

# **N and P limitation of fynbos plants and the nutritional status of legume habitats in the Cape Floristic Region.**

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**Supervisors**

**Dr. S.B.M. Chimphango and Prof. M.D. Cramer.**

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## **Declaration**

I declare that all of the work in the document, save for that which is properly acknowledged, is my own. The thesis is submitted for the degree of Master of Science in the Department of Botany of the University of Cape Town. It has not been submitted for any degree or examination at any other university.

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# **N and P limitation of fynbos plants and the nutritional status of legume habitats in the Cape Floristic Region.**

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## **Abstract**

In general, terrestrial ecosystems are limited by both N and P, but, as legumes fix atmospheric nitrogen, I hypothesized that fynbos legumes would be P limited and non-legumes would be N limited, and that the degree to which these plants respond to N and P would depend on the levels of each of these two nutrients in the growth media. To test these hypotheses, three legumes and three non-legumes native to the fynbos were grown in a complete factorial arrangement of four levels of N and P in a glass house. Nitrogen was supplied at 20, 40, 80 and 150 mg kg<sup>-1</sup> soil and P at 0.8, 5, 15, and 31 mg kg<sup>-1</sup>. Overall, the results showed that the responses of the legume and non-legume species to N and P supply were species specific, but that the legume species seemed to be more limited by N supply than the non-legumes. There was no N x P interaction in this study, which implied that the plant response to N and P, did not depend on the levels of each other.

Given the nitrogen fixing capabilities of legumes and high nutrient demanding lifestyle, I made the hypothesis that, on a given landscape, the soils on which the fynbos legumes occur were more nutrient rich than the bulk non-legume soils. Related to this hypothesis is the question of whether the more nutrient rich soils within the legume populations would be mirrored by leaves with both a higher specific leaf area and higher nutrient concentration, than the leaves of the non-legumes. To test these hypotheses, I determined the nutrient levels of both the soils and plants within both the legume- and non-legumes stands at eight different sites in the CFR. The data were subjected to multifactorial discriminant function analysis and Nested ANOVA analyses. The results of the analyses led to the conclusion that the soil on which legume stands occurred were rarely more fertile than the non-legumes on the landscape in the CFR, and, except for leaf N, there seemed to be no differences between leaf nutrient concentrations and the SLA of the legume and non-legume plants.

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## CHAPTER 1

### GENERAL INTRODUCTION

#### 1.1 The geology and soil types of the Cape Floristic Region (CFR)

The southern Cape is dominated by the Cape Folded Belt (Cape Supergroup) consisting mostly of hard, resistant, quartzitic rocks (Lambrachts, 1979) which is a 6–10 km thick siliciclastic sequence and divided into the Table Mountain, Bokkeveld and Witteberg Groups (Lambrachts, 1979; Tinker, *et al.*, 2008). Underlying the fynbos biome is mainly the Table Mountain- and, in some areas, the Witteberg Groups (Lambrachts, 1979). Due to a wide range of environmental conditions, the fynbos is characterized by wide range of soil types and ten broad soil associations are recognised, using parent material, rockiness, soil depth, texture, and acidity as differentiating criteria (Lambrachts, 1979). The ten broad soil associations are quartzites, with very little soil, quartzites with varying amounts of shallow sandy soils, quartzites with varying amounts of deeper loamy soils, acid sandy soils mainly along the coast, calcareous sandy soils also mainly along the coast, heavy textured and highly leached granite, slates and phyllite soils, heavy textured and moderate to poorly leached slates and phyllite soils, and lastly, dominantly duplexes soils. Mucina and Rutherford (2006) identified different fynbos types occurring on the varying soil types: sandstone, quartzite, shale, silcrete, ferricrete, conglomerate, alluvium, granite and limestone bedrocks. Even within a single region, the levels of soil nutrients can vary considerably (Day, 1983). An example of different ratios of nutrient availability, especially N and P, contributing to this mosaic of soil types, with consequently different communities, can be found in a study by Richards, *et al.*, (1996). He found a total of three communities with distinct boundaries in each of two transects of only 300m at a fynbos site on the Soutanysberg, within the Agulhas National Park near Bredasdorp. One transect showed large decreases in total N and P and the second showed a decreasing trend of total P. So the number of ecological niches available to the plants is multiple and often in close proximity to each other (Witkowski and Mitchell, 1987; Richards, *et al.*, 1995; Richards, *et al.*, 1997; Goldblatt and Manning, 2002; Van der Niet and Johnson, 2009). Based on differences in nutrient content, depth and texture, altitude, topography, precipitation and changes in seasonality from the west to the east of the Cape region, a system of land classes, termed Broad Habitat Units (BHU's), as

biodiversity entities for conservation planning, was developed (Cowling and Heijnis, 2001). The BHU's are essentially surrogates for plant biodiversity and were derived by uniquely intersecting layers of vegetation, homogeneous climate zones, geology and topography in a geographic information system (GIS) and using expert knowledge (Rouget, *et al.*, 2003). Within the CFR, the Fynbos biome covered the largest area (81.5%) and included the highest number of primary (8) and secondary (69) BHUs (Cowling and Heijnis, 2001).

## 1.2 Soil N and P

Mediterranean ecosystems, one of which is the CFR, are often characterised by low primary productivity which is constrained by water and nutrient availability and are frequently disturbed by fire (Castell and Terradas, 1994; Sánchez-Díaz, 2001). The CFR, which includes the fynbos habitat, presents with a severe lack of especially N and P, due to, mostly, infertile parent material and an extremely slow and incomplete breakdown of organic litter (Larcher, 2003; Mitchell and Gibson, 2006).

The CFR is a natural habitat in an old, climatically buffered, infertile landscape (OCBLS) (Lambers *et al.*, 2010) which has ancient and deeply weathered soil (Lambers, *et al.*, 2006; Lambers *et al.*, 2010) and very slow rates of chemical weathering and rejuvenation (Rundel, *et al.*, 1980; Lambers *et al.*, 2010), due to its high quartz content (Soderberg and Compton, 2007). In addition, the Table Mountain Group, which occupy much of the landscape of the Mediterranean region of South Africa, are acidic and nutrient poor (Campbell, 1986) and highly infertile (Cowling, 1990), with very low concentrations of elements other than Si and O, thus they contribute very little in the way of nutrients, more specifically P, to the soil (Rundel, *et al.*, 1980; Soderberg and Compton, 2007). As P is supplied to a large extent by the parent material (Groves, *et al.*, 1983; Lavelle and Spain, 2001), soil P in the fynbos biome is low. The mean P content of the earth's crust is  $1.2\text{g kg}^{-1}$ , while the soils of the fynbos have between 3 and  $40\text{ mg P kg}^{-1}$  soil (Hawkins, *et al.*, 2005; Lambers *et al.*, 2010). There does seem to be an enrichment of available P after fire, as organic P is converted to orthophosphate, the form of phosphate available to plants (Certini, 2005). This enrichment declines shortly thereafter as, in acid soils; orthophosphate is adsorbed to Al, Fe and Mn (Certini, 2005). This reiterates the findings of Brown and Mitchell (1986) who found an immediate post fire increase in resin-extractable P at a fynbos study area

at Pella, near to Cape Town, South Africa, whilst the total P showed no significant change. The resin-extractable P then declined to pre-fire levels during the first four months after the fire.

N input into Mediterranean systems are primarily due to wet deposition and biological nitrogen fixation (BNF), which may be restricted by a low availability of P (Day, 1983), as well as ash deposition following fire (Rundel, *et al.*, 1980; McMaster *et al.*, 1982; Day, 1983). Smithwick, *et al.*, (2005), in a review of post fire N cycling, points out the various effects fire has in changing the total soil N levels. This can range from 65% to 310% of pre-fire levels. On the other hand, Wan *et al.*, (2001); Certini, (2005) and Boerner, *et al.*, (2009) found that (repeated) fires seem to have no effect on total soil N. Wan, *et al.*, (2001), in a meta-analysis of N response related to fire, found that  $\text{NH}_4$  increased immediately after fire and then gradually declined to pre-fire levels after one year, where-as the  $\text{NO}_3$  pool was small immediately after the fire and then significantly increased to three-fold the pre-fire level in 6 months to one year after the fire. These results showing immediate and longer term soil N changes after fire closely mirror similar results found by Stock and Lewis (1986) in an investigation into how fire affects the soil N in a coastal fynbos ecosystem. Decomposition of litterfall serves to recycle N within Mediterranean systems (Stock, *et al.*, 1995; Rapp *et al.*, 1999). This transference of dead plant material to the soil may not be significant because of climatic restrictions and the chemical nature of the decaying tissues (Rundel, *et al.*, 1980; McMaster *et al.*, 1982; Day, 1983).

### **1.3 N and P Limitation to Plant Growth**

In plants, growth is particularly important because both survival and reproduction depend on plant size, and thus on the growth rate (Shipley, 2006) and differences in growth rate are caused by, among other factors, nutrient availability (Freijsen and Veen 1990). Plant growth rate is the increment in dry mass (DM), volume, length or area (per time unit) that results from the division, expansion and differentiation of cells (Lambers, *et al.*, 2008) and if an increase in the availability of a particular resource brings about an increase in the plant's growth rate, that resource is limiting (Tilman, 1986; Freijsen and Veen, 1990). N limitation is driven by N being almost absent from new soils, lost to ecosystems by means of leaching, volatilization (fires), runoff, erosion and aeolian particulate removal from unconsolidated ash beds after fires, denitrification, and/or by

organic N being directly carbon bonded, often in structural or complexed forms that need multiple enzyme systems for its breakdown to its available forms (Rundel, *et al.*, 1980; McMaster *et al.*, 1982; Day, 1983; Stock and Lewis, 1986; Vitousek and Howarth, 1991; Sánchez-Díaz, 2001; LeBauer and Treseder 2008; Schafer and Mack, 2010). Of the mechanisms underlying P limitation are P depletion which can occur during long term soil and ecosystem development (Vitousek, *et al.*, 2010), low P parent rock (Vitousek, *et al.*, 2010) and P sinks, when P is adsorbed onto the surfaces of other minerals (Vitousek and Howarth, 1991; Lambers *et al.*, 2006; Vitousek, *et al.*, 2010). Over geological time, P can become unavailable to plants when mineral-sorbed P becomes occluded by iron and aluminium (Lambers *et al.*, 2006). High soil N:P ratios can also make P unavailable to plants (Vitousek, *et al.*, 2010).

There is conflicting evidence about whether terrestrial Mediterranean ecosystems are N, P or N and P co-limited: According to Dias, *et al.*, (2012) the Mediterranean maquis in Portugal is N limited, while phosphorus and, to a lesser extent, nitrogen limit the growth of sclerophyllous shrubs on nutrient-poor soils in southern Australia (Groves, *et al.*, 1986). N seems to be the limiting resource in Californian chaparral (Padgett and Allen, 1999) and Mc Master, *et al.*, (1982) found N and P to co-limit chaparral. In the Mediterranean Basin, P limited the growth of both pine and holm oak (Sardans, *et al.*, 2004). In the fynbos ecosystem, general consensus has yet to be reached regarding the status on N and/or P limitation, as results of factorial experiments give contradictory results (Witkowski, 1989a; Witkowski, 1989b; Lamb and Klausner 1988). In the above discussion of nutrient limitation in the Mediterranean ecosystems, one has to bear in mind that extrapolating data from one Mediterranean-type ecosystem to another, or even from one habitat to another in the same ecosystem, should be used with caution, as soils may differ in fertility (Dias, *et al.*, 2012). This is particularly true of the CFR, as it is a mosaic of different soil types (Mucina and Rutherford, 2006).

### **3. Plant Responses to N and P Supply**

#### **3.1 Biomass accumulation**

Legumes tend to be more responsive to P supply, than non-legumes. In a study, Grove (1988) applied increasing P levels to a karri (*Eucalyptus diversicolor*) stand in which *Bossiaea laidlawiana* was a dominant legume of the under storey. The biomass of the

legume increased and that of the non-legumes decreased. In a factorial designed experiment involving enhanced CO<sub>2</sub> and P to two communities, one with and the other without legumes, Stöcklin and Körner (1999) found that P fertilization did not influence the biomass of graminoids and forbs, but that legumes increased significantly ( $p < 0.05$ ) by 29%. On the other hand, legumes tended to be less responsive than non-legumes to N supply with regard to biomass and/or growth rate (Reich, *et al.*, 2003. Graham and Vance, 2003; Lee *et al.*, 2003; Guénaëlle, *et al.*, 2007)

One of the factors that may lead to low effects of fertilizer treatments is the practice of applying N, P and potassium separately, not considering the possible interactive effects these fertilizers may have (Engelbrecht, *et al.*, 2010). According to Craine and Jackson, (2010) and Harpole, *et al.*, (2011) possible responses by plant species to different combinations of N and P concentrations, which are supplied concurrently, could be that N and P simultaneously limit primary production, i.e. N and P function as a single collective resource. In this case (simultaneous or classic co-limitation) there is no response in primary production to either N or P when added on their own, but a significant increase in primary production with simultaneous addition of N and P. A second possible scenario of nutrient interaction response could be when each nutrient elicits a primary production response on their own, and an even greater response to simultaneous addition of N and P. This is known as independent co-limitation or co-limitation by trade off. Harpole, *et al.*, (2011) also defines a third possible response known as serial co-limitation, where the response to the addition of the second nutrient only occurs after the first (primary) nutrient had elicited a response. These three responses are classified as synergistic (Harpole, *et al.* 2011). Davidson and Howarth (2007) define synergistic limitation as N and P limitation alternating in many small increments of primary production, which ultimately produces a synergistic effect. Interesting to note that the response to simultaneous addition of N and P could also have an antagonistic response, as demonstrated in a factorial experiment by Wallace, (1990), where N and P applied to Valencia orange trees simultaneously, caused a decrease in yield, as opposed to the response in yield of these trees to N and P individually. Siddique, *et al.*, (2010) found a similar antagonistic effect in studying tree species diversity in a tropical forest in which N + P addition had a weaker effect on tree biomass and diversity than N- or P- only additions.

Results of factorial designed fertilization experiments in nutrient poor soils, other than fynbos, showed a mixed effect of N and P fertilization. Kachi and Hirose (1983) did an experiment in which a dune plant, *Oenothera erythrosepala*, was subjected to fertilization experiments in which N, P and K were administered singly and in various combinations, both *in situ* and in a pot culture. The field experiment indicated that *O. erythrosepala* was P limited, whereas the pot culture experiment indicated that the plant growth was limited by N. In a field experiment within chaparral vegetation, one dominant shrub, *Adenostoma fasciculatum*, responded to all treatments on the first year and only to the N treatment in the second year, while the other dominant shrub, *Ceanothus griggii*, responded to the P treatment (McMaster *et al.*, 1982). In a factorial experiment by Von Oheimb, *et al.*, (2010), a heathland cover plant, *Calluna* sp. from north-west Germany showed a positive response to N and N+P and no response to P. On the other hand, Falk *et al.*, (2010) found that *Molinia caerulea*, encroaching in the same dry heathland of the previously discussed experiment, responded positively to P fertilization, with no response to N fertilization nor was there any N and P interaction.

### **3.2 Biomass allocation**

There are trade-offs between investment in structures that lead to a high growth potential and those associated with conservation of nutrients (Lambers and Poorter, 1992). Research into these trade-offs have led to the formulation of an optimal partitioning model that predicts that plants allocate more resources to tissues and processes which lead to the acquisition of the relatively limiting resources in such a way, that the acquisition of all resources become equally limiting (Bloom, *et al.*, 1985). In line with this model, Gedroc, *et al.*, (1996) and Marschner, *et al.*, (1996) found that the root vs. shoot organ allocation by plants was determined by available resources. An example would be that of Bonifas, *et al.*, (2005) who reported that corn and velvetleaf increased biomass partitioning to roots when N was limiting, while Boutraa (2009) found that *Phaseolus vulgaris*, a legume, also increased biomass partitioning to the roots, but under limiting available P.

## **4. Legumes as a population within plant communities**

In areas of the fynbos where legumes persist, many (primarily seeders) (Schutte *et al.*, 1995) tend to occur in distinct populations (hereafter called legume populations) which

are meters, to tens of meters, in diameter, with discernable boundaries between the legume populations and the adjacent fynbos vegetation. There are a number of possible reasons for their occurrence in these patches. One reason could be that some legumes have short-distance seed dispersal mechanisms and it is assumed that these plants cannot afford the cost of allocating resources to long-distance seed dispersal mechanisms, such as protein rich (and thus edible) seeds (Wilson and Travaset, 2000; Goldblatt and Manning, 2002). Some of the other dispersal methods they use are ant dispersal (myrmecochory) which has a dispersal distance of less than six meters (Auld, 1996; Holmes and Richardson, 1999; Goldblatt and Manning, 2002; Pierce and Cowling, 1991; Linder *et al.*, 2010), self dispersal such as autochory (explosive mechanisms) and barochory (heavy seeds dropping close to the parent plant), as well as low canopy height which restrict seed dispersal distances (Auld, 1996; Holmes and Richardson, 1999; Valbuena and Trabaud, 2001; Lavergne, *et al.*, 2004). All of these methods disperse the seeds for only short distances.

Another possible explanation for this patchy distribution could be a result of post fire changes in the species composition due to heterogeneous deposition of ash, which may lead to complex differences in nutrient availability within the landscape (Hanley and Fenner, 1997). Legume seedlings seem to proliferate after fire (Goergen and Chambers, 2009; Knox and Clarke, 2006), probably due to higher levels of soil nutrients associated with ash deposits after a fire (Stock and Lewis, 1986; Pausas and Vallejo, 1999; Schafer and Mack, 2010). This is also consistent with the report of Ritchie and Tilman, (1995) who stated that legumes may require a higher than usual amount of nutrients to maintain the cost of N-fixation and paying this cost is only advantageous under conditions of low soil N availability (Crews, 1999). Support for the statement by Ritchie and Tilman, (1995) comes from two forest studies, that of a lowland rain forest in south western Cameroon, where Newbery, *et al.* (1988) found a positive correlation between a group of caesalpinoid legumes and a relatively high concentration of soil phosphorus, and a study of soil nutrients and tree species richness of a Costa Rican forest, in which Husston (1980) found a negative correlation between available soil N and the percentage frequency of legumes, a result also reported by Campbell (1927), Carlsson, *et al.*, (2009) and Roscher, *et al.*, (2011). Fire also enhances seed germination of legumes, as the heat from the fires scarifies the seeds, leading to successful germination (Bradstock and Auld, 1995; Auld, 1996).

A third possible reason for legumes occurring in distinct populations could be niche construction mechanisms, through nutrient cycling (Binkley and Giardina, 1998). These are effects plants can have on the soil properties (Yang, *et al.*, 2011) and may lead to formation of islands of fertility (Yang, *et al.*, 2011), sometimes referred to as niche construction. These islands of fertility are created when higher organic matter contents and a higher cation exchange capacity are brought about by the nutrient cycling of the organic matter found under the relevant plants (Yang, *et al.*, 2011). Dong *et al.*, (2009) call this a “typical shrub fertile island phenomenon”. The fertile island effect is even more pronounced if the dominant plants in the patch are legumes (Rodríguez, *et al.*, 2011), due to the high nutrient levels in the biomass (Bashan and De-Bashan, 2010). However, it is also possible that these sites were already nutrient rich before the legumes colonised the areas, because legumes are associated with high nutrient lifestyle (Vitousek and Howarth, 1991; Vitousek, *et al.*, 2002) favouring nutrient rich habitats, followed by decline in their numbers in the field about 10 years after fire (Cocks, 1994).

### **Rationale, Hypotheses and Objectives**

There is conflicting evidence about whether Mediterranean ecosystems are N, P, or N and P co-limited (Mc Master, *et al.*, 1982; Groves, *et al.*, 1986; Padgett and Allen, 1999; Sánchez-Díaz, 2001; Sardans, *et al.*, 2004; Dias, *et al.*, 2012). In the fynbos ecosystem, general consensus has yet to be reached regarding the status on N and/or P limitation, because results of factorial experiments are also contradictory (Witkowski, 1989 a; Witkowski, 1989 b; Lamb and Klausner 1988). Furthermore, studies have shown that legumes respond positively to the addition of P, with both enhanced leaf nutrients and shoot dry weight (Kolawole and Kang, 1997; Stöcklin, *et al.*, 1998; Mapfuno, *et al.*, 2005; Amba, *et al.*, 2011). With this in mind, I postulated two hypotheses: The first that legumes are limited by P and non-legumes by N and the second, that the degree to which legume and non-legume respond to N and P would depend on the levels of each of these two nutrients in the growth media. A glass house experiment was designed to address these hypotheses.

With the observation that legumes tended to occur in distinct populations, I proposed the hypothesis that on a given landscape, the soils on which the fynbos legumes occur were more nutrient rich than that of the surrounding non-legume populations, and that

the legume leaves would have higher nutrient concentrations and SLA's than the non-legume leaves. A field study was conducted to address these hypotheses.

Thus, the objectives of the study were to determine:

1. Whether legumes native to the Cape Floristic Region are limited by phosphorus and non-legumes by nitrogen.
2. The levels of N and P supply for high growth and nutrient concentration of legume and non-legume plants (i.e. N and P interaction).
3. Whether soil nutrients in the legume patches are higher than those from adjacent non-legumes on a specific landscape.
4. Whether fynbos legumes have higher SLA and leaf nutrient concentration than non-legumes.

The research chapters have been written as full chapters, each with an abstract and introduction. Thus repetitions of some sections were unavoidable.

