Does variability in soil P influence the distribution of *Podalyria* species in the CFR?

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Abstract

Cape species show considerable variation in their distribution ranges throughout the Cape Floristic Region. Some species show a restricted distribution range while others are more widely spread. It was hypothesised that *Podalyria* species with a restricted distribution in the Cape are adapted to higher levels of soil P. These species would therefore be limited to 'pockets' of high P soils found in the Cape. Conversely, widespread species were hypothesised to be adapted to the low levels of soil P which dominate the Cape and should therefore show P toxicity under higher P levels. Response to P supply was compared between two restricted species (*Podalyria calyptrata* and *P. leipoldtii*) and two widespread species (*P. burchellii* and *P. myrtillifolia*) when supplied with 10, 25, 50, 100 or 150 mg P kg^{-1} dry sand. No significant differences were found in total biomass accumulation, investment in below-ground biomass or nodulation between restricted and widespread species for all P levels. There were however species effects. All species except *P. leipoldtii* increased biomass accumulation with the supply of 25 or 50 mg P kg^{-1} compared to 10 mg P kg^{-1}. Both *P. leipoldtii* and *P. myrtillifolia* showed a reduction in biomass at 100 mg P kg^{-1} while *P. calyptrata* and *P. burchellii* showed a reduction in biomass at 150 mg P kg^{-1}. All species except *P. leipoldtii* showed an increase in investment in below-ground biomass at 10 mg P kg^{-1} and nodulation also varied with species. *P. calyptrata* showed a down regulation of P uptake with an increase in soil P levels and data obtained from a field survey of soil P provided evidence for the growth of *P. calyptrata* populations in a wide range of soil P. From the information gathered the hypothesis was rejected and it was concluded that *Podalyria* species distribution across the CFR landscape did not seem to be a result of variability in soil P.

Introduction

The Western Cape region of Southern Africa is characterised by ancient, highly weathered, infertile soils (Lambers *et al.* 2006, Lambers *et al.* 2008, Lambers *et al.* 2010). For millions of years these soils have been above sea level and have been unexposed to glaciation or to natural disturbances (Lambers *et al.* 2008, Lambers *et al.* 2010, Vitousek *et al.* 2010). In the early stages of soil development N availability is very low and P availability is high (Raich *et al.* 1996, Lambers *et al.* 2008, Vitousek *et al.* 2010). As soils age however, the process of N_2

fixation by soil microbial activity increases soil N levels (Lambers *et al.* 2008, Vitousek *et al.* 2010). At the same time P is lost by the processes of weathering, erosion and leaching (Lambers *et al.* 2008). The remaining P available for use by plants is then further reduced when inorganic P is converted to organic P by soil micro-organisms. P may also be sorbed onto the surfaces of soil minerals such as calcium. These are later occluded by Fe and Al and in the process are made unavailable for plant uptake (Raich *et al.* 1996, Vance *et al.* 2002, Lambers *et al.* 2006). The ancient soils of the South Western Cape are therefore characteristically low in both total and available P (Lambers *et al.* 2006, Lambers *et al.* 2008, Lambers *et al.* 2010, Cramer 2010).

The Cape Floristic Region in South Africa consists of 9000 species in an area of 90 000km^2 (Goldblatt and Manning 2000). These species show considerable variability in their distribution patterns with some species widely spread throughout the region while others are confined to specific areas (Goldblatt and Manning 2000). What is causing this variability in distribution? According to Cowling *et al.* (1994), Richards *et al.* (1997a) and Shane *et al.* (2008) the Cape is characterised by landscape-level mosaics of soil nutrient availability. Although the ancient soils of the Cape are predominantly P impoverished, a gradient in soil P may still occur between different soil types (Cowling *et al.* 1994, Richardson *et al.* 1997b). Low soil P levels are dominant throughout the Cape (Lambers *et al.* 2006, Lambers *et al.* 2008, Lambers *et al.* 2010) but pockets of higher P soils may exist. These pockets may be found in areas of disturbance or along river banks where nutrients are consistently replenished. The small forested areas of the Cape are also known to contain soil with higher nutrient contents and therefore higher P values than those found in the fynbos (Manders 1990). This gradient in soil nutrient levels has lead to differential adaptations among species. These adaptations enable plants to exploit soil resources and often involve changes in root architecture, growth rate, biomass, length and distribution (Raghothama 1999, Vance *et al.* 2002, Lambers *et al.* 2006, Richardson *et al.* 2009). Differential adaptations to changing soil nutrient levels often leads to the evolution of new species (Cowling *et al.* 1994, Cowling *et al.* 1996). Mosaics of soil nutrient availability in the CFR are therefore associated with a high level of beta diversity or species turnover (Cowling *et al.* 1996, Shane *et al.* 2008).

Goldblatt and Manning (2000) divided the Cape into six phytogeographic regions and divided species distributions among these regions (Appendix 1). The phytogeographic regions of the Cape include the South West (SW), North West (NW), Agulhas Plain (AP), Karoo Mountain (KM), Langeberg (LB) and the South East (SE). These phytogeographic regions were used to define the restricted and widespread species of the Cape. Species confined to one of the regions were considered to be restricted while those in two or more were considered to be widespread (Table 1).

It was hypothesised that species with a restricted distribution in the Cape are adapted to higher levels of soil P. These species would therefore be limited to 'pockets' of high P soils found in the Cape. Conversely, widespread species were hypothesised to be adapted to the low levels of P which dominate the soils of the Cape and should therefore show P toxicity under higher soil P levels.

