Organic amendments: A recipe for the restoration of *Acacia* invaded fynbos?

By
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Abstract

The Working for Water initiative which aims to remove water-demanding alien trees from South African watersheds has prompted research into the restoration of fynbos once alien plants, specifically Australian *Acacia*, have been cleared. Yelenik (2000) reported that the high soil nitrogen levels found under *Acacia saligna* stands could hamper restoration efforts. The high nitrogen levels in these soils could potentially inhibit the re-establishment of fynbos in the cleared stands by having a direct negative effect on fynbos plants, which are adapted to nutrient poor soils and by promoting a secondary invasion of weedy grasses which would increase seedling stress through competition. It has been suggested that a mulch with a high C:N ratio be added to the soil to overcome the problem of high soil nitrogen in stands recently cleared. The mulch would have the effect of increasing microbial activity and the subsequent immobilization of plant available Nitrogen. This study attempted to test whether such action would be successful through a glass-house bioassay, in which five different fynbos types were planted in soil taken from an old *Acacia saligna* stand and treated with different organic amendments. The bioassay showed that firstly competition between seedlings of fynbos plants and invading weedy grasses was more likely to hamper restoration efforts than the direct effect of high soil Nitrogen levels on plant growth and secondly that the addition of bark chips to the soil could potentially facilitate restoration efforts.

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INTRODUCTION

The question this paper attempts to answer is whether organic amendments can be used to enhance the re-establishment of native fynbos species on stands cleared of *Acacia* invading aliens. Since the inception of the Working for Water programme in 1995, which aimed to enhance the water-delivery of water-sheds by clearing water demanding aliens (van Wilgen *et al.* 1998), restoration ecologists have grappled with the problem of restoring these cleared stands to their native fynbos vegetation (Holmes 2002, Yelenik *et al.* 2003). Simply removing the aliens does not guarantee that native fynbos plants will be able to re-establish in these heavily disturbed systems (Holmes 2002). In fact Yelenik *et al.* (2003) predicted that the high soil Nitrogen (N) levels typical of recently cleared *Acacia* stands (see Stock *et al.* 1995) would impede the recolonisation of these stands by fynbos species. *Acacia* species are nitrogen fixers and both *Acacia cyclops* (Stock *et al.* 1995) and *Acacia saligna* (Yelenik *et al.* 2003) have been shown to increase soil N. *Acacia cyclops* and *Acacia saligna* are two prime culprits of alien invasion in the Western Cape (Hoffman *et al.* 1999). Yelenik *et al.* (2003) suggested two possible mechanisms of how high N levels could impede re-colonisation by native species. High soil nitrogen can either directly inhibit the growth of fynbos species or indirectly affect seedling survival as nitrophilous grasses prosper in the nitrogen rich soil and out-compete the native seedlings. Lamb and Klaussner (1988) found that vegetative growth of five-year old *Protea repens* shrubs was lower in nitrogen fertilised compared to shrubs in unfertilised soil. Yelenik *et al.* 2003 showed that growth rates of *Ehrharta calycina* grown in soil taken from *Acacia* stands were higher than *E. calycina* plants grown in fynbos soils.

Organic amendments effectively lower the concentrations of inorganic nitrogen in soil (Zink & Allen 1998). Although some plants can utilise organic nitrogen generally it is accepted the Ammonium and Nitrate are the primary sources of N for higher plants (Stock *et al.* & Lewis 1984, Lewis 1986, Lambers et al 1998, Schimel & Chapin 1996). If soil microrganisms cannot meet their nutritional requirements through the decomposition of organic matter then they will absorb nutrients from the soil solution (Lambers *et al.* 1998). The process whereby micro-organisms remove nutrients from the soil solution is known as immobilisation (Lambers *et al.* 1998). Once the nutrients are immobilised in the tissues of the microbial biomass they are unavailable to plants (Zink & Allen 1998). The addition of organic matter to soils increases the microbial activity in the soil, however if the organic matter is low in nutrients, i.e. has a high C:N ratio (greater than 20:1), then the soil microrganisms will not be able to meet their nutritional demand through the decomposition of the added organic matter and

will therefore absorb nutrients from the soil solution (Lambers *et al.* 1998). In summary the addition of nutrient poor organic amendments increases microbial activity, which subsequently increases soil nitrogen immobilisation. Based on the above Yelenik *et al.* proposed that to immobilise the excess Nitrogen is soils of recently cleared *Acacia* stands a mulch with a high C:N ratio could be added to the soil and thus facilitate the restoration of the stand. It is this suggestion made by Yelenik *et al.* 2003 which formed the basis of this project.

The aims of the project

The study aimed to answer the following questions. How do fynbos species, including *E. calycina*, grown in soil taken from an *Acacia* infested stand respond to different organic amendments? How do the organic amendments affect the soil inorganic nitrogen levels? Can soil organic amendments facilitate the restoration of fynbos in plots that have been cleared of alien *Acacias*?

METHODS

Project design

Five species, namely *Protea repens* (Proteaceae), *Leucadendron salicifolium* (Proteaceae), *Phylica ericoides* (Rhamnaceae), *Rhodocoma fruticosa* (Restionaceae) and *Ehrharta calycina* (Poaceae) were included in the project. These five species gave a range of different fynbos types and thus enabled us to consider the different responses of the types included to the treatments. The average starting size of the plants and the number of plants per treatment is shown in Table 3. There were five treatments and a control. All the treatments were made up by adding a carbon source or a combination of carbon sources to soil collected from an old *Acacia saligna* stand, hereafter referred to as the original soil. The original soil was collected in a dense *Acacia saligna* stand in the Atlantis region, Western Cape. In the stand the average height of the trees was estimated at 6m, the average stem diameter was estimated to be ~8cm, and the trees were spaced approximately 1m to 1.5m apart. Plant litter was cleared away from the soil and the top five cm of the soil layer were removed into 45cm x 30cm x 20cm tubs. Later the soil in the tubs was thoroughly mixed together and put through a 2mm sieve, which removed large organic particles contained in the soil. The carbon sources used were bark chips, charcoal and white sugar. These three sources were used to give a range of carbon sources from a soluble and easily accessible source in the case of sugar to a recalcitrant source in the case of charcoal.

The aim of adding the carbon sources was to roughly double the amount of carbon matter in the original soil thus increase the C:N ratio, which would hopefully result in the immobilisation of available Nitrogen in the soil by the microbial community. The organic content of the original soil and the carbon sources was determined by recording the mass loss after incinerating the soil and the carbon sources at 420°C in a muffle furnace overnight (Yelenik, 2000). The percent mass organic matter of the different substances is shown in Table 1.

The five treatments and the quantities of the carbon supplement added to the original soil is shown in Table 2. In the case of the bark and charcoal treatments the carbon source was first ground to a 2mm powder in a hammer-mill and then thoroughly mixed into the original soil before the plants were planted into the soil. In the case of the sugar treatments roughly half the total amount of sugar to be added was mixed into the soil before the plants were transferred to the soil and the rest was added topically half-way through the experiment. The second batch of sugar was simply sprinkled over the surface of the soil in the pots before the pots were watered.

In addition to the pots with plants 18 pots of soil, three pots per treatment, were stored in the glass house with the other pots and watered on the same schedule as the other pots. Any plants that germinated in these pots were removed, as were plants that germinated in the pots with the chosen species.

	Percent OM by mass
Soil	5.8 ± 0.2% (N=5)
Charcoal	92.1 ± 0.9% (N=3)
Bark chips	63.8 ± 1.7% (N=3)
Sugar	*100% (assumed)*

Table 1: The percent organic matter (OM) by mass in the original fresh soil and the carbon sources used. The values were determined by recording the percent mass lost over combustion at 405°C in a muffle furnace.

Treatment	Initial g/5kg soil	Top-ups (g sugar/pot)
Charcoal	315	-
Bark	455	-
Charcoal & sugar	160 & 60	10
Bark & Sugar	230 & 60	10
Sugar	125	20

Table 2: The amount of the different C sources added and mixed into to 5kg of original soil before the plants were transferred into their new pots and the subsequent sugar additions to the sugar treatments approximately half-way through the experiment.

	N	N/treatment	Mean (mm)	Minimum (mm)	Maximum (mm)
Protea repens	42	7	41	15	70
Leucadendron salicifolium	36	6	48	25	104
Phylica ericoides (Ht)	42	7	99	30	150
Phylica ericoides (Wd)	42	7	137	65	250
Rhodocoma fruticosa	35	6	28	10	44
Ehrharta calycina	-	8	-	-	-

Table 3: The number and initial size of the different species, excluding *Ehrharta calycina*, used in the experiment. Size is expressed in terms of height, unless otherwise indicated, which was recorded just after the plants were transferred to the treated soil. *E. calycina* were planted as seeds, 10 per pot, and 8 pots per treatment. Of the *E. calycina* seeds planted the first to germinate was monitored while the others were noted but removed as they germinated.