## CHAPTER 2

### Are the Cape Fynbos legumes limited by phosphorus and non-legumes by nitrogen?

#### Abstract

Vegetation productivity is thought to be influenced partly by soil N and P in Mediterranean ecosystems, such as the CFR. It was hypothesized that legumes native to fynbos are limited by P and non-legumes by N and that plant response to P and N would depend on the levels of each of these nutrients in the soil. The hypotheses were tested by a glass house experiment, in which three fynbos legumes and three Proteaceae species (here after referred to as non-legumes), were given factorial combinations of four levels of N and P, where the N was supplied at 20, 40, 80 and 150 mg kg<sup>-1</sup> soil and that of P, 0.8, 5, 15 and 31 mg kg<sup>-1</sup>. After about four months, the plants were harvested and the dry weight of the leaves, stems, and roots of the legumes and non-legumes were determined, as well as that of the nodules of the legumes and the cluster roots of the non-legumes. The ratio of the roots to shoots, nodules to roots and cluster roots to roots, as well as specific leaf area were calculated. Percentage leaf N and P, and leaf N to P ratio of *Podalyria calyptrata* and *Leucospermum cordifolium* were determined and gas exchange measurements of all six species were recorded. The main effects with regards to biomass accumulation and biomass allocation were more pronounced at the higher levels of N supply, than that of P supply in both legumes and non-legumes, while leaf gas exchange parameters showed very limited responses to the higher levels of N and P. The changes in the SLA also varied with species. Measurements of tissue N and P showed that *P. calyptrata* did not respond with significant ( $p < 0.05$ ) differences in leaf N and P. *Leucospermum cordifolium* responded with changes in leaf N and P with both the higher levels of N and P supply. Overall, the results show that the responses of the legume and non-legume species to N and P supply were species specific, but the legume species were more limited by N supply than P supply. The lack of N x P interaction implied that none of the 16 N and P combinations supplied to the plants had a synergistic affect on the biomass accumulation (growth) of either the legumes or the non-legumes, i.e. neither were co-

limited by N and P.

## Introduction

Previous studies have shown that legumes tend to be more responsive to P and less responsive to N supply, than non-legumes. In a glasshouse study on specifically fynbos species by Power *et al.*, (2010), eight legumes and six non-legumes were given either 1 or 10 mg P kg<sup>-1</sup> soil. Five legumes presented with an increase in biomass with the higher P fertilization, whilst the biomass of one did not change and the biomass of two decreased. The biomass of only one of the non-legumes increased at the higher P supply, but that of the other 4 non-legumes did not change. The question I asked is: Would concurrent applications of different levels of both N and P to legumes and non-legumes have different effects on their growth responses? In an endeavour to answer this question, I grew three legumes and three non-legumes indigenous to the fynbos in a complete factorial arrangement of four levels of N and P for about 18 weeks, after which their biomass, concentration of N and P of their leaves and gas exchange measurements were recorded. The expected results were that the non-legumes would make better use of available P by responding with higher biomass production, if higher levels of N were supplied and that fynbos legumes, which can supply their own N by means of biological nitrogen fixation (BNF), would respond with a similar change in biomass, with an adequate supply of P. As mycorrhizal infection, nodule development, leaf SLA and biomass accumulation and allocation seem to be regulated largely by nutrient availability, a further aspect to this study was to determine if, and to which degree, would these plant traits be influenced by the manipulation of available N and P.

Modifications of gas exchange are usually the first response to environmental stress, which is then followed by biomass allocation (Yin *et al.*, 2009). Bradbury and Malcolm, (1977); Da Silva, *et al.*, (2008) and Graciano, *et al.*, (2005) found no response of water use efficiency (WUE) to P fertilization, where as Korol *et al.*, (1999) found that a positive response to P fertilization is associated with water stress, whilst the photosynthetic rate of durum wheat in an experiment involving N fertilization, by Cabrera-Bosquet, *et al.*, (2007), showed a decrease. According to Cramer, *et al.*, (2009), soil nutrient availability may partially regulate the transpiration rate, although other available experimental data are contradictory. For instance, there was generally no response in the transpiration rate of Sitka spruce to P and/or N fertilization

(Bradbury and Malcolm, 1977), *Pseudotsuga menziesii* (Guehl and Garbaye, 1990; Korol *et al.*, 1999), spring wheat (Li, *et al.*, 2001), *Eucalyptus grandis* (Graciano, *et al.*, 2005), maritime pine (Loustau, *et al.*, 1999), *Pseudotsuga menziesii* and *Populus x euroamericana* (Ripullone, *et al.*, 2004), *Helianthus* seedlings (Ripullone, *et al.*, 2004) and *Pseudotsuga menziesii* and *Populus x euroamericana* (Howard and Donovan, 2007). Though some researchers found an increased transpiration rate in plants with the addition of P: *Grevillia robusta*, *Calliandra calothyrsus* and *Senna spectabilis* (Gunga, *et al.*, 2003), *Eucalyptus grandis* seedlings (Graciano, *et al.*, 2005) and *Viamia japurensis*, *Bellucia grassularoides* and *Laetia procera* (Da Silva, *et al.*, 2008), others found decreased transpiration rates with N fertilization (Guehl, *et al.*, 1995; Ludwig *et al.*, 2006; Scholz, *et al.*, 2006; Cabrera-Bosquet, *et al.*, 2007; Cernusak, *et al.*, 2007; Cramer, *et al.*, 2008; Garrish, *et al.*, 2010).

The objectives of this experiment were:

- To determine whether legumes native to the Cape Floristic Region are limited by phosphorus and non-legumes by nitrogen.
- To determine the levels of N and P supply for optimum growth and nutrient concentration of legume and non-legume plants (i.e. N and P interaction)

## Materials and Methods

The experiment was conducted in a glass house at the University of Cape Town (UCT), from 17 November, 2009 to 23 March, 2010. Soil N and P supply were manipulated, using a factorial experimental design. Four levels of N in the form controlled release granules of urea (Multicote TM(4+)42.00) and four levels of P as 75% FePO<sub>4</sub> and 25% Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> in powder form were combined as shown in Table 2.1 and added to the pots, giving a total of 16 different treatments. Each treatment was replicated 5 times.

**Table 2.1.** The levels of N and P of the 16 treatments.

Treatment	N (mg kg <sup>-1</sup> soil)	P (mg kg <sup>-1</sup> soil)
1	20	0.8
2	20	5
3	20	15
4	20	31
5	40	0.8
6	40	5
7	40	15
8	40	31
9	80	0.8
10	80	5
11	80	15
12	80	31
13	150	0.8
14	150	5
15	150	15
16	150	31

### Plant Culture

Three Fabaceae: *Podalyria calyptata*, *Otholobium striatum* and *Aspalathus linearis* and three Proteaceae species: *Leucadendron coniferum*, *Leucospermum cordifolium* and *Protea repens* were chosen as they are found in the Cape Peninsula (Western Cape Province, S.A.) and grow in nutrient poor soil. *P. calyptata*, *O. striatum* and *A. linearis* are nodulating legumes and *A. linearis* also forms cluster roots, while all three Proteaceae species form cluster roots. The Fabaceae species are hereafter referred to as legumes, and the Proteaceae species as non-legumes.

Seedlings of similar size were transplanted to 18 cm pots containing 2.8 kg of acid washed silica sand which were mixed with 200g of soils collected to a depth of 20 cm from legume and non-legume stands within the fynbos. The added soil was to serve as rhizobia and mycorrhizal inoculate. The acid washed silica sand and inoculate soil were mixed in a cement mixer for 10 minutes in a ratio of silica sand:legume soil:non legume soil of 18:0.5:0.5. The pots were placed on trolleys in the glasshouse in a completely randomized layout and watered to 70% field capacity every four days with a complete nutrient cocktail ( $\text{mg l}^{-1}$ ):  $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ , 0.0474;  $\text{K}_2\text{SO}_4$ , 0.0218;  $\text{NaH}_2\text{PO}_4$ , 0.00016;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.0133;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.0007;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.0005;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.0005;  $\text{H}_3\text{BO}_3$ , 0.0025;  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.0001; FeEDTA, 0.0601. On very hot days the plants were given extra de-ionized water to prevent water/heat stress. After two weeks, the nutrients were removed from the pots by flushing with de-ionised water. There-after the experiment began by modifying the nutrient cocktail to the specifications as set out in Table 2.1.  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  was replaced with  $\text{CaCl}_2$  and  $\text{NaH}_2\text{PO}_4$  omitted. The pots were watered to 70% field capacity every four days with this modified nutrient cocktail for the duration of the experiment. The trolleys were randomly rearranged every two weeks during the experiment to minimize edge effects. The plants were treated with a fungicide and an insecticide as the need arose.

#### Gas exchange measurements

Measurements of gas exchange were taken at harvest. The photosynthetic and transpiration rates, stomatal conductance, and intercellular  $\text{CO}_2$  concentration of the youngest, fully expanded leaves of each plant were measured, using a LI-6400 Portable Photosynthesis System (Licor, Lincoln, NE, USA) with the conifer chamber attached. A conifer chamber is a "hollow" cuvette, into which a number of small or narrow leaves, such as pine needles, could be put. The  $\text{CO}_2$  concentration of the reference air entering the leaf chamber was adjusted with a  $\text{CO}_2$  mixer control unit to 400 ppm, relative humidity was between 29 and 50%, cuvette temperature at 23 to 24°C and air flow rate was  $500 \mu\text{mol s}^{-1}$ . Measurements were made under irradiance of  $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Stomatal density of one expanded leaf from each plant was determined by making an impression of the leaf surface using nail varnish and counting the number of stomata under a light-microscope at x400 in three different inter-venal areas and calculating an average.

## Measurement of biomass.

After recording the gas exchange measurements, the plants were removed from the pots and the sand gently washed off the roots under running water, blotted dry with paper towel and separated into roots, stems, and leaves. The SLA of each plant was determined by removing ten (*P. calyptрата* and *L. cordifolium*) or more (the other four species) new, fully formed leaves from each plant and then measuring the combined leaf area of the leaves using a LI-3100 Area Meter, Li-cor Inc. The leaves were put into a paper bag and oven dried at 70°C for 54 hours and the dry matter (DM) recorded. Using all the leaves of each plant, the specific leaf area (SLA) was calculated by dividing the leaf area by the dry mass. A randomly selected 0.5 – 1.0 cm piece of the roots of each plant was cut off and preserved in 10% ethanol for assessment of mycorrhizal colonization (%) of the roots, using the grid-line intersect method. Nodules were removed from the roots of the legumes and the cluster roots from the roots of the Proteaceae. The individual fresh mass (FM) of all of the above parts of the plant were recorded and the materials put into paper bags and oven dried at 70°C for 54 hours. This dry matter (DM) mass was also recorded. Using these DM weights, the following ratios were calculated: root:shoot and root:nodule (legumes), root:cluster roots (non-legumes).

Only *P. calyptрата* and *L. cordifolium* were analysed for percentage leaf N and P, because these species showed biomass response to either N or P supply. The leaves were ground using a Wiley mill (Arthur H Thomas, Philadelphia, PA, USA) and sent to BEMLab Private Laboratory, Somerset, South Africa for nutrient analyses. The concentration of N was determined by a Leco FP-528 N Analyzer (Leco Corporation) and the concentration of P was determined by ashing the leaves at 480°C for 8 hours and then dissolving the ash in 16% HCl. The concentration of P in the dissolved ash was determined, using inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista MPX, Mulgrave, Australia).

Data were log transformed, when necessary, to meet assumptions of homogeneity and subjected to factorial ANOVA analysis, using Statistica, Version 9. The Duncan's post hoc test was performed when  $p < 0.05$  among means. Correlations between total biomass of the plants with the different levels of supplied N and P were determined. Statistical analyses were considered significant at  $p < 0.05$ .

## Results

The total biomass of two legumes and one non legume species showed a significant ( $P < 0.01$ ) response to the levels of N. In *P. calyptata* and *O. striatum*, the total biomass of plants receiving 20, 40 and 80 mg N kg<sup>-1</sup> was less ( $P < 0.05$ ) than that of plants supplied with 150 mg N kg<sup>-1</sup> (Table 2.2), whereas the biomass of *L. coniferum* plants receiving 20 mg N kg<sup>-1</sup> recorded a lower biomass than those receiving 40, 80 or 150 mg N kg<sup>-1</sup> which showed similar biomass amounts (Table 2.3). With regards to P supply, not one legume responded with a change in biomass (Table 2.2). On the other hand, the *P. repens* receiving 31 mg P kg<sup>-1</sup> soil had a higher total biomass than those supplied with 0.08, 5, 15 mg P kg<sup>-1</sup> (Table 2.3). However, *L. cordifolium* and *L. coniferum* showed no response to P supply (Table 2.3).

There were no differences in the biomass of any of the legumes, irrespective of the levels of supplied P, while the nodule biomass of all three legumes were less ( $P < 0.01$ ) at the N supply of 80 and 150 mg N kg<sup>-1</sup>, than those from N supply of 20 and 40 mg N kg<sup>-1</sup> (Table 2.2). The higher levels of N supply led to increasingly lower ( $P < 0.05$ ) cluster root biomass in all three non-legume species (Table 2.3), whilst the higher P supply levels had no effect on the cluster root biomass of the non-legumes

Neither N nor P nutrition altered the root:shoot biomass allocation in any of the legumes. With regards to the non-legume species, the supply of 150 mg N kg<sup>-1</sup> to *L. cordifolium* and *P. repens*, led to lower root to shoot ratios than those of the lower levels of N (Tables 2.2 and 2.3). The nodule to root ratio of all the three legumes were lower at the higher levels of N, in contrast, there was no effect on the ratio by the different levels of P (Tables 2.2 and 2.3). A similar pattern manifested itself with the N supply to the non-legumes with regards to their cluster root to root ratios; in that the cluster root to root ratios were lower at the higher levels of N but the supply of 5 and 15 mg P kg<sup>-1</sup> to *P. repens* led to a higher ( $P < 0.01$ ) cluster root to root ratio relative to those supplied with 0.8 and 31 mg P kg<sup>-1</sup> (Table 2.3). Only *P. calyptata* responded with less mycorrhizal infection at the higher levels of P (Table 2.2). The non-legumes, as expected, did not form any mycorrhizal associations

Of the six species, three showed significant ( $P < 0.05$ ) SLA changes in response to the N or P supply. *Podalyria calyptata* (legume) and *L. cordifolium* (non-legume) showed a

higher ( $P < 0.05$ ) SLA at  $31 \text{ mg P kg}^{-1}$ , than those at  $5 \text{ mg P kg}^{-1}$  (Tables 3.2 and 3.3). *Otholobium striatum* (legume) showed a significantly ( $P < 0.01$ ) lower SLA of  $1.76 \text{ m}^2 \text{ kg}^{-1} \text{ DM}$  at  $150 \text{ mg N kg}^{-1}$ , than the  $1.99 \text{ m}^2 \text{ kg}^{-1} \text{ DM}$  at  $80 \text{ mg N kg}^{-1}$  (Table 2.2).

There were very few differences ( $P < 0.05$ ) between gas exchange measurements of stomatal densities, photosynthetic rates and water use efficiency of the six species at the various levels of N and P supply, with significant ( $P < 0.05$ ) differences in only four of the 32 parameters measured, thus these data were not shown. Furthermore, there were limited responses in the transpiration rates of the plants to the changes in the levels of soil N and P, with only *P. calyptrata* showing the highest transpiration rate at the highest P level of  $31 \text{ mg P kg}^{-1}$  and *O. striatum* recorded a lower transpiration rate at  $80 \text{ mg N kg}^{-1}$  than at the other three levels of soil N (Table 2.4)..

There were only five instances (i.e. 5%) of significant ( $p < 0.05$ ) interactions between supplied N and P out of the 86 parameters analysed. These interactions pertained to the SLA and growth rate of *L. cordifolium*, the nodule DM of *A. linearis* and the transpiration rate of *P. calyptrata* and *O. striatum*. (Appendix 1 and 2). These interactions did not show any meaningful pattern.

**Table 2.2** Effects of the addition of the four levels of N and P on the SLA, the biomass accumulation of the total plant and nodules, root to shoot ratio, nodule to root ratio, transpiration rate, and mycorrhizal infection of the legumes at harvesting. Values are mean ( $\pm$  S.E.). Data annotated with either \*, \*\* or \*\*\* indicate p-values of <0.05, <0.01 or <0.001 respectively. Means followed by different letters are significantly different. Means for significant N x P interaction are presented in figure 3.1

Sites	Nutrient	Levels	SLA (m <sup>2</sup> kg <sup>-1</sup> DM)	Total Plant (g DM)	Nodules (g DM)	Root : shoot	Nodule : root	Mycorrhizal infection (%)
<i>P. calypttrata</i>	N	20	21.3 $\pm$ 0.7	12.00 $\pm$ 0.9 a	0.36 $\pm$ 0.05 b	0.30 $\pm$ 0.02	0.15 $\pm$ 0.02 b	49.36 $\pm$ 3.8
		40	21.8 $\pm$ 1.1	14.41 $\pm$ 0.8 ab	0.39 $\pm$ 0.03 b	0.31 $\pm$ 0.02	0.13 $\pm$ 0.01 ab	57.41 $\pm$ 4.3
		80	21.2 $\pm$ 0.9	13.95 $\pm$ 1.0 a	0.30 $\pm$ 0.04 ab	0.30 $\pm$ 0.02	0.11 $\pm$ 0.01 a	55.32 $\pm$ 4.1
		150	19.9 $\pm$ 0.6	18.42 $\pm$ 2.6 c	0.21 $\pm$ 0.02 a	0.29 $\pm$ 0.02	0.07 $\pm$ 0.01 a	48.08 $\pm$ 3.6
		F-Statistic	0.76 (3,60)	4.744(3,61) **	4.3137(3,61) **	0.660	2.2557 **	1.543 (3,55)
	P	0.8	18.8 $\pm$ 0.7 a	14.65 $\pm$ 0.8	0.35 $\pm$ 0.04	0.32 $\pm$ 0.02	0.13 $\pm$ 0.02	55.69 $\pm$ 3.7 b
		5	20.0 $\pm$ 0.7 ab	18.97 $\pm$ 2.9	0.37 $\pm$ 0.04	0.27 $\pm$ .02	0.12 $\pm$ 0.01	52.11 $\pm$ 4.5 ab
		15	22.4 $\pm$ 1.0 bc	12.97 $\pm$ 0.8	0.26 $\pm$ 0.03	0.29 $\pm$ 0.01	0.10 $\pm$ 0.01	57.16 $\pm$ 3.6 b
		31	22.9 $\pm$ 0.6 c	12.71 $\pm$ 1.0	0.29 $\pm$ 0.04	0.30 $\pm$ 0.02	0.11 $\pm$ 0.01	43.96 $\pm$ 3.4 a
			F-statistic	6.01 ** (3,60)	3.832(3,61)	1.0182(3,61)	0.782(3,60)	0.0909(3,60)
	NxP F-statistic	0.43(9,60)	0.1003(9,61)	0.8803(9,61)	1.067(9,60)	0.4823(9,60)	0.618(9,55)	
<i>O. striatum</i>	N	20	20.5 $\pm$ 0.6 b	8.73 $\pm$ 0.57 a	0.20 $\pm$ 0.02 c	0.28 $\pm$ 0.01	0.12 $\pm$ 0.01 c	39.35 $\pm$ 2.95
		40	20.0 $\pm$ 0.5 b	9.92 $\pm$ 0.35 b	0.24 $\pm$ 0.04 c	0.28 $\pm$ 0.01	0.12 $\pm$ 0.02 c	46.20 $\pm$ 3.25
		80	19.9 $\pm$ 0.6 b	11.42 $\pm$ 0.37 ab	0.13 $\pm$ 0.01 b	0.30 $\pm$ 0.01	0.06 $\pm$ 0.01 b	46.46 $\pm$ 3.47
		150	17.6 $\pm$ 0.1 a	12.68 $\pm$ 0.67 c	0.04 $\pm$ 0.01 a	0.31 $\pm$ 0.01	0.01 $\pm$ 0.00 a	43.46 $\pm$ 3.40
		F-statistic	6.1 ** (3,63)	8.1414 ***	15.7254 ***	0.7374	26.5955 ***	1.27 (3,56)
	P	0.8	19.2 $\pm$ 0.6	10.91 $\pm$ 0.6	0.14 $\pm$ 0.02	0.30 $\pm$ 0.01	0.066 $\pm$ 0.01	40.93 $\pm$ 3.7
		5	20.3 $\pm$ 0.5	10.72 $\pm$ 0.5	0.17 $\pm$ 0.05	0.29 $\pm$ 0.01	0.090 $\pm$ 0.02	41.34 $\pm$ 3.1
		15	18.6 $\pm$ 0.5	10.76 $\pm$ 0.5	0.15 $\pm$ 0.02	0.29 $\pm$ 0.01	0.076 $\pm$ 0.01	47.39 $\pm$ 3.7
		31	21.1 $\pm$ 0.7	10.18 $\pm$ 0.7	0.16 $\pm$ 0.02	0.28 $\pm$ 0.02	0.084 $\pm$ 0.01	45.45 $\pm$ 2.5
		F-statistic	2.3 (3,63)	1.0151(3,61)	0.2594(3,61)	1.0880(3,63)	0.8942(3,63)	1.54 (3,56)
	NxP F-statistic	0.8 (9,63)	1.0109(9,61)	0.9213(9,61)	0.8379(9,63)	1.7722(9,63)	1.99 (9,56)	
<i>A. linearis</i>	N	20	42 $\pm$ 0.1	6.28 $\pm$ 0.4	0.23 $\pm$ 0.03 b	0.33 $\pm$ 0.02	0.20 $\pm$ 0.03 b	27.19 $\pm$ 5
		40	45 $\pm$ 0.1	5.58 $\pm$ 0.4	0.23 $\pm$ 0.09 b	0.35 $\pm$ 0.02	0.33 $\pm$ 0.21 b	35.59 $\pm$ 5
		80	45 $\pm$ 0.1	6.67 $\pm$ 0.4	0.06 $\pm$ 0.01 a	0.31 $\pm$ 0.02	0.05 $\pm$ 0.01 a	25.77 $\pm$ 5
		150	44 $\pm$ 0.1	8.05 $\pm$ 0.6	0.01 $\pm$ 0.01 a	0.25 $\pm$ 0.01	0.01 $\pm$ 0.00 a	19.72 $\pm$ 5
		F-statistic	0.1398 (3,54)	1.4216	12.0234 ***	1.3013	4.0944 *	2.6596 (3,54)
	P	0.8	43 $\pm$ 0.1	6.63 $\pm$ 0.4	0.13 $\pm$ 0.04	0.35 $\pm$ 0.02	0.11 $\pm$ 0.04	31.30 $\pm$ 5.2
		5	43 $\pm$ 0.1	6.27 $\pm$ 0.4	0.24 $\pm$ 0.09	0.32 $\pm$ 0.02	0.34 $\pm$ 0.21	29.29 $\pm$ 5.0
		15	43 $\pm$ 0.1	7.08 $\pm$ 0.6	0.09 $\pm$ 0.02	0.29 $\pm$ 0.01	0.07 $\pm$ 0.02	23.73 $\pm$ 4.6
		31	46 $\pm$ 0.1	6.41 $\pm$ 0.5	0.09 $\pm$ 0.02	0.30 $\pm$ 0.02	0.07 $\pm$ 0.01	24.74 $\pm$ 4.9
		F-statistic	0.0646 (3,54)	1.3617(3,61)	2.5274(3,61)	2.3089(3,54)	1.434(3,54)6	0.7861 (3,54)
	NxP F-statistic	0.3969 (9,54)	0.4605(9,61)	2.9503(9,61) ***	1.5093(9,54)	0.7722(9,54)	0.8594 (9,54)	

**Table 2.3** Effects of the addition of the four levels of N and P on the SLA, the biomass accumulation of the total plant and cluster roots, root to shoot ratio, cluster root to root ratio, and transpiration rate of the non-legumes at harvesting. Values are mean ( $\pm$  S.E.). Data annotated with either \*, \*\* or \*\*\* indicate p-values of <0.05, <0.01 or <0.001 respectively. Means followed by different letters are significantly different.

