Resource use differentiation as a mechanism facilitating coexistence of plants with similar growth forms

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Abstract

Differential resource use as a possible mechanism of coexistence was investigated for nine fynbos shrub species with similar growth forms. Stands comprising a different combination of coexisting proteoid species were studied at three sites on the Cape Peninsula. Additional data were provided for seven proteoid species at Cape Agulhas, of which only two species were coexisting. A new approach involving stable isotopes of C and N was used to determine species differences in water use and source of nitrogen, respectively. $\delta^{13}C$ and $\delta^{15}N$ values were plotted together on a two-way scatter plot to determine the degree of niche separation. Support for the concept of resource differentiation varied between sites. At the Silvermine site, 2 of the 3 species separated from each other along both axes. At the Table Mountain site, one species displayed a difference in water use but the other three species showed no niche separation. At the Constantiaberg site, two species showed no niche differentiation when coexisting, but separated along the $\delta^{13}C$ axis when growing in monospecific stands. At the Cape Agulhas site, the coexisting species showed no niche separation. Other coexistence mechanisms are discussed for each site. Differential resource use is not suitable as a unitary explanation of coexistence, but should be considered in conjunction with other mechanisms.

Introduction

Within the field of community ecology, the mechanisms which enable plants to coexist have received much attention and have been the focus of considerable debate. Theories of coexistence originated in the field of animal ecology (e.g. Gause 1934; Sale 1977) and the extension of these principles into plant ecology has resulted in the refinement of old theories and the development of new ones.

Coexistence theory has developed along two lines of contradictory thinking. Classical theory predicts that the most similar species cannot coexist unless every species in a community occupies a distinct niche (Grubb 1977; Newman 1982; Cody 1986; Tilman 1986) but recently, a number of other theories have arisen which predict that the most similar species are the most likely to coexist (Shmida and Ellner 1984; Warner and Chesson 1985; Hubbell and Foster 1986).

A central tenet of classical theory is the concept of niche differentiation which holds that if different species are to coexist, each must occupy a distinct niche. This niche is defined relative to limiting resources (e.g. nutrients, light, space), to which species respond differentially (Cowling *et al.* 1994). Differential use of mineral nutrients and water is frequently invoked to explain coexistence of non-similar species.

- **Coexistence of similar species**

Shmida and Ellner (1984) have derived several mechanisms for coexistence in which plant species are considered to be 'trophically equivalent' (in terms of their relations to habitats, resources and exploiters). They show, by means of a model, that spatial heterogeneity incorporating differences in life history, seed dispersal and environmental variation is sufficient to ensure coexistence of species which do not differ in their trophic requirements. Other scientists have challenged the view that species must differ in their ecologies and argue that the relationship between niche overlap and competitive interactions between species, on which their coexistence depends, is complex and that apparent niche differences may be misinterpretations (Silvertown and Law 1987; Chesson 1991). Consequently, a wide variety of alternatives to the concept of niche differentiation have been proposed. Reproductive traits play a large role in these models of trophic equivalence: Sale (1977) developed the concept of the 'lottery model' for fish on coral reefs whereby recruits compete for space which is randomly allocated to propagules of different species, and this concept has been adopted by many plant ecologists (e.g. Grubb 1977; Shmida and Ellner 1984; Warner and Chesson 1985; Fagerstrom 1988). Hubbell and Foster (1986) present a coexistence model which assumes that species are ecologically identical to, but reproductively isolated from, each other, and that recruitment success is determined by chance. Chesson (1986) proposed that environmental variability in any system slows the trends to competitive exclusion, thereby promoting coexistence.

• Coexistence of non-similar species

Much time has been spent searching for new niche axes but the suggested importance of resource use as a niche axis has rarely been tested empirically. Plants require essentially the same basic resources (light, water, carbon dioxide, mineral nutrients) and it has been argued that there is limited potential for specialisation along this axis (e.g. Connell 1978; Davis 1991). Tests of the niche differentiation hypothesis involve studies of whether species overlap in their resource requirements or mode of resource acquisition and use. It has been proposed that variation in aspects of morphology associated with nutrient use (e.g. root depth, leaf area) provide indirect measures of niche differentiation (Cody 1986), but these measures may reflect a resource for which the species are not actually competing (Connell 1978). Direct measures, such as in this study, offer more robust interpretations of resource use, as does the selection of resources known to be limiting in a given ecosystem.

The fynbos biome is characterised by nutrient-poor soils and dry summers and it is a commonly held view that competition plays an important role in structuring communities. Classical theory is based on the competitive exclusion principle (Gause 1934) which states that where two species exist in the same environment, competition will result in the eventual elimination of one of the species. It is logical to hypothesise that coexisting species must avoid or reduce the influence of this competition and in order to do this, each species should occupy a distinct niche (Newman 1982). Cody (1986) argues that differences in plant growth form reflect different strategies of resource utilisation and that plants with similar morphologies should be segregated by habitat or display resource use differentiation. Cody highlighted the need to identify axes along which related species segregate in order to determine whether resource partitioning exists and thereby gain a better understanding of coexistence. As a test of Cody's hypotheses, this study deals with species from the same family (Proteaceae), all of which have essentially the same growth form.

Previous work in fynbos

Previous studies of coexistence on a basis of resource use have yielded varying results. It has been demonstrated that species differ in their use of nitrogen resources (Gray and Schlesinger 1983; Pate *et al.* 1993; Stewart *et al.* 1993) and in their water relations (Poole and Miller 1975; Wieland and Bazzaz 1975; Thomas and Davis 1989; Richards *et al.* 1995a). *Banksia* species in SW Australia were shown to differ in their ability to reduce nitrate and it has been suggested that the preference of some species for nitrate over ammonium reduces competition between these species and those which preferentially use ammonium (Pate *et al.* 1993; Stewart *et al.* 1993). More emphasis has been placed on attempts to show how differences in the water relations of species can facilitate their coexistence, especially in Mediterranean-type ecosystems where water is often seasonally limiting. Davis and Mooney (1986) examined the seasonal water use patterns of four co-occurring chaparral shrubs and proposed that root stratification promotes sharing of soil moisture resources and in this way creates a form of below-ground niche separation. Their results are consistent with those of Poole and Miller (1975) who reported differences in water relations in mixed stands of chaparral shrubs with different rooting depths. Deep rooted plants, which have access to water in the dry summer, fix most of their carbon in summer, whereas shallow rooted species are more active in winter (Davis and Mooney 1986). In contrast to these studies, in an investigation of three co-dominant *Banksia* shrub species in SW Australia, Lamont and Bergl (1991) did not find sufficient differences in water relations, architecture or vegetative phenology to explain coexistence.

