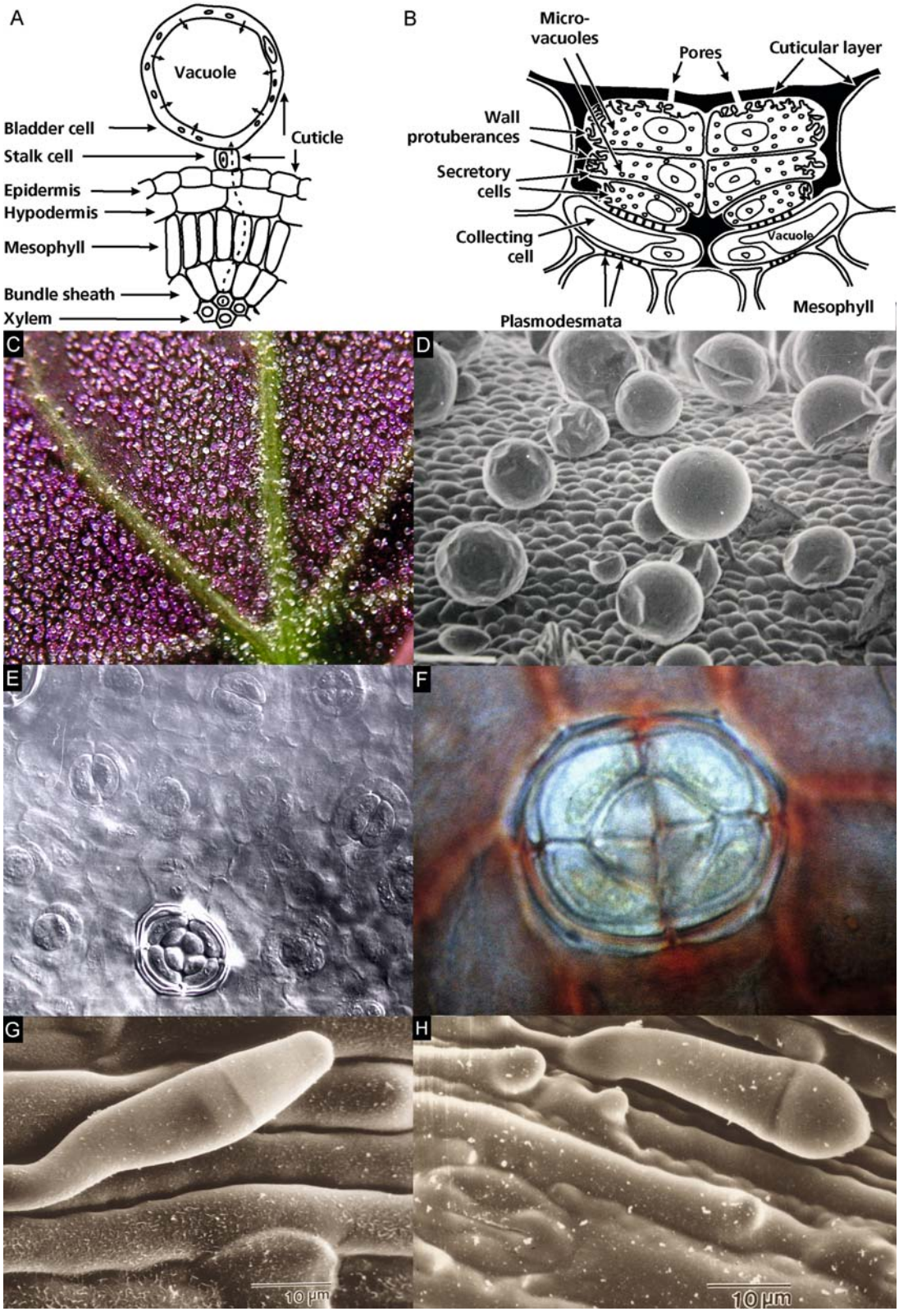


FIGURE 30. (A) Leaf salt gland Na-excretion rate and (B) leaf sap Na concentration plotted against relative salinity tolerance of 35 *Cynodon*(bermudagrass) turfgrass cultivars. Relative salinity tolerance is the salinity level resulting in 50% shoot dry weight relative to that of control; a broad range in salinity tolerance exists within the *Cynodon* genus (Marcum & Pessarakli 2006). Copyright Crop Science Society of America.

the Cl^- concentration in the root environment. It is substantial in *Avicennia marina* (gray mangrove), increasing from 90% at the lowest salinity level to 97% at 500 mM NaCl (Ball 1998). Exclusion may be due to active excretion from the roots, as in *Plantago* species (Table 17), or be associated with highly impermeable membranes. Active excretion must incur respiratory costs, as discussed in Sect. 4.2 of Chapter 2B on plant respiration.

Salt removal from the leaves involves specialized structures, specialized **trichomes** (**salt bladders**) (Schirmer & Breckle 1982) or **salt glands** (Wiehe & Breckle 1990). In *Atriplex* species, salt that arrives in the transpiration stream is transported via plasmodesmata to the cytosol of epidermal cells and then to bladder-like cells on stalks (special trichomes) on the epidermal surface (Fig. 31). The salt is pumped into the large vacuole of this bladder cell. In the end, the bladder may collapse and the salt is deposited on the leaf surface, where it gives the leaves a white appearance until washed away by rain. The salt may also be **excreted** in such a way that concentrated droplets fall from the leaves, as in some *Tamarix* species. True salt glands, as opposed to the trichomes of *Atriplex*, are found in *Tamarix aphylla* (Fig. 31). The salt glands of *Tamarix aphylla* consist of eight cells, six of which are involved in pumping the salt to the leaf surface. Salt is transported from mesophyll cells via plasmodesmata to two basal collecting cells that transport it to the secreting cells. The secreting cells are surrounded by a lipophilic layer, except where they are connected to the basal cells via plasmodesmata. In these secreting cells, salt is pumped into microvacuoles. These merge with the plasma membrane and the salt is then exported to the apoplast. The invaginations in these cells suggest that active membrane transport is involved as well. The salt diffuses via the apoplast to a pore in the cuticle, where it is deposited on the leaf surface. The waxy layer that surrounds the secreting cells prevents back diffusion to the mesophyll cells (Popp 1995).

What might be the advantage of salt excretion from the leaves over salt excretion from the roots? If all the salt that arrives via mass flow at the root surface were excluded, then the salt concentration in the rhizosphere would rapidly rise to very high levels. In the absence of a substantial removal from the rhizosphere by bulk flow of less saline water, the local accumulation of salt would continue to reduce water potential and aggravate the problems associated with water uptake (Passioura et al. 1992).


A
Vacuole
Bladder cell
Cuticle
Stalk cell
Epidermis
Hypodermis
Mesophyll
Bundle sheath
Xylem
B
Micro-
vacuoles
Pores
Cuticular layer
Wall
protuberances
Secretory
cells
Collecting
cell
Vacuole
Plasmodesmata
Mesophyll
C
D
E
F
G
H
10 µm
10 µm

A high water-use efficiency in combination with salt exclusion therefore has advantages over active or passive exclusion only. Mangrove species with the highest water-use efficiency are also the most salt-resistant ones (Ball 1988).

3.4.5 Compartmentation of Salt Within the Cell and Accumulation of Compatible Solutes

Salt resistance also involves the **compartmentation** of the potentially toxic ions in the vacuole and the capacity to produce nontoxic, **compatible solutes** in the cytoplasm (Sect. 3 of Chapter 3 on plant water relations). Compartmentation in the **vacuole** is achieved by an active mechanism that is induced in halophytes such as *Plantago maritima* (sea plantain) (Fig. 32) and *Mesembryanthemum crystallinum* (common iceplant) (Barkla et al. 1995), but not in glycophytes, such as *Plantago media* (hoary plantain), in the presence of NaCl in the root medium. A specific Na^+ transporter is involved in compartmentalizing Na^+ in the vacuole (Apse & Blumwald 2007).

Some moderately salt-resistant glycophytes, for example, *Hordeum vulgare* (barley) cultivars, also accumulate some salt in their leaves. Using X-ray diffraction, it can be shown that Cl^- predominantly accumulates in the vacuoles of the epidermis cells of leaf blades and sheaths. To a smaller extent Cl^- is also found in the mesophyll cells of the leaf sheath, whereas the concentration remains low in the mesophyll cells of the leaf blade, even after exposure to 50 mM NaCl in the root environment for 4 days (Huang & Van Steveninck 1989).

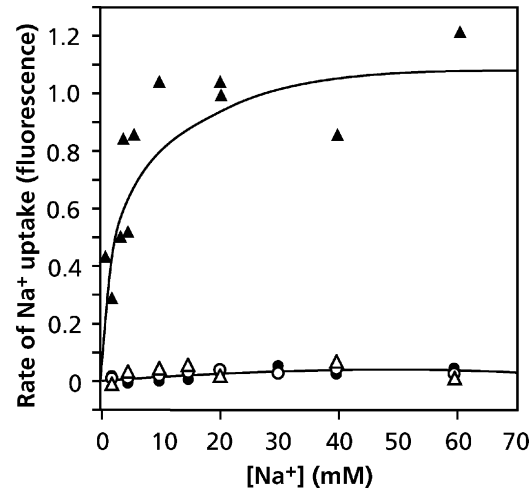


FIGURE 32. Uptake of Na^+ in tonoplast vesicles of the glycophyte *Plantago media* (hoary plantain, *circles*) and the halophyte *Plantago maritima* (sea plantain, *triangles*). Tonoplast vesicles were isolated from plants grown in the absence (*open symbols*) or in the presence (*filled symbols*) of 50 mM NaCl (Staal et al. 1991). Copyright Physiologia Plantarum.

3.5 Flooded Soils

The absence of oxygen in the soil causes a drop in redox potential, due to microbial activity. At a low redox potential, NO_3^- rapidly disappears due to its use as an electron acceptor by **denitrifying bacteria**, and $\mathbf{NH_4}^+$ is the predominant source of inorganic N for the plant. Fe and Mn are similarly reduced. These reduced forms are much more soluble and potentially toxic to the plant. SO_4^{2-} is also used as an alternative electron acceptor by specialized bacteria, leading to the formation of $\mathbf{S^{2-}}$, which is an inhibitor of cytochrome oxidase (Sect. 3.6 of Chapter 2B on plant respiration). Thus, the availability of many ions is affected by the redox potential which leads to shortage of some nutrients and potentially toxic levels of others.

FIGURE 31. Two schematic diagrams of structures involved in the excretion of salt to the leaf surface. (A) Diagram of a trichome of a leaf of an *Atriplex* (saltbush) species. (B) Diagram of a salt-excreting gland of *Tamarix aphylla* (tamarisk) (after Esau 1977; reprinted with permission from John Wiley & Sons, Inc.). (C) Lower leaf surface of fresh leaves of *Atriplex pratovii* with dense cover of salt bladders. Courtesy M. Wennemann & S.-W. Breckle, Department of Ecology, University of Bielefeld, Bielefeld, Germany. (D) Scanning electron micrograph showing salt bladders on a leaf of *Atriplex hortensis*.- Courtesy U. Schirmer & S.-W. Breckle, Department of Ecology, University of Bielefeld, Bielefeld, Germany. (E) Stages of development of salt glands on a leaf of *Limonium ramosissimum*. Courtesy W.Wiehe & S.-W. Breckle, Department of Ecology, University of Bielefeld, Bielefeld, Germany. (F) Salt gland on upper leaf surface *Acantholimon ulicinum* var. *creticum*, stained with Sudan red. Courtesy W. Wiehe & S.-W. Breckle, Department of Ecology, University of Bielefeld, Bielefeld, Germany. (G) Scanning electron micrograph showing a salt hair on a leaf of *Bouteloua eriopoda*(black grama). (H) Scanning electron micrograph showing a salt hair on a leaf of *Buchloe dactyloides*(buffalograss). G and H: courtesy K.B. Marcum, Department of Applied Biological Sciences, Arizona State University, Mesa, USA.

Toxicity is largely prevented by oxidation, possibly followed by precipitation of these ions in the oxygenated rhizosphere. **Oxygenation of the rhizosphere** of flooding-resistant species is due to the presence of an **aerenchyma**, which allows root respiration to continue and leads to detoxification of potentially toxic ions in the rhizosphere (Sect. 4.1.4 of Chapter 2B on plant respiration and Sect. 5.6.1 of Chapter 7 on growth and allocation; Kirk & Kronzucker 2005).

4. Plant Nutrient-Use Efficiency

Plants differ both in their capacity to acquire nutrients from the soil (Sect. 3) and in the amount of nutrients they need per unit growth, the nutrient concentrations in their tissue, and the time and extent to which they withdraw nutrients during leaf senescence before leaf abscission. In this section, we discuss several approaches for analyzing the efficiency with which plants utilize nutrients to produce new biomass. Whole-plant nutrient-use efficiency (NUE) addresses processes related to carbon gain and loss, whereas photosynthetic nitrogen-use efficiency (Sect. 6 of Chapter 2A on photosynthesis) addresses only the instantaneous use of N for photosynthetic carbon gain.

4.1 Variation in Nutrient Concentration

4.1.1 Tissue Nutrient Concentration

Plants differ in the concentration of mineral nutrients in their tissue, depending on environment, allocation to woody and herbaceous tissues, developmental stage, and species (Fig. 33). N, P, and K are the nutrients that most frequently limit plant growth. However, as explained in Sect. 2.1.1 and Fig. 1A, N tends to limit plant productivity on young soils, whereas P becomes increasingly limiting as soils age. The presence of a specific mineral in plant tissues does not imply that the plant needs this mineral for growth. For example, Cd is found in tissues of plants growing on Cd-polluted soil, but it is *not* an essential nutrient for any plant. Similarly, high Na concentrations are not required for growth.

