

**The role of water and nutrient availability in determining above- and below-ground allocations in a C4 grass *Stipagrostis ciliata* (Desf.) de Winter.**

TIMOTHY E. MOORE<sup>1</sup>

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<sup>1</sup> *University of Cape Town, Private Bag, 7701 Rondebosch, South Africa*

Primary supervisor: Dr Edmund February

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University of Cape Town

KD MOOR

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## Abstract

In order to understand the impacts climate change will have on plants it is important to understand the role of functional diversity in determining plant success across a range of environments. Two populations of *Stipagrostis ciliata* were compared at two sites -drier coastal and wetter inland- that varied in their water and nutrient availability. Analysis an inverse relationship between rainfall and N availability, with the drier coastal site having significantly lower soil and plant  $\delta^{15}\text{N}$  ( $Z_{\text{adi}} = -1.964$ ,  $p < 0.05$ ). Plant percent N decreased by 63% between the coastal and inland site. Mean root:shoot ratios also differed significantly between sites ( $Z_{\text{adi}} = -1.964$ ,  $p < 0.05$ ). Although total rooting depth did not appear to differ between sites, in total more root material was found per plant at the inland site, with 40% of all root material occurring directly below the plant. At the coastal site, a greater proportion of root material was allocated laterally in the upper 10cm of soil. As expected, water use efficiency, based on  $\delta^{13}\text{C}$ , was higher at the drier coastal site. It is proposed that plants will alter above and below- ground allocation depending on the nature of the limiting resource. In dry environments, more root material in upper soil layers, and a faster growth rate associated with higher shoot allocation, may enhance water uptake. Where nutrients are limiting, increased root biomass might increase nutrient, especially N interception. Competition may also be higher at low nutrient sites.

## **Introduction**

Plant allocations above and below ground are greatly influenced by the environments in which they grow. Specifically, plants are expected to allocate biomass to regions in their environments that optimize resource uptake. This would suggest that in environments that differ in terms of water and nutrient availability, plants will differ in their allocations to roots and shoots. For example, plants are predicted to have larger root: shoot ratios in more arid than in more mesic environments (Walter 1963; Pallardy 1981; Chapin *et al.* 1993). In order to understand the impacts variation in climate will have on plants it is important to understand the role of functional diversity in determining plant success across a range of environments.

In water-limited environments, the availability of water and nutrients to plants depends on environmental conditions, sizes and shapes of their root systems, and root competition (Schenk and Jackson, 2002). In dry environments it may be necessary for plants to increase their allocation below-ground to increase the amount of water available to them. The same is true for nutrients; plants may need to allocate more resources below ground in more nutrient poor environments in order to secure available nutrients.

The use of stable isotopes in ecology has increased in popularity (see review by Dawson *et al.*, 2002). The relative ease of sampling and the diversity of inferences that can be made from the results of isotope analyses make isotopes an ideal tool for studying plant ecology and, more specifically, interactions between plants and their environments.

The principle source of variation in stable C isotopic signatures in plants is caused by differences in photosynthetic pathways, resulting in uneven discrimination between heavier and lighter isotopes. Specifically, plants with C3 pathways are expected to be more depleted of the heavier  $^{13}\text{C}$  isotope, due to greater discrimination of the Rubisco enzyme during  $\text{CO}_2$  uptake (Dawson *et al.*, 2002). A further source of variation in  $\delta^{13}\text{C}$  values between plants is water availability (McCulley *et al.*, 2004). Decreasing water availability, which can lead to plant closing their stomata, is linked to a decrease in C isotope discrimination by leaves (Ehleringer and Cooper, 1988).

This leads to plants with higher water use efficiency (WUE) being enriched relative to those with lower WUE (Farquhar and Richards, 1984).

The discrimination of plants against different N isotopes is related to the availability of nutrients and water (Tilman, 1988). There is generally an inverse relationship between  $\delta^{15}\text{N}$  and nutrient availability, i.e. nutrient rich ecosystems tend to be isotopically enriched (Vitousek and Walker, 1989). This is because plants can afford to discriminate when the concentration of N in the soil is high relative to a plants N requirements (Högberg, 1997). Similarly, soil and plant  $\delta^{15}\text{N}$  enrichment has been associated with areas that experience lower precipitation (McCulley *et al*, 2004).