Timing of the project and glass-house protocol

All the plants and seeds were acquired in the week starting on the 16 June 2003. The *P. repens*, *L. salicifolium* and *R.fruticosa* plants were acquired as seedlings from the Kirstenbosch nursery. These plants were planted into speedling trays with Consol acid wash sand and kept in the glass-house where they where watered daily. The *P. ericoides* individuals were bought as cuttings from a whole-sale fynbos nursery (Good Hope Nursery, Scarborough). The plants were left in their plastic soil jackets until they had to be transferred to the treated soil. The *P. ericoides* plants were also left in the glasshouse but did not need to be watered daily, but rather approximately once every three days. The *E. calycina* individuals were bought as seeds (and were stored in a cool dry drawer until they were planted in the treated soil.) After a period of acclimatisation to the glass-house environment the plants were transferred to the treated soil in the week starting on the 14 July 2003. The *E. calycina* seeds were also planted in the treated soil in the same week, as the other plants were transferred.

The second batch of sugar was added to the sugar treatments in the week starting on the 25 August. Before the sugar was added the size and health status of each plant was recorded. Soil for the final soil available nitrogen analysis was removed in the week starting on the 2 October 2003, as the soil sample was removed the size and health status of the plant was recorded.

Altogether the plants were in the treated soil from approximately the 14 July to the 2 October. During this time the plants were watered if the soil medium was considered dry, which was determined by pressing one's thumb against the surface of the soil and noting

moisture or the lack of it. The plants were organised onto, which were rearranged within the glass-house at the beginning of each week to negate any glass-house position effect.

Transferring the plants to the treated soil

Except in the case of *E. calycina* each plant was housed in its own pot, which were 10cm deep, had a top diameter of 12.5cm and a bottom diameter of 8.5cm. The original soil or sand housing the plants' roots was removed by submerging the speedling trays or soil jacket in water and then gently rinsing all soil or sand away from the plants' roots. The naked plant was then held at the base of its stem over its new pot and the treated soil was poured into the pot over the plant's roots. Once in their new soil the plants were returned to the glasshouse and watered according to need, which was normally every two days. In the case of *E. calycina*, 10 seeds were planted per pot. The first seed to germinate per pot was monitored while other individuals that germinated later in the same pot were noted but removed to prevent any complications that could have arisen due to competition for resources and space.

Plant size and health status

The plant features that were recorded for all the plants during the project were height, width and health status. The width of the *E. calycina* individuals were not recorded but rather the number of shoots per individual was recorded. These features were recorded three times during the project. The first measurements were taken once the plants had been transferred into the treated soil. The second measurements were recorded just before the second batch of sugar was added to the sugar treatments. The last measurements were taken at the end of the experiment at the same time as the soil for the soil nitrogen analysis was collected. Heights and widths were measured with a ruler marked at 0.5 cm intervals. To get a measure of the plants size that included height and width simultaneously the volume of the plants were calculated using the formula for the volume of a parabola, i.e.:

$$\text{Volume} = \pi(\text{Width})^2(\text{Height})/8$$

This value was then cube-rooted so that it could be expressed in millimetres rather than millimetres cubed, which made the interpretation of the values simpler.

The health status of the plant was decided qualitatively by noting any visible sign of stress such as discolouring of the leaves, wilting or the dropping of leaves. A three-category scheme was used - plants were recorded as being in a good state of health, in a poor state of health or dead.

Soil Nitrogen analysis

The soil inorganic nitrogen levels were recorded at the end of the experiment using manual colorimetric methods. Fresh soil samples were collected from five of the pots in every treatment for each species. The soil samples were preferably taken from pots where the plant was still alive so that inorganic nitrogen concentrations in the soil could be compared against the final height of the plant. The soil samples were taken by boring a small plastic tube with a diameter of 2.5cm into the soil and then removing soil that was more than 5mm below the surface for the analysis. To extract the inorganic nitrogen 10g of the soil sample was added to 1M KCl and shaken for one hour(Stock 1983). The solution was then filtered through filter paper and the filtrate was stored in the zero-degree room until it was analysed for nitrate, nitrite and ammonium. In addition to the fresh samples taken from the pots that had been in the glass house 5 soil samples were taken from the original soil that had been stored in a dry laboratory. The Comprised Cadmium Reduction Analysis was used to determine Nitrate levels, the Griess-Ilosvay method to determine Nitrite levels and the Manual Indo-Phenol Blue Determination to determine Ammonium levels (Stock 1983). In each case 15 samples were run against 5 standards and three blanks. The blanks were made up from the same stock of KCl solution that was used to extract the soil inorganic nitrogen. The KCl solution used for the blanks was also passed through the same type of filter paper that was used in the inorganic nitrogen extraction so that any contribution the filter paper could have made to the ammonium levels was eliminated (Stock 1983). The Nitrate concentrations were calculated using a standard curve constructed between 0.1 and 1.5 μg N ml^{-1}. The Ammonium concentrations were calculated from a standard curve constructed between 0.1 and 3.0 μg N ml^{-1} (Stock 1983). The R^2 values from the standard curves used to calculate Nitrate and Ammonium concentrations are shown in Table 4.

	N	Mean	Minimum	Maximum
Ammonium	12	0.9947	0.9806	0.9992
Nitrate	12	0.9928	0.9589	0.9997

Table 4: The mean, maximum and minimum R^2 values for the standard curves used to determine the concentration of ammonium and nitrate in the soil. For each nitrogen type there was a total of 12 batches,2 batches were run for each species and 2 for the pots with only soil and the original unmixed soil. In each batch there were 5 standards, three blanks and 15 samples.

Data analysis

For each plant the average heights of the plant in the different treatments were tested for significant differences using a One-Way ANOVA. The average soil inorganic nitrogen concentrations in the different treatments were tested for significant difference for the plants separately and then combined also using a One-Way ANOVA. ANOVA assumptions of

normality were tested graphically, using normal probability plots, and assumptions of homoscedasticity were tested using Levene's Test for Homogeneity of Variances (Zar 1999; StatSoft, Inc. (2002)). If data failed on one of these tests then the data was log transformed but if the data transformation was unsuccessful at improving the normality or decreasing the variance then the averages was tested for significant differences using a Kruskal-Wallis ANOVA. Significant differences are reported at $p < 0.05$.

Linear regressions were calculated between the change in height of each plant over the experiment and the final soil inorganic nitrogen concentrations, with the soil nitrogen as the independent variable. The linear regressions were used to determine if soil Nitrate concentrations, Ammonium concentrations or a combined value were the best predictor of growth, if indeed any of them were good predictors of growth.

RESULTS

Plant health and size

According to the health categorisation used, generally plants from all the species in the control, charcoal and bark treatments were healthier than plants in the sugar or sugar combination treatments (Figure 1). The difference in the health status of the plants in the treatments without sugar compared to the plants in the treatments with sugar was especially marked after the second batch of sugar was added. Whereas the percentage of plants that had died was low up to the point when the second batch of sugar was added it had increased substantially after the second batch was added. In most cases all the plants in the control, charcoal and bark treatments were classified as healthy at the end of the experiment. In the case of *P. ericoides* and *R.fruticosa* plants in the bark treatments were fared slightly worse than the plants in the Control and charcoal treatments.

For all the species the plants in the control and charcoal treatments were bigger than the plants in the pots where sugar was added (Figure 2). The heights of the *P. repens* and *L. salicifolium* individuals in the bark treatments were more similar to the heights of the plants of the same species in the control and charcoal treatments. For the other species the plants in the bark treatments were more similar in size to the plants in the sugar or sugar combination treatments. Although the *E. calycina* individuals in the bark treatments were considered healthy at the end of the experiment, like the plants in the charcoal treatment and the control, they were significantly smaller (Figure 3c) than plants in the control and charcoal treatments, and were not significantly taller than the plants in the plants in the charcoal & sugar combination treatment and the sugar treatment (ANOVA, $F = 49.94$, $df = 5$, $p < 0.05$).

