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Phosphorus deficiency affects the allocation of below-ground resources to combined cluster roots and nodules in *Lupinus albus*

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ABSTRACT

Lupins can rely on both cluster roots and nodules for P acquisition and biological nitrogen fixation (BNF), respectively. The resource allocation (C, N and P) between cluster roots and nodules has been largely understudied during P-deficient conditions. The aim of this investigation was therefore to determine the changes in resource allocation between these organs during fluctuations in P supply. *Lupinus albus* was cultivated in sand culture for 3 weeks, with either sufficient (2 mM high) or limiting (0.1 mM low) P supply. Although variation on P supply had no effect on the total biomass, there were significant differences in specialised below-ground organ allocation to cluster roots and nodule formation. Cluster root formation and the associated C-costs increased during low P supply, but at sufficient P-supply the construction and growth respiration costs of cluster roots declined along with their growth. In contrast to the cluster root decline at high P supply, there was an increase in nodule growth allocation and corresponding C-costs. However, this was not associated with an increase in BNF. Since cluster roots were able to increase P acquisition under low P conditions, this below-ground investment may also have benefited the P nutrition of nodules. These findings provide evidence that when lupins acquire N via BNF in their nodules, there may be a trade-off in resource allocation between cluster roots and nodules.

Introduction

Phosphate (P) is one of the most limiting mineral nutrients for plant growth (Plaxton and Carswell, 1999; Raghothama, 1999, 2000). Its availability to the plant is limited by various properties of the soil itself and is largely determined by solubilisation of P containing compounds and P diffusion rates in the soil (Silberbush and Barber, 1983). Phosphate readily chelates to metal cations, clay particles and organic soil material rendering it unavailable for plant uptake (Jungk et al., 1993; Richardson, 1994; Abel et al., 2002; Vance et al., 2003). Soil P is also influenced by pH, ionic strength, adsorption and dissolution from these particles (Vance et al., 2003). Slow soil diffusion rates and fast root uptake transporters, causes a rapid depletion of P in the rhizosphere, leading to irregular P distribution in the soil (Lambers et al., 2006). Organic and inorganic compounds readily interact and bind to P (Raghothama, 1999). Plants display great phenotypic plasticity in acquisition strategies for macro-nutrients such as N and P, and can respond to P deficiency by means of a suite of adaptations at the morphological and biochemical level (Keerthisinghe et al., 1998; Vance et al., 2003; Lambers et al., 2006).

It is well established that plants preferentially allocate resources to increase below ground biomass and growth under P limitation. This is often at the expense of growth and photosynthesis (Cakmak, 1994; Raghothama, 1999; Vance et al., 2003; Lambers et al., 2006) cluster or proteoid roots are a combined physiological and morphological below-ground adaptation for phosphate (P) acquisition in P-deficient soils (Dinkelaker et al., 1995). The production of cluster roots will incur a C and nutrient (N and P) cost to the plant. Moreover, the cost of cluster root production must be kept to a minimum to decrease negative growth effects at the whole plant level. The root system alone can consume 11–14% of fixed carbon to maintain functionality (Kaschuk et al., 2009). Under P-limitation, cluster roots can constitute more than 50% of the root system (Reddell et al., 1997; Lamont, 2003). P must ultimately be transported from the cluster roots to other plant organs if plant P status is to be maintained. It was shown by Keerthisinghe et al. (2002) that plant growth could be maintained, if cluster roots constitute more than 50% of the root system. The exact costs of cluster roots *vs.* roots in relation to respiratory costs are currently unknown (Lamont, 2003); however, cluster root growth and

Abbreviations: P Max, maximum rate of photosynthesis; PNUE, photosynthetic nitrogen use efficiency; PPUE, photosynthetic phosphate use efficiency; %NDFA, nitrogen derived from atmosphere; RGR, relative growth rates; SNAR, specific nitrogen acquisition rate; SNUR, specific nitrogen utilisation rate; SPAR, specific phosphate acquisition rate; SPUR, specific phosphate utilisation rate.

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function must incur a large C burden on the plant (Lambers et al., 2006). Most species of plants associated with cluster root formation can symbiotically fix atmospheric nitrogen via biological nitrogen fixation (BNF) (Skene, 1998), but interestingly do not form mycorrhizal associations (Skene, 1998; Neumann and Martinoia, 2002).