Materials and Methods

Glasshouse study

Species and growth conditions

Four *Podalyria* species indigenous to fynbos vegetation with restricted and widespread distribution were selected for the study. These included *Podalyria calyptrata* (Retz.) Willd. and *Podalyria leipoldtii* L.Bolus ex A.L.Schutte ined which represented species with restricted distribution ranges and *Podalyria burchellii* DC. and *Podalyria myrtillifolia* (Retz.) Willd. which represented species with widespread distribution ranges (Goldblatt and Manning 2000). Each pair of species being compared were closely related and showed a shared historical background. *P. calyptrata* and *P. burchellii* are from closely related clades and *P. myrtillifolia* and *P. leipoldtii* are from the same clade (Boatwright *et al.* 2008).

All species were grown from seed (Silverhill Seeds and Books, Cape Town, South Africa). The seeds were soaked overnight in boiling water to break dormancy after which they were sown into vermiculite and acid washed sand.

Four weeks after emergence, four seedlings of each species were removed and transplanted into 3 kg pots containing an acid washed mix of coarse and fine sand (1:1). The substrate mix was mechanically mixed with either 10, 25, 50, 100 or 150 mg P kg^{-1} of insoluble P consisting of 75% FePO4 (AR, Sigma, F1523) and 25% Ca5(PO4)3(OH) (AR, Sigma C3161), which resembles the approximate ratio found in Table Mountain Sandstone-derived soils (Witkowski and Mitchell 1987). During transplantation, seedlings were inoculated with 1mL broth culture of rhizobium which was supplemented with 2mg of CFR soil inoculum that was sprinkled at the base of each seedling.

The pots were supplied with a University of Western Australia (UWA) nutrient solution containing (μM): Ca(NO3)2, 400; K2SO4, 200; MgSO4., 54; MnSO4, 0.24; ZnSO4, 0.10; CuSO4, 0.02; H3BO3, 2.4; NaMoO4, 0.03; Fe-EDTA, 10 to 70% field capacity twice a week. The pots were also wetted to 70% field capacity once a week with water. All the pots were randomly arranged on trolleys in a well ventilated glasshouse situated at the University of Cape Town (S 33° 57.353'; E 018° 27.742'). Three weeks after transplanting, seedlings were thinned to one per pot. Pots and trolleys were re-arranged every 2 weeks. Plants were grown for 4 months from March to July.

Plant biomass

At harvest, the root systems were gently excavated under running water and the plants were separated into leaves, stems, nodules and roots. Each organ was weighed for fresh biomass weight and dried at 60°C for 48 h. After drying, the material was re-weighed and milled using a Wiley mill (Model no 3, Arthur H. Thomas, Philadelphia USA) and a ball mill (MM200, Retsch®, Haan, Germany).

Foliar nutritional analysis

Pulverised leaf material was sent to Bemlab for analysis. Foliar [P] was measured by dry-ashing pulverized leaf material at 480°C for 8 h and dissolving with a 1:1 (v/v) of HCl according to Kalra (1998). Assessment of the element concentrations in solution was performed using inductively coupled plasma atomic emission spectrometry (Varian Vista MPX, Mulgrave, Australia). Foliar [N] was determined by a LECO FP-528 nitrogen analyzer (Leco Corporation, St. Joseph, USA).

Field study

Podalyria species were collected in the field in May 2011. Species were collected from a total of 11 sites in the Cape. Sites included the Rondebosch Common, Vogelgat Nature Reserve (x2), Marloth Nature Reserve (x2), a roadside between Stanford and Caledon, Signal Hill, Tafelberg Road (Table Mountain), Camps Bay, Kirstenbosch Forest and Silvermine Dam. At each site three soil samples were taken and plant samples were collected for voucher specimens and for identification.

All collected soils were air dried and sieved (1 mm mesh) prior to analyses at Bemlab for pH, total and available P and total N. pH was determined by shaking 2g of material in 20 mL 1M KCl at 180 rpm for 60 min, centrifuging at 10000g for 10 min and measuring the pH of the supernatant (Powers *et al.* 2010). Total N was determined by digestion with a Leco FP528 N Analyzer (Leco Corporation). Available P was determined by extracting 2 g of soil in Bray II solution (Bray and Kurtz 1945) and then filtering through Whatman No.2 filter paper. The filtrate was analyzed colourmetrically using the Malachite Green method (Motomizu *et al.* 1983).

Statistical analysis

All variables, apart from percentage data were log transformed. Percentage data were arcsine square root transformed (Sokal and Rolhf 1995). All statistical analyses were conducted in STATISTICA (Statsoft 2009). A three-way nested ANOVA was used to test differences between species distributions, species and P treatments. The fixed factor was species distributions and the random factors were species and P treatments.

A one-way ANOVA was used to determine the effects of P treatments on tissue [N] and [P] and tissue N:P ratios of individual species. A one-way ANOVA was also used to determine the effects of different sites on the soil nutrient characteristics of *P. calyptrata* habitats.

Results

Biomass accumulation

A significant interaction between species and P treatments was evident on biomass accumulation ($F_{16,92}$=15.82, P<0.001) (Figure 1). All species except *P. leipoldtii* showed a higher biomass accumulation at 25 and 50 mg P kg^{-1} than at 10 mg P kg^{-1} and 150 mg P kg^{-1}. Specifically, total biomass at 25 and 50 mg P kg^{-1} were similar, but were higher (P<0.05) than total biomass at 10 mg P kg^{-1} and 150 mg P kg^{-1} in *P. calyptrata* and *P. burchellii* (Figure 1). The supply of 100 and 150 mg P kg^{-1} to *P. leipoldtii* and *P. myrtillifolia* decreased total biomass relative to plants receiving 25 or 50 mg P kg^{-1}. Thus, total biomass of *P. leipoldtii* did not change with increasing levels of P up to 100 mg P kg^{-1}, but the biomass declined with application of 150 mg kg^{-1}.