The Cape Floral Kingdom displays an extraordinarily high diversity of plant species, and associated with this is a high degree of coexistence. Plant communities show strong edaphic specificity (Mustart and Cowling 1993; Richards *et al.* 1995b, 1997) and the edaphic heterogeneity of the region plays a large role in promoting this diversity (Cowling 1987). In light of the relationship between soil type and vegetation composition, Richards *et al.* (1997) state that differences between species in resource use suggests that complex differences in soil nutrient availability are important in determining the spatial distribution of species in nutrient-poor habitats, and can thus be implicated in studies of coexistence. Despite the high diversity in fynbos, coexistence among species from the same guild are relatively rare. In particular, Midgley and Watson (1992) and Mustart and Cowling (1993) have stated that mixed stands of truly coexisting members of the Proteaceae are uncommon. Members of this family show essentially the same morphology and this makes the mechanism of their coexistence particularly interesting.

This study aims to identify resource differentiation as a niche axis facilitating coexistence in species with the same growth form. Both nitrogen and water are considered to be limiting resources in fynbos and it is expected that differential use of these resources has the effect of reducing interspecific competition to the extent that similar species are able to coexist.

A new technique involving the use of stable light isotopes was developed to determine whether coexisting species use resources in a different way. The $\delta^{15}N$ natural abundance method has previously been used to distinguish between N-fixers and non-N-fixers (Shearer and Kohl 1989; Stock *et al.* 1995) as well as determining the ectomycorrhizal habit of trees in a miombo woodland in Tanzania (Högberg 1990). In this study, the principle behind the use of nitrogen isotopes is that the $\delta^{15}N$ value of a plant reflects the $\delta^{15}N$ value of its nitrogen source and in this way indicates any differential resource use between species. The $^{13}C/^{12}C$ ratio has been shown to be linearly related to water use efficiency and use has been made of stable carbon isotopes in studies of water use strategies in a variety of habitats (e.g. Ehleringer 1993; le Roux *et al.* 1996). In this study, in which the effects of variable habitat conditions were minimised, differences in water use efficiency between species imply genetically programmed differences in water use strategies. An advantage of the isotope technique is that environmental and plant physiological conditions are integrated over a long period and therefore the influence of daily or seasonal variation is eliminated. The use of stable light isotopes enables easy measurement of two resource variables and is a potentially superior method over studying the use of only one resource in isolation.

Methods

• Study sites and species

The study was conducted at 3 sites on the Cape Peninsula, namely Table Mountain, Constantiaberg and Silvermine (see Figure 1). This region, at the south-western tip of the fynbos biome, experiences a Mediterranean-type climate and summers are typically hot and dry while winters are cool and wet. Each site consisted of a mixed stand of two or more proteoids in which the species intermingled completely, forming truly coexisting stands in which no conspecific clumping was evident (see Plates 1 and 2). At each site, the proteoids were the dominant species, although the density of proteoids varied between sites. The stands were not very extensive and it is assumed that soil conditions were relatively constant throughout the stand. Within each stand, the influence of environmental variability, such as moisture content of the soil, was kept to a minimum by collecting samples along the contour where possible, rather than down a slope.

Constantiaberg

This site was on a north-east facing slope below Constantiaberg Peak (34° 02' 13" S, 18° 23' 32" E), at an altitude of 440 m. Two *Protea* species, *Protea coronata* Lam. and *Protea lepidocarpodendron* (L.) L., were present at this site, in both monospecific and mixed stands. The *P. coronata* stand was higher up the slope than the *P. lepidocarpodendron* stand and between the two stands was a broad interface where both species coexisted. Leaf samples were taken from each of the monospecifc stands as well as from the mixed stand. Both species are tall, erect shrubs 2-3 m in height and often form dense, almost inpenetrable stands (Rourke 1980). They both grow best in damp, sheltered areas and have a preference for heavy, clay soils (Rourke 1980). Both species flower in late autumn-winter (Rourke 1980).

