Section I
INTRODUCTION

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Chapter 1

PHOSPHORUS: BACK TO THE ROOTS

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Abstract: Phosphorus (P) is a pivotal nutrient for all life on Earth. It is poorly mobile in soil and inorganic P concentrations in the soil solution are <0.6 to 11 μM. Organic P concentrations in the soil solution tend to be higher, but organic P needs to be hydrolysed before it can be taken up by plant roots. Such hydrolysis involves phosphatases that are either released from the roots or derived from microorganisms in the rhizosphere. A large fraction of soil P is sorbed onto soil particles, and hence is unavailable to most plants. Roots that release large amounts of P-solubilising carboxylates can access some of this sorbed P. Rates of P uptake from the soil solution are determined predominantly by the movement of P in soil. Root traits that enhance P movement in soil increase P acquisition; however, the kinetic properties of P transporters that take up this P have little effect on net P uptake. The downregulation of genes encoding these transporters is important to avoid P toxicity at a high P supply. Species or genotypes that lack the capacity to downregulate their P-uptake capacity typically show P-toxicity symptoms at a high P supply. Mycorrhizal symbionts increase the soil volume that is available for P acquisition. Attempts to select or engineer genotypes with greater P-uptake capacities should consider both root and soil characteristics, including soil microorganisms such as mycorrhizal fungi.

Keywords: Peak phosphorus, phosphorus mobility in soil, phosphorus toxicity, rhizosphere, soil phosphorus pools, sorption

1.1   Introduction

Phosphorus (P) is one of the major elements required by all living cells to grow and develop. Phosphorus does not occur naturally as a free element,

because it is too reactive, combining rapidly with other elements such as oxygen or hydrogen. When P is oxidised to the fullest extent possible, the product is orthophosphate (PO_4^{3-}; Pi), in which four oxygen atoms have bonded with a single P atom. At neutral pH, the Pi anion is present as a mixture of HPO_4^{2-} and $H_2PO_4^-$; it is predominantly as $H_2PO_4^-$ that P is transported into plant cells. The assimilated P is intimately involved with cellular bioenergetics and metabolic regulation, and is also an important structural component of essential biomolecules such as DNA, RNA, phospholipids (Chapter 9), ATP, and sugar-phosphates. Unlike in some bacterial cells, however, Pi cannot be reduced within plant cells to lower oxidation states. Rather, assimilated Pi is either sequestered in the cell vacuole or rapidly incorporated into organic form (e.g. initially as ATP) via photophosphorylation or oxidative phosphorylation.

Phosphorus plays a central role in virtually all major metabolic processes in plants, particularly photosynthesis and respiration, but it is also one of the least available macronutrients in many terrestrial and aquatic ecosystems. The massive use of P-containing fertilizers in agriculture, currently estimated to be in excess of 160 million metric tonnes (Mt) of rock phosphate (which equates to approximately 21 million Mt of P) per year worldwide (Fixen & Johnston, 2012), demonstrates how the plant-available P level of many soils is suboptimal for crop growth (Johnston *et al.*, 2014). The use of P-fertilizers can also be quite inefficient, with less than 20% of P applied to land that was recently converted to be used for crops or pastures typically being absorbed by plants during their first growing seasons (Simpson *et al.*, 2011). The judicious use of P fertilizer on established fields, however, allows 80–90% of applied P to be used by the crop and removed at harvest (Johnston *et al.*, 2014). The remaining P is sorbed onto soil particles or erodes and leaches (Andersson *et al.*, 2013; Smith & Schindler, 2009). Agricultural P runoff is a primary factor in the eutrophication of lakes and estuaries, and has also resulted in blooms of toxic cyanobacteria. With the world's population continuing its rapid increase, humankind faces a daunting challenge to produce sufficient food crops in the face of dwindling supplies of P-fertilizers. Thus, research on plant metabolic adaptations to suboptimal soil P availability, which is the focus of the present volume, is of significant practical importance. This will help to facilitate the development of effective tools and strategies for the rational application of biotechnology to reduce agriculture's current heavy reliance on expensive, polluting, and unsustainable P-fertilizers.

