The characterization of Rooibos tea soils and their effects on nitrogen nutrition of the plant

Botany honours project
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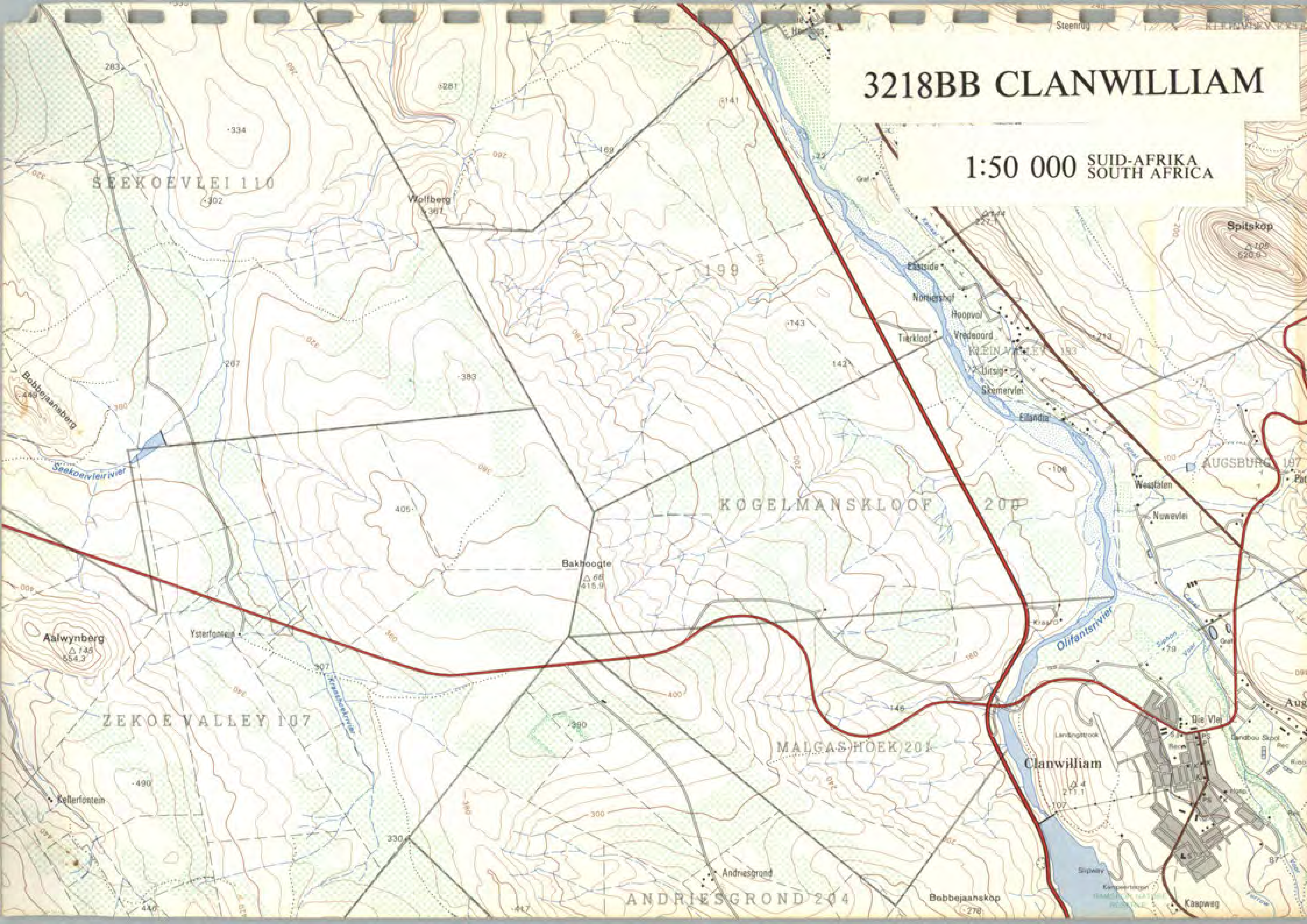
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ABSTRACT

The effects of soil characteristics and crop age on the nitrogen nutrition of the Rooibos tea plant *(Aspalathus linearis)* were studied, in order to elucidate the interaction between the plant and its environment in the cultivated state. The effects of Rooibos cropping over time on the soil parameters pH, organic matter, nitrogen content, and phosphorus content were investigated. These soil properties were also studied along a 400m toposequence supporting a 2-year old Rooibos crop. Leaf and stem N contents along the toposequence were used as an index to demonstrate the effect of topography on N nutrition. In addition, N_2-fixation was studied in 1- and 2-year old plants using the N-difference method, and the allocation of N to component organs determined. Soil organic matter, derived both from the Rooibos and previous crops, is purported to be the driving force behind the remaining soil and plant parameters studied in relation to crop age and topography, specifically through its effect on soil acidity. Nitrogen derived from fixation and soil N uptake increased with age, both as a result of improved soil conditions and greater nitrogen and carbon allocation to roots and nodules. It is proposed that premature death of cultivated Rooibos plants may be related to soil P deficiencies after the third year, and that patches of low productivity in Rooibos fields are either the result of ancient termitaria (heuweltjies) or increased fungal infection.

