

## Low-phosphorus conditions affect the nitrogen nutrition and associated carbon costs of two legume tree species from a Mediterranean-type ecosystem

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**Abstract.** The role of phosphorus nutrition in two-legume tree species from the Mediterranean-type ecosystem of the Cape Floristic Region (CFR) in South Africa was investigated. There is very little information about the functional adaptations of nitrogen (N) and phosphorus (P) nutrition in these legume trees growing in nutrient-poor soils. Nodulated *Virgilia divaricata* and *V. oroboides* tree saplings were grown in sterilised sand and supplied with Long Ashton nutrient solution, which was modified to contain either sufficient-phosphate (500 µM) or low-phosphate (5 µM) nutrient solution for 90 days. During low-P conditions, the growth of *V. divaricata* was not affected, whereas *V. oroboides* showed a decrease in growth. The decrease in *V. oroboides* under low-P conditions was related to the lower P uptake, which resulted in an alteration in belowground biomass allocation, which consequently affected on the N nutrition and carbon (C) cost of growth. In this regard, *V. oroboides* plants allocated less biomass to roots and nodules, as a proportion of whole plant growth. The impact of this was a decline in N nutrition, growth respiration and photosynthetic costs in *V. oroboides*. In contrast, *V. divaricata* maintained its P concentrations, photosynthetic costs and increased its nodule allocation under low-P conditions, to the benefit of N nutrition. The two CFR tree legumes appear to have different adaptations to low-P conditions, which may influence their N and P acquisition in their naturally low-P environment.

**Additional keywords:** acidic soils, biological nitrogen, fynbos, nutrient deficiencies fixation, *Virgilia*.

Received 29 October 2013, accepted 11 February 2014, published online 14 April 2014

### Introduction

The Mediterranean-type ecosystem of the Cape Floristic Region (CFR) in South Africa mainly grows on sandstone-derived soils (Goldblatt and Manning 2000), which are typically very acidic and nutrient poor (Kruger *et al.* 1983). The soils of this region bear a resemblance to the soils of the Western Australian heathlands rather than to those of other Mediterranean-climate regions (Groves 1983; Mitchell *et al.* 1984). *Virgilia* is one of tree legume genera that are endemic to these CFR (fynbos) acidic soils. Mediterranean acidic soils usually have different concentrations of elements, coupled with related nutrient deficiencies, associated with nitrogen (N) and phosphorus (P) deficiencies (Bordeleau and Prevost 1994; von Uexkull and Mutert 1998; Grigg *et al.* 2008). Soil acidity is a significant problem facing legume and agricultural production in many areas of the world, including southern Africa (Graham 1992; Bordeleau and Prevost 1994; Marschner 1995; Correa and Barneix 1997). Most legume plants require neutral to slightly acidic soils for growth, but experience problems with nodulation if the pH drops to a very acidic state (Lie 1981; Munns 1986; Graham 1992; Bordeleau and Prevost 1994; Marschner 1995; Correa and Barneix 1997). Both soil acidity and nutritional disorder adversely affect the survival, growth and N fixation of

microorganisms because they affect legume–rhizobia symbiosis (Lie 1981; Munns 1986; Graham 1992). P remains mostly unavailable for plant uptake, specifically on acid-weathered soils in both tropical and subtropical regions (Bieleski 1973; Schachtman *et al.* 1998; von Uexkull and Mutert 1998; Vance 2001).

Phosphate is quite abundant in many soils, but because it forms insoluble complexes with cations (calcium and iron) and is bound to organic compounds by microbial action, it is often unavailable for plant uptake (Jungk *et al.* 1993; Richardson 1994; Hinsinger 2001; Dakora and Phillips 2002; Vance 2001). Low-P soils will limit legume growth to a greater extent than do low-N soils, because during symbiosis with rhizobia, legumes can utilise both atmospheric N and soil N acquired through rhizobia in their nodules (Mortimer *et al.* 2008). It has been reported that host legume nodules require comparatively high amounts of P and energy; thus, P deficiency can impair both nodulation and symbiotic N fixation, affecting photosynthesis, respiration, growth, organic-acid supply and production of the host (Drevon and Hartwig 1997; Vadez *et al.* 1997; Almeida *et al.* 2000; Olivera *et al.* 2004; Lynch and Ho 2005; Harrison *et al.* 2009). Despite these complications, legume species endemic to fynbos preferentially grow specifically in such