There was no significant difference in total plant biomass between restricted and widespread species (P>0.05). Both the restricted *P. calyptrata* and the widespread *P. burchellii* showed a significant decrease in biomass only at 150 mg P kg^{-1} while the restricted *P. leipoldtii* and the widespread *P. myrtillifolia* showed a significant decrease in biomass from 100 mg P kg^{-1} (P<0.001). There was no significant difference between total biomass of the restricted *P. leipoldtii* and the widespread species *P. burchellii* and *P. myrtillifolia* at both 100 mg P kg^{-1} and the 150 mg P kg^{-1} (P>0.05).

Biomass allocation

There was a significant difference between biomass allocation to roots and shoots for different P treatments in all species ($F_{16,92}$=22.65, P<0.0001). The root:shoot ratios for all species, except P. *leipoldtii* were higher (P<0.001) at 10 mg P kg^{-1} than at all other P treatments (Figure 2). A significant difference was found between the root:shoot ratios of the restricted species *P. calyptrata* and *P. leipoldtii* at 10 mg P kg^{-1} while no significant difference was evident between root:shoot ratios of restricted *P. leipoldtii* and widespread *P. myrtillifolia* (Figure 2).

Nodulation

There were no differences in nodule dry weight in *P. calyptrata* for all P levels. However, *P. leipoldtii* showed a decrease in nodule dry weight from 50 to 100 mg P kg^{-1} whereas *P. burchellii* had a lower nodule dry weight at 10 mg P kg^{-1} than at all other levels. Both *P. leipoldtii* and *P. myrtillifolia* showed a decrease in nodule dry weight from 50 to 150 mg P kg^{-1} (Figure 3). When looking at a correlation between nodule dry weight and plant biomass (Table 1) it was observed ~~we can see~~ that an increase in nodule dry weight results in an increase in plant biomass.

Leaf tissue analysis

An increase in the levels of P supply resulted in an increase in tissue [P] in *P. calyptrata* ($F_{4,24}$=11.140, P<0.001), *P. burchellii* ($F_{4,23}$=39.246, P<0.001) and *P. myrtillifolia* ($F_{3,12}$=10.319, P<0.01) but not in *P. leipoldtii* . Tissue [P] was significantly lower at 10 mg P kg^{-1} for *P. calyptrata* and for *P. burchellii* (P<0.001). A significant increase in tissue [P] was evident in *P. burchellii* at 100 mg P kg^{-1} and 150 mg P kg^{-1} and in *P. myrtillifolia* at 100 mg P kg^{-1} (Figure 4).

P. calyptrata ($F_{4,24}$=14. 45, P<0.001), *P. burchellii* ($F_{4,19}$=24.58, P<0.001) and *P. myrtillifolia* ($F_{3,174}$=21.3, P<0.001) showed a higher (P<0.001) tissue [N] at 25 mg P kg^{-1} than at 10 mg P kg^{-1} (Figure 4). Levels of P of 150 mg P kg^{-1} caused a decrease in tissue [N] in both *P. calyptrata* and *P. burchellii.* An increase in P levels to 50 mg P kg^{-1} caused a decrease in tissue [N] in *P. myrtillifolia.* Tissue [N] for *P. myrtillifolia* recovered once again at 100 mg P kg^{-1} (Figure 5).

The tissue N:P ratios were significantly higher (P<0.05) in plants supplied with 10 mg P kg^{-1} than those receiving the higher levels of P in all species except *P. leipoldtii* (Figure 6). Plants of *P. calyptrata* showed a decrease (P<0.001) in tissue N:P ratios from 10 to 25 mg P kg^{-1}, thereafter N:P ratios remained unchanged for all P levels. *P. burchellii* showed a decrease (P<0.001) in N:P ratios from 10 to 25 mg P kg^{-1} and again from 50 to 100 mg P kg^{-1}. The N:P values for *P. myrtillifolia* were low (below 20) at 10 mg P kg^{-1} and there was no difference in N:P ratios at 10 and 25 mg P kg^{-1}. However, a decrease in N:P ratios occurred from 25 to 100 mg P kg^{-1} in *P. myrtillifolia. P. leipoldtii* showed a decrease in N:P ratios from 10 to 25 mg P kg^{-1} but showed an increase from 25 to 50 mg P kg^{-1} (Figure 6).

Field study

From the soil data analysis it was confirmed that all 6 populations of *P. calyptrata* grew in soils with similar available P content (P Bray II) (Table 3). The two populations of *P. sericea* (Andrews) R.Br., another species showing a restricted distribution, were also found to grow in soils with similar available P content (Table 3). Furthermore, *P. calyptrata, P. sericea* and other unidentified species all grew in a similar range (1.67 to 13 mg P kg^{-1}) of soil available P (Table 3).

However, total [P] of soils of *P. calyptrata* sites were very broad ranging from a low of 42.49 mg P kg^{-1} to a high of 242.99 mg P kg^{-1} (Table 3). *P. calyptrata* population 1 (Vogelgat) and 6 (Silvermine Dam) grew in soils with significantly lower total [P] than populations 2 (Vogelgat), 3 (Tafelberg Road) and 4 (Camps Bay) and *P. calyptrata* populations 2 and 3 grew in soils with total [P] significantly lower than populations 4 (Camps Bay) and 5 (Kirstenbosch Forest) (Table 3). *P. sericea* populations were also found growing in soils containing varying ($P<0.001$) total [P] ranging from 53.68 mg P kg^{-1} to 236.89 mg P kg^{-1} (Table 3). Unidentified species grew in soils with total [P] ranging from 30.72 mg P kg^{-1} to 174.75 mg P kg^{-1} (Table 3).