1.2 Phosphorus or phosphorous?

Phosphorus (P) must be the most frequently misspelled nutrient on our planet. In mainstream journals dealing with plant or soil science, the element is often misspelled as *phosphorous*. Spell-checkers do not pick up this mistake, however, because *phosphorous* is an existing word. It is not an
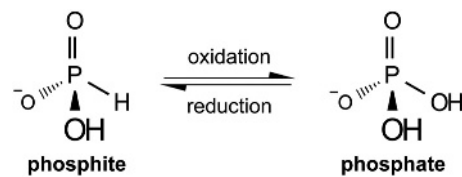
Figure 1.1   Phosphite and phosphate. Phosphite is less-oxidised than phosphate (Pi), and is not a direct source of P for plant nutrition. In soil, phosphite can be oxidised by microbes to Pi, which then makes it available for uptake by plant roots.

alternative spelling for *phosphorus*, however, and this is not a matter of British or American English. *Phosphorous acid* ($HPO(OH)_2$) is a reduced form of Pi in which an oxygen bonded to the P atom is replaced by a hydrogen (Figure 1.1). Phosphite ($H_2PO_3^-$; also known as phosphonate) is an alkali metal salt of phosphorous acid that represents an important but highly controversial agronomic commodity that is being widely marketed either as an agricultural fungicide or as a superior source of P for crop nutrition (McDonald *et al.*, 2001; Thao & Yamakawa, 2009). Published research conclusively indicates that phosphite functions as an effective control agent for a number of crop diseases caused by various species of pathogenic oomycetes belonging to the genus *Phytophthora* (e.g. Dunne *et al.*, 2010; McDonald *et al.*, 2001; Ratjen & Gerendás, 2009; Thao & Yamakawa, 2009). However, evidence that phosphite can be used directly by plants as a source of nutritional P is lacking. Phosphite can have direct effects on plants, because phosphite concentrations comparable to those required to control plant infection by pathogenic *Phytophthora* species are extremely phytotoxic to P-deprived plants; it is much less phytotoxic to P-fertilised plants (Carswell *et al.*, 1996; McDonald *et al.*, 2001; Ratjen & Gerendás, 2009; Thao & Yamakawa, 2009). This is because phosphite treatment effectively blocks the signal-transduction pathway by which plants (and yeast) perceive and respond to P-deprivation at the molecular level (Chapter 2). Thus, phosphite intensifies the deleterious effects of P-deficiency by 'tricking' P-deprived plant cells into sensing that they are P-sufficient when, in fact, their cellular P concentration is very low. Names are important, and so is characterisation of the mechanisms by which growth-enhancing substances actually work. Calling phosphite an agricultural fungicide in order to register it involves abiding by time-consuming and costly regulatory protocols. Calling phosphite a 'plant P fertilizer' can avoid the substantial expenses and tests associated with registering it as a fungicide. Whilst a number of agrochemical companies continue to market phosphite as a 'superior P fertilizer', a compound that suppresses P-starvation responses deserves the term of an 'anti-fertilizer' and should not be allowed to be advertised as an alternative to Pi (McDonald *et al.*, 2001; Ratjen & Gerendás, 2009; Thao & Yamakawa, 2009). However, as discussed in Chapter 2, the phosphite anion represents a useful tool to help dissect the signal-transduction

pathways by which plants respond to nutritional P deprivation at the molecular level.

1.3   Phosphorus on a geological time scale

The cycling of P in the global environment is an important biogeochemical process. Phosphorus is present in only minute quantities in the Earth's crust (0.9 mg g^{-1} dry soil) (Filippelli, 2008). This is less than half of what is found in Martian rocks (Greenwood & Blake, 2006). Both, on Earth and on Mars, P is derived from the weathering of igneous rocks. It has been suggested that the greatly increased oxygen concentration of the Earth's atmosphere that occurred between 2.3 and 2.4 billion years ago (the so-called 'Great Oxidation Event') (Canfield *et al.*, 2013), may have been the cause of enhanced oxidative weathering on land. This oxidative weathering liberated more P to the oceans and stimulated primary production and organic carbon burial (Bekker & Holland, 2012). Because of the relatively small quantities in the Earth's crust, it took about three billion years before enough P was weathered from igneous rock in which it was entrapped for the seas to become saturated (Griffith *et al.*, 1977). This led to the formation of phosphate rock reserves that are now mined to produce P fertilizers (Cooper *et al.*, 2011).