Legumes are well known for their symbiotic relationship with rhizobia (Valentine et al., 2011). This symbiosis allows for N acquisition through BNF, bypassing the need for direct N uptake. BNF is a notoriously energetically expensive process, consuming on average 20 ATP (including obligatory H_2 evolution), per reaction, for the production of two NH_3 molecules (Schulze et al., 1999). Production of ATP is a high P requiring reaction, consuming phosphate per nitrogenase reaction. Comparatively, nitrate reduction to ammonia, after direct transporter uptake, indirectly consumes 15 ATPs (Valentine et al., 2011). It is furthermore, also known that nodulated plants expend more P on BNF when compared to direct N uptake mechanisms (Sa and Israel, 1991). Nodules act as strong sinks for P even under adequate P supply (Drevon and Hartwig, 1997). This is compounded during P deficiency where nodules often exhibit higher P content when compared to roots and shoots (Drevon and Hartwig, 1997). Høgh-Jensen et al. (2002) also showed that P is preferentially partitioned to nodules for maintenance of BNF rates under P-deficiency, sometimes at the expense of plant growth. Apart from a strong P sink, nodules must also be supplied with photosynthate in the form of malate. Nodules thus incur a large C and P burden on the plant. The model legume, white lupin (*Lupinus albus*) readily nodulates with *Bradyrhizobium sp*. to form effective nodules (Schulze et al., 2006) and is also one of the best documented, cluster root forming species (Watt and Evans, 1999; Neumann et al., 2000; Neumann and Martinoia, 2002; Lamont, 2003; Cheng et al., 2011). It is therefore an ideal model to use for the investigation of the costs associated with nutrient acquisition via nodules and cluster roots.

Overall, there is very little known about the costs of combined cluster roots and nodules under P deficiency in any lupin species. Therefore, the aim of this study was to investigate the below-ground allocation of C, N and P to nutrient acquisition organs (roots, nodules and cluster roots), during P deficiency in the model legume *L. albus*. In this regard, the carbon costs of both cluster roots and nodule development during P limitation was assessed, via biomass and growth kinetics, nutrient acquisition efficiencies, respiratory and photosynthetic costs.

Materials and methods

Plant growth conditions

Lupinus albus (*L. albus* cv. Andromeda) seeds were germinated in vermiculite before transplantation to sand culture. Seeds were sterilised and then inoculated with a commercially available inoculum (StimuPlant cc) containing *Bradyrhizobium* sp (*Lupinus*) and grown in vermiculite for 10 days. Thereafter, plants were transplanted into 20 cm pots and cultivated in quartz sand for 21 days. The plants were divided into two treatment groups, low phosphate (LP) and high phosphate (HP), each receiving a modified Long Ashton (Smith et al., 1983) solution containing either 2 mM (HP) or 0.1 mM (LP) $NaH_2PO_4{\cdot}2H_2O$ as phosphate source (Keerthisinghe et al., 1998; Le Roux et al., 2006, 2009). The pH of the solution was adjusted to 6.5, and 400 ml was applied to the plants once a week, furthermore, the plants received distilled H_2O every other day. No N source was added to ensure nodulation and BNF. Plants were grown under glasshouse conditions in a north-facing glasshouse at the University of Stellenbosch between the months of April and June. The range of midday irradiances was between 400 and 600 $\mu mol\,m^{-2}\,s^{-1}$ and the average night/day temperatures were 13–22 °C.

Photosynthesis and gas exchange measurements

The youngest fully expanded leaf was used for photosynthetic measurements. Light–response curves were used to determine the appropriate photon flux density (800 $\mu mol\,m^{-2}\,s^{-1}$) at which to conduct photosynthetic measurements. Readings were taken between 11 am and 4 pm, using the LI-6400XT portable photosynthesis and fluorescence system (Li-Cor, Lincoln, Nebraska,USA).

Photosynthetic CO_2 response curves were carried out in order to determine the maximum photosynthesis rate (Pmax), Rubisco activity and electron transport. Measurements were performed on the youngest fully expanded leaves (5 replicates in each treatment per species), using a Li-6400 gas exchange system (LI-COR Inc., IRGA, Lincolin, NE, USA). Measurements were taken between 9 am and 4 pm. A full response curve took 45 min to 1 h to complete. The leaves were enclosed in a leaf chamber (6 cm^2), which received a steady light of 800 $\mu mol\,m^{-2}\,s^{-1}$ at a leaf temperature of 24 °C. CO_2 concentrations increased according to the following increments: 50 and 100 ppm.

Harvesting and nutrient analysis

Seedlings were harvested at 30 days after transplantation into the sand culture. Upon harvesting, the plants were separated into nodules, roots, stems and leaves. The harvested plant material was placed in a drying oven, at 40°C for 3 days and their dry weights (DW) were recorded. The dried material was milled with a ball mill. The milled samples were analysed for their respective C, N and P concentrations by a commercial laboratory, using inductively coupled mass spectrometry (ICP-MS) and a LECO-nitrogen analyser with suitable standards (BemLab, De Beers Road, Somerset West, SA).

Carbon and nutrition cost calculations

Construction costs, C_W ($mmol\,C\,g^{-1}\,DW$), were calculated according to the methods of Mortimer et al. (2005), modified from the equation used by Peng et al. (1993):

$$C_W = \left[C + kN \times \frac{180}{24}\right]\left(\frac{1}{0.89}\right)\left(\frac{6000}{180}\right)$$

where C_W is the construction cost of the tissue ($mmol\,C\,g^{-1}\,DW$), C is the carbon concentration (mmol C/g), k is the reduction state of the N substrate ($k = -3$ for NH_3) and N is the organic nitrogen content of the tissue (g/DW) (Williams et al., 1987). The constant (1/0.89) represents the fraction of the construction costs that provides reductant that is not incorporated into the biomass (Williams et al., 1987; Peng et al., 1993) and (6000/180) converts units of g glucose/DW to mmol C g/DW.