Sites	Nutrient Levels (mg kg <sup>-1</sup> )		SLA (m <sup>2</sup> kg <sup>-1</sup> DM)	Total Plant (g DM)	Cluster roots (g DM)	Root:shoot	Cluster root:root
<i>Leucodendron coniferum</i>	N	20	10.6 $\pm$ 0.4	7.24 $\pm$ 0.3 a	0.09 $\pm$ 0.02 b	0.28 $\pm$ 0.01	0.059 $\pm$ 0.01 b
		40	14.9 $\pm$ 0.4	10.16 $\pm$ 0.7 b	0.10 $\pm$ 0.04 b	0.31 $\pm$ 0.02	0.060 $\pm$ 0.03 b
		80	10.3 $\pm$ 0.6	10.54 $\pm$ 0.8 b	0.03 $\pm$ 0.01 ab	0.27 $\pm$ 0.01	0.016 $\pm$ 0.01 ab
		150	11.2 $\pm$ 0.5	11.32 $\pm$ 0.8 b	0.01 $\pm$ 0.00 a	0.25 $\pm$ 0.01	0.003 $\pm$ 0.00 a
	F-statistic		0.98 <sub>(3,50)</sub>	7.040 <sub>(3,61)</sub> ***	2.9649 <sub>(3,61)</sub> **	2.111 <sub>(3,50)</sub>	2.964 <sub>(3,50)</sub> *
	P	0.8	10.8 $\pm$ 0.6	9.16 $\pm$ 0.7	0.09 $\pm$ 0.02	0.26 $\pm$ 0.01	0.054 $\pm$ 0.01
		5	15.1 $\pm$ 4.4	10.28 $\pm$ 0.7	0.08 $\pm$ 0.02	0.27 $\pm$ 0.01	0.041 $\pm$ 0.01
		15	10.2 $\pm$ 0.6	9.70 $\pm$ 0.7	0.03 $\pm$ 0.01	0.29 $\pm$ 0.03	0.020 $\pm$ 0.01
		31	11.3 $\pm$ 0.6	9.73 $\pm$ 0.9	0.05 $\pm$ 0.04	0.29 $\pm$ 0.01	0.034 $\pm$ 0.03
	F-statistic		1.14 <sub>(3,50)</sub>	0.725 <sub>(3,61)</sub>	1.6070 <sub>(3,61)</sub>	1.012 <sub>(3,50)</sub>	1.6070 <sub>(3,50)</sub>
NxP -statistic		0.43 <sub>(9,60)</sub>	1.113 <sub>(9,61)</sub>	0.4881 <sub>(9,61)</sub>	0.550 <sub>(9,50)</sub>	0.4881 <sub>(9,50)</sub>	
<i>Leucospermum cordifolium</i>	N	20	9.2 $\pm$ 0.3	6.93 $\pm$ 0.43	0.09 $\pm$ 0.03 b	0.29 $\pm$ 0.01 b	0.069 $\pm$ 0.03 c
		40	9.2 $\pm$ 0.3	8.70 $\pm$ 0.51	0.02 $\pm$ 0.01 a	0.27 $\pm$ 0.01 b	0.014 $\pm$ 0.00 c
		80	9.6 $\pm$ 0.3	9.09 $\pm$ 0.06	0.01 $\pm$ 0.00 a	0.25 $\pm$ 0.01 b	0.003 $\pm$ 0.00 b
		150	10.1 $\pm$ 0.4	8.73 $\pm$ 0.85	0.00 a	0.17 $\pm$ 0.01 a	0.002 $\pm$ 0.00 a
	F-statistic		1.70 <sub>(3,63)</sub>	2.207 <sub>(3,61)</sub>	5.8136 <sub>(3,61)</sub> **	22. $\pm$ 046 <sub>(3,63)</sub> ***	5.5882 <sub>(3,63)</sub> **
	P	0.8	8.8 $\pm$ 0.4 a	9.49 $\pm$ 0.6 b	0.04 $\pm$ 0.02	0.24 $\pm$ 0.01	0.023 $\pm$ 0.02
		5	9.0 $\pm$ 0.3 a	8.91 $\pm$ 0.6 b	0.03 $\pm$ 0.01	0.26 $\pm$ 0.02	0.017 $\pm$ 0.00
		15	10.3 $\pm$ 0.3 b	7.00 $\pm$ 0.5 a	0.01 $\pm$ 0.01	0.24 $\pm$ 0.02	0.011 $\pm$ 0.00
		31	9.9 $\pm$ 0.2 b	8.03 $\pm$ 0.7 ab	0.05 $\pm$ 0.03	0.025 $\pm$ 0.02	0.038 $\pm$ 0.03
	F-statistic		5.45 <sub>(3,63)</sub> *	3.508 <sub>(3,61)</sub> *	0.5645 <sub>(3,61)</sub>	0.44 <sub>(3,63)</sub> 5	0.5100 <sub>(3,63)</sub>
NxP F-statistic		2.06 <sub>(9,63)</sub> *	0.4084 <sub>(9,61)</sub>	0.8384 <sub>(9,61)</sub>	0.793 <sub>(9,63)</sub>	0.8756 <sub>(9,63)</sub>	
<i>Protea repens</i>	N	20	5.6 $\pm$ 0.3	3.42 $\pm$ 0.3	0.40 $\pm$ 0.08 c	0.72 $\pm$ 0.05 b	0.39 $\pm$ 0.07 b
		40	5.7 $\pm$ 0.2	3.20 $\pm$ 0.2	0.38 $\pm$ 0.06 ab	0.74 $\pm$ 0.05 b	0.41 $\pm$ 0.06 b
		80	6.3 $\pm$ 0.2	3.41 $\pm$ 0.4	0.21 $\pm$ 0.04 ab	0.62 $\pm$ 0.03 b	0.20 $\pm$ 0.03 a
		150	6.2 $\pm$ 0.4	3.21 $\pm$ 0.4	0.08 $\pm$ 0.01 a	0.49 $\pm$ 0.02 a	0.09 $\pm$ 0.01 a
	F-statistic		0.90 <sub>(3,61)</sub>	0.5710 <sub>(3,61)</sub>	12.8623 <sub>(3,61)</sub> ***	14.7245 <sub>(3,61)</sub> ***	13.9880 <sub>(3,61)</sub> ***
	P	0.8	6.1 $\pm$ 0.3	3.14 $\pm$ 0.3 a	0.21 $\pm$ 0.08	0.59 $\pm$ 0.04	0.22 $\pm$ 0.08 a
		5	6.2 $\pm$ 0.2	2.99 $\pm$ 0.2 a	0.33 $\pm$ 0.07	0.69 $\pm$ 0.06	0.34 $\pm$ 0.07 b
		15	5.2 $\pm$ 0.3	2.94 $\pm$ 0.2 a	0.29 $\pm$ 0.05	0.67 $\pm$ 0.03	0.31 $\pm$ 0.05 b
		31	6.3 $\pm$ 0.3	4.19 $\pm$ 0.5 b	0.23 $\pm$ 0.04	0.61 $\pm$ 0.05	0.20 $\pm$ 0.04 a
	F-statistic		2.65 <sub>(3,61)</sub>	2.8013 <sub>(3,61)</sub> *	2.2560 <sub>(3,61)</sub>	1.8491 <sub>(3,61)</sub>	4.3816 <sub>(3,61)</sub> **
NxP F-statistic		1.56 <sub>(9,61)</sub>	1.6396 <sub>(9,61)</sub>	0.8687 <sub>(9,61)</sub>	0.7470 <sub>(9,61)</sub>	1.1698 <sub>(9,61)</sub>	

**Table 2.4** Effects of the addition of the four levels of N and P on the transpiration rate of the legumes and non-legumes at harvesting. Values are mean ( $\pm$  S.E.). Data annotated with either \*, \*\* or \*\*\* indicate p-values of <0.05, <0.01 or <0.001 respectively. Means followed by different letters are significantly different.

Sites	Nutrient Levels (mg kg <sup>-1</sup> soil)		Transpiration (mmol H <sub>2</sub> O <sup>-2</sup> s <sup>-1</sup> )	Sites	Nutrient Levels (mg kg <sup>-1</sup> soil)		Transpiration (mmol H <sub>2</sub> O <sup>-2</sup> s <sup>-1</sup> )	
<i>P. calyptrata</i>	N	20	5.15 $\pm$ 1.55	<i>L. coniferum</i>	N	20	0.95 $\pm$ 0.09	
		40	4.74 $\pm$ 1.37			40	0.87 $\pm$ 0.07	
		80	5.04 $\pm$ 1.61			80	0.98 $\pm$ 0.09	
		150	4.51 $\pm$ 0.97			150	0.91 $\pm$ 0.07	
	F-Statistic <sub>C(3,63)</sub>		0.282		F-Statistic <sub>C(3,57)</sub>		0.0968	
	P	0.8	4.34 $\pm$ 1.39 a		P	0.8	1.04 $\pm$ 0.06	
		5	4.49 $\pm$ 1.21 a			5	0.89 $\pm$ 0.08	
		15	4.73 $\pm$ 1.33 a			15	0.97 $\pm$ 0.09	
		31	5.85 $\pm$ 1.20 b			31	0.81 $\pm$ 0.07	
	F-statistic <sub>C(3,63)</sub>		4.347**		F-Statistic <sub>C(3,57)</sub>		1.4263	
NxP F-statistic <sub>C(9,63)</sub>		2.058*	NxP F-statistic <sub>C(9,57)</sub>		0.5808			
<i>O. striatum</i>	N	20	2.01 $\pm$ 0.13 b	<i>L. cordifolium</i>	N	20	4.10 $\pm$ 0.33	
		40	1.91 $\pm$ 0.11 b			40	5.24 $\pm$ 0.33	
		80	1.61 $\pm$ 0.09 a			80	5.43 $\pm$ 0.28	
		150	1.91 $\pm$ 0.08 b			150	5.16 $\pm$ 0.77	
	F-statistic <sub>C(3,62)</sub>		3.6322*		F-Statistic <sub>C(3,64)</sub>		1.8526	
	P	0.8	1.90 $\pm$ 0.17		P	0.8	5.22 $\pm$ 0.50	
		5	1.86 $\pm$ 0.06			5	4.40 $\pm$ 0.38	
		15	1.79 $\pm$ 0.10			15	4.84 $\pm$ 0.33	
		31	1.89 $\pm$ 0.09			31	5.46 $\pm$ 0.65	
	F-statistic <sub>C(3,62)</sub>		0.2824		F-Statistic <sub>C(3,64)</sub>		1.202	
NxP F-statistic <sub>C(9,62)</sub>		2.3747*	NxP F-statistic <sub>C(9,64)</sub>		0.1808			
<i>A. linearis</i>	N	20	4.04 $\pm$ 0.48	<i>P. repens</i>	N	20	1.69 $\pm$ 0.08	
		40	4.15 $\pm$ 0.49			40	1.57 $\pm$ 0.10	
		80	4.52 $\pm$ 0.52			80	1.88 $\pm$ 0.14	
		150	3.82 $\pm$ 0.45			150	1.76 $\pm$ 0.13	
	F-statistic <sub>C(3,56)</sub>		0.4577		F-Statistic <sub>C(3,62)</sub>		0.8445	
	P	0.8	3.97 $\pm$ 0.48		P	0.8	1.78 $\pm$ 0.12	
		5	3.81 $\pm$ 0.45			5	1.91 $\pm$ 0.11	
		15	4.50 $\pm$ 0.45			15	1.57 $\pm$ 0.08	
		31	4.25 $\pm$ 0.52			31	1.64 $\pm$ 0.13	
	F-statistic <sub>C(3,56)</sub>		0.6732		F-Statistic <sub>C(3,62)</sub>		1.7236	
NxP F-statistic <sub>C(9,56)</sub>		0.6742	NxP F-statistic <sub>C(9,62)</sub>		1.1890			

Concentrations of N and P in the leaves were assessed only on *P. calyprata* and *L. cordifolium*, because these species showed biomass response to either N or P supply. There was no change in the leaf concentrations of N and P, or their N:P ratios of the *P. calyprata* with the supply of different levels of N or P (Table 2.5). On the other hand, the higher levels of P supplied to the plants of *L. cordifolium* resulted in higher levels of leaf N and P, but the N:P ratio was unchanged (Table 2.5). In contrast, the higher levels of N supplied led to higher concentrations of leaf N, but lower concentrations of leaf P, which resulted in higher N:P ratios (Table 2.5).

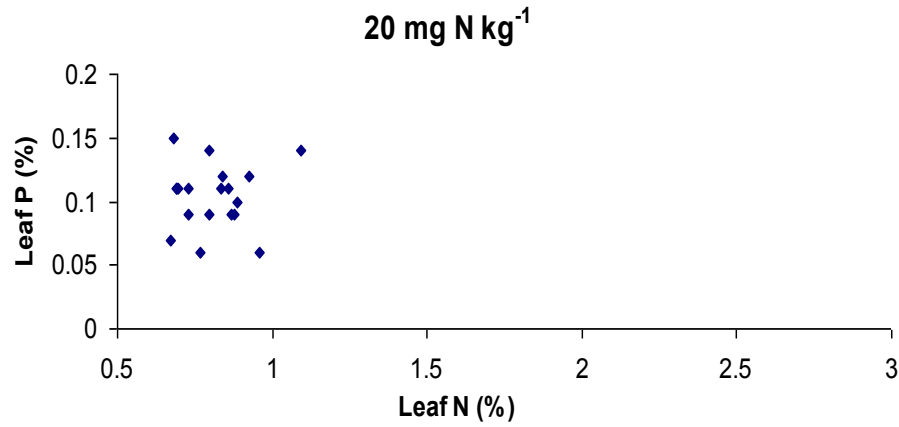
**Table 2.5**

Effects of the addition of four levels of N and P on the leaf N, Leaf P and N to P ratio of *P. calyprata* and *L. cordifolium* at harvesting. Values are mean ( $\pm$  S.E.). Data annotated with either \*, \*\* or \*\*\* indicate p-values of <0.05, <0.01 or <0.001 respectively. Means followed by different letters are significantly different.

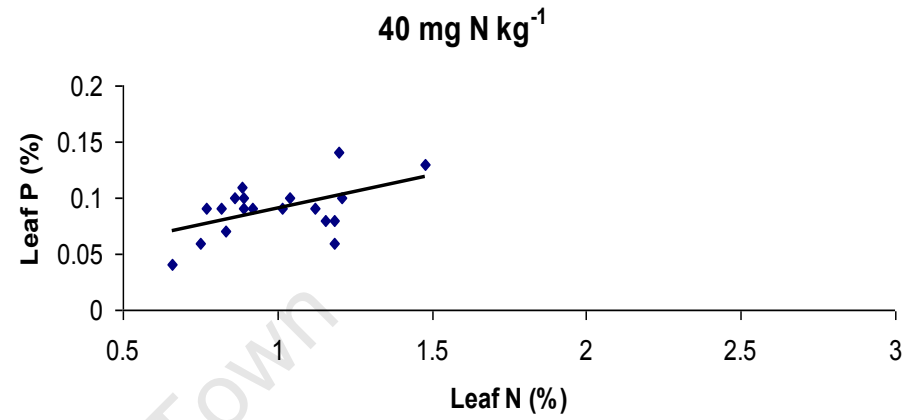
		Leaf N and P (%)			
	Nutrients	Nutrient Levels	Leaf N (%)	Leaf P (%)	N:P Ratio
<i>P. calyprata</i> (Legume)	N (mg kg <sup>-1</sup> )	20	2.40 $\pm$ 0.05	0.14 $\pm$ 0.02	20.45 $\pm$ 1.68
		40	2.32 $\pm$ 0.07	0.10 $\pm$ 0.01	23.68 $\pm$ 1.31
		80	2.33 $\pm$ 0.06	0.13 $\pm$ 0.02	21.00 $\pm$ 1.82
		150	2.33 $\pm$ 0.06	0.10 $\pm$ 0.01	24.13 $\pm$ 1.15
		F-statistic		0.14	2.160
	P (mg kg <sup>-1</sup> )	0.8	2.25 $\pm$ 0.04	0.10 $\pm$ 0.01	24.68 $\pm$ 1.44
		5	2.31 $\pm$ 0.07	0.11 $\pm$ 0.01	23.82 $\pm$ 1.19
		15	2.41 $\pm$ 0.07	0.12 $\pm$ 0.01	22.23 $\pm$ 1.66
		31	2.42 $\pm$ 0.06	0.14 $\pm$ 0.01	19.79 $\pm$ 1.44
		NXP Interaction			
	F-statistic		0.77	0.719	0.727
<i>L. cordifolium</i> (Non-legume)	N (mg kg <sup>-1</sup> )	20	0.79 $\pm$ 0.03 a	0.11 $\pm$ 0.01 b	8.0 $\pm$ 0.63 a
		40	0.99 $\pm$ 0.05 b	0.09 $\pm$ 0.01 ab	11.5 $\pm$ 0.71 a
		80	1.29 $\pm$ 0.06 c	0.08 $\pm$ 0.01 a	16.3 $\pm$ 0.84 b
		150	1.77 $\pm$ 0.09 d	0.09 $\pm$ 0.00 a	20.7 $\pm$ 0.96 c
		F-statistic		63.720 ***	3.137 *
	P (mg kg <sup>-1</sup> )	0.8	1.10 $\pm$ 0.1 a	0.08 $\pm$ 0.01 a	15.9 $\pm$ 1.78
		5	1.12 $\pm$ 0.09 a	0.09 $\pm$ 0.00 ab	12.9 $\pm$ 0.98
		15	1.28 $\pm$ 0.11 b	0.10 $\pm$ 0.01 b	14.3 $\pm$ 1.34
		31	1.35 $\pm$ 0.12 b	0.10 $\pm$ 0.01 b	13.7 $\pm$ 1.21
		F-statistic		5.469 **	2.929 *
	NXP Interaction				
	F-statistic		0.605	0.502	1.2482

There were significant positive correlations between the concentration of leaf N and P of *L. cordifolium* only at the higher levels of N supply at 40-, 80- and 150 mg N kg<sup>-1</sup>, but no correlations at N supply of 20 mg N kg<sup>-1</sup> (Figure 2.1). There were no leaf N and P correlations at any levels of P supply (data not shown). The concentration of leaf N and -P of *P. calyptata* showed no correlations at any of the N and P levels supplied (Data not shown). The biomass of *P. calyptata*, *O. striatum* and *L. coniferum* showed weak, but significant ( $P < 0.05$ ) positive correlations with supplied N, but *P. calyptata* showed a negative correlation, whilst *P. repens* showed a positive correlation with supplied P (Table 2.6).