The roots of early terrestrial plants, which occurred on soils with relatively low availability of P, evolved symbioses with mycorrhizal fungi in order to acquire sufficient soil P more than 400 million years ago (Chapter 14) (Remy *et al.*, 1994). Weathering of soil enhanced the P availability for plants and microorganisms. During soil development (pedogenesis), however, soil P concentrations decline to much lower levels, due to long-term weathering, erosion and leaching (Turner & Condron, 2013; Walker & Syers, 1976). As a result, alternative mechanisms evolved to enhance the P-acquisition efficiency of plants on severely P-impoverished soils, including the root secretion of hydrolytic enzymes such as nucleases and acid phosphatases that mobilise inorganic-P (Pi) from the soil's organic-P pool (Chapter 10) (Plaxton & Tran, 2011), as well as the release of carboxylates that mobilise P from both organic-P and (Pi) sources (Chapter 11) (Lambers *et al.*, 2006; Lambers *et al.*, 2008).

Once P is liberated from minerals during weathering, it is quickly sequestered into a number of more recalcitrant phases (Walker & Syers, 1976), limiting its availability to plants and microorganisms (Chapter 13) (Lambers *et al.*, 2009; Porder *et al.*, 2007). Unlike the situation for nutrients such as nitrogen and carbon, ecosystems depend entirely on the aqueous transfer of P. Notable exceptions are P-impoverished ecosystems that rely on the import of Aeolian dust, for example the Amazon Basin (Bristow *et al.*, 2009; Bristow *et al.*, 2010) and old volcanic islands in Hawaii (Chadwick *et al.*, 1999).

Variations in the global P cycle occurred not only when the atmospheric oxygen concentration was increased (Canfield *et al.*, 2013), but also, for

example, during the uplift of the Himalayan-Tibetan Plateau which increased chemical weathering, leading to an enhanced input of P to the oceans. Additionally, P is redistributed on glacial timescales, resulting from loss of the continental margin during glacial low sea levels (Filippelli, 2008). Global phosphate rock reserves are the result of the formation of P-bearing minerals in marine sediments; this process occurs both in continental margin sediments and in deep sea sediments (oozes) (Filippelli, 2011). Current rock phosphate reserves have been formed over millions of years, and are gradually being depleted (Cordell *et al.*, 2009). As rock P reserves decline (Fixen & Johnston, 2012), the costs of P fertilizers will rise, and this is a significant issue for farmers in developing nations that have limited excess to P fertilizers (Lynch, 2007; Scholz & Wellmer, 2013). Improvements in the efficiency of cropping systems to acquire and use P are therefore urgently needed (Johnston *et al.*, 2014; Rose *et al.*, 2011).

1.4 Phosphorus as an essential, but frequently limiting, soil nutrient for plant productivity

Soil P concentrations would have been low soon after terrestrial life on Earth began, as outlined above for soils following thousands of years of pedogenesis. In the current era, in young soils, for example following the retreat of glaciers (Richardson *et al.*, 2004) or the deposition of coastal dunes (Laliberté *et al.*, 2013), soil P concentrations are relatively high and P is not the key limiting nutrient for plant growth. However, during pedogenesis, P rapidly becomes the major macronutrient limiting plant productivity (Vitousek *et al.*, 2010).

Pierre and Parker (1927) measured an average Pi concentration in the soil solution of 3 μM in a study of 21 different soils from the South and Middle West of the USA (range: <0.6 to 11 μM), far lower than the intracellular Pi concentrations (5–20 mM) required for optimal crop growth (Fang *et al.*, 2009; Vance *et al.*, 2003). The soils were chosen to represent a wide range in texture and organic matter content, and the average organic-P concentration was considerably higher: 15 μM (Pierre & Parker, 1927). More recent publications, including those dealing with soils fertilised with P at rates that are common agronomic practice, confirmed the range of soil P concentrations reported in this early work (Hossner *et al.*, 1973; Johnston *et al.*, 2014; Ron Vaz *et al.*, 1993; Yanai, 1991).

The Pi in the soil solution to sustain near-maximum growth of pearl millet (*Pennesitum typhoides*) is 6.5 μM (Fox & Kamprath, 1970), very similar to what has been found for a range of other crop and pasture species grown in nutrient solution (Asher & Loneragan, 1967; Breeze *et al.*, 1984; Breeze *et al.*, 1985) Even lower concentrations were found to saturate the growth of perennial ryegrass (*Lolium perenne*) in pot trials using phosphated-goethite as the source of P (Parfitt, 1979).