*Stipagrostis ciliata* (Desf.) de Winter. is a C4 grass with a widespread distribution in the central and western parts of South Africa (Milton and Dean, 2000). Associated with this distribution is adaptation to arid conditions; Bushmanland, where *S. ciliata* forms one of the dominant species, receives a mean annual rainfall of less than 100mm (Dean and Milton, 1999). *S. ciliata* is a perennial grass, which dies back during the dry season (summer in Namaqualand). Although believed not to be native to Namaqualand, large populations exist there, generally associated with sheep and other livestock farming. Its widespread distribution across a range of habitats makes it ideal for studying functional variation in response to water and nutrient availability.

The aim of this study was to, using stable C and N isotopes, address the function of water and nutrient availability in determining above- and below-ground resource allocation in *Stipagrostis ciliata*. Specifically, three key questions will be addressed.

Firstly, is there an interaction between rainfall and nutrient availability, and what is the nature of this interaction? It is predicted that a higher rainfall site will have lower nutrient availability due to higher plant productivity and, possibly, increased loss of due to leaching from the system. Thus it is expected that  $\delta^{15}\text{N}$  values at a site with higher rainfall will be higher than at a lower rainfall site.

Secondly, is there a difference in WUE between plants found at sites with different amounts water available (in the form of annual precipitation)? It is expected that plants at sites of lower water availability will be more efficient in terms of water use.

As a consequence, plant  $\delta^{13}\text{C}$  values where less water is available will be more negative (enriched with  $^{13}\text{C}$ ).

Finally, how do differences in rainfall and nutrient availability affect plant allocation above- and below- ground? Specifically, does rainfall and nutrient availability influence the density of root material in the soil profile and, if so, how? It is generally expected that plants in lower rainfall areas will have higher root:shoot ratios. It is believed that this increased root area, often associated with increased root depth, in areas of low precipitation enable plants to access more water.

## **Methods**

### *Study sites*

The study was carried out in the winter rainfall, Namaqualand region of South Africa, towards the end of the rainy season (August, 2007). Both study sites were located in the Succulent karoo biome. A steep rainfall gradient exists as one moves inland from the coast in this region. Sites were selected that were as pure in species composition as possible, in order to avoid the effects of interspecific competition on plant allocations. The coastal site was located 30 km from the coast on the road between Wallekraal and Soebatsfontein. The inland site was located 60km from the coast, just north of the town Garies.

Percent cover of *S. ciliata* was characterised by sampling three 5X5m quadrats at each site. The presence of other species in the quadrats was also noted. Rainfall for each site was obtained from agrohydrological data for South Africa (Schultz, 1997).

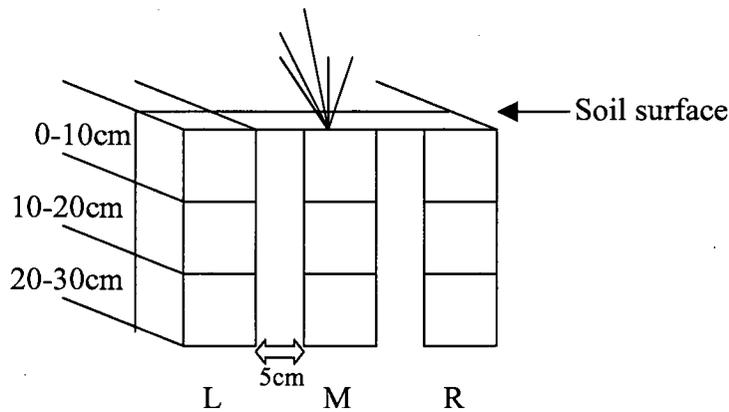
Vegetation type was also characterised based on the vegetation types identified by Low and Rebelo (1996). Both sites were characterised by shale-based, clay-rich soils. The GPS locations and environmental details of each site are summarised in Table 1.

