Plant Nutrition 2: Macronutrients (N, P, K, S, Mg, and Ca)

The study of plant nutrition requires an experimental approach; little can be learned merely by direct observation. From the time of the Greeks until the 17th century, it was assumed based on observations that plants were composed mainly of water, and the role of mineral nutrients was poorly understood. A key study published in 1699 changed that, when John Woodward showed that plants grew better in water drawn from the Thames River (with its obvious “mineral matter” that he describes in some detail) compared with those grown in much purer and clearer springwater or rainwater. From these studies, Woodward correctly concluded that “Earth, and not water, is the matter that constitutes vegetables.”

From Woodward’s time to the present, countless scientists have contributed to our understanding of plant nutrition. Marschner (2012) is an excellent textbook and source for historical studies. An older but equally significant textbook is Justus von Liebig’s 1841 “Die Organische Chemie in ihre Anwendung auf Agrikultur und Physiologie” (published in English as “Organic Chemistry in its Applications to Agriculture and Physiology”). Von Liebig described the importance of N, P, and S for plant nutrition and also popularized the idea, based on Carl Sprengle’s work, that a plant’s growth is limited by the nutrient in shortest supply. This idea is known as the Law of the Minimum, and examples of it at work will be found in this article.

From 1843 onwards, Joseph Gilbert and John Lawes experimented extensively on the nutrient requirements of plants on Lawes’ estate, which is now known as Rothamsted Research Station (UK) and is the longest running agricultural research station in the world. Their collected works were published in 1895 as “The Rothamsted Experiments.” One of their major findings was that phosphates in bones and minerals could be made more available for uptake by plants by acid treatment. Their experiments were largely funded by sales of the resulting “superphosphate,” the first commercially produced artificial fertilizer.

By the start of the 20th century it was well established that the six elements we know as macronutrients, N, P, K, S, Mg, and Ca, are each indispensable for growth. Macronutrients are required in relatively large amounts: 1 to 20 mg/g fresh weight, compared with micronutrients that are required on the order of μg/g fresh weight (and which are discussed in a separate article). Here, we examine how macronutrients contribute to plant growth. Specifically, we look at (1) the availability of nutrients in the soil along with the effects of soil microbes and physical properties on their availability; (2) nutrient uptake from the external environment, across plasma membranes and into plant cells; (3) in some cases, the assimilation of the nutrient into organic molecules; (4) the distribution and redistribution of nutrients throughout the plant; and (5) regulation of these processes. In parallel, we will examine the genetic basis of a plant’s nutrient use efficiency (NUE) and evaluate strategies by which to replenish the nutrients that growing plants extract from soil.

NUTRIENT USE EFFICIENCY

NUE is usually defined as the amount of grain or other harvested material divided by the amount of nutrient supplied to the plant (in other words, yield per unit input), but there are several other ways to calculate NUE depending on the purpose (see Good et al., 2004). As an example, only ~30 to 50% of the nitrogen applied to a field ends up in the harvested grain, and this value can be much lower. Because of the high costs of nutrient fertilizers, considerable effort goes toward improving crop plant NUEs through breeding and agronomic practices. NUE varies by species but also by variety. High NUE varieties can be particularly important for farmers growing in resource-poor environments, where large inputs of fertilizers are not feasible.

NUE is determined by a very large number of genes and processes and can be difficult to select for directly, but as more is learned about the factors involved, it becomes increasingly feasible to identify and select for improvements. NUE depends on the physiology, anatomy, and morphology of the plant as well as the nutrient fertilization regime. It is frequently considered to be the product of two separate processes: nutrient uptake efficiency and nutrient utilization efficiency.

Macronutrients are usually taken up as inorganic ions from the soil or surrounding environment, which in vascular plants takes place largely through roots. Root systems have a large surface area to maximize contact with the soil. Nutrient uptake is determined by root system architecture and morphology, root exudations, mycorrhizas, and the activities of various transporters, as well as the availability and distribution of nutrients in the soil.

Nutrient utilization usually involves the assimilation of the nutrient into organic forms and the recycling and remobilization of the nutrient to maximize plant productivity within the context of finite resources. Across the plant’s lifespan, NUE is affected by how the nutrient is used when it is abundant and how the plant responds when it is limiting. Responses to nutrient limitation involve conserving and optimizing nutrient use through decreased growth rate, metabolic shifts that minimize requirements for the nutrient, or remobilization of the nutrient from less critical to more critical tissues (e.g., seeds and growing tissues). For years, modeling approaches have been used to understand this complex “trait” and judge when and how to apply supplemental nutrients. With modern sensors and assays, -omics approaches, and systems biology methods, these models are becoming ever more sophisticated and are helping to reveal the molecular parameters involved, which will be useful for breeding efforts.
SOIL-NUTRIENT HETEROGENEITY

Although our topic focuses primarily on the processes controlled by the plant, the understanding of plant nutrition also requires an understanding of soil properties and the roles of soil-dwelling microbes. The physical, chemical, and biological properties of soils are tremendously heterogeneous and dynamic. Soils are composed of crushed rocks from various geological eras, oceanic and aquatic sediments, volcanic debris, and decomposing organic materials. The science of soils incorporates an immensely broad and specialized vocabulary with which to describe these diverse soil types (for example, see IUSS Working Group WRB, 2014). Young soils such as those found at sites of volcanic activity (e.g., Hawaii) are often rich in P but poor in N. As soils age, P leaches away and becomes less abundant, whereas N levels increase due to microbial N fixation. Beyond simply aging, soil nutrient availability changes with human or natural activities. Soils can be rejuvenated through the addition of new materials as a consequence of glaciation or dust borne by wind, and soils can lose nutrients as a consequence of erosion. Ice age glaciations contributed to the relatively rich soils in the northern hemisphere. By contrast, parts of Australia, South America, and southern Africa have geologically ancient soils that are frequently P deficient. Many species endemic to these regions have evolved special adaptations for survival on nutrient-poor soils.

Soil pH is an important consideration for the availability of nutrients, particularly those that are taken up as positively or negatively charged ions. Soil pH can affect the electrochemical gradient across plant membranes, as well as the ionic form of some nutrients. Many ions form insoluble complexes with other minerals in a pH-dependent manner. All things considered, for some nutrients, particularly those that are taken up as positively or negatively charged ions, a neutral or slightly acidic soil pH is optimal. Further, soil microbes have direct and indirect effects on plant nutrition. Nitrogen-fixing bacteria and mycorrhizal fungi are important contributors to nutrient uptake for some or most plants, respectively, and are discussed in detail in Teaching Tools in Plant Biology 19: Plants and Their Microsymbionts.