Nutrient concentrations change predictably with plant development. In especially woody plants, the **C:N ratio** increases with increase in plant age, as the ratio of woody mass to active mass increases. Nutrients associated with **metabolism** (e.g., N, P, and K) have highest concentrations when a leaf or other organ is first produced, then concentrations decline, first as the concentration becomes diluted by increasing quantities of cell-wall material during leaf expansion, then by resorption of nutrients during senescence (Fig. 34). **Ca**, which is largely associated with cell walls and is phloem immobile

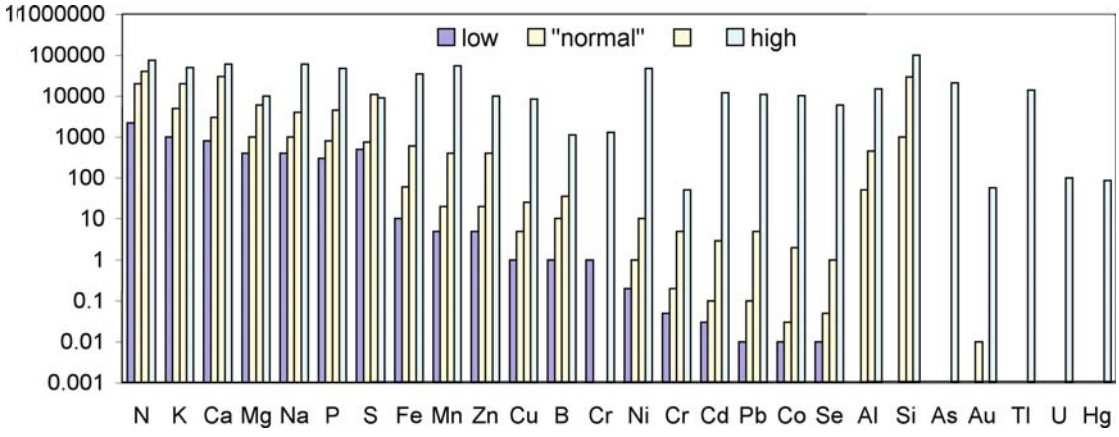


FIGURE 33. The range of concentrations of minerals as determined in plant dry matter. The two middle bars refer to concentrations commonly observed in healthy plants; the bar at the left refers to either plants that are very efficient at using a specific nutrient or plants that exhibit a low concentration because their leaves are severely deficient or senescent, or because the plants exclude certain elements; the bar at the right refers to plants exhibiting exceptionally high concentrations of an element, e.g., in halophytes or metallophytes. Based on numerous references, including Biddulph et al. (1956), Foulds (1993), Bell (1997), Anderson et al. (1998), Baker et al. (2000), Reeves & Baker (2000), Broadley et al. (2003) and Osaki et al. (2003a,b).

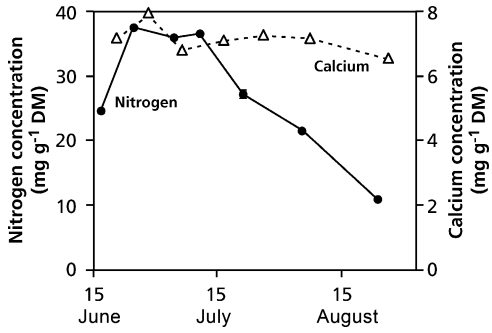


FIGURE 34. Typical seasonal pattern of leaf N and Ca concentrations of leaves of *Salix pulchra* (willow) from an Alaskan arctic tundra meadow (Chapin et al. 1980).

(Sect. 2 in Chapter 3 on long-distance transport) and therefore not resorbed (Sect. 4.3), increases continuously through leaf development.

Tissues differ predictably in nutrient concentrations: leaves have higher concentrations of nutrients associated with **metabolism** (N, P, and K) and lower concentrations of Ca than do **woody stems**; roots have intermediate concentrations. Whole-plant nutrient concentrations, therefore, differ among species and environments, depending on relative allocation to these tissues.

Environment strongly affects plant nutrient concentration by changing both allocation among organs and the composition of individual tissues. The major environmental effect on tissue nutrient composition is to alter the concentration of nutrients associated with **metabolism**. Plants have high concentrations of N, P, and K when conditions are favorable for growth (e.g., with adequate water and nutrients) (Niklas et al. 2005). The balance of available nutrients in the environment then alters the proportions of these nutrients. Whole-plant biomass **N:P ratios** [g N (g P)$^{-1}$] may vary up to 50-fold, due to differences in root allocation, nutrient uptake, biomass turnover, and reproductive output (Aerts & Chapin 2000). At the vegetation level, N:P ratios <10:1 and >20:1 tend to correspond with N- and P-limited biomass production, respectively, as evidenced by short-term fertilization experiments. N:P ratios are, on average, higher in graminoids than in forbs and higher in stress-tolerant species than in ruderals; they correlate negatively with the maximum relative growth rates of species and with their N-indicator values (Sect. 3 of Chapter 7 on growth and allocation). At the vegetation level, N:P ratios tend to correlate negatively with biomass production; high N:P ratios promote graminoids and stress-tolerating species, relative to other species (Güsewell 2004). In general, leaf N and P concentrations decline, and the N:P ratio increases toward the equator as average temperature and growing season length increase (Reich & Oleksyn 2004). This trend persists across taxonomic groups, and presumably reflects both acclimation and adaptation. Higher leaf N and P concentrations compensate for reduced metabolic rates at low temperatures, and soils may differ in relative N and P supply across tropical to temperate regions (Hedin 2004).

There are no striking differences among species in biochemical allocation of N and P among classes of chemical compounds (e.g., protein N, nucleic acid N, lipid N) (Chapin 1988). The major differences among species relate to accumulation of certain compounds in the cytoplasm for osmotic functions (N-containing compatible solutes) and in vacuoles for storage functions (e.g., P_i, NO_3^-, and vegetative storage proteins; Sect. 4.3 of Chapter 7 on growth and allocation) or chemical defense (e.g., alkaloids and cyanogenic glycosides; Sect. 3 of Chapter 9B on ecological biochemistry).

When nutrient supply declines relative to plant demand, most plants show the following sequence of events (Chapin 1980): (1) decrease in vacuolar reserves with little effect on growth; (2) continued reduction in tissue nutrient concentrations, especially in older leaves and stems, reduced rates of leaf growth and photosynthesis (in that order), increased nonstructural carbohydrate concentrations, senescence of older leaves, and reallocation of reserves to compensate for reduced nutrient status (increased root mass ratio and increased root absorption capacity); (3) greatly reduced photosynthesis and nutrient absorption, dormancy, or death of meristems.

4.1.2 Tissue Nutrient Requirement

Species differ in their nutrient requirement for maximum growth, but the physiological mechanisms for this are not always known. For example, the tissue **calcium concentration** at which 90% of the maximum yield is achieved is about twice as high for **dicots** as for **monocots** (Table 18). In addition, when comparing graminoids and forbs at similar sites, the forbs invariably have higher concentrations of both Ca and Mg (Meerts 1997). The reason for this difference is likely the greater cation exchange capacity of the cell walls of dicotyledonous species (i.e., the amount of free Ca-binding carboxylic acid groups in pectins) (Woodward et al. 1984). The tissue **P concentration** at which 90% of the maximum yield

Table 18. Effect of the calcium concentration in the nutrient solution on the growth and the calcium concentration in the shoots of a monocotyledonous [*Lolium perenne* (perennial ryegrass)] and a dicotyledonous [*Solanum lycopersicum* (tomato)] species.

	Calcium supply (μM)				
Species	**0.8**	**2.5**	**10**	**100**	**1000**
Growth rate (% of maximum value)					
Lolium perenne	**42**	**100**	**94**	**94**	**93**
Solanum lycopersicum	**3**	**19**	**52**	**100**	**80**
Calcium concentration (μmol g^{-1} dry mass)					
Lolium perenne	**15.0**	**17.5**	**37.4**	**92.3**	**269.5**
Solanum lycopersicum	**49.9**	**32.4**	**74.9**	**321.9**	**621.3**

***Source*: Loneragan 1968 and Loneragan & Snowball 1969, as cited in Marschner 1983.**

occurs is greater for many **crop legumes** than for **nonlegumes** (Fig. 5 in Chapter 9A on symbiotic associations). The physiological basis of this difference is not quite clear, but it is likely associated with the fast-growing strategy of many legumes, as well as with the high energetic requirement and use of phosphorylated intermediates to fix N_2 in legume nodules (Sprent 1999).

Some slow-growing species from severely P-impoverished soils maintain relatively high rates of photosynthesis at extremely low leaf P concentrations (Wright et al. 2004, Denton et al. 2007), presumably because they contain very little P in their vacuoles. From a biochemical point of view, all species will need similar amounts of N, P, S, and so on, to make a unit of growth, simply because they are constructed in a similar manner (Sterner and Elser 2002). Thus, the idea that there are different *metabolic* requirements is erroneous, except that specific enzymes may require a specific ion. For instance, **Ni** is an essential element for **urease**, which hydrolyzes urea to CO_2 and H_2O. Urease is required in all plants, but in greater amounts in those legumes that produce ureides when grown symbiotically with rhizobia (Sect. 3.4 of Chapter 9A on symbiotic associations) (Walker et al. 1985). Apart from these exceptional differences, variation in nutrient requirement and nutrient productivity (Sect. 4.2.1) depends much more on the balance between requirements for protein synthesis for new growth and N storage (Sect. 4 of Chapter 7 on growth and allocation).

4.2 Nutrient Productivity and Mean Residence Time

4.2.1 Nutrient Productivity

A useful measure of the efficiency of nutrient use to produce new biomass is **nutrient productivity** (Ingestad 1979), the ratio of relative growth rate (RGR, mg g^{-1} day^{-1}) to whole-plant nutrient concentration in the plant tissue (NP, mol g^{-1}). For example, N productivity (NP, mg mol^{-1} N day^{-1}) is

$$NP = RGR/PNC \quad (1)$$

where PNC is the plant N concentration (i.e., total plant N per total plant mass). When grown at an optimum nutrient supply, plants differ widely in their N productivity (Fig. 35). A higher N productivity is associated with rapid growth, a relatively large investment of N in photosynthesizing tissue, an efficient use of the N invested in the leaves for the process of photosynthesis, and a relatively small use of carbon in respiration (Fig. 35). C_4 species also have a high N productivity under optimal N supply which is apparently a result of their lower N requirement for photosynthesis (high PNUE) (Sect. 6.1 of Chapter 2A on photosynthesis).

N productivity shows saturation and sometimes an optimum curve, when plotted as a function of the N supply to the plant (Fig. 36). The decrease in NP above the maximum value for NP is due to a decrease in the rate of photosynthesis per unit of N in the leaf at high leaf N which reflects increased allocation of N to storage (Sect. 4 of Chapter 7 on growth and allocation). The decrease when the N supply is less than that at the maximum value for NP is largely due to greater investment of N in nonphotosynthetic tissue (Sect. 5.4 of Chapter 7 on growth and allocation).

4.2.2 The Mean Residence Time of Nutrients in the Plant

Although the nutrient productivity gives a good indication of a plant's **instantaneous** NUE, it does not provide insight into a plant's **long-term performance** in a natural habitat. To develop such insight, we expand the concept of **nutrient-use efficiency** to consider the time during which nutrients remain in the plant to support productivity. Plant NUE (g g^{-1} N), which is defined in this way,

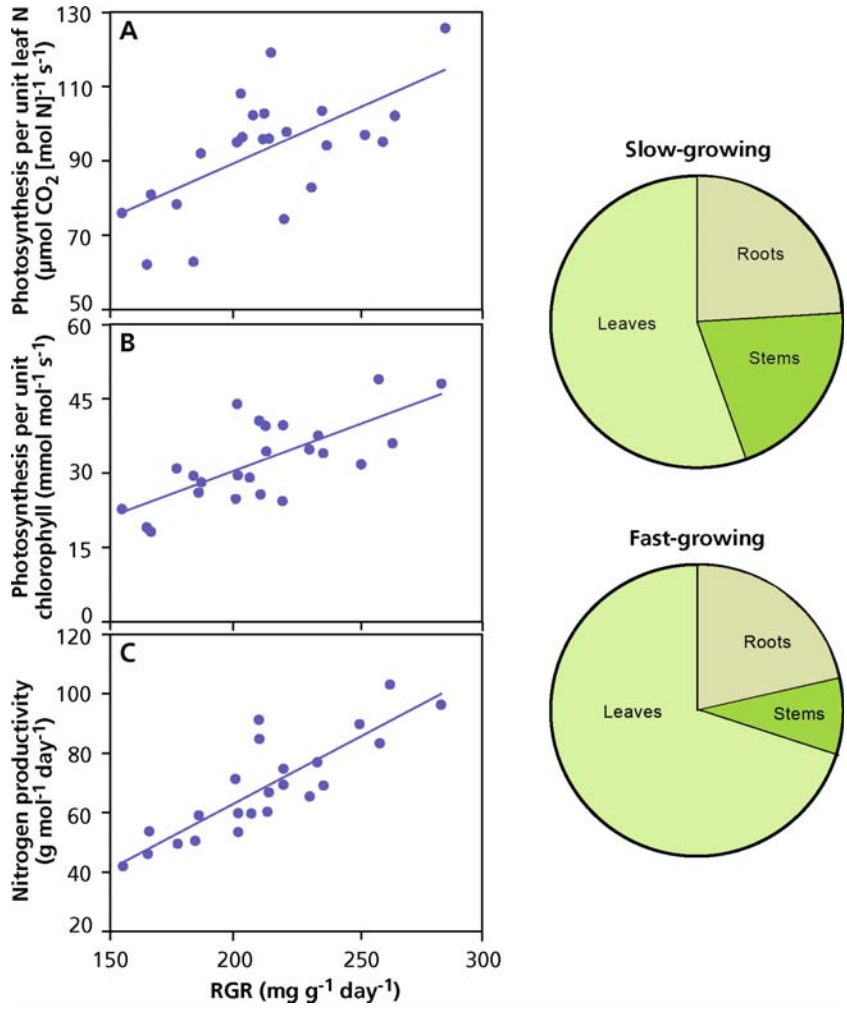


FIGURE 35. The N productivity (*bottom panel*) of fast- and slow-growing herbaceous plant species, grown with free access to nutrients in a growth room. The physiological background of the higher N productivity of fast-growing species is their greater investment of N in leaves, as opposed to roots and stems (*circles* at the right), and their higher rate of photosynthesis per unit N in the leaves (photosynthetic N-use efficiency, PNUE) (*top panel*). The rate of photosynthesis per unit chlorophyll in the leaves is also higher for the fast-growing herbaceous species (*middle panel*) (after Poorter et al. 1990). Copyright American Society of Plant Biologists.

is the product of the NP (g g^{-1} N yr^{-1}) (as defined earlier, but is now determined over much longer periods; say 1 year), and the **mean residence time** (MRT; yr) of that nutrient in the plant (Berendse & Aerts 1987):

$$\text{NUE} = \text{NP} \cdot \text{MRT} \qquad (2)$$

The mean residence time is the average time the nutrient remains in the plants, before it is lost due to leaf shedding, herbivory, root death, and so on.