acidic soils. They must, therefore, have evolved adaptations to function optimally under these limiting P conditions (Vance *et al.* 2003). Some strategies are aimed at conserving the use of P, whereas others are directed toward enhanced acquisition and uptake of P (Lajtha and Harrison 1995; Raghothama 1999; Horst *et al.* 2001; Vance 2001). Adaptations that conserve the use of P involve a decrease in growth rate, increased growth per unit of P uptake, remobilisation of internal inorganic P (Pi), modification in carbon (C) metabolism that bypass P-requiring steps and alternative respiratory pathways (Schachtman *et al.* 1998; Plaxton and Carswell 1999; Raghothama 1999; Uhde-Stone *et al.* 2003a, 2003b). In legumes, adaptations leading to enhanced P acquisition entail the expression of genes that result in the production of cluster roots. Cluster roots increase the root surface area. This enhances nodule efficiency for P utilisation (Le Roux *et al.* 2009), root exudation of organic acids and acid phosphatase, as well as the induction of numerous transporters (Gilbert *et al.* 1999; Gilroy and Jones 2000; Lynch and Brown 2001; Neumann and Martinoia 2002; Lamont 2003; Uhde-Stone *et al.* 2003a; Vance *et al.* 2003). The exudation of organic acids such as malate and citrate stimulated by P stress has mostly been reported in non-mycorrhizal species such as lupin (Dinkelaker *et al.* 1995; Jones 1998; Hinsinger 2001; Ryan *et al.* 2001; Le Roux *et al.* 2008).

The high sensitivity of legume plants, and indeed the N<sub>2</sub>-fixation process to environmental conditions such as acidic soils associated with P deficiency, may result in higher C costs (Mengel 1994). This concurs with Le Roux *et al.* (2009), who showed that lupin nodules under P stress acted as stronger C sinks. Nodules are known to have a strong sink capacity for P assimilation during P starvation (Hogh-Jensen *et al.* 2002). The enhanced nodule cost for P utilisation is considered to be an essential coping strategy during P stress (Le Roux *et al.* 2009). The C sink was found to be more pronounced in plants during double symbiosis under low-P conditions (Mortimer *et al.* 2008). This was shown by a greater growth respiration of low-P plants than high-P plants (Mortimer *et al.* 2008). The sink effect was also evidenced by the higher photosynthetic rates of host plant (Mortimer *et al.* 2008). In the case of P stress, the most direct currency is P itself or growth parameters related to P accumulation (Koide *et al.* 2000).

*Virgilia* is a small tree genus that includes two species (*V. divaricata* (Adamson) and *V. oroboides* (P.J.Bergius) Salter) and two subspecies. It is confined to the south-western and southern coastal regions of the CFR (Greinwald *et al.* 1989). Studies have been conducted on growth and adaptations of legume species native to Mediterranean-type fynbos ecosystems that occur on naturally acidic soils (Muofhe and Dakora 1999; Spriggs and Dakora 2008; Power *et al.* 2010; Kanu and Dakora 2012). However, information on the physiology of N and P uptake, efficiency and utilisation in legume trees in fynbos soils is largely unknown.

The mechanisms of legume adaptations to acquire N from both the atmosphere and soil in a low-P environment will assist in the understanding of the distribution of these trees in their native environment of the CFR. The aim of the present study was, therefore, to investigate how low P nutrition affects the N acquisition pathways and their associated costs in two indigenous fynbos legumes, *V. divaricata* and *V. oroboides*.

## Materials and methods

### *Plant material and growth conditions*

Seeds of both *V. oroboides* and *V. divaricata* were obtained from Kirstenbosch Botanical Gardens, Cape Town, South Africa, and scarified using an acid-scarification method that entails soaking the seeds in sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) for 30 min and then rinsing them 10 times in distilled water. Hereafter, seeds were treated overnight with smoke water, also obtained from Kirstenbosch. The seeds were germinated in natural fynbos soil (natural inoculation), obtained from Stellenbosch Mountain, Stellenbosch, South Africa. Plants were grown under glasshouse conditions at the University of Stellenbosch, South Africa, and they were watered daily. The range of midday irradiances was between 600 and 800 μmol m<sup>-2</sup> s<sup>-1</sup> and the average night and day temperature ranges were 15–25°C. After 40 days, the plants were transferred to clean sand and initially watered with distilled water for a week to acclimatise. Hereafter, seedlings were supplied with low N (500 μM), quarter-strength Long Ashton nutrient solution modified to sufficient P (500 μM) and low P (5 μM) (pH 5.8) once a week and watered with distilled water in between nutrient-solution supply. The experiment was split between the two species, so that each species was subjected to low- and sufficient-P treatments. The combination of species and P concentrations resulted in four treatments, with eight replicates each.

### *Harvesting and nutrient analysis*

Seedlings were harvested at 90 days after transplanting into the sand culture. On 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 the dry weights (DWs) of plant parts 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 plasma–mass spectrometry (ICP-MS) and a LECO-nitrogen analyser with suitable standards (BemLab, De Beers Road, Somerset West, South Africa).