The Global Nitrogen Cycle

Nitrogen is one of the most abundant elements on Earth, but it is largely inaccessible to life because although more than three-quarters of the Earth’s atmosphere is nitrogen, it is found in the very stable triple-bonded form of dinitrogen gas (N₂). Nitrogen enters the biosphere when it is “fixed” (reduced), which occurs by the process of biological nitrogen fixation (by free-living or symbiotic nitrogen-fixing prokaryotes), by physical processes such as lightning or by industrial nitrogen fixation. Currently, 120 million tons of N₂ are fixed annually to produce ammonium for fertilizer; this accounts for roughly half of terrestrial nitrogen fixation and accounts for ~2% of the world’s fossil fuel consumption. The annual production of industrial fixed ammonium has increased 9-fold since 1960 as a consequence of increasing population growth and increasing meat consumption; without the input of nitrogen-containing fertilizers, it would be impossible to produce enough food for today’s human population. Only 30 to 50% of nitrogen in fertilizers is taken up by plants. The remainder is lost back to the atmosphere as N₂ or nitrogen oxides (which are significant greenhouse gases) or washed away to contaminate waterways. The energy used to produce fixed nitrogen and its downstream effects are serious environmental concerns that drive our need to understand how nitrogen is taken up and used by plants as well as our need to improve the efficiency of these processes.

NITROGEN: THE MOST ABUNDANT MINERAL ELEMENT OF A PLANT

After C, H, and O, nitrogen is the most abundant element in a plant or animal body, and under some conditions (e.g., post-glacial North America and Europe) nitrogen is the mineral nutrient that most limits plant growth. For many crops, optimal growth demands the application of large amounts of nitrogen, on the order of 20 to 50 g per kg harvested. Nitrogen is an essential component of amino acids for protein synthesis, nucleic acids for DNA/RNA synthesis, chlorophyll, and a large number of other nitrogen-containing compounds that are required for defense and other functions. There are four routes to N assimilation: uptake as nitrate; uptake as ammonium; uptake as organic molecules such as amino acids, peptides, proteins, and nucleic acids; and fixation of gaseous N₂. N₂ fixation only occurs in association with microbes and is discussed in Teaching Tools in Plant Biology 19: Plants and Their Microsymbionts.

REPLENISHING NUTRIENTS EXTRACTED FROM SOIL

In agricultural systems with low productivity, soil can be replenished naturally, by lying fallow or by growing legume rotation crops that enrich soil. In agricultural lands, soil nutrients that are depleted by high-yielding crops must be replaced by some sort of fertilizer, which can be composed of composts and animal manures, blends of inorganic salts, or a combination of these. The three major elements found in most fertilizers are N, P, and K, and we will examine the sources of these fertilizer compounds as we discuss them individually. Suffice it to say that the global trade in fertilizers is a multi-billion-dollar business annually and a considerable contributor to food prices. Their costs, their sometimes limited availability, and the environmental impacts associated with their use make it imperative that fertilizer application be managed appropriately. Furthermore, it is important to note that the health of the humans and animals that eat plants is affected by the nutrient status of the plant, and it is common for animals that eat nutrient-deficient plants to themselves experience poor nutritional health.
Nitrogen Uptake and Transporters

Nitrogen in soil varies in its form as well as its quantity. Industrial or biological nitrogen fixation produces a highly reduced form of nitrogen, ammonium (NH$_4^+$), which in solution is in a pH-dependent equilibrium with ammonia (NH$_3$). Nitrogen fertilizers can be applied to soils as urea [CO(NH$_2$)$_2$], NH$_4^+$, and/or NO$_3^-$.

Plant residue or animal waste can be in organic forms, including proteins and peptides, amino acids, or urea, or as their degradation product NH$_4^+$. As a highly reduced, energy-storing molecule, NH$_4^+$ can be oxidized to NO$_3^-$ by soil-dwelling nitrifying bacteria. Therefore, in soils containing oxygen, NO$_3^-$ is the major form of nitrogen available to and acquired by plants, although plants generally fare best when both ionic forms are present. Some plants, particularly those adapted to acid or waterlogged soil, preferentially take up NH$_4^+$. When NH$_4^+$ levels are elevated, for example, in flooded soils in which anaerobic conditions prevent nitrification, plants can experience ammonium toxicity, which occurs due to futile cycling of NH$_4^+$ and NH$_3$ across cell membranes.

As charged molecules, NH$_4^+$ and NO$_3^-$ require transporters to move across membranes. Many transporter proteins have been identified that can transport NO$_3^-$ or NH$_4^+$ with varying affinities and degrees of specificity. For example, both low- and high-affinity transporters have been identified for each, enabling nitrogen to be taken up under a wide range of soil conditions. Some transporters are localized in the plasma membrane and function in uptake from the soil. Others are located in the vacuolar membrane and transport ions into or out of this important storage compartment. Still others are efflux transporters that move ions outward from the cytosol to the apoplast; these are critical for moving ions into the xylem stream for long-distance transport and for the remobilization of nutrients during periods of starvation or organ senescence.

Several different families of nitrate transporters have been identified. To accommodate the tremendous range of concentrations at which nitrate can be found in soil, some of these are specialized for very high affinity uptake (at low nitrate concentrations of ~1 μM to 1 mM) and some for low affinity uptake (at high nitrate concentrations >1 mM).

The NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER FAMILY is a large gene family of nitrate/peptide transporters, some of which can transport other molecules including the hormones indole-3-acetic acid or abscisic acid. Most members of this large gene family have not been functionally characterized. NPF6.3 (also known as NRT1.1 or CHL1) was the first nitrate transporter to be identified, through a screen for chloride resistance. Chlorate is a nitrate analog that can be taken up into a plant and reduced to chlorite, which is toxic. CHL1/NRT1.1/NPF6.3 is particularly interesting for several reasons. It is expressed in the root epidermis, cortex, and endodermis and is involved in nitrate uptake from the soil. It is a dual-affinity transporter, meaning that it can take up nitrate with both low and high affinity depending on its phosphorylation status. Furthermore, it has been identified as a nitrate sensor, as will be discussed further below. Another family of nitrate uptake transporters is the nitrate-induced, high-affinity transporters encoded by the NRT2 genes, of which there are seven in Arabidopsis thaliana. NRT2 transporters were first identified by homology with nitrate transporters in fungi and algae.

Nitrate transport out of cells and into subcellular compartments is just as important as its import into the cytosol. Another member of the NPF family (NRT1.5; new name NPF7.3) pumps nitrate from xylem parenchyma into xylem for transport to the shoot. The energy for nitrogen assimilation comes from photosynthesis, so much of the nitrogen acquired from the soil is carried to the shoot as inorganic ions. In the shoot, it can be stored in the vacuole (pumped in by chloride-channel transporters CLCa and CLCb) or assimilated in the cytoplasm. As an anion, nitrate can be transported by some nonselective anion channels as well as some in the chloride channel family. Nitrate also can be transported by some members of the slow anion channel-associated 1 homolog 3 SLAC/SLAH family.

Plants also have specific and passive transporters for other nitrogen-containing compounds, including transporters for urea, amino acids, and ammonium; see Nacry et al. (2013) for more information on these transporter families.