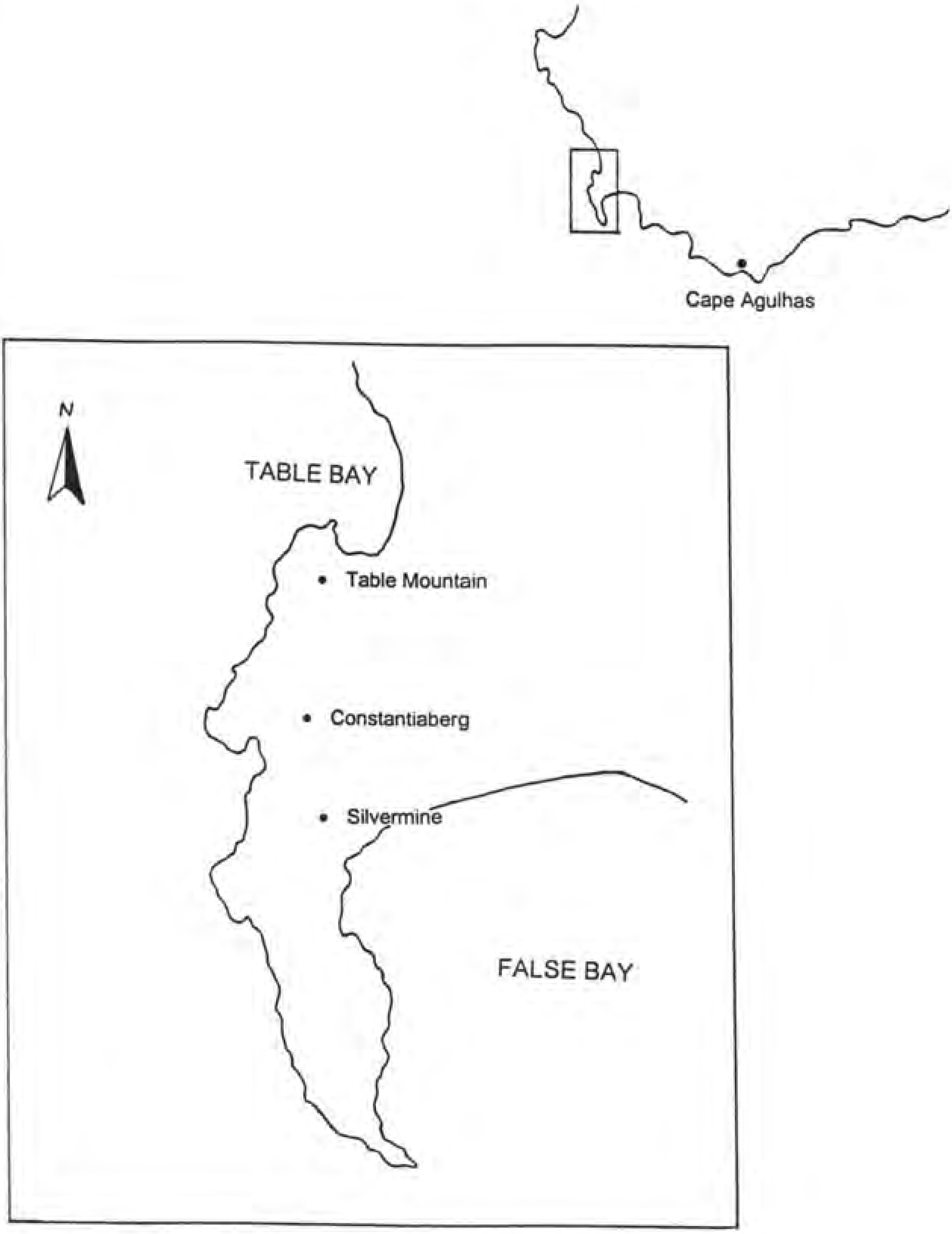

Figure 1. Map of the Cape Peninsula showing the location of the three study sites at which stands of coexisting proteoids occur. The location of the Cape Agulhas site, from which preliminary data were obtained, is also shown.

Table Mountain

This site consisted of a dense stand of four coexisting *Protea* species, growing on the northern slope of Table Mountain near the lower cable station (33° 56' 10" S, 18° 24' 09" E), at an altitude of 260 m (see Plate 1). Leaves were collected from *Protea compacta* R. Br., *Protea neriifolia* R. Br. and *Protea burchellii* Stapf. which were completely intermingled, and from *Protea repens* (L.) L. which occurred lower down the slope. Although *P. repens* was collected from a slightly different locality to the other species, this is still considered to be one stand as the other species were present with *P. repens*, albeit at a lower density. Despite the fact that *P. repens* is the only species to occur naturally on Table Mountain (Rourke 1980), this planted stand has persisted for more than 20 years (W.D. Stock, pers. comm.). All four species flower in winter, although the length of flowering time varies between species.

Silvermine

Three species from different proteoid genera were sampled at this site: *Leucospermum conocarpodendron* (L.) H. Beuk, *Leucodendron laureolum* (Lam.) Fourc. and *Mimetes fimbriifolius* Salisb. ex Knight (see Plate 2). They occurred on a western slope at the top of Silvermine Mountain (34° 05' 34" S, 18° 25' 19" E) at an altitude of 260 m. This area has been unburnt for many years and the stand consisted of very large individuals at a lower density than stands at the other sites. Leucadendrons are dioecious plants and the sex of the individual was noted as it was sampled. Both *L. conocarpodendron* and *M. fimbriifolius* flower in the spring and summer months, whereas *L. laureolum* flowers in June (Rebelo 1995).

Plate 1. Stand of four *Protea* species coexisting at the Table Mountain site, indicating the dense nature of the stand. A: *P. compacta*, B: *P. burchellii*, C: *P. repens*, D: *P. neriifolia.*

Plate 2. Two proteoid species of similar growth form coexisting at the Silvermine site. A: *Mimetes fimbriifolius*, B: *Leucospermum conocarpodendron*.

Cape Agulhas

Additional data were obtained from a preliminary study done by W.D. Stock, on a number of different proteoids occurring at two sites near Cape Agulhas. Although not all the species were coexisting at these two sites, this was the pilot study in which the new method of isotopic analysis was tested, and these data provide additional information and support for the validity of the method. At the first site, leaf samples were taken from *Protea obtusifolia* H. Beuk ex Meisn., growing on the limestone soils, and *Protea susannae* E. Phillips and *Protea compacta*, growing in separate stands on the acid soils. At the second site, four *Leucadendron* species were sampled: *Leucadendron meridianum* I. Williams from the limestone soils, *Leucadendron coniferum* (L.) Meisn. from soils with an intermediate pH, and *L. laureolum* and *Leucadendron xanthoconus* (Kuntze) K. Schum. which were coexisting on acid sands. This preliminary study relates directly to the work done by Richards *et al.* (1995a, 1995b, 1997) on fynbos vegetation-environment relationships in the Cape Agulhas area.

- **Sampling methods**

At each site, five individuals of each species were sampled. In the case of the Constantiaberg site five individuals from each species in each stand were sampled). Care was taken not to sample on the edge of a stand and to ensure that species were truly coexisting, heterospecific nearest-neighbours were selected wherever possible. Between 5-8 leaves were removed from each individual, always from the same aspect and the same level of the plant but from different branches, to minimise micro-site differences. The most recent fully mature leaves were sampled in preference to old leaves.

The leaves were dried in an oven at 70°C for 48 hours and then ground to pass a size 40 mesh to obtain a homogenous sample for isotopic analysis. Care was taken to clean the grinder thoroughly between samples as isotopic analysis requires such small amounts of sample that even a little contamination can influence results greatly.

- **Isotopic analyses**

Biochemical and physical processes occuring during photosynthesis have different tendencies to discriminate between ^{12}C and ^{13}C (Berry 1988). Both carbon dioxide entering the plant and water leaving the plant do so through stomata, resulting in a trade-off between fixing carbon for growth and the risk of desiccation (Farquhar *et al.* 1988). Species differ in their water use efficiency (WUE), defined as the ratio of dry matter produced to water used (Farquhar *et al.* 1988) and it has been demonstrated that this WUE is linearly related to $\delta^{13}C$. Plants in which the stomata are open discriminate more against $\delta^{13}C$ and therefore have a lower $\delta^{13}C$ value which indicates a less water use efficient life-style. Species differing in their WUEs indicate that they are utilising the available water resource differently and in this way, WUE is considered a potential niche axis for resource differentiation.