### *Fieldwork and data collection*

Three plants of *Stipagrostis ciliata* were sampled at each site. Plants were selected that were at least 40cm away from their nearest neighbour to prevent overlapping of root material. Plants were sectioned using a spade with a 20cm wide blade; with all sections at a site being done in the same direction (coastal site: north side of plant, inland site: down-slope side of the plant). For each plant, a trench roughly 40cm deep

and 140X60cm in area was removed from the side of the plant opposite to the sampling side enabling access to deeper cores and for the removal excess root material from the side of the plant that was not sampled. All aboveground material of the plants was sampled, dried at 70°C, and weighed prior to isotope analysis.

10cm<sup>3</sup> samples of soil were removed from the soil profile at three depths, from directly under and on either side of the plant, in order to evaluate the spatial distribution of the roots. (Figure 1). Samples were taken down to a depth of 30cm.



**Figure 1.** Diagram of procedure for sampling root material from down the soil profile. 10cm<sup>3</sup> sections of soil were removed from each depth. Three samples were taken per depth, one directly below the plant and one on either side. Root and soil material collected was later used for stable N and C isotope analyses.

Root material was removed from each sample by sieving through a 2.0mm sieve. The roots were then washed in distilled water and any excess soil material was removed. Root samples were then dried at 70°C. Once dry, root samples were weighed on a four-point Mettler AE200 electronic balance (Mettler-Toledo, Greifensee, Switzerland). A sub-sample from each section, along with a sample of soil from the same section was analysed used for isotopic analysis. Total root weights and ratios of root:shoot were calculated for each individual in order to test for a significant shift in allocation between sites.

#### *Isotope analysis*

Leaf and root samples were ground into a homogenous powder using a Retsch MM200 ball mill (Retsch Inc. GmbH&Co KG, Haan, Germany). Powdered leaf and

root samples were then weighed into tin cups with an accuracy of 1 microgram, using a Sartorius microbalance. Soil samples were not ground prior to analysis.

Leaf, root and soil samples were then combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy). The gases created during this combustion were then passed to a Delta Plus XP Isotope Ratio Mass Spectrometer (IRMS) (Thermo electron, Germany), via a ConFlo III gas control unit (Thermo Finnigan, Germany). Stable isotope values are then given as:

$$d (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 1000$$

Where R is the ratio of the heavy to light isotope ( $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$ ).

The values are expressed relative to a standard, that is, atmospheric air for Nitrogen and Vienna Pee Dee Belemnite (VPDB) for Carbon. Higher d values indicate enrichment relative to the heavier isotope (either  $^{15}\text{N}$  or  $^{13}\text{C}$ ). The deviation from the standard is denoted by the term d and the results expressed as parts per thousand (‰).

C4  $\delta^{13}\text{C}$  values should average around -12.5‰ (Vogel *et al*, 1990). As a result, some root samples from site 1 with  $\delta^{13}\text{C}$  values greater than -18‰ were excluded from further analyses.

### *Statistical analysis*

All statistical analysis was carried out in STATISTICA 7.0 (Statsoft<sup>®</sup> Inc. USA). Because of the generally small sample sizes and non-normality of the data, non-parametric Mann-Whitney U tests (ZAR, 1996) were used for all two-sample comparisons.

### **Results**

Sites differed in mean annual rainfall, percent cover and the presence of other species (Table 1). The drier inland site was more mixed in terms of species composition, with lower *S. ciliata* cover (33%). In contrast, the wetter inland site had higher cover of *S. ciliata* (38%); also no other plant species were found in the sampled quadrats.

### *Rooting distribution*

At both sites, no *S. ciliata* roots were observed below 30cm. Table 2 shows the mean density of root material for each sampled layer. In total, the inland site contained significantly higher total root biomass per plant ( $Z_{adi} = -1.964$ ,  $p < 0.05$ ) when compared to the coastal site (Figure 2, and 3). Sites differed in the proportion of root material allocated to the top 20cm of soil (Figure 3). For all six samples taken up to that depth, significant differences were found between sites ( $Z_{adi} = -1.964$ ,  $p < 0.05$ ). At the coastal site, most root material was concentrated in the top 10cm of soil (Figure 3). At the inland site, almost 40% of all root material was found directly below the plant. Root:shoot ratios doubled between sites ( $Z_{adj} = -1.964$ ,  $p < 0.05$ , Figure 3). <sup>4</sup>

Mean leaf  $\delta^{13}C$  differed significantly between sites ( $Z_{adj} = -1.964$ ,  $p < 0.05$ , Table 3). At the drier inland site, leaf  $\delta^{13}C$  ranged between  $-11.37\text{‰}$  and  $-11.85\text{‰}$ , while at the wetter coastal site  $\delta^{13}C$  ranged between  $-10.64\text{‰}$  and  $-11.04\text{‰}$  for leaf tissue. Also, leaf percent N and C:N ratio differed significantly between sites ( $Z_{adj} = -1.964$ ,  $p < 0.05$ , Table 3).