Specific nitrogen absorption rate (SNAR) ($mg\,N\,g^{-1}\,root\,DW\,d^{-1}$) is the calculation of the net N absorption rate per unit root DW (Nielson et al., 2001):

$$SNAR = \left[\frac{M_2 - M_1}{t_2 - t_1}\right] \times \left[\frac{\log_e R_2 - \log_e R_1}{R_2 - R_1}\right]$$

where M is the N content per plant, t is the time and R is the root DW.

Specific nitrogen utilisation rate (SNUR) ($g\,DW\,mg^{-1}\,N\,d^{-1}$) is a measure of the DW gained for the N taken up by the plant (Nielson et al., 2001): $SNUR = \left[\frac{W_2 - W_1}{t_2 - t_1}\right] \times \left[\frac{\log_e M_2 - \log_e M_1}{M_2 - M_1}\right]$

Specific P absorption rate (SPAR) ($mg\,N^{-1}\,g\,root\,DW^{-1}\,d^{-1}$) is the calculation of the net P absorption rate per unit root DW (Nielson et al., 2001): $SPAR = \left[\frac{M_2 - M_1}{t_2 - t_1}\right] \times \left[\frac{\log_e R_2 - \log_e R_1}{R_2 - R_1}\right]$ where M is the P content per plant, t is the time and R is the root DW.

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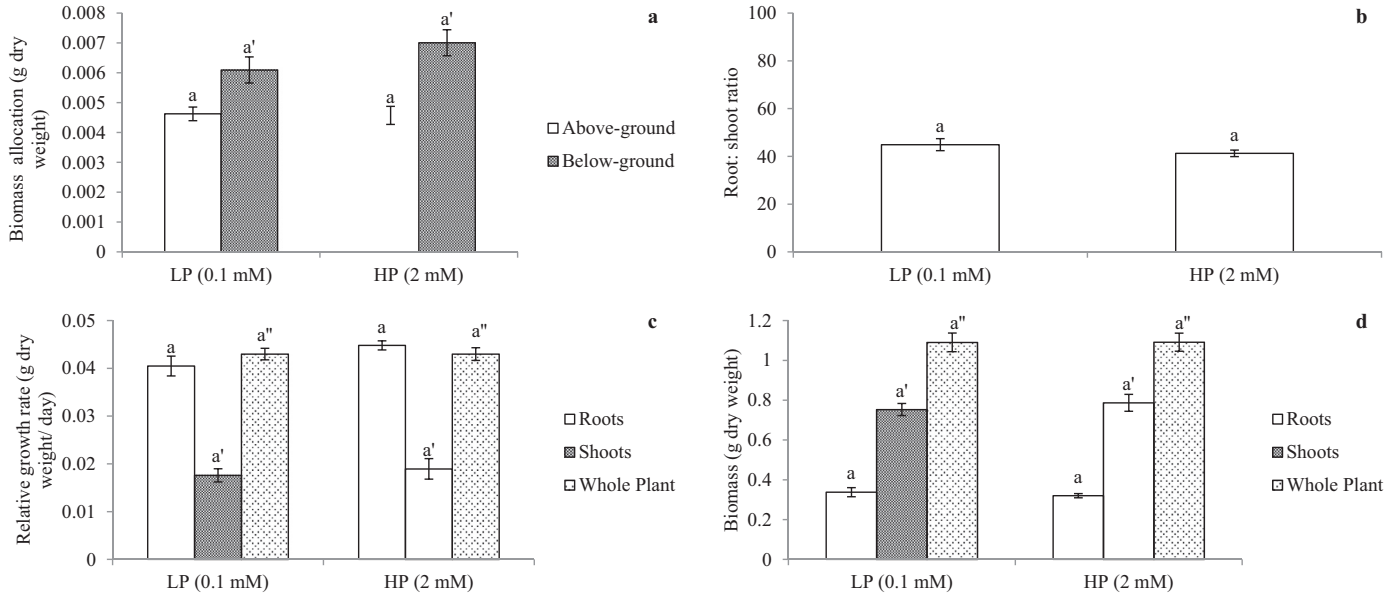

Fig. 1. Above and below ground allocation (a), root:shoot ratio (b), relative growth rates (c) and root, shoot and whole plant biomass (d) of LP (0.1 mM) and HP (2 mM) treated *Lupinus albus* plants. Values are presented as means ($n = 6$–8) with standard error bars, The different letters indicate significant differences among the treatments, where the prime lettering indicates the comparisons between the same organ ($P \leq 0.05$).

Specific phosphate utilisation rate (SPUR) (g DW mg^{-1} P d^{-1}) is a measure of the DW gained for the P taken up by the plant (Nielson et al., 2001):

$$\text{SPUR} = \left[\frac{W_2 - W_1}{t_2 - t_1}\right] \times \left[\frac{\log_e M_2 - \log_e M_1}{M_2 - M_1}\right]$$

where M is the P content and W is the plant DW.