P. calyptrata populations were found to grow in acidic soils with a pH ranging from 2.97 to 4.67 KCl (Table 3). Available calcium in the soils of *P. calyptrata* populations ranged from 1.80 to 11.35 cmol(+)/kg. Once again there was a strong overlap of ranges of pH and calcium availability between the different populations of *P. calyptrata, P. sericea* and the unidentified species (Table 3).

Discussion

We hypothesised that *Podalyria* species showing a restricted distribution throughout the CFR would be confined to pockets of high P and would have a higher response to P supply than widespread species. In this study, it was found that 10 mg P kg^{-1} was deficient for the growth of all species except *P. leipoldtii*. An increase in P levels from 10 to 25 mg P kg^{-1} increased biomass accumulation in all species except *P. leipoldtii* and appeared to be adequate for the growth of all species. An increase in P levels to 50 mg P kg^{-1} however, caused no change in plant growth. *P. calyptrata* (restricted) and *P. burchellii* (widespread) were able to tolerate P

levels up to 100 mg P kg^{-1} without the corresponding P toxicity and decrease in growth found in *P. leipoldtii* (restricted) and *P. myrtillifolia* (widespread). *P. calyptrata* and *P. burchellii* are therefore less sensitive to increasing levels of P which implies that they are better able to down-regulate their P uptake. An excessive increase in P supply in nutrient deficient environments can result in the well documented occurrence of P toxicity in species adapted to low P (Lambers *et al.* 2006, Lambers *et al.* 2008, Lambers *et al.* 2010). When soil P levels are too high, P accumulates in plant tissue and becomes toxic. This may have caused the decrease in total plant growth found here (Raghothama 1999, Lambers *et al.* 2008, Lambers *et al.* 2010). *P. leipoldtii* does not appear to be adapted to high levels of P and shows a similar response to changing P levels as the widespread *P. myrtillifolia* while *P. burchellii*, a widespread species, shows a similar response to changing P levels as *P. calyptrata*, a restricted species. However, the CFR is known for its nutrient deficient soils and low P availability (Lambers *et al.* 2008, Lambers *et al.* 2010). Species adapted to these low P environments may therefore react differently to increasing P supplies.

According to Lamont (1982), Vitousek and Fields (1999), Vitousek *et al.* (2002) and Olivera *et al.* (2004), N_2 fixing plants have a greater sensitivity to a deficiency in P supply than non N_2 fixers. N_2 fixers require greater concentrations of P in order to maintain symbiotic relations and the N_2 fixation process (Vitousek 2002). In accordance with the reports of Vitousek (2002), the low level of 10 mg P kg^{-1} was associated with a decrease in nodulation for both *P. calyptrata* and *P. burchellii* (Figure 3). A correlation between nodule dry weight and plant biomass (Table 2) lends support to the theory that an increase in P availability increases nodulation (Olivera *et al.* 2004). As P levels increased nodulation, a corresponding increase in plant biomass followed.

McConnaughay and Coleman (1998) and Powers *et al.* (2010) indicated that differences in root:shoot biomass allocation have repeatedly been associated with resource availability. P supply has an effect on carbon partitioning between the roots and shoots of plants. This is known as the optimal foraging hypothesis (Gedroc *et al.* 1996, McConnaughay and Coleman 1998). An increase in root:shoot biomass is a well documented response of plants to P deficiency (Lamont 1982, Gedroc *et al.* 1996, McConnaughay and Coleman 1998, 1999, Raghothama 1999, Vitousek 2002, Vance 2002, Evans and Watts 2003, Olivera *et al.* 2004, Powers *et al.* 2010), as was observed in this study (Figure 6). The root:shoot ratios for all

species except *P. leipoldtii* were highest at the lowest level of P supply (10 mg P kg^{-1}). *P. calyptrata*, *P. burchellii* and *P. myrtillifolia* therefore showed changes to biomass allocation in response to a low P environments. However, *P. leipoldtii* did not share this characteristic. There is a possibility that *P. leipoldii* may have acquired other adaptations for enhanced P uptake such as mychorizal associations or an increase in root hair proliferation. As nutrient availability decreases, plants place more of their energy into root production to increase root surface area and to increase the area covered by roots for nutrient aquisition (Gedroc *et al.* 1996, McConnaughay and Coleman 1998, 1999, Raghothama 1999, Vance 2002, Watt and Evans 2003). A more extensive root system increases P aquisition efficiency and so is better able to exploit soil P (McConnaughay and Coleman 1998, 1999, Raghothama 1999).

Lambers *et al.* (2010) reported that many species in the Cape are adapted to low nutrient availability and are often prone to P toxicity when P levels are increased. For all species except *P. calyptrata* there was a decrease in biomass with an increase in foliar [P] which is associated with P toxicity. In contrast, *P. calyptrata* showed a down regulation of P uptake with an increasing soil P level. Tissue [P] remained constant at 25, 50, 100 and 150 mg P kg^{-1} despite a decline in biomass at 150 mg P kg^{-1}. Therefore, *P. calyptrata* can be associated with a better adaptation to higher levels of soil P than the other species. Both *P. burchellii* and *P. myrtillifolia* showed a decrease in biomass coupled with an increase in tissue P at 100 mg P kg^{-1}. This is an indication of P toxicity (Shane *et al.* 2004). These species demonstrate characteristics of species that lack the ability to down regulate P uptake, even when [P] reaches toxic levels in the tissues (Lambers *et al.* 2008, Shane *et al.* 2008, Lambers *et al.* 2010).