At both sites root  $\delta^{15}N$  values were significantly lower than soil  $\delta^{15}N$  values from corresponding samples at each depth ( $Z_{adj} = -1.964$ ,  $p < 0.05$ , Table 4). Root  $\delta^{15}N$  values ranged between  $6.09\text{‰}$  and  $6.79\text{‰}$  at the lower rainfall, coastal site and between  $9.51\text{‰}$  and  $10.19\text{‰}$  at the higher rainfall, inland site.

### **Discussion**

Plants trade off above- and below-ground allocations relative to the availability of water and nutrients (Caldwell, 1986). In water-limited environments, the availability of water and nutrients to plants depends on environmental conditions, sizes and shapes of their root systems, and root competition for the limited resources (Caldwell, 1986; Schenk and Jackson, 2002). This paper sought to investigate how water and nutrient availability influence plant growth and allocation to above- and below-ground material.

### *Rainfall and Nutrients*

Numerous studies have illustrated the inverse relationship between rainfall and nutrient availability shown in this study, the inland site having higher  $\delta^{15}\text{N}$  and %N values (Table 3, and 4; Vogel *et al*, 1990; Evans and Ehleringer, 1993; Swap *et al*, 2004; Aranibar, 2004). It is generally accepted that decreased nutrients, particularly N, with rainfall is due to the flow of nutrients to inaccessible regions in the soil caused by increased leaching in more mesic areas (Högberg, 1997; Aranibar, 2004). This relationship between water and nutrient availability seems to explain the pattern observed in the allocations and structure of the plants found at the two sites studied. What is suggested here is that plant allocations are dependent on which resource is limiting. At the drier coastal site water is what limits and restricts plant growth, while at the more mesic inland site, it is nutrient availability that limits and determines plant allocation.

#### **Where water is limiting**

As expected, plants at the coastal site were more water use efficient than plants at the more mesic inland site (Table 3). Lower water availability at the coastal site forces plants to close stomata, and thus discriminate less towards the heavier  $^{13}\text{C}$  isotope. The effect that rainfall has on plants is influenced primarily by the availability and location of soil water in relation to root distribution (Walter, 1971). The smaller total root mass per plant (Figure 2) and lower root:shoot ratios (Table 3) found here do not agree with other studies. For example, in a study across a rainfall gradient in Patagonia Schulze *et al* (1998) found that root:shoot ratios increased with an increase in aridity. A possible explanation for this difference is that what might be more important in this system is the ability to capture water where and when it is available. Walter (1971) showed that small precipitation events recharge only shallow soil layers, where shallow-rooted grasses readily take up water. Namaqualand is characterized by brief, episodic rainfall events (Dean and Milton, 1999). This suggests that it is important to allocate a greater proportion of roots to the upper soil layers in arid environments where rainfall events are small. This is borne out by the significantly higher proportion of root material found in the surface layers on either side of the plant at the coastal site versus the inland site (Figure 3).

Also, lower root:shoot ratios are indicative of higher plant growth rates (Schulze *et al*, 1998). It is possible that due to the brief and episodic nature of rainfall in the region, plants that are water-limited need to be able to capitalize quickly on and rainfall that becomes available to have a chance to grow and reproduce before the end of the wet season. This higher allocation to growth is borne out by the lower C:N ratio of the coastal plants (Table 3).