INTRODUCTION

Aspalathus linearis (Burm. fil.) R. Dahlgr. (ssp. *linearis*), commonly known as Rooibos, is found in its natural state only on the slopes of the Cedarberg and Olifants River mountain ranges of the Western Cape. In the early 1920's, a Russian immigrant, Benjamin Ginsberg, became the first distributor of Rooibos tea, which was sold under the trade mark Eleven O'Clock. He purchased the tea from the inhabitants in the mountains who cut and processed the wild plants. Increased demand for Rooibos tea in the 1930's led many farmers in the Clanwilliam district to begin cultivating Rooibos as a crop.

In recent years, a profitable export market for Rooibos tea has developed, with Japan, Germany and the US being the largest purchasers. Rooibos tea is caffeine-free, and contains far less tannin than Ceylon tea. It is therefore often marketed as a health drink. In Japan, Rooibos tea can be prescribed for nervous tension, allergies, and digestive problems, and as a source of anti-oxidants to counteract the free radicals in the body and thus delay ageing (Yoshikawa et al. 1990).

The soil in the Clanwilliam district is derived from Table Mountain sandstone, and tends to be sandy, well-drained and acidic. The main area of Rooibos tea production receives almost 80% of its rain in the winter, with an annual rainfall of approximately 375mm. However, the active growth period for Rooibos is in the spring. Therefore, the plants use their 2-3 metre long tap root to acquire below-ground water, and are fairly drought resistant.

Fields intended for cultivation are prepared after the first winter rains (April/May) by means of deep ploughing. Juvenile plants are outplanted from well-irrigated seed-beds in June/July. The first harvest takes place roughly 18 months after planting, and two more profitable crops are usually obtained over the following two years. However, the fourth and fifth crops are generally much lower in yield. This decline in productivity after the third crop is commonly associated with fungal infection that intensifies with plant maturity.

Despite the economic importance of this crop, very little research has been done on it. So far, the only published studies on Rooibos relate to the taxonomy of its nodule-forming bradyrhizobia and its medicinal properties. Therefore, if yields are to be increased, there is a need to understand the physiology of this species, especially the interaction between the plant and its environment.

In this study, two approaches were adopted. In one, the effects of Rooibos cropping on various aspects of soil properties were investigated. Soil parameters, such as pH, organic matter, nitrogen content and phosphorus content, were studied in three different fields, comprising 1-, 2- and 3-year old Rooibos plants. Additionally, these soil properties were also studied along a 400m toposequence supporting 2-year old Rooibos plants.

The second approach examined the N nutrition of Rooibos tea plants. In this study, a number of 1- and 2-year old Rooibos plants were dug up for assessing whole-plant N content and N allocation to component organs in relation to age. Furthermore, as the Rooibos tea plant is an N_2-fixing species, attempts were made to estimate the plant's relative dependence on soil N and symbiotic fixation. This was done by using a non-legume of equal age and with similar rooting pattern to estimate N uptake by the legume. Additionally, leaf and stem N contents were measured along the toposequence, and used as an index to demonstrate the effect of topography on N nutrition in Rooibos.

All experiments were performed on a farm approximately 10km west of Clanwilliam (see map). The farmer, Mr. Willie Nel, follows the same rotational cropping scheme on all Rooibos fields. Oats are sown for two years prior to planting Rooibos, with the first oats crop being harvested and the second lightly grazed by sheep. An inorganic phosphate fertilizer is evenly applied to the oats fields in both years prior to the Rooibos, at a dose of 300kg per hectare. Rooibos is then cultivated for about five years, followed by wheat and oats for another five to eight years. This rotational cropping system is designed to break the cycle of the fungal infection to which Rooibos plants are prone.

MATERIALS AND METHODS

Soil Parameters and Cropping Length

Soil pH

Soil samples were randomly collected from the 1-, 2-, and 3-year fields. The samples were taken from a depth of 0-30cm and mixed thoroughly. After sieving (2mm size) 25g soil subsamples were shaken in 50ml of 0.01M $CaCl_2$ for 15 min, and the pH of the suspension measured on a pH meter.

Soil Organic Matter

10g soil subsamples (2mm-sieve) were oven-dried at 80°C overnight to expel all moisture. After weighing, they were transferred to a muffle furnace at 450°C for 16 hours and then weighed after cooling. The organic matter content was determined by subtracting the final weight from the oven-dried weight, and dividing by the original sample weight (10g).

Soil Nitrogen Content

The total nitrogen concentrations of 1g soil samples were determined by the Kjeldahl procedure (Nelson and Le Sommers 1973). A digestion step, involving salicylic acid, sulphuric acid, sodium thiosulphate and a selenium catalyst, was used to convert organic and nitrate N to ammonium. A set of standards, containing 0.7 to 4.9 mg N in the form of Titriplex III, were also digested. After digestion, each tube was made up to 25ml using distilled water, and a 2ml aliquot distilled until all the nitrogen condensed into 2ml of 0.02M hydrochloric acid. This distillate was then titrated against 0.005M NaOH, and the result recorded. The nitrogen content represented by the resultant value was read off the standard curve.