### *Gas-exchange measurements*

The photosynthetic response to varying levels of light was determined to measure maximum photosynthetic rate ( $P_{max}$ ) on a light-response curve. Measurements were performed on the youngest fully expanded leaves (5 replicates in each treatment per species), using an open gas-exchange system Li-6400 (LI-COR, IRGA, Lincoln, NE, USA). Measurements were taken from 0900 hours to 1600 hours. A full light-response curve took 60–90 min to complete. The leaves were enclosed in a leaf chamber and at a leaf temperature of 24°C, the light was varied from 0, 50, 100, 150, 200, 250, 350, 500, 650, 800, 900, 1000, 1500 to 2000 μmol m<sup>-2</sup> s<sup>-1</sup>.

### *Carbon and nutrition cost calculations*

- (1) Construction costs,  $C_W$  (mmol C g<sup>-1</sup> DW), were calculated according to the methods of Mortimer *et al.* (2005), modified from the equation used by Peng *et al.* (1993), as follows:

$$C_w = [C + kN/14 \times 180/24](1/0.89)(6000/180),$$

where  $C_w$  is the construction cost of the tissue (mmol C g<sup>-1</sup> DW),  $C$  is the carbon concentration (mmol C g<sup>-1</sup>),  $k$  is the reduction state of the N substrate ( $k = -3$  for NH<sub>3</sub>) and  $N$  is the organic-N content of the tissue (g DW<sup>-1</sup>) (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 per DW to mmol C per g DW.

- (2) Specific N-absorption rate (SNAR; mg N g<sup>-1</sup> root DW day<sup>-1</sup>) is the calculation of the net N-absorption rate per unit root DW (Nielsen *et al.* 2001), as follows:

$$\text{SNAR} = [(M_2 - M_1/t_2 - t_1)] \\ \times [(\log_e R_2 - \log_e R_1)/(R_2 - R_1)],$$

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

- (3) Specific N-utilisation rate (SNUR; g DW mg<sup>-1</sup> N day<sup>-1</sup>) is a measure of the DW gained for the N taken up by the plant (Nielsen *et al.* 2001), as follows:

$$\text{SNUR} = [(W_2 - W_1/t_2 - t_1)] \\ \times [(\log_e M_2 - \log_e M_1)/(M_2 - M_1)],$$

where  $M$  is the N content and  $W$  is the plant DW.

- (4) Growth respiration  $R_g(t)$  (μmol CO<sub>2</sub> day<sup>-1</sup>) is the daily growth respiration of the plant (Peng *et al.* 1993), as follows:

$$\text{growth respiration } R_g(t) = C_t - \Delta W_c,$$

where  $C_t$  (μmol CO<sub>2</sub> day<sup>-1</sup>) is the C required for daily construction of new tissue.  $C_t$  was calculated by multiplying the root growth rate (g DW day<sup>-1</sup>) by tissue construction cost ( $C_w$ ).  $\Delta W_c$  (μmol C day<sup>-1</sup>) is the change in root C content and was calculated by multiplying the root C content and root growth rate.

- (5) Belowground allocation represents the fraction of new biomass partitioned into new roots and nodules over the given growth period. This was calculated according to Bazzaz (1997), as follows:

$$df/dt = \text{RGR}(\delta - B_r/B_t),$$

where RGR is the relative growth rate (mg g<sup>-1</sup> day<sup>-1</sup>) and  $\delta$  is the fraction of new biomass gained during the growth period.  $B_r/B_t$  is the root weight ratio, based on total plant biomass ( $B_t$ ) and root biomass ( $B_r$ ).

#### Calculations of percentage N derived from the atmosphere (%Ndfa)

The  $\delta^{15}\text{N}$  analyses were carried out at the Archeometry Department, University of Cape Town, South Africa. The isotopic ratio of  $\delta^{15}\text{N}$  was calculated as  $\delta = 1000\text{‰} (R_{\text{sample}}/R_{\text{standard}})$ , where  $R$  is the molar ratio of the heavier to the lighter isotope of the samples and standards are as described

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, 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  $\delta^{15}\text{N}$  values for the N 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, namely, two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

%Ndfa was calculated according to Shearer and Kohl (1986), as follows:

$$\% \text{Ndfa} = 100((\delta^{15}\text{N}_{\text{reference plant}} - \delta^{15}\text{N}_{\text{legume}}) / \\ (\delta^{15}\text{N}_{\text{reference plant}} - B)),$$

where the reference plant was non-nodulated *V. divaricata*, planted 2 weeks later than the experimental plants and grown under the same glasshouse conditions using 500 μM N in a Long Ashton nutrient solution (25% strength). The B value is the  $\delta^{15}\text{N}$  natural abundance of the N derived exclusively from biological N-fixation of nodulated *V. divaricata*. The seeds were germinated in the natural inoculum and, thereafter, the seedlings were grown with N-free 25% strength Long Ashton nutrient solution in sterile-sand culture. The B value of *V. divaricata* was determined as -2.58.