The concentrations in the soil solution and used in experimental glasshouse experiments are remarkably low compared with those commonly used in experiments on P nutrition of, for example, *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*), in which 250 µM (Dong *et al.*, 1998; Muchhal *et al.*, 1996; Muchhal & Raghothama, 1999), 1 mM (Narang *et al.*, 2000), or even 2.5 mM (Wu *et al.*, 2003) is 'normal' and 0 mM is commonly used as 'low'. Similarly high P concentrations (300–320 µM or 1 mM) are frequently used for the experimental growth of rice (*Oryza sativa*) (Li *et al.*, 2010; Ni *et al.*, 1996; Secco *et al.*, 2013; Seo *et al.*, 2008) under 'normal' conditions, and 16 or 35 µM for growth at a 'low' P availability (Ni *et al.*, 1996; Seo *et al.*, 2008). However, it would be incorrect to assume that these high reported concentrations are really present as free Pi ions in solution (either in agar or in the liquid culture medium). It is impossible to achieve such high concentrations whenever similarly high calcium concentrations are used in the culture medium, because calcium phosphates precipitate. The real Pi concentration in solution, as opposed to the total amount of P, can be calculated using Geochem-PC (Parker *et al.*, 1995; Shaff *et al.*, 2010). In agar, the adsorption of Pi onto the gel, as well as the agar being a potential source of additional P at low P loadings, offer additional complications; Eurobio agarose may contain up to 43 µg P g^{-1} dry weight of agarose (Irshad *et al.*, 2012) and other sources of agar even more (U. Irshad and C. Plassard, pers. comm.). With respect to the 'low-P' plants, in reality, these will have been exposed to a concentration supplied at the start (e.g. 16 or 35 µM in the cited examples for rice) and 'zero P' when they exhausted all P in the nutrient solution. It takes growing conditions with a very large volume or very frequent replacement of nutrient solutions to really maintain a low P concentration in solution for extended periods (Asher & Loneragan, 1967; Asher, 1981; Breeze *et al.*, 1984). An alternative approach is that developed by Ingestad (1970; 1982), who imposed an exponential growth rate on seedlings by increasing the relative addition rate of a limiting nutrient exponentially. In this way, plants can be grown in a steady state whilst being limited by a specific nutrient, such as P (De Groot *et al.*, 2001; Ericsson & Ingestad, 1988).

The 'normal' Pi concentrations used in nutrient solution, for example 0.1 mM Pi for the growth of barley (*Hordeum vulgare*) or barleygrass (*H. leporinum*), may not cause P-toxicity problems because the Pi-uptake systems in these species are downregulated, but they do lead to an excessive accumulation of Pi in leaves (Chapin & Bieleski, 1982). In species such as *Hakea prostrata*, that do not have a less-pronounced capacity to downregulate P-uptake systems, there may well be a risk of P toxicity. In other species, such as *Trifolium subterraneum* (subterranean clover) and *Lupinus digitatus* (blue lupin) (Asher & Loneragan, 1967), a range of tropical food legumes (*Cajanus cajan* (pigeonpea), *Pachyrrhizus erosus* (Mexican yam bean), *Psophocarpus tetragonolobus* (winged bean), *Vigna angularis* (adzuki bean), *V. mungo* (black gram), *V. unguiculata* (cowpea) (Bell *et al.*, 1990) and *Medicago*

truncatula (Tang *et al.*, 2001), toxicity symptoms occur at 15 or 25 µM Pi in nutrient solution, especially – but not exclusively – when the nitrogen supply is suboptimal. Toxicity symptoms are often observed when plants, for example *Solanum tuberosum* (potato) (Cogliatti & Clarkson, 1983) or *H. vulgare* (Green *et al.*, 1973), are pregrown in a low-P solution and then exposed to a 'normal' P solution. If we seek to study plants with a realistic P status, it is essential to supply Pi in a manner that avoids both an accumulation of Pi to very high levels and a depletion of Pi in the nutrient solution. In addition to the approaches discussed in the preceding paragraph, plants can be grown in soil or sand, but even then care must be taken not to provide unrealistically high Pi concentrations.

1.5   Soil phosphorus pools

The Pi pool in the soil solution tends to be small (Pierre & Parker, 1927), but the total inorganic soil P pool varies over three orders of magnitude, mainly depending on soil age (Turner *et al.*, 2013). In ancient weathered soils in south-western Australia, the readily available Pi concentration can be as low as 1 mg kg^{-1} or less (Lambers *et al.*, 2012), whereas in young volcanic soil in Chile, values of 1000 mg kg^{-1} are common (Borie & Rubio, 2003). The Pi pool in the soil solution is readily available for uptake by plants, but that is not the case for a large fraction of the Pi pool, which can be strongly sorbed to oxides and hydroxides of iron and aluminium (Barrow, 1999; Borie & Rubio, 2003). The extent to which the soil's total Pi pool can be accessed by plants using different P-acquisition strategies will be explored in detail in several chapters of this book (see Chapters 10, 11, 13, 14).