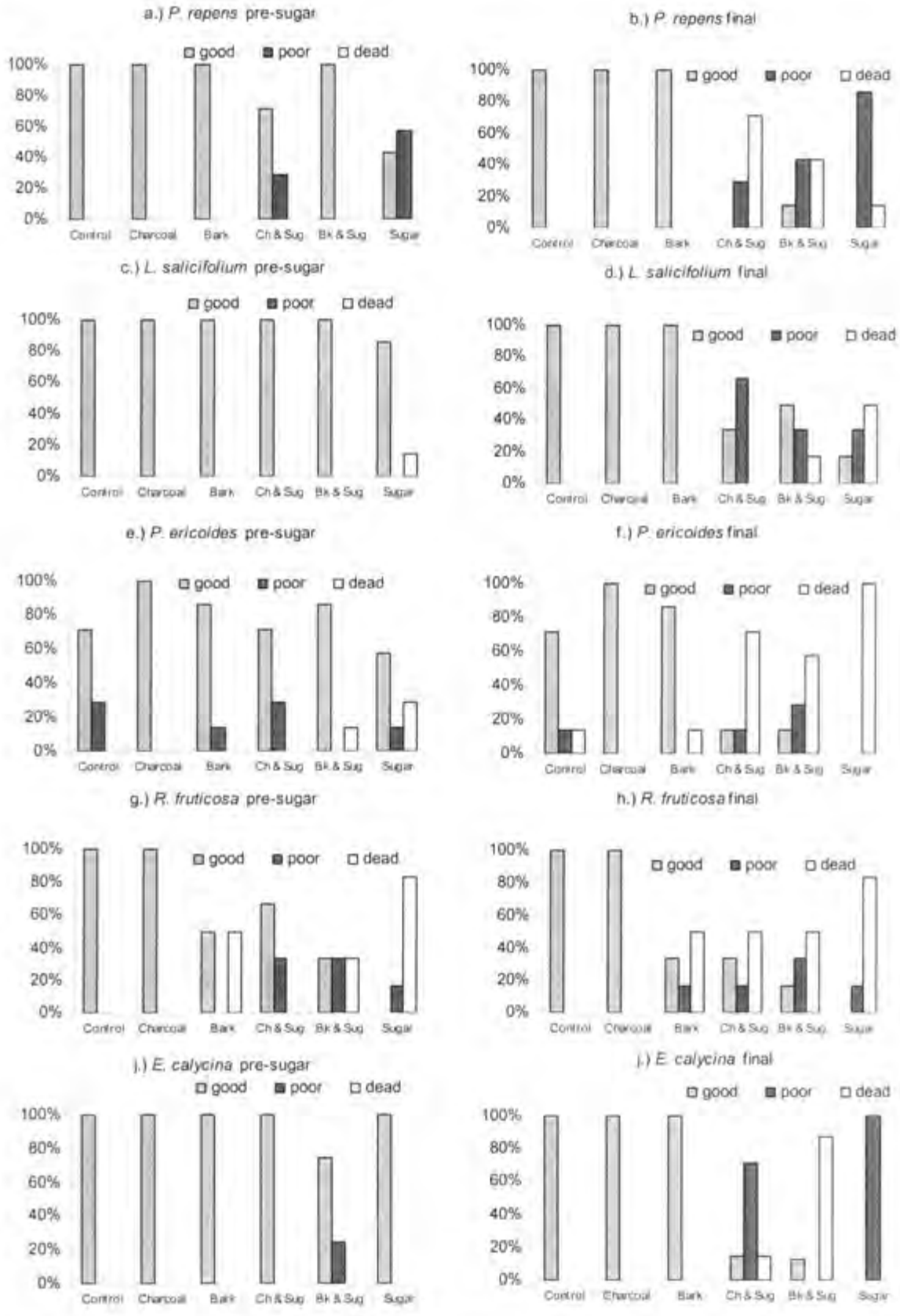

Figure 1: Bar-graphs showing the percentages of plants in the three health categories for the different treatments, before the second batch of sugar was added to the sugar treatments and at the end of the experiment. The data for the different species is shown separately.

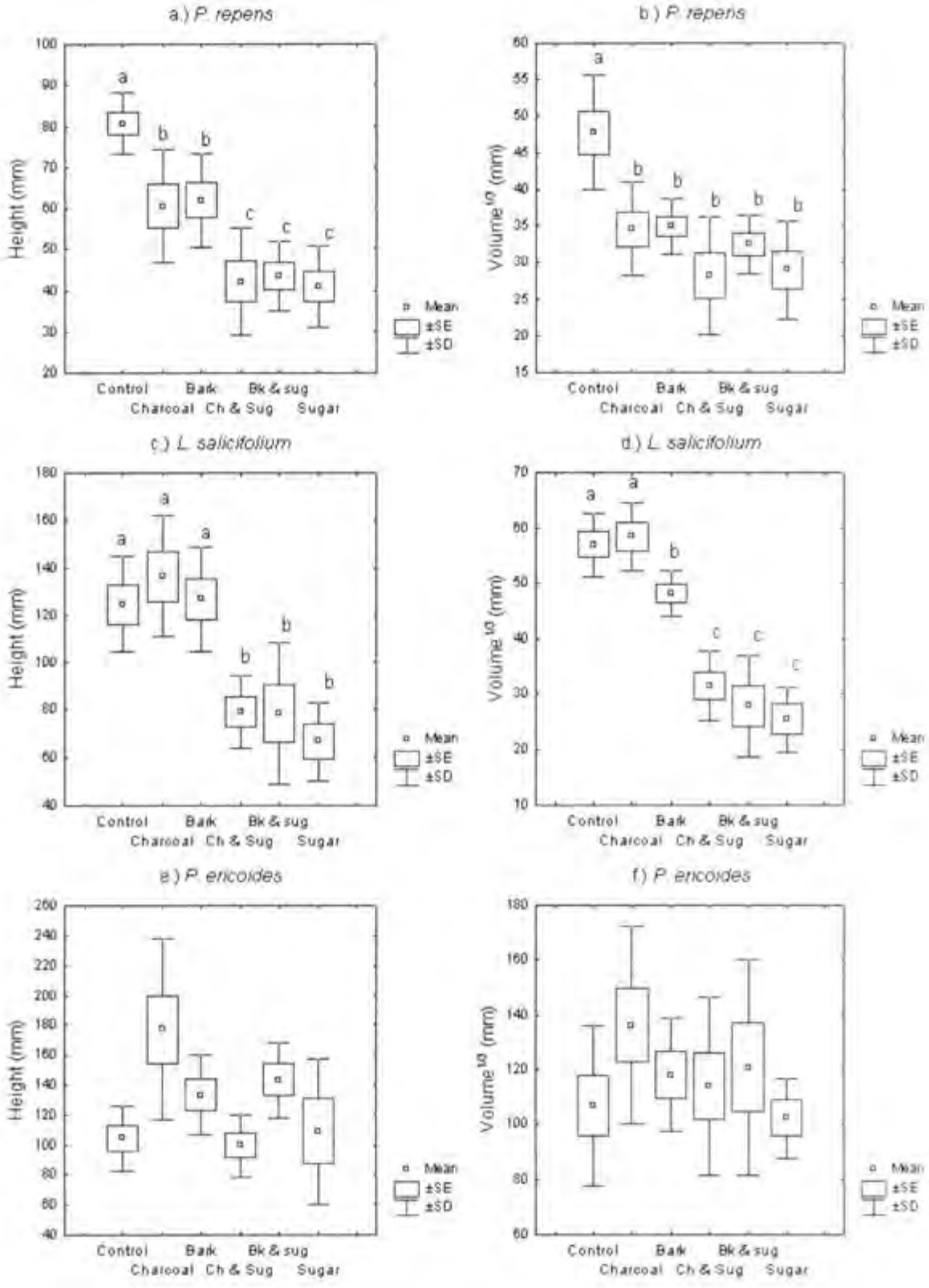

Figure 2: The average heights and cube root of the volumes of *P. repens*, *L. salicifolium* and *P. ericoides* in the various treatments at the end of the experiment. ANOVA showed that the average size of the *P.repens* and *L. salicifolium* plants at the end of the experiments were significantly different but not for the *P. ericoides* plants (see Table 5) . Letters indicate significant difference according to Fisher's LSD post-hoc test.

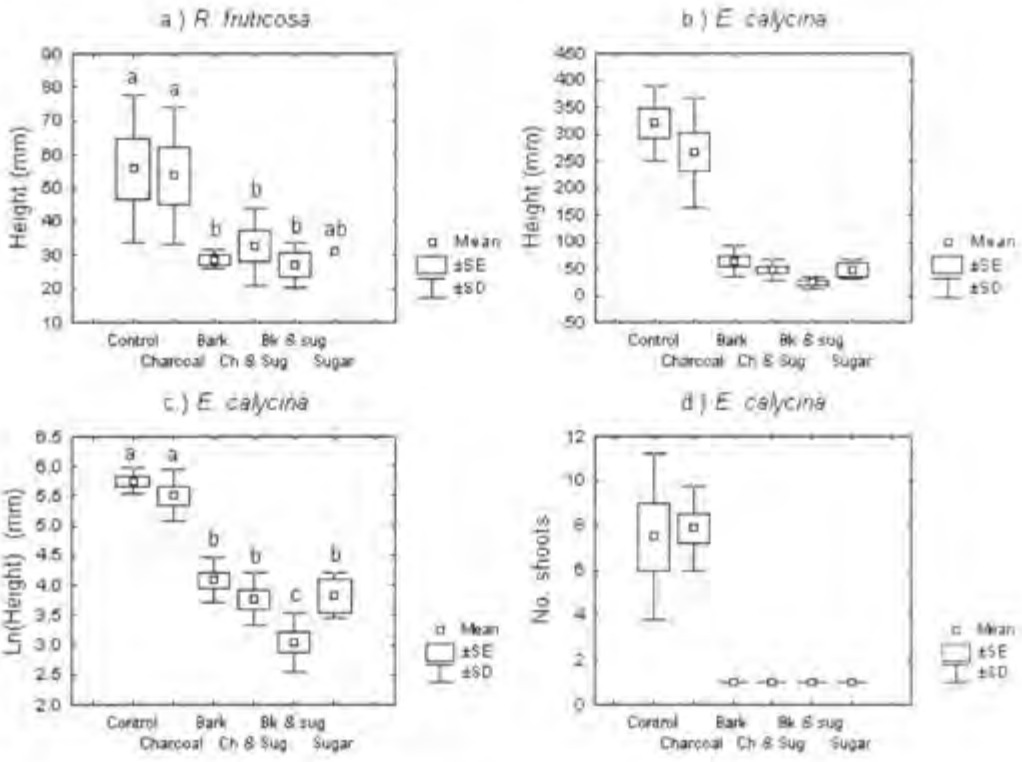

Figure 3: The final mean heights of *R.fruticosa* and *E. calycina* and the mean number of shoots per *E. calycina* individual in the various treatments. The results from the ANOVA tests are shown in Table 1. Letters indicate significant difference ($p < 0.05$).