The N-use efficiencies of evergreen heathland shrub species and that of a co-occurring deciduous grass species are remarkably similar, but the underlying components differ (Table 19). **Evergreen** species achieve their NUE with a low N productivity and a high mean residence time, whereas **deciduous** species have a considerably higher N productivity, but a lower mean residence time. In competition experiments with the species from Table 19, the grass wins at a relatively high N supply, because of its higher N productivity. At a low N supply, the

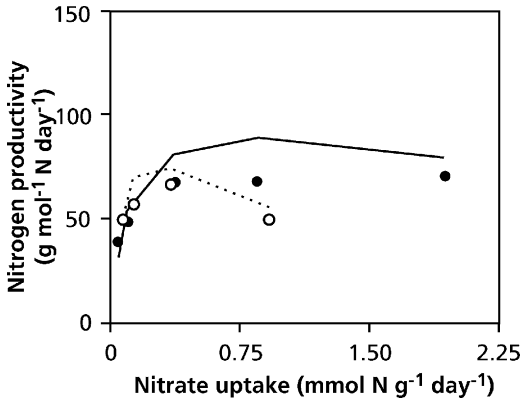


FIGURE 36. The N productivity of *Briza media* (quacking grass, *open symbols* and *broken line*) and *Dactylis glomerata* (cocksfoot, *filled symbols* and *continuous line*), as a function of the rate of NO_3^- uptake. The NO_3^- uptake was varied through different exponential rates of N addition in order to maintain a constant RGR at each rate of NO_3^- supply. The symbols give the actual experimental data and the lines refer to results of a simulation model (Van der Werf et al. 1993). Copyright Blackwell Science Ltd.

competitive ability of the evergreen shrub is higher, because of its long mean residence time of N in the plant. A high mean residence time is the most important mechanism for nutrient conservation in infertile sites (Eckstein et al. 1999). Both adaptation and acclimation contribute to the greater mean residence time in infertile sites. These sites are typically dominated by evergreen shrubs and trees, and both grasses and evergreen trees and shrubs adapt to low nutrient supply through increases in leaf longevity (Westoby et al. 2002).

TABLE 19. The long-term nitrogen productivity (NP), the mean residence time of nitrogen (MRT), and the nitrogen-use efficiency (NUE) of an evergreen heathland shrub species [*Erica tetralix* (crossleaf heath)] and a co-occurring deciduous grass species [*Molinia caerulea* (purple moorgrass)].

	Erica tetralix	*Molinia caerulea*
Nitrogen productivity ($g\ g^{-1}\ N\ yr^{-1}$)	77	110
Mean residence time (yr)	1.2	0.8
Nitrogen-use efficiency ($g\ g^{-1}\ N$)	90	89

Source: Aerts 1990.

It is interesting that the plant features that favor a low rate of nutrient loss (high mean residence time) also decrease the rate of **decomposition** of the leaf litter. This tends to aggravate the low availability of nutrients in the already nutrient-poor environments (Sect. 3.2 of Chapter 10A on decomposition). As will be discussed in Sect. 2.4 of Chapter 9A on symbiotic associations and in Chapter 10A on decomposition, however, some species can make use of nutrients in leaf litter even before it is fully decomposed.

4.3 Nutrient Loss from Plants

Nutrient loss is just as important as nutrient uptake in determining the **nutrient budgets** of perennial plants; however, much less is known about the controls over nutrient loss.

4.3.1 Leaching Loss

Leaching accounts for about 15% of the N and P and half the K returned from above-ground plant parts to soil (Table 20), with the remainder coming from senesced leaves and stems. Use of experimental "mini-umbrellas" to prevent rain from contacting leaves suggests that leaching losses can be an even larger proportion (25–55% of nutrient loss from leaves) (Chapin & Moilanen 1991). Leaching occurs most readily when there are high concentrations of soluble nutrients in the intercellular spaces of leaves, for example, during rapid leaf production or senescence and when plants grow under conditions of high **nutrient availability**. Leaching rate is highest when rain first hits a leaf, then declines exponentially with continued exposure to rain (Tukey 1970). The frequency of rainfall is, therefore, more important than its intensity in determining

TABLE 20. Nutrients leached from the canopy (throughfall) as a percentage of the total aboveground nutrient return from plants to the soil for 12 deciduous and 12 evergreen forests.

	Throughfall (% of annual return)	
Nutrient	Evergreen forests	Deciduous forests
N	1	15
P	15	15
K	59	48
Ca	27	24
Mg	33	38

Source: Chapin 1991.

leaching loss. **Deciduous** leaves have a higher rate of leaching loss than do **evergreens** because of their higher tissue nutrient concentrations. This is compensated, however, by the greater time of exposure to leaching in evergreen plants (Thomas & Grigal 1976), so that leaching constitutes a similar proportion of above-ground nutrient loss by evergreen and deciduous forests (Table 20).

The magnitude of nutrient loss by leaching decreases in the order K > Ca > N = P which reflects the greater mobility of monovalent than divalent cations, and the greater susceptibility to loss of inorganic than of organically bound nutrients. It was initially thought that one explanation for the scleromorphic leaves with thick cuticles in nutrient-poor sites was prevention of leaching loss (Loveless 1961); however, there is no clear relationship between cuticle thickness or scleromorphy and the susceptibility of leaves to leaching loss (Sects. 5.4.5 and 8.2 of Chapter 3 on plant water relations). These leaf traits are more likely selected for their importance in withstanding unfavorable conditions during the nongrowing season and reducing leaf loss to herbivores and pathogens (Sect. 3.2 of Chapter 9B on ecological biochemistry; Read et al. 2006).

Acid rain increases leaching of cations, particularly of Ca (Fig. 37), because hydrogen ions in the rain exchange with cations held on the cuticular exchange surface and because acidity alters the chemical nature of the cuticle so that it is more susceptible to diffusion and mass flow of nutrients to the leaf surface (Shriner & Johnston 1985, Reuss & Johnson 1986).

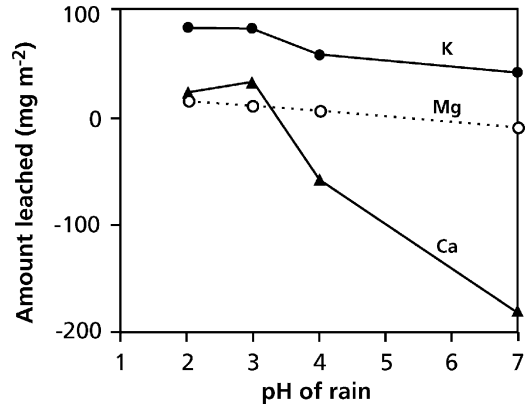


FIGURE 37. Effects of pH of simulated rain (containing 315, 35, and 35 mg m^{-2} of Ca, Mg, and K, respectively) on the leaching of Ca, K, and Mg from spruce crowns. At high pH, spruce needles absorb Ca, and to a lesser extent Mg, from the rain (after Chapin 1991).

4.3.2 Nutrient Loss by Senescence

Approximately half of the N and P content of **leaves** is resorbed during senescence and is used to support further plant growth (Aerts 1996, Killingbeck 1996). By contrast, Ca, which is immobile in the phloem (Sect. 2 of Chapter 2C on long-distance transport), is not resorbed and reutilized. N- and P-**resorption efficiency** (proportion of maximum nutrient pool resorbed) ranges from 0 to 80% among species and environmental conditions (Reich et al. 1995, Killingbeck 1996) (Table 21).

TABLE 21. Nutrient withdrawal from senescing leaves of trees growing on nutrient-poor sandy sites.*

Species	Location	Leaf longevity	Resorption (%)	
			P	N
Goupia glabra	Guyana	Lower	53	0
Cecropia obtusa	Guyana	Lower	63	0
Dicymbe altsonii	Guyana	Higher	60	33
Chlorocardium rodiei	Guyana	Higher	51	0
Banksia menziesii	Australia	Higher	82	73
Eucalyptus gomphocephala	Australia	Lower	55	61
Larix laricina	Minnesota, USA	Lower	0	48
Populus tremuloides	Minnesota, USA	Lower	42	65

* For the tree species from a rainforest in Guyana, nitrogen was not a factor limiting growth. Phosphorus was available in critically low amounts which may have been limiting for growth; however, productivity may also have been limited by other nutrients or the low pH of the soil. For the Australian tree species from an open sclerophyll nutrient-poor woodland, where bushfires regularly remove large amounts of nitrogen from the ecosystem, growth of the investigated trees was limited by nitrogen (Raaimakers 1995). In Minnesota, nitrogen was the growth-limiting nutrient (Tilton 1977, Verry & Timmons 1976). In addition most other nutrients were also scarcely available. Nutrient resorption was calculated from the amount of P or N present in senesced leaves and the peak amount found in the leaves of each species.

Similar variation occurs with respect to the terminal nutrient concentration in senesced leaves (**resorption proficiency**). Concentrations of 3 mg N g^{-1} dry mass and 100 μg P g^{-1} dry mass in senesced leaves are considered the ultimate potential resorption of these nutrients in woody perennials; however, some Western Australian *Banksia*species from the world's most severely P-impoverished habitats show even greater resorption proficiency, down to 27 μg P g^{-1} dry mass (Denton et al. 2007). Resorption efficiency of both N and P is highest in graminoids; N-resorption efficiency is higher in deciduous shrubs and trees than it is in evergreens, although the differences are small compared with differences in mean residence time (Aerts 1996) (Table 22). Evergreen species have a greater ability to reduce the mass-based P concentration in senescing leaves than do deciduous species (greater P-resorption proficiency) (Table 23). In spite of the large range observed in nutrient resorption and the importance of resorption to plant nutrient budgets, no clear patterns of physiological and ecological controls over nutrient resorption have emerged. About 60% of studies show no relationship of resorption efficiency to nutrient availability, with most of the remaining studies showing small decreases in resorption efficiency in fertile sites (Aerts 1996, Demars & Boerner 1997). In nutrient-rich sites, larger *quantities* of nutrients are generally withdrawn from the leaves and larger *quantities* remain in senesced leaves, compared with leaves of plants growing in infertile sites (Killingbeck 1996, Richardson et al. 2005), but the proportion of N and P resorbed is similar across sites (Aerts 1996, Wright & Westoby 2003). Thus, the nutrient concentration of litter is higher in more fertile sites, which has important consequences for decomposition (Chapter 10A on decomposition).

Resorption is the net result of several processes: enzymatic breakdown of N- and P-containing compounds in the leaves, phloem loading and transport, and the formation of an abscission layer that cuts off the transport path and causes the leaf to fall. Resorption is positively correlated with leaf mass loss during senescence which suggests a link with export via the phloem (Chapin & Kedrowski 1983). Leaves that are darkened during senescence to reduce source strength have low resorption, whereas leaves with strong sinks (e.g., nearby developing fruits or new leaf growth) have high resorption which again suggests a role for source–sink interactions and phloem transport in explaining proportional resorption (Nambiar & Fife 1987, Chapin & Moilanen 1991). Both graminoids (Aerts 1996) and evergreens (Nambiar & Fife 1987) that have active growth of new leaves (a strong sink) at the time of leaf senescence have high resorption efficiency. Comparing different species, all major N and P chemical fractions are broken down to the same extent during autumn senescence (Chapin & Kedrowski 1983). It is therefore unlikely that there is some recalcitrant nutrient fraction that limits resorption efficiency in some species more than in others. Strong winds, water stress, and frosts can reduce resorption efficiency, but leaves typically abscise only after resorption has ceased (Boerner 1985, Chapin & Moilanen 1991). Species with gradual leaf fall may have low resorption efficiencies (del Arco et al. 1991).

There is very little information on nutrient resorption from senescing stems and **roots**. In the few studies of roots, no resorption has been reported (Nambiar 1987, Aerts 1990), again with a distinct exception for the Western Australian *Hakea prostrata* (harsh hakea) from a severely P-impoverished habitat which efficiently mobilizes P from peak concentrations of 2500 μg P g^{-1} root dry mass down to 96 μg P g^{-1} root dry mass (Shane et al. 2004c).

Table 22. N- and P-resorption efficiency of different growth forms (mean values, with number of species in parentheses).

	Resorption efficiency (% of maximum pool)	
Growth form	**N**	**P**
All data	**50 (287)**	**52 (226)**
Evergreen trees and shrubs	**47 (108)**	**51 (88)**
Deciduous trees and shrubs	**54 (115)**	**50 (98)**
Forbs	**41 (33)**	**42 (18)**
Graminoids	**59 (31)**	**72 (22)**

***Source*: Aerts 1996.**
***Note*: Results are mean values, with the number of species in parentheses.**

4.4 Ecosystem Nutrient-Use Efficiency

Our definitions of **nutrient-use efficiency** (NUE) have so far been based on individual plants. The same concept has been applied to ecosystems that are approximately in steady state [i.e., where aboveground production is approximately equal to litterfall (leaves, twigs, small branches, and reproductive parts)]. Ecosystem NUE is the ratio of litterfall mass to litterfall nutrient content (i.e., the inverse of the

Table 23. Ranges of N and P concentrations representing complete and incomplete resorption, which are synonymous with high and low resorption proficiency, respectively.

Resorption proficiency	
Complete resorption	**Incomplete resorption**
Based on nutrient concentrations per unit mass in senescent leaves	
<7 mg N g^{-1} dry weight	>10 mg N g^{-1} dry weight
<0.5 mg P g^{-1} dry weight (deciduous species)	>0.8 mg P g^{-1} dry weight (deciduous species)
<0.4 mg P g^{-1} dry weight (evergreen species)	>0.5 mg P g^{-1} dry weight (evergreen species)
Based on nutrient concentrations per unit area in senescent leaves	
<0.5 μg N mm^{-2} leaf area	>0.75 μg N mm^{-2} leaf area
<0.3 μg P mm^{-2} leaf area	>0.8 μg P mm^{-2} leaf area

***Source*: Killingbeck 1996.**
***Note*: Mass-based P concentrations are segregated between deciduous and evergreen species because of the large difference between these life forms with ability to reduce P in senescing leaves.**

nutrient concentration of litterfall) (Vitousek 1982). This is equivalent to the biomass produced per unit of nutrient gained or lost. Defined in this way, ecosystem NUE is generally greater in sites with low availability of nutrients (particularly for N, which is the element that most strongly limits productivity in most terrestrial ecosystems in young landscapes). The data for ecosystem NUE, however, must be interpreted with care: NUE and nutrient concentration in the litter are inversely and negatively correlated and are not independent. All else being equal, a high nutrient concentration of the litter (i.e., a low dry mass: N ratio) is associated with a high N loss in litterfall.