Phosphorus is not distributed homogeneously through the soil profile, except in very young soils such as recently deposited sand dunes (Laliberté *et al.*, 2012). As soils develop and plants remove nutrients from them, plant litter is deposited on the soil surface and thus a soil P profile develops, with highest concentrations in the surface layers (Laliberté *et al.*, 2012; Smeck, 1973; Walbridge *et al.*, 1991). In agricultural soils, a similar profile is to be expected, as a result of manure or inorganic-P fertilizer being spread on top of the soil (Dick, 1983; Holanda *et al.*, 1998), especially under no-tillage conditions (Cade-Menun *et al.*, 2010; Guertal *et al.*, 1991). Weathering and pedogenesis result in a decrease of total P, especially Pi, with organic-P becoming relatively more important (Walker & Syers, 1976). This is most pronounced in the topsoil, which is the most weathered soil horizon in a soil profile, relative to deeper soil horizons (Turner *et al.*, 2013).

The organic-P concentration in the soil solution can be about fivefold greater than the Pi pool (Pierre & Parker, 1927). The total organic-P pool as a fraction of the total P concentration is very small (<0.1%) on very young soils along the Franz Josef chronosequence in New Zealand, but

that fraction increases to about half of the total P concentration in older soils (Turner *et al.*, 2013). In lowland tropical rainforest soils, organic-P represents 26% of total soil P (Turner & Engelbrecht, 2011). whereas in high-P volcanic soils in Chile organic-P constitutes about half of the total soil P (Borie & Rubio, 2003). The organic-P pool comprises a range of chemical compounds: phosphate monoesters (e.g. inositol phosphates, sugar phosphates and mononucleotides) and phosphate diesters (e.g. phospholipids, DNA), organic-polyphosphates, phosphonates, and phytates (Cade-Menun *et al.*, 2010; Chapuis-Lardy *et al.*, 2001; Turner *et al.*, 2005; Turner & Engelbrecht, 2011).

Although plants take up Pi (as discussed in Chapter 5), they can also access a fraction of the organic-P pool, following hydrolysis of some organic-P compounds by secreted nucleases, phosphodiesterases, purple acid phosphatases (Chapter 10), and phytases (George *et al.*, 2006a; George *et al.*, 2006b; Maruyama *et al.*, 2012; Plaxton & Tran, 2011; Tarafdar & Claassen, 1988). These Pi-releasing enzymes may be excreted by the roots themselves, or they may be of microbial origin (Chapter 13) (Kitayama, 2013).

1.6   Soil phosphorus mobility

Because of the low concentrations of Pi in the soil solution and the high reactivity of Pi, minute amounts of Pi move via mass flow towards the root surface of transpiring plants. Mass flow typically delivers as little as 1–5% of a plant's P demand (Barber, 1962; Oliveira *et al.*, 2010; Prenzel, 1979), and the amount intercepted by growing roots is even less than that (Barber *et al.*, 1963; Clarkson, 1981). Organic-P concentrations in the soil solution tend to be higher than Pi concentrations (Pierre & Parker, 1927), which likely contributes to their greater mobility in soil (Hannapel *et al.*, 1964a). In addition, microbial activity – presumably the conversion of immobile organic-P into mobile organic-P – may account for greater mobility of organic-P, because the stimulation of microbial activity enhances the mobility of organic-P, whereas its inhibition decreases its mobility (Hannapel *et al.*, 1964b).

Most Pi arrives at the root surface by diffusion (Bhadoria *et al.*, 1991a; Bhat & Nye, 1973; Drew & Nye, 1970), followed by active transport across the plasma membrane of root hair and root epidermal cells (Chapter 5) or of mycorrhizal fungal hyphae (Chapter 14). However, the diffusion coefficient of Pi in soil is relatively low, compared to that of some other nutrients, typically of the order of $0.1 - 5 \times 10^{-13}$ m^2 s^{-1}. Since this diffusion coefficient declines with decreasing soil moisture content (Bhadoria *et al.*, 1991b), any root activity that increases the moisture content in the rhizosphere will potentially increase plant P acquisition (as discussed in Section 1.7). Plants have a range of mechanisms, which enhance the acquisition of sufficient Pi to sustain their growth, many of which will be elaborated on in subsequent chapters (Chapters 11–14).