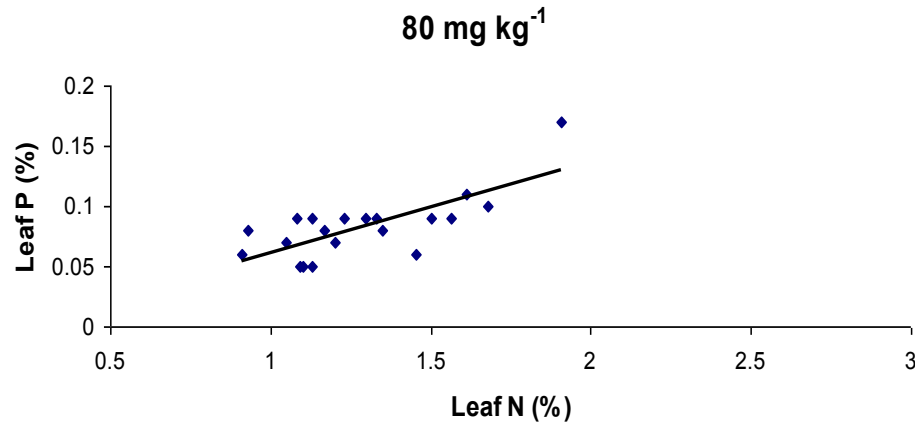
University of Cape Town



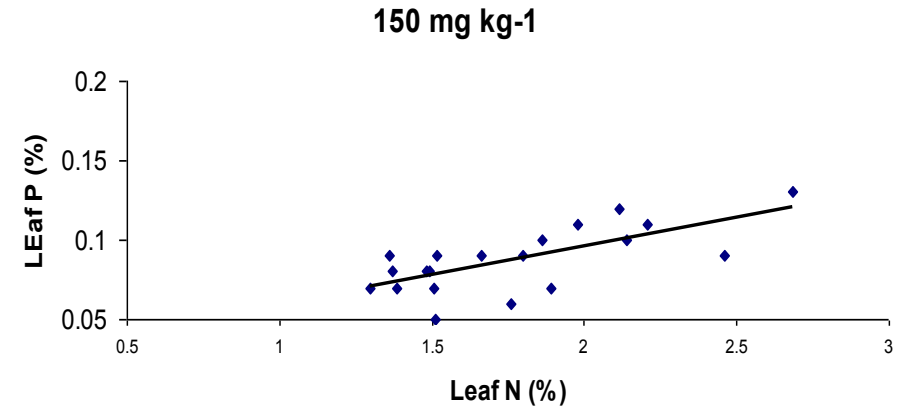
a)  $r^2 = 0.0247$ ;  $p = 0.5081$



b)  $r^2 = 0.2747$ ;  $p = 0.0213$



c)  $r^2 = 0.5387$ ;  $p = 0.0002$



d)  $r^2 = 0.4890$ ;  $p = 0.0006$

**Figure 2.1** Correlations between the concentration of leaf N and P of *L. cordifolium* at a) N supply of 20 mg N kg<sup>-1</sup>, b) N supply of 40 mg N kg<sup>-1</sup>, c) N supply of 80 mg N kg<sup>-1</sup> and d) N supply of 150 mg N kg<sup>-1</sup>

**Table 2.6** Correlation between the total biomass of each of the six species with the different levels of supplied N and P. Differences are significant at  $p < 0.05$  \*,  $0.01^{**}$  and  $0.001^{***}$  respectively. ,

Parameters	Functional group	Species	r	r <sup>2</sup>
N	Legumes	P. calytrata	0.3426	0.1174**
		O. striatum	0.4401	0.1937***
		A. linearis	0.0637	0.0041
	Non-legumes	L. coniferum	0.3983	0.1586***
		L. cordifolium	0.1159	0.0134
		P.repens.	-0.1210	0.0146
P	Legumes	P. calytrata	-0.2830	0.0801*
		O. striatum	-0.1472	0.0218
		A. linearis	0.1224	0.0150
	Non-legumes	L. coniferum	-0.0224	0.0006
		L. cordifolium	-0.2278	0.0519*
		P. repens	0.2681	0.0719*

## Discussion

The hypotheses of this study was that the growth of legumes is limited by P and non-legumes by N and that plant response to N and P would depend on the levels of N and P in the growth media. A factorial experiment was conducted to determine whether N and P interacted on the growth of legume and non-legume species. Looking at the overall results, only 34 % of the parameters measured (Tables 2.3, 2.4 and 2.5) showed significant ( $p < 0.05$ ) main effects at the higher levels of N and P and only 6% (5 out of the possible 81 instances of N and P interactions) showed significant ( $p < 0.05$ ) interactions between the different levels of soil N and P. The reasons for the lack of interaction on most of the parameters are not clear. However these results mirrored the results that Witkowski (1989b) reported in a complete, factorial designed fertilizer experiment conducted at the CSIR fynbos biome intensive study site at Pella, 62 km north of Cape Town. Of the nine growth forms assessed in the study by Witkowski (1989b), none responded to P and N\*P fertilizer combination, and only three forms responded to increased N supply: the restoids species positively and the graminoids, and annuals negatively. The observations reported by Witkowski (1989b) and in this study may imply that CFR species are not responsive to high levels of nutrient supply, a general characteristic of plants that are adapted to low nutrient environment (Chapin, 1980; Aerts, 1999; Hawkins *et al.*, 2005), or that other nutrients were also limiting.

The main effects of N and P on most of the parameters measured varied with species in both legume and non-legume plants. For instance, there was an overall lack of responses on the biomass accumulation and root : shoot ratio by all three legume species, as well as by *L. coniferum*, a non-legume, to the different levels of P supplied. On the other hand, the biomass of *P. repens* plants receiving the highest level of P ( $31 \text{ mg kg}^{-1}$ ) was higher than those receiving the lower levels, in contrast to the biomass of *L. cordifolium* that was highest at the low levels of P supply. The positive response of the *P. repens* to high level of P supply was consistent with the reports of Witkowski (1989c) who showed a positive response of *P. repens* to an increasing P supply of  $12.8 \text{ g m}^{-2}$  from the  $0 \text{ g m}^{-2}$ . This confirms that *P. repens* has potential to respond positively to higher levels of P. With limited interactions between N and P supply, these results suggest that the levels of P used in this study could influence a change of biomass accumulation only in the non-legumes, but not the legumes. The lack of legume species response to the levels of P used in this study ( $0.8$  to  $31 \text{ mg kg}^{-1}$ ) was surprising, because in an earlier study involving species of *Aspalathus*, *Cyclopia*,

*Indigofera* and *Podalyria*, biomass accumulation was higher in plants receiving 10 mg P kg<sup>-1</sup> than those receiving 1 mg kg<sup>-1</sup> (Power, *et al.* 2010).

The lack of a clear pattern of biomass response to P supply of the non-legumes was also reported by Witkowski, *et al.*, (1990) working on a proteoid, *Leucospermum parile*, as well as an ericoid evergreen shrub, *Phyllica cephalantha*. Their results showed reduced shoot biomass accumulation on *L. Parile*, but no response on *P. cephalantha* with P fertilization. The tendency of high levels of P to reduce growth might be a result of a nutrient imbalance, where increasing the non-limiting P exacerbates the N limitation (Grundon, 1972; Witkowski, *et al.*, 1990; Akamine, *et al.*, 2007,) or the result of P toxicity due to an inability to down regulate P uptake (Hawkins, *et al.*, 2006, Shane, *et al.*, 2008; Lambers *et al.*, 2010). Furthermore, the lack of changes in biomass accumulation with P fertilization might be a characteristic of low-P adapted plants (Poorter and Remkes, 1990; Poorter and De Jong, 1999; Sultan, 2000; Lambers, *et al.*, 2008).

The effects of the N treatments on biomass accumulation of *P. calyptрата* and *O. striatum* showed a significantly ( $p < 0.05$ ) higher total plant biomass at the higher (150 mg kg<sup>-1</sup>) level of N supply (Table 2.2). This is in agreement with the report of Muofhe and Dakora (1999) on field cultivated *A. linearis*, where more biomass was accumulated when fertilized with N compared to their unfertilized counterparts. The response of the legumes to N supply was unexpected, as these plants were nodulated and the expectation would be that the symbiosis with rhizobia would lead to sufficient fixation of N<sub>2</sub>. A possible explanation could be that legumes have, in general, a higher N-demanding lifestyle and so have a greater demand for N (Vitousek, *et al.*, 2002), and hence the presence of nodules is not a guarantee of sufficient N supply for their N nutritional needs. Several studies have shown that Nitrogen fixation capacity differed amongst different strains of rhizobia (Wynne, *et al.*, 1980; Mostasso, *et al.*, 2002; Romdhane, *et al.*, 2008). Among the non-legume species, the biomass accumulation of only *L. coniferum* responded positively to the higher levels of N (40 – 80 mg kg<sup>-1</sup>) (Table 2.3), suggesting that there is species variation in the potential of both the legumes and non legumes to increase biomass with N fertilization, with the legume species seemingly more N limited than the non-legumes.

Plants have to balance the acquisition of light and soil resources by allocating resources to the production of roots and shoots in appropriate proportions (Taub, 2007) and low availability of nutrients and water enhance allocation of biomass to roots (Chapin, *et al.*, 1980; Chapin, 1980; Poorter, 1990; Lambers and Poorter, 1992; Poorter and Nagel, 2000; Taub, 2007). One would thus expect that the plants in this study to show a lower root to shoot ratio at the higher levels of N and P supply, which was, indeed, the response of the two non-legumes, *L. cordifolium* and *P. repens* to the higher levels of N supply. The root to shoot ratios of the legume species and the *L. coniferum* did not change with the higher supply of either N and/or P, again indicating species variation in biomass allocation in response to changes in nutrient availability, with the non-legumes more affected than the legume species.

The observed lower nodule biomass in legume species, leading to a lower nodule to root ratio at the higher levels of N, with similar results on cluster roots in the non-legumes species at higher levels of N, was expected, as high rates of nitrogen inhibit nodule and cluster root production (Da Silva *et al.*, 1993; Yinbo *et al.*, 1997; Lima *et al.*, 2006; Dinkelaker, *et al.*, 1995). It was surprising that the cluster root biomass of the three non-legumes showed no response to the increased P supply, as high levels of P supply should also have an inhibitory effect on the development of cluster roots (Lamont, *et al.*, 1984; Marschner, *et al.*, 1987; Lambers, *et al.*, 2002; Shane and Lambers, 2005). The possible explanations for this observation is that levels of P supply used in this study was probably not high enough to depress cluster root formation (Marschner, *et al.*, 1987; Dinkelaker, *et al.*, 1995; Neuman, *et al.*, 1999; Hawkins, *et al.*, 2005). On the other hand, it has been demonstrated that cluster roots may form at low, moderate, or even sufficient levels of both P and N (Marschner *et al.*, 1987; Johnson *et al.*, 1996; Keerthisinghe, *et al.*, 1998; Sas, *et al.*, 2002; Schmidt, *et al.*, 2003).

As expected, mycorrhizal infection was observed in the legumes, but not the non-legumes, because mycorrhizas are very rare or absent in Proteaceae (Brundrett, 2002). Only *P. calyptрата* responded with the expected lower infection at the highest P level of 31 mg P kg<sup>-1</sup> soil (Sanders and Tinker, 1973; Chambers, *et al.*, 1980; Abbott, *et al.*, 1984). Mycorrhizal colonization did not decrease in the other legume species at the higher levels of P supply, while two of the legumes in this experiment seemed to be N limited based on their biomass response to the supplied N. Mosse and Philips (1971)

and Sylvia and Neal (1990) have found that root colonization by VAM was only reduced when both N and P were available in sufficient concentrations. On the other hand, these varied results might indicate species variation in response to N and P fertilization.

Transpiration-driven mass flow is an important nutrient acquisition strategy, and soil nutrient availability may partially regulate transpiration (Cramer, *et al.*, 2009). Except for *P. calypttrata* which showed a significantly higher ( $p < 0.01$ ) transpiration rate at the P level of 31 mg P kg<sup>-1</sup> soil, than at the lower three P levels, the other species showed no response to the different levels of N and P (Tables 2.3 and 2.4), indicating that, overall, soil nutrient levels in this study did not regulate the transpiration rate. Lack of regulation of transpiration by soil nutrient availability were also reported by Guehl and Garbaye, (1990), Li, *et al.*, (2001), Ripullone, *et al.*, (2004), Graciano, *et al.*, (2005) and Howard and Donovan, (2007).

The analysis of leaf N and P were conducted only in *P. calypttrata* (a legume) and *L. cordifolium* (a non-legume). The leaf N and P and N:P ratio of *P. calypttrata* showed no change ( $p < 0.05$ ) at the different levels of N and P supply (Table 2.5). *Leucospernum cordifolium* showed higher concentrations of both leaf N and P with P supply, whereas N supply increased leaf N, but decreased leaf P. In line with the concept of luxury consumption, when plants maximise available nutrient uptake, which is stored for later use (Chapin, 1980; Aerts and Chapin, 2000; Van Wijk, *et al.*, 2003; Lambers, *et al.*, 2008), without changing their biomass production and allocation pattern (Zhang *et al.*, 2004), *L. cordifolium* showed an increase in leaf N with increasing N supply (Table 2.5), but without a corresponding increase in biomass. In contrast, this non-legume also had a greater % leaf P at higher levels of P supply (Table 2.5), but with lower overall plant biomass accumulation. The higher leaf P might be due to the reduced biomass accumulation (Table 2.3) which is a classical example of P toxicity effects associated with lack of ability to down regulate P uptake (Hawkins, *et al.*, 2008; Shane, *et al.*, 2008). The general lack of correlations between the leaf N and P of *P. calypttrata* and *L. cordifolium*, especially at the various levels of P supply could be due to luxury uptake of the nutrient with the higher supply (Shaver and Chapin, 1980) or low P uptake due to the a possible low P content in the growth medium. This view is supported by the observed positive correlation between N and P in the leaf tissue of *L. cordifolium* at the 40 mg N kg<sup>-1</sup> or higher, but not at the low levels of 20 mg N kg<sup>-1</sup> (Figure 2.1). The higher levels of both N and P in the leaves of *L. cordifolium* at the higher levels of P

supply suggest synergistic effect of P on N where high levels of P stimulated N uptake. There are a number of experimental results in which an increase in available N led to an increase in tissue P (and N) concentration (Boto and Wellington, 1983) and visa versa (Boto and Wellington, 1983; Zhang *et al.*, 2004; Stamford *et al.*, 2006). However, Reichardt *et al.*, (1991) found a decrease in leaf P of balsam poplars with increasing N supply in their factorial designed experiment, a result that is similar to the observation in this study where leaf P in *L. cordifolium* was lower at the higher levels of N supply of 80 and 150 mg N kg<sup>-1</sup>. The mechanism for this suggested negative leaf N to P correlation is unclear.

## Conclusion

In conclusion, there were no discernable trends in the responses of the legume and non-legume species to the manipulated N and P supply, i.e. no demonstration of definite limitation of either N or P to the growth of these two functional groups. The responses were species specific with some legume species responding to a higher N supply than the non-legumes.

The lack of N x P interaction implies that the plants' response to N and P, in this study, did not depend on the levels of each other in the growth medium.

## CHAPTER 3

### **Are the soils in which fynbos legume populations grow more fertile than those of non-legumes?**

#### **Abstract**

Many legume species in the CFR grow in distinct populations in habitats which range from water seeps, river valleys and mountain slopes to quite arid areas, and this distribution could be associated with water and/or soil nutrient availability and/or niche construction. I hypothesized that soils in the legume habitat contain a greater nutrient availability than the adjacent non-legume soils and that the leaves of the legumes have higher specific leaf areas (SLA), as well as higher concentration of nutrients than those of the non-legumes. In order to address these hypotheses, I collected two sets of data; the first were used to compare the soil nutrient status of legume populations to the soil nutrient status of the adjacent non-legume soil at eight sites across the Fynbos. Cederberg and De Hoop were the only two sites in which there were significant differences in some of the nutrients between legume and non-legume soils. Secondly, data on leaf nutrient concentrations and of legume and non-legume plants were collected at each site. In general, the legumes showed higher concentrations of leaf N, while the levels of P, K, Ca, Mg and Na and the SLA were, in most cases, statistically the same as those of the non-legumes. It was concluded that fynbos legumes rarely occur in fertile islands. Except for leaf N, there were no differences in leaf nutrient concentrations and SLA between the legumes and non-legumes.

## Introduction

A population of plants on a specific landscape has effects on soil properties and may lead to formation of islands of fertility (Yang, *et al.*, 2011), sometimes referred to as niche construction. This effect is even more pronounced if the dominant plants in the patch are legumes (Rodrigues, *et al.*, 2011), due to the high nutrient levels in the biomass (Bashan and De-Bashan, 2010). One of the important requirements for niche construction in ecosystems is the quality and quantity of litterfall (Binkley and Giardina, 1998). Litterfall is one way in which nutrients are transferred from the standing phytomass to the soil. There are numerous studies indicating that the litterfall of legumes enhance the soil fertility and influence the soil nutrient cycle, due to both its quality and quantity (Holmes and Cowling, 1997; Stock, *et al.*, 1995; Cocks and Stock, 2000; Spehn, *et al.*, 2002; Mulder, *et al.*, 2002; Yelenik, *et al.*, 2007). Both the quantity and quality of litter are species specific as Spehn, *et al.*, (2002), Marchante, *et al.*, (2008) and Jeddi, *et al.*, (2009) found that even amongst legumes, the rate at which the soil conditions are changed were species specific. An example of such a study was done by He, *et al.*, (2011) who studied fertile island development under the canopies of four *Acacia* species. They found that there were significantly different total N and total P levels under the canopies of the respective acacias and that there were significantly higher levels of these two nutrients under the tree canopies, than in the gaps. Mechanisms that can explain soil nutrient enrichment beneath canopies include the redistribution of nutrients absorbed by the root system to the area (Rossi and Villagra, 2003), biological fixation of nitrogen by symbiotic rhizobia (Rossi and Villagra, 2003) and higher microbial activities as a result of relatively high soil humidity and organic matter content which speeds up N cycling (Scheaffer and Evans, 2005). Another factor that impacts on increasing soil nutrients is time constraints for a build-up of organic residues (Binkley and Giardina, 1998). On the other-hand, it is possible that legume plants occupied patches that are already nutrient rich because legumes are known to be high nutrient demanding (Vittousek, *et al.*, 2002; Sprent, *et al.*, 1996; Powers and Tiffen, 2010) as evidenced by their ability to dominate some parts of the CFR after fire.

There is limited information as to whether variation in soil chemistry translates into variation in plant foliar nutrient concentrations (Neff, *et al.*, 2006; Ordoñez, *et al.*, 2009) under natural conditions. One factor that complicates the evaluation of this possible

relationship is a lack of quantitative measurements to evaluate leaf tissue nutrient variation across soil nutrient gradients (Neff, *et al.*, 2006). Another complicating factor is that leaf nutrients vary yearly, seasonally, with water availability, according to plant type, and thus year x season x plant species interactions determine mineral concentrations (Castell and Terradas, 1994; Ramírez-Orduña *et al.*, 2005). However, strong correlations have been reported between regional variation in soil N or P and foliar N:P ratio with lower N:P ratio associated with areas of high soil fertility (Stock and Verboom, 2012, Fourqurean and Zieman, 1992). This observation shows that, in some areas, there is a relationship between nutrient status of soil and foliar N:P ratio.

SLA is the amount of leaf area per unit (dry) leaf weight (Lambers, *et al.*, 2008), and is very plastic (Dijkstra, 1990; Knops and Reinhart, 2000). It is widely accepted that the productivity of the habitat in which the plant grows, determines its SLA (Poorter and De Jong, 1999). Plants growing in fertile habitats have a high SLA, and the opposite is true of plants growing in infertile habitats (Dijkstra and Lambers, 1989; Poorter and De Jong, 1999; Wilson, *et al.*, 1999; Shaver *et al.*, 2001). It is interesting to note that Poorter and De Jong, (1999) have found that data concerning SLA from comparative laboratory experiments could be extrapolated to infer valid conclusions about SLA ranking in the field

The question asked was whether fynbos legumes occur in fertile islands, and, if so, do the leaves of the legumes reflect this higher level of nutrients by a higher SLA and increased levels of nutrients in their leaves? This study examines whether soil nutrient concentrations in legume patches are higher than the soils immediately adjacent to the legume patches at eight sites scattered across the CFR. It was hypothesized that:

1. Soil nutrients in the legume patches are higher than those from adjacent non-legumes on a specific landscape.
2. Fynbos legumes have higher SLA's and leaf nutrient concentrations than non-legumes.

## **Materials and methods**

## Site selection

The study was conducted in eight sites in the Cape Floristic Region (CFR). The sites were chosen based on soil types and included two sites from each of four soil types within the fynbos: sandstone derived, limestone derived, granitic and sandy soils. The sites (Figure 3.1) were as representative as possible of the fynbos. The characteristics of each site, with regard to the legume vegetation and micro habitat are summarized in Table 3.