A field study was run in order to determine whether the restricted species were indeed growing in soils with higher levels of P than the widespread species. By determining the soil [P] of the natural habitats of species, the field study could show the natural range of P in which species grew. *P. calyptrata* populations were found growing in soils with P levels ranging from low (40 mg P kg^{-1}) to high (242 mg P kg^{-1}) (Table 3). This shows a tolerance of *P. calyptrata* to a wide range of P availabilities. This supports the observations of high P tolerance in the glasshouse study where the plants also showed down regulation of P uptake. Similarly, plants of *P. sericea*, another restricted species, were also found to grow in soils

with a wide range of total [P] (53.7 – 236.9 mg P kg^{-1}). Although species in the widespread group were not identified in the field study, the pattern of *Podalyria* species response to increased P supply in the glasshouse study, coupled with the wide range of total P in the habitats of the restricted species suggest that the hypothesis that restricted species are found in pockets of high P soils in the CFR was not supported. Therefore, based on these results, the distribution of *Podalyria* species in the CFR may not be influenced by soil P. There were however, some species effects. For example, *P. calyptrata* showed a characteristic down regulation of P uptake with an increase in soil P levels and in the field study was found across a wide range of total soil P. On the other hand, a substantial difference was found in the response of *P. leipoldtii* to the increasing P levels supplied. Plants of *P. leipoldtii* did not show an increase in biomass with increased soil P levels. Biomass allocation was also not changed with increased P levels. This lack of a response to increased levels of P supply as well as the lack of down regulation of P uptake observed in *P. burchellii* and *P. myrtillifolia* are characteristic of species that are adapted to low P environments (Shane *et al.* 2008).

When looking at the results obtained there is a need to consider the fact that the study used Goldblatt and Manning (2000)'s phytogeographic regions to define restricted and widespread species. One phytogeographic region may contain a range of P levels. These P levels may vary with the natural landscape or may change with changes in soil type. The restriction of species to one phytogeographic region therefore does not necessarily mean that it is confined to one level of P.

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Table 1. Distribution of *Podalyria* species in the Cape Floristic Region. Species that occur in only one phytogeographic region are considered restricted (R) species and presence at multiple regions refers to widespread (W) species. (Goldblatt and Manning 2000) SW = South West, NW = North West, AP = Agulhas Plain, KM = Karoo Mountain, LB = Langeberg, SE = South East

Podalyria Species	Widespread/Restricted	Location in the CFR
burchellii	W	KM, LB, SE
myrtillifolia	W	NW, SW, AP, LB, SE
rotundifolia	W	NW, SW, LB
biflora	W	NW, SW, LB
oleaefolia	W	SW, AP
hirsuta	W	SW, LB
calyptrata	R	SW
leipoldtii	R	NW
glauca	R	SE
microphylla	R	SW
orbicularis	R	SW
pearsonii	R	NW
argentea	R	SW
cordata	R	SW
lanceolata	R	LB
reticulata	R	SW
sericea	R	SW
variabilis	R	SW

Table 2. Correlations between plant biomass and nodule dry weight of *P. calyptrata, P. leipoldtii, P. burchellii* and *P. myrtillifolia* where r^2 shows the strength of the relationship (red font indicates a significant correlation).

	r^2	t	P
P. calyptrata	0.857158	12.72869	0.000000
P. leipoldtii	0.856688	12.46686	0.000000
P. burchellii	0.975301	32.04183	0.000000
P. myrtillifolia	0.938876	33.48582	0.00

Table 3. Soil data (mean ± S.E.) from 11 sites of *Podalyria* populations in the CFR. Numbers followed by the same letters are not significantly different. Species f, g and h were not yet identified.

	P Brayll (mg/kg)	Total P (mg/kg)	pH (KCl)	Ca (cmol(+)/kg)
***P. calyptrata* 1**	1.67±0.88 (ab)	42.49±2.09 (ab)	3.6±0.06 (abcd)	3.87±0.82 (cde)
***P. calyptrata* 2**	4.67±1.45 (abcd)	89.58±1.74 (cd)	3.47±0.09 (abc)	11.35±2.39 (ef)
***P. calyptrata* 3**	6.00±1.15 (bcd)	88.70±22.69 (cd)	4.2±0.15 (cde)	1.80±0.43 (abc)
***P. calyptrata* 4**	6.67±2.40 (bcd)	142.76±24.79 (de)	4.67±0.54 (ef)	7.49±3.59 (cde)
***P. calyptrata* 5**	6.67±1.21 (bcd)	242.99±15.17 (e)	4.33±0.18 (de)	6.45±2.11 (cde)
***P. calyptrata* 6**	3.67±0.67 (abc)	53.63±3.20 (abc)	2.97±0.03 (a)	2.53±0.60 (bcd)
***P. sericea* 1**	9.66±1.76 (bcd)	53.68±3.68 (abc)	3.97±0.22 (bcd)	0.52±0.13 (a)
***P. sericea* 2**	13.00±1.15 (d)	236.89±20.73 (e)	4.73±0.09 (ef)	9.71±0.41 (de)
species f	12.00±1.00 (cd)	174.75±22.71 (e)	7.43±0.07 (g)	42.58±1.23 (f)
species g	11.33± 1.20 (cd)	64.63±7.26 (bc)	5.43±0.03 (f)	2.26±0.18 (bc)
species h	1.33± 0.33 (a)	30.72±1.66 (a)	3.37±0.07 (ab)	1.02±0.34 (ab)