The three processes that might cause differences in ecosystem NUE are

(1) photosynthesis per unit nutrient (PNUE)
(2) mean residence time (MRT) during which the nutrient contributed to production
(3) proportion of nutrients resorbed prior to senescence

PNUE is low in slow-growing plants from low N environments (Fig. 2A.34, Sect. 6 of Chapter 2A on photosynthesis; Reich et al. 1992, 1995). This is offset to an unknown extent by greater mean residence time of N in infertile sites (Westoby et al. 2002). Resorption is similar across sites or slightly higher in infertile sites (Sect. 4.3). Although these patterns are well documented at the scale of individual plants or leaves, we have insufficient information to quantify their net effect on NUE at the ecosystem scale. It is therefore currently impossible to provide an independent confirmation from physiological measurements of Vitousek's (1982) conclusion that NUE is greatest in infertile sites. Current uncertainties include (1) the effect of herbivory on nutrient loss (which is greater in fertile sites and removes nutrient-rich tissues, leading to an over-estimate of NUE in fertile sites), (2) leaching losses (which are generally similar between fertile and infertile sites), and (3) the omission of below-ground dynamics, for which few data are available.

In summary, plants vary in their capacity for nutrient uptake and efficiency of nutrient use. Genetic adaptation and acclimation, however, vary in their relative importance to different processes. Acclimation is probably the major factor that accounts for the high root mass ratio in infertile sites. Due to low availability, rates of nutrient acquisition are low for plants in infertile sites. These plants generally have low leaf N concentrations and a low photosynthetic N-use efficiency (Table 22), due primarily to effects of environment on tissue concentration and to both genetic and phenotypic differences in PNUE. Plants on infertile sites generally keep their nutrients for a longer period; for example, the mean residence time of nutrients is higher for evergreens than it is for deciduous leaves, and any given species retains its leaves longer on infertile sites. Plants also differ in the extent to which they withdraw nutrients from senescing leaves, but the variation in the extent to which nutrients are withdrawn shows a less consistent difference between fertile and infertile sites. The high ecosystem NUE in infertile sites reflects low tissue N concentrations and high mean residence time.

5. Mineral Nutrition: A Vast Array of Adaptations and Acclimations

Nutrients move in the soil to root surfaces by mass flow and diffusion, but selective systems (channels, carriers) are then needed to transport the nutrients into the symplast. Because anion transport mostly occurs up an electrochemical potential gradient, metabolic energy is required to import these nutrients from the rhizosphere. Although cation transport may occur down an electrochemical potential gradient, metabolic energy is also required to import these nutrients from the rhizosphere, because the maintenance of the electrochemical potential gradient requires ATP. When essential nutrients move too slowly to the roots' surface, adaptive mechanisms are required, especially for the acquisition of P, Fe, and Zn.

Species have adapted to adverse or favorable soil conditions, and individual plants have some capacity to acclimate to a range of soil conditions. Some of these acclimations are physiological (e.g., an induction of ion-uptake systems when nutrients are in short supply, and excretion of phosphate-hydrolyzing enzymes). Others are anatomical (e.g., the formation of more or longer root hairs when P_i is in short supply), or morphological (e.g., the increase in root mass ratio when N is limiting for growth). These anatomical and morphological acclimations also have a physiological basis, however, and often require induction of specific genes, after a shortage of nutrients has been sensed.

Plants need many macronutrients and micronutrients, but the concentration of the various elements in plant tissues does not necessarily give us a correct estimate of a plant's requirements. Rather, elements may accumulate because the plant lacks mechanisms to keep these out and stores these elements in compartments where they are least harmful. In this chapter, we have encountered numerous species that occupy sites that are practically inaccessible to others. These adapted plants include halophytes, metallophytes, calcifuges, and calcicoles. Halophytes and metallophytes do not need high concentrations of NaCl and heavy metals, respectively, for maximum growth, but they are among the few species that can cope with such adverse soil conditions; that is, their ecological amplitude is much narrower than their physiological amplitude. Calcifuges are largely restricted to acid soils, because they lack the capacity to acquire some nutrients from alkaline soils. On the other hand, calcicoles are restricted to alkaline soils, because they are adversely affected by toxic compounds in acid soils (Al). Understanding plant distribution as dependent on soil type clearly requires an appreciation for a breadth of physiological mechanisms.

Plants differ in the mechanisms employed to acquire nutrients from various soils, as well as in the requirement for these nutrients and in their long-term nutrient-use efficiency. Plants from nutrient-rich sites tend to produce more biomass per unit nutrient in the plant, whereas plants from nutrient-poor sites tend to keep the nutrients they have acquired for a longer time. There is less variation among species in the extent to which they resorb nutrients from senescing leaves, but some species from severely nutrient-impoverished habitats show remarkable resorption proficiency. Variation in nutrient availability sometimes influences resorption (i.e., a smaller proportion of the N invested in leaf mass tends to be remobilized on N-rich sites than on N-poor sites).

Knowledge of a plant's mineral nutrition is pivotal to understanding the distribution of plant species and the high diversity of plant species in nutrient-impoverished soils. It is also essential for modern agriculture and forestry (e.g., to avoid nutrient deficiency disorders or to breed for plants that can acquire nutrients from soils of low nutrient availability). It is also important to resolve environmental problems (e.g., through phytoremediation). Mixed cultures and crop rotations can be highly beneficial in cropping situations. Intercrop species (i.e., plants that are used because of their favorable effect on the actual crop that is of agronomic interest), can be selected on the basis of ecophysiological information presented in this chapter. For example, if the intercrop plant solubilizes Fe or rock phosphate that becomes available to the crop, then it might prevent chlorosis or reduce the need for phosphate fertilization, respectively. This chapter should inspire us to think of traits that might be exploited in future agriculture.

References

Adams, M.A. & Pate, J.S. 1992. Availability of organic and inorganic forms of phosphorus to lupins (*Lupinus* spp.). *Plant Soil* **145:** 107–113.

Aerts, R. 1990. Nutrient use efficiency in evergreen and deciduous species from heathlands. *Oecologia* **84:** 391–397.

Aerts, R. 1996. Nutrient resorption from senescing leaves of perennials: are there general patterns? *J. Ecol.* **84:** 597–608.

Aerts R, & Chapin III, F.S. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv. Ecol. Res.* **30**: 1–67.

Albuzzio, A. & Ferrari, G. 1989. Modulation of the molecular size of humic substances by organic acids of the root exudates. *Plant Soil* **113:** 237–241.

Al-Hiyaly, S.A.K., McNeilly, T., & Bradshaw, A.D. 1990. Theeffect of zinc contamination from electricity pylons. Contrasting patterns of evolution in five grass species. *New Phytol.* **114:** 183–190.

Anderson, C.W.N., Brooks, R.R., Stewart, R.B., & Simcock, R. 1998. Harvesting a crop of gold in plants. *Nature* **395:** 553–554.

Andrews, M. 1986. The partitioning of nitrate assimilation between root and shoot of higher plants. *Plant Cell Environ.* **9:** 511–519.

Apse, M.P. & Blumwald, E. 2007. Na^+ transport in plants. *FEBS Lett.* **581:** 2247–2254.

Arianoutsou, M., Rundel, P.W., & Berry, W.L. 1993. Serpentine endemics as biological indicators of soil elemental concentrations. In: Plants as biomonitors, B. Markert (ed). VCH Weinheim, New York, pp. 179–189.

Aslam, M., Travis, R.L., & Rains, D.W. 1996. Evidence for substrate induction of a nitrate efflux system in barley roots. *Plant Physiol.* **112:** 1167–1175.

Assunção, A.G.L., Schat, H., & Aarts, M. 2003. *Thlaspi caerulescens*, an attractive an attractive model species to study heavy metal hyperaccumulation in plants. *New Phytol.* **159:** 351–360.

Atkin, O.K. 1996. Reassessing the nitrogen relations of arctic plants: a mini-review. *Plant Cell Environ.* **19:** 695–704.

Baker, A.J.M., McGratch, S.P., Reeves, R.D., & Smith, J.A.C. 2000. Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soil. In: Phytoremediation of contaminated soil and water, N. Terry & G.S. Banuelos (eds). CRC Press Inc., Boca Raton, pp. 85–107.

Ball, M.C. 1988. Ecophysiology of mangroves. *Trees* **2:** 129–142.

Barber, S.A. 1995. Soil nutrient bioavailability, 2nd edition. Wiley, New York.

Barber, S.A. & Ozanne, O.G. 1970. Audioradiographic evidence for the differential effect of four plant species in altering the calcium content of the rhizosphere soil. *Soil Sci. Soc. Am. Proc.* **34:** 635–637.

Barkla, B.J., Zingarelli, L., Blumwald, E., Smith, A.C. 1995. Tonoplast Na^+/H^+ antiport activity and its energization by the vacuolar H^+-ATPase in the halophytic plant *Mesembryanthemum crystallinum*. *Plant Physiol.* **109:** 549–556.

Barrow, N.J. 1984. Modeling the effect of pH on phosphate sorption by soils. *J. Soil Sci.* **35:** 283–297

Bates, T.R. & Lynch, J.P. 1996. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. *Plant Cell Environ.* **19:** 529–538.

Bell, R.W. 1997. Diagnosis and prediction of boron deficiency for plant production. *Plant Soil* **193**: 149–168.

Berendse, F. & Aerts, R. 1987. Nitrogen-use efficiency: a biologically meaningful definition? *Funct. Ecol.* **1:** 293–296.

Bhat, K.K.S. & Nye, P.H. 1973 Diffusion of phosphate to plant roots in soil. I. Quantitative autoradiography of the depletion zone. *Plant Soil* **38:** 161–175.

Biddulph, O., Cory, R. & Biddulph, S. 1956. The absorption and translocation of sulfur in red kidney bean. *Plant Physiol.* **33:** 293–300.

Blom-Zandstra, M., Vogelzang, S., & Veen, B. 1998. Sodium fluxes in sweet pepper exposed to varying sodium concentrations. *J. Exp. Bot.* **49**, 1863–1868.

Bloom, A.J., Sukrapanna, S.S., & Warner, R.L. 1992. Root respiration associated with ammonium and nitrate absorption and assimilation by barley. *Plant Physiol.* **99:** 1294–1301.

Boerner, R.E.J. 1985. Foliar nutrient dynamics, growth, and nutrient use efficiency of *Hamamelis virginiana* in three forest microsites. *Can. J. Bot.* **63:** 1476–1481.

Bolan, N.S., Hedley, M.J., & White, R.E. 1991. Processes of soil acidification during nitrogen cycling with emphasis on legume based pastures. *Plant Soil* **134:** 53–63.

Boyd, R.S. & Martens, S.N. 1998. Nickel hyperaccumulation by *Thlaspi montanum* var. *montanum* (Brassicaceae): a constitutive trait. *Am. J. Bot.* **85:** 259–265.

Britto, D.T. & Kronzucker, H.J. 2005. Nitrogen acquisition, PEP carboxylase, and cellular pH homeostasis: new views on old paradigms. *Plant Cell Environ.* **28:** 1396–1409.

Britto, D.T. & Kronzucker, H.J. 2006. Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. *Trends Plant Sci.* **11:** 529–534.

Broadley, M.R., Bowen, H. C., Cotterill, H.L., Hammond, J.P., Meacham, M.C., Mead, A., & White, P.J. 2003. Variation in the shoot calcium content of angiosperms. *J. Exp. Bot.* **54:** 1431–1446.

Brooks, R.R. (ed.) 1998. Plants that hyperaccumulate heavy metals. Their role in phytoremediation, microbiology, archaeology, mineral exploitation and phytomining. CAB International, Wallingford.

Brooks, R.R., Lee, J., Reeves, R.D. &, Jaffrré, T. 1977. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochem. Explor.* **7**: 49–57.

Brooks, R.R., Chambers, M.F., Nicks, L.J., & Robinson, B.H. 1998. Phytomining. *Trends Plant Sci.* **3:** 359–362.

Brouwer, R. 1962. Nutritive influences on the distribution of dry matter in the plant. *Neth. J. Agric. Sci.* **10:** 399–408.

Brown, G. & Brinkmann, K 1992. Heavy metal tolerance in *Festuca ovina* L. from contaminated sites in the Eifel Mountains, Germany. *Plant Soil* **143:** 239–247.

Brown, G., Mitchell, D.T., & Stock, W.D. 1984. Atmospheric deposition of phosphorus in a coastal fynbos ecosystem if the south-western Cape, South Africa. *J. Ecol.* **72:** 547–551.

Brune, A., Urbach, W., Dietz, K.-J. 1994. Compartmentation and transport of zinc in barley primary leaves as basic mechanisms involved in zinc tolerance. *Plant Cell Environ.* **17:** 153–162.

Bucher, M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytol.* **173:** 11–26

Burgess, S.S.O. & Dawson, T.E. 2004. The contribution of fog to the water relations of *Sequoia sempervirens*

(D. Don): foliar uptake and prevention of dehydration. *Plant Cell Environ.* **27:** 1023–1034.

Cakmak, I., Sari, N., Marschner, H., Ekiz, H., Kalayci, M., Yilmaz, A., & Braun, H.J. 1996. Phytosiderophore release in bread wheat genotypes differing in zinc efficiency. *Plant Soil* **180:** 183–189.

Callahan, D.L., Baker, A.J.M., Kolev, S.D., & Wedd, A.G. 2005. Metal ion ligands in hyperaccumulating plants. *J. Biol. Inorg. Chem.* **11:** 2–12.

Campbell, W.H. 1996. Nitrate reductase biochemistry comes of age. *Plant Physiol.* **111:** 355–361.

Casimiro, I., Beeckman, T., Graham, N., Bhalerao, R., Zhang, H., Casero, P., Sandberg, G., & Bennett, M.J. 2003. Dissecting *Arabidopsis* lateral root development. *Trends Plant Sci.* **8:** 165–171.

Chang, Y.-C., Ma, J.F., & Matsumoto, H. 1998. Mechanism of Al-induced iron chlorosis in wheat (*Triticum aestivum*). Al-inhibited biosynthesis and secretion of phytosiderophores. *Physiol. Plant.* **102:** 9–15.