**Figure 3.1** Google map, showing the spatial distribution of the eight sites within the CFR.

**Table 3.1** A summary of the characteristics, relevant to the study, of each site. ND = not determined.

Site	GPS Co-ordinates	Soil Type	Surface water relative to legume stand	Legume Species	Legume cover (%) and density (plants m <sup>-2</sup> )	Vegetation age (years since last fire)
<b>Cederberg</b>	S: 32 <sup>0</sup> 24' 58.9" E: 19 <sup>0</sup> 06' 62.6"	Sand stone derived	Seasonal run-off streams: 3. One above legume stand, one through the middle and one, still flowing, at the bottom	<i>Psoralea vigilans</i>	27 1.3	ND
<b>Bainskloof</b>	S: 33 <sup>0</sup> 35' 57.00" E: 19 <sup>0</sup> 07' 38.00"	Sand stone derived	No surface water	<i>Indigofera frutescence</i>	40 0.45	20
<b>Elim Sandberg Fynbos Reserve</b>	S: 34 <sup>0</sup> 35' 56.4" E: 19 <sup>0</sup> 42' 04.4"	Sandy	No surface water	<i>Aspalathus</i> sp.	ND	5
<b>Hopefield</b>	S: 33 <sup>0</sup> 02' 66.4" E: 18 <sup>0</sup> 20' 30.9"	Sandy	No surface water	<i>Wiborgia obcordata</i>	10 0.01	25+
<b>De Hoop Nature Reserve</b>	S: 34 <sup>0</sup> 26' 03.3" E: 20 <sup>0</sup> 25' 09.8"	Lime stone derived	No surface water	<i>Aspalathus pallescens</i>	35 2.76	15
<b>Heidehof Fynbos Farm</b>	S: 34 <sup>0</sup> 36' 33.8" E: 19 <sup>0</sup> 30' 44.0"	Lime stone derived	No surface water	<i>Otholobium bracteolatum</i>	19 0.5	8
<b>Grootbrak</b>	S: 34 <sup>0</sup> 02' 04.6" E: 22 <sup>0</sup> 13' 73.1"	Granitic	No surface water	<i>Aspalathus cinerascens</i>	19 0.96	4
<b>Botlierskop Game Reserve</b>	S: 34 <sup>0</sup> 01' 62.7" E: 22 <sup>0</sup> 08' 33.9"	Granitic	No surface water	<i>Aspalathus submissa</i>	ND	5

Once an isolated population of indigenous legumes with a distinct boundary between the legumes (here after called a legume population) and the adjacent fynbos vegetation, was identified at a particular site, three transects were set up in such a way that each one started within the legume stand and extended into the adjacent non legume area. Each transect had six equidistant soil sampling points: three within the legume stand, and three in the adjacent fynbos vegetation. The distances between the soil sampling points on each of the three transects of one site was the same. Between sites, these distances were adjusted according to the diameter of the legume stands and varied from 2m (Hopefield) to 7 m (Elim). At each soil sampling point along the transect, three soil samples (20cm deep) were taken, bulked, put into a plastic bag and labelled. A total of 18 soil samples were taken from each site. The soil samples were taken to the laboratory, air dried and sieved through a 0.84 mm sieve to remove stones and plant residues, then sent to BEMlab Private Laboratory, Somerset, South Africa, for nutrient analyses. Soil pH was determined by shaking 2g of material in 20 ml 1M KCl at 180 rpm for 60 min, centrifuging at 10 000 g for 10 min and measuring the pH of the supernatant. Total N was determined by the combustion method using a LECO FP528 N Analyzer (Leco Corporation, St. Joseph, USA). NH<sub>4</sub> was determined by means of the Salicylate- and NO<sub>3</sub> by means of the Cadmium Reduction Method. Total P was determined by acid digestion using a mixture of nitric and hydrochloric acid and the extract analysed by the inductively coupled plasma atomic emission spectrometry (ICP-AES; Varian Vista MPX, Mulgrave, Australia). Exchangeable cations were displaced from 10 g of soil sample with 25 ml of 0.2 M ammonium acetate. The samples were filtered through Whatman No. 2 filter paper and made to 200 ml before concentrations of Ca, K, Mg and Na were determined using ICP-AES analysis. Available P was determined by extracting 2 g of soil in Bray II solution and then filtering through Whatman No.2 filter paper. The Bray II filtrates were all colorimetrically analyzed using the Malachite Green method. Percentage C was determined using the Walkley Black method.

At each site, plant material was sampled by collecting a leafy branch from four different plants of each legume species and similar branches from at least four non-legume species outside the legume population. The non-legume species that were abundant and/or flowering were chosen for easier identification.

The leaf areas of ten fully mature leaves from plants with relatively large leaves, e.g. the *Protea* species, and many leaves in the case of plants with relatively small leaves, such as the *Aspalathus* species, were measured using a Photoshop program as described by

Powers, *et al.*, 2010. The above mentioned leaves from each plant were put into a paper bag and oven dried, at 70°C, for 48 hrs, then their weight recorded, using a Shimadzu AUY 220 scale. The specific leaf area (SLA) of the plants were determined by dividing the leaf areas by the dry mass (DM) of the same leaves, which gave the SLA in m<sup>2</sup> kg<sup>-1</sup>.

For nutrient analyses, leaves were separated from the leafy branch of each plant, put into an envelope and oven dried at 70°C for 56 hrs. The leaves were ground using a Wiley mill (Arthur H Thomas, Philadelphia, PA, USA) or a steel ball mill (MM200, Retsch®, Haan, Germany) and sent to BEMLab Private Laboratory, Somerset, South Africa, for nutrient analyses. The concentration of N was determined by a Leco FP-528 N Analyzer (Leco Corporation) and the concentrations of P, K, Ca and Mg were measured by dry-ashing the ground leaf material at 480°C for 8 h and dissolving the ash in 16% HCl. Assessment of the element concentrations in solution was performed using the ICP-AES.

### **Statistical analysis**

All analyses were conducted with statistical analysis software (Statistica, Version 10). Data were natural log transformed when necessary, to meet assumptions of homogeneity. All analyses were considered significant at  $p < 0.05$ . A three-way nested ANOVA was used on the soil data in order to study the variation in soil nutrients among the soil sampling points on the transect at each site, between the legume and non-legume populations, and between the sites in the fynbos. The sampling points were nested in the legume population and the sampling points and the legume population was nested in the site. Sampling points and legume population were used as fixed factors and the site as a random factor. Plant data were subjected to a two-way nested ANOVA in order to study the variation in the nutrient levels of the leaves of the different species (species), and between the nutrient levels of the legume and non-legume species (plant type). The species were nested in the plant type (legume and non-legume) Plant type was used as the fixed factor and the species as the random factor.

The Duncan's *post hoc* test was performed to separate means that were significantly different at  $p < 0.05$ . A multivariate analysis using Discriminate Function Analyses (forward stepwise approach) was used to measure separation between legume and non-legume sites and to determine which parameters were influential in the separation of the sites. The Partial Wilk's Lambda was used to determine which of the influential parameters contributed significantly to the separation of the sites. A Pearson correlation analysis was done between

the measured soil nutrients and the corresponding leaf nutrients of each site to determine if leaf nutrients were influenced by soil nutrients.

## Results

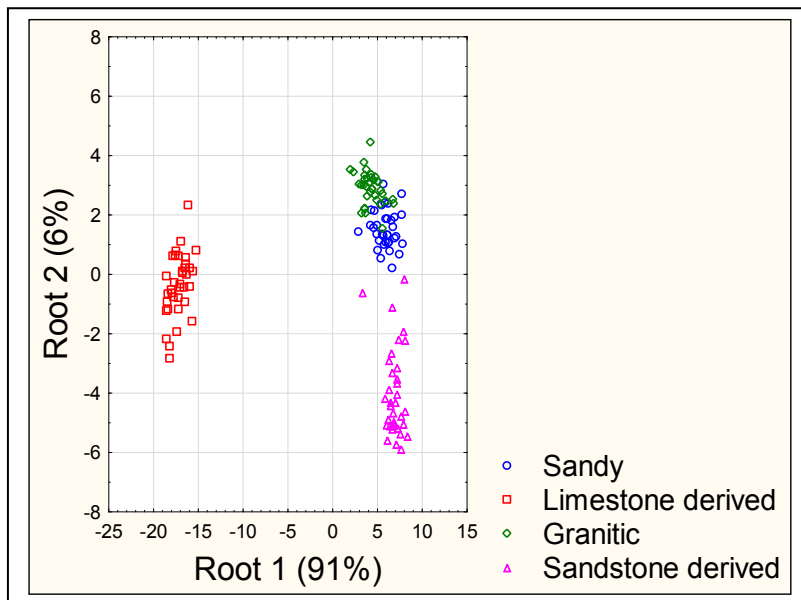
### Variation in concentration of soil nutrients

The results of the Three-way Nested ANOVA revealed significant differences between all twelve parameters with regard to the eight sites, significant differences between C, total N, NH<sub>4</sub>, K, Ca, Mg, Na and T-value between the legume- and non-legume soils and significant total N differences between the six sampling points along the transects (Table 3.2).

**Table 3.2** F-values and probability levels from the three-way nested ANOVA of different sites, soil patches and transects on soil nutrient parameters. The values with \*, \*\*, and \*\*\* indicate P-levels of <0.05, <0.01 and <0.001 respectively.

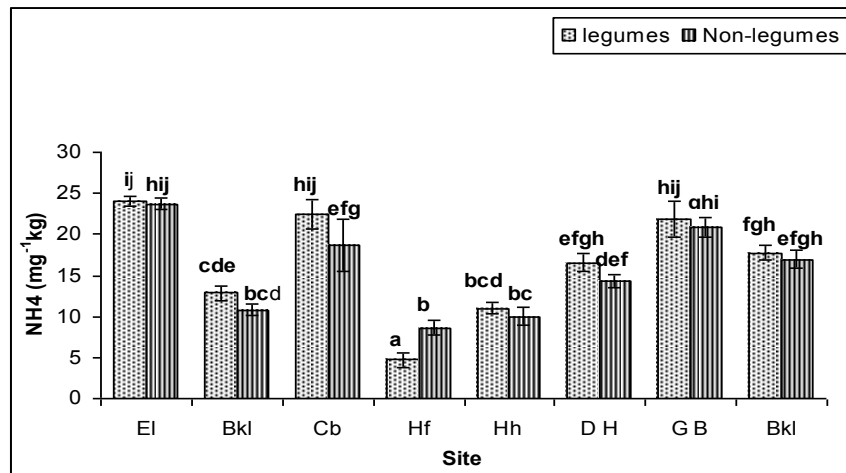
		pH	C	Total N	NH <sub>4</sub>	NO <sub>3</sub>	Total P
Site	F <sub>(7,96)</sub>	602 ***	23.47 ***	23.78 ***	9.63 **	25.23 ***	15.60 ***
Legume population(site)	F <sub>(8,96)</sub>	2.1	4.85 ***	4.88 ***	3.39 **	1.26	0.76
Sampling points(site*Legume population)	F <sub>(32,96)</sub>	0.5	1.21	1.87 *	1.35	1.02	0.83
		Bray II P	K	Ca	Mg	Na	T-value
Site	F <sub>(7,96)</sub>	45.95 ***	73.65 ***	275.37 ***	61.00 ***	44.22 ***	130.66 ***
Legume population(site)	F <sub>(8,96)</sub>	1.78	2.85 *	2.64 *	2.67 *	3.16 **	5.32 ***
Sampling points(site*Legume population)	F <sub>(32,96)</sub>	0.75	0.96	0.57	1.01	1.01	0.95

The significant differences among the sites in all the parameters measured (Table 3.2) was supported by a Discriminant Function Analysis (DFA), which was categorized by soil type. The analysis clearly shows a generally wide separation between the soil types: sandstone derived, granitic, and limestone derived, with sandy soil partially separated from granite derived (Figure 3.3). The first two roots from the DFA explained 96% of the total variance, with the variables T-value, Mg and pH (Root 1) and T-value, Mg, NO<sub>3</sub> and Na (Root 2) contributing most to the total variance. The classification matrix of the soil types from the DFA was indicated to be 98% correct.

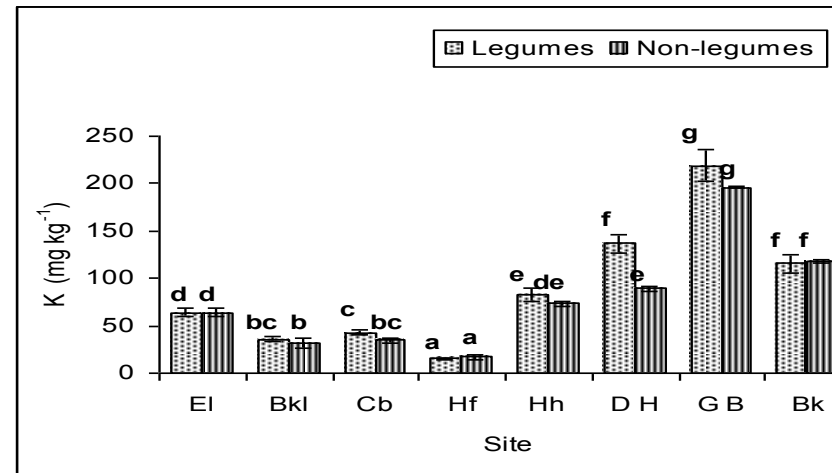


**Figure 3.3** Discriminant Function Analysis (FDA) scatter diagram showing the separation of the soil types based on Roots 1 that explains 91 and Root 2 explaining 6% of the variance. The classification matrix is 98% correct.

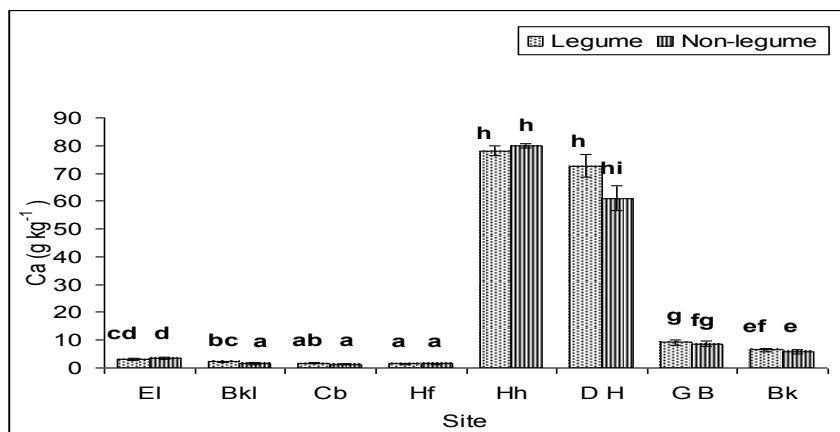
Statistical output suggests that differences in the parameters measured between legume and non-legume soils were not evident at all sites (Figure 3.2). The following four sites showed higher mineral contents in their legume soils than in their non-legume soils: Cederberg with  $\text{NH}_4$ , Na and C, De Hoop with K, Na and K, Bainskloof with Ca and Mg and Botlierskop with C (Figure 3.2 a – e and g). The legume soils of Cederberg, De Hoop and Bainskloof also had a higher T-Value than that of their non-legume soils (Figure 3.2 f). A surprising result is that the  $\text{NH}_4$  level of the non-legume soil at Hopefield was higher than that of its legume soil (Figure 3.2 a). Four of the parameters, pH,  $\text{NO}_3$ , Bray II P and Total P were statistically the same for the legume and non-legume soils at all of the sites (Data not shown).



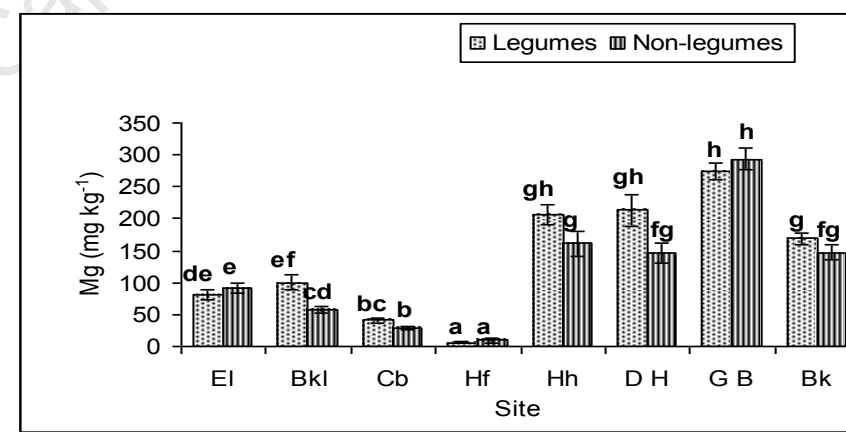
a



b

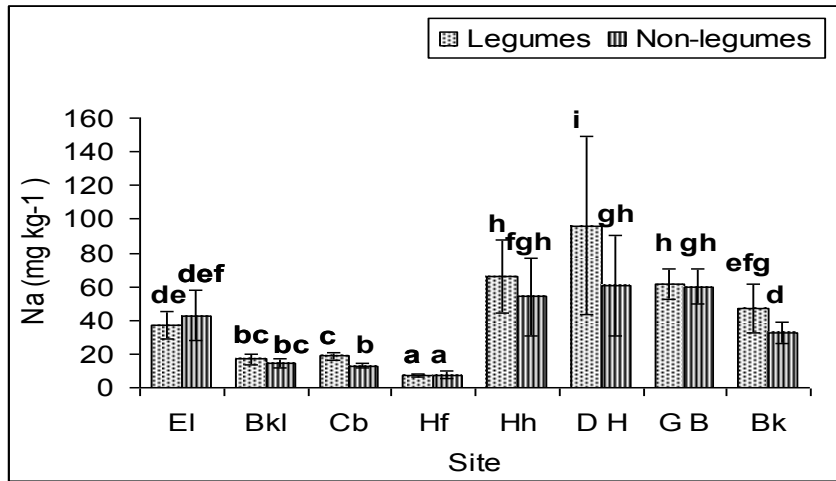


c

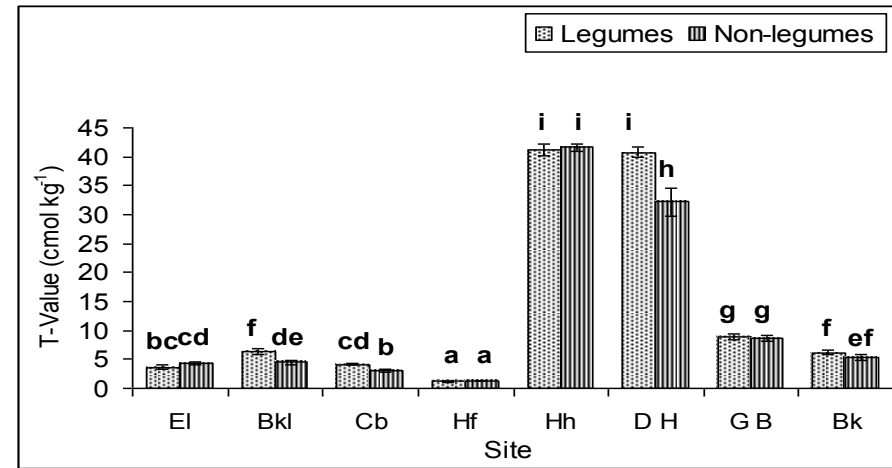


d

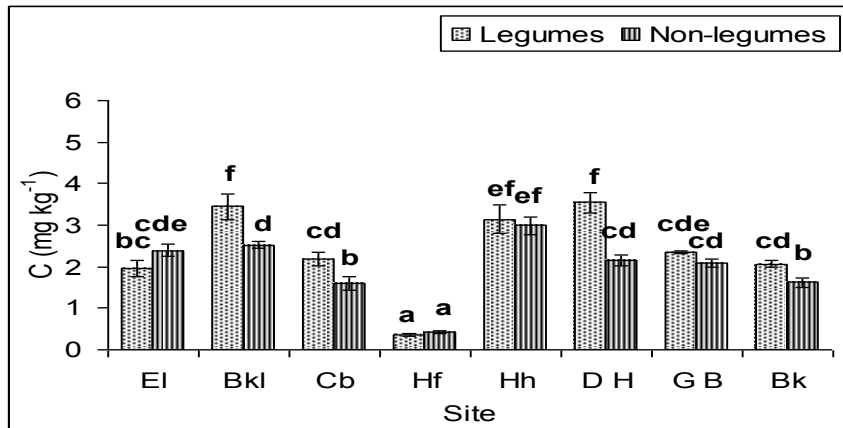
**Figure 3.2** Differences in the concentrations of a) NH<sub>4</sub>, b) K, c) Ca and d) Mg between legume and non-legume soils of the eight sites. Different letters above the bars indicate significant ( $P < 0.05$ ) differences between means. Vertical lines on bars indicate  $\pm$  Standard error. EI = Elim, Bkl = Bainskloof, Cb = Cederberg, Hf = Hopefield, Hh = Heidehof, DH = De Hoop, GB = Grootbrak, Bk = Botlierskop.



e



f



g

**Figure 3.2 (Continued)** Differences in the concentrations of e) Na, f) T-Value and g) C between legume and non-legume soils of the eight sites. Different letters above the bars indicate significant ( $P < 0.05$ ) differences between means. Vertical lines on bars indicate  $\pm$  Standard error. EI = Elim, Bkl = Bainskloof, Cb = Cederberg, Hf = Hopefield, Hh = Heidehof, DH = De Hoop, GB = Grootbrak, Bk = Botlierskop.

The sites that showed differences between legume and non-legume soils of at least one of the soil parameters from the ANOVA analyses (Cederberg, Bainskloof, Hopefield, De Hoop and Botlierskop) (Figure 3.2) were subjected to a DFA to determine which parameters contributed most to the separation of the legume and non-legume populations. The DFA analysis indicated that the separation of legume and non-legume soils on the basis of contribution by soil nutrient concentration using the multivariate analysis was significant at only Cederberg and De Hoop (Table 3.3). At Cederberg, Na, Total P and T-value significantly contributed to the separation of the legume and non-legume soils and Total P, Mg, Total N and NH<sub>4</sub> at De Hoop.