Soil Phosphorus Content

Soil samples (1g in each case) were digested in 1ml of triacid mixture (HNO_3:H_2SO_4:HCl) at 180°C. When the samples cleared, they were cooled and diluted to 25ml with distilled H_2O. The phosphorus content of 10ml aliquots were then determined by the molybdenum blue method (Murphy & Riley 1962), by reading at a wavelength of 882nm on a bench spectrophotometer. A set of standards containing known amounts of P in the range 2-25μg were included, and their optical density readings used to generate a simple linear regression equation. This standard curve was then used to calculate the phosphorus levels in the samples. Since the standards in the Murphy & Riley assay are not included in the digestion procedure, the P concentrations of the soil samples were multiplied by a correction factor of 250.

Soil Parameters and Topography

A 400m transect along a sloping field bearing a 2-year old Rooibos crop was divided into 8 stretches of 50m each. A soil sample was taken from each 50m stretch, and the pH, organic matter, and nitrogen and phosphorus levels were determined using the same methods described for the 1- and 3-year Rooibos fields.

Nitrogen Nutrition and Crop Age

Total N Content

Three 2-year old and four 1-year old Rooibos plants were dug up, and separated into leaves, stems, roots and nodules. They were then oven-dried to constant weight at 80°C before being weighed and ground to 40 μm size. The nitrogen contents of these samples were determined by the Kjeldahl procedure.

Non-Legume N Content

Three non-leguminous plants belonging to the Asteraceae were dug up from the 1-year and 2-year fields, and the nitrogen contents of the leaves, stems and roots determined as for the Rooibos plants. The total amount of nitrogen in these reference plants was averaged for each of the two fields.

N Fixed by Rooibos

The amount of nitrogen fixed by the Rooibos plants was then estimated by the N-difference method as:

N Fixed = Total N in Legume - Total N in Reference Plant

A basic assumption inherent in this method is that the legume and control crops assimilate the same amount of soil N (Peoples & Herridge 1990). In order to comply with this assumption, reference plant types that appeared to be exploring the same rooting volume were chosen.

Leaf and Stem N Content with Age

Stems were taken randomly from more than 20 plants of each of the three age groups. They were then oven-dried for 48 hours at 80°C, and the leaves separated from the stems. Stems and leaves were then ground separately to a size of 40 μm, and the nitrogen contents of 0.1 g samples of leaves or stems determined by the Kjeldahl procedure as described above.

N Nutrition and Topography

A 400m transect along a sloping field bearing a 2-year old Rooibos crop was divided into 8 stretches of 50m each. A stem sample was collected from every plant in three rows of Rooibos in each 50m stretch. After oven-drying the samples for 48 hours at 80°C, the leaves and stems were separated and ground to a size of 40μm. The nitrogen content of 0.1g subsamples was then determined by the Kjehdahl procedure as described above.

Statistical Analyses

All data analyses were performed on a statistical software package, Statgraphics V5. When normal probability plots indicated parametric data, one-way Analyses of Variances (ANOVA) were performed. Where data sets did not show normal distributions, the Kruskall-Wallis Test was used. When two data sets with small sample sizes were being compared, such as in the nitrogen and biomass allocation experiments, the Mann Whitney-U Test was performed. In addition, regression analyses were done on both the age and catena data by plotting each soil and plant parameter studied against each of the remaining parameters. Significance levels were determined by reading off the probabilities on a 1-tailed table of critical *r* values.

RESULTS

Effects of Cropping Length on Soil Characteristics

Soil pH

The soil from the 1-year old Rooibos field was strongly acidic, and had a pH that was significantly different ($p<0.05$) from the soil pH of the 2- and 3-year fields (Figure 1.1).

Soil Organic Matter

The soil organic matter also increased significantly ($p<0.05$) from the 1- to the 2-year Rooibos fields (Figure 1.1). Regression analyses revealed a significant positive correlation ($P<0.025$) between the soil organic matter content and the soil pH over the three years (Table 1).

Soil Nitrogen Content

The soil nitrogen content of the 2-year soil was significantly higher ($p<0.05$) than that of the 1- and 3-year soil values. Soil N correlated positively with soil organic matter ($P<0.05$), but not with pH (Table 1).

	Soil pH	Organic Matter	Soil N	Stem N	Leaf N	Soil P
Soil pH	___	0.737	0.404	0.864	0.829	0.723
Organic Matter	Sig. 2.5%	___	0.777	0.834	0.867	0.889
Soil N		Sig. 1%	___	0.581	0.686	0.786
Stem N	Sig. 1%	Sig. 1%		___	0.881	0.826
Leaf N	Sig. 1%	Sig. 1%	Sig. 2.5%	Sig. 1%	___	0.861
Soil P	Sig. 2.5%	Sig. 1%	Sig. 1%	Sig. 1%	Sig. 1%	___

Table 1: r-values for the regression equations of each soil and plant characteristic for the 1-, 2- & 3-year Rooibos fields plotted against each of the other characteristics. Significance levels are indicated where they apply.