Chaney, R.L, Malik, M., Li, Y.M., Brown, S.L, Angle, J.S., & Baker A.J.M. 1997. Phytoremediation of soil metals. *Curr. Opin. Biotech.* **8:** 279–284.

Chapin III, F.S. 1974. Morphological and physiological mechanisms of temperature compensation in phosphate absorption along a latitudinal gradient. *Ecology* **55:** 1180–1198.

Chapin III, F.S. 1980. The mineral nutrition of wild plants. *Annu. Rev. Ecol. Syst.* **11:** 233–260.

Chapin III, F.S. 1988. Ecological aspects of plant mineral nutrition. *Adv. Min. Nutr.* **3:** 161–191.

Chapin III, F.S. 1991. Effects of multiple environmental stresses on nutrient availability and use. In: Response of plants to multiple stresses, H.A. Mooney, W.E. Winner, & E.J. Pell (eds). Academic Press, San Diego, pp. 67–88.

Chapin III, F.S. & Bloom, A. 1976. Phosphate absorption: adaptation of tundra graminoids to a low temperature, low phosphorus environment. *Oikos* **26:** 111–121.

Chapin III, F.S. & Kedrowski, R.A. 1983. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. *Ecology* **64:** 376–391.

Chapin III, F.S. & Moilanen, L. 1991. Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. *Ecology* **72:** 709–715.

Chapin III, F.S. & Slack, M. 1979. Effect of defoliation upon root growth, phosphate absorption, and respiration in nutrient-limited tundra graminoids. *Oecologia* **42:** 67–79.

Chapin III, F.S., Johnson, D.A., & McKendrick, J.D. l980. Seasonal movement of nutrients in plants of differing growth form in an Alaskan tundra ecosystem: Implications for herbivory. *J. Ecol.* **68:** 189-209.

Chapin III, F.S., Fetcher, N., Kielland, K., Everett, K.R., & Linkins, A.E. 1988. Productivity and nutrient cycling of Alaskan tundra: enhancement by flowing soil water. *Ecology* **69:** 693–702.

Chapin III, F.S., Moilanen, L., & Kielland, K. 1993. Preferential use of organic nitrogen for growth by non-mycorrhizal arctic sedge. *Nature* **361:** 150–153.

Chardonnens, A.N., Koevoets, P.L.M., Van Zanten, A., Schat, H., & Verkleij, J.A.C. 1999. Properties of enhanced tonoplast zinc transport in naturally selected zinc-tolerant *Silene vulgaris*. *Plant Physiol.* **120:** 779–785.

Cheeseman, J.M. 1988. Mechanisms of salinity tolerance in plants. *Plant Physiol.* **87:** 547–550.

Cheeseman, J.M. & Hanson, J.B. 1979. Energy-linked potassium influx as related to cell potential in corn roots. *Plant Physiol.* **64:** 842–845.

Cheng, W. & Johnson, D.W. 1998. Elevated CO2, rhizosphere processes, and soil organic matter decomposition. *Plant Soil* **202:** 167–174.

Chiou, T.-J. 2007. The role of microRNAs in sensing nutrient stress. *Plant Cell Environ.***30:** 323–332.

Clarkson, D.T. 1981. Nutrient interception and transport by root systems. In: Physiological factors limiting plant productivity, C.B. Johnson (ed). Butterworths, London, pp. 307–314.

Clarkson, D.T. 1996. Root structure and sites of ion uptake. In: Plant roots: the hidden half, 3rd edition, Y. Waisel, A. Eshel, & U. Kafkaki (eds). Marcel Dekker, Inc., New York, pp. 483–510.

Clarkson, D.T., Lüttge, U., & Kuiper, P.J.C. 1986. Mineral nutrition: sources of nutrients for land plants from outside the pedosphere. *Progr. Bot.* **48:** 80–96.

Clemens, S. 2001. Molecular mechanisms of plant metal tolerance and homeostasis. *Planta* **212:** 475–486.

Clemens, S., Palmgren, M.G., Kramer, U. 2002. A long way ahead: understanding and engineering plant metal accumulation. *Trends Plant Sci.* **7**, 309–315.

Clement, C.R., Hopper, M.J., Jones, L.H.P., & Leafe, E.L. 1978. The uptake of nitrate by *Lolium perenne* from flowing nutrient solution. II. Effect of light, defoliation, and relationship to CO_2 flux. *J. Exp. Bot.* **29:** 1173–1183.

Clijsters, H. & Van Assche, F. 1985. Inhibition of photosynthesis by heavy metals. *Photosynth. Res.* **7:** 31–40.

Davenport, R.J. & Tester, M. 2000. A weakly voltage-dependent, nonselective cation channel mediates toxic sodium influx in wheat. *Plant Physiol.* **122:** 823–834.

Davenport, R.J., Muñoz-Mayor, A., Jha, D., Essah, P.A., Rus, A., & Tester, M. 2007. The Na^+ transporter AtHKT1; 1 controls retrieval of Na^+ from the xylem in *Arabidopsis*. *Plant Cell Environ.* **30:** 497–507.

De Boer, A.H. 1985. Xylem/symplast ion exchange: Mechanism and function in salt-tolerance and growth. PhD Thesis, University of Groningen, Groningen, the Netherlands.

De Boer, A.H. & Wegner, L.H. 1997. Regulatory mechanisms of ion channels in xylem parenchyma cells. *J. Exp. Bot.* **48:** 441–449.

Degenhardt, J., Larsen, P.B., Howell, S.H., & Kochian, L.V. 1998. Aluminum resistance in the *Arabidopsis* mutant *alr*-104 is caused by an aluminum-induced increase in rhizosphere pH. *Plant Physiol.* **117:** 19–27.

Deiana, S., Gessa, C., Manunza, B., Marchetti, M., & Usai, M. 1992. Mechanism and stoichiometry of the redox reaction between iron (III) and caffeic acid. *Plant Soil* **145:** 287–294.

del Arco, J.M., Escudero, A., & Garrido, M.V. 1991. Effects of site characteristics on nitrogen retranslocation from senescing leaves. *Ecology* **72:** 701–708.

Delhaize, E. & Ryan, P.R. 1995. Aluminum toxicity and tolerance in plants. *Plant Physiol.* **107:** 315–321.

Delhaize, E., Ryan, P.R., & Randall, P.J. 1993. Aluminum tolerance in wheat (*Triticum aestivum* L.). II. Aluminum-stimulated excretion of malic acid from root apices. *Plant Physiol.* **103:** 695–702.

Delhaize, E., Gruber, B.D., & Ryan P.R. 2007. The roles of organic anion permeases in aluminium resistance and mineral nutrition. *FEBS Lett.* **581:** 2255–2262.

Demars, B.G. & Boerner, R.E.J. 1997. Foliar nutrient dynamics and resorption in naturalized *Lonicera maackii* (Caprifoliaceae) populations in Ohio, USA. *Am. J. Bot.* **84:** 112–117.

Demidchik, V., Davenport, R.J. & Tester, M. 2002. Nonselective cation channels in plants. *Annu. Rev. Plant Biol.* **53:** 67–107.

Denton, M.D., Veneklaas, E.J., Freimoser, F.M., & Lambers, H. 2007. *Banksia* species (Proteaceae) from severely phosphorus-impoverished soils exhibit extreme efficiency in the use and re-mobilisation of phosphorus. *Plant Cell Environ.* **30:** 1557–1565.

De Silva, D.L.R., Hetherington, A.M., & Mansfield, T.A. 1996. Where does all the calcium go? Evidence of an important regulatory role for trichomes in two calcicoles. *Plant Cell. Environ.* **19:** 880–886.

Diaz, S.A., Grime, J.P, Harris, J., & McPherson, E. 1993. Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* **364:** 616–617.

Dong, B., Ryan, P.R., Rengel, Z., & Delhaize, E. 1999. Phosphate uptake in *Arabidopsis thaliana*: dependence of uptake on the expression of transporter genes and internal phosphate concentration. *Plant Cell Environ.* **22:** 1455–1461.

Drew, M.C. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytol.* **75:** 479–490.

Drew, M.C., Saker, L.R., & Ashley, T.W. 1973. Nutrient supply and the growth of the seminal root system in barley. I. The effect of nitrate concentration on the growth of axes and laterals. *J. Exp. Bot.* **24:** 1189–1202.

Driscoll, C.T., Lawrence, G.B., Bulger, A.J., Butler, T.J., Cronan, C.S., Eagar, C., Lambert, K.F., Likens, G.E., Stoddard, J.L. & Weathers. K.C. 2001. Acidic deposition in the northeastern United States: sources and inputs, ecosystem effects and management strategies. *BioSci.* **51**: 180–198.

Duffus, J.H. 2002. "Heavy metals"—a meaningless term? *Pure Appl. Chem.* **74:** 793–807.

Eckstein, R.L., Karlsson, P.S., & Weih, M. 1999. Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. *New Phytol.* **143:** 177–189.

Epstein, E. & Hagen, C.E. 1952.A kinetic study of the absorption of alkali cations by barley roots. *Plant Physiol.* **27:** 457–474.

Erskine, P.D., Stewart, G.R., Schmidt, S., Turnbull, M.H., Unkovich, M.H., & Pate, J.S. 1996. Water availability—a physiological constraint on nitrate utilization in plants of Australian semi-arid mulga woodlands. *Plant Cell Environ.* **19:** 1149–1159.

Esau, K. 1977. Anatomy of seed plants, 2nd edition. John Wiley & Sons, New York.

Eviner, V.T. & Chapin III, F.S. 1997. Plant-microbial interactions. *Nature* **385:** 26–27.

Föhse, D., Claassen, N., & Jungk, A. 1991. Phosphorus efficiency of plants. *Plant Soil* **132:** 261–272.

Forde, B.G. 2002. Local and long-range signaling pathways regulating plant responses to nitrate. *Annu. Rev. Plant Biol.* **53:** 203–224.

Foulds W. 1993. Nutrient Concentrations of foliage and soil in south-western Australia. *New Phytol.* **125:** 529–546.

Franken, B., Blijjenberg, J., & De Kroon, H. 1999. Root morphological and physiological plasticity of perennial grass species and the exploitation of spatial and temporal heterogeneous nutrient patches. *Plant Soil* **211:** 179–189.

Frey, B., Keller, C., Zierold, K., & Schulin, R. 2000. Distribution of Zn in functionally different leaf epidermal cells of the hyperaccumulator *Thlaspi caerulescens*. *Plant Cell Environ.* **23:** 675–687.

Furukawa, J., Yamaji, N., Wang, H., Mitani, N., Murata Y., Sato, K., Katsuhara, M., Takeda, K., & Ma, J.F. 2007. An aluminum-activated citrate transporter in barley. *Plant Cell Physiol.* **48:** 1081–1091.

Gahoonia, T.S. & Nielsen, N.E. 2004. Barley genotypes with long root hairs sustain high grain yields in low-P field. *Plant Soil* **262:** 55–62.

Garnett, T.V. & Smethurst, P.J. 1999. Ammonium and nitrate uptake by *Eucalyptus nitens*: effects of pH and temperature. *Plant Soil* **214:** 133–140.

Gerendás J. & Schurr, U. 1999. Physicochemical aspects of ion relations and pH regulation in plants—a quantitative approach. *J. Exp. Bot.* **50:** 1101–1114.

Gersani, M. & Sachs, T. 1992. Development correlations between roots in heterogeneous environments. *Plant Cell Environ.* **15:** 463–469.

Gilbert, G.A., Allan, D.A., & Vance, C.P. 1998. Phosphorus deficiency in white lupin alters root development and metabolism.In: Radical biology: advances and perspectives in the function of plant roots, H.E. Flores, J.P. Lynch, & D.M. Eissenstat (eds). Current topics in plant physiology, Vol. 17. American Society of Plant Physiology, Rockville, MD, pp. 92–103.

Godbold, D.L., Horst, W.J., Marschner, H., & Collins, J.C. 1983. Effect of high zinc concentrations on root growth and zinc uptake in two ecotypes of *Deschampsia caespitosa* differing in zinc tolerance. In: Root ecology and its practical application, W. Böhm, L. Kutschera, & E. Lichtentegger (eds). Bundesanstalt für alpenländische Landwirtscaft, Gumpenstein, pp. 165–172.

Gressel, N. & McColl, J.G. 1997. Phosphorus mineralization and organic matter decomposition: A critical review. In: Driven by nature: plant litter quality and decomposition, G. Cadisch & K.E. Giller (eds). CAB International, Wallingford.

Güsewell, S. 2004. N:P ratios in terrestrial plants: variation and functional significance. *New Phytol.* **164:** 243–266.

Gutierrez, F.R. & Whitford, W.G. 1987. Chihuahuan desert annuals: importance of water and nitrogen. *Ecology* **68:** 2032–2045.

Hairiah, K., Stulen, I., & Kuiper, P.J.C. 1990. Aluminium tolerance of the velvet beans *Mucuna pruriens* var. *utilis* and *M. deeringiana*. I. Effects of aluminium on growth and mineral composition. In: Plant nutrition—physiology and applications, M.L. Van Beusichem (ed). Kluwer Academic Publishers, Dordrecht, pp. 365–374.

Hall, J.L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.* **53:** 1–11.

Harper, S.M., Edwards, D.G., Kerven, G.L., & Asher, C.J. 1995. Effects of organic acid fractions extracted from *Eucalyptus camaldulensis* leaves on root elongation of maize (*Zea mays*) in the presence and absence of aluminium. *Plant Soil* **171:** 189–192.

Hayes, J.E., Simpson, R.J., & Richardson, A.E. 2000. The growth and phosphorus utilisation of plants in sterile media when supplied with inositol hexaphosphate, glucose 1-phosphate or inorganic phosphate. *Plant Soil* **220:** 165–174.

Hedin, L.O. 2004. Global organization of terrestrial plant-nutrient interactions. *Proc. Natl. Acad. Sci. USA* **101:** 10849–10850.

Hedin, L.O., Granat, L., Likens, G.E., Buishand, A., Galloway, J.N., Butler, T.J., & Rodhe, H. 1994. Steep declines in atmospheric base cations in regions of Europe and North America. *Nature* **367:** 351–354.

Henry, H.A.L. & Jefferies, R.L. 2003. Plant amino acid uptake, soluble N turnover and microbial N capture in soils of a grazed arctic salt marsh. *J. Ecol* **91:** 627–636.