	Height			Volume$^{1/3}$		
	F	df	p	F	df	p
P. repens	14.69	5	**0.000**	8.68	5	**0.000**
L. salicifolium	10.66	5	**0.000**	31.32	5	**0.000**
P. ericoides	-	-	-	0.97	5	0.453
R. fruticosa	2.78	5	**0.048**	-	-	-
*E. calycina**	46.94	5	**0.000**	-	-	-

Table 5: The results of the ANOVAs comparing the means heights and volumes of the plants in the different treatments at the end of the experiment. The heights of the *E. calycina* plants were log transformed (natural logarithm) to homogenize the variance.

Fisher's LSD test was used as opposed to the Tukey HSD post-hoc test because it is a less conservative test ~~and~~ than the Tukey test. The Tukey HSD test failed to show the significant difference between the mean heights of *R.fruticosa* in the different treatments even though an ANOVA had showed that generally the means were significantly different and thus in this case the less conservative LSD test was necessary. It was therefore used in all other cases where ANOVA showed significant differences for the sake of consistency.

Soil inorganic Nitrogen

Figure 4 shows the final mean soil inorganic levels in the different treatments. The data is based on samples taken from all the pots. Nitrate levels were higher in the control and charcoal treatments compared to the sugar and sugar combination treatments. This pattern is similar to the pattern of height differences in the different treatments for the *R.fruticosa, E. calycina* and *P. ericoides* plants. Soil nitrate levels in the bark treatments were more similar to the nitrate levels in the sugar and sugar combination treatments than the nitrate levels in the control and the charcoal treatment. The final soil nitrate levels in the treatments with sugar where all similar and approximately 1 $\mu g\ N\ g^{-1}$. The values in the control and charcoal treatment were approximately 6.5$\mu g\ N\ g^{-1}$ and 9.5 $\mu g\ N\ g^{-1}$, respectively. There was not as much variation in the soil ammonium levels compared to the soil nitrate. The range in the soil ammonium levels across the treatments was roughly 2 $\mu g\ N\ g^{-1}$, compared to the roughly 9 $\mu g\ N\ g^{-1}$ for the soil Nitrate levels. Most of the variation in the soil Nitrate levels was caused by the high levels in the control and the charcoal treatment. The sugar treatments had the second lowest amounts of soil Nitrate, the bark treatments had the lowest, but the highest levels of soil Ammonium. Both the soil nitrate and Ammonium levels of the original soil were approximately five $\mu g\ N\ g^{-1}$, which was higher than the values in the bark, sugar and sugar combination treatments but lower than the values in the control and the charcoal treatment. The soil nitrate and ammonium values were combined to give total soil inorganic nitrogen levels (Figure 4b). The pattern between total soil inorganic nitrogen was more similar to that of the soil nitrate levels compared to that of the soil ammonium levels, except that the values in the sugar treatments were relatively higher for the combined value because of the relatively high levels of soil ammonium in the sugar treatments.

ANOVA assumptions of homoscedasticity were not met for the soil Nitrate and Ammonium values if they were treated separately. By combining the values the variance was reduced and the means could be tested for significant differences using ANOVA. The test showed that the means for the different treatments were significantly different ($F= 41.22$, $df= 5$, $p < 0.05$). Significant differences between the means were then tested using Fisher's LSD post-hoc test. The mean for the control was significantly higher than the means in the other treatments, except for the mean in the charcoal treatment which was the highest (Figure 5). The mean soil inorganic levels in the bark treatments was significantly lower than the means in the charcoal and sugar combination treatment and the sugar treatment, but not significantly lower than the mean of the bark and sugar combination treatment. The mean in the bark and sugar combination treatment was significantly lower than the mean for the sugar treatment. The means from the charcoal and sugar combination treatment and the sugar treatment were not significantly different.

In all the species either an ANOVA or a Kruskal-Wallis ANOVA by ranks showed that the mean soil nitrate levels in the different treatments were significantly different, when the data from the soil with different species was kept separate (Table 6). Except in the case of *P. ericoides* the mean soil Nitrate levels in the control and charcoal treatment where higher than in the other treatments (Figure 6). The soil nitrate levels in the bark treatments were comparatively low in all cases and the lowest in the pots with *P. repens* and *L. salicifolium* individuals.

On the whole soil Nitrate levels in the pots with plants was slightly lower than the plants with no plants. The pattern between the treatments with regard to both soil Nitrate and soil Ammonium was similar in the soil with no plants as it was in the soil with plants, except that the soil nitrate in control treatments in the pots with the *L. salicifolium*, *E. calycina* and especially the *P. ericoides* individuals was lower than it was in the pots with no plants.

A pattern of soil Ammonium levels between the different treatments that fitted for all the species was less obvious to discern (Figure 7). In fact there was no significant difference between the means in the different treatments in the pots with *L. salicifolium* and *E. calycina* individuals. In the pots with *P. repens*, *P. ericoides* and *E. calycina* plants an ANOVA showed that the mean soil ammonium levels in the different treatments were significantly different (Table 7). A Kruskal-Wallis ANOVA by ranks showed that the means in the pots with *R.fruticosa* plants were also significantly different. One similarity between the pots with the different plants was that the soil Ammonium levels were comparatively high in the sugar treatments compared to the other treatments. Contrasting to the relationships between the mean soil Nitrate levels in the pots with no plants and the pots with plants, the mean soil Ammonium levels in the pots with plants is lower than it is in the pots with no plants.

Figures 4 to 7 indicate that there is a closer match between the differences in mean plant heights across the treatments and the differences in mean soil nitrate levels across the treatments as there exists between the mean soil Ammonium levels across the treatments. The plants in the control and charcoal treatments are generally taller than the plants in the sugar and sugar combination treatments. Similarly the mean soil Nitrate levels are generally highest in these two treatments. Final plant heights were smaller in the sugar combination and sugar treatments as were the soil Nitrate levels. The inconsistency in this observation that plant heights matched soil Nitrate levels was that even though soil nitrate levels were low in the bark treatments *P. repens* and *L. salicifolium* heights were relatively high. Correlation analyses showed that there was a positive and significant relationship between increase in plant height over the experiment and final soil Nitrate levels (Figure 9), i.e. higher final soil nitrate levels were associated with higher growth levels. Although the r-values were significant for the data from all the species the R^2 values were generally less than 0.5, which

meant that although the relationship between final soil nitrate levels and growth over the experiment was significant, soil nitrate levels alone was not a good predictor of growth.

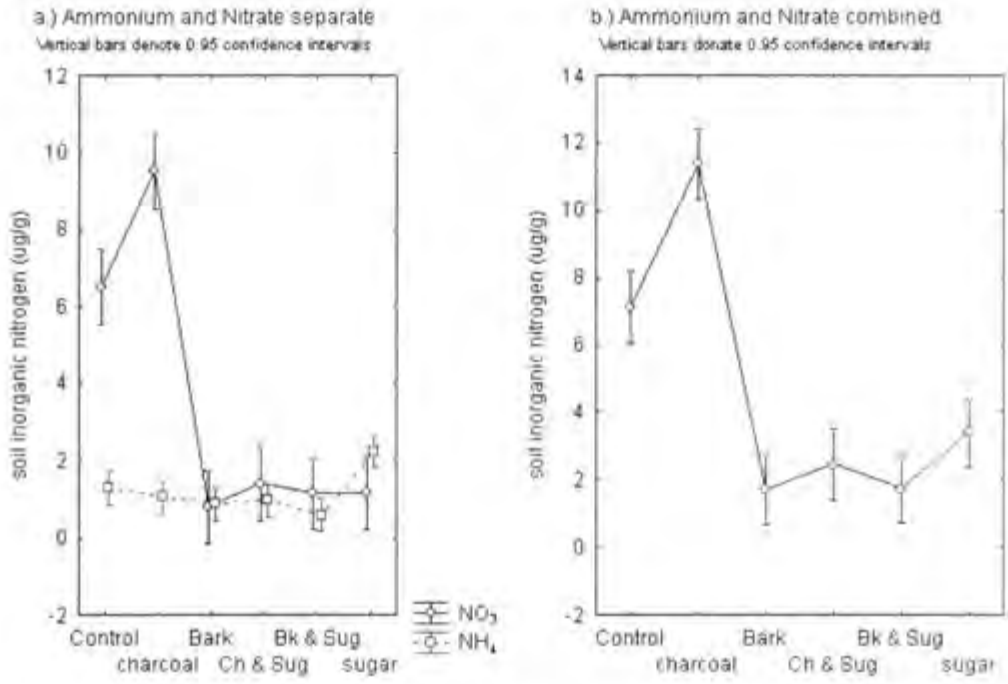

Figure 4: The soil inorganic levels in the different treatments combined across the species at the end of the experiment.