Primary Nitrogen Assimilation

Ammonium is a reduced form of nitrogen ready for assimilation into amino acids, whereas nitrate must first be reduced to ammonium, either in the root or after transport to the shoot; where these processes take place depends on the species and on various environmental conditions. NO$_3^-$ is reduced by the sequential action of nitrate reductase in the cytosol and nitrite reductase in the plastid. Nitrate reductase is a large enzyme with a complex catalytic scheme; electrons from the electron donor are passed to FAD then heme, with the final transfer to NO$_3^-$ involving an essential covalently attached molybdenum cofactor. Nitrite reductase uses ferredoxin, an electron acceptor in the light-harvesting photosynthetic reactions, as an electron donor. Genes encoding theses enzyme are generally upregulated by increasing tissue nitrate concentration.

NH$_4^+$ produced by the action of nitrate reductase/nitrite reductase is assimilated into the amino acids glutamine and glutamate by the action of glutamine synthase (GS) and glutamine-2-oxoglutarate aminotransferase (GOGAT). This energy-demanding step requires an ample supply of inorganic nitrogen and available carbon skeletons, and it is highly regulated. In angiosperms, GS is encoded by multiple genes, with GST genes encoding cytosolic enzymes and the single GS2 gene encoding a plastid-localized enzyme. Different isoforms have slightly different physiological roles that can include primary assimilation as well as reassimilation during senescence and photorespiration. The regulation of these enzymes, including their posttranscriptional control, has been studied extensively because the overall nitrogen use efficiency of a plant is determined to a large extent by GS activity. Once assimilated into organic form, nitrogen is transported throughout the plant and used in the synthesis of other nitrogen-containing metabolites.

Nitrogen Remobilization

Plants have evolved several strategies by which to optimize their allocation of assimilated nitrogen. For example, after its assimilation,
Regulation of Nitrogen Uptake and Assimilation

Plants are very responsive to their nitrogen status as well as to the availability of nitrogen in their environment. This sensitivity is evident from studies of transcriptional changes associated with changes in N status as well as changes in root growth pattern. Under conditions of N deficit, these changes contribute to an enhancement of N uptake, for example, by transcriptional induction of certain N transporters, and changes in N utilization, for example, the acceleration of senescence and accompanying remobilization of nitrogen to younger leaves or reproductive tissues.

In addition to downstream N-containing metabolites and hormones, NO₃⁻ itself is a signal that regulates diverse responses such as lateral root development, leaf expansion, and the transcription of nitrate-regulated genes. A nitrate reductase mutant allows responses to nitrate to be distinguished from responses to downstream metabolites. Nitrate sensing occurs by way of the nitrate transporter/receptor (transceptor) CHL1/NRT1.1/NPF6.3, and a mutation has been identified in this protein that affects its nitrate-transport capacity but not its nitrate-sensing capacity, greatly facilitating the study of this unique protein.

Metabolic and Transcriptional Responses to Nitrogen

Plants respond to nitrogen deficiency by a dramatic repogramming of transcription; up to 10% of the genome is responsive to nitrogen resupply after N deprivation. Genes transcriptionally regulated by N include N transporters and enzymes involved in N assimilation, such as nitrate reductase and glutamine synthase, as well as genes involved in carbon metabolism (demonstrating the close link between N and C metabolism). Beyond transcription, many components of the nitrogen uptake and metabolism pathways are further regulated by posttranscriptional controls, including the actions of microRNAs, phosphorylation, and subcellular localization. Genetic studies show some but not all of the N responses are mediated by NO₃⁻ by way of CHL1/NRT1.1/NPF6.3.

The plant’s responses to nitrogen are also sensitive to the availability of other metabolites such as sucrose and organic nitrogen. These metabolites indicate how effectively N can be assimilated, as well as how great the demand is. Nitric oxide also contributes to the regulation of nitrogen uptake and assimilation. Hormones can be long-distance signals of nitrogen status, particularly cytokinins (CKs), the accumulation of which generally correlate with nitrogen availability. For example, the current model proposes that when nitrogen-starved roots are resupplied with nitrate, CK synthesis is increased in the roots, CKs are translocated to the shoot in the xylem sap, and nitrogen-responsive genes are induced in the shoot.

Root Responses to Local and Systemic Signals

It is well established that both local and systemic signals contribute to root as well as transcriptional responses to nitrogen. In a landmark study by Drew (1975), when plants were grown in chambers with different segments of the primary root exposed to different concentrations of NO₃⁻, lateral roots on the root segment in the region of higher NO₃⁻ grew much longer as compared with those on the lower-NO₃⁻ root segment. This study shows that roots respond strategically to N-rich patches by stimulating growth.

Split root systems have proven to be effective models in which to investigate local versus systemic signals. For example, when a plant is grown with one-half of the root system subjected to low-nitrogen stress, the other half grown with KNO₃ grew more vigorously than a control plant in which both halves were grown with KNO₃. This study suggests that the roots in the low-N environment broadcast an N demand signal to which the other roots respond by proliferation. When the split-root plants are decapitated, this effect is not observed, nor is it observed in plants that are deficient in the production of cytokinin. Analysis of this system also provides support for a systemic N repletion signal. Other studies have identified small peptides and their cognate receptors as well as a transcription factor involved in systemic N signaling.

In a locally mediated lateral root outgrowth response, high NO₃⁻ perception by the CHL1/NRT1.1/NPF6.3 transceptor activates a transcription factor that promotes lateral root outgrowth. This protein also acts as an auxin transporter that is oriented in such a way that it can transport auxin away from a developing lateral root tip and its auxin transport activity is suppressed by nitrate. When nitrate levels are low, auxin is transported efficiently and lateral root outgrowth is minimized. When nitrate levels are high, the auxin transport activity is suppressed, and auxin accumulation in the developing lateral root supports its growth.

Strategies to Mitigate the Environmental Consequences of N Fertilizers

High yields demand the use of fertilizers, but much of the nitrogen applied to soils eventually ends up as pollutants of water and air.
Field-Based Practices to Minimize the Effects of N Fertilizers

The simplest and cheapest strategy to minimize the effects of N fertilizers is to avoid using them. The traditional method for resupplying soil with N is to use nitrogen-fixing crops as companion or rotation crops; this approach was used widely until chemically synthesized fertilizers became affordable in the mid-20th century and continues to be used in regions where fertilizer costs are prohibitive. Traditional methods mean that the land produces less grain or is less amenable to mechanization, so they are not widely used in the most productive grain-growing regions.

The second best strategy to minimize N fertilizer effects is to use them only when and where they are needed. Toward this end, there are several methods by which plant or soil nitrogen status can be determined. Some methods measure chlorophyll concentration, which is closely correlated with nitrogen status. Transmission methods measure the ratio of red light (absorbed by chlorophyll) to infrared light (not absorbed) that passes through a leaf. Other sensors measure various wavelengths of reflected or fluoresced light and can be used at the plant or field scale. Plant or soil nitrate levels can be determined by nitrate-selective electrodes or dipsticks. These methods can inform a farmer when the plant needs nitrogen. An alternative strategy can be to use slow-release or deeply buried fertilizers that prolong the time that the fertilizer remains in the soil. Each of these strategies adds cost or labor to food production, but also brings environmental benefits.