The ^{15}N natural abundance technique can be used to determine whether species differ in their source of nitrogen or in the way they assimilate available nitrogen (Handley and Raven 1992). All proteoids are non-nitrogen-fixers (Rebelo 1995) and no species exhibit any known mycorrhizal associations (Allsopp and Stock 1993). Consequently, the $\delta^{15}N$ value for each plant indicates the form in which nitrogen is taken up by the plant or the depth at which it is acquired from the soil (Handley and Raven 1992). There is evidence that soil ^{15}N enrichment increases with vertical depth in the profile (Shearer and Kohl 1989; Gebauer and Schulze, cited by Handley and Raven 1992). Different $\delta^{15}N$ values for different species could reflect their preference for different forms of nitrogen, i.e. nitrate or ammonium, and although higher $\delta^{15}N$ values indicate the uptake of ammonium rather than nitrate (Handley and Raven 1992), without a known reference plant it is not possible to state confidently that species with a low $\delta^{15}N$ value are using nitrate in preference to ammonium. However, it is clear that different $\delta^{15}N$ values reflect a different source of nitrogen and this can provide another niche axis.

Leaf samples in the range of 2.5-3.0 mg were analysed in the mass spectrometer (Finnegan MAT 252; Bremen, Germany) to obtain the $^{15}N/^{14}N$ ratio which is expressed relative to the isotopic composition of atmospheric nitrogen. A second run with leaf samples of 0.05-0.08 mg yielded the $^{13}C/^{12}C$ ratio which is expressed relative to the isotopic composition of the PDB (limestone of the Pee Dee Belemnite formation) standard. Deviations from the standards are written as $\delta^{13}C$ and $\delta^{15}N$ respectively and these values are expessed in per mil (‰).

The isotope values of the species at each site were plotted on a two-way scatter plot with $\delta^{13}C$ on the y-axis and $\delta^{15}N$ on the x-axis. In this way, replicates from each species group together and patterns of differential resource use are determined by the degree of separation of these groups along one or both axes. As patterns were readily apparent statistical analyses were not performed. In order to make the degree of niche overlap more distinct, boundaries were drawn around the replicates of those species which show separation from any other species at each site.

Results

From the preliminary study there is evidence for differentiation of resource use along both axes. *P. compacta, P. obtusifolia* and *P. susannae* all separate out along both axes (Figure 2). The limestone species, *P. obtusifolia*, has a lower δ^{15}N value than the other two species, which grow on acid soils. The lower δ^{13}C value for *P. obtusifolia* indicates that it is less water efficient than the other two species.

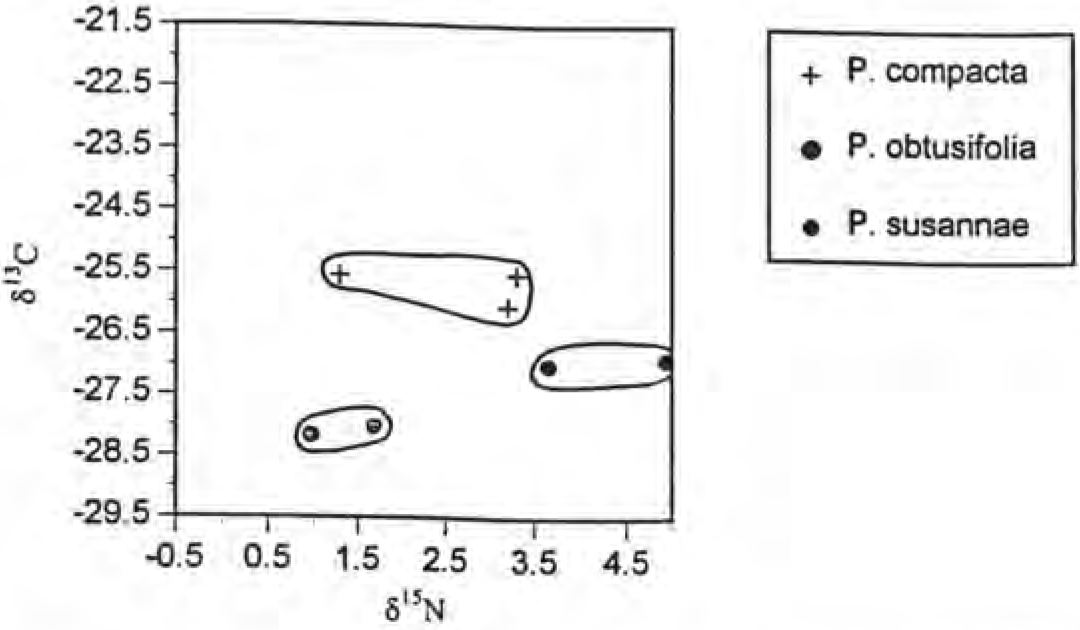

Figure 2. Two-way scatter plot illustrating the δ^{13}C and δ^{15}N values of leaves from three *Protea* species collected near Cape Agulhas. *P. obtusifolia* grows on limestone soils, *P. compacta* and *P. susannae* grow on acid soils. Boundaries encircle the replicates of each species considered to display resource use differentiation.

Results from the other Cape Agulhas site are not as clear-cut but similar patterns are evident (Figure 3). It appears that *L. meridianum*, on the limestone soils, is more water use efficient and utilises a different source of nitrogen. *L. laureolum* and *L. xanthoconus*, which coexist on the acid soils, have a slightly higher δ^{15}N value than *L. meridianum* and a similar δ^{13}C value, although the result suggest that *L. xanthoconus* is less water efficient than the other two species. *L. coniferum*, on soils of an intermediate pH, has the highest δ^{15}N values but does not differ from the other species in terms of water use efficiency.