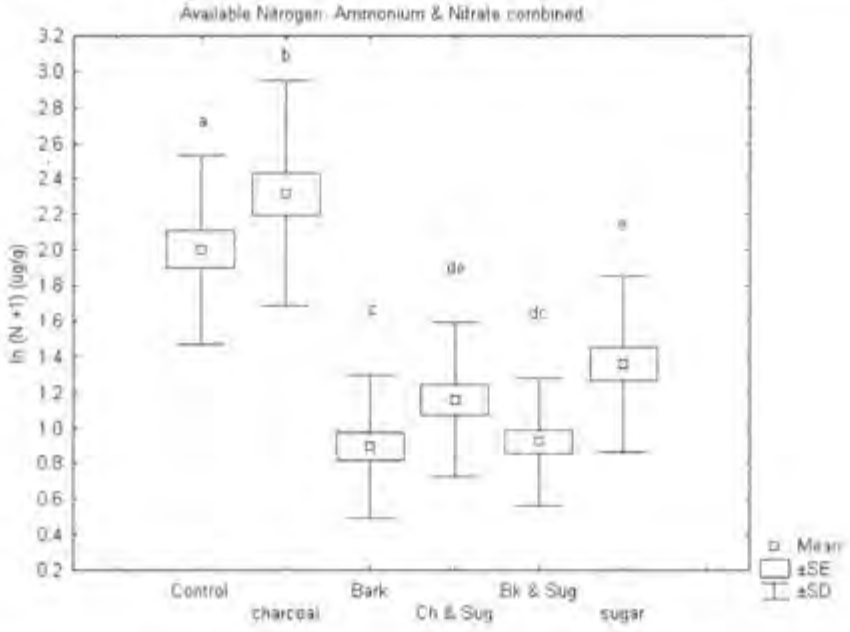

Figure 5: The mean soil available nitrogen (inorganic nitrogen) at the end of the experiment in the various treatments. The means were found to significantly different using a one-way ANOVA ($F = 41.22$, $d.f. = 5$, $p<0.05$). Letteres indicate significant difference accodrding to Fisher's LSD post-hoc test.

Nitrate

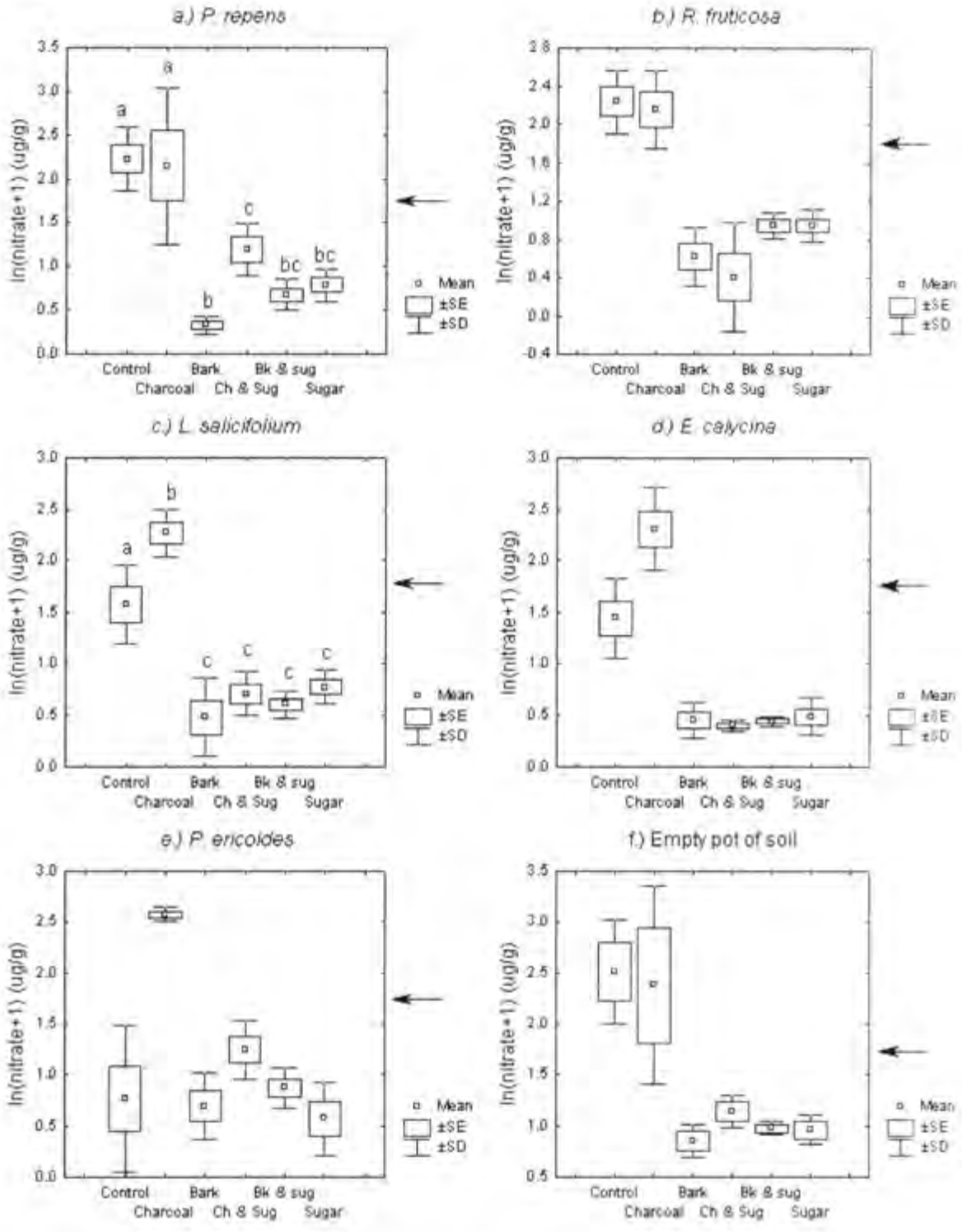

Figure 6: Box and whisker plots showing the mean, standard error and standard deviation of the soil nitrate levels at the end of the experiment ANOVA assumptions were only met for the *P. repens* and *L. salicifolium* pots, the letters indicate significant differences. Kruskal-Wallis ANOVA by ranks showed that the means in for the other four study species were also significantly different within species. The arrow indicates the level of nitrate in the original soil (dry). . Refer to Table 6 for the results of the ANOVA and Kruskal-Wallis ANOVA tests.

Nitrate						
	ANOVA			Kruskal-Wallis ANOVA		
	F	Df	p	H	df, N	P
P. repens	16.79	5	**0.000**			
L. salicifolium	34.27	5	**0.000**			
P. ericoides				15.37	5, N = 29	**0.009**
R.fruticosa				21.56	5, N = 30	**0.001**
E. calycina				20.9	5, N = 30	**0.001**
Empty pot				13.4	5, N = 18	**0.001**

Table 6: The results of the ANOVAs (Analyses of Variance) and the Kruskal-Wallis ANOVA by ranks testing the significant difference between the mean soil Nitrate levels in the different treatments. The data for the different species was kept separate (StatSoft, inc 2002).

Ammonium						
	ANOVA			Kruskal-Wallis ANOVA		
	F	Df	p	H	df, N	P
P. repens	7.71	5	**0.000**			
L. salicifolium				*6.27*	*5, N = 29*	*0.281*
P. ericoides	11.34	5	**0.000**			
R.fruticosa				15.06	5, N =30	**0.0101**
E. calycina	1.20	5	0.338			
Empty pot	3.36	5	**0.040**			

Table 7: The results of the ANOVAs (Analyses of Variance) and the Kruskal-Wallis ANOVA by ranks testing the significant difference between the mean soil Ammonium levels in the different treatments (StatSoft, inc 2002).

Plant Available Nitrogen						
	ANOVA			Kruskal-Wallis ANOVA		
	F	df	p	H	df, N	P
P. repens	16.01	5	**0.000**			
L. salicifolium	29.03	5	**0.000**			
P. ericoides	18.96	5	**0.000**			
R.fruticosa	19.76	5	**0.000**			
E. calycina	22.73	5	**0.000**			
Empty pot				12.79	5, N = 18	**0.025**
All combined	41.22	5	**0.000**			

Table 8: The results of the ANOVAs (Analyses of Variance) and the Kruskal-Wallis ANOVA by ranks testing the significant difference between the mean soil inorganic Nitrogen (NO_3 and NH_4 combined) levels in the different treatments (StatSoft, inc 2002).