Nitrifying bacteria oxidize soil NH$_4^+$ to NO$_3^-$, which is more prone to leaching away from the root zone. Nitrification inhibitors are compounds that interfere with this process and maintain soil N availability. Some tropical grasses such as Brachiaria humidicola exude nitrification inhibitors, in this case brachialactone. Studies are ongoing to determine the feasibility of extending this trait to crops; already sorghum (Sorghum bicolor) genotypes and some species related to commonly grown crop plants have been identified that inhibit nitrification.

Finally, the damage caused when NO$_3^-$ washes away from fertilized fields can be mitigated by actively cultivating soil denitrifying bacteria. Denitrifying bioreactors are simple structures that filter field runoff through a bed of bacteria that capture NO$_3^-$ and convert it to harmless N$_2$.

Breeding for Enhanced Nitrogen Use Efficiency

The environmental and economic costs of food production can be lowered by increasing plant NUE. To accomplish this, we have to understand where the bottlenecks to nitrogen uptake and physiological utilization are and identify how to make these reactions occur more efficiently. To a first approximation, we can surmise that a crop plant optimized for NUE would have a high rate of uptake of scarce N from soil, a high rate of N incorporation into organic forms, and a high efficiency of N use, recycling, and remobilization into grain.

These assumptions have been largely borne out. Key genes have been identified through forward and reverse genetic approaches as well as modern -omics methods and experimentally validated using transgenic plants. Through these approaches, two of the key NUE-modulating enzymes have been identified as GS and GOGAT, which are involved in primary N assimilation as well as remobilization. In fact, it has been observed that each nitrogen atom passes through the catalytic cycle of GS/GOGAT many times between its primary assimilation and its ultimate departure from the plant, so it is reasonable that small changes in these activities can add up to large NUE effects. Promising results also come from expression of the enzyme alanine amino transferase, which regenerates 2-oxoglutarate by transferring an amino group from glutamate to pyruvate, producing alanine. Alanine can serve as a storage form of nitrogen, and transgenic plants overexpressing alanine amino transferase have been shown to have increased NUE.

Other identified genes affecting NUE include various transporters, transcription factors, regulatory proteins, and metabolic enzymes. As an example, a recent report that started with a quantitative trait mapping study in rice (Oryza sativa) identified a single amino acid substitution in a plant-specific component of a heterotrimeric G protein as contributing to NUE, opening a new avenue for research. Several other studies indicate that root systems that are better able to assimilate N from soil contribute to enhanced NUE.

Transgenic strategies have had mixed results (for a comprehensive summary, see McAllister et al., 2012). Several candidate genes have been investigated, and although these studies often show some positive effects in laboratory settings, their results seem to be variable in the field. The confounding effects of the plants’ regulatory mechanisms, including the important contributions of posttranscriptional regulatory controls, may be responsible for this observation or the fact that more than one gene may need to be altered to obtain a significant increase in NUE. Ongoing studies are addressing both of these possibilities. Considering that the global market in chemically synthesized nitrogen fertilizers is worth $100 billion annually, as well as its well-documented downstream consequences, a small increase in plant nitrogen use efficiency could have big payoffs.
PHOSPHORUS: THE MOST DIVERSE SET OF FUNCTIONS

In many soils, particularly younger soils, phosphorus (P) is the second most limiting nutrient for plant growth after nitrogen; in older soils, such as those found in parts of Australia and South America, P can be the most limiting nutrient. In biological systems, it is found in a large number of organic forms as well as in the form of inorganic phosphate (Pi), an oxidized cation that is generally written as PO$_4^{3-}$. The exact formula of inorganic phosphate is pH dependent, ranging from H$_3$PO$_4$ in very acidic conditions through H$_2$PO$_4^-$, HPO$_4^{2-}$, and PO$_4^{3-}$ with increasing pH. Of all the mineral nutrients, P has the most diverse set of cellular functions. It is involved in structure (e.g., phospholipid membranes), energy storage (ATP), information storage (DNA), and information transfer (RNA, protein kinase, and phosphorelay cascades).

P Reserves Are Limited and Being Used Quickly

Globally, 40 to 60% of arable land crop yields are limited by P availability. In some cases, soil P levels are absolutely low, but in many others, P is present but in an unavailable form (see below). Crop yields can be enhanced by the application of P fertilizer that is mined from rock deposits, but there are economic and ecological costs associated with this practice. The term “peak phosphorus” has been used to describe the present situation because P reserves are finite resources. Estimates for when P reserves will be depleted vary and range from 50 to 400 years. The depletion of easily accessible P reserves will drive up costs for the extraction process and ultimately food. Serious environmental problems occur as a consequence of phosphate runoff, which can threaten the biodiversity of natural environments and in aquatic environments can lead to harmful algal or cyanobacterial blooms. For these reasons, it is important to learn about the uptake and use of P by plants, to develop plants with better P usage efficiencies, and to develop methods for improved P management, including methods to reclaim it from agricultural and waste sources.

Phosphate Acquisition

**Phosphate Mining: Root Exudates and Mycorrhizal Symbioses**

Most of the P in soil is found either as insoluble phosphate sorbed (adsorbed or absorbed) onto soil particles or as organic compounds. Only a tiny amount of the P is present in the soil solution as Pi, the only form that can be taken up by plants. Therefore, plants have evolved strategies by which to “scavenge” or “mine” phosphorus, including the production of root exudates and the establishment of symbioses with mycorrhizal fungi.

Root exudates act on inaccessible phosphorus to increase its availability; estimates vary but as much as 30% of the carbon fixed by the plant might be exuded into the rhizosphere. Low molecular weight organic acids (e.g., citrate, malate, and oxalate) displace and replace phosphate from insoluble complexes, such as calcium phosphate and phosphates of aluminum and iron oxides and hydroxides. Soil can be very rich in P in the form of organic molecules, which are substrates for exuded enzymes such as phosphatases and phytases (phytate is inositol hexaphosphate). These enzymes release phosphate in a form suitable for uptake by the plant.

The symbiotic association with mycorrhizal fungi is a critical strategy for Pi uptake used by most plants (~80% of species, with Arabidopsis being a familiar exception). In this association, the fungal partner scavenges Pi, which is then taken up into the fungal cell and transferred to the plant (the fungus in exchange is provided with carbon). The fungus greatly increases the effective surface area of the root as well as the volume of soil from which Pi can be assimilated. This is particularly important because Pi has an extremely low mobility in soils; the zone immediately surrounding a root quickly becomes depleted of Pi, but the fungus extends beyond this zone to forage more effectively. The intricate signaling choreography upon which the mycorrhizal symbiosis depends is described in detail in Teaching Tools in Plant Biology 19: Plants and Their Microsymbionts.