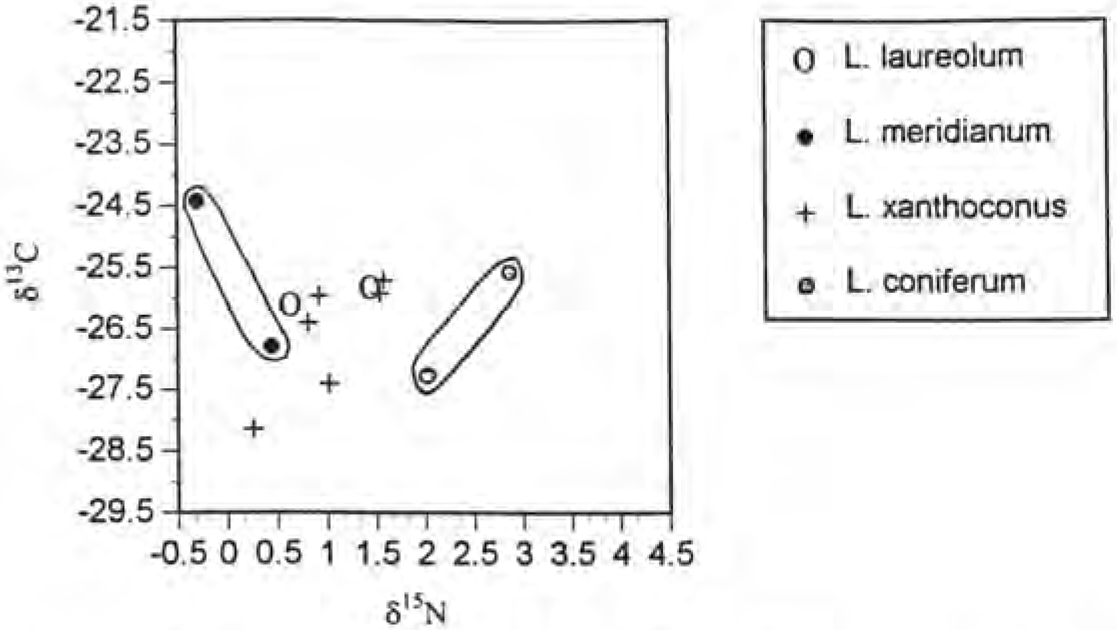

Figure 3. Two-way scatter plot illustrating the $\delta^{13}C$ and $\delta^{15}N$ values of leaves from four *Leucadendron* species collected near Cape Agulhas. *L. meridianum* grows on limestone soils, *L. laureolum* and *L. xanthoconus* coexist on acid soils, and *L. coniferum* grows on soils of intermediate pH. Boundaries encircle the replicates of each species considered to display resource use differentiation.

From the true coexistence sites on the Cape Peninsula, a certain degree of separation along each axis is apparent, although evidence for niche differentiation varies between sites. At each site, clumping of the samples into relatively distinct groups occurred, indicating that environmental conditions were constant within each site.

In the monospecific stands at the Constantiaberg site (Figure 4), *P. coronata* and *P. lepidocarpodendron* share the same range of $\delta^{15}N$ values, but separate out along the $\delta^{13}C$ axis, with *P. coronata* as the less water use efficient species. However, the data from the mixed stand show both species to have the same range of $\delta^{13}C$ values (intermediate to the values obtained as single species), and the same range of $\delta^{15}N$ values, but lower than when they occurred in monospecific stands.

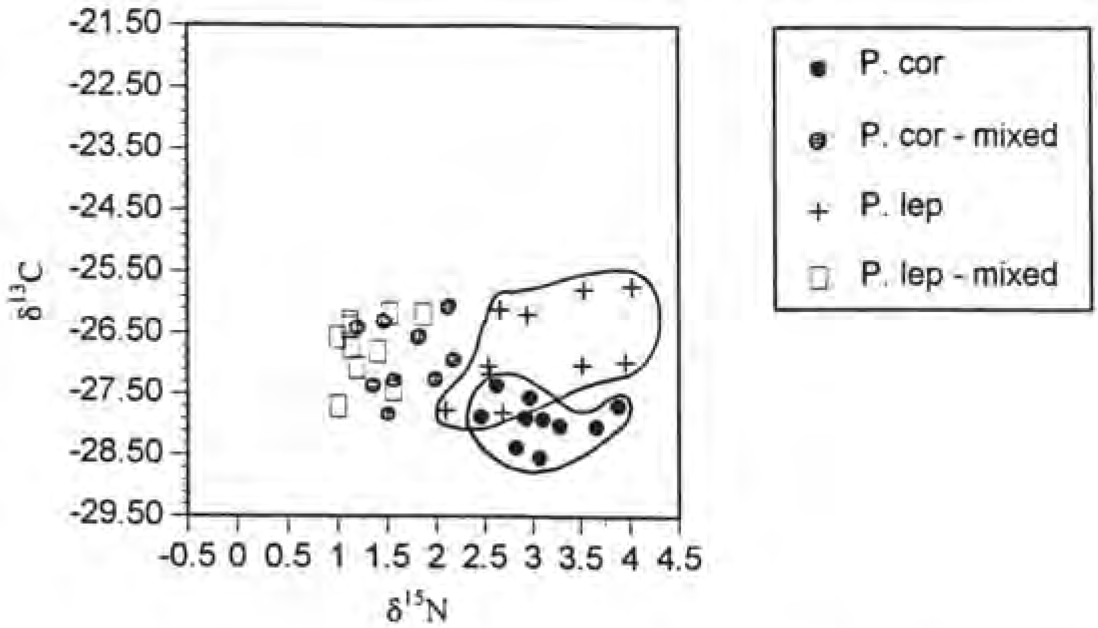

Figure 4. Two-way scatter plot illustrating the $\delta^{13}C$ and $\delta^{15}N$ values of leaves from two *Protea* species collected from monospecific and mixed stands on Constantiaberg Mountain. P. cor = *P. coronata* in monospecific stand; P. cor - mixed = *P. coronata* in mixed stand; P. lep = *P. lepidocarpodendron* in monospecific stand; P. lep - mixed = *P. lepidocarpodendron* in mixed stand. Boundaries encircle the replicates of each species considered to display resource use differentiation.

At the Table Mountain site (Figure 5), *P. neriifolia*, *P. compacta* and *P. burchellii* all have similar $\delta^{13}C$ values, but *P. neriifolia* differs slightly in its $\delta^{15}N$ value. No pattern in nitrogen utilisation is evident for *P. repens* but, although it covers a wide range of $\delta^{13}C$ values, it differs remarkably from the other species in terms of its water use efficiency, indicating that it uses less water than the other species.