Ammonium

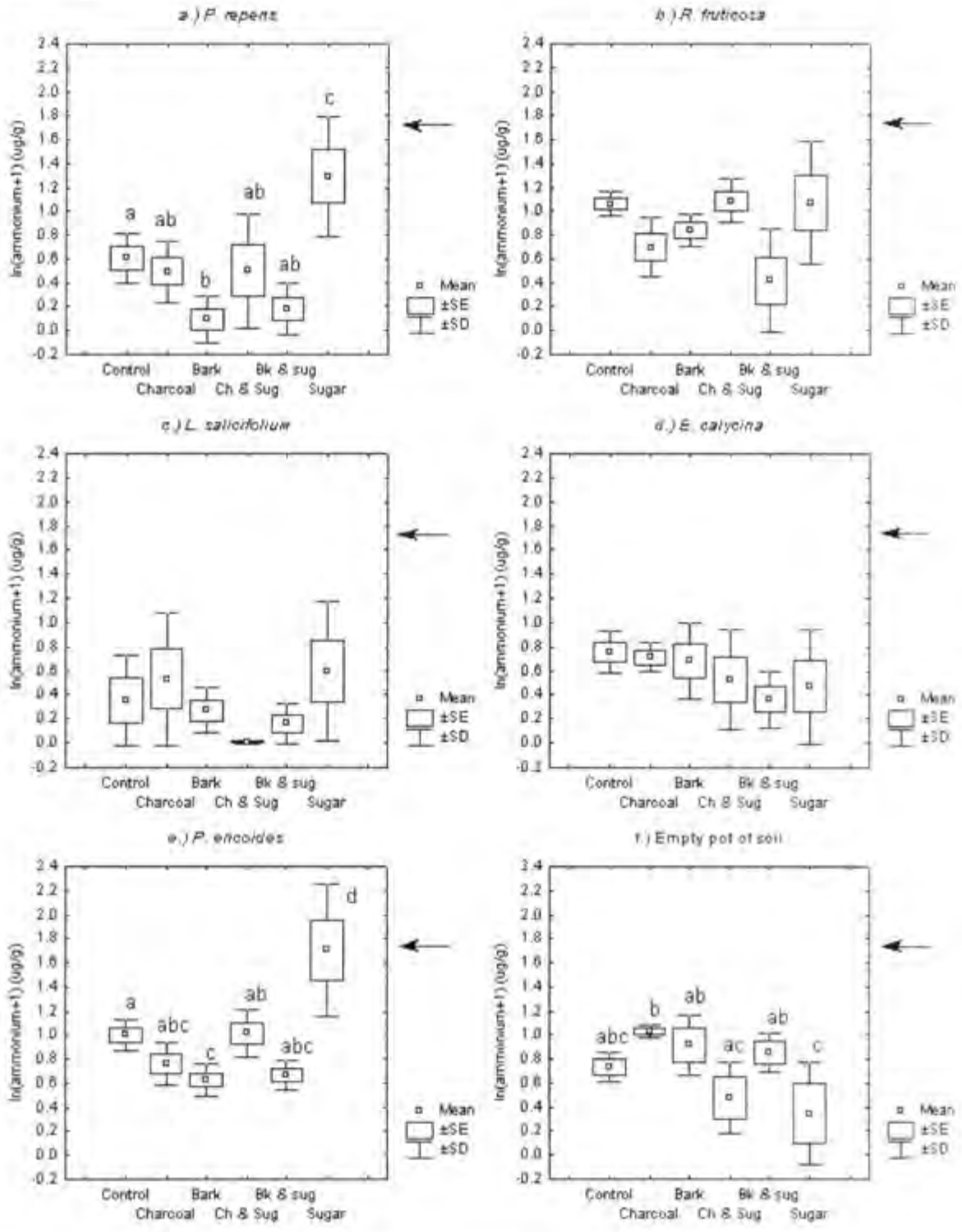

Figure 7: Box and whisker plots showing the mean, standard error and standard deviation of the soil ammonium levels at the end of the experiment. ANOVA assumptions were met for the *P. repens*, *P. ericoides*, *E. calycina* and Empty Pot values. Except for the *E. calycina* values the means within the treatments for other species were significantly different, the letters indicate significant differences. Kruskal-Wallis ANOVA by ranks showed that the means in the *R.fruticosa* pots were significantly different ($p < 0.05$), however they were not in the *L. salicifolium* pots. The arrow indicates the level of ammonium in the original soil (dry). Refer to Table 6 for the results of the ANOVA and Kruskal-Wallis ANOVA tests..

Total Available Nitrogen

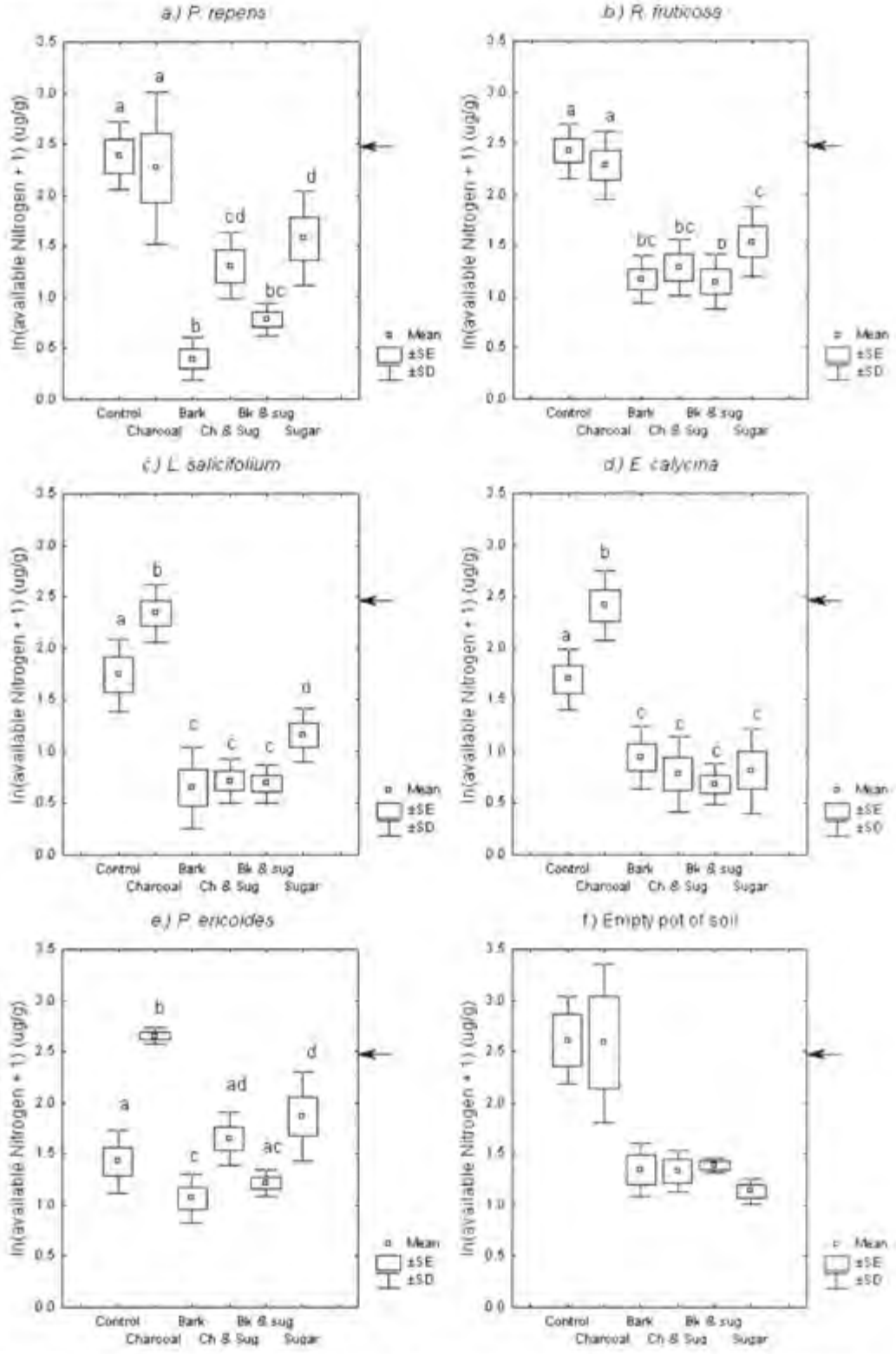

Figure 8: Box and whisker plots showing the mean, standard error and standard deviation of the soil available nitrogen levels at the end of the experiment. ANOVA assumptions were met for all the experiments except for the Empty Pot experiment, the letters indicate significant differences. Kruskal-Wallis ANOVA by ranks showed that the means in the Empty Pot experiment were significantly different ($p < 0.05$). The arrow indicates the level of available nitrogen the original soil (dry).