	Soil pH	Organic Matter	Soil N	No. of Plants	Stem N	Leaf N	Soil P
Soil pH	___	0.707	0.762	0.557	0.425	0.665	0.421
Organic Matter	Sig. 2.5%	___	0.835	0.623	0.632	0.749	0.562
Soil N	Sig. 2.5%	Sig. 1%	___	0.456	0.735	0.799	0.245
No. of Plants		Sig. 5%		___	0.263	0.439	0.705
Stem N		Sig. 5%	Sig. 2.5%		___	0.944	0.109
Leaf N	Sig. 5%	Sig. 2.5%	Sig. 2.5%		Sig. 1%	___	0.044
Soil P				Sig. 5%			___

Table 2: r-values for the regression equations of each soil and plant characteristic along the toposequence plotted against each of the other characteristics. Significance Levels are indicated where they apply.

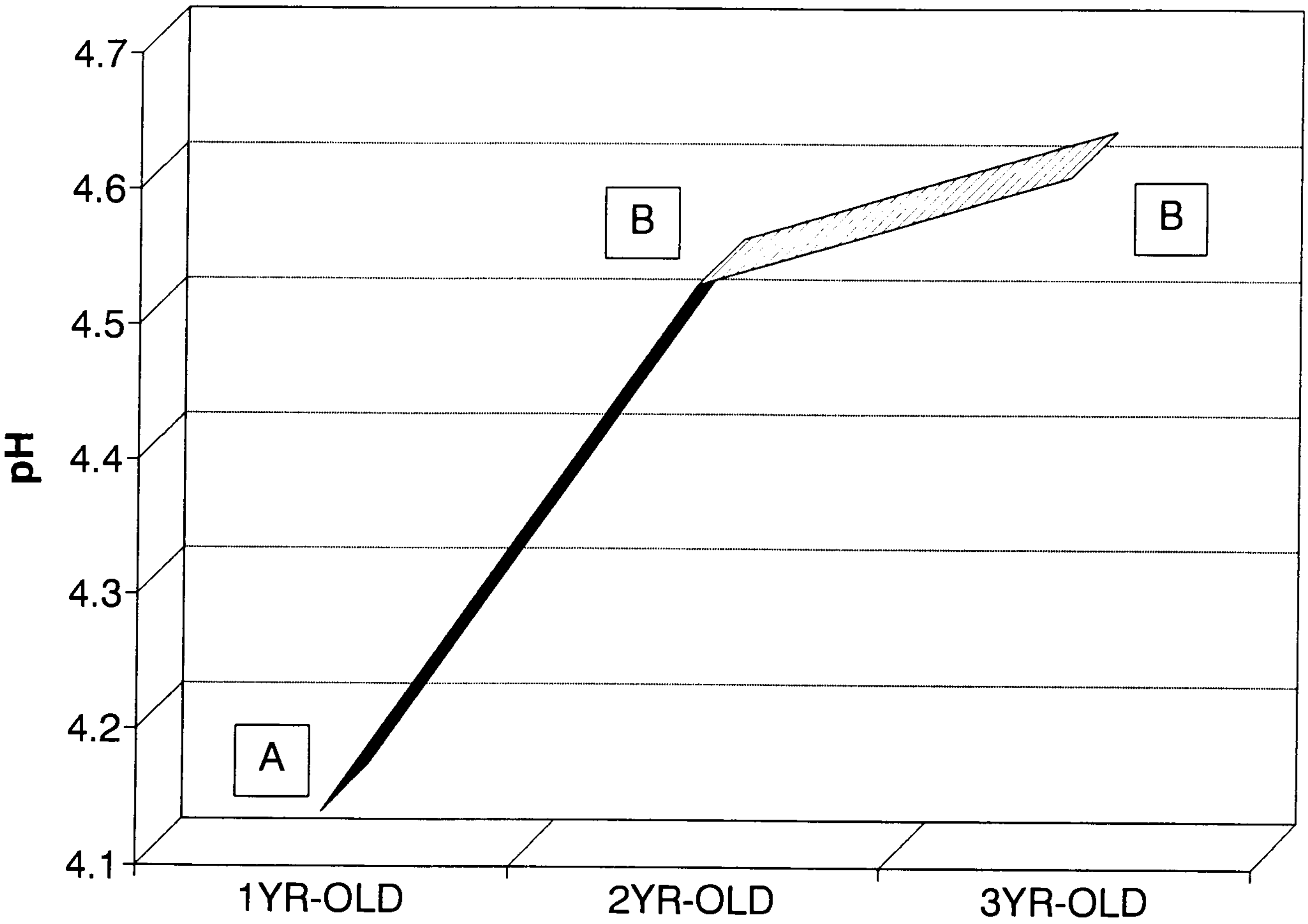

Figure 1.1 Soil pH levels of fields supporting 1-, 2- & 3-year old Rooibos plants. Different letters indicate significant differences ($p < 0.05$) between values.