Although all three species at the Silvermine site show similar values along both axes, *L. conocarpodendron* and *M. fimbriifolius* show a degree of niche separation (Figure 6). *L. conocarpodendron* appears to use slightly more water than the other two species and there is an indication that it may be using a different source of nitrogen. There is no apparent sexual differentiation of leucadendrons.

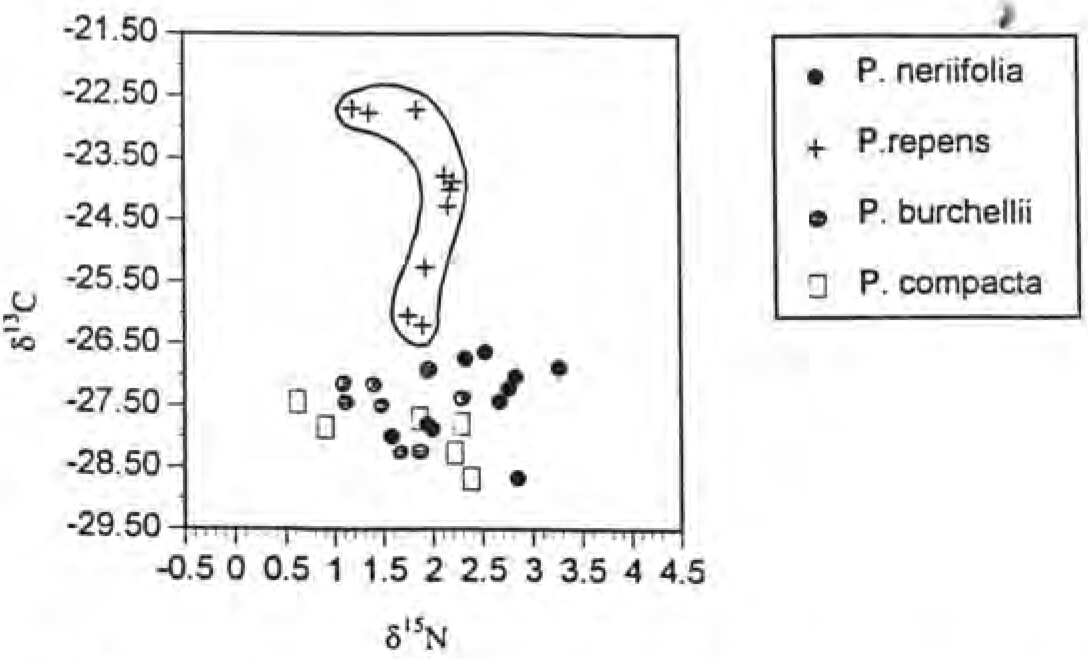

Figure 5. Two-way scatter plot illustrating the δ^{13}C and δ^{15}N values of leaves from four *Protea* species collected from a mixed stand on Table Mountain. Boundaries encircle the replicates of each species considered to display resource use differentiation.

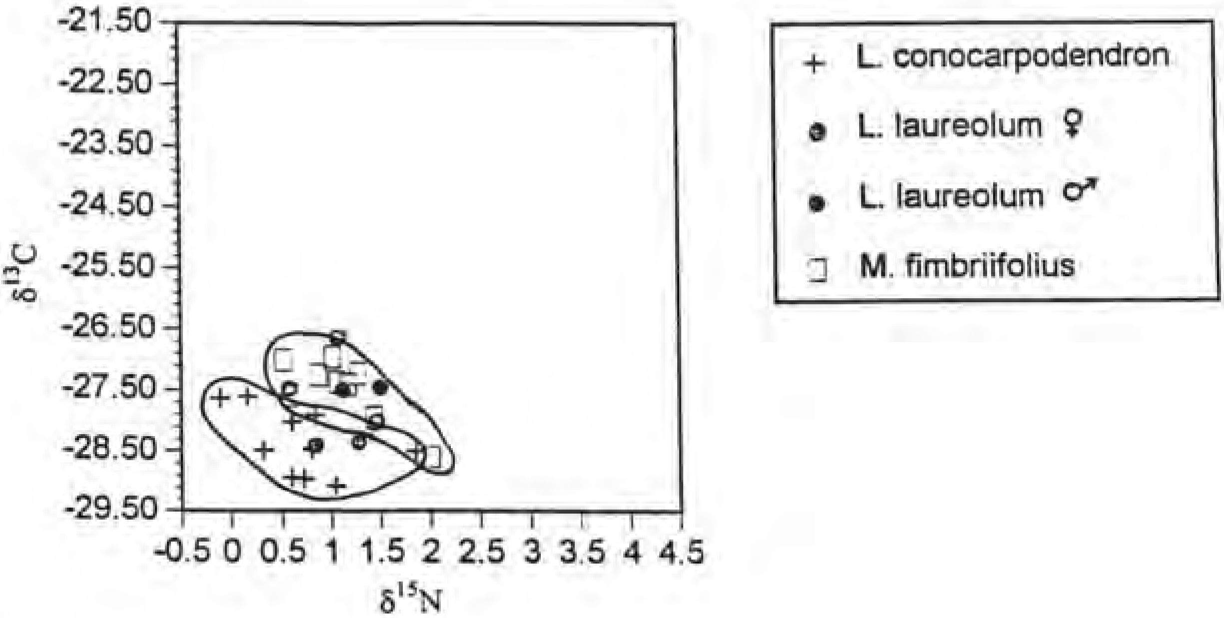

Figure 6. Two-way scatter plot illustrating the δ^{13}C and δ^{15}N values of leaves from three proteoid species, *Leucospermum conocarpodendron*, *Leucadendron laureolum* (males and females) and *Mimetes fimbriifolius*, collected on Silvermine Mountain. Boundaries encircle the replicates of each species considered to display resource use differentiation.

Discussion

Despite suggestions by various authors that coexisting species should differ in terms of their resource use (Tilman 1986; Davis and Mooney 1991; Richards *et al.* 1997), evidence from this study suggests that resource use is not a suitable niche axis for explaining coexistence in proteoids. From the slight differences in $\delta^{13}C$ and $\delta^{15}N$ obtained for some species, it appears that each species has specialised in its mode of nutrient acquisition and use, but this differentiation is not sufficiently distinct for resource use to be considered as a major niche axis.