#### Statistical analysis

The effects of the factors and their interactions were tested with an ANOVA (Kaleidagraph, Synergy Software, Reading, PA, USA). Where the ANOVA revealed significant differences among treatments, the means (6–8) were separated using *post hoc* Tukey's l.s.d. (SuperAnova for Macintosh, Abacus Concepts, Berkeley, CA, USA) ( $P \leq 0.05$ ).

## Results

### Biomass

Under normal P conditions (HP), *V. oroboides* is evidently better adapted than is *V. divaricata*, because it had greater total biomass for the duration of the experiment (Table 1). The greater biomass resulted from the shoot DW, whereas root and nodule DWs were consistently lower for the duration of the experiment. There were no significant differences between the leaf areas (Table 1) of the two species. Under low-P conditions (LP), *V. divaricata* maintained a constant plant biomass, whereas *V. oroboides* showed a 38% decline in plant DW (Table 1). This decline resulted from a 70% decline in nodule DW, and a 63% decline in shoot DW, respectively. Low-P conditions may have inhibited the growth of nodules, resulting in a decline in biological N fixation. Furthermore, *V. oroboides* also showed an increase in its root:shoot ratio, whereas *V. divaricata* remained unchanged (Table 1).

**Table 1. Biomass parameters and growth nutrition of 90-day-old *Virgilia divaricata* and *V. oroboides* saplings grown in sand culture under sufficient-phosphorus (500  $\mu\text{M}$ ) and low-phosphorus (5  $\mu\text{M}$ ) concentrations for 90 days**Values are means ( $n=6-8$ )  $\pm$  s.e. The values within a row followed by the same letter are not significantly different between the treatments ( $P=0.05$ )

Parameter	500 $\mu\text{M}$ phosphorus		5 $\mu\text{M}$ phosphorus	
	<i>V. divaricata</i>	<i>V. oroboides</i>	<i>V. divaricata</i>	<i>V. oroboides</i>
<b>Biomass</b>				
Plant dry weight (g)	0.49 $\pm$ 0.024a	1.27 $\pm$ 0.188b	0.39 $\pm$ 0.051a	0.61 $\pm$ 0.057a
Shoot dry weight (g)	0.27 $\pm$ 0.015a	0.82 $\pm$ 0.127b	0.25 $\pm$ 0.033a	0.36 $\pm$ 0.047a
Root dry weight (g)	0.15 $\pm$ 0.015a	0.35 $\pm$ 0.064b	0.12 $\pm$ 0.019a	0.22 $\pm$ 0.023a
Nodule dry weight (g)	0.07 $\pm$ 0.028ab	0.10 $\pm$ 0.029b	0.02 $\pm$ 0.005a	0.03 $\pm$ 0.003a
Leaf area (m <sup>2</sup> )	0.004 $\pm$ 0.001ab	0.01 $\pm$ 0.002b	0.004 $\pm$ 0.001ab	0.003 $\pm$ 0.0004ab
Root : shoot	0.57 $\pm$ 0.055ab	0.42 $\pm$ 0.024a	0.49 $\pm$ 0.039a	0.62 $\pm$ 0.090b
<b>Growth nutrition</b>				
Specific N-absorption rate (mmol N g <sup>-1</sup> day <sup>-1</sup> )	0.02 $\pm$ 0.005a	0.06 $\pm$ 0.014b	0.05 $\pm$ 0.025ab	0.01 $\pm$ 0.003a
Specific N-utilisation rate (g dw g <sup>-1</sup> N <sup>-1</sup> day <sup>-1</sup> )	0.04 $\pm$ 0.011b	0.01 $\pm$ 0.001a	0.01 $\pm$ 0.001ab	0.03 $\pm$ 0.004ab