Higginbotham, N., Etherton, B., & Foster, R.J. 1967. Mineral ion contents and cell transmembrane electropotentials of pea and oat seedling tissue. *Plant Physiol.* **42:** 37–46.

Hinsinger, P. 1998. How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. *Adv. Agron.* **64:** 225–265.

Hinsinger, P., Plassard, C., Tang, C., & Jaillard, B. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. *Plant Soil* **248:** 43–59.

Hodge, A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol.* **162:** 9–24.

Hodge, A., Robinson, D., Griffiths, B.S., & Fitter, A.H. 1999. Why plants bother: Root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. *Plant Cell Environ.* **22:** 811–820.

Hoffland, E., Findenegg, G.R., & Nelemans, J.A. 1989. Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to P-starvation. *Plant Soil* **113:** 161–165.

Huang, C.X. & Van Steveninck, R.F.M. 1989. Maintenance of low Cl-concentrations in mesophyll cells of leaf blades of barley seedlings exposed to salt stress. *Plant Physiol.* **90:** 1440–1443.

Huang, N.-C., Chiang, C.-S., Crawford, N.M., & Tsay, Y.F. 1996. *Chl1* encodes a component of the low-affinity nitrate uptake system in *Arabidopsis* and shows cell type-specific expression in roots. *Plant Cell* **8:** 2183–2191.

Hübel, F. & Beck, F. 1993. In-situ determination of the P-relations around the primary root of maize with respect to inorganic and phytate-P. *Plant Soil* **157:** 1–9.

Ingestad, T. 1979. Nitrogen stress in birch seedlings II. N, P, Ca and Mg nutrition. *Physiol. Plant.* **52:** 454–466.

Jenny, H. 1980. The soil resources. Origin and behavior. Springer-Verlag, New York.

Johnson, M.N, Reynolds, R.C., & Likens, G.E. 1972. Atmospheric sulfur: Its effect on the chemical weathering of New England. *Science* **177:** 514–515.

Johnson, A.H., Frizano, J., & Vann, D.R. 2003. Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. *Oecologia* **135:** 487–499.

Jones, D.L. 1998. Organic acids in the rhizosphere—a critical review. *Plant Soil* **205:** 25–44.

Jones, D.L., Darrah, P.R., & Kochian, L.V. 1996a. Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in iron uptake. *Plant Soil* **180:** 57–66.

Jones, D.L., Prabowo, A.M., & Kochian, L.V. 1996b. Kinetics of malate transport and decomposition in acid soils and isolated bacterial populations: The effects of microorganisms on root exudation of malate under Al stress. *Plant Soil* **182:** 239–247.

Jones, D.L., Healey, J.R., Willett, V.B., Farrar, J.F., & Hodge, A. 2005. Dissolved organic nitrogen uptake by plants—an important N uptake pathway? *Soil Biol. Biochem.* **37:** 413–423.

Kaiser, W.M. & Huber, S.C. 2001. Post-translational regulation of nitrate reductase: mechanism, physiological relevance and environmental triggers. *J. Exp. Bot.* **52:** 1981–1989.

Kamh, M., Horst, W.J., Amer, F., Mostafa, H., & Maier, P. 1999. Mobilization of soil and fertilizer phosphate by cover crops. *Plant Soil* **211:** 19–27.

Kamh, M., Abdou, M., Chude, V., Wiesler, F., & Horst, W.J. 2002. Mobilization of phosphorus contributes to positive rotational effects of leguminous cover crops on maize grown on soils from northern Nigeria. *J. Plant Nutr. Soil Sci.* **165:** 566–572.

Keerthisinghe, G., Hocking, P., Ryan, P.R., & Delhaize, E. 1998. Proteoid roots of lupin (*Lupinus albus* L.): Effect of phosphorus supply on formation and spatial variation in citrate efflux and enzyme activity. *Plant Cell Environ.* **21:** 467–478.

Keltjens, W.G. & Tan, K. 1993. Interactions between aluminum, magnesium and calcium with different monocotyledonous and dicotyledonous plant species. *Plant Soil* **155/156:** 485–488.

Kielland, K. 1994. Amino acid absorption by arctic plants: Implications for plant nutrition and nitrogen cycling. *Ecology* **75:** 2373–2383.

Kielland, K., McFarland, J., & Olson, K. 2006. Amino acid uptake in deciduous and coniferous taiga ecosystems. *Plant Soil* **288:** 297–307.

Killingbeck, K.T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology* **77:** 1716–1727.

King, B.J., Siddiqui, N.Y., Ruth, T.J., Warner, R.L., & Glass, A.D.M. 1993. Feedback regulation of nitrate influx in barley roots by nitrate, nitrite, and ammonium. *Plant Physiol.* **102:** 1279–1286.

Kinraide, T.B. 1993. Aluminium enhancement of plant growth in acid rooting media. A case of reciprocal alleviation of toxicity by two toxic cations. *Physiol. Plant.* **88:** 619–625.

Kirk, G.J.D. & Kronzucker, H.J. 2005. The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: a modelling study. *Ann Bot* **96:** 639–646.

Kochian, L. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46:** 237–260.

Kochian, L.V., Piñeros, M.A., & Hoekenga, O.A. 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant Soil* **274:** 175–195.

Krämer, U. **2005.** Phytoremediation: novel approaches to cleaning up polluted soils. *Curr. Opin. Biotechnol.* **16:** 133–141.

Krämer, U., Cotter-Howels, J.D., Charnock, J.M., Baker, A.J.M., & Smith, J.A. 1996. Free histidine as a metal chelator in plants that accumulate nickel. *Nature* **379**: 635–638.

Krämer, U., Smith, R.D., Wenzel, W.W., Raskin, I., & Salt, D.E. 1997. The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesingense* Halacsy. *Plant Physiol.* **115:** 1641–1650.

Krämer, U., Pickering, I.J., Prince, R.C., Raskin, I., & Salt, D.E. 2000. Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. *Plant Physiol.* **122:** 1343–1354.

Krishnamurti, G.S.R., Cieslinnski, G., Huang, P.M., & Van Rees, K.C.J. 1997. Kinetics of cadmium release from soils as influenced by organic acids: Implications in cadmium availability. *J. Environ. Qual.* **26:** 271–277.

Kronzucker, H.J., Siddiqi, M.Y., & Glass, A.D.M. 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. *Nature* **385:** 59–61.

Kronzucker, H.J., Siddiqi, M.Y. Glass, A.D.M., & Kirk, G.J.D. 1999a. Nitrate-ammonium synergism in rice. A subcellular flux analysis. *Plant Physiol.* **119:** 1041–1046.

Kronzucker, H.J., Glass, A.D.M. & Siddiqi, M.Y. 1999b. Inhibition of nitrate uptake by ammonium in barley. Analysis of component fluxes. *Plant Physiol.* **120:** 283–292.

Krupa, Z., Oquist, G., & Huner, N.P.A. 1993. The effect of cadmium on photosynthesis of *Phaseolus vulgaris*—a fluorescence analysis. *Physiol. Plant.* **88:** 626–630.

Lacan, D. & Durand, N. 1994. Na^+ and K^+ transport in excised soybean roots. *Physiol. Plant.* **93:** 132–138.

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J., & Veneklaas, E.J. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Ann. Bot.* **98:** 693–713.

Lambers, H., Shaver, G., Raven, J.A., & Smith, S.E. 2008. N- and P-acquisition change as soils age. *Trends Ecol. Evol.*, **23:** 95–103.

Larsen, P.B., Degenhardt, J., Tai, C.-Y., Stenzler, L.M., Howell, S.H., & Kochian, L.V. 1998. Aluminum resistance *Arabidopsis* mutants that exhibit altered patterns of aluminum accumulation and organic acid release from roots. *Plant Physiol.* **117:** 7–18.

Lasat, M.M., Baker, A.J.M., & Kochian, L.V. 1996. Physiological characterization of root Zn^{2+} absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. *Plant Physiol.* **112:** 1715–1722.

Lata, J.-C., Degrange, V., Raynaud, X., Maron, P.-A., Lensi, R., & Abbadie, L. 2004. Grass populations control nitrification in savanna soils. *Funct. Ecol.* **18:** 605–611.

LeNoble, M.E. Blevins, D.G., Sharp, R.E., & Cumbie, B.G. 1996a. Prevention of aluminium toxicity with supplemental boron. I. Maintenance of root elongation and cellular structure. *Plant Cell Environ.* **19:** 1132–1142.

LeNoble, M.E. Blevins, D.G., & Miles, R.J. 1996b. Prevention of aluminium toxicity with supplemental boron. II. Stimulation of root growth in an acidic, high-aluminium subsoil. *Plant Cell Environ.* **19:** 1143–1148.

Li, L., Li, S.-M., Sun, J.-H., Zhou, L.-L., Bao, X.-G., Zhang, H.-G., & Zhang, F.-S. 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. *Proc. Natl. Acad. Sci. USA* **104:** 11192–11196.

Lipson, D. & Näsholm, T. 2001. The unexpected versatility of plants: Organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* **128:** 305–316.

Liu, K.-H. & Tsay, Y.-F. 2003. Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. *EMBO J.* **22:** 1005– 1013.

Lodhi, M.A.K. & Killingbeck, K.T. 1980. Allelopathic inhibition of nitrification and nitrifying bacteria in a ponderosa pine (*Pinus ponderosa* Dougl.) community. *Am. J. Bot.* **67:** 1423– 1429.

Lolkema, P.C., Doornhof, M., & Ernst, W.H.O. 1986. Interaction between a copper-tolerant and a copper-sensitive population of *Silene cucubalus*. *Physiol. Plant.* **67:** 654–658.

Loneragan, J.F. 1968. Nutrient requirements of plants. *Nature* **220:** 1307–1308.

Loveless, A.R. 1961. A nutritional interpretation of sclerophylly based on differences in chemical composition of sclerophyllous and mesophytic leaves. *Ann. Bot.* **25:** 168–184.

Ma, J.F. 2000. Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiol.* **41:** 383–390.

Ma, J.F. 2005. Plant root responses to three abundant soil minerals: silicon, aluminum and iron. *Crit. Rev. Plant Sci.* **24:** 267–281.

Ma, J.F., Hiradata, S., Nomoto, K., Iwashita, T., & Matsumoto, H. 1997. Internal detoxification mechanisms of Al in hydrangea. Identification of Al forms in the leaves. *Plant Physiol.* **113:** 1033–1039.

Ma, J.F., Hiradata, S., & Matsumoto, H. 1998. High aluminum resistance in buckwheat. II. Oxalic acid detoxifies aluminum internally. *Plant Physiol.* **117:** 753–759.

Ma, J.F., Ryan, P.R., & Delhaize, E. 2001a. Aluminium tolerance in plants and the complexing role of organic acids. *Trends Plant Sci.* **6:** 273–278

Ma, J.F., Goto, S., Tamai, K., & Ichii, M. 2001b. Role of root hairs and lateral roots in silicon uptake by rice. *Plant Physiol.* **127:** 1773–1780.

Ma, L., Komar, K.M., Tu, C., Zhang, W., Cai, Y., & Kennelley E.D. 2001c. A fern that hyperaccumulating arsenic. *Nature* **409:** 579.

Ma, J.F., Ueno, H., Ueno, D., Rombola, A.D., Iwashita, T. 2003. Characterization of phytosiderophore secretion under Fe deficiency stress in *Festuca rubra*. *Plant Soil* **256:** 131–137.

Ma, J.F., Nagao, S., Huang, C.F., & Nishimura, M. 2005. Isolation and characterization of a rice mutant hypersensitive to Al. *Plant Cell Physiol.* **46:** 1054–1061.

Ma, J.F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y., Yano, M. 2006. A silicon transporter in rice. *Nature* **440:** 688–691.

Ma, J.F., Yamaji, N., Mitani, N., Tamai, K., Konishi, S., Fujiwara, T., Katsuhara, M., Yano, M. 2007. An efflux transporter of silicon in rice. *Nature* **448:** 209–212.

Macduff, J.H., Hopper, M.J., & Wild, A. 1987. The effect of root temperature on growth and uptake of ammonium and nitrate by *Brassica napus* L. cv. bien venu in flowing solution culture: II. uptake from solutions containing NH_4NO_3. *J. Exp. Bot.* **38:** 53–66.

Macklon, A.E.S., Mackie-Dawson, L.A., Sim, A., Shand, C.A., & Lilly, A. 1994. Soil P resources, plant growth and rooting characteristics in nutrient poor upland grasslands. *Plant Soil* **163:** 257–266.

Macfie, S.M. & Taylor, G.J. 1992. The effect of excess manganese on photosynthetic rate and concentration of chlorophyll in *Triticum aestivum* grown in solution culture. *Physiol. Plant.* **85:** 467–475.

Magalhaes, J.V., Liu, J., Guimaraes, C.T, Lana, U.G.P., Alves, V.M.C., Wang, Y.-H., Schaffert, R.E., Hoekenga, O.A., Pineros, M.A., Shaff, J.E., Klein, P.E., Carneiro, N.P., Coelho, C.M., Trick, H.N., & Kochian, L.V. 2007. A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nature Genetics* **39:** 1156–1161.

Marcum, K.B. & Pessarakli, M. 2006. Salinity tolerance and salt gland excretion efficiency of bermudagrass turf cultivars. *Crop Sci.* **46:** 2571–2574.

Marschner, H. 1983. General introduction to the mineral nutrition of plants. In: Encyclopedia of plant physiology, N.S., Vol 15A, A. Läuchli & R.L. Bieleski (eds). Springer-Verlag, Berlin, pp. 5-60.

Marschner, H. 1991a. Root-induced changes in the availability of micronutrients in the rhizosphere. In: Plant roots: the hidden half, 3rd edition, Y. Waisel, A. Eshel, & U. Kafkaki (eds). Marcel Decker, Inc., New York, pp. 503–528.

Marschner, H. & Römheld, V. 1996. Root-induced changes in the availability of micronutrients in the rhizosphere. In: Plant roots: the hidden half, 3rd edition, Y. Waisel, A. Eshel, & U. Kafkaki (eds). Marcel Decker, Inc., New York, pp. 557–580.

Martinoia, E., Heck, U., & Wiemken, A. 1981. Vacuoles as storage compartments for nitrate in barley leaves. *Nature* **289:** 292–294.