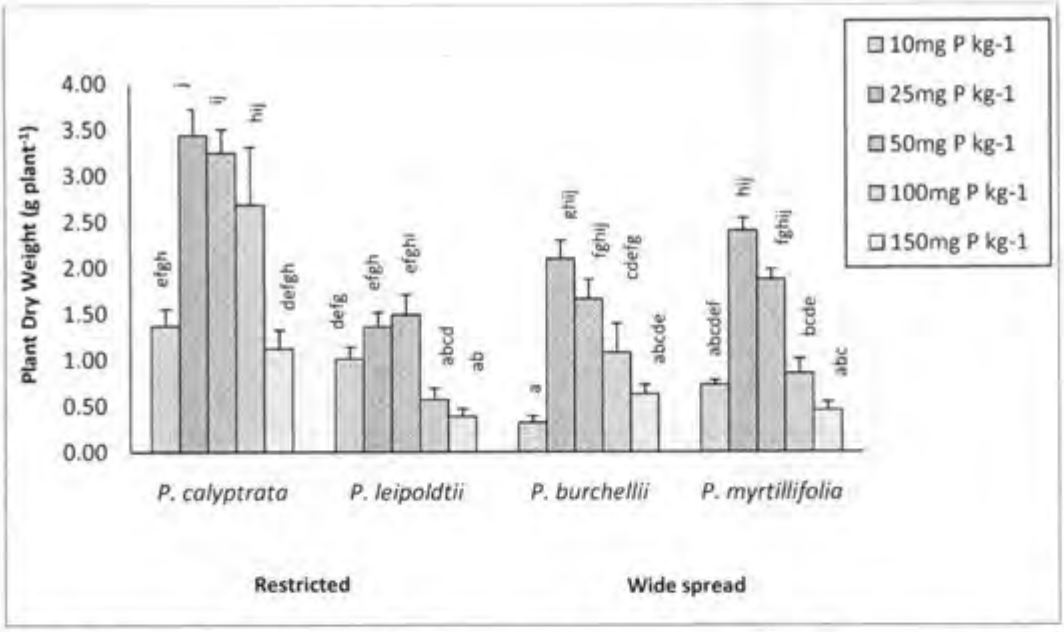

Fig 1. Biomass accumulation of *P. calyptrata, P. leipoldtii, P. burchellii* and *P. myrtillifolia* supplied with 10, 25, 50, 100 or 150 mg P kg^{-1}. Bars and errors represent mean ± SE. Letters in figure denote significant differences between species and P treatments ($P<0.05$) from a 3 way nested ANOVA.

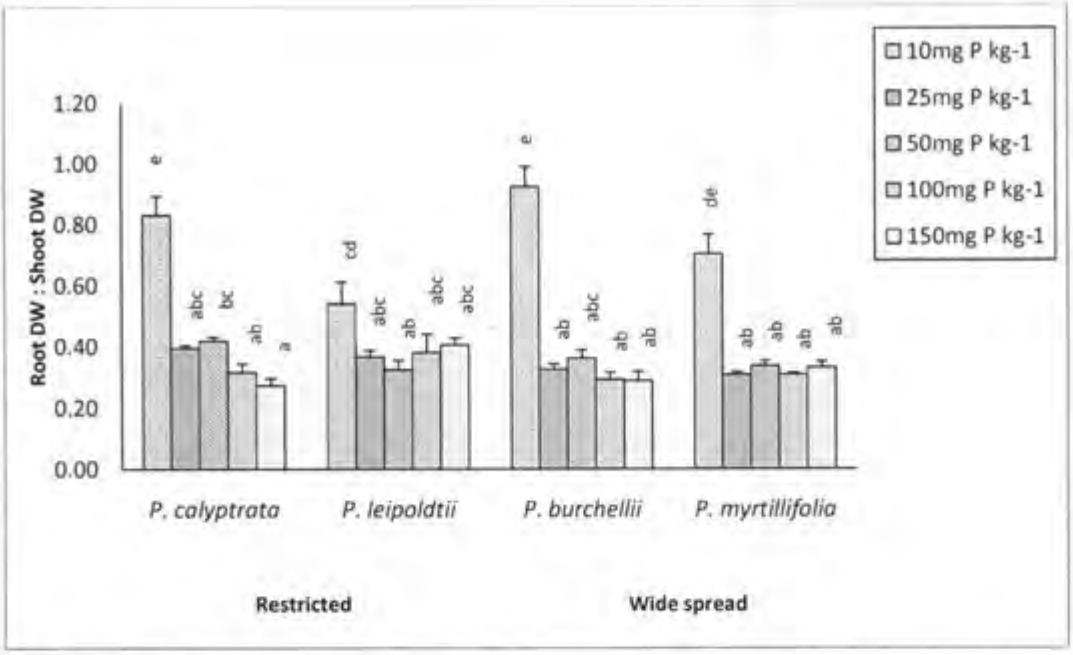

Fig 2. Root:shoot ratio of *P. calyptrata, P. leipoldtii, P. burchellii* and *P. myrtillifolia* supplied with 10, 25, 50, 100 or 150 mg P kg^{-1}. Bars and errors represent mean ± SE. Letters in figure denote significant differences between species and P treatments ($P<0.05$) from a 3 way nested ANOVA.