### P and N Nutrition

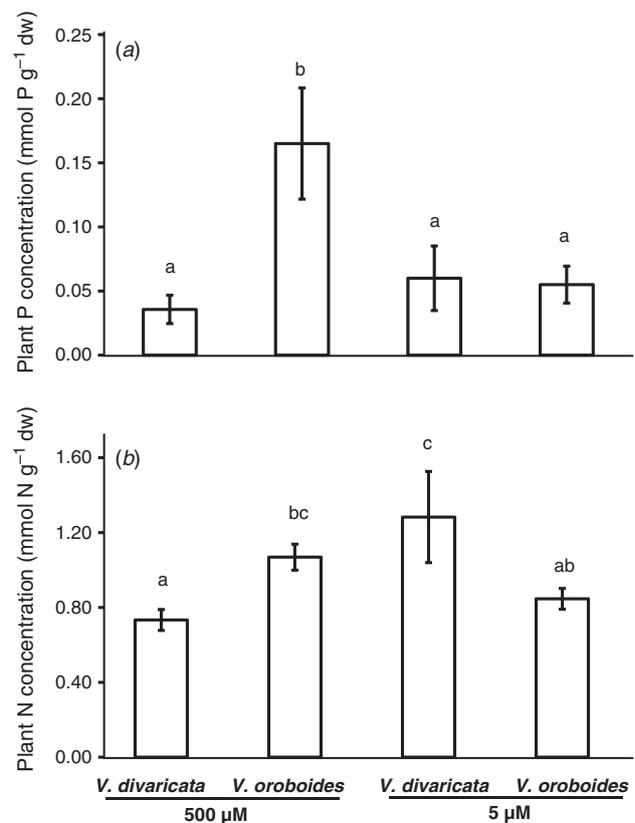
The P concentration (Fig. 1a) of plants exposed to adequate P concentrations followed a similar pattern as did the plant biomass. *V. oroboides* accumulated more P than did *V. divaricata*. The increased nodule DW of both *Virgilia* species and the sufficient plant P concentration of *V. oroboides* appear to favour biological N fixation (BNF), as shown by the increase in %NDFa (Fig. 2a). There was a significant difference in N uptake between the species during adequate P conditions. *V. oroboides* showed a greater SNAR than did *V. divaricata* (Table 1). This was also reflected by the higher plant N concentration (Fig. 2b). Specific N-utilisation rate was lower in *V. oroboides* than in *V. divaricata* under adequate P conditions (Table 1), suggesting that *V. divaricata* is more efficient at utilising N resources.

Phosphorus-stressed *V. oroboides* plants showed a decrease in P concentration, following a similar pattern of development of nodules, whereas *V. divaricata* maintained a constant P concentration. The decrease in P concentration of *V. oroboides* concurred with the decrease in %NDFa (Fig. 2a), which is an indicative decrease in BNF. Although *V. divaricata* maintained its P concentration during P stress, there was a decrease in % NDFa, whereas there was an increase in N concentration. This suggests that *V. divaricata* may be dependent on other N sources, e.g. soil N. In addition, *V. oroboides* had a lower specific N uptake (SNAR) under P stress than under adequate P conditions, but showed greater efficiency in N utilisation (SNUR) (Table 1). Although plants under P stress had a decreased BNF, they were more efficient in utilising atmospheric N per nodule, as indicated by the BNF-derived N per nodule (Fig. 2b).

### Photosynthetic rate

There was a significant difference in photosynthetic rate between the two species under sufficient P conditions. *V. oroboides* showed a higher photosynthesis rate (Fig. 3) to maintain its increased growth. The higher photosynthetic rate of *V. oroboides* was complemented by an increase in plant-growth respiration (Fig. 4a).

During P stress, *V. oroboides* had a decreased photosynthetic rate, shown by the significant difference

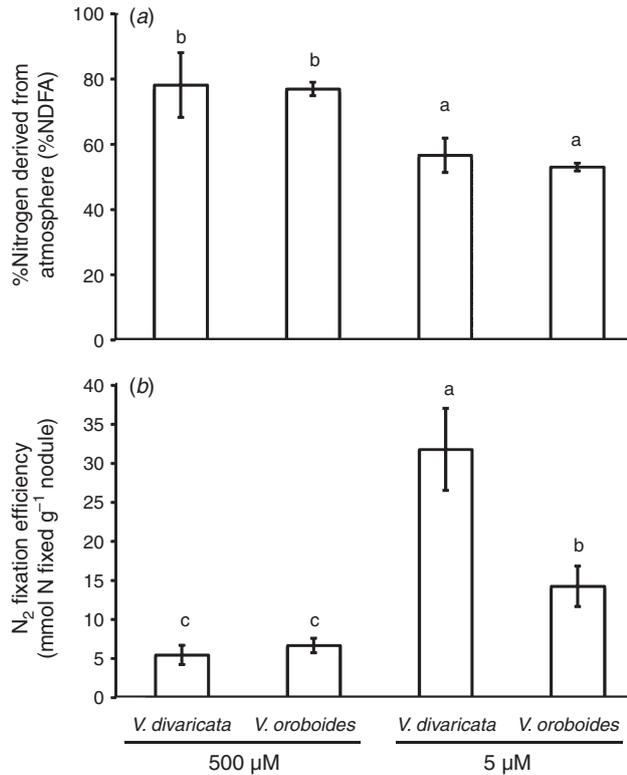


**Fig. 1.** Plant (a) phosphorus (P) and (b) nitrogen (N) concentration of *Virgilia divaricata* and *V. oroboides* saplings grown in sand culture under sufficient-P (500  $\mu\text{M}$ ) and low-P (5  $\mu\text{M}$ ) concentrations for 90 days. Values are means ( $n=6-8$ ) with standard error bars. The same letter indicates that the treatments are not significantly different from each other. \* $P \leq 0.05$ .