1.7 Factors determining rates of phosphorus uptake by roots

Given the very low mobility of Pi in dry soil, plant activities that enhance this mobility are expected to increase plant Pi uptake. The release of water by roots into superficial dry soil layers, taken up from moist deeper layers (hydraulic lift), is therefore expected to increase Pi acquisition (Prieto *et al.*, 2012). Likewise, the release of phospholipid surfactants by roots changes the biophysical properties of the rhizosphere and increases soil solution Pi concentration (Read *et al.*, 2003).

Whilst mass flow contributes very little to the acquisition of Pi by crop plants, it is possible that this situation is different for slow-growing plants in sandy soils (Cernusak *et al.*, 2011; Matimati *et al.*, 2014). However, such sandy soils contain very little Pi, so if mass flow is to have an effect on P acquisition, it is likely to involve organic-P forms, some of which are more mobile in soil (Frossard *et al.*, 1989; Hoffman & Rolston, 1980). Unless the concentration of organic-P in the soil solution is very high and the plant's P demand is relatively low, it is unlikely that mass flow can deliver a substantial component of the plant's P requirement. Root activities that enhance Pi in the soil solution can be expected to have a major impact on Pi uptake (see Chapters 11 and 13) (Lambers *et al.*, 2006).

Simulation models of plant P uptake that take into account both soil and root characteristics have been used to assess which traits have a major impact on net P uptake (Figure 1.2) (Schenk & Barber, 1979; Silberbush & Barber, 1983). The rate of root elongation and root diameter, including root hairs (Bhat & Nye, 1973), are among the most important root traits. What was not included in the early models was the nonhomogeneous distribution of P in the soil profile, but this aspect has been addressed recently (Dunbabin *et al.*, 2013). Jonathan Lynch and coworkers acknowledged the distribution of P in the soil profile, and suggested selecting for genotypes with shallow roots that would readily access poorly mobile nutrients in shallow soil layers (Lynch & Brown, 2008; Postma & Lynch, 2012; York *et al.*, 2013). What was also not included in the early simulation models is the role of mycorrhizal hyphae in P acquisition. When roots are colonised by arbuscular mycorrhizal fungi, the Pi transporters in their epidermal cells that are responsible for Pi uptake from the rhizosphere are downregulated, and Pi uptake by the mycorrhizal hyphae is the dominant pathway for P acquisition (Chapter 14) (Smith *et al.*, 2003). Transporters that are inducible by arbuscular mycorrhizal fungi are expressed in cortical cells, acquiring Pi released by arbuscules (Karandashov & Bucher, 2005; Paszkowski *et al.*, 2002). The mycorrhiza-inducible Pi transporter genes are downregulated at high Pi supply (Nagy *et al.*, 2009). Beyond mycorrhizal hyphae, P acquisition by plants largely relies on processes of mobilisation of poorly soluble forms of inorganic and organic-P in the rhizosphere – that is, on many biogeochemical and biochemical processes driven by roots or rhizosphere microbiota (Chapter 13) (Clarkson, 1985; Hinsinger
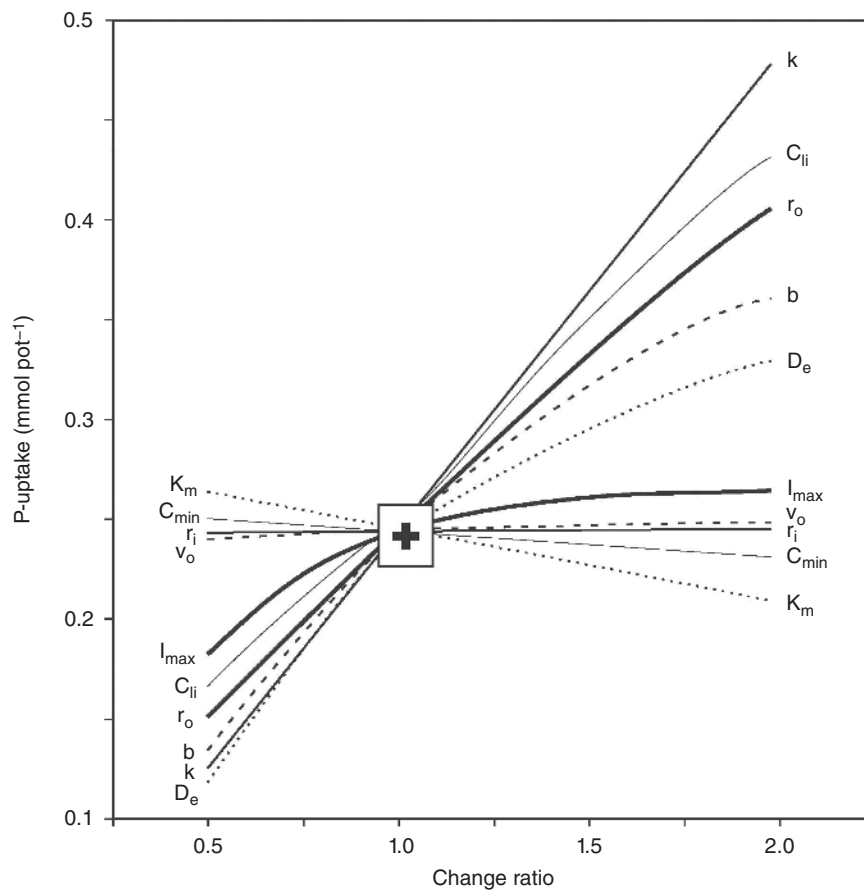
Figure 1.2 Effects of changing parameter values (from 0.5- to 2.0-fold the standard value) on simulated inorganic phosphate (Pi) uptake by roots of soybean (*Glycine max*). k is the rate of root elongation; C_{li} is the initial Pi concentration in the soil solution; r_o is the root diameter; b is the buffer power of the soil; D_e is the diffusion coefficient of Pi in the soil; I_{max} is the maximum Pi 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 Pi uptake is possible; and K_m is the Pi concentration at which the rate of Pi uptake is 50% of I_{max} (Silberbush & Barber, 1983).