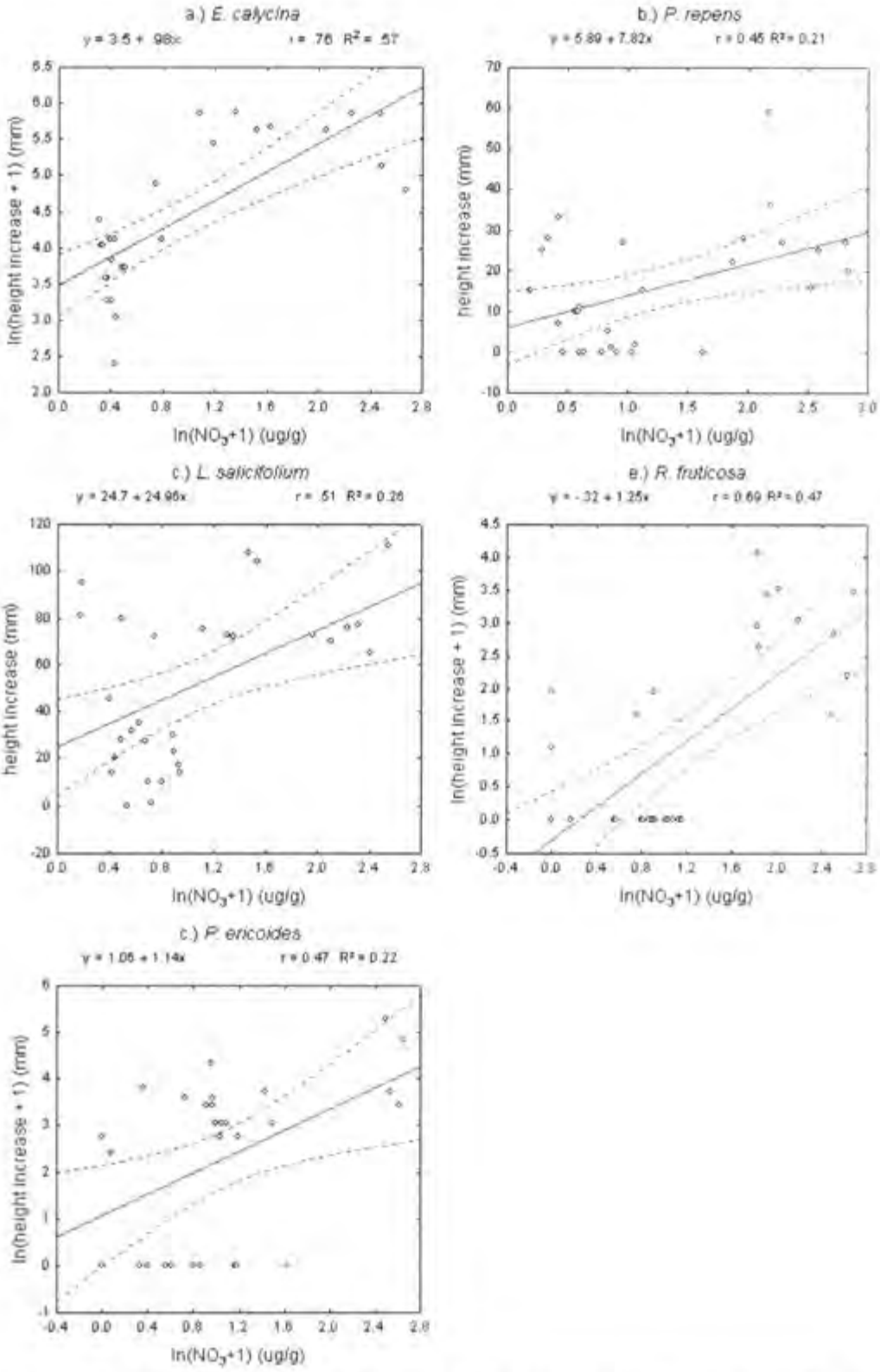

Figure 9: Scatterplots showing the relationship between soil Nitrate levels at the end of the experiments and the change in height for the five different species. All the relationship were significant ($p < 0.05$), and each graph was made up of between 29 to 30 points (Statsoft, inc 2002).

DISCUSSION

Plants in the control and charcoal experiments *were* the largest and healthiest plants at the end of the experiment. This implies that the high soil inorganic Nitrogen levels in the soil taken from the *Acacia* stand do not impede growth of fynbos types but rather seem to promote it. Neither were the soil inorganic levels of the *Acacia* soil an under-estimate of the potential for *Acacia* saligna species to enrich the soil with Nitrogen, as the values were even higher than those measured by Stock *et al.* (1995) in *A. saligna* soil, also on the West Coast. This is not to say that N enrichment of the soil by N fixing aliens cannot reach levels where it suppresses growth rates of fynbos species, as Stock *et al.* (1995) has shown that other *Acacia* species, specifically *A. cyclops* are more capable of increasing soil N levels than A. saligna. It might be that soil N concentrations in *A. cyclops* invaded fynbos can limit growth of fynbos species. Nevertheless it seems that in *A. saligna* soil once the aliens are cleared, subsequent invasion by weedy grass species is more detrimental to the restorations of these areas than the direct affect of high soil N on growth.

Yelenik *et al.* (2003) have already reported the propensity for stands cleared of *A. saligna* to be quickly colonised by *E. calycina*. *E. calycina* is a native fynbos grass but is not regarded as a dominant component of undisturbed fynbos communities. Yelenik has already shown that growth rates of *E. calycina* are elevated in the N rich *Acacia* soil compared to fynbos soil. Weedy grass species are often encountered as an obstacle in the restoration of artificially nitrogen enriched habitats (Zink & Allen 1998; Wilson & Gerry 1995, Powers *et al.* date unknown). For example the replacement of *Calluna* dominated-heathlands, in the British lowlands, by grasses such as *Molinia* has been attributed to increasing levels of atmospheric Nitrogen deposition (Power *et al.* date unknown). The competitive edge the extra nitrogen in the *Acacia* soils gives *E. calycina* over seedlings of other fynbos plants is apparent if one considers the respective growth rates of *E. calycina* in the untreated soil, i.e. the control, compared to that of the other species in the untreated soil. Whereas at the beginning of the experiment the *E. calycina* individuals were planted as seeds while the other species were planted as either seedlings or cuttings by the end of the experiment the *E. calycina* individuals in the control pots towered over individuals of the other species in the other pots. Based on this one can predict that *E. calycina* individuals would easily out compete seedlings of other fynbos species in stands recently cleared of A. saligna, if no organic amendments were added to the soil.

Bark, sugar or charcoal?

One of the aims of the study was to determine whether soil organic amendments can be used to facilitate the restoration of fynbos after it has been cleared of alien *Acacias*? The response of the plants to the different carbon sources varied and thus the question becomes which

organic amendment could work. Which organic amendment could prevent the establishment of E.calycina without negatively effecting the establishment of other more desired fynbos species. Sugar would not work because although it drastically reduces the growth and germination of E.calycina it also negatively affects the growth of other fynbos types. The same can be said about the sugar combination treatments. Charcoal would not work in that it does not seem to be an effective agent of nitrogen immobilisation, and both *E. calycina* growth rates and soil nitrate levels were as high in the Charcoal treatment as they were in the control. Bark chips could potentially work as they suppress the growth rate of *E. calycina* but overall has only a mild impact on the growth rates and health status of the other species, excluding *R.fruticosa*. Even though bark had a negative effect on *R.fruticosa*, if the addition of bark chips to the soil facilitated the establishment of fynbos shrubs such as *P. repens, L. salicifolium* and *P. ericoides* once these plants mature *E. calycina* would be excluded from the habitat as it is not known to prosper in the late successional stages of fynbos (Yelenik *et al.* 2003). Interestingly in other experiments that were successful at showing that organic amendments can promote the restoration of areas invaded by weedy grassy species bark chips and saw dust were also found to be the most appropriate carbon source (Zink & Allen 1998, Wilson & Gerry 1995).

Immobilisation, soil Nitrate and growth

The low growth rates in the sugar treatments can be explained by the corresponding low Nitrate levels in the sugar treated soil, compared to the control and charcoal treatment where final Nitrate levels and growth levels were high. Alternatively one could argue that argue that the relatively high Ammonium levels in the sugar treatments were toxic to the plants. Ammonium (NH_4) is toxic to plants cells as it is in equilibrium with Ammonia (NH_3), which rapidly diffuses across membranes, picks up Hydrogen ions and so disrupts the Hydrogen ion gradient across membranes, therefore if it is not rapidly assimilated to amino acids it becomes toxic to the plant itself (Lambers *et al.* 1998). The low total soil inorganic levels in the sugar and sugar combination treatments implies that sugar was an effective agent at promoting microbial activity and subsequently nitrogen immobilisation. Sugar is soluble in water and presumably could be easily accessed by the microbial community. Charcoal on the other hand is highly recalcitrant, which could explain why the inorganic levels in the charcoal treatment were still so high and similar to the control at the end of the experiment. The carbon in charcoal is no longer in its original organic form, but has been transformed to almost pure carbon, and therefore the soil microrganisms are unable to break it down. Therefore additions of charcoal do not promote microbial activity or nitrogen immobilisation.

The fact that the heights of *P. repens* and *L. salicifolium* plants in the bark treatment were significantly greater than the heights of the plants in the sugar and sugar combination treatments is surprising. Generally high growth rates were associated with high soil Nitrate

levels however in the bark treatment growth rates of *L. salicifolium* and *P. repens* were high despite low soil Nitrate levels. As stated in the introduction inorganic forms of nitrogen are generally considered to be more important as N sources for plants than inorganic Nitrogen. However in some situations where inorganic nitrogen levels are low organic nitrogen can become a potentially important source of Nitrogen (Schimel & Chapin 1996, Nasholm *et al.* 1998, Chapin *et al.* 1993). *P. repens* and *L. salicifolium* are both members of the Proteaceae family, and many members in this family are known to produce cluster roots in response to low nutrient conditions (Lambers *et al.* 1998 Schmidt *et al.* 2003). Schmidt *et al.* (2003) suggested that cluster roots have a special function in accessing soil organic N, and were able to show that *Hakea actities*, also a member of Proteaceae, produced more cluster roots when fertilised with Nitrate, glutathione or protein compared to plants fertilised with Ammonium or glutamine or plants not fertilised at all. Thus it seems plausible that *P. repens* and *L. salicifolium* were able to sustain relatively high growth rates by accessing organic nitrogen that originated from the bark added to the soil. Sugar is not made up of any organic nitrogen and thus its addition to the soil would not increase the concentration of organic nitrogen in the soil.