**Phosphate Foraging: Root System Architecture**

Because of Pi’s immobility in soil, root growth patterns are extremely Pi sensitive. Phosphate reserves can be more abundant in the topsoil, so plants growing in low-P soil often show decreased primary root elongation and enhanced lateral root outgrowth, enhanced production of adventitious roots (produced by the shoot), as well as a change in root angle so that the lateral roots spread in a more horizontal pattern. Root hairs tend to become elongated and form more densely, increasing the area of the root/soil interface in low-P soils. Because investing in root growth can be metabolically expensive, some roots remain slender and do not expand radially, whereas others produce air-filled cavities known as aerenchyma.

Many members of the predominantly Southern Hemisphere plant family Proteaceae have adapted to low-P soils and reveal mechanisms for survival with severe P limitation. One common adaptation is the production of cluster roots that exude copious amounts of carboxylates or phosphatases to enhance Pi assimilation. The balance between investing in root growth versus root exudation may be determined by the absolute versus available levels of phosphorus. White lupin (Lupinus albus), a legume native to Mediterranean regions, also makes cluster roots and provides a genetically tractable model for studies of cluster-root development and function.

**Phosphate Transporters**

Pi uptake into the negatively charged cytosol is electrically unfavorable and requires the cotransport of two to four protons, so it is energetically expensive compared with cation uptake. Transport into the plant occurs by way of the PHT1 family of plant Pi transporters that were identified by homology and functional similarity to fungal Pi transporters. The PHT1 family has nine members in Arabidopsis and 13 in rice. Different family members have different affinities for Pi and expression patterns, although most are expressed in roots, particularly the root hairs and cortical cells, and in mycorrhizal species some are expressed at the fungal-plant interface. In general, their transcription level is...
determined by Pi availability, and the transcription factor PHR1 (PHOSPHATE STARVATION RESPONSE1) is involved in their regulation. Other types of Pi transporters have been identified that reside in the mitochondrial, plastid, or Golgi membranes. Once phosphate enters the cell, it is part of a large pool of free phosphate that is incorporated into diverse organic compounds by the action of hundreds of enzymes.

**Metabolic Adjustments to Low Phosphate**

Plants can acclimate to mild Pi deficiency through a variety of metabolic adjustments. One set collectively reallocates Pi to high-priority targets, for example, by mobilizing Pi from vacuolar reserves, though as yet the molecular identity of vacuolar Pi transporters remains obscure. Other metabolic reallocation responses include the activation of RNases, acid phosphatases, and Pi transporters in older or senescing tissues and the translocation of released Pi to younger tissues. PHO1 is a putative Pi efflux carrier that has been implicated in long-distance Pi transport from source to sink by exporting it to the apoplasm for transport in the xylem. Phosphate starvation also leads to increased production of root-exuded carboxylates and phosphatases to enhance Pi uptake.

Longer term Pi limitation leads to global metabolic changes that enable the plant to function in spite of the less than optimal availability of Pi. For example, phospholipids in membranes can be replaced by sulfolipids and galactolipids, and the number of ribosomes can be lowered (rRNAs represent the largest pool of organic phosphorus). Decreasing ribosomal pools can make plants slower to grow or respond but can be an effective strategy for survival when Pi is limiting. Accelerated leaf senescence and anthocyanin accumulation are also signs of Pi deficiency.

An extreme example of ribosome rationing can be seen in some Proteaceae that maintain many fewer ribosomes than other plants. Although mature leaves operate with ~20% of the rRNA of other plants, they have similar rates of photosynthesis. Furthermore, through a developmental strategy known as delayed greening in which leaf expansion precedes chloroplast maturation, growth is temporally separated from photosynthesis; cellular phosphate reserves can be employed first in cytosolic rRNAs (during growth) and subsequently in chloroplastic rRNAs (during greening).

**Regulatory Controls of P Uptake**

The metabolic and transcriptional shifts in response to Pi starvation are under tight control. A core group of Pi starvation-induced (PSI) genes have been identified that include genes involved in Pi uptake and remobilization as well as genes encoding phosphatases that can increase Pi availability. PHR1 and its orthologs are transcription factors that bind to a conserved DNA element (P1BS) found in the PSI genes, ensuring that they are downregulated when Pi is abundant and inducible by Pi starvation. Recently, the interaction between PHR1 and this DNA element was shown to be inhibited in the presence Pi through the action of proteins of the SPX family. When Pi is present, SPX proteins bind to PHRs and prevent them from activating the transcription of PSI genes. This interaction is particularly interesting because in yeast, proteins related to SPX are known to act as Pi sensors; this interaction therefore may indicate how plants sense Pi availability.

In the regulation of Pi nutrition, it is similarly important to maintain the Pi assimilation pathways in a downregulated state when this nutrient is not limiting. An example of this comes from the study of PHO2. When Pi levels are sufficient, PHO2 mRNA is translated to produce PHO2, a ubiquitin ligase that targets PHT1 and PHO transporters for degradation. Under Pi starvation, miR399 is expressed, which is a small RNA that targets PHO2 mRNA for degradation; thus, the transporters are stable and Pi is taken up and translocated to the shoot. Interestingly, a target mimicry that interferes with miR399 action is also upregulated by Pi deficiency, providing a fine-tuning mechanism. PHT1 transporter activity is further regulated by endoplasmic reticulum retention and phosphorylation.

Several mutants with disruptions in Pi regulatory controls show Pi hyperaccumulation in the shoot and a corresponding decrease in growth rate, suggesting that in terms of Pi accumulation, plants have mechanisms in place to assimilate not too little and not too much but just the right amount.

In addition to miR399 and other miRNAs, many hormones have been implicated in Pi starvation responses, including auxin, ethylene, gibberellins, cytokinins, and particularly strigolactones, the synthesis of which is stimulated by Pi starvation. Strigolactones promote associations with arbuscular mycorrhizal fungi, alter root system architecture, and are translocated to the shoot where they suppress branching and so lower the plant’s demand for Pi by slowing the growth rate.

**Breeding Crops for Improved Phosphate Use Efficiency**

The identification of genes that are involved in root exudation and architecture, Pi uptake and remobilization, and regulatory controls of these processes opens the door to genetic approaches to improve Pi use efficiency. Several strategies have been examined in lab and field studies, including the overexpression of Pi regulatory transcription factors and transporters and increased exudation of carboxylates, phosphatases, and phytases to the rhizosphere. The results have had mixed success. For example, in nutrient solution, overexpression of a PHT1 transporter leads to plants with increased Pi content but smaller stature, possibly because Pi accumulates to inhibitory levels; whether this approach contributes to Pi uptake in crops growing in P-limited soils remains to be seen. The fact that the regulatory machinery seems to be as important in avoiding excess Pi uptake as it is in ensuring adequate Pi uptake suggests that efforts to enhance Pi uptake will require a subtle approach.

Breeding strategies that alter patterns of root growth architecture have been somewhat more promising. New phenotyping methods make it possible to select directly for root architecture in breeding programs. Already varieties with root system architectures favorable for Pi uptake have been developed and shown to be more efficient at Pi uptake.