#### **Where nutrients are limiting**

Success in acquiring soil-based resources is determined by placement of roots in resource-rich and low-competition patches (Pechácková, *et al* 2004). Overall root biomass (Figure 2), and higher root:shoot ratios suggest that plants in nutrient-limited (in this case N) systems are faced with increased root competition for whatever nutrients are available. Hodge *et al* (1999) found that plants with more prolific root growth captured more N. This suggests that more root material increases access to nutrients. At the same time, it is believed that below-ground competition for nutrients increases as nutrient availability decreases (Casper and Jackson, 1997). And so increasing root material might improve a plant's competitive ability when nutrients are limiting. The lack of other species present in sampled quadrats (Table 1) at the inland site may be accounted for if *S. ciliata* is out-competing them for the limited nutrients that are available at the site. Milton and Dean (2000) found that *S. ciliata* adults competitively excluded seedlings for establishing in the absence of disturbance.

#### **Conclusion**

In this study, *Stipagrostis ciliata* has displayed remarkable plasticity in above- and below-ground allocation relative to rainfall and nutrient availability. This may explain why it is so successful over such a wide geographic and climatic range. Swap *et al* (1994) failed to find significant relationships between stable N and C isotopes in C4 grasses and environmental variables. Amazingly, despite only two sites being assessed, and over a relatively short geographic scale, significant differences were found between C and N isotopic ratios between sites.

Within the context of a changing climate, an understanding role the environment plays in shaping and influencing plant functional diversity is vital. It has been suggested that because of the role that rainfall plays in governing both water and

nutrient availability, arid and semiarid systems may be among the most sensitive to precipitation fluctuations caused by climate change (Schwinning *et al*, 2005). Plant allocations, and in particular plant allocations to roots, are important parameters to consider when understanding plant reactions to change in rainfall amount and seasonality. The Namaqualand system may be an ideal system for the study of the impacts of climate change on functional and biological diversity, because of the steep climatic and environmental gradients, as well as the high diversity of plant species and functional types in the region.

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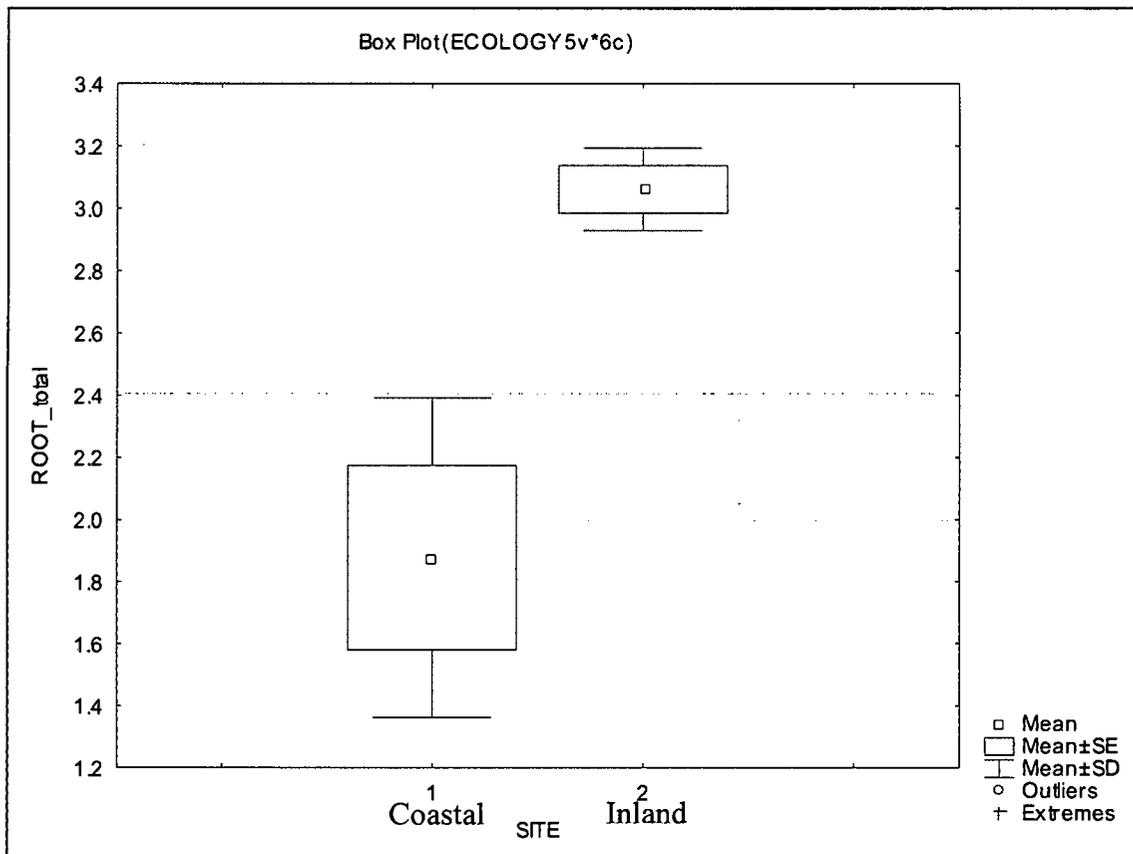
## Figures and Tables