The stable light isotope technique seems to be a suitable way of determining whether species differ in their resource requirements and in their use of these resources. An advantage of using stable isotopes is that fractionation is integrated over the life-time of the plant, thereby eliminating seasonal influences. However, nitrogen and phosphorus levels in the soil vary greatly during the year (Stock *et al.* 1988) and this technique does not distinguish between species using resources at different times of the year, i.e. species which are temporally rather than spatially separated in their resource use. It is also not possible to pick up any differences in resource use at the juvenile stage, which has been shown to be the stage in which competition for water is the most intense (Lamont and Bergl 1991). One negative aspect of using the isotope technique is the expense and time spent on each sample which limits the number of replicates possible. However, even with five replicates per site, the Cape Peninsula sites yielded sufficiently precise results for patterns to be distinct. These patterns are a little more tenuous for the Cape Agulhas sites. Each symbol in Figures 2 and 3 represents five plants which were grouped together before anaylsis (W.D. Stock, pers. comm.) This averaging of replicates yields patterns which are less obvious and hence results which are less robust than those from the other sites.

Despite this, the preliminary study provides support for the concept that species use resources in a different way. It is not possible to say, though, whether these differences are due to processes occurring within the plant or during uptake of nutrients, or whether they are a function of the soil type on which each species grows. Limestone soils have a higher nitrate and moisture content (Richards *et al.* 1997) and this could explain the lower $\delta^{15}N$ and $\delta^{13}C$ values of *P. obtusifolia.* Nitrification is inhibited at low pH values (Haynes and Goh 1978, cited by Richards *et al.* 1997) and it is therefore not surprising that *P. compacta* and *P. susannae*, growing on the acid sands, have higher $\delta^{15}N$ values.

These results are consistent with those of Richards *et al.* (1995b) who concluded that vegetation composition is strongly associated with soil type. In a further study, Richards *et al.* (1997) suggested that differences in plant nutrient demands may be important in determining species distributions and that these differences may facilitate coexistence. However, in the second site at Cape Agulhas, the two species coexisting on the acid sands do not show any substantial differentiation along either axis, although *L. xanthoconus* shows a fairly wide range of $\delta^{13}C$ indicating that it may be using water resources in a different way to *L. laureolum*. An interesting pattern is that the species growing on the soil of intermediate pH (*L. coniferum*), has the highest $\delta^{15}N$ value. Bearing in mind that low pH inhibits nitrification, we expect the species growing on the acid sands to have the highest $\delta^{15}N$ values as we expect them to be the best adapted to that soil type. Although no reference plant was available as proof, it is predicted that the species most suited to utilising ammonium will be dominant on acid soils whereas those species on the limestone soils will be adapted to high nitrate conditions. However, nitrification is strongly influenced by temperature and moisture availability and this may be partly responsible for the observed patterns. The species growing on the acid sands did not differ in $\delta^{13}C$ from *L. coniferum* growing on the soil of intermediate pH which may be interpreted to mean that there is no difference in moisture availability between these two soil types.

Contrary to our predictions, the greatest similarity in resource use is demonstrated by the coexisting species. In addition to this, they show unexpectedly low $\delta^{15}N$ values, following the logic presented above. This indicates that by growing together they are forced to use nitrogen resources in a different way, but that they respond to the pressure of growing together in the same way. Alternatively, it could mean that stresses imposed by intense competition induce a higher degree of fractionation within the plant and consequently the leaves will have a lower $\delta^{15}N$ value.

Similar patterns were observed at the Constantiaberg site. When growing in single-species stands, *P. coronata* and *P. lepidocarpodendron* are clearly separated along the $\delta^{13}C$ axis, implying that one (*P. lepidocarpodendron*) is more water use efficient than the other (*P. coronata*). They show no differentiation along the $\delta^{15}N$ axis when growing separately or intermingled, but when growing together in the mixed stand, they both have the same, lower, range of $\delta^{15}N$ values. The complete mixing of individuals within the same range along both axes provides clear evidence that species do not need to differ in either their water use efficiency or source of nitrogen in order to coexist. The reason why the $\delta^{15}N$ values should differ for the monospecific and the mixed stands is not clear from this study. Midgley and Watson (1992) found that intraspecific competition in proteas was more intense than interspecific competition. Competition between conspecifics could have the effect of decreased root length relative to that in mixed stands. The result is that nitrogen resources are being utilised from different levels of the soil, with different isotopic signatures, in each stand. A deeper root system suggests better access to water and consequently a lower water use efficiency, but the mixed stand has a higher $\delta^{13}C$ value than that for the *P. coronata* stand. This apparent anomaly may be explained by the fact that the *P. coronata* stand occurred in a slightly wetter area where it is likely that water is not a limiting factor and consequently the plants have no need to be efficient in their use of water.

There are distinct boundaries between the mixed stand and monospecific stands at the Constantiaberg site, and this implies that the plants in each stand experience different environmental conditions, or that they have been subjected to different disturbance regimes. I suggest that differential response to fire, or a similar variable, is responsible for the community structure observed, and that resource differentiation in the mixed stand is a secondary effect induced by competition between the two coexisting species.

Results from the Table Mountain site are consistent with the notion that resource differentiation is not a prerequisite for coexistence. *P. neriifolia, P. compacta* and *P. burchellii* show a large degree of overlap, and although they are more spread out on the nitrogen axis, no distinct trends of resource differentiation are apparent. An obvious exception at the Table Mountain site is *P. repens* which shows a strong differentiation along the δ^{13}C axis. However, this species was collected lower down the slope than the other three species and it is possible that differences in water use strategy reflect differences in the availability of water. One would predict a species growing lower on the mountain slope, where there is presumably more water, to be less water use efficient. Contrary to this, *P. repens* has a higher water use efficiency than the other species. It may be that *P. repens* is inherently efficient in its water use and this may explain the success of the species; it is the most widespread *Protea* species in the Cape and is adapted to growing in a wide variety of habitats (Rebelo 1995). *P. repens* is the only one of the four species which occurs naturally on Table Mountain, and the fact that these species have grown together for so long on soils to which they are not optimally suited, suggests that some factor other than resource use is responsible for coexistence.