Interestingly, a strategy that started with a rice variety with enhanced Pi uptake ability has led to the discovery of a gene...
involved in regulating root system architecture. Analysis of the stress-tolerant aus rice variety grown in regions with very low soil P availability led to the identification of a major quantitative trait locus Pup1 (Phosphate Uptake 1) that confers enhanced growth in P-limiting conditions. This locus proved difficult to work with, but after some effort the gene PSTOL1 (Phosphate starvation tolerance 1), encoding a protein kinase, was identified. Although the direct targets for PSTOL1 are not yet known, overexpression of this protein causes an increase in grain yield on P-deficient soils, due at least in part to an enhancement of root growth.

Strategies to Mitigate the Environmental Consequences of P Fertilizers

As discussed previously, there are compelling environmental and economic reasons to employ greater precision when applying fertilizers. Monitoring soil and plants for signs of P limitation can ensure that it is applied at the appropriate rate, time, location, and chemical form. In particular, the very low mobility of Pi in the soil means that it should be applied where the roots are. As Pi reserves become more limiting, price increases are likely to drive a greater precision in Pi applications.

At the other end of the chain, it is important to try to decrease the amount of P that ends up in waterways as a consequence of fertilizer runoff, contamination from farmed animal excreta, and urban water treatments. At the farm level, greater care in the timing and amount of Pi fertilizer applications can help, as can the adoption of no-till practices that decrease soil erosion and nutrient runoff. Human urine is a rich source of Pi that can be collected separately from solid waste through special two-compartment toilets and used as fertilizer. Pi can be reclaimed from sewage and farm wastewater through chemical methods such as the selective precipitation of magnesium-phosphate or other salts, or adsorption or ion exchange methods. Biological methods take advantage of the ability of microbes or algae to extract and concentrate Pi from dilute systems, reclaiming it for reuse as well as cleaning the water prior to discharge.

Phytate accumulates in seeds but is indigestible to monogastric animals (e.g., pigs, as opposed to ruminant animals such as cows that have four-chambered stomachs); monogastric animals excrete P-rich manure that is an environmental pollutant. Furthermore, micronutrients such as zinc and iron are chelated by phytate and rendered inaccessible. For more efficient Pi and micronutrient assimilation, low-phytate plant varieties have been developed through conventional and genetic engineering methods, including through the overexpression of bacterial or fungal phytase genes. Another approach is to apply the enzyme phytase directly to animal foods. Finally, genetically engineered pigs that produce phytase in their saliva have been developed, which although effective at phytate metabolism still await regulatory approval.

Potassium Is an Essential Macronutrient

Potassium is an essential nutrient in plants and in all living cells. It is not incorporated into other molecules but instead functions as an uncharged ion. Although not covalently attached, it has essential roles in stabilizing the structures of many proteins and macromolecules. Potassium also has essential roles in water and ionic balance. Plant movements, including phototropism, the rapid movements of the sensitive plant (Mimosa pudica), and the guard cell volume changes that regulate transpiration, are mediated by the transport of potassium across cell membranes and the accompanying movement of water. Additionally, potassium is a cofactor for some enzymes, including pyruvate kinase, starch synthase, and plasma membrane H+-ATPase, and through this and other functions, potassium contributes to diverse metabolic processes. Potassium deficiency is particularly associated with small stature, wilting, decreased rates of photosynthesis and growth, and increased susceptibility to stress.

Potassium Uptake and Remobilization

Plants take up potassium from soils against a steep concentration gradient. Plant cells maintain a cytosolic K+ concentration of ~100 mM, but soil concentrations are typically 0.1 to 1 mM, 100- to 1000-fold lower. More than 50 years ago, classic studies by Epstein and colleagues identified a biphasic potassium uptake response. Using a wide range of K+ concentrations, they measured the rate of uptake of radioactively labeled potassium.
Their results showed different kinetic properties at low and high external $[\text{K}^+]$, which they interpreted to mean that different mechanisms are involved. When potassium is scarce, a high-affinity system is required for uptake, whereas when potassium is abundant a low-affinity system is sufficient. We now know that, to a first approximation, potassium/proton cotransporters that consume the energy equivalent of pumping two protons across the membrane are primarily involved in high-affinity uptake, and potassium channels that consume the energy equivalent of pumping one proton are primarily involved in low-affinity uptake. The channels and carriers involved in potassium uptake as well as recycling and remobilization strategies are described in more detail in *Teaching Tools in Plant Biology 29: Membrane Transport and Energetics, Potassium Nutrition, and Sodium Toxicity*.

**SULFUR: CLEAN AIR LEADS TO DEFICIENT PLANTS**

**Sulfur in Global Cycles and Cells**

Sulfur can be found in many redox forms: elemental sulfur (S), reduced forms including sulfide ($\text{H}_2\text{S}$) and organic sulfur ($\text{R-SH}$), and oxidized forms including sulfate ($\text{SO}_4^{2-}$), sulfite ($\text{SO}_3^{-}$), and sulfur dioxide ($\text{SO}_2$). Plants assimilate sulfur primarily from soil as sulfate and to a lesser extent from the atmosphere as $\text{SO}_2$ or $\text{H}_2\text{S}$. Until recently, sulfur was not considered limited for plant growth in most soils, but since air pollution control measures were effected in the 1980s, sulfur deposition into soil from the atmosphere has decreased dramatically and uncovered the fact that some plants benefit from sulfur fertilizer.

There are several similarities between the nitrogen and sulfur cycles. Both N and S are found in the atmosphere as well as the soil, and both are subject to oxidative and reductive reactions by soil microbes. In the soil, a reduced form of each ($\text{NH}_4^{+}$ or $\text{H}_2\text{S}$) is readily oxidized to a form assimilated by plants ($\text{NO}_3^{-}$ or $\text{SO}_4^{2-}$), which then must be reduced by the plant for incorporation into organic molecules.

In plants, sulfur makes up $\approx 1$ mg/g dry tissue mass, which is small compared with carbon (450 mg/g) or nitrogen (15 mg/g). Sulfur in plants is found mainly as methionine and cysteine in proteins, with the third major form being glutathione, a small molecule made from glutamate, cysteine, and glycine. Glutathione is a redox agent that cycles between reduced and oxidized forms and is involved in many enzymatic reactions as well as protection from damaging reactive oxygen species. Sulfur is also found in some defense compounds such as glucosinolates, cysteine-rich proteins including defensins and metallothioneins (metal binding proteins), and flavor compounds such as allium.

**Sulfate Uptake**

Sulfur uptake and transport is primarily mediated by the SULTR family of sulfate transporters, which are divided into four subfamilies with varied tissue and membrane specificities. Much of the primary uptake that occurs in roots occurs through SULTR1;1 and SULTR1;2, which are expressed in the root epidermal and cortical cell layers. Some of the other SULTR transporters have other functions, including efflux from the vacuole to cytosol, or are involved in long-distance transport via expression in the phloem or xylem parenchyma. In higher plants, transport into plastids also appears to involve this family of transporters.