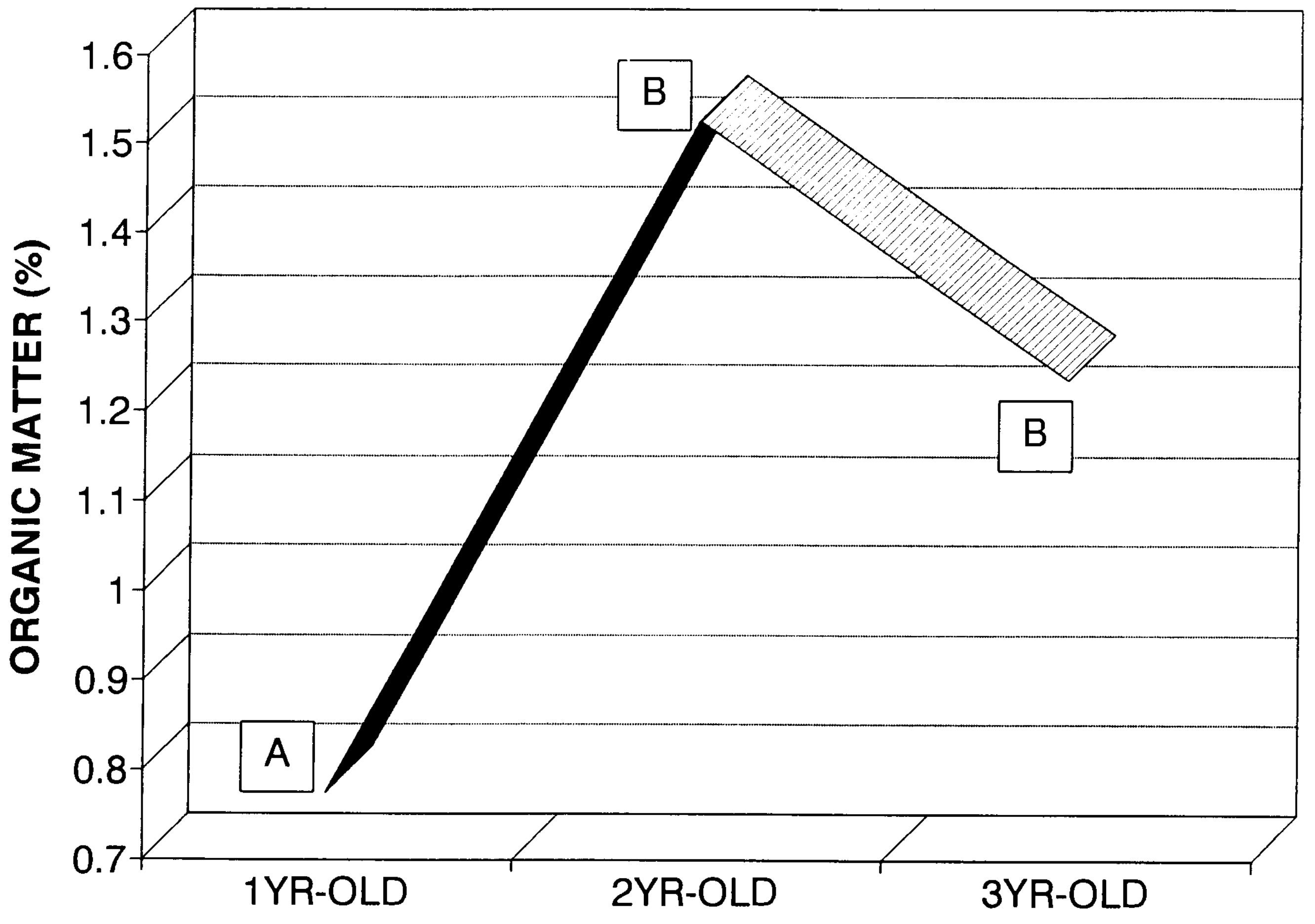

Figure 1.2 Soil organic matter levels of fields supporting 1-, 2- & 3-year old Rooibos plants. Different letters indicate significant differences ($p<0.05$) between values.