**Table 3.3.** Summary of Discriminant Function Analysis showing the Partial Wilks' Lamda of the nutrients which contributed significantly to the separation of the legume- and non-legume soils of Cederberg and De Hoop. Sites not shown in the table showed no differences in the measured soil parameters between the legume and non-legume populations at the sited

Site	Nutrient parameter	Partial Wilks' Lamda
Cederberg	Na	0.13 ***
	Total P	0.48 **
	T-value	0.34 **
De Hoop	Total P	0.38 **
	Mg	0.38 **
	Total N	0.52 *
	NH <sub>4</sub>	0.61*

The observed significant differences between the sampling points on the transects points by total N (Table 3.2) led to a two-way nested ANOVA, where the differences between legume population and sampling points were analysed separately for each site (Table 3.4). There were significant ( $P < 0.01$ ) differences in the soil Total N between the legume and non-legume populations at only the Cederberg and De Hoop sites (Table 3.4), with Total N being higher in the legume populations than the non-legume soil at both sites (Table 3.5).

**Table 3.4.** F-values and probability levels from the Two-way Nested ANOVA on Total N. The asterisks \*, and \*\* indicate P-levels of < 0.05 and < 0.01 respectively. Cb – Cederberg; Bkl – Bainskloof; EI – Elim; Hf – Hopefield; D H – De Hoop; Hh – Heidehof; Bk – Botlierskop; G B – Grootbrak.

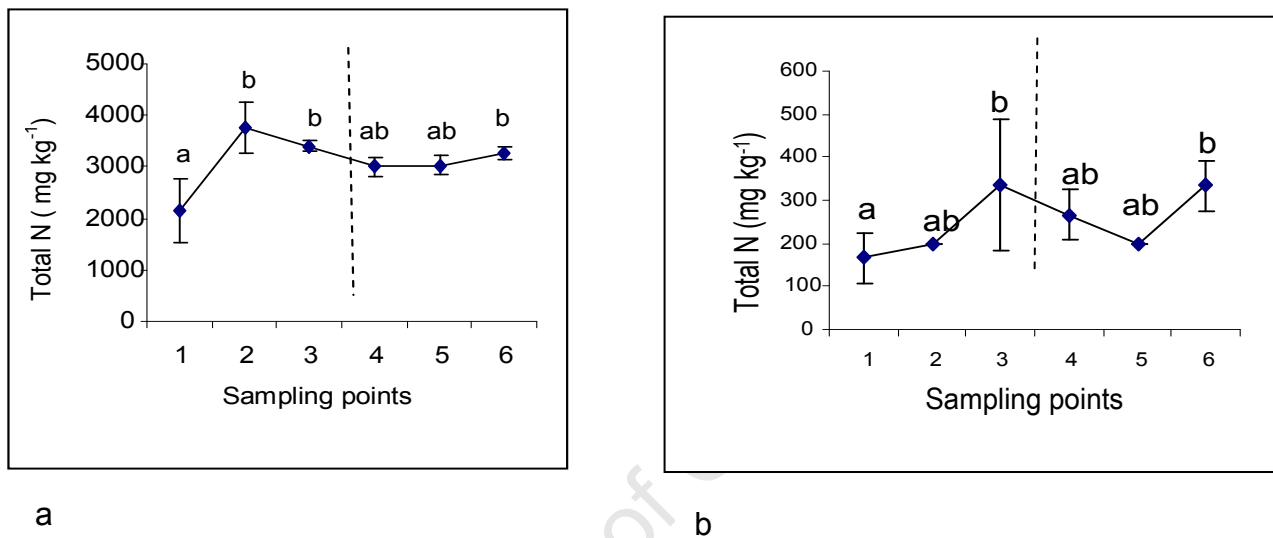
		Cb	Bkl	EI	Hf	D H	Hh	Bk	G B
Legume population	F <sub>(1,12)</sub>	13.11 *	6.19	4.37	0.59	24.45 **	0.07	6.19	0.95
Sampling points(Legume population)	F <sub>(4,12)</sub>	0.71	0.64	2.80	3.56 *	1.82	3.41 *	0.64	0.82

**Table 3.5.** Summary of the results of the Two-way Nested ANOVA analysis of Total N of the legume populations and adjacent areas of each site. Values in bold font indicate significant differences between the legume population and adjacent non-legume area. \*, and \*\* indicate P levels of <0.05, and <0.01 respectively and values are mean ± SE

Site	Patch	Soil Type	Total N (mg kg <sup>-1</sup> Soil)	F-Statistic <sub>(4,12)</sub>
Cederberg	Legume	Sandstone derived	<b>1411 ± 71 b</b>	<b>13.11*</b>
	Non-legume	Sandstone derived	<b>1022 ± 90 a</b>	
Bainskloof	Legume	Sandstone derived	2077 ± 182	6.19
	Non-legume	Sandstone derived	1488 ± 136	
Elim	Legume	Sandy	733 ± 62	4.37
	Non-legume	Sandy	988 ± 66	
Hopefield	Legume	Sandy	233 ± 37	0.59
	Non-legume	Sandy	266 ± 24	
De Hoop	Legume	Limestone derived	<b>3488 ± 214 b</b>	<b>24.45*</b>
	Non-legume	Limestone derived	<b>1766 ± 168 a</b>	
Heidehof	Legume	Limestone derived	3111 ± 332	0.71
	Non-legume	Limestone derived	3100 ± 90	
Botlierskop	Legume	Granite derived	1188 ± 87	2.44
	Non-legume	Granite derived	1033 ± 70	
Grootbrak	Legume	Granite derived	1744 ± 72	0.95
	Non-legume	Granite derived	1644 ± 120	

In addition, there were significant differences on total N between the sampling points on the transects of only Hopefield and Heidehof (Table 3.4). At Heidehof, the concentration of total N at sampling point 1 were lower (P<0.05) than sampling points two and three within the legume population and lower than sampling point six and the

same as sampling points four and five within the non-legume population (Figure 3.4 a). At Hopefield, the concentration of total N at sampling point 1 were the same as sampling point two and lower ( $P<0.05$ ) than that of sampling point three within the legume population, and the same as sampling points four and five and lower ( $P<0.05$ ) than that of sampling point six within the non-legume population (Figure 3.4 b). This suggested that there were no progressive changes of Total N along the transects, ranging from higher levels at point 1 to lower levels at point six, as expected.



**Figure 3.4.** Differences in the total soil N between six sampling points along the transects from within the legume- and non legume populations of (a) Heidehof and (b) Hopefield. Different letters above the bars indicate significant ( $P<0.05$ ) differences between means. Vertical lines on bars indicate  $\pm$  Standard error. Broken line indicates border between sampling points within the legume population (1, 2 and 3) and sampling points within the non-legume population (4, 5 and )

### Comparison of leaf traits between legume and non-legume plants

The results of a two-way nested ANOVA for plant types and species, with species nested in plant type at each site, showed that there were significant differences ( $P<0.05$ ) between the legumes and non-legumes pertaining to the N:P ratio and concentrations of N, Ca and Mg at a number of sites (Table 3.7). A more detailed comparison between leaf traits of the legumes and non-legumes at each individual site are shown in Tables 3.8 to 3.11

**Table 3.7** F-values from two-way nested ANOVA on leaf traits of plant species nested in plant types at each site. \*, \*\* and \*\*\* indicate P-levels of <0.05, <0.01 and <0.001 respectively.

Site	Parameter	F-Stat.	SLA	N:P	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Cederberg	Plant type	F <sub>(1,15)</sub>	0.11	0.01	10.60*	2.79	1.86	0.03	0.00	0.96
	Species(Plant type)	F <sub>(3,15)</sub>	48.66***	28.82**	33.06***	20.27***	12.27***	14.95***	14.09***	29.81***
Bainskloof	Plant type	F <sub>(1,12)</sub>	0.61	0.42	0.66	0.01	0.07	2.17	1.15	13.80
	Species(Plant type)	F <sub>(2,12)</sub>	27.79***	52.99***	51.96***	154.93***	29.68***	29.97***	18.76***	42.71***
Elim	Plant type	F <sub>(1,12)</sub>	1.49	20.36*	4.70	0.75	5.75	0.06	0.01	1.12
	Species(Plant type)	F <sub>(2,12)</sub>	35.23***	0.82	2.83	1.71	1.35	33.58***	32.29***	5.01*
Hopefield	Plant type	F <sub>(1,12)</sub>	0.74	20.93*	21.02*	7.13	4.60	0.04	2.45	0.48
	Species(Plant type)	F <sub>(2,12)</sub>	99.61***	0.62	32.84***	4.80*	17.28***	28.97**	1.34	10.14**
De Hoop	Plant type	F <sub>(1,15)</sub>	0.04	2.27	18.41*	0.23	0.17	15.55*	11.82*	0.00
	Species(Plant type)	F <sub>(3,15)</sub>	160.35***	7.79**	29.35***	2.86	12.88***	11.78***	8.51***	290.07***
Heidehof	Plant type	F <sub>(1,19)</sub>	1.64	7.80*	12.65*	1.18	0.06	4.63	7.41*	0.15
	Species(Plant type)	F <sub>(5,19)</sub>	7.00***	3.67*	31.66***	3.87*	7.20***	29.84***	11.23***	56.54***
Botlierskop	Plant type	F <sub>(1,15)</sub>	0.85	0.88	0.29	0.62	0.43	4.93	0.09	1.06
	Species(Plant type)	F <sub>(3,15)</sub>	153.30***	73.59***	47.48***	18.842***	9.49***	15.31***	27.15***	12.83***
Groothorak	Plant type	F <sub>(1,15)</sub>	0.71	5.60	5.36	0.08	0.33	0.24	0.14	0.38
	Species(Plant type)	F <sub>(3,15)</sub>	13.38***	3.41*	23.15***	8.02**	14.44***	22.86***	2.95	18.03***

A more detailed legume versus non-legume comparison of the species at Cederberg and Bainskloof, both with sandstone derived soils, showed that the two legume species, *Psoralea vigilans* and *Aspalathus* species, growing at Cederberg, had higher concentrations of leaf N than that of the non-legumes and that the legume species, *Indigofera frutescens*, growing in Bainskloof had either the same or a lower N concentration than that of the three non-legumes (Tables 3.8 a and b). The SLA, Na and the rest of the nutrients of the legumes of both sites showed either the same or lower values than those of the non-legumes of the respective sites (Tables 3.8 a, and b).

**Table 3.8.** Leaf traits analysed using Two-way nested ANOVA with species nested in plant type at Cederberg and Bainskloof. Different letters within the columns indicate significant ( $P < 0.05$ ) differences between means. Values are mean  $\pm$  Standard errors

**(a) Cederberg**

Plant Type	Species	SLA g m <sup>-2</sup>	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Legume	<i>Psoralea vigilans</i>	4.58 $\pm$ 0.62 b	2.06 $\pm$ 0.11 e	0.073 $\pm$ 0.01 c	0.366 $\pm$ 0.05 b	0.38 $\pm$ 0.03 a	0.160 $\pm$ 0.03 ab	1451 $\pm$ 76 a
	<i>Aspalathus</i> sp	10.08 $\pm$ 0.43 d	1.63 $\pm$ 0.10 d	0.088 $\pm$ 0.01 c	0.548 $\pm$ 0.08 b	1.07 $\pm$ 0.09 c	0.215 $\pm$ 0.01 b	3974 $\pm$ 660 c
Non-legume	<i>Clutia alaternoides</i> (L)	6.46 $\pm$ 0.22 c	1.04 $\pm$ 0.03 c	0.070 $\pm$ 0.00 c	0.455 $\pm$ 0.06 b	0.52 $\pm$ 0.03 ab	0.315 $\pm$ 0.02 c	2391 $\pm$ 146 b
	<i>Berzelia squarrosa</i>	8.62 $\pm$ 0.66 d	0.87 $\pm$ 0.04 b	0.020 $\pm$ 0.00 a	0.168 $\pm$ 0.06 a	0.58 $\pm$ 0.07 b	0.168 $\pm$ 0.03 ab	4830 $\pm$ 378 c
	<i>Colozza</i> Thun.							
	<i>Protea nitida</i> Mill.	3.51 $\pm$ 0.12 a	0.56 $\pm$ 0.02 a	0.033 $\pm$ 0.01 b	0.034 $\pm$ 0.01 a	0.74 $\pm$ 0.14 b	0.095 $\pm$ 0.02 a	4462 $\pm$ 285 c
	F <sub>(3,15)</sub>	48.66***	33.06***	20.27***	12.27***	14.95***	14.09***	29.81***

**(b) Bainskloof**

Plant Type	Species	SLA g m <sup>-2</sup>	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Legume	<i>Indigofera frutescens</i> L. f.	7.66 $\pm$ 0.18 a	1.69 $\pm$ 0.06 c	0.073 $\pm$ 0.00 b	0.57 $\pm$ 0.04 ab	0.48 $\pm$ 0.08 a	0.140 $\pm$ 0.02 a	913 $\pm$ 38 a
	<i>Ursinia</i> sp.	13.20 $\pm$ 0.35 b	1.28 $\pm$ 0.04 b	0.078 $\pm$ 0.00 b	0.61 $\pm$ 0.08 b	0.82 $\pm$ 0.04 b	0.293 $\pm$ 0.01 b	12635 $\pm$ 1167 c
Non-legume	<i>Othonna quinqueidentata</i> Thun.	13.99 $\pm$ 1.25 b	1.67 $\pm$ 0.08 c	0.223 $\pm$ 0.03 c	1.51 $\pm$ 0.24 c	2.66 $\pm$ 0.25 c	0.975 $\pm$ 0.19 c	5194 $\pm$ 381 b
	<i>Metalasia muralifolia</i> DC	7.08 $\pm$ 0.76 ab	0.83 $\pm$ 0.06 a	0.033 $\pm$ 0.00 a	0.31 $\pm$ 0.04 a	1.96 $\pm$ 0.23 c	0.360 $\pm$ 0.06 b	5784 $\pm$ 387 b
		F <sub>(2,12)</sub>	27.79***	59.96***	154.93***	29.69***	29.98***	18.76***

At Elim and Hopefield, both with sandy soils, the legume species *Aspalathus. sp.* growing at Elim, had the same concentrations of leaf N, P, K and Na than those of the non-legumes, and the concentrations of leaf Ca and Mg was either lower or the same (Table 3.9 a). At Hopefield, the leaf N of the legume, *Wiborgia obcordata*, was higher than that of the non-legumes, while the level of its leaf Na was either higher or the same and the levels of P, K and Ca was either lower or the same at those of the non-legumes (Table 3.9 b). The SLA of both legumes at both sites were lower than that of the non-legumes growing on the respective sites (Table 3.9 a, and b).

**Table 3.9.** Leaf traits analysed using Two-way nested ANOVA with species nested in plant type at Elim and Hopefield. Different letters within the columns indicate significant ( $P < 0.05$ ) differences between means. Values are mean  $\pm$  Standard errors

**(a) Elim**

Plant Type	Species	SLA g m <sup>-2</sup>	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Legume	<i>Aspalathus</i> sp.	5.48 $\pm$ 0.26 c	0.89 $\pm$ 0.16	0.010 $\pm$ 0.00	0.150 $\pm$ 0.06	0.83 $\pm$ 0.09 b	0.180 $\pm$ 0.03 b	6047 $\pm$ 646
	<i>Leucadendron platyspermum</i> R. Br.	4.54 $\pm$ 0.17b	0.36 $\pm$ 0.01	0.010 $\pm$ 0.00	0.005 $\pm$ 0.01	0.36 $\pm$ 0.01 a	0.063 $\pm$ 0.02 a	4141 $\pm$ 235
Non-legume	<i>Leucospermum cordifolium</i> Salis. ex Knight.	4.33 $\pm$ 0.15 b	0.52 $\pm$ 0.04	0.015 $\pm$ 0.00	0.020 $\pm$ 0.02	1.18 $\pm$ 0.13 c	0.388 $\pm$ 0.06 b	5929 $\pm$ 584
	<i>Protea compacta</i> R. Br.	2.89 $\pm$ 0.12 a	0.56 $\pm$ 0.06	0.013 $\pm$ 0.00	0.080 $\pm$ 0.04	0.79 $\pm$ 0.10 b	0.048 $\pm$ 0.01 a	3972 $\pm$ 355
F <sub>(2,12)</sub>		35.23***	2.82	1.71	1.35	33.58***	32.29***	5.01

**(b) Hopefield**

Plant Type	Species	SLA g m <sup>-2</sup>	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Legume	<i>Wiborgia obcordata</i> P.J. Rennie	12.42 $\pm$ 1.28 c	4.90 $\pm$ 0.19 d	0.253 $\pm$ 0.02 c	0.958 $\pm$ 0.05 c	0.63 $\pm$ 0.05 a	0.198 $\pm$ 0.02	934 $\pm$ 170 b
	<i>Trichocephalus stipularis</i> L. Bronn.	2.86 $\pm$ 0.06 b	1.05 $\pm$ 0.06 b	0.113 $\pm$ 0.02 b	0.050 $\pm$ 0.04 a	1.23 $\pm$ 0.13 b	0.180 $\pm$ 0.02	299 $\pm$ 48 a
	<i>Diospyros austro-africana</i> De Winter.	3.35 $\pm$ 0.06 b	1.41 $\pm$ 0.04 c	0.120 $\pm$ 0.01 b	0.508 $\pm$ 0.14 b	1.20 $\pm$ 0.05 b	0.145 $\pm$ 0.02	1171 $\pm$ 260 b
	<i>Phylca</i> sp.	0.02 $\pm$ 0.02 a	0.79 $\pm$ 0.05 a	0.065 $\pm$ 0.01 a	0.035 $\pm$ 0.02 a	0.51 $\pm$ 0.07 a	0.133 $\pm$ 0.03	461 $\pm$ 85 a
F <sub>(2,12)</sub>		99.61***	32.84***	4.80*	17.28***	28.97***	1.34	10.14**

At De Hoop and Heidehof, both with limestone derived soils, all four the legume species, *Aspalathus pallescens* at De Hoop and *Aspalathus salteri*, *Otholobium bracteolatum* and *Indigofera brachystachya* at Heidehof had higher leaf N than the non-legumes at the respective two sites, with the legumes at both sites also presenting with a general trend of higher leaf Ca and Mg than those of the non-legumes (Tables 3.10 a, and b). The concentration of leaf P, K, and Na of the legumes at Heidehof are lower than those of the legumes (Table 3.10 b), while the legume leaf concentration of P and K at De Hoop are the same as those of the non-legumes (Table 3.10 a). The SLA of the legume at De Hoop was higher than that of the non-legume, *Protea obtusifolia*, and lower than that of the non-legumes *Euchaetis meridionalis*, *Oedera capensis* and

*Leucadendron meridianum*, whereas the SLA of the legumes *Aspalathus salteri* and *Indigofera brachystachya* at Heidehof were the same as that of the non-legumes at this site and the legume (Tables 3.10 a, and b). The legume *Otholobium bracteolatum* had a higher SLA than that of the non-legumes at Heidehof (Table 3.10 b).