Volatile sulfur compounds can be taken up by plants. These include the gaseous $\text{H}_2\text{S}$ and $\text{SO}_2$ from atmospheric sources and compounds produced by plant growth-promoting bacteria. Atmospheric $\text{SO}_2$ enters the plant through stomata and is then converted to sulfite ($\text{SO}_3^{-}$) or oxidized to sulfate. Although $\text{H}_2\text{S}$ and $\text{SO}_2$ can serve as nutrients, too much can be toxic. Mosses and other bryophytes don’t have a protective cuticle and are vulnerable to damage to elevated atmospheric sulfur dioxide.

**Assimilation into Organic Compounds**

Once inside the cell, the first step in sulfate assimilation is incorporation into adenosine 5’-phosphosulfate (APS) by ATP sulfurylase (ATPS). APS is the entry point to two pathways. In the primary metabolic pathway, APS reductase separates adenosine monophosphate from the sulfur moiety, releasing sulfite ($\text{SO}_3^{-}$), which is further reduced to sulfide ($\text{H}_2\text{S}$). In a two-step reaction, sulfide replaces serine’s hydroxyl group to produce cysteine. This reaction is catalyzed by a complex known as cysteine synthase, which is composed of serine acetyltransferase (SAT; also known as SERAT) and O-acetylserine(thiol)lyase (OAS-TL). The cysteine synthase complex seems to act as a metabolic sensor that fine-tunes the flux of the S assimilation pathway. The other pathway downstream from APS involves its phosphorylation to produce an activated form of sulfur 5’-phosphoadenosine 3’-phosphosulfate, which is subsequently incorporated into defense and other compounds.

**Regulation of Sulfur Uptake and Metabolism**

Sulfate uptake is upregulated by sulfur deficiency and down-regulated by reduced sulfur compounds in a form of negative feedback regulation. Uptake is determined by transcriptional and posttranslational controls of the transporters. For example, low-S status induces transcription of several transporter genes. Similarly, transcriptional and posttranslational mechanisms contribute to the regulation of S assimilation. Expression of genes encoding ATPS (the first step in S assimilation) is regulated by miR395, which is induced under low-S conditions and targets ATPS mRNA for degradation. The next enzyme in the assimilatory pathway, APS reductase is thought to control much of the assimilatory flux. Its expression is subject to both positive and negative regulatory controls involving various metabolites, but which of these act as direct versus indirect signals remains a matter of debate. O-acetylserine (OAS) also contributes to the regulatory control of the Cys synthase complex, by destabilizing it and inactivating serine acetyltransferase when S levels are low (with the net result being a switching off of the pathway from Ser to Cys via OAS). Interestingly, the Cys synthase complex functions in the cytosol, mitochondria, and plastids, suggesting that the production of this amino acid occurs in the compartment in which it is used.
Several additional contributors to the S regulatory network have been identified, with a complex and not fully resolved interaction network that modulates activity of uptake transporters and assimilatory enzymes as well as root-to-shoot translocation. Ethylene, miR395, and several transcription factors, including SULFUR LIMITATION (SLIM), contribute to this network which under S deficiency promotes sulfur uptake and translocation to the shoot as well as recycling of S-containing compounds. S deficiency also affects root growth by way of auxin. Specifically, primary root elongation is promoted in low-sulfate conditions and is inhibited by elevated cellular levels of cysteine.

Addressing S Deficiency in Plants
With less combustion-derived sulfur dioxide entering the atmosphere, S deficiency in plants is becoming more common. This condition is intensified in soils that are fertilized with NPK fertilizers (recall the “law of the minimum”). S deficiency can affect yields and quality of many crop plants, particularly wheat (*Triticum aestivum*) and oilseed rape (*Brassica napus*), which has a high S requirement. S demands also increase with increasing demand for defense compounds or exposure to heavy metals, which are chelated by S-containing metallothioneins. Sulfur can be added to soils as ammonium sulfate fertilizer, as elemental S, or as a liquid fertilizer containing ammonium thiosulfate; no mining is required as there is an ample supply of S available as by-products of petroleum refining processes. The possibility of increasing the uptake, assimilation, and remobilization of S through breeding or genetic engineering is also being explored.

MAGNESIUM: THE “FORGOTTEN ELEMENT”

Magnesium in Rocks and Cells
Magnesium, “the forgotten element,” has received less attention than some of the other macronutrients. It is the eighth most abundant element on Earth and (other than H and O) the third most abundant element in seawater, after Na and Cl. Mg in soil usually comes from weathered rock (dolomite, magnesite, and serpentine) and evaporated seawater (*Triticum aestivum*) and oilseed rape (*Brassica napus*), which has a high S requirement. S demands also increase with increasing demand for defense compounds or exposure to heavy metals, which are chelated by S-containing metallothioneins. Sulfur can be added to soils as ammonium sulfate fertilizer, as elemental S, or as a liquid fertilizer containing ammonium thiosulfate; no mining is required as there is an ample supply of S available as by-products of petroleum refining processes. The possibility of increasing the uptake, assimilation, and remobilization of S through breeding or genetic engineering is also being explored.

Mg2+ deficiency directly limits photosynthesis and causes leaf chlorosis that is aggravated by high light due to the production of reactive oxygen species.

Interestingly, in many species, Mg2+ deficiency causes a dramatic increase in the shoot to root ratio; this is opposite to the effect of most mineral nutrient deficiencies, which preferentially restrict shoot growth. The restriction on root growth in Mg-deficient plants is thought to be caused by a deficiency of sucrose loading into the phloem in photosynthesizing source leaves that causes the leaves to accumulate sucrose and effectively starves the root system; sugar signaling may also be involved.

Uptake and Assimilation
A family of Mg2+ transporters known in plants as MRS or MGT has been identified by homology to Mg2+ transporters in yeast, and there is evidence that these proteins support Mg2+ transport in plants. These transporters are distinct from other cation transporters but are conserved among the domains of life. Like other mineral elements, excess Mg2+ is stored in the vacuole, and tonoplast members of the MRS/MGT family have been identified as well as MGHX1, a tonoplast-localized Mg/ proton exchanger.

Magnesium deficiency is common in soils that are acidic as well as soils that are prone to leaching because these conditions promote the elimination of Mg2+ from upper soil profiles. High levels of Al3+ contribute to Mg2+ deficiency through competition for nonspecific binding in the apoplast as well as specific competition for uptake transporters. Conversely, adequate soil Mg2+ or enhanced rate of Mg2+ uptake by a plant can protect it from the harmful effects of Al3+ toxicity, which is a major inhibitor of plant growth on acidic soils.

Humans require dietary consumption of Mg2+ for bone construction, and deficiencies can occur when their diet consists mainly of Mg2+-poor grains. Mg deficiency also can occur in animals; cattle fed on young, rapidly growing spring grass can develop a sometimes fatal ailment called grass tetany as a consequence of inadequate Mg nutrition. Humans should ensure that their diet includes whole grains, nuts, and legumes as well as leaves to ensure adequate Mg. Adding Mg2+ fertilizers to soils can enhance Mg uptake by the plant, and genetic studies are underway to develop staple crop varieties with enhanced Mg2+ accumulation in the grain. Unlike some of the other macronutrients, there appears to be no significant downstream environmental impact from using Mg2+ as a fertilizer in agriculture.