Growth respiration R_g (t) (μmol CO_2 d^{-1}) is the daily growth respiration of the plant (Peng et al., 1993):

$$R_g(t) = C_t - \Delta W_c$$

C_t (μmol CO_2 day^{-1}) is the C required for daily construction of new tissue. C_t was calculated by multiplying the root growth rate (g DW day^{-1}) by tissue construction cost (C_w). ΔW_c (μmol C day^{-1}) is the change in root C content and was calculated by multiplying the root C content and root growth rate.

Calculations of $\delta^{15}N$

The $\delta^{15}N$ analyses were carried out at the Archeometry Department, University of Cape Town. The isotopic ratio of $\delta^{15}N$ was calculated as $\delta = 1000‰$ ($R_{sample}/R_{standard}$), where R is the molar ratio of the heavier to the lighter isotope of the samples and standards is as defined by Farquhar et al. (1989). Between 2.100 and 2.200 mg of each milled sample were weighed into 8 mm × 5 mm tin capsules (Elemental Micro-analysis Ltd., Devon, UK) on a Sartorius microbalance (Goettingen, Germany). The samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyser (Fisons instruments SpA, Milan, Italy). The $\delta^{15}N$ values for the nitrogen gas released were determined on a Finnigan Matt 252 Mass Spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyser by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift: two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard $(NH_4)_2SO_4$.

Nitrogen derived from atmosphere (%NDFA) was calculated according to Shearer and Kohl (1986):

$$\%\text{NDFA} = 100\left(\frac{\delta^{15}N_{\text{reference plant}} - \delta^{15}N_{\text{legume}}}{\delta^{15}N_{\text{reference plant}} - B}\right)$$

where the reference plant was wheat (*Triticum aestivum*) grown under the same glasshouse conditions. The B-value is the $\delta^{15}N$ natural abundance of the N derived from biological N-fixation of the above-ground tissue of *Virgilia divaricata*, grown in a N-free solution. The B value of was determined as −0.71‰.

Statistical analysis

The effects of the factors and their interactions were tested with an analysis of variance (ANOVA) (Super-Anova, Statsgraphics Version 7, 1993, Statsgraphics Corporation, USA). Where the ANOVA revealed significant differences between treatments, the means (6–8) were separated using *post hoc* Student–Newman–Kuehl's (SNK) multiple-range test ($P \leq 0.05$). Different letters indicate significant differences between treatments, where the prime lettering indicates the comparisons between the same organs.

Results

Plant biomass, relative growth rates (RGR) and construction costs

There was no difference in above and below ground allocation of biomass between treatment groups (Fig. 1a) and thus no change was observed in the root:shoot ratio (Fig. 1b). Furthermore, there was no significant difference in the relative growth rates for roots, cluster roots, nodules or shoots between treatment groups (Fig. 1c). Biomass allocation to roots, shoots and on a whole plant basis, was unchanged between treatments (Fig. 1d).

Biomass allocation within the root system did however show a marked change between treatments. Plants subjected to P deprivation produced more cluster root biomass and less nodule biomass, when compared to plants supplied with sufficient P (Fig. 2a). Cluster roots furthermore constituted up to 24% of the root system in P deficient plants, but less than 5% in plants supplied with sufficient P