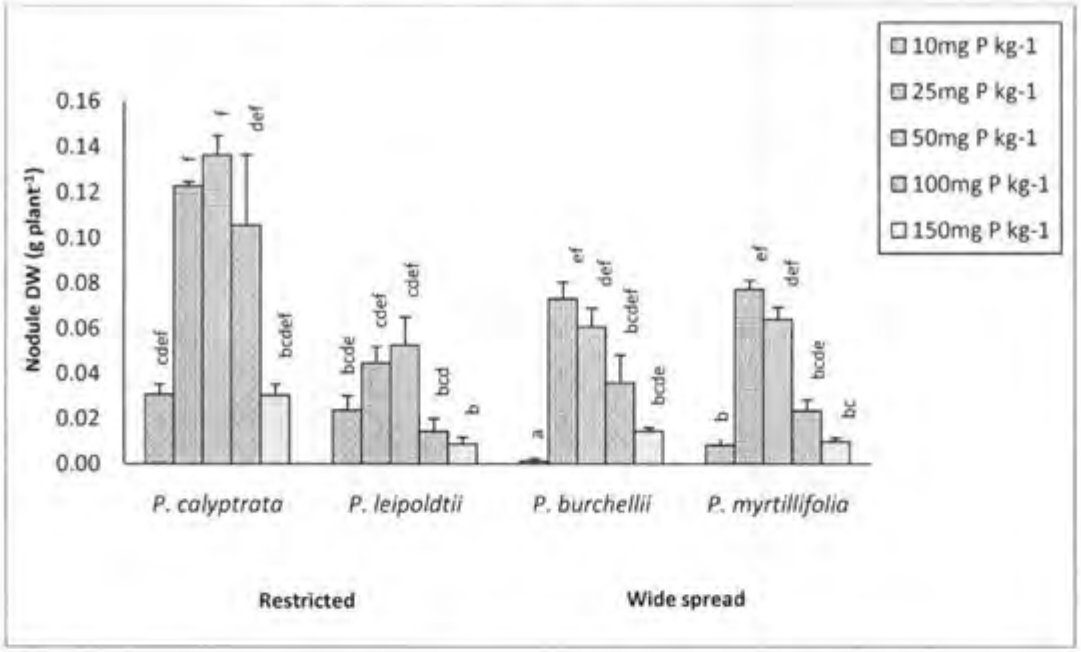

Fig 3. Nodule Dry weight of *P. calyptrata*, *P. leipoldtii*, *P. burchellii* and *P. myrtillifolia* supplied with 10, 25, 50, 100 or 150 mg P kg^{-1}. Bars and errors represent mean ± SE. Letters in figure denote significant differences between species and P treatments ($P<0.05$) from a 3 way nested ANOVA.

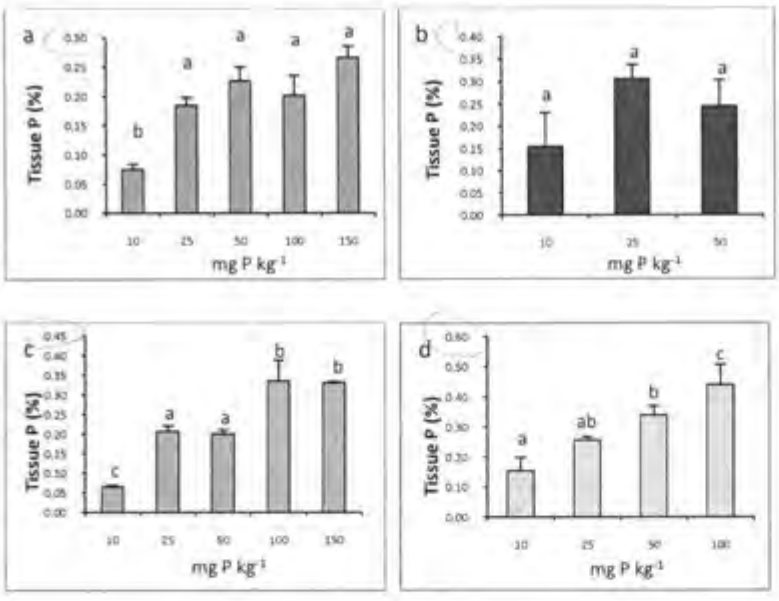

Fig 4 : Tissue P of (a) *P. calyptrata*, (b) *P. leipoldtii*, (c) *P. burchellii* and (d) *P. myrtillifolia* supplied with 10, 25, 50, 100 or 150 mg P kg^{-1}. Leaf nutrient data for *P. leipoldtii* could not be calculated for 100 mg P kg-1 and 150 mg P kg^{-1} due to small sample sizes. Bars and errors represent mean ± SE. Significant differences between P treatments ($P<0.05$) are indicated by different letters, from a one-way ANOVA.

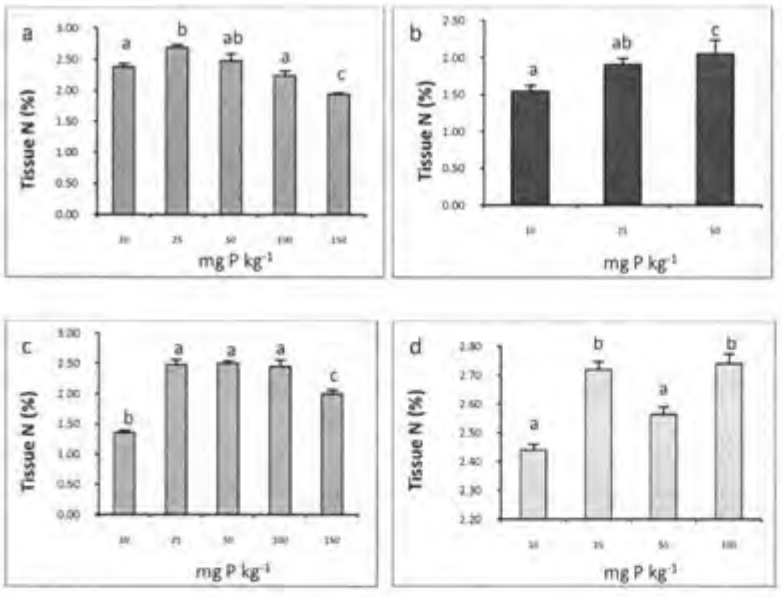

Fig 5: Tissue N of (a) *P. calyptrata*, (b) *P. leipoldtii*, (c) *P. burchellii* and (d) *P. myrtillifolia* supplied with 10, 25, 50, 100 or 150 mg P kg^{-1}. Leaf nutrient data for *P. leipoldtii* could not be calculated for 100 mg P kg-1 and 150 mg P kg^{-1} due to small sample sizes. Bars and errors represent mean ± SE. Significant differences between P treatments ($P<0.05$) are indicated by different letters, from a one-way ANOVA.