McKane, R.B., Johnson, L.C., Shaver, G.R., Nadelhoffer, K.J., Rastetter, E.B., Fry, B., Giblin, A.E., Kielland, K., Kwiatkowski, B.L., Laundre, J.A. & Murray, G. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* **415:** 68–71.

McNaughton, S.J., & Chapin III, F.S. 1985. Effects of phosphorus nutrition and defoliation on C_4 graminoids from the Serengeti Plains. *Ecology* **66:** 1617–1629.

McNeilly, T. 1968. Evolution in closely adjacent plant populations III. *Agrostis tenuis* on a small copper mine. *Heredity* **23:** 99–108.

Meerts, P. 1997. Foliar macronutrient concentrations of forest understorey species in relation to Ellenberg's indices and potential relative growth rate. *Plant Soil* **189:** 257–265.

Meharg, A.A. & Macnair, M.R. 1992. Suppression of the high affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L. *J. Exp. Bot.* **43:** 519–524.

Miller, A.J. & Cramer, M.D. 2005. Root nitrogen acquisition and assimilation. *Plant Soil* **274:** 1–36.

Min, X., Siddiqi, M.Y., Guy, R.D., Glass, A.D.M., & Kronzucker, H.J. 1999. A comparative study of fluxes and compartmentation of nitrate and ammonium in early-successional tree species. *Plant Cell Environ.* **22:** 821–830.

Mistrik, I. & Ullrich, C.I. 1996. Mechanism of anion uptake in plant roots: Quantitative evaluation of H^+/NO_3^- and $H^+/H_2PO_4^-$ stoichiometries. *Plant Physiol. Biochem.* **34:** 621–627.

Morikawa, H., Higaki, A., Nohno, M., Takahashi, M., Kamada, M., Nakata, M., Toyohara, G., Okamura, Y., Matsui, K., Kitani, S., Fujita, K., Irifune, K., & Goshima, N. 1998. More than 600-fold variation in nitrogen dioxide assimilation among 217 plant taxa. *Plant Cell Environ.* **21:** 180–190.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.* **25:** 239–250.

Munns, R. 2005. Genes and salt tolerance: bringing them together *New Phytol.* **167:** 645–663.

Murphy, A. & Taiz, L. 1995. Comparison of metallothionein gene expression and nonprotein thiols in ten *Arabidopsis* ecotypes. *Plant Physiol.* **109:** 945–954.

Nair, V.D. & Prenzel, J. 1978. Calculations of equilibrium concentration of mono- and polynuclear hydroxyaluminium species at different pH and total aluminium concentrations. *Z. Pflanzenernähr. Bodenk.* **141:** 741–751.

Nambiar, I.K.S. 1987. Do nutrients retranslocate from fine roots? *Can. J. For. Res.* **17:** 913–918.

Nambiar, I.K.S. & Fife, D.N. 1987. Growth and nutrient retranslocation in needles of radiata pine in relation to nitrogen supply. *Ann. Bot.* **60:** 147–156.

Neumann, G. & Römheld, V. 1999. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. *Plant Soil* **211:** 121–130.

Neumann, G., Massonneau, A., Langlade, N., Dinkelaker, B., Hengeler, C., Römheld, V., & Martinoia, E. 2000. Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (*Lupinus albus* L.) *Ann. Bot.* **85:** 909–919.

Nian, H., Yang, Z.M., Ahn, S.J., Cheng, Z.J., & Matsumoto, H. 2002. A comparative study on the aluminium- and copper-induced organic acid exudation from wheat roots. *Physiol. Plant.* **116:** 328–335.

Niklas, K.J., Owens, T., Reich, P.B., & Cobb, E.D. 2005. Nitrogen/phosphorus leaf stoichiometry and the scaling of plant growth. *Ecol. Lett.* **8:** 636–642.

Nuruzzaman, M., Lambers, H., Bolland, M.D.A., & Veneklaas, E.J. 2005. Phosphorus benefits of different legume crops to subsequent wheat grown in different soils of Western Australia. *Plant Soil* **271:** 175–187.

Nye, P.H. & Tinker, P.B. 1977. Solute movement in the soil-root system. Blackwell, Oxford.

Ohwaki, Y. & Sugahara, K. 1997. Active extrusion of protons and exudation of carboxylic acids in response to iron deficiency by roots of chickpea (*Cicer arietinum*L.). *Plant Soil* **189:** 49–55.

Osaki, M., Yamada, S., Ishizawa, T, Watanabe, T. & Shinano, T. 2003a. Mineral characteristics of leaves of plants from different phylogeny grown in various soil types in the temperate region. *Plant Foods Human Nutr.* **58**: 117–137.

Osaki, M., Yamada, S., Ishizawa, T, Watanabe, T. & Shinano, T. 2003b. Mineral characteristics of the leaves of 166 plant species with different phylogeny in the temperate region. *Plant Foods Human Nutr.* **58**: 139–152.

Parfitt, R.L. 1979. The availability of P from phosphate-goethite bridging complexes. Desorption and uptake by ryegrass. *Plant Soil* **53:** 55–65.

Passioura, J.B., Ball, M.C., Knight, J.H. 1992. Mangroves may salinize the soil and in so doing limit their transpiration rate. *Funct. Ecol.* **6:** 476–481.

Pate, J.S. Verboom, W.H., & Galloway, P.D. 2001. Co-occurrence of Proteaceae, laterite and related oligotrophic soils: coincidental associations or causative inter-relationships? *Aust. J. Bot.* **49:** 529–560.

Pearse, S.J., Veneklaas, E.J., Cawthray, G.R., Bolland, M.D.A. & Lambers, H. 2006. Carboxylate release and other root traits of wheat, canola and 11 grain legume species as affected by P status. *Plant Soil* **288:** 127–139.

Pellet, D.M., Papernik, L.A., & Kochian, L.V. 1996. Multiple aluminum-resistance mechanisms in wheat (roles of root apical phosphate and malate exudation). *Plant Physiol.* **112:** 591–597.

Pérez Corona, M.E., Van der Klundert, I., & Verhoeven, J.T.A. 1996. Availability of organic and inorganic phosphorus compounds as phosphorus sources for *Carex* species. *New Phytol.* **133:** 225–231.

Poorter, H., Remkes, C., & Lambers, H. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiol* **94:** 621–627.

Popp, M. 1995. Salt resistance in herbaceous halophytes and mangroves. *Progr. Bot.* **56:** 416–429.

Poschenrieder, C., Tolra, R., & Barceló, J. 2006. Can metals defend plants against biotic stress? *Trends Plant Sci.* **11:** 88–295.

Prenzel, J. 1979. Mass flow to the root system and mineral uptake of a beech stand calculated from 3-year field data. *Plant Soil* **51:** 39–49.

Przybylowicz, J., Pineda, C.A., Prozesky, V.M., &. Mesjasz-Przybylowicz, J. 1995. Investigation of Ni hyperaccumulation by true elemental imaging. *Nucl. Instr. Meth.* **B104:** 176–181.

Purnell, H.M. 1960. Studies of the family Proteaceae. I. Anatomy and morphology of the roots of some Victorian species. *Aust. J. Bot.* **8:** 38–50.

Raaimakers, T.H.M.J. 1995. Growth of tropical rainforest trees as dependent on P-availability. Tree saplings differing in regeneration strategy and their adaptations to a low phosphorus environment in Guyana. PhD Thesis, Utrecht University, Utrecht, the Netherlands.

Rauser, W.E. 1995. Phytochelatins and related peptides. Structure, biosynthesis, and function. *Plant Physiol.* **109**: 1141–1149.

Rawat, S.R., Silim, S.N., Kronzucker, H.J. Siddiqi, M.Y., & Glass, A.D.M. 1999. AtAMT1 gene expression and NH_4^+ uptake in roots of *Arabidopsis thaliana*: evidence for regulation by root glutamine levels. *Plant J.* **19:** 143–152.

Read, J., Sanson, G.D., Garine-Wichatitsky, M.d., & Jaffre, T. 2006. Sclerophylly in two contrasting tropical environments: low nutrients vs. low rainfall. *Am. J. Bot.* **93:** 1601–1614.

Reddell, P., Yun, Y., & Shipton, W.A. 1997. Cluster roots and mycorrhizae in *Casuarina cunninghamiana*: their occurrence and formation in relation to phosphorus supply. *Aust. J. Bot.* **45:** 41–51.

Reeves, R.D. & Baker, A.J.M. 2000. Metal-accumulating plants. In: Phytoremediation of toxic metals: using plants to clean up the environment, I. Raskin & B.D. Ensley (eds). John Wiley & Sons, New York, pp. 193–229.

Reich, P.B. & Oleksyn, J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc. Natl. Acad. Sci. USA* **101:** 11001–11006.

Reich, P.B., Walters, M.B., & Ellsworth, D.S. 1992. Leaf lifespan in relation to leaf, plant and stand characteristics among diverse ecosystems. *Ecol. Monogr.* **62:** 365–392.

Reich, P.B., Ellsworth, D.S., & Uhl, C. 1995. Leaf carbon and nutrient assimilation and conservation in species of differing succession status in an oligotrophic Amazonian forest. *Funct. Ecol.* **9:** 65–76.

Remans, T., Nacry, P., Pervent, M., Filleur, S., Diatloff, E., Mounier, E., Tillard, P., Forde, B.G., & Gojon, A. 2006. The *Arabidopsis* NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. *Proc. Natl. Acad. Sci. USA* **103:** 19206–19211.

Rengel, Z. & Römheld, V. 2000. Root exudation and Fe uptake and transport in wheat genotypes differing in tolerance to Zn deficiency. *Plant Soil* **222:** 25–34.

Reuss, J.O. & Johnson, D.W. 1986. Acid deposition and the acidification of soils and waters. Springer-Verlag, New York.

Richardson, A.E. 1994. Soil microorganisms and phosphorus availability. In: Soil biota. Management in sustainable farming systems, C.E. Pankhurst, B.M. Doube, V.V.S.R. Gupta, & P.R. Grace (eds). CSIRO, East Melbourne, pp. 50–62.

Richardson, A.E., Hadobas, P.A., & Hayes, J.E. 2000. Acid phosphomonoesterases andphytase activities of wheat (*Triticum aesticum*) roots and utilization of organic phosphorus substrates by seedlings grown in sterile culture. *Plant Cell Environ.* **23:** 397–405.

Richardson, S.J. Peltzer, D.A., Allen, R.B., McGlone, M.S., & Parfitt, R.L. 2004. *Oecologia* **139:** 267–276.

Richardson, S.J., Peltzer, D.A., Allen, R.B., & McGlone, M.S. 2005. Resorption proficiency along a chronosequence: responses among communities and within species. *Ecology* **86:** 20–25.

Richardson, A.E., George, T.S., Jakobsen, I., & Simpson, R.J. 2007. Plant utilization of inositol phosphates. In: Inositol phosphates: linking agriculture and the environment, B.L. Turner, A.E. Richardson, & E.J Mullaney (eds). CABI Publishing, Wallingford. pp. 242–260.

Roberts, S.K. 2006. Plasma membrane anion channels in higher plants and their putative functions in roots. *New Phytol.* **169:** 647–666.

Robinson, D. 1994. The responses of plants to non-uniform supplies of nutrients. *New Phytol.* **127:** 635–674.

Robinson, D. 1996. Variation, co-ordination and compensation in root systems in relation to soil variability. *Plant Soil* **187:** 57–66.

Robinson, N.J., Tommey, A.M., Kuske, C., & Jackson, P.J. 1993. Plant metallothioneins. *Biochem. J.* **295:** 1–10.

Robinson, B.H., Leblanc, M., Petit, D., Brooks, R.B., Kirkman, J.H., & Gregg, P.E.H. 1998. The potential of *Thlaspi caerulescens* for phytoremediation of contaminated spoils. *Plant Soil* **203:** 47–56.

Robinson, B.H., Brooks, R.R., & Clothier, B.E. 1999. Soil amendments affecting nickel and cobalt uptake by *Berkheya coddii*: potential use for phytomining and phytoremediation. *Ann. Bot.* **84:** 689–694.

Römer, W., Kang, D.-K., Egle, K., Gerke, J., & Keller, H. 2000. The acquisition of cadmium by *Lupinus albus* L., *Lupinus angustifolius* L., and *Lolium multiflorum* Lam. *J. Plant Nutr. Soil Sci.* **163:** 623–628.

Römheld, V. 1987. Different strategies for iron acquisition in higher plants. *Physiol. Plant.* **70:** 231–234.

Ryan, P.R., Kinraide, T.B., & Kochian, L.V. 1994. Al^{3+}–Ca^{2+} interactions in aluminium rhizotoxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake. *Planta* **192:** 98–103.

Ryan, P.R., Reid, R.J., & Smith, F.A. 1997. Direct evaluation of the Ca^{2+}-displacement hypothesis for Al toxicity. *Plant Physiol.* **113:** 1351–1357.

Sakaguchi, T., Nishizawa, N.K., Nakanishi, H., Yoshimura, E., & Mori, S. 1999. The role of potassium in the secretion of mugineic acids family phytosiderophores fro iron-deficient barley roots. *Plant Soil* **215:** 221–227.

Salt, D.E. & Rauser, W.E. 1995. MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. *Plant Physiol.* **107:** 1293–1301.

Salt, D.E., Prince, R.C., Pickering, I.J., & Raskin, I. 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.* **109:** 1427–1433.

Salt, D.E., Kato, N., Krämer., U., Smith, R.D., & Raskin, I. 2000. The role of root exudates in nickel hyperaccumulation and tolerance in accumulator and non-accumulator species of *Thlaspi*. In: Phytoremediation of contaminated soil and water, N. Terry & G.S. Bañuelos (eds). CRC Press, Boca Raton, pp 191–202.

Sardans, J., Peñuelas, J., & Estiarte, M. 2007. Seasonal patterns of root-surface phosphatase activities in a Mediterranean shrubland. Responses to experimental warming and drought. *Biol. Fertil. Soils* **43:** 779–786.

Scheurwater, I., Clarkson, D.T., Purves, J., Van Rijt, G., Saker, L., Welschen, R., & Lambers, H. 1999. Relatively large nitrate efflux can account for the high specific respiratory costs for nitrate transport in slow-growing grass species. *Plant Soil* **215:** 123–134.

Schimel, J. P. & Bennett, J. 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* **85:** 591–602.

Schirmer, U. & Breckle, S.-W. 1982. The role of bladders for salt removal in some Chenopodiaceae (mainly *Atriplex* species). In: Tasks for vegetation science, Vol. 2, D.N. Sen & K.S. Rajpurokit (eds). Dr W. Junk Publishers, The Hague, pp. 215–231.