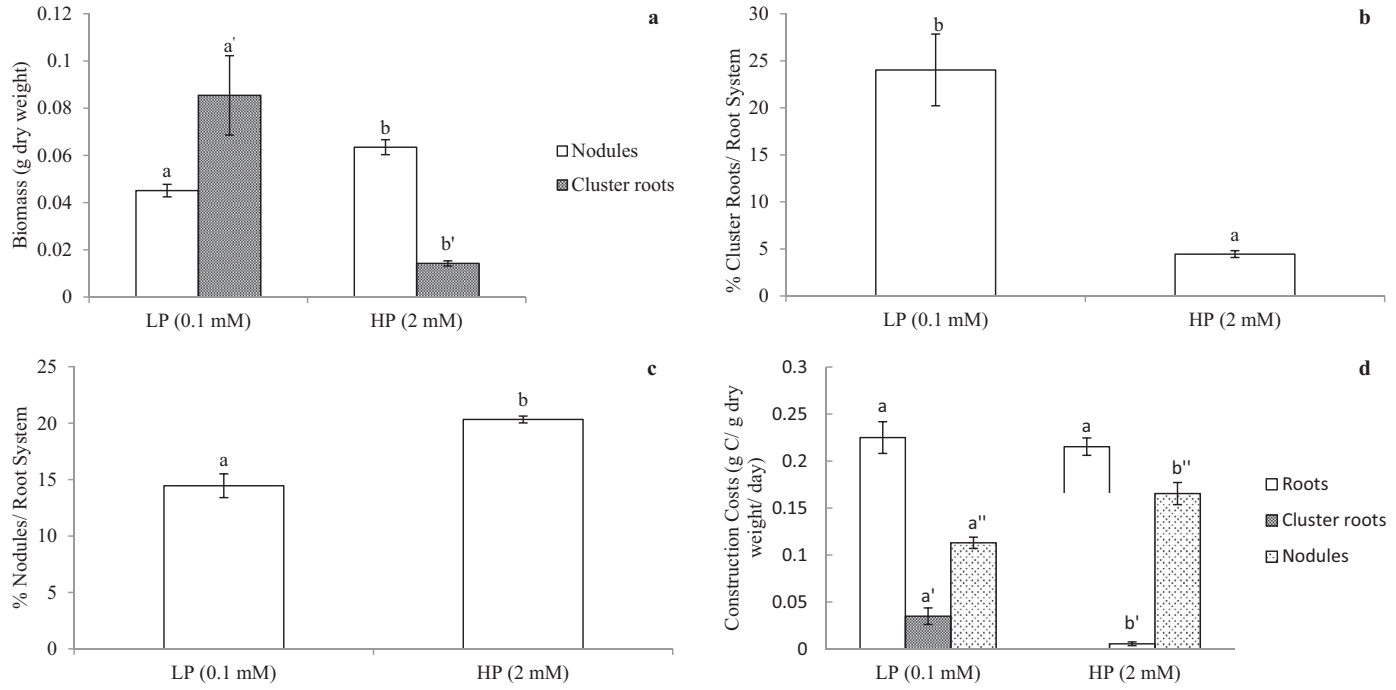

Fig. 2. Nodule and cluster root biomass (a), percentage (%) of cluster roots (b) and nodules (c) per root system and growth respiration (d) of LP (0.1 mM) and HP (2 mM) treated *Lupinus albus* plants. Values are presented as means ($n = 6–8$) with standard error bars. The different letters indicate significant differences among the treatments, where the prime lettering indicates the comparisons between the same organs ($P \leq 0.05$).

(Fig. 2b). Nodules constituted up to 20% of the root system of plants supplied with sufficient P, while only 14% in the P deficient treatment group (Fig. 2c). The construction costs for cluster roots were significantly increased in P deficient plants, while nodule construction costs were decreased. Construction costs of roots were similar for both treatment groups (Fig. 2d).

Photosynthesis and growth respiration

Maximum photosynthetic capacity was significantly lowered in P deficient plants (Fig. 3a). This is coupled with reduced photosynthetic phosphate (Fig. 3b) and nitrogen use efficiencies (Fig. 3c). Nodules of plants supplied with adequate P showed a significant increase in nodule growth respiration (Fig. 3d). There were no significant differences in the growth respiration rates for roots, cluster roots and shoots between treatment groups. It must however be noted that the majority of growth respiration investment was in shoots and the least in cluster roots (Fig. 3d).

Mineral nutrition

The concentration of N and P did not significantly differ for roots, cluster roots or shoots between treatment groups (Fig. 4a and b). There was however an increase in P concentration in the nodules of plants supplied with sufficient P (Fig. 4a). Shoots on average contained the largest concentration of N, while roots the largest concentration of P (Fig. 4a and b). No significant difference in Nitrogen derived from atmosphere (%NDFA) was observed between treatment groups in any plant organ (Fig. 4c). BNF efficiency was also similar for both treatment groups (Fig. 4d).

Cluster roots of P deficient plants showed a significant increase in P acquisition rates when compared to cluster roots of plants supplied with sufficient P (Fig. 5c). The utilisation rates of both N and P were however not significantly different for roots and shoots between treatments (Fig. 5b and d). Cluster roots of plants supplied with adequate P had a significantly increased P utilisation rate (Fig. 5d).

Discussion

When plants are dependent on BNF via symbiotic nodules, there may be a trade-off in resource allocation between cluster roots and nodules. Both cluster roots and nodules require resources, in the form of P, N and C for maintenance, growth and functionality. When any of these resources are deficient, competition can arise between sinks organs for valuable resources (Lynch and Ho, 2005). This is evident from the increase in cluster root formation during P deficiency, but decline during P sufficient conditions. The opposite is true of nodules, where more nodules were formed during adequate P supply.

During P-deficiency, the absence of any effects on gross biomass accumulation or allocation to below and above-ground organs in *L. albus*, may indicate the extreme adaptation of lupins to P-deficient conditions. This concurs with previous work, where Schulze et al. (2006) found that *L. albus* grown with no added P, showed no effect on biomass accumulation when compared with plants supplied with sufficient P for up to 27 days of growth. Similar results have also been reported by Abdolzadeh et al. (2010), where there was no significant difference in gross biomass for *L. albus* before 32 days of LP treatment. This could be attributed to cluster root mobilisation and uptake of P, compensating for decreased P supply. These temperate legumes are also mainly found in nutrient-poor, acidic soils, conditions that would limit growth in most plants (Gilbert et al., 1998).