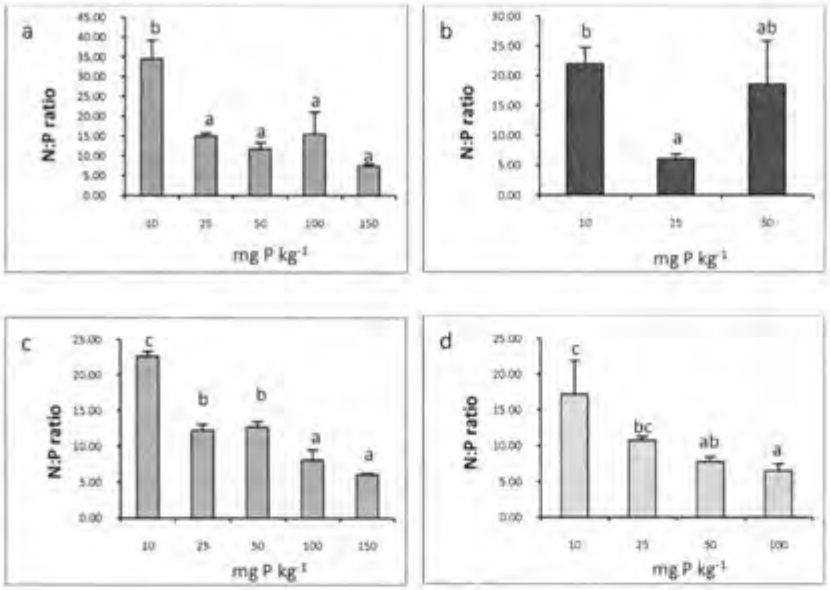

Fig 6: Tissue N:P of (a) *P. calyptrata*, (b) *P. leipoldtii*, (c) *P. burchellii* and (d) *P. myrtillifolia* supplied with 10, 25, 50, 100 or 150 mg P kg^{-1}. Leaf nutrient data for *P. leipoldtii* could not be calculated for 100 mg P kg-1 and 150 mg P kg^{-1} due to small sample sizes. Bars and errors represent mean ± SE. Significant differences between P treatments ($P<0.05$) are indicated by different letters, from a one-way ANOVA.

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Appendix

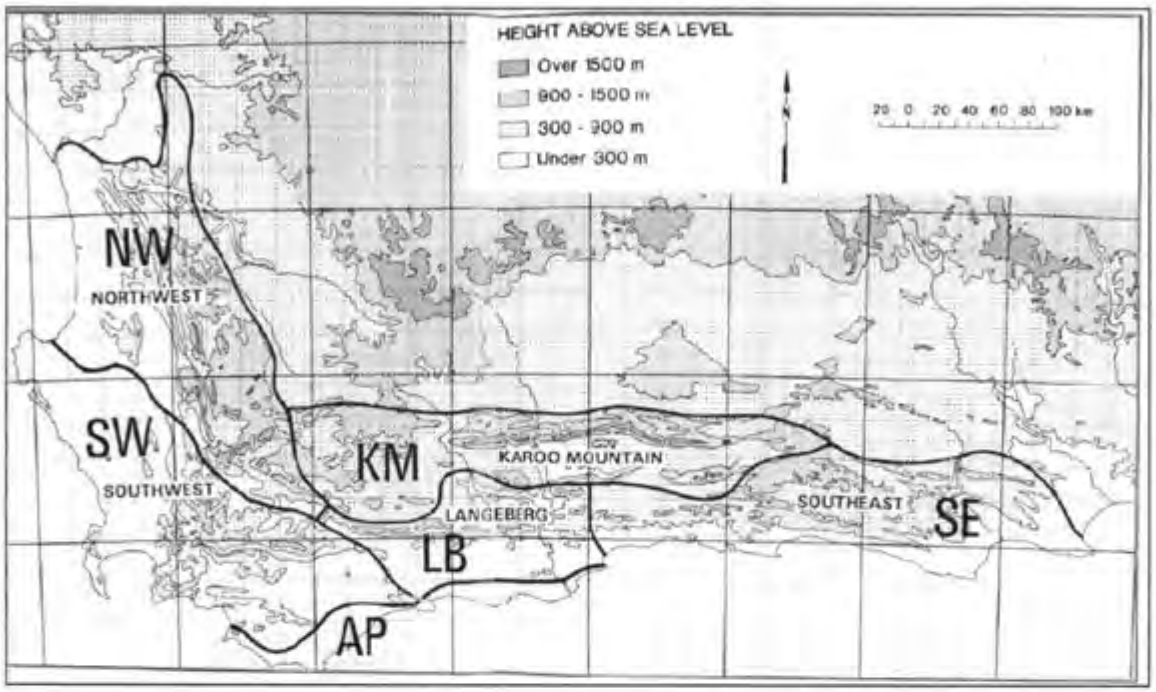

Fig 1. The six phytogeographic regions of the Cape. SW = South West, NW = North West, AP = Agulhas Plain, KM = Karoo Mountain, LB = Langeberg, SE = South East (Goldblatt and Manning 2000).