et al., 2001). Most plant nutrition models do not account for these key processes, however, which explains why they strongly underestimate the actual P acquisition under limiting soil P conditions (Hinsinger *et al.*, 2011).

The least important traits for P acquisition from soil, according to the simulation model of Silberbush and Barber (1983), are the kinetic properties of the root's P-uptake system (Figure 1.2). This is to be expected, given that P mobility in soil is a major constraint for P uptake from soil (Clarkson, 1985; Tinker & Nye, 2000). Remarkably, this major result is not taken on board by many

who work on P-starvation responses. Of course, high-affinity Pi transporters are crucially important for Pi uptake from the rhizosphere. As discussed in Chapter 5, the enhanced expression of genes encoding high-affinity Pi transporters during P starvation is considered important to increase Pi uptake, whereas the results shown in Figure 1.2 indicate it is obviously not. How can this discrepancy be resolved? The actual significance of the change in expression of the genes encoding Pi transporters that are involved in Pi uptake from the rhizosphere is not their 'upregulation' at limiting P supply, but their 'downregulation' at very high Pi supply. Upregulations and downregulations are simply two ends of the same continuum. Species that occur naturally in P-impoverished environments with a very low capacity to downregulate their Pi-uptake system are very sensitive to P toxicity (de Campos *et al.*, 2013; Shane *et al.*, 2004). Interestingly, one such P-sensitive species, *Eucalyptus marginata* (jarrah), only shows severe P-toxicity symptoms following a toxic Pi pulse when it is not colonised by mycorrhizal fungi (Kariman *et al.*, 2014), which suggests that its Pi-uptake systems are downregulated only when the plants are mycorrhizal. Other Pi-sensitive plants include the *Pho2* mutant of *Arabidopsis thaliana* (Dong *et al.*, 1998), which is defective in a ubiquitin-conjugating E2 enzyme (Liu *et al.*, 2012) and a transgenic overexpressing miR399 (Aung *et al.*, 2006). These plants do not downregulate their Pi-uptake systems, and accumulate excessive amounts of Pi in their shoots (Chapter 2) (Lin *et al.*, 2008). In soils where the mobility of P is very high, such as rice paddy fields, the kinetic properties of the Pi-uptake system may be important (Park *et al.*, 2007; Seo *et al.*, 2008), but further studies are required to confirm this point.