Despite the absence of changes in total biomass, significant difference in specialised below-ground organ allocation to cluster roots and nodules, suggest that these adaptations may have underpinned the plant's tolerance of P-deficient conditions. The greater biomass allocation to cluster roots during P-deficiency specifically concurs with previous work. Keerthisinghe et al. (1998), Watt and Evans (1999) and Neumann et al. (2000), all report increased

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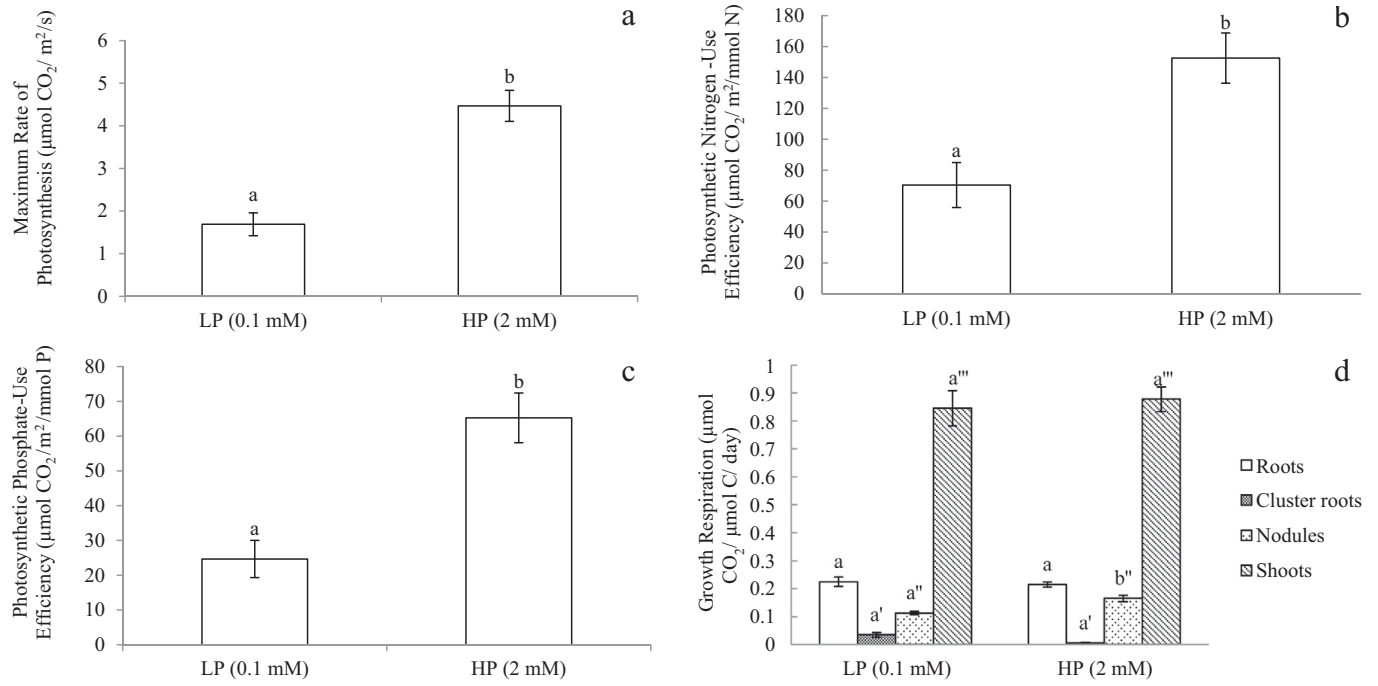

Fig. 3. Maximum photosynthetic capacity (a), photosynthetic nitrogen (b) and phosphate (c) use efficiency and growth respiration of roots, cluster roots, nodules (d) and shoots of LP (0.1 mM) and HP (2 mM) treated *Lupinus albus* plants. Values are presented as means ($n = 6–8$) with standard error bars. The different letters indicate significant differences among the treatments, where the prime lettering indicates the comparisons between the same organs ($P \leq 0.05$).

biomass allocation to cluster roots during P-deprivation. The reduction of cluster root growth during sufficient P supply, thus points to the plasticity of these specialised roots for P-acquisition. This functional plasticity is however associated with C-costs, since with sufficient P-supply, construction and growth respiration costs of cluster roots declined. It has been shown by Shane et al. (2003) and Li and Liang (2005) that cluster root formation is reduced in plants supplied with sufficient P. This decline in cluster root formation can be attributed to a threshold shoot P level that suppresses cluster root formation during sufficient P supply (Shen et al., 2005) leading to decreased demand for C. The main functions of cluster roots are the location and acquisition of P during low P supply (Lamont, 2003). Once favourable P conditions return, cluster root production declines, since roots themselves are capable of P acquisition via P-transporters (Marschner et al., 1996). Cluster roots consumed more P during favourable conditions instead of acquiring P. This can be