**Table 1.** Site locations and characteristics. Percent cover estimated from three 5X5m quadrats at each site. Both sites were situated on clay-rich soils.

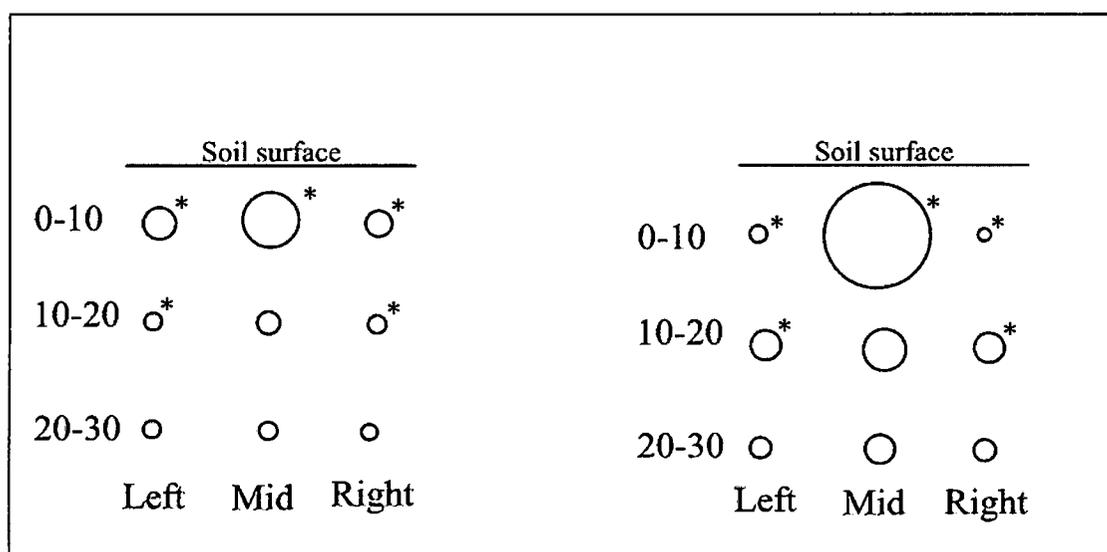
Site	GPS	Elevation(m)	MAR(mm)	Vegetation Type	% Cover	Other plants present
Coastal	30.21.57.2S 17.34.45.5E	151.5	131	Namaqualand Blomveld	33.33	<i>Tetragonia fruticosa</i> ? ? <i>Erharta calycina</i> ? Unknown Legume ?
Inland	30.31.52.0S 17.59.28.0E	236.6	228	Namaqualand Klipkoppe Shrubland	38.1	NA

**Table 2.** Mean root densities for each sampled soil layer. Density calculated as total root material per 10cm<sup>3</sup> block. (Note: L=left hand side of plant; M= directly below plant; R= to right hand side of plant).