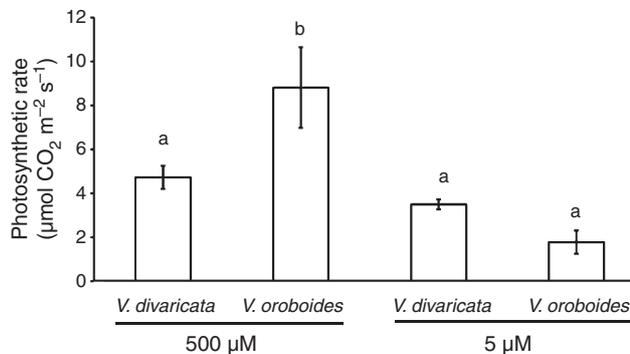
between the treatments, whereas *V. divaricata* maintained its photosynthetic rate.

### Respiratory carbon costs

Both species showed greater construction costs (Fig. 4b) during P stress. There was no significant difference in growth

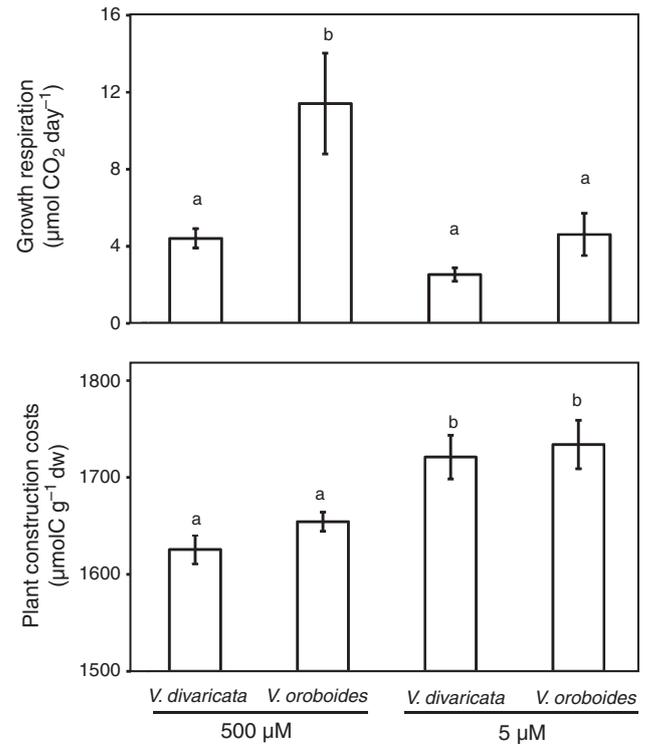


**Fig. 2.** (a) Percentage nitrogen (N) derived from the atmosphere (%Ndfa) and (b)  $\text{N}_2$  fixation efficiency of *Virgilia divaricata* and *V. oroboides* saplings grown in sand culture under sufficient-P (500  $\mu\text{M}$ ) and low-P (5  $\mu\text{M}$ ) concentrations for 90 days. Values are means ( $n=6-8$ ) with standard error bars. The same letter indicates that the treatments are not significantly different from each other. \* $P \leq 0.05$ .



**Fig. 3.** Photosynthetic rates of *Virgilia divaricata* and *V. oroboides* saplings grown in sand culture under sufficient-P (500  $\mu\text{M}$ ) and low-P (5  $\mu\text{M}$ ) concentrations for 90 days. Values are means ( $n=6-8$ ) with standard error bars. The same letter indicates that the treatments are not significantly different from each other. \* $P \leq 0.05$ .

respiration (Fig. 4a) in *V. divaricata* during the two treatments. This may be because *V. divaricata* was able to maintain its DW and P nutrition during the experiment. In contrast, *V. oroboides* decreased growth respiration by 60% under P stress. During P stress, both plant species showed both decreased Rubisco and electron-transport activities (Fig. 4b, c) during photosynthesis.



**Fig. 4.** (a) Plant growth respiration and (b) plant construction costs of *Virgilia divaricata* and *V. oroboides* saplings grown in sand culture under sufficient-P (500  $\mu\text{M}$ ) and low-P (5  $\mu\text{M}$ ) concentrations for 90 days. Values are means ( $n=6-8$ ) with standard error bars. The same letter indicates that the treatments are not significantly different from each other. \* $P \leq 0.05$ .