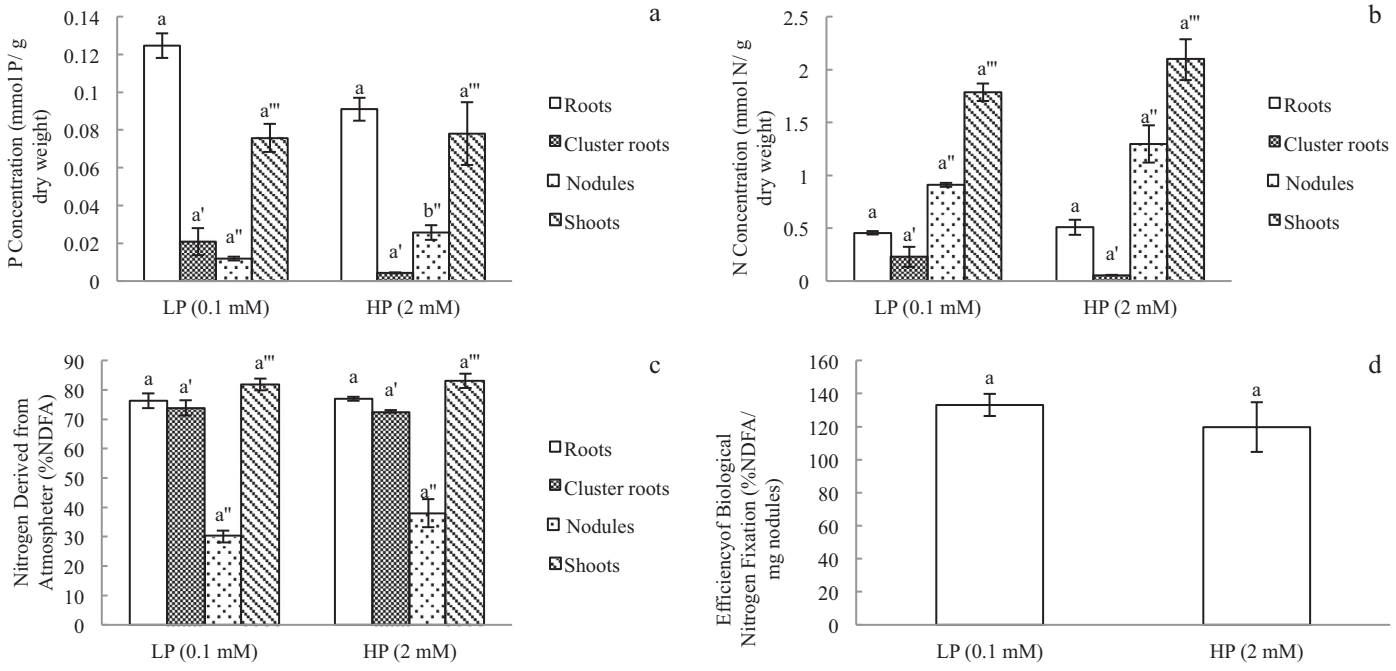

Fig. 4. mmol P (a), mmol N (b) and nitrogen derived from atmosphere (c) of roots, cluster roots, nodules and shoots, and biological nitrogen fixation efficiency (BNF) (d) of LP (0.1 mM) and HP (2 mM) treated *Lupinus albus* plants. Values are presented as means ($n = 6–8$) with standard error bars. The different letters indicate significant differences among the treatments, where the prime lettering indicates the comparisons between the same organs ($P \leq 0.05$).

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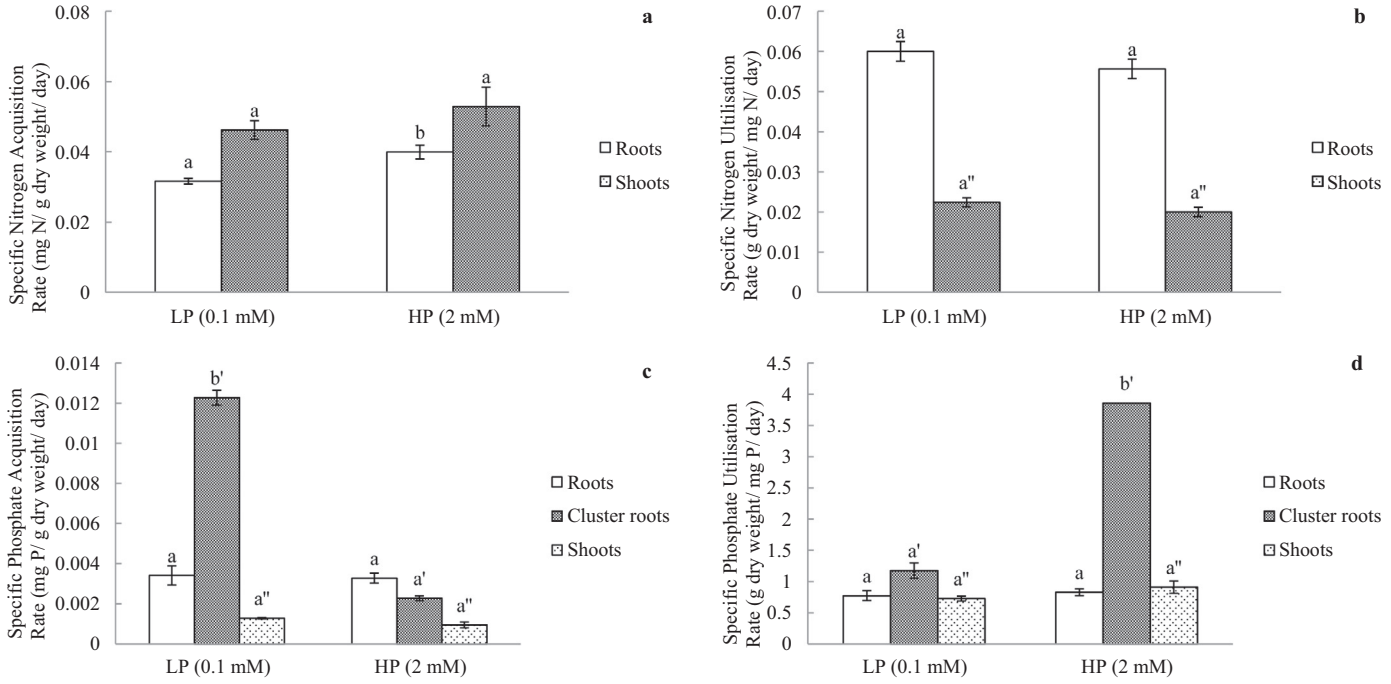