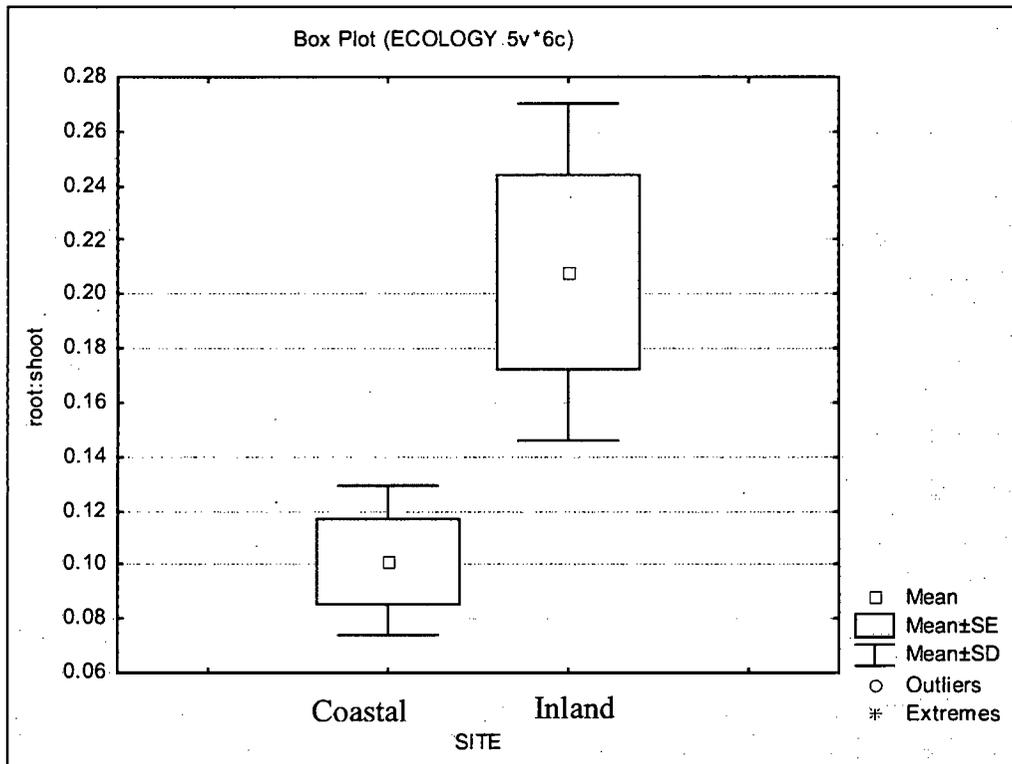
	Inland			Coastal		
	L Mean±SE	M Mean±SE	R Mean±SE	L Mean±SE	M Mean±SE	R Mean±SE
0-10cm	0.0312±0.008	0.0616±0.011	0.0226±0.015	0.0115±0.002	0.1285±0.015	0.0054±0.002
10-20cm	0.0109±0.002	0.0179±0.007	0.012±0.003	0.0283±0.003	0.0441±0.0143	0.0285±0.002
20-30cm	0.0107±0.0015	0.012±0.005	0.0089±0.002	0.0155±0.004	0.0271±0.005	0.0173±0.004



**Figure 2.** Box-and-Whisker plot of mean root mass per plant at each site. Boxes correspond to mean±SE. Whiskers correspond to mean±SD. Mann-Whitney U test was significant (at  $\alpha= 0.05$ )



**Figure 3.** Mean root weights for each site. Circle diameters proportional to root mass found in each layer. Marked circles are significantly different from circles of corresponding samples at other site ( $Z_{adj} = -1.964, p < 0.05$ ).



**Figure 4.** Box-and- Whisker Plot of mean root:shoot ratios between sites. Boxes correspond to mean±SE. Whiskers correspond to mean±SD. Mann-Whitney U test was significant (at  $\alpha= 0.05$ )

**Table 3.** Leaf composition and structure between sites. All variables are significantly different ( $Z_{adj} = -1.964$ ,  $p < 0.05$ )

	Leaf %N	Leaf d13C	Leaf C:N
Coastal	1.04	-11.61	43.61
Inland	0.66	-10.77	68.1

**Table 4.** Mean  $\delta^{15}\text{N}$  (‰) values for soil and root samples at each depth. Lower  $\delta^{15}\text{N}$  values are associated with higher discrimination against the heavier isotope where N is more abundant.

	Coastal site		Inland site	
	Soil $\delta^{15}\text{N}$ (‰)	Root $\delta^{15}\text{N}$ (‰)	Soil $\delta^{15}\text{N}$ (‰)	Root $\delta^{15}\text{N}$ (‰)
0-10cm	11.15±0.2	6.79±0.74	14.66±0.69	10.19±0.88
10-20cm	10.49±0.12	6.64±0.56	14.54±0.62	9.47±0.71
20-30cm	10.20±0.22	6.09±0.22	13.20±0.48	9.51±0.64