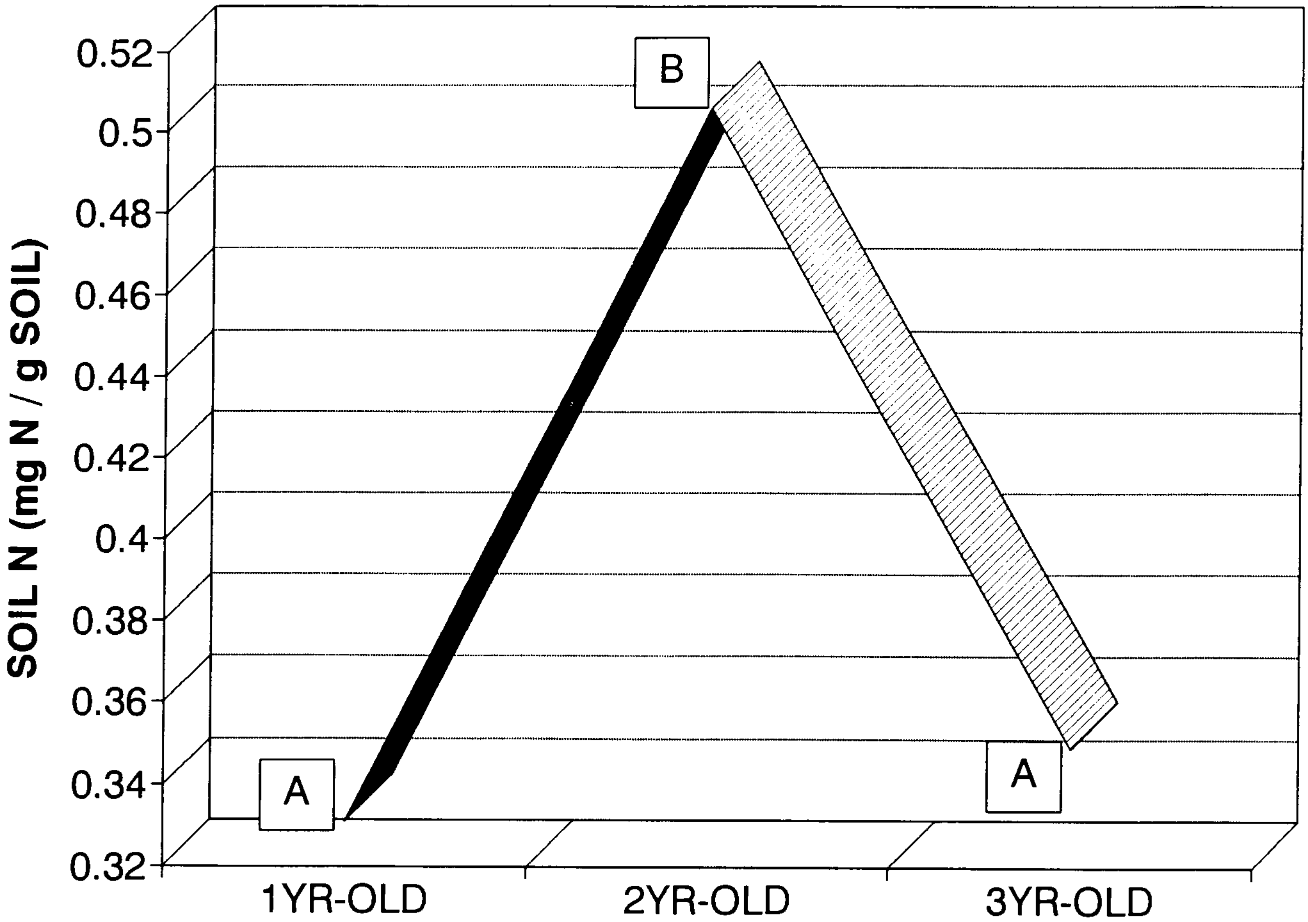

Figure 1.3 Soil nitrogen contents of fields supporting 1-, 2- & 3-year old Rooibos plants. Different letters indicate significant differences ($p<0.05$) between values.