Fig. 5. Specific nitrogen acquisition rate (a), specific nitrogen utilisation rate (b), specific phosphate acquisition rate (c) and specific phosphate utilisation rate (d) of roots, cluster roots and shoots of LP (0.1 mM) and HP (2 mM) treated *Lupinus albus* plants. Values are presented as means ($n = 6–8$) with standard error bars. The different letters indicate significant differences among the treatments, where the prime lettering indicates the comparisons between the same organs ($P \leq 0.05$).

seen from the large increase in P utilisation rate, yet decrease in P acquisition rate and biomass of cluster roots under sufficient P supply. Cluster roots thus appear to function optimally at low P supply. During high P supply nutrient resources are diverted from cluster root production and function to other plant organs, mainly nodules.

Increased C-costs under deficient P-supply can be associated with more than just growth and construction costs for cluster roots; it also includes the functional advantage of these organs under nutrient deficient conditions. The improved P acquisition in cluster roots is underpinned by the specific P acquisition rates (SPAR) of these organs. In this regard, the high uptake rate of P might be a factor of increased surface area or high P-transporter activity in cluster roots as shown in previous studies (Lui et al., 2001; Lamont, 2003). Keerthisinghe et al. (1998) showed that cluster roots increased the uptake of P from soils, by increased mining of the rhizosphere depletion zone. Cluster roots also increase the absorptive area of nutrient uptake by an increased (more than a 100-fold) surface/area ratio due to their fine structure and bottlebrush-like architecture (Schulze et al., 2002; Lamont, 2003). Lui et al. (2001) reported increased expression of phosphate transporters, LaPT1 and LaPT2, in cluster roots of P-deficient *L. albus*. LaPT1expresssion was dramatically increased in cluster roots (compared to normal roots) during P deficiency and lead to enhance P acquisition by cluster roots. LaPT1 is further only expressed under P-deficient conditions in both normal roots and cluster roots, whilst LaPT2 is uniformly expressed in both roots and cluster roots, irrespective of P supply (Lui et al., 2001). The functional and growth costs of these cluster roots were however not reflected as a sink stimulation of leaf photosynthesis. Instead, increased nodule growth, once cluster root growth subsided at sufficient P levels, appears to have been a larger sink for photosynthesis stimulation. Nodules can cause a sink stimulation of photosynthesis due to increased phloem loading of photosynthates, associated with the C-costs of this symbiosis (Kaschuk et al., 2009). This is verified by the absence of any increases in leaf P, during sufficient P conditions.

The increase in nodule biomass investment coincides with a decrease in cluster root production, during adequate P supply. This increase however, did not translate into an increased plant dependence on BNF, or improvement of the plant's nitrogen nutrition. The unchanged BNF efficiencies under P supply may imply optimal functioning of nodules under low P supply, as found in *Lupinus angustifolius* where no change in BNF efficiencies or %NDFA was observed during short term P deprivation (Le Roux et al., 2006, 2009). Legumes are known to produce more, but smaller nodules during P-deficiency (Schulze et al., 2006). These nodules are less effective due to increased O_2 diffusion caused by an increase in the surface/area ratio. Due to the number of nodules produced however, similar BNF rates, comparable to non-stressed plants, can be maintained (Schulze et al., 2006). It may also be possible that the cluster roots may have improved the P nutrition to the nodules as evidenced by previous work on white lupins (Schulze et al., 2006; Mortimer et al., 2008). In spite of their unchanged function, the increase in nodule growth allocation may have imposed sink stimulation, due to increased construction costs and growth respiration at high P supply. Nodules are known to act as strong sinks for both C and P resources, even under sufficient P supply. Nodulated legumes have been shown to require more P compared to unnodulated legumes (Høgh-Jensen et al., 2002), with nodules consuming up to 4–16% of recently fixed photosynthate (Kaschuk et al., 2009). This can lead to sink stimulation via photosynthesis for increased C supply, as is evident in this study.

These findings suggest that when plants are dependent on BNF via nodules, a physiological trade-off may exist between cluster roots and nodules during low P supply. Valuable resources in the form of C and P are redirected from cluster roots to nodules during adequate P supply. This was evident in the increased photosynthetic rates, P concentration and growth of nodules during high P supply. Nodules thus appear to have adapted to maintain function and efficiency of BNF, despite changes in P nutrition and costs associated with cluster roots, during fluctuations in P supply. This increase in the costs of cluster roots during P deficiency appears to have improved the P nutrition of nodules in order to maintain their function under P stress.

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