CONCLUSION

The relatively high growth rates of all the fynbos species, but especially *E. calycina*, in untreated soil taken from an old *A. saligna* stand suggests that the restoration of native fynbos in stands recently cleared of *A. saligna* will more likely be impeded by invasions of weedy grass species, particularly *E. calycina*, than the negative effects of high nitrogen levels on the growth of certain fynbos species. Sugar and bark chips were found to be successful at immobilising N in N rich soil taken from an old *A. saligna* stand. Charcoal was found to be ineffective at promoting N immobilisation. Bark chips were seen as the best choice of an organic amendment as it reduced both the number of shoots and the growth rate of *E. calycina* while it did not have a negative effect on the health of *P. repens*, *L. salicifolium* or *P. ericoides*.

REFERENCES

CHAPIN FS III, L. MOILANEN, K. KEILLAND. 1993. PREFERENTIAL USAGE OF ORGANIC NITROGEN FOR GROWTH BY A NON-MYCORRHIZAL SEDGE. NATURE 361:150 – 152.

HOLMES. P.M. 2002. DEPTH DISTRIBUTION AND COMPOSITION OF SEED-BANKS IN ALIEN-INVADED AND UNINVADED FYNBOS VEGETATION. AUSTRAL ECOLOGY VOL. 27:110-120

LAMB A.J., & KLAUSSNER E. 1988. RESPONSE OF THE FYNBOS SHRUNS *PROTEA REPENS* AND *ERICA PLUKETII* TO LOW LEVELS OF NITROGEN AND PHOSPHOROPUS APPLICATIONS. SOUTH AFRICAN JOURNAL OF BOTANY. 54(6):558-564.

LAMBERS H, F.S. CHAPIN III, T.L. PONS. 1998. PLANT PHYSIOLOGICAL ECOLOGY. SPRINGER. NEW YORK.

LEWIS, O.A.M. 1986. PLANTS AND NITROGEN. EDWARD ARNOLD LTD. GREAT BRITAIN.

NASHOLM T., A. EKBALD, A. NORDIN, R. GIESLER, M. HOGBERG & P. HOGBERG. 1998. BOREAL FOREST PLANTS TAKE UP ORGANIC NITROGEN. NATURE 392:914-916.

POWER, S.A., M.R. ASHMORE & D.A. COUSINS. *DATE UNKNOWN* IMPACTS AND FATE OF EXPERIMENTALLY ENHANCED NITROGEN DEPOSITION ON A BRITISH LOWLAND HEATH. *SOURCE UNKNOWN*.

STATSOFT, INC. (2002). STATISTICA (DATA ANALYSIS SOFTWARE SYSTEM), VERSION 6. WWW.STATSOFT.COM.)

SCHIMEL J.P. & F.S. CHAPIN III. 1996. TUNDRA PLANT UPTAKE OF AMINO ACID AND NH_4^+ NITROGEN IN SITU: PLANTS COMPETE WELL FOR AMINO ACID N. ECOLOGY 77:2142-2147.

SCHMIDT S., M. MASON, T. SANGTIEAN & G.R. STEWART. 2003. DO CLUSTER ROOTS OF *HAKEA ACTITTES* (PROTEACEAE) ACQUIRE COMPELX ORGANIC NITROGEN? PLANT AND SOIL. 248: 157 – 165.

STOCK, W.D. 1983. AN EVALUATION OF SOME COLORIMETRIC METHODS FOR THE DETERMINATION OF INORGANIC NITROGEN IN SOIL EXTRACTS. COMMUNICATIONS IN SOIL SCIENCES AND PLANT ANALYSIS. 14(10), 925-936.STOCK *ET AL.* W.D., K.T. WIENAND & A.C. BAKER. 1995. IMPACTS OF INVADING N_2-FIXING *ACACIA* SPECIES ON PATTERNS OF NUTRIENT CYCLING IN TWO CAPE ECOSYSTEMS: EVIDENCE FROM SOIL INCUBATION STUDIES AND ^{15}N NATURAL ABUNDANCE VALUES. OECOLOGIA 101:375-382.

VAN WILGEN B.W., D.C. LE MAITRE & R.M. COWLING. 1998. ECOSYTEM SERVICES,EFFICIENCY, SUSTAINABILITY AND EQUITY: SOUTH AFRICA'S WORKING FOR WATER PROGRAMME. TRENDS IN EVOLUTION AND ECOLOGY. 13:378-379.

WILSON S.D., A.K. GERRY. 1995. STRATEGIESFOR MIXED-GRASS PRAIRIE RESTORATION: HERBICIDE, TILLING AND NITROGEN MANIPULATION.

YELENIK, S. 2000. ECOSYSTEM LEVEL IMPACTS OF ANNUAL AND PERENNIAL N_2-FIXING INVASIVE ALIEN PLANTS IN THE FYNBOS VEGETATION OF SOUTH AFRICA. UNPUBLISHED MASTER'S THESIS, UNIVERSITY OF CAPE TOWN, SOUTH AFRICA.

YELENIK S.G., W.D. STOCK *ET AL.*, D.M. RICHARDSON. *IN PRESS*. ECOSYSTEM-LEVEL IMPACTS OF INVASIVE *ACACIA SALIGNA* IN THE SOUTH AFRICAN FYNBOS.

ZINK T.A., M.F. ALLEN. 1998. THE EFFECTS OF ORGANIC AMENDMENTS ON THE RESTORATION OF A DISTURBED COASTAL SAGE SCRUB HABITAT. RESTORATION ECOLOGY. 6:52-58

ZAR, J.H. 1999. BIOSTATISTICAL ANALYSIS. PRENTICE HALL, ENGLEWOOD CLIFFS, NEW JERSEY, USA.

APPENDIX A: A photographic essay of fynbos responses to organic amendments

Figure 1: The plants at the end of the experiment. The pots are organised in to rows and each row represents a different treatment. Besides the trolley holding the *R. fruticosa* pots the pots on the left are the controls and the pots on the right are the sugar treatments. From left to right the rows represent the control, the charcoal treatment, the bark treatment, the charcoal and sugar combination treatment, the bark and sugar combination treatment and the sugar treatment.

Figure 2: The *E. calycina* individuals at the end of the experiment.

Figure 3: The *P. repens* individuals at the end of the experiment.

Figure 4: The *L. salicifolium* individuals at the end of the experiment.

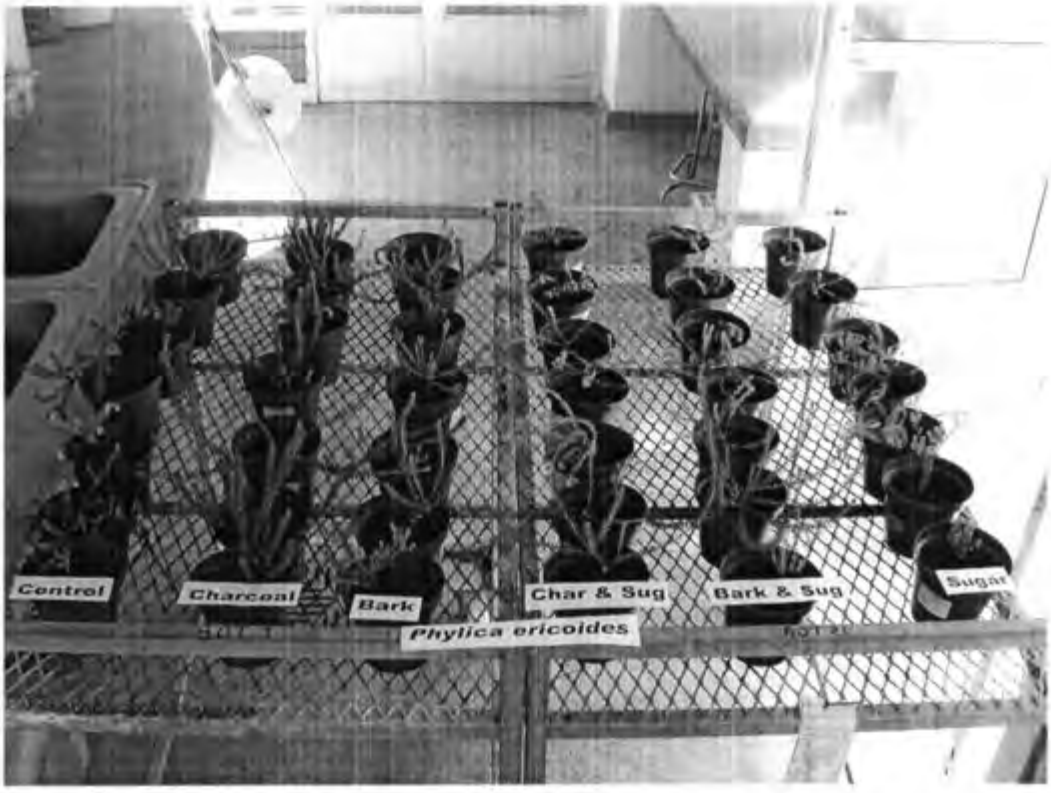

Figure 5: The *P. ericoides* individuals at the end of the experiment.

Figure 6: The *R. fruticosa* individuals at the end of the experiment.