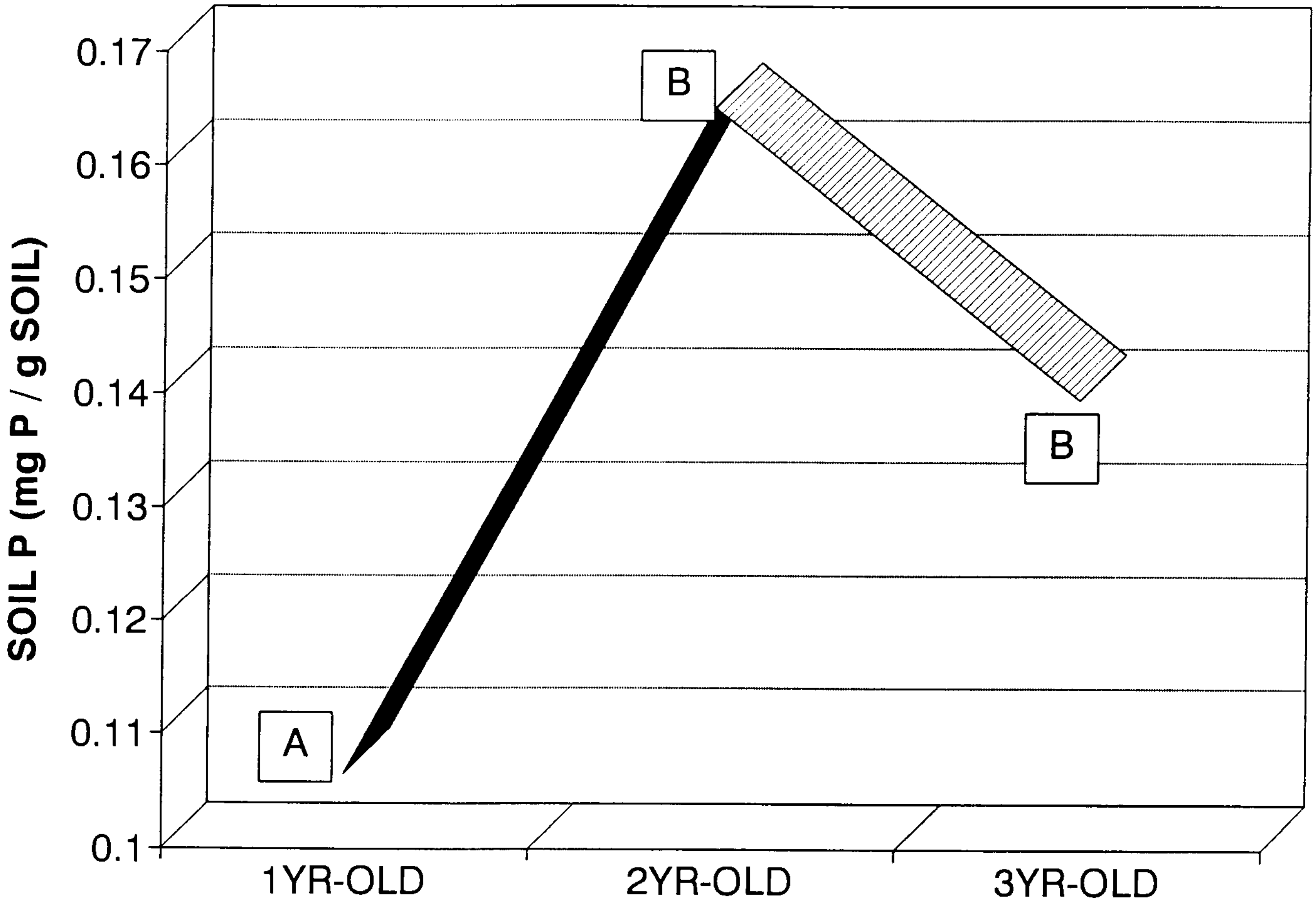

Figure 1.4 Soil phosphorus levels of fields supporting 1-, 2- & 3-year old Rooibos plants. Different letters indicate significant differences ($p<0.05$) between values.