**Table 3.10** Leaf traits analysed using Two-way nested ANOVA with species nested in plant type at De Hoop and Heidehof . Different letters within the columns indicate significant ( $P < 0.05$ ) differences between means. Values are mean  $\pm$  Standard errors

**(a) De Hoop**

Plant Type	Species	SLA g m <sup>-2</sup>	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Legume	<i>Aspalathus</i> <i>pallens</i> Eckl & 7evh	3.52 $\pm$ 0.07 b	1.37 $\pm$ 0.04 c	0.043 $\pm$ 0.00	0.375 $\pm$ 0.15 b	3.78 $\pm$ 0.26 d	0.445 $\pm$ 0.04 c	2545 $\pm$ 2.17 c
	<i>Euchaetis</i> <i>meridionalis</i> l. Williams.	4.30 $\pm$ 0.05 c	0.67 $\pm$ 0.01 b	0.035 $\pm$ 0.01	0.280 $\pm$ 0.05 b	2.12 $\pm$ 0.04 c	0.173 $\pm$ 0.02 b	603 $\pm$ 49 a
	<i>Oedera</i> <i>capensis</i> (L) Bruce.	4.95 $\pm$ 0.13 d	0.66 $\pm$ 0.03 b	0.033 $\pm$ 0.00	0.660 $\pm$ 0.13 c	1.28 $\pm$ 0.10 b	0.188 $\pm$ 0.02 b	14705 $\pm$ 957 e
Non-legume	<i>Leucadendron</i> <i>meridianum</i> l. Williams	4.88 $\pm$ 0.10 d	0.47 $\pm$ 0.02 a	0.058 $\pm$ 0.01	0.000 $\pm$ 0.00 a	0.94 $\pm$ 0.14 a	0.160 $\pm$ 0.03 b	3756 $\pm$ 218 d
	<i>Protea</i> <i>obtusifolia</i> H. Buek.	2.16 $\pm$ 0.13 a	0.50 $\pm$ 0.01 a	0.033 $\pm$ 0.00	0.045 $\pm$ 0.02 a	1.30 $\pm$ 0.14 b	0.038 $\pm$ 0.01 a	1493 $\pm$ 144 b
	F <sub>(3,15)</sub>	160.35***	29.35***	2.86	12.89***	11.78***	8.51**	290.07 ***

**(b) Heidehof**

Plant Type	Species	SLA g m <sup>-2</sup>	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Legume	<i>Aspalathus</i> <i>salteri</i> . L. Bolus.	5.09 $\pm$ 0.46 a	1.20 $\pm$ 0.07 e	0.035 $\pm$ 0.00 ab	0.178 $\pm$ 0.06 ab	1.94 $\pm$ 0.32 b	0.393 $\pm$ 0.04 c	4827 $\pm$ 871 c
	<i>Otholobium</i> <i>bracteolatum</i> Fckl & 7evh	7.97 $\pm$ 0.53 b	1.82 $\pm$ 0.07 f	0.070 $\pm$ 0.01 bc	0.535 $\pm$ 0.13 cd	2.84 $\pm$ 0.18 c	0.180 $\pm$ 0.04 b	3704 $\pm$ 389 a
	<i>Indigofera</i> <i>brachystachya</i> E. Mev.	4.16 $\pm$ 0.10 a	0.99 $\pm$ 0.03 d	0.020 $\pm$ 0.00 a abc	0.230 $\pm$ 0.04 abc	7.27 $\pm$ 0.24 d	0.348 $\pm$ 0.05 c	796 $\pm$ 74 bc
	<i>Protea</i> <i>obtusifolia</i> H. Ruek	4.05 $\pm$ 0.54 a	0.60 $\pm$ 0.03 a	0.123 $\pm$ 0.02 c	0.048 $\pm$ 0.02 a	0.96 $\pm$ 0.06 a	0.023 $\pm$ 0.01 a	2669 $\pm$ 186 b
	<i>Disparago</i> sp.	4.37 $\pm$ 0.38 a	0.72 $\pm$ 0.03 b	0.043 $\pm$ 0.01 ab	0.383 $\pm$ 0.15 bcd	1.93 $\pm$ 0.15 b	0.028 $\pm$ 0.01 a	8626 $\pm$ 909 d
Non-legume	<i>Searsia</i> <i>laevigata</i> (l) F.A. Barklev.	4.99 $\pm$ 0.52 a	0.84 $\pm$ 0.01 c	0.040 $\pm$ 0.01 ab	0.705 $\pm$ 0.28 d	1.96 $\pm$ 0.32 b	0.155 $\pm$ 0.03 b	1095 $\pm$ 18 a
	<i>Leucadendron</i> <i>meridianum</i> l. Williams	4.40 $\pm$ 0.15 a	0.54 $\pm$ 0.02 a	0.098 $\pm$ 0.05 bc	0.035 $\pm$ 0.02 a	0.99 $\pm$ 0.05 a	0.185 $\pm$ 0.02 b	3446 $\pm$ 139 bc
	F <sub>(5,19)</sub>	7.00***	31.67 ***	3.87*	7.21***	29.84 ***	11.24***	56.54***

Three of the four legumes, *Aspalathus asparagoides*, *Aspalathus cinerascens*, and *Indigofera nigromontana*, at the two sites with granitic soils, Botlierskop and Groot Brak, had an SLA that were the same as that of the non-legumes of their respective sites, while the SLA of the legume, *Aspalathus submissa* of Botlierskop was higher than that of the non-legumes of this site (Tables 3.11 a, and b). With regards to leaf N, only one of the three non-legumes at Botlierskop, *Rhus glauca* had a higher concentration than the two legumes growing at this site (Table 3.11 a), whilst both legumes growing at Groot Brak had higher concentrations than the non-legumes at the site (Table 3.11 b). Except for *Aspalathus submissa* (Botlierskop) with a higher concentration of leaf Mg than that of the non-legumes at this site, the rest of the leaf nutrients of the legumes of both sites were either the same as, or lower than that of the non-legumes of the respective sites (Tables 3.11 a, and b).

**Table 3.11** Leaf traits analysed using Two-way nested ANOVA with species nested in plant type at Botlierskop and Groot Brak . Different letters within the columns indicate significant ( $P < 0.05$ ) differences between means. Values are mean  $\pm$  Standard errors

**g) Botlierskop**

Plant Type	Species	SLA g m <sup>-2</sup>	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Legume	<i>Aspalathus asparagoides</i> L.	6.70 $\pm$ 13 a	1.24 $\pm$ 0.06 c	0.025 $\pm$ 0.00 a	0.318 $\pm$ 0.04 a	0.49 $\pm$ 0.03 a	0.078 $\pm$ 0.01 a	2698 $\pm$ 247 bc
	<i>Aspalathus submissa</i> R. Dahlaren.	20.50 $\pm$ 1.28 c	1.54 $\pm$ 0.08 d	0.060 $\pm$ 0.00 c	0.185 $\pm$ 0.05 a	0.60 $\pm$ 0.06 ab	0.263 $\pm$ 0.01 c	1955 $\pm$ 191 a
	<i>Rhus glauca</i> Thun.	7.40 $\pm$ 0.30 a	1.93 $\pm$ 0.18 e	0.045 $\pm$ 0.01 b	0.343 $\pm$ 0.12 a	1.35 $\pm$ 0.17 c	0.178 $\pm$ 0.04 b	2134 $\pm$ 210 ab
Non-legume	<i>Syncarpha vestita</i> (L.) B. Nord.	6.62 $\pm$ 0.10 a	1.03 $\pm$ 0.01 b	0.060 $\pm$ 0.00 c	0.750 $\pm$ 0.12 b	1.39 $\pm$ 0.12 c	0.188 $\pm$ 0.01 b	3224 $\pm$ 221 c
	<i>Leucadendron salignum</i> P.J. Beraius.	9.69 $\pm$ 0.28 b	0.74 $\pm$ 0.03 a	0.050 $\pm$ 0.00 bc	0.155 $\pm$ 0.02 a	0.67 $\pm$ 0.04 b	0.063 $\pm$ 0.01 a	4766 $\pm$ 547 d
	F <sub>(3,15)</sub>	153.30***	47.48***	18.42***	9.49***	15.31***	27.15***	12.83***

**(h) Grootbrak**

Plant Type	Species	SLA g m <sup>-2</sup>	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg <sup>-1</sup> )
Legume	<i>Aspalathus cinerascens</i> E. Mev	5.99 ± 0.48 a	1.68 ± 0.01 c	0.048 ± 0.00 a	0.620 ± 0.07 ab	0.41 ± 0.01 a	0.253 ± 0.01 b	4156 ± 438 c
	<i>Indigofera nigromontana</i> E. Mev.	8.23 ± 0.65 b	2.44 ± 0.23 d	0.118 ± 0.04 c	0.515 ± 0.02 ab	1.32 ± 0.19 c	0.128 ± 0.06 a	1723 ± 362 b
	<i>Searsia lucida</i>	6.52 ± 0.35 a	0.94 ± 0.02 a	0.048 ± 0.00 a	0.650 ± 0.09 b	1.20 ± 0.15 c	0.255 ± 0.04 b	3351 ± 646 bc
	<i>Polygala fruticosa</i> P.J.	10.28 ± 0.51 c	1.39 ± 0.04 b	0.068 ± 0.00 ab	0.438 ± 0.08 a	0.78 ± 0.05 b	0.158 ± 0.04 b	539 ± 29 a
Non-legume	<i>Berrius Hermannia holosericea</i>	8.59 ± 0.34 bc	1.39 ± 0.06 b	0.080 ± 0.00 bc	1.090 ± 0.06 c	0.85 ± 0.09 b	0.220 ± 0.03 ab	2677 ± 693 bc
	Jacq F <sub>(3,15)</sub>	13.38***	23.16***	8.02**	11.44***	22.86***	3.37*	18.03***

A correlation analysis between leaf N and leaf P of the species at each site, indicated that there were significant ( $P < 0.05$ ) correlations between the leaf N and P at only four sites namely Cederberg, Bainskloof, Hopfield and Grootbrak (Table 3.12).

**Table 3.12.** Correlations between the Leaf N and P of the species sampled at each site. \* and \*\*\* indicate P-levels of  $< 0.01$  and  $< 0.001$  respectively.

Site	r	r <sup>2</sup>	t
Cederberg	0.743	0.553	4.716***
Bainskloof	0.662	0.387	2.947*
Elim	0.035	0.001	0.131
Hopfield	0.926	0.858	9.188***
De Hoop	0.026	0.001	0.109
Heidehof	-0.172	0.030	-0.963
Botlierskop	0.053	0.003	0.224
Grootbrak	0.738	0.544	4.637***

The N:P ratio of the legume and non-legume plants in this study were put into groups of ratios greater than 20 to show P limitation (high N and low P in the tissue), less than 10 to show N limitation (low N and high P), and between 10 and 20 to represent optimum ratio or N and P co-limitation (Güsewell, 2004). The results showed that about 77% of the legumes and 42% of the non-legumes sampled had N:P ratio greater than 20, about 23% legumes and 42% non-legumes had N:P ratio between 10 and 20, and only about 15% of the non-legumes and no legumes recorded N:P ratio less than 10 (Tables 3.13 and 3.14).

**Table 3.13** N:P ratio of legume leaves and their implication on N or P limitation according to Gusewell (2004). N:P ratio values are mean  $\pm$  SE. N limited refers N:P Ratio  $<10$ , P limited refers N:P Ratio  $> 20$ , N and P co-limited refers N:P Ratio between 10 to 20. Values are means  $\pm$  SE

Legumes	N:P Ratio	N - Limited	P - Limited	N & P Co-limited
<i>Aspalathus cinerascens</i>	16.60 $\pm$ 0.72			*
<i>Aspalathus</i> sp. Cederberg	18.66 $\pm$ 0.56			*
<i>Wiborgia obcordata</i>	19.60 $\pm$ 0.94			*
<i>Indigofera frutescens</i>	23.37 $\pm$ 1.21		*	
<i>Indigofera nigromontana</i>	23.7 $\pm$ 1.21		*	
<i>Aspalathus submissa</i>	25.58 $\pm$ 1.34		*	
<i>Otholobium bracteolatum</i>	27.03 $\pm$ 2.82		*	
<i>Psoralea vigilans</i>	29.79 $\pm$ 3.23		*	
<i>Aspalathus pallescens</i>	32.45 $\pm$ 1.39		*	
<i>Aspalathus salteri</i>	34.38 $\pm$ 1.164		*	
<i>Indigofera brachystachya</i>	49.63 $\pm$ 1.61		*	
<i>Aspalathus asparagoides</i>	51.21 $\pm$ 4.58		*	
<i>Aspalathus</i> sp. Elim	89.25 $\pm$ 16.32		*	

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**Table 3.14 N:P ratio** of non-legumes and their implication on N or P limitation according to Güsewell (2004). N:P ratio values are mean  $\pm$  SE. N limited refers N:P Ratio <10, P limited refers N:P Ratio >20, N and P co-limited refers N:P Ratio between 10 to 20. Values are means  $\pm$  SE

Non-legumes	N:P Ratio	N - Limited	P - Limited	N & P Co-limited
<i>Protea obtusifolia</i> Heidehof	5.24 $\pm$ 0.72	*		
<i>Othonna quinquedentata</i>	7.84 $\pm$ 0.95	*		
<i>Searsia lucida</i>	7.84 $\pm$ 0.95	*		
<i>Leucodendron meridianum</i>	8.52 $\pm$ 0.90	*		
<i>Trichocephalus stipularis</i>	10.80 $\pm$ 2.9			*
<i>Phylca</i> sp.	12.48 $\pm$ .38			*
<i>Diospyros austro-africana</i>	12.04 $\pm$ 1.24			*
<i>Leucadendron salignum</i>	14.75 $\pm$ 0.61			*
<i>Leucadendron meridianum</i> Heidehof	14.85 $\pm$ 6.75			*
<i>Clutia alaternoides</i>	14.91 $\pm$ 0.61			*
<i>Protea obtusifolia</i> De Hoop	16.35 $\pm$ 2.39			*
<i>Protea obtusifolia</i> sp.	16.60 $\pm$ 0.72			*
<i>Syncarpha vestita</i>	17.42 $\pm$ 1.26			*
<i>Disparago</i> sp.	17.69 $\pm$ 1.9			*
<i>Protea nitida</i>	18.70 $\pm$ 2.94			*
<i>Polygala fruticosa</i>	20.83 $\pm$ 1.19		*	
<i>Oedera capensis</i>	21.3 $\pm$ 2.68		*	
<i>Searsia laevigata</i>	22.2 $\pm$ 5.43		*	
<i>Hermannia holosericea</i>	25.83 $\pm$ 2.51		*	
<i>Metalasia muraliifolia</i>	25.83 $\pm$ 2.51		*	
<i>Leucadendron platyspermum</i>	35.75 $\pm$ 1.11		*	
<i>Eucheatis meridionalis</i>	32.65 $\pm$ 5.98		*	
<i>Leucospermum cordifolium</i>	37.75 $\pm$ 6.24		*	
<i>Berzelia squarrosa</i> Colozza	43.38 $\pm$ 2.11		*	
<i>Rhus glauca</i>	43.89 $\pm$ 2.25		*	
<i>Protea compacta</i>	47.50 $\pm$ 6.30		*	

## Discussion

### Comparison of soil nutrients from legume and non-legume populations

Scientific literature abounds with the soil characterization of the CFR as a distinct mosaic of distinct soil types, based on differences in nutrient content, topography, and precipitation, which plays a pivotal role in the species composition and boundaries of communities (Witkowski and Mitchell, 1987; Richards, *et al.*, 1997; Goldblatt and Manning, 2002; Van der Niet and Johnson, 2009). This soil nutrient mosaic concept is in agreement with the results of the soil analysis of the eight sites, as there were significant ( $p < 0.001$ ) differences in the soil pH,  $\text{NO}_3$ , Total P, Bray II, and  $\text{NH}_4$  at ( $p < 0.01$ ), between the sites (Table 3.2). The results also indicate that legumes occupy soil types with variable nutrient levels.

Analysis of the soil data using Nested ANOVA showed differences between the legume and non-legume soils at Hopefield, Cederberg, Bainskloof, Botlierskop and De Hoop in some of the parameters measured (Figure 3.2 and Table 3.5). In order to determine if these differences were significant, a Discriminant Function Analysis on the soil data was done. This analysis showed that differences between the legume and non-legume soils were only significant at Cederberg and De Hoop (Table 3.3). Thus, the hypothesis that legume plants occupy more fertile soil than the non-legumes is weakly supported. These results are partly contradictory to those of a number of researchers who indicated that legumes enrich the soil in which they grow (Stock, *et al.*, 1995; Holmes and Cowling, 1997; Cocks and Stock, 2000; Spehn, *et al.*, 2002; Mulder, *et al.*, 2002; Yelenik, *et al.*, 2007; Dong, *et al.*, 2009). Soil enrichment due to the presence of plants depends firstly on site specific abiotic factors, which include optimum soil moisture, temperature, and pH and secondly on specific biotic factors brought about by the presence of legumes. These include the particular legume species, the age of the plants and the amount and quality of the litter. The interactions between these two in turn determine the biomass and activity of soil microbes (Day, 1983; Enríques, *et al.*, 1993; Wedin and Tilman, 1990; Binkley and Giardina, 1998; Spehn, *et al.*, 2000; Spehn, *et al.*, 2002; Zak, *et al.*, 2003; Wardle, *et al.*, 2003; Goberna, *et al.*, 2006; Marchante, *et al.*, 2008; Van der Heijden *et al.*, 2008; Weixing, *et al.*, 2009; Jeddi, *et al.*, 2009).

The most probable reasons why the soil of the legume populations of Elim, Grootbrak, Heidehof, and Botlierskop were not enriched with nutrients include the age of vegetation which was eight years old or less (Table 3.1), so there had not been sufficient time for decomposition of litter for nutrient accumulation to take place (Binkley and Giardina, 1998). At these sites, there were also no visible signs of surface water near the legume stands and thus the soil moisture content was most probably low, especially during the hot dry summer periods, which may have inhibited the growth and survival of decomposing soil organisms. Moreover, the legumes growing in the patches at Elim, Grootbrak, and Botlierskop were sparsely populated *Aspalathus* sp (< 1 plant per m<sup>2</sup>) with very small leaves, which means that the biomass of the litter was in all probability too small to affect nutrient levels in the soil. Similarly, at Hopefield site, the legume density of small leaved *W. obcordata* trees were also sparse (0.5 plant per m<sup>2</sup>). This would produce a relatively low quantity of litter and, together with the very dry, sandy soil, most likely inhospitable to many soil microbes, decomposition would be very limited. This could explain why this legume soil had not been enriched, notwithstanding the fact that *W. obcordata* had a vegetation age of more than 25 years and the best quality litter of all the legumes plants sampled, with the highest leaf N and P at 4.51 and 0.24% respectively (Table 3.9a).

Soil nutrient levels in the legume populations at De Hoop and Cederberg were significantly higher than the non-legume populations, with Total P, Mg, Total N, NH<sub>4</sub><sup>+</sup> identified as major contributors to the separation of the patches at De Hoop and Total P, Na and T-value at Cederberg (Table 3.3). Possible explanations for the higher nutrient status in legume population than non-legume population at De Hoop could be that the age of the vegetation was more than 15 years (Table 3.1), which, in all probability, was enough time for the decomposition of the legume litter and a subsequent enrichment of the soil. The plant density of *A. pallescens* at De Hoop was 2.76 plants per m<sup>2</sup> which provided approximately 35% ground cover. The age of the legume population after fire at the Cederberg site could not be determined, but there were no signs of fire damage to the vegetation in the recent past. The Cederberg site had relatively plenty of soil moisture available, as there were seasonal run-off mountain streams that were flowing through the legume population, and a stream at the bottom of the site (about 20 m away) still flowing in mid summer (Table 3.1). This moister habitat would have created an ideal habitat for the activities of decomposing soil microbes. Growing in the area were two legume species, *P. vigilans* at a density of 1.3 plants per

m<sup>2</sup> with about 27% ground cover and an *Aspalathus* sp. (plant density not measured). Together, the two species could have provided relatively large amounts of litter fall which might have led to the enhanced soil nutrient status of the legume populations.

The Bainskloof site was an enigma. The site had a number of characteristics in common with De Hoop and Cederberg which had enriched legume soil relative to the non-legume soil. The Bainskloof vegetation was more than 20 years old, a fern species, *Pteridium quilinum*, was found growing among the *I. frutescence*, indicating the availability of soil moisture due to possibly a periodically high water table (Cucchi, *et al.*, 2004; Marrs and Watt, 2006), and a seasonal run-off mountain stream that was about 10 m away. Plants of *I. frutescence* have reasonably sized leaves with a density that covered about 40% of the ground. Although all the above factors should have lead to soil nutrient enrichment, the soil pH at Bainskloof was the most acidic at pH 3.15 (Table 3.7) which could have hindered the activity and proliferation of decomposing microbes communities (Anderson and Nilsson 2000; White *et al.*, 2005; Fernández-Calviño *et al.*, 2011).

### **Comparison of leaf traits between legume and non-legume plants.**

Another research question was whether the fynbos legume leaves have higher nutrient levels and SLA than those of the non-legumes. It was found that there were no definite trends in either the concentrations of the leaf nutrients, other than N, nor the SLA, of the two plant groups. Sixty nine percent of the legumes in this study had higher concentration of leaf N than that of the non-legumes. Higher N concentration in the leaves of legumes compared to that of non-legumes were also found by Tuohy, *et al.*, (1991) and Powers and Tiffen (2010). The latter attempted to classify plant functional types in tropical dry forests in Costa Rica. The higher concentration of leaf N of the legumes at Cederberg, Hopefield, Heidehof, De Hoop and Grootbrak are in line with the findings of several investigations which found that N<sub>2</sub> fixation in legumes translates into increased leaf N (Forester, *et al.*, 2007; Freitas *et al.*, 2010). The possible explanation could be that legumes have, in general, a higher N-demanding lifestyle and so have a greater demand for N (Vitousek, *et al.*, 2002; Sprent, *et al.*, 1996; Powers and Tiffen, 2010). This high demand would then be met by means of biological nitrogen fixation (Döbereiner, 1978; Vitousek, *et al.*, 2002; Lambers, *et al.*, 2008; Powers and Tiffen, 2010) and/or forming mycorrhizal associations (Larcher, 2003; Bledsoe and Zasoski,

1983; Brundrett, 2002; Chalot, *et al.* 2006). Thus higher leaf N of legumes, as compared to that of non-legumes, might be a property of legumes, rather than a reflection of soil N. Golluscio *et al.*, (2006) found a discrepancy between indirect evidence of N<sub>2</sub> fixation in the leguminous *Adesmia volckmanni* and the fact that their  $\delta^{15}\text{N}$  signatures were the same as non-fixing species in the same xeric site. They came to the conclusion that N<sub>2</sub> fixation might also be an episodic phenomenon linked to seasonality, when environmental conditions promote nodulation and/or fixation. This is a possible explanation why there were no differences between the leaf N of the legume and non-legume species at Bainskloof, Elim and Botlierskop.

Other than leaf N, there was a poor relationship between soil and leaf nutrients. The concentrations of Na at Cederberg, De Hoop and Botlierskop, as well as Ca and Mg at Bainskloof and K at De Hoop were higher within the legume soils than in the non-legume soils (Figures 3.2), yet the leaves of the legumes growing at these sites had statistically the same, or even lower levels of these nutrients than the non-legumes (Tables 3.8 a and b, 3.9a, and 3.10a). Furthermore, with the exception of leaf N, the measured leaf nutrients (including P) of the various legumes were lower or the same as those of the non-legumes within their specific sites (Tables 3.8 to 3.11), irrespective of the difference in nutrient levels of the soils at the site (Table 3.2). Similarly, there were no clear distinctions between the legumes and non-legumes based on their SLA. Of the 13 legume species sampled at the eight sites, only four species had higher SLA values than the non-legume species, one species each at Elim (*Aspalathus sp.*), Hopfield (*W. obcordata*), Heidehof (*O. bractealatum*) and Botlierskop (*A. submissa*) whilst the SLA values of the other legume species were similar or lower than that of some of the non-legumes (Tables 3.8 to 3.11). Surprisingly, the SLA of legume plants in the identified nutrient rich sites of Cederberg and De Hoop (Table 3.3) were lower than some of the non-legume plants growing on the lower nutrient patch at the site. This observation does not agree with the reports in literature which showed that plants growing in nutrient rich soils have higher SLA's than those growing in nutrient poor soils (Dijkstra and Lambers, 1989; Poorter and De Jong, 1999; Wilson, *et al.*, 1999; Shaver, *et al.*, 2001; Wright *et al.*, 2001).