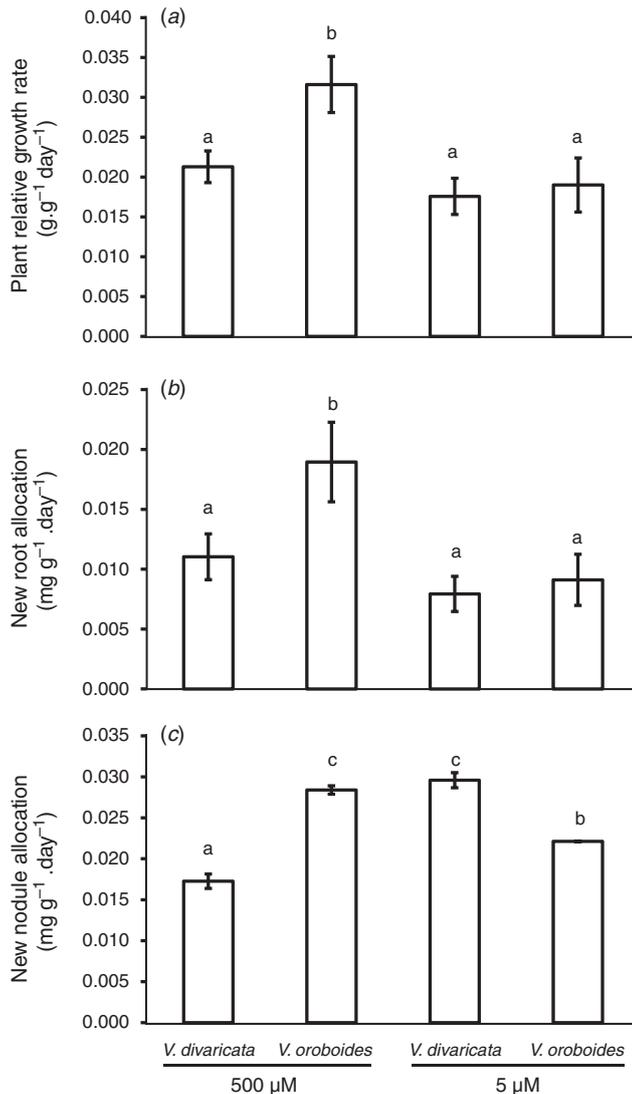
#### Belowground allocation

During sufficient P supply, *V. oroboides* showed an increase in plant relative growth rate relative to *V. divaricata* (Fig. 5a). Under P stress, however, *V. oroboides* had a lower relative growth rate, whereas *V. divaricata* maintained its relative growth rate. Furthermore, *V. oroboides* increased its allocation to new root tissue during sufficient P supply, whereas there was no significant difference in both treatments in *V. divaricata* (Fig. 5b). In contrast, *V. divaricata* allocated more to new nodule-tissue growth during limited P supply than did *V. oroboides* (Fig. 5c).

#### Discussion

The two tree legumes appear to have different adaptations to P starvation, in that during low-P supply, *V. divaricata* maintained its biomass relative to *V. oroboides*, by altering its biomass allocation to the belowground nutrient-acquiring structures.

The limited P supply reduced plant growth and may have limited the N demand and, consequently, BNF of the two legumes. This is consistent with results from previous studies where limited P nutrition reduced nodule growth, metabolism and  $\text{N}_2$  fixation and, subsequently, also plant growth (Sa and Israel 1991; Al Niemi *et al.* 1998; Almeida *et al.* 2000; Olivera *et al.* 2004; Hernández *et al.* 2007; Le Roux *et al.* 2008; Le Roux *et al.* 2009; Mortimer *et al.* 2009). The difference between these two indigenous legumes is that *V. oroboides*



**Fig. 5.** (a) Plant relative growth rate, (b) new root allocation and (c) new nodule allocation of *Virgilia divaricata* and *V. oroboides* saplings grown in sand culture under sufficient-P (500  $\mu\text{M}$ ) and low-P (5  $\mu\text{M}$ ) concentrations for 90 days. Values are means ( $n=6-8$ ) with standard error bars. The same letter indicates that the treatments are not significantly different from each other.  $*P \leq 0.05$ .

accumulated more P under P-sufficient conditions, which promoted nodular growth, BNF and SNAR. Consequently, it is likely that this improved BNF-derived N nutrition supported the increased biomass accumulation of *V. oroboides*, because it has been demonstrated in soybean that symbiotic  $\text{N}_2$  fixation requires more P for optimal functioning than for plant growth (Israel 1987). However, during P deficiency, *V. divaricata* appears to be physiologically better adapted for maintaining biomass and macro-element nutrition than is *V. oroboides*.