Schmidt, W. 2003. Iron solutions: acquisition strategies and signaling pathways in plants. *Trends Plant Sci.***8:**188–193.

Schmidt, S. & Stewart, G.R. 1999. Glycine metabolism by plant roots and its occurrence in Australian plant communities. *Aust. J. Plant Physiol.* **26:** 253–264.

Scholz, G., Becker, R., Pich, A., & Stephan, U.W. 1992. Nicotinamine—a common constituent of strategies I and II of iron acquisition by plants: a review. *J. Plant Nutr.* **15:** 1647–1665.

Shane, M.W. & Lambers, H. 2005. Cluster roots: A curiosity in context. *Plant Soil* **274:** 99–123.

Shane, M.W. & Lambers, H. 2006. Systemic suppression of cluster-root formation and net P-uptake rates in *Grevillea crithmifolia* at elevated P supply: a Proteaceae with resistance for developing symptoms of "P toxicity". *J. Exp. Bot.* **57:** 413–423.

Shane, M.W. McCully, M., & Lambers, H. 2004a. Tissue and cellular phosphorus storage during development of "phosphorus toxicity" in *Hakea prostrata* (Proteaceae). *J. Exp. Bot.* **55:** 1033–1044.

Shane, M.W., Szota, C. & Lambers, H. 2004b. A root trait accounting for the extreme phosphorus sensitivity of *Hakea prostrata* (Proteaceae). *Plant Cell Environ.* **27:** 991–1004.

Shane, M.W., Cramer, M.D., Funayama-Noguchi, S., Millar, A.H., Day, D.A., & Lambers, H. 2004c. Developmental physiology of cluster-root carboxylate synthesis and exudation in harsh hakea: expression of phospho*enol*-pyruvate carboxylase and the alternative oxidase. *Plant Physiol.* **135:** 549–560.

Shane, M.W., Dixon, K.W. & Lambers, H. 2005. The occurrence of dauciform roots amongst Western Australian reeds, rushes and sedges, and the impact of P supply on dauciform-root development in *Schoenus unispiculatus* (Cyperaceae). *New Phytol.* **165:** 887–898.

Sharples, J.M., Meharg, A.M., Chambers, S.M., & Cairney, J.W.G. 2000. Mechanism of arsenate resistance in the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. *Plant Physiol.* **124:** 1327–1334.

Shaver, G.R. & Chapin III, F.S. 1991. Production:biomass relationships and element cycling in contrasting arctic vegetation types. *Ecol. Monogr.* **61:** 1–31.

Shriner, D.S. and & Johnston Jr., J.W. 1985. Acid rain interactions with leaf surfaces: a review. In: Acid deposition: environmental, economic, and policy issues, D.D. Adams & W.P Page (eds). Plenum Publishing Corporation, New York, pp. 241–253.

Siddiqi, M.Y., Glass, A.D.M., & Ruth, T.J., & Rufty, T.W. 1990. Studies of the nitrate uptake system in barley. I. Kinetics of $^{13}NO_3^-$ influx. *Plant Physiol.* **93:** 1426–1432.

Siddiqi, M.Y., Glass, A.D.M., & Ruth, T.J. 1991. Studies of the uptake of nitrate in barley. III. Compartmentation of NO_3^-. *J. Exp. Bot.* **42:** 1455–1463.

Silberbush, M. & Barber, S.A. 1983. Sensitivity of simulated phosphorus uptake to parameters used by a mechanistic-mathematical model. *Plant Soil* **74:** 93–100.

Silva, I.R., Smyth, T.J., Israel, D.W., Raper, C.D. & Rufty, T.W. 2001a. Altered aluminum inhibition of soybean root elongation in the presence of magnesium. *Plant Soil* **230:** 223–230.

Silva, I.R., Smyth, T.J., Israel, D.W., & Rufty, T.W. 2001b. Magnesium ameliorates aluminum rhizotoxicity in soybean by increasing citric acid production and exudation by roots. *Plant Cell Physiol.* **42:** 546–554.

Smart, C.J., Garvin, D.F., Prince, J.P., Lucas, W.J., & Kochian, L.V. 1996. The molecular basis of potassium nutrition. *Plant Soil* **187:** 81–89.

Smirnoff, N., Todd, P., & Stewart, G.R. 1984. The occurrence of nitrate reduction in the leaves of woody plants. *Ann. Bot.* **54:** 363–374.

Soderberg, K. & Compton, J. 2007. Dust as a nutrient source for fynbos ecosystems, South Africa. Ecosystems **10:** 550–561.

Sprent, J.I. 1999. Nitrogen fixation and growth of non-crop legume species in diverse environments. *Perspect. Plant Ecol. Evol. Syst.* **2:** 149–162.

Staal, M., Maathuis, F.J.M., Elzenga, T.J.M., Overbeek, J.H.M., & Prins, H.B.A. 1991. Na^+/H^+ antiport activity in tonoplast vesicles from roots of the salt-tolerant *Plantago maritima* and the salt-sensitive *Plantago media*. *Physiol. Plant.* **82:** 179–184.

Stark, J.M. & Hart, S.C. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous ecosystems. *Nature* **385:** 61–64.

Sterner, R.W. and J.J. Elser. 2002. Ecological Stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton.

Sunarpi, Horie, T., Motado, J., Kubo, M., Yang, H., Yoda, K., Horie, R., Chan, W.-Y., Hattori, K., Osumi, M., Yamagami, M., Schroeder, J., & Uozmi, N. 2005. Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na+ unloading from xylem vessels to xylem parenchyma cells. *Plant J.* **44:** 928–938.

Szczerba, M.W., Britto, D.T., & Kronzucker, H.J. 2006a. Rapid, futile K^+ cycling and pool-size define low-affinity potassium transport in barley. *Plant Physiol.* **141:** 1494–1507.

Szczerba M W, Britto D T and Kronzucker H J 2006b. The face value of ion fluxes: the challenge of determining influx in the low-affinity transport range. *J. Exp. Bot.* **57:** 3293–3300.

Tarafdar, J.C. & Jungk, A. 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biol. Fert. Soils* **3:** 199–204.

Tester, M. & Davenport, R. 2003. Na^+ tolerance and Na^+ transport in higher plants. *Ann. Bot.* **91:** 503–527.

Thomas, W.A. & Grigal, D.F. 1976. Phosphorus conservation by evergreenness of mountain laurel. *Oikos* **27:** 19–26.

Tilton, D.L. 1977. Seasonal growth and foliar nutrients of *Larix laricina* in three wetland ecosystems. *Can. J. Bot.* **55:** 1291–1298.

Tinker, P.B.H. & Nye, P.H. 2000. Solute transport in the rhizosphere. Oxford University Press, Oxford.

Touraine, B., Clarkson, D.T., & Muller, B. 1994. Regulation of nitrate uptake at the whole plant level. In: A whole-plant perspective on carbon-nitrogen interactions, J. Roy & E. Garnier (eds). SPB Academic Publishing, The Hague pp. 11–30.

Trueman, L.J., Richardson, A., & Forde, B.G. 1996a. Molecular cloning of higher plant homologues of the high-affinity nitrate transporters of *Chlamydomonas reinhardtii* and *Aspergillus nidulans*. *Gene* **175:** 223–231.

Trueman, L.J., Onyeocha, I., & Forde, B.G. 1996b. Recent advances in the molecular biology of a family of eukaryotic high affinity nitrate transporters. *Plant Physiol. Biochem.* **34:** 621–627.

Tukey Jr., H.B. 1970. The leaching of substances from plants. *Annu. Rev. Plant Physiol.* **21:** 305–324.

Turner B.L. (2006) Inositol phosphates in soil: amounts, forms and significance of the phosphorylated inositol stereoisomers. In: Inositol phosphates: linking agriculture and the environment, B.L. Turner, A.E. Richardson, & E.J Mullaney (eds). CABI Publishing, Wallingford, pp. 186–206.

Turner, B.L. & Richardson, A.E. 2004. Identification of *scyllo*-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Sci. Soc. Am. J.* **68:** 802–808.

Ueno, D., Rombola, A.D., Iwashita, T., Nomoto, K., Ma, J.F. 2007. Identification of two novel phytosiderophores secreted by perennial grasses. *New Phytol.* **174:** 304–310.

Van der Werf, A., Visser, A.J., Schieving, F., & Lambers, H. 1993. Evidence for optimal partitioning of biomass and nitrogen at a range of nitrogen availabilities for a fast- and slow-growing species. *Funct. Ecol.* **7:** 63–74.

Van Hoof, N.A.L.M, Koevoets, P.L.M., Hakvoort, H.W.J., Ten Bookum, W. M., Schat, H., Verkleij, J.A.C. & Ernst, W.H.O. 2001. Enhanced ATP-dependent copper efflux across the root cell plasma membrane in copper-tolerant *Silene vulgaris*. *Physiol. Plant.* **113:** 225–232.

Van Vuuren, M.M.I., Robinson, D., & Griffiths, B.S. 1996. Nutrient inflow and root proliferation during the

exploitation of a temporally and spatially discrete source of nitrogen in the soil. *Plant Soil* **178:** 185–192.

Verry, E.S. & Timmons, D.R. 1976. Elements in leaves of a trembling aspen clone by crown position and season. *Can. J. For. Res.* **6:** 436–440.

Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. *Am. Nat.* **119:** 553–572.

Vitousek, P.M. 2004. Nutrient cycling and limitation: Hawaii as a model system. Princeton University Press, Princeton.

Von Ballmoos, P., Amman, M., Egger, A., Suter, M., & Brunold, C. 1998. NO_2-induced nitrate reductase activity in needles of Norway spruce (*Picea abies*) under laboratory and field conditions. *Physiol. Plant.* **102:** 596–604.

Vögeli-Lange, R. & Wagner, G.J. 1990. Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. *Plant Physiol.* **92:** 1086–1093.

Walker, T.W. & Syers, J.K. 1976. The fate of phosphorus during pedogenesis. *Geoderma* **15:** 1–9.

Walker, C.D., Graham, R.D., Madison, J.T., Cary, E.E., & Welch, R.M. 1985. Effects of Ni deficiency on some nitrogen metabolites in cowpea (*Vigna unguiculata* L. Walp). *Plant Physiol.***79:** 474–479.

Wang, B.L., Shen, J.B., Zhang, W.H., Zhang, F.S., & Neumann, G. 2007. Citrate exudation from white lupin induced by phosphorus deficiency differs from that induced by aluminum. *New Phytol.* **176:** 581–589.

Warren, C.R. 2006. Potential organic and inorganic N uptake by six *Eucalyptus*species. *Funct. Plant Biol.* **33:** 653–660.

Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A., & Wright, I.J. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annu. Rev. Ecol. Syst.* **33:** 125–159.

White, P.J. 1999. The molecular mechanism of sodium influx to root cells. *Trends Plant Sci.* **4:** 277–278.

Wiehe, W. & Breckle, S.-W. 1990. The ontogenesis of the salt glands of *Limonium*(Plumbaginaceae). *Bot. Acta* **103:** 107–110.

Wolt, J.D. 1994. Soil solution chemistry. John Wiley & Sons, New York.

Woodward, R.A., Harper, K.T., & Tiedemann, A.R. 1984. An ecological consideration of the significance of cation-exchange capacity of roots of some Utah range plants. *Plant Soil* **79:** 169–180.

Wright, I.J. & Westoby, M. 2003. Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. *Funct. Ecol.* **17**: 10–19.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J. Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., & Villar, R. 2004. The worldwide leaf economics spectrum. *Nature* **428:** 821–827.

Yanai, J., Robinson, D., Young, I.M., Kyuma, K., & Kosaki, T. 1998. Effects of the chemical form of inorganic nitrogen fertilizers on the dynamics of the soil solution composition and on nutrient uptake by wheat. *Plant Soil* **202:** 263–270.

Yang, Y.-Y., Jung, J.-Y., Song, W.-Y, Suh, H.-S., & Lee, Y. 2000. Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. *Plant Physiol.* **124:** 1019– 1026.

Zak, D.R., Pregitzer, K.S., Curtis, P.S., Teeri, J.A., Fogel, R., & Randlett, D.A. 1993. Elevated CO_2 and feedback between carbon and nitrogen cycles. *Plant Soil* **151:** 105–117.

Zerihun, A., McKenzie, B.A., & Morton, J.D. 1998. Photosynthate costs associated with the utilization of different nitrogen-forms: influence on the carbon balance of plants and shoot-root biomass partitioning. *New Phytol.* **138:** 1–11.

Zhang, H. & Forde, B.G. 1998. An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. *Science* **279:** 407–409.

Zhang, H. & Forde, B.G. 2000. Regulation of *Arabidopsis* root development by nitrate availability. *J. Exp. Bot.* **51:** 51–59.

Zhang, H., Jennings, A., Barlow, P.W., & Forde, B.G. 1999. Dual pathways for regulation of root branching by nitrate. *Proc. Natl. Acad. Sci. USA* **96:** 6259–62534.

Zhang, W.-H., Ryan, P.R., & Tyerman, S.D. 2004. Citrate-permeable channels in the plasma membrane of cluster roots from white lupin. *Plant Physiol.* **136:** 3771–3783.

Zhao, F.J., Lombi, E., Breedon, T., & McGrath, S.P. 2000. Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. *Plant Cell Environ.* **5:** 507–514.

Zheng, S.J., Ma, J.F., & Matsumoto, H. 1998. High aluminum resistance in buckwheat. I. Al-induced specific secretion of oxalic acid from root tips. *Plant Physiol.* **117:** 745–751.

Zohlen, A. & Tyler, G. 1997. Differences in iron nutrition of two calcifuges, *Carex pilulifera* L. and *Veronica officinalis*L. *Ann. Bot.* **80:** 553–559.

Zohlen, A. & Tyler, G. 2000. Immobilisation of tissue iron on calcareous soil—differences between calcicole and calcifuge plants. *Oikos* **89:** 95–106.

Zohlen, A. & Tyler, G. 2004. Soluble inorganic tissue phosphorus and calcicole–calcifuge behaviour of plants. *Ann. Bot.* **94:** 427–432.

Zuo, Y., Zhang, F., Li, X., & Cao, Y. 2000. Studies on the improvement in iron nutrition of peanut by intercropping with maize on a calcareous soil. *Plant Soil* **220:** 13–25.