CALCIUM: LOW FREE CYTOSOLIC LEVELS

In its free-cation form, calcium is maintained at very low levels in the cytosol, on the order of 0.1 to 0.2 μM. The overall concentration of calcium in the cytosol can be in the millimolar range, but most of it is not a free cation but instead buffered by binding to organic compounds or proteins such as calmodulin. In many plants, calcium is also found in the form of calcium oxalate crystals that are formed by specialized cells called idioblasts and accumulate in vacuoles and cell walls. Calcium
Calcium Uptake and Transport

Most plants generally are able to obtain sufficient calcium from the soil, although this ability is affected by the presence of other competing cations (Al$^{3+}$ and Na$^+$) and soil pH. At the membranes, the challenge is to maintain submicromolar free Ca$^{2+}$ concentrations in the face of much greater free Ca$^{2+}$ concentrations in the apoplast, vacuole, and other organelles; the Ca$^{2+}$ concentration may be thousands of times higher outside the cells than in. Calcium transport across the plasma membrane occurs by way of several highly regulated calcium channels that control inward flux, calcium pumps (Ca$^{2+}$-ATPases) that pump it outwards, and Ca$^{2+}/$H$^+$ antiporters. Similar families of channels, pumps, and carriers are present in the tonoplast membrane surrounding the vacuole where a sizable pool of Ca$^{2+}$ is sequestered.

Ca$^{2+}$ transport within a plant primarily occurs within the apoplas, where most of the free Ca$^{2+}$ is located. Because the Casparian strip at the root endodermis or exodermis blocks apoplastic transport, much of the uptake takes place at the tip of the root where the Casparian strip is not yet developed. From there, Ca$^{2+}$ can move into the xylem stream for transport throughout the rest of the plant. Phloem transport takes place via the symplast, so it presents a challenge to a plant’s ability to remobilize Ca$^{2+}$ from older to younger tissues. Without the ability to draw on internal stores, young growing tissues can be damaged by even transient interruptions in their exogenous supply of calcium.

Calcium’s Structural Role

Ca$^{2+}$ has structural roles in the cell wall and at the surface of membranes. In membranes, it is thought to interact with the negatively charged phosphate groups of membrane phospholipids and to regulate numerous ion transporters. In the cell wall, it stabilizes pectin, which is a polymer of galacturonic acids with various modifications. Ca$^{2+}$ forms cross-bridges between negative charges and so gels and stabilizes pectin. (This function can be observed in molecular gastronomy in the formation of “spherical,” in which a pectin-like alginate solution is exposed to a Ca$^{2+}$-containing solution to form gel balls.) Pectin forms the middle lamella between adjacent cells and also the newly forming cell wall laid down during pollen tube growth. Ca$^{2+}$ deficiency weakens the middle lamella layer and allows cells to separate, which can result in unsightly cracked skins of rapidly growing fruits, but also can result in cell death, in syndromes known as blossom end rot, tip burn, and bitter tip.

Calcium signaling

Ca$^{2+}$ waves are secondary signals of many abiotic stresses, light and gravity cues, and biotic interactions. The large Ca$^{2+}$ concentration difference between the cytosol and other cellular compartments ensures that when Ca$^{2+}$ channels open, the ion moves rapidly into the cytosol. Calcium is quickly removed from the cytosol through the action of calcium/proton antiporters and Ca$^{2+}$-ATPases that are related to plasma membrane H$^+$-ATPases. How these protein activities are coordinated to produce Ca$^{2+}$ waves and how this oscillating signal is decoded remains incompletely understood, but several constituents of the signaling pathway have been identified including numerous Ca$^{2+}$ binding proteins. Interestingly, long-distance spreading of Ca$^{2+}$ signals have been identified, but these may be transduced from cell to cell by a different signal than Ca$^{2+}$ itself.

PLANT MACRONUTRIENTS: SUMMARY AND ONGOING RESEARCH

Aside from C, H, and O, the six elements most abundant in a plant body are K, N, P, S, Mg, and Ca, each of which is usually assimilated from soil as a charged molecule and transferred across plant membranes by way of specialized transporter proteins. The gene sequences of many nutrient transporters have been identified, although much remains to be done to characterize their kinetic and regulatory properties in vivo.

N, P, and S are assimilated covalently into a large number of organic molecules, whereas K, Mg, and Ca function by way of ionic interactions; nevertheless each is completely indispensable. Uptake and assimilation are energetically demanding and closely regulated to ensure that supply matches demand. Current efforts examine how plants juggle their needs for each of these nutrients; rarely is only one limiting and the others replete.

Our understanding of plant nutrition has been established largely through studies that focus on one nutrient at a time in defined conditions, but increasingly it is clear that a broader approach is necessary to identify how plants cope with the more heterogeneous field environment. The growth of the root system is dictated by local and systemic information about all required nutrients (as well as water availability, physical properties of soil, etc.). As an example, elongation of the apical root region is most sensitive to inhibition by low Pi but is inhibited still further by a limitation of another nutrient. Such an analysis can also identify specific genes that respond to multiple nutrients, which will be important in efforts to breed plants for success in nutrient-poor soils. The effects of soil microbes in nutrient availability and the effects of the plant on soil microbe activities are often overlooked in laboratory-based studies.

The ultimate goal of much of the research described here is the development of greener, more sustainable agricultural systems. The enhancement of plant growth and yield by application of fertilizers contributes one of the major impacts of
food production. Although the traditional methods of applying complex organic (manure) fertilizers are less prone to leaching and runoff than chemically synthesized fertilizers, high-intensity crop farming areas are often not located close enough to livestock farming regions to warrant the shipping costs. As the environmental costs of overfertilizing have become more evident, farmers are increasingly incorporating strategies to mitigate these effects, often with the encouragement of local governments, which can help to offset increased production costs. In the coming decades, the tension between the demands for higher crop yields and the desire for cleaner food production will grow. It is a challenge for today’s students to work toward innovative solutions to the challenge of feeding the plants that feed us.

Mary Williams
mwilliams@aspb.org
Features Editor, The Plant Cell
American Society of Plant Biologists
c/o Laboratory of Plant Physiology and Biophysics
University of Glasgow

RECOMMENDED READING
(This is a representative list of sources to help the reader access a huge body of literature. We apologize in advance to those whose work is not included.)

Overview, History, Soils, and Fertilizers


Nitrogen Cycle and Global Impacts


Nitrogen Uptake, Assimilation, and Remobilization


Regulation of Nitrogen Uptake and Assimilation


Nitrogen Use: Mitigation and Breeding Strategies


Phosphorus Uptake and Regulation


Phosphorous Mitigation and Breeding Strategies


Potassium


Sulfur


Magnesium


Calcium


Multiple Nutrients and Nutrient Interactions