These observations indicate that neither the leaf nutrient levels, nor the SLA, necessarily mirror the nutrient richness of the soils in which they grow. As pointed out in the discussion of Chapter Two, the CFR species may not be responsive to high levels

of nutrient supply (Chapin, 1980; Aerts, 1999; Hawkins *et al.*, 2005). The most likely explanation could be that the plant resources are optimized through homeostatic mechanisms in such a way that all resources limit growth simultaneously (Bloom, *et al.*, 1985). This direct control is known as multiple resource limitation (MRL) (Cleland and Harpole, 2010). Both Neff, *et al.*, (2006) and Castle and Neff, (2009) found that plants did not vary leaf nutrient levels according to soil nutrient levels, and that dominant species had a remarkable strong capacity to maintain foliar chemistry ratios despite large underlying differences in soil nutrient availability (Ordoñez, *et al.*, 2009). In addition, Craven, *et al.* (2007) and White and Broadly, (2009) indicated that tissue concentrations of mineral elements can show great differences between plant species growing in the same environment. The study by Fisher, *et al* (2006) also found that in a western Australian *Banksia* community, some local plant species, but not the *Banksia*, reflected an increased leaf P concentration with increased soil P of invaded sites, but other leaf nutrients such as N, K, Na, Ca, S, Fe, Zn, Cu, Mn and Mg were not altered. The choice of plant organ sampled could also confound the results, because even though Mediterranean woody evergreen trees tend to store nutrients in their leaves (Massei, *et al.*, 2000), there are some plants that store nutrient in parts other than the leaves. For example *Banksia ericifolia* tend to store P in their stems, especially at elevated P supply (Parks, *et al.*, 2000). It is also worth noting that leaf nutrient concentrations might show yearly or seasonally temporal variations (Ramírez-Orduña, *et al.*, 2005; Chapin, *et al.*, 1980). In line with the above, the SLA of fynbos species may not reflect the nutrient richness of the soil they grow in, because of this capability of plants to regulate tissue concentration of nutrients independent of soil nutrients, especially under low nutrient conditions. Also, plants that are adapted to low nutrient environments tend to accumulate more quantitative secondary compounds, as found by Bell (2005) who grew 3 slow growing perennial legumes, *Medicago sativa*, *Dorycnium rectum* and *D. hirsutum* under controlled conditions, with a resultant decrease in SLA (Lambers and Poorter, 1992). Thus, there is evidence of species-specific nutrient use traits and responses, rather than flexible nutrient use (Ordoñez, *et al.*, 2009)

Several authors have found that that N and P uptake are related and have synergistic effects on plant growth (Shaver and Melillo, 1984; Lajtha and Klein, 1988; Elser *et al.*, 2007; Zhang *et al.*, 2007). For example in an experiment by Akamine *et al.*, (2007) N application to *Curcuma longa* enhanced the effect of P on the yield of turmeric. This

relationship between N and P also means that any increases in either nutrient will induce a limitation by the other (Shaver and Melillo, 1984; Lajtha and Klein, 1988; Elser *et al.*, 2007). The optimum N:P ratio for photosynthesis, as determined by Güsewell (2004) is from 10 to 20 and plants tend to accumulate these two nutrients in such a way that their internal N:P ratios are within this value (Lajtha and Klein, 1988). This implies that N:P ratio outside 10 and 20 is an imbalanced ratio associated with either N or P limitation. Therefore, using the Güsewell (2004) model, 77% of the legumes, and 42% of the non-legumes sampled at the eight sites showed P limitation from the growth environment, whereas only 15% of the non-legumes and none of the legumes showed N limitation. Thus, soil P availability and/or uptake might be limiting for plant growth for both legumes and non-legumes in the CFR, while only non-legumes may be N limitation. These results are in contrast to the results of the glass house experiment, which indicated that some legume species responded to a higher N supply. This phenomenon can be explained in that other reports have shown that variation in N:P value in tissue of plants is a species specific trait (Stock and Verboom, 2012; Güsewell and Koerselman, 2002; Neff, *et al.*, 2006; Townsend, *et al.*, 2007), with limited or no relationship with soil N or P. However, inferred N and P limitation of plants as measured by the leaf N:P ratio is presently widely used (Vitousek, *et al.*, 2010; Han, *et al.*, 2005; Hättenschwiler *et al.*, 2008; Johnson, 2009). Take note that this method of determining possible N and/or P limitation is only robust when carried out within a defined group of plants and ecosystems (Vitousek, *et al.*, 2010) and when only N and/or P limit plant growth (Koerselman and Meuleman, 1996). Thus, this technique may be inappropriate when applied across plant species with wide differences in phylogeny, when plant species have an underlying difference in their stoichiometry, or where there is a possibility of other nutrients interactively co-limiting growth along with N and P. (Drenovsky and Richards, 2004; Neff, *et al.*, 2006; Craine, *et al.*, 2008; Cleland and Harpole, 2010; Vitousek, *et al.*, 2010; Stock and Verboom, 2012). Thus the use of foliar N:P ratios to determine N and P limitation has to be used with caution.

## **Conclusions and recommendations**

Soil from only two of the eight legume sites were more nutrient rich than the adjacent fynbos soil, therefore I conclude that fynbos legumes rarely enrich the soil in which they grow. Except for leaf N, there seems to be no differences in leaf nutrient concentrations and SLA between legume and non-legume species. Variations in soil

nutrient concentrations do not necessarily translate into corresponding variations in leaf nutrient concentrations in the CFR and I agree with Neff *et al.*, (2006) in that differences in foliar chemistry may represent differences in plant adaptations to particular environments, rather than being an indication of underlying differences in soil nutrient levels.

The CFR is a biodiversity hotspot (Frazee, *et al.*, 2003) and extremely rich in endemic plant species (6200) (Goldblatt and Manning, 2002). Soil fertility is reported to be one of the key drivers in maintaining and generating biodiversity in the CFR (Rouget, *et al.*, 2003; Van der Heijden, *et al.*, 2008). Thus, most of the CFR legumes may have evolved with traits that lack the ability to alter the nutritional status of the soil, and therefore the biodiversity of the region is maintained. The presence of legumes that increases soil fertility poses the danger of changing the plant community composition (Van der Heijden *et al.* 2006) and, ultimately may lead to reduced plant diversity due to soil fertility enrichment (Tilman, 1986).

Research into factors, other than soil nutrient availability, should be undertaken to possibly explain the occurrence of isolated legume stands within the Cape Floristic Region, such as the presence of compatible rhizobia, seed dispersal mechanisms and distances, disturbance, proximity to water, heterogeneous deposition of ash, pollinators and the scarification of legume seeds by heat, leading to rapid post fire germination after fires and effective soil depth (Haridasan, 2008).

## CHAPTER 4

### General Discussion and Synthesis

The world is finite, so biological growth is ultimately limited by resources, with plant growth often constrained by the availability of essential nutrients, as well as other factors such as water, environmental stresses, predators, pathogens, and space (Harpole, *et al.*, 2007). In terrestrial environments, primary production is often limited by N and/or P (Elser, *et al.*, 2007; Vitousek *et al.*, 2010). With regards to Mediterranean ecosystems, there are several studies showing that these ecosystems are N, P or N and P co-limited (McMaster, *et al.*, 1982; Groves, *et al.*, 1986; Allen Padgett, 1999; Sardans, *et al.*, 2004; Diaz, *et al.*, 2012). In contrast, other studies showed a tendency of no pattern or trend of plant growth limitation by N, P or N and P co-limitation in the fynbos vegetation. For example, according to Witkowski, (1989a), the sand-plain lowland fynbos seems to be limited by N, as well as by one or more other nutrients which was not specified, but not P. Another factorial designed *in situ* fertilizer experiment, also by Witkowski, (1989b) indicated that none of nine growth forms were P limited, and only three growth forms responded to N supply: one positively and the other two growth forms negatively. The third factorial experiment by Witkowski, *et al.*, (1990) found that *Leucospermum parile* also had neither N x P interactions nor multiple main effects, but that *Phyllisia cephalantha* showed a synergistic N x P interaction. Data is available from a field experiment by Lamb and Klausner (1988) that indicated P limitation and possible toxicity from N supply to two fynbos species *Protea repens* and *Erica plukenetii*, together with a synergistic N x P interaction in *Erica plukenetii*, and an antagonistic interaction in *Protea repens*. The results of the glass house experiment also showed no N x P interactions. These observations seem to support the view that CFR plants are adapted to growing in low productive habitats, and thus they have a low relative growth rate and would show limited response to nutrient enrichment. However, the glasshouse data did not support the view that the CFR plants are multiple resource limited because of lack of significant interactions between N and P supply and the absence of main effects of N and P (Harpole, *et al.*, 2007; Raich, *et al.*, 1996). It is worth noting that, the number of nutritional studies on N and P interaction within the fynbos ecosystem are insufficient to establish a response pattern and to ascertain with some degree of certainty whether fynbos plants are multiple resource limited or not.

Fynbos plants could negate the effect of nutrient poor soils on the growth of plants by varying their transpiration rate, allocating resources to roots rather than to shoots, and forming cluster roots or mycorrhizal-roots in order to enhance nutrient uptake. However, increasing transpiration rate (and thus water mass flow) as a adaptation for enhancing nutrient uptake is a contentious issue, as there are arguments for; where available N and P had an influence on the transpiration rate of plants (Guehl, *et al.*, 1995; Gunga, *et al.*, 2003; Da Silva, *et al.*, 2003; Graciano, *et al.*, 2005; Ludwig *et al.*, 2006; Scholz, *et al.*, 2006; Cabrera-Bosquet, *et al.*, 2007; Cernusak, *et al.*, 2007; Cramer, *et al.*, 2008; Garrish, *et al.*, 2010) and arguments against; where the transpiration rate was not influenced by manipulating available N and P (Bradbury and Malcolm, 1977; Guehl and Garbaye, 1990; Korol *et al.*, 1999); Loustau, *et al.*, 1999; Li, *et al.*, 2001; Graciano, *et al.*, 2005; Howard and Donovan, 2007; Table 2.5 of Chapter Two).

The reallocation of biomass or resources towards organs associated with enhanced acquisition of a limiting resource is consistent with the optimal partitioning theory, where in the case of nutrient limitation, more resources would be channelled to the roots (Gleeson and Good, 2003; Hermans, *et al.*, 2006). The inverse to this should also hold true, i.e. if the nutrient supply is adequate, then resources will be withheld from the root and be allocated to the shoot. This would then lead to a decrease in the root to shoot ratio, as shown by the lower root to shoot ratio of both *P. repens* and *L. cordifolium* at the higher levels of N supply (Table 2.3). With regard to legumes, nitrogen fixation could make biomass allocation to roots independent of soil nitrogen supply (Markham and Zekveld, 2007), so a change in the root to shoot ratio does not necessarily follow a change in soil N supply (Table 2.2). Nodulation, thus BNF, (Döbereiner, 1978; Dixon and Wheeler, 1986; Sprent, 1999) and root colonization by mycorrhiza (Mosse and Philips, 1971; Marsner, *et al.*, 1996) of the roots of legumes, as well as the development of cluster roots (of Proteaceae, Cyperaceae and Restionaceae) (Skene, 1998; Sylvia and Neal, 1990; Vance *et al.*, 2003; Hawkins, 2005; Neumann and Martinoia, 2002) are sensitive to the levels of N and/or P in the soil, i.e. generally decreasing with increasing levels of soil N and P (same references as above). In line with the above, the biomass of the nodules of all three legumes and that of the cluster roots of all three non-legumes of the glass house study (Tables 2.3 and 2.4) was significantly ( $P < 0.05$ ) lower at the  $150 \text{ mg kg}^{-1}$  N supply than at the  $20 \text{ mg kg}^{-1}$ . There was, however, a very limited response in these root traits, as well as mycorrhizal

colonization, to the higher ( $31 \text{ mg P kg}^{-1}$ ), levels of P supply, indicating that for this study, N was the most limiting resource for growth.

Legume leaves have a high concentration of N. This is often significantly higher than the concentration of leaf N found in non-legumes (Sprent, *et al.*, 1996; Seneviratne, 2000; Palm, *et al.*, 2001; Spehn, *et al.*, 2002; Teklay, 2004; Tables 3.8 to 3.11). The concentration of leaf P of the legumes and non-legumes does not seem to be significantly different (Ramírez-Orduña, *et al.*, 2005; He, *et al.*, 2008; Tables 3.8 to 3.11), and in some reports, legumes have been found to have a lower P concentration than that of non-legumes (Palm, *et al.*, 2001; Teklay, 2004). With regards to the other macronutrients (K, Ca, Mg and Na) in leaves, there seems to be no clear pattern of differences between these nutrients in legumes and non-legumes (Ramírez-Orduña, *et al.*, 2005; Tables 3.8 to 3.11). Creating fertile islands within a landscape requires litter with high levels of nutrients especially N, (Binkley and Giardina, 1998), time (Binkley and Giardina, 1998; Marchante, *et al.*, 2008) and an ideal environment of soil moistness, correct pH and suitable temperatures for soil organisms (Stark and Firestone 1995; Barros, *et al.*, 1995; Anderson and Nilsson 2000; White *et al.*, 2005; Weixing, *et al.*, 2009; Fernández-Calviño *et al.*, 2011). A limitation of one or several of these factors at a site would inhibit the formation of these fertile islands, as was the case in the six sites of this thesis that did not manifest with enhanced soil nutrient concentration in their legume populations. Therefore the hypothesis “Soil nutrients in the legume populations are higher than those from adjacent non-legumes on a specific landscape” was weakly supported. As mentioned in Chapter Three, most of the CFR legumes might have evolved with traits that lack the ability to alter the nutritional status of the soil and therefore the biodiversity of the region is maintained. The presence of legumes that increases soil fertility poses the danger of changing the plant community composition (Van der Heijden *et al.* 2006) and, ultimately may lead to reduced plant diversity due to soil fertility enrichment (Tilman, 1986). This is particularly true of the invasive *Acacia cyclops* and *A. saligna*, which have led, and still leads, to community changes due to, among other factors, altering the soil properties of the fynbos ecosystem (Stock, *et al.*, 1995; Yelenik, *et al.*, 2004; Fornara, 2005; Yelenik *et al.*, 2007).

## Conclusion

According to the results of this study, the fynbos species' growth limitations by N and/or P varied with functional groups and species, with no NxP interactions. The lack of pattern of the results of the NxP factorial experiments involving fynbos plant species in the field and glasshouse investigations is in part due to species adaptation to infertile CFR soils, contrary to many legumes in other ecosystems, indigenous legume stands within the fynbos rarely create their own nutrient rich niches. Except for N, there was no discernable pattern in the differences between the macro-nutrients concentrations or SLA of legumes and non-legumes, irrespective of sites and different soil types. The relatively high N concentration in the legume leaves compared to that of the non-legumes, was probably due to either nitrogen fixation or due to N enriched leaves being a phylogenetic property of Leguminoceae.

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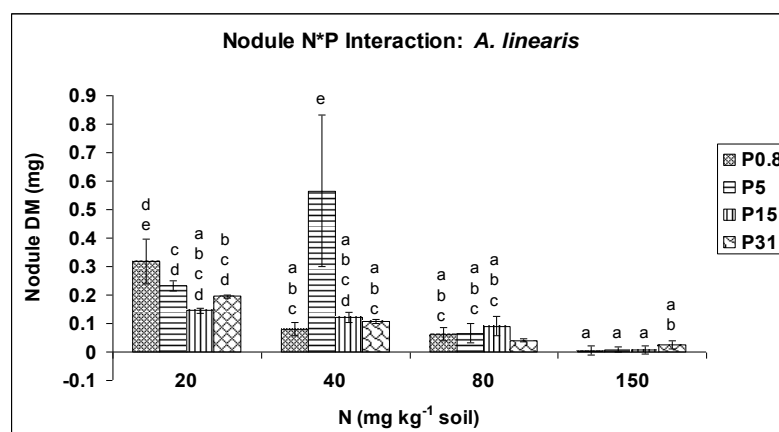
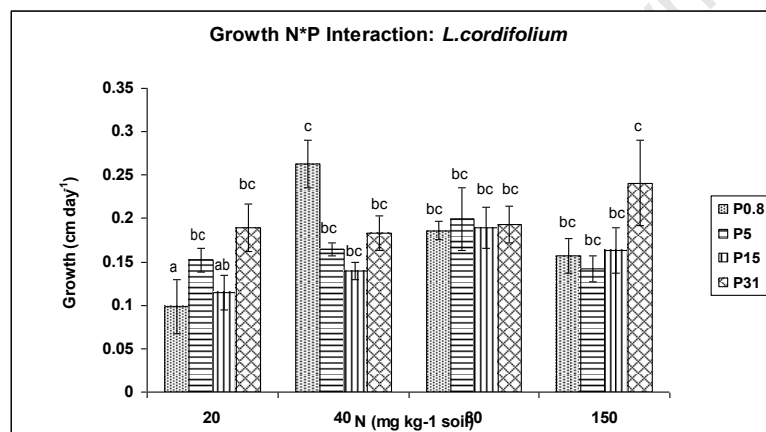
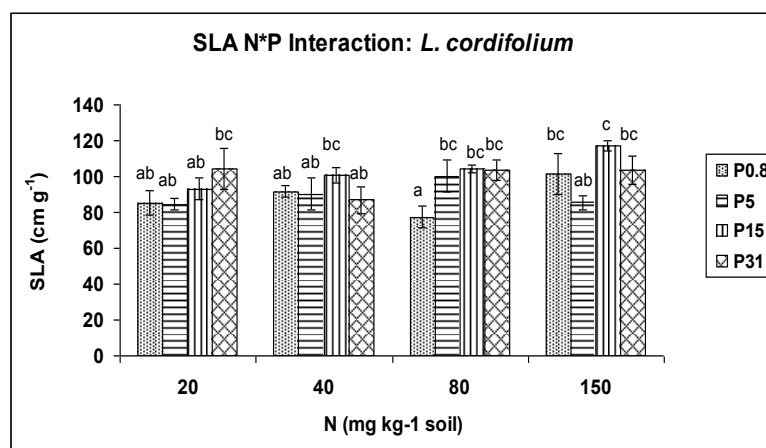
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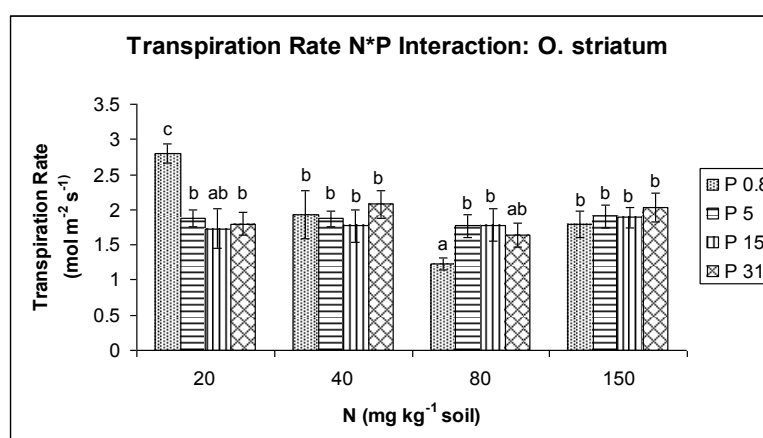
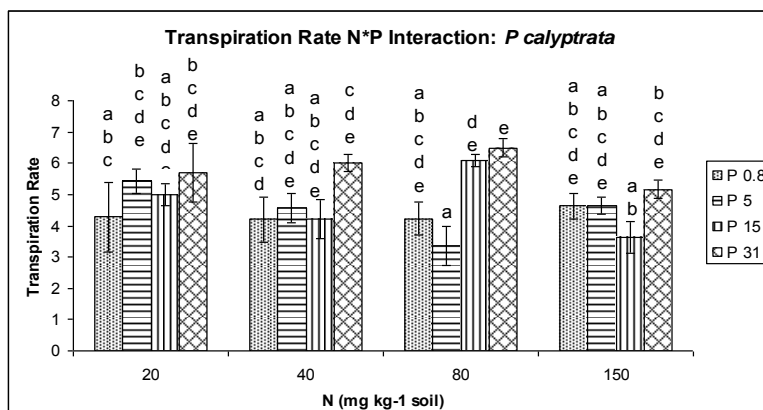
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## Appendix 1



**Appendix 1** Interactions of the four levels of N and P on the SLA and growth rate of *L. cordifolium* and the nodule DM of *A. linearis* at harvesting. Different letters above the columns indicate significant differences (p < 0.05) between means. Vertical lines indicate ± S.E.

## Appendix 2



**Appendix 2** Interactions of the four levels of N and P on the transpiration rate of *P. calyptрата* (a) and *O. striatum* at harvesting. Different letters above the columns indicate significant differences ( $p < 0.05$ ) between means. Vertical lines indicate  $\pm$  S.E.

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