1.8 Phosphorus-starvation responses: does phosphorus homeostasis exist?

Plants show a range of responses to a low P supply which are generally referred to as 'P-starvation responses' (e.g. Karthikeyan *et al.*, 2014; Plaxton & Tran, 2011; Ticconi *et al.*, 2001; Yang & Finnegan, 2010). These P-starvation responses minimise plant P deficiency, and include decreased growth and increased root/shoot ratio, root-hair density and carboxylate exudation (Lambers *et al.*, 2006). They also involve a decrease in the uptake and metabolism of nitrogen (Chapter 7) (e.g. Gniazdowska *et al.*, 1999; Rufty *et al.*, 1993). It is often claimed that these P-starvation responses lead to P homeostasis, but does that suggestion make sense? Before that question can be answered, it is important to confirm what 'homeostasis' really means. Based on the suggestions of Cannon (1929), who coined the term but gives full credit to Claude Bernard for the concept, Wikipedia captures it in an excellent manner: "From homoeostasis or homœostasis (from Greek: ὅμοιος, 'hómoios', 'similar', and στάσις, '*stásis*', 'standing still') — is the property of a system in which variables are regulated so that inter-

nal conditions remain stable and relatively constant. Examples of homeostasis include the regulation of temperature and the balance between acidity and alkalinity (pH). It is a process that maintains the stability of the human body's internal environment in response to changes in external conditions" (http://en.wikipedia.org/wiki/Homeostasis). Following this perfectly clear definition, there can be no P-starvation responses and homeostasis at the same time. When plants are grown at a limiting P supply, they dramatically decrease their tissue P concentration, especially the Pi concentration (Veneklaas *et al.*, 2012), and thus do not maintain homeostasis. Initially, there is a depletion of the vacuolar Pi pool, followed by a large (up to 50-fold) reduction in cytosolic Pi and organic-P levels (Veneklaas *et al.*, 2012). What the P-starvation response does, therefore, is to help stave off rapid cell death that would otherwise ensue, as for example reported for P-sufficient *Brassica napus* suspension cells cultured into media without Pi. The cells stop growing after a few days, but otherwise remain at least 90% viable for about three to four weeks. Blocking the P-starvation response by adding 1 mM phosphite to the medium causes them to enter programmed cell death within a few days, and the cells die within a week (Singh *et al.*, 2003). There is nothing wrong with either Claude Bernard's concept of homeostasis or with the concept of a P-starvation response, but the two terms are incompatible, and it is not helpful to use the term homeostasis when dealing with plant functioning in response to low-P conditions. If it were to be specified that homeostasis refers to the cytosolic Pi concentration, that would be a different matter, but that would need to spelled out, which is commonly not done.

1.9   Concluding remarks

When it comes to plant P nutrition, which is pivotal for much what is discussed throughout this book, it is clear that there is not much P on planet Earth, it does not rapidly move in the soil and, as a result, soil P is not readily accessible for most plants. Modern agriculture relies heavily on mined phosphate rock to produce fertilizer, but this is obviously a nonrenewable resource that is being depleted (Cordell *et al.*, 2009; Vance *et al.*, 2003). Whilst it is unlikely that 'peak phosphorus' will be reached during the next few decades (Fixen & Johnston, 2012), this does not negate the need to work towards more P-efficient and sustainable food-production systems (Johnston *et al.*, 2014; Scholz & Wellmer, 2013) with less impact on natural ecosystems (Heckrath *et al.*, 1995; Smith & Schindler, 2009). Moreover, we only have to go back to when rock phosphate was first used to fertilise crops to significantly enhance crop yield. It was soon discovered that some sources of rock phosphate contained significant amounts of cadmium found naturally in the organic-rich marine sediments that are precursors to rock phosphate (Filippelli, 2002). Whilst these cadmium levels do not affect plant productivity, they are harmful for consumers of the products from these plants, and hence the

food chain (Chaney, 2012; Chaney, 2013; Grant & Sheppard, 2008). Having plenty of phosphate rock reserves does not mean that these resources are of similar quality as what is used today; some may contain high concentrations of heavy metals, or may be difficult and expensive to mine, and increasing fertilizer prices can therefore be anticipated (Scholz & Wellmer, 2013). Peak P therefore deserves a place on the political agenda, and represents a subject that plant and soil scientists need to bring to the attention of a wider audience.

The various chapters in this book explore plant P nutrition from various angles, enhancing our fundamental understanding of how plants acquire and use this essential nutrient (Chapters 2–4), and how they remobilise P from senescing tissues (Chapters 6 and 10). This will, in turn, allow the development of effective biotechnological strategies to produce plants that are more efficient at either acquiring or using P, and thus enhance the efficiency of cropping systems.

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