During P deficiency, the 48% decline in *V. oroboides* plant DW, coupled with a 64% decline in plant P concentration, concurs with results of previous work on P concentration in plants grown under P-deficient conditions (Hernández et al. (2007). Furthermore, the increase in the root:shoot ratio of

*V. oroboides* during P deficiency represents a typical P-stress response of plants under P limitation (Rychter and Mikulka 1990; Juszczuk and Rychter 2002). This reduced growth was associated with a decline in the nodule DW, which concurs with previous findings (Gordon et al. 1990; Almeida et al. 2000; Tang et al. 2001; Høgh-Jensen et al. 2002). The decrease in biomass of *V. oroboides* may have reduced the reliance on BNF as a source of N for growth, as supported by findings of previous work (Sa and Israel 1991; Gordon et al. 1997; Almeida et al. 2000) where limiting P supply reduced the growth and nodule biomass, and caused a reduction in N demand and  $\text{N}_2$  fixation in the host plant. The higher N accumulation in *V. divaricata* under low-P conditions, in spite of similar reliance on BNF as for *V. oroboides*, suggested that *V. divaricata* may employ additional strategies for N acquisition under these conditions. One mechanism is the high specific N-absorption rate of the *V. divaricata* root system, which reflects the contribution of both roots and nodules. Another mechanism is the improved efficiency of BNF under low-P conditions in *V. divaricata*. In this regard, *V. divaricata* increased its allocation of resources to new nodule-tissue growth under P starvation, and also showed greater efficiency than did *V. oroboides* in acquiring atmospheric  $\text{N}_2$ . It is known that enhanced nodule efficiency for nutrient utilisation is considered a pivotal coping strategy during P deficiency (Vadez et al. 1997; Raghothama 1999; Høgh-Jensen et al. 2002; Vance et al. 2003; Lynch and Ho 2005; Mortimer et al. 2008, 2009; Le Roux et al. 2009). However, enhanced nodule function may impose a cost on host reserves.

The increase in tissue-construction costs in both species under low P concurs with the findings of previous studies, which have shown an increase in C budget during low-P conditions (Lynch and Beebe 1995; Nielsen et al. 1998; Mortimer et al. 2008). This indicates that the belowground structures required more C per gram of tissue produced, although it is unclear what the precise structural nature of this additional C is (Mortimer et al. 2008). This increased construction did not appear to incur a large sink demand on photosynthetic products and instead it seems evident that growth respiration, representing the costs associated with the new tissue growth, had a larger sink effect. In *V. oroboides*, the higher photosynthetic rates at sufficient P were associated with the enhanced growth-respiration rates of the belowground nutrient-acquiring structures. The combined sink effect of the host plant roots and nodules can incur a drain of the host C reserves so as to maintain N and P nutrition. This may increase the photosynthetic rate of the host plant (Ainsworth et al. 2004; Kaschuk et al. 2009, 2010a, 2010b). However, under low-P conditions, the decline in photosynthesis in *V. oroboides* concurs with the lower investment in belowground growth, as evidenced by the decreased allocation and growth respiration of the root and nodule nutrient-acquiring structures. In contrast to this, the unchanged photosynthetic and growth-respiration rates of *V. divaricata* at both sufficient- and low-P treatments indicated an adaptation to low-P environments. In this regard, the increase in nodule allocation appears to have been achieved at the expense of root allocation. This trade-off has clearly benefited the efficiency of N nutrition of *V. divaricata* during low P supply.

Although the current study showed that legume performance in nutrient-poor soils may be dependent on different allocations of biomass to nutrient-acquiring structures such as root and nodules, other biological and biochemical factors may be equally important. In this regard, it is very likely that biological factors, such as mycorrhiza formation (Maseko and Dakora 2013), along with biochemical factors, such as the secretion of organic acids (Dakora and Phillips 2002) and acid phosphatases (Maseko and Dakora 2013), may also play important roles in nutrient poor soils.

## Conclusions

During P starvation, *V. divaricata* saplings were better adapted than those of *V. oroboides*, by the alteration in biomass allocation to nodules and their improved efficiency of N acquisition and utilisation. The results of the present study suggested that *V. divaricata* is a more resilient tree, and would do well both in habitats with high- or low-P availability. This is interesting because *V. oroboides* trees are more specifically associated with P-poor CFR soils than is *V. divaricata*, the latter species often growing in richer P-nutrient soils. The implication of these findings is that the *V. divaricata* tree species may have a competitive advantage, if it should invade the more P-poor habitat of *V. oroboides*.

## Acknowledgements

This work was funded by the DST/NRF–Center of Excellence for Tree Health and Biotechnology, based at the University of Pretoria. We acknowledge the Department of Botany and Zoology at the University of Stellenbosch for their research facilities.

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