At the Silvermine site, all three species have similar values of δ^{13}C and δ^{15}N. However, the two most morphologically similar species, *M. fimbriifolius* and *L. conocarpodendron,* show a degree of separation along both axes. There is an indication the *L. conocarpodendron* is more water use efficient, and this species also shows a slightly lower δ^{15}N value, indicating that it is utilising a different pool of soil nitrogen to the other species. *L. conocarpodendron* and *M. fimbriifolius* are very similar in nearly every respect, including morphology, fire response, recruitment strategies and phenology which are all factors considered in other theories of coexistence. It is therefore interesting that these two species do show a certain degree of resource use differentiation which may be at least a partial explanation of their coexistence. These two species often occur in the same habitat, although they do not always intermingle in the same stand (Bond *et al.* 1990). It may be that *M. fimbriifolius* has a higher water requirement than *L. conocarpodendron* and consequently establishes in patches of soil with a higher soil water availability. Despite the fact that *L. laureolum* females require more nutrients to produce bigger cones, no differences between the sexes is evident along either axis. The main energy source for cone production is carbohydrates which occur in excess in fynbos plants and therefore the lack of sexual differentiation is not surprising.

- **Alternative explanations for coexistence**

Although evidence for resource differentiation in species from Mediterranean-type ecosystems has previously been presented (e.g. Davis and Mooney 1986; Pate *et al.* 1993), these studies have tended to concentrate on 2-4 species, some of which are not truly coexisting, and only deal with one aspect of resource use. This study, which deals with a variety of different genera in different combinations at each site, and with more than one limiting resource, does not reveal resource use as a major niche axis explaining coexistence in fynbos. The high diversity (and consequently high degree of coexistence) in fynbos has promoted several studies of coexistence, most of which invoke reasons other than resource use as the most important parameter determining coexistence.

Yeaton and Bond (1991) explained coexistence in the apparently trophically equivalent (in terms of their morphologies, habitats and life histories) proteoids *P. lepidocarpodendron* and *L. conocarpodendron* by invoking differences in their dispersal mechanisms. Both species rely on open space for their recruitment, and although *P. lepidocarpodendron* was found to be competitively dominant over *L. conocarpodendron*, the latter species is antdispersed and is therefore better able to colonise open spaces made available after fire; *P lepidocarpodendron* is wind dispersed and its seeds tend to accumulate at the base of adult plants rather than in the open. Thus dispersal is responsible for determining community structure by facilitating coexistence through the utilisation of different establishment sites.

All *Protea* species are serotinous to some degree (Rebelo 1995) and are wind-dispersed, and therefore dispersal cannot be responsible for the patterns at the Table Mountain and Constantiaberg sites. However, the Silvermine site consists of species from three different genera, one of which is *L. concocarpodendron*. Another of the species, *M. fimbriifolius*, is also myrmecochorous (Bond and Slingsby 1983) and consequently these two species have an advantage over *L. laureolum* in being better able to colonise the post-fire environment. In addition to this, *L. laureolum* differs from the other two species in its phenology; *L. conocarpodendron* and *M. fimbriifolius* flower in spring and summer whereas *L. laureolum* flowers earlier, in June. Flower production requires large amounts of energy and subsequently more resources are utilised during these periods. Different phenologies represent temporal segregation which has been implicated by several authors as a mechanism facilitating coexistence (Shmida and Ellner 1984; Davis and Mooney 1986).

With the exception of *P. repens*, the Table Mountain proteas are very similar in most respects. They do not appear to use resources in a different way, they all flower in winter and are all serotinous, releasing their seeds after fire. The rarity of natural stands comprising truly coexisting *Protea* species may reflect the lack of distinct differences between *Protea* species. The interesting thing about this site is that, having been artificially planted, these species are able to tolerate potentially unsuitable environmental conditions. This tolerance, and the lack of any resource use differentiation, suggests that the recruitment stage is more important than resource use in determining community structure and hence coexistence in proteas.

Cowling *et al.* (1994) studied mechanisms of coexistence in *P. obtusifolia* and *L. meridianum*, two non-sprouting, serotinous proteoids which grow in the same habitat and have similar sized seed banks, patterns of germination and seedling establishment. Following fires, each species competes for space which, in this case, comprises small, soil-filled holes in limestone outcrops. Recruitment is on a basis of chance, with environmental variation influencing the relative success of species in each recruitment event. It is possible that this form of 'lottery theory' is applicable to the Table Mountain site, where the species share similar regeneration traits.

Disturbance has been implicated as an important process creating opportunities for coexistence (e.g. Connell 1978) and this is of particular importance in fire-prone Mediterranean ecosystems (see Cowling 1987; Davis 1991; Lamont and Bergl 1991; Yeaton and Bond 1991). Each fire favours a different suite of species, depending on components of their regeneration niche (see Grubb 1977), interrupting trends towards competitive exclusion and thereby facilitating coexistence. Having found no evidence for differential use of water resources, Lamont and Bergl (1991) postulated that coexistence in *Banksia* species in fire-prone kwongan is determined by the number of individuals surviving a fire (thereby pre-empting space) and the propagules released per species in response to fire together with the outcome of competition between seedlings immediately after fire. They noted that any competitive exclusion occurred within the first few years after fire, after which communities stabilised. This highlights the importance of considering differential resource use at the juvenile stage.

Without additional information regarding the fire responses, in terms of survival and post-fire recruitment strategies, of the species dealt with in this study, it is not possible to identify where fire applies to coexistence in these communities. Cowling (1987) has stated the importance of fire as a determinant of community structure in fynbos and it is likely that it is a primary mechanism of coexistence in fire-prone Mediterranean-type ecosystems (Cowling 1987; Davis 1991; Lamont and Bergl 1991).

The host of alternative explanations to resource use differentiation supports the ideas of Shmida and Ellner (1984) and other advocates of the theory that trophically equivalent species are able to coexist. However, it is my opinion that the importance of resource use differentiation should not be disregarded and that concepts from the two opposing schools of thought should be integrated so that further explanations of coexistence take into consideration the inherent complexity of interactions in communities.

This study was an attempt to show that resource use differentiation enables the coexistence of morphologically similar species. There is evidence that not all species coexisting in the same stand display differentiation along the same niche axis, and that interactions between coexisting species are complex and variable. Differentiation in resource use is not suitable as a unitary explanation of coexistence in proteoids, but evidence suggests that it works in conjunction with other mechanisms so that in each community a different explanation of coexistence is appropriate.

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