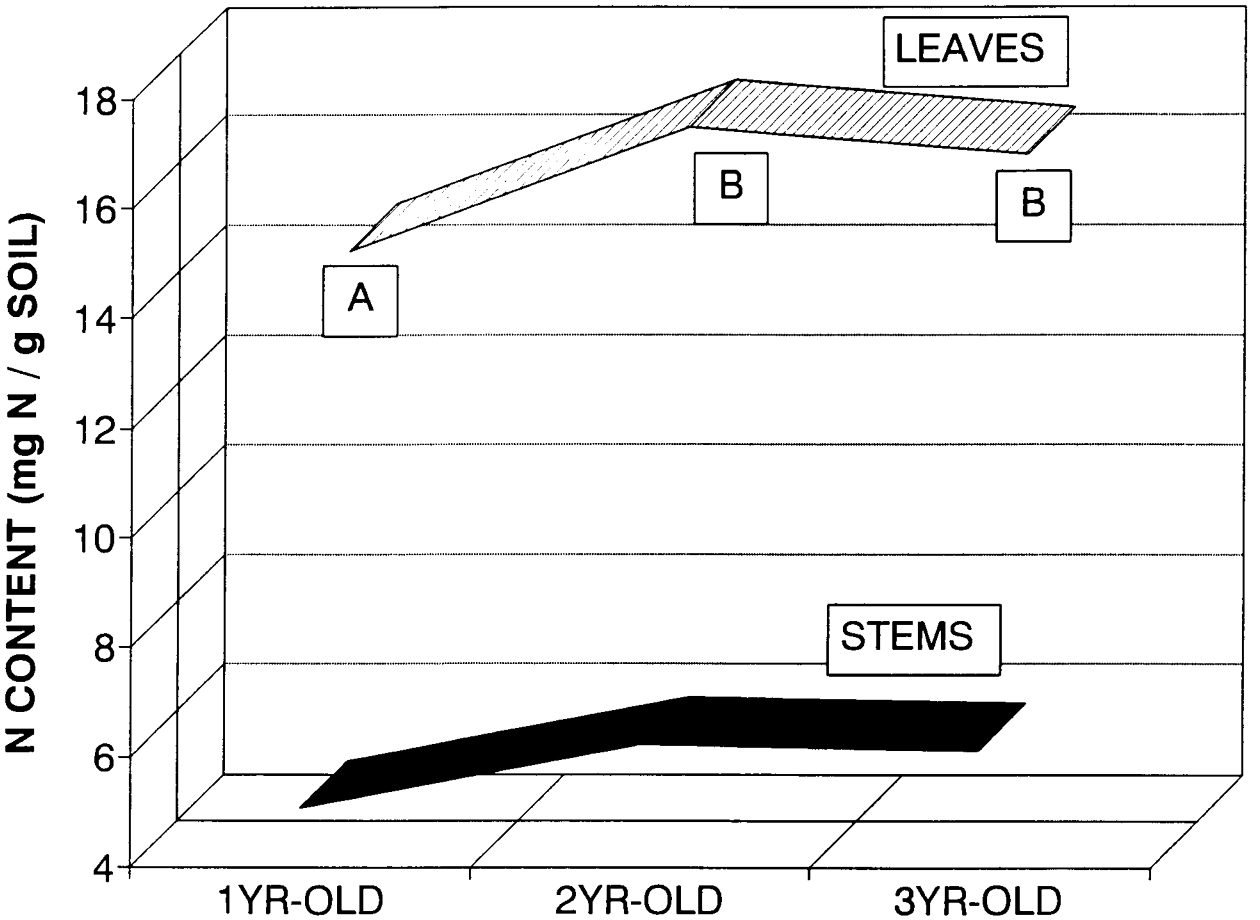

Figure 1.5 Leaf and stem nitrogen contents of 2-year old Rooibos plants down a toposequence. Different letters indicate significant differences ($p < 0.05$) between values.

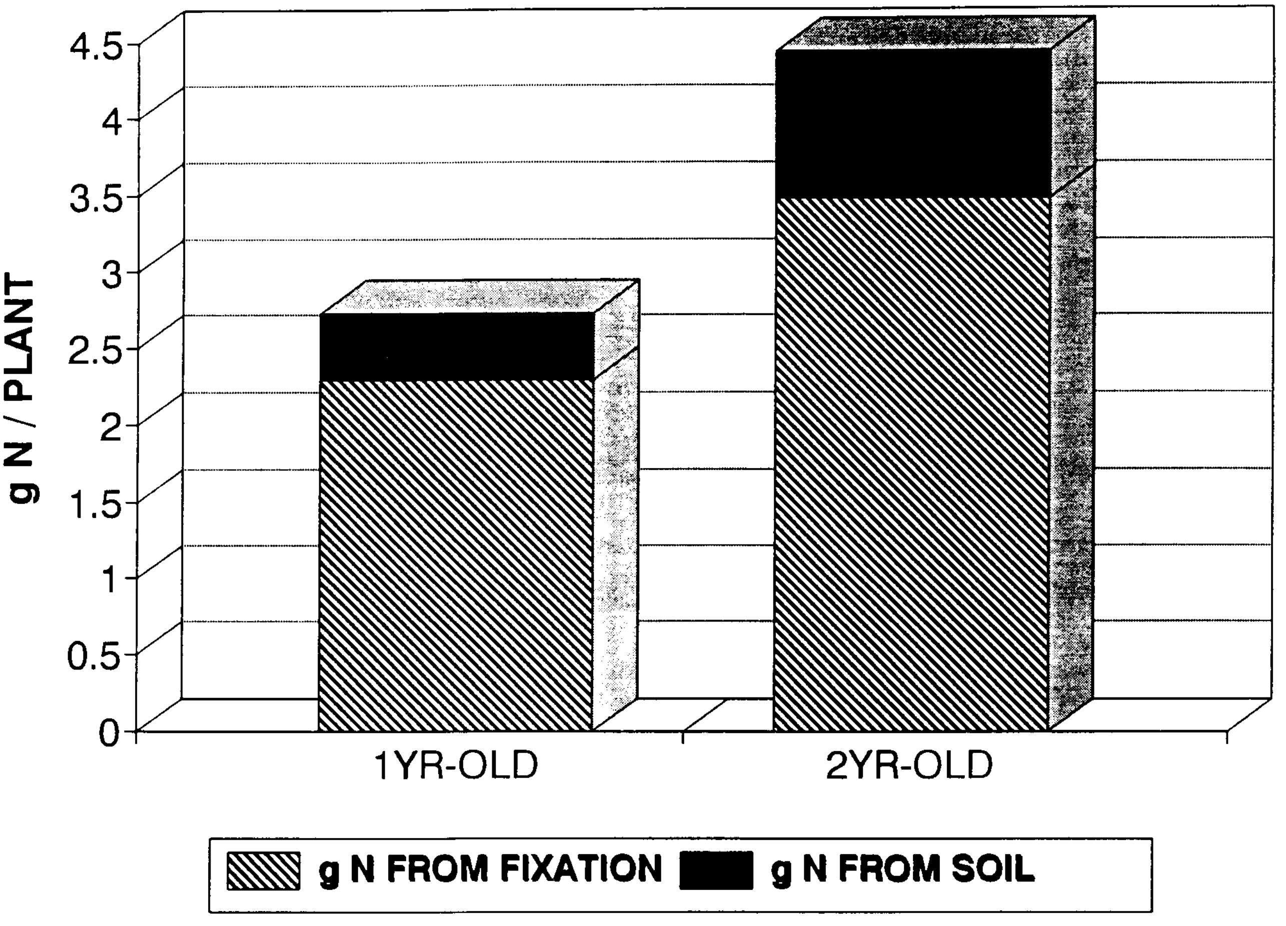

Figure 1.6 The sources of nitrogen in 1- & 2-year old Rooibos plants. The g N from the soil is the amount of N in the reference plants. The g N from fixation is total N minus g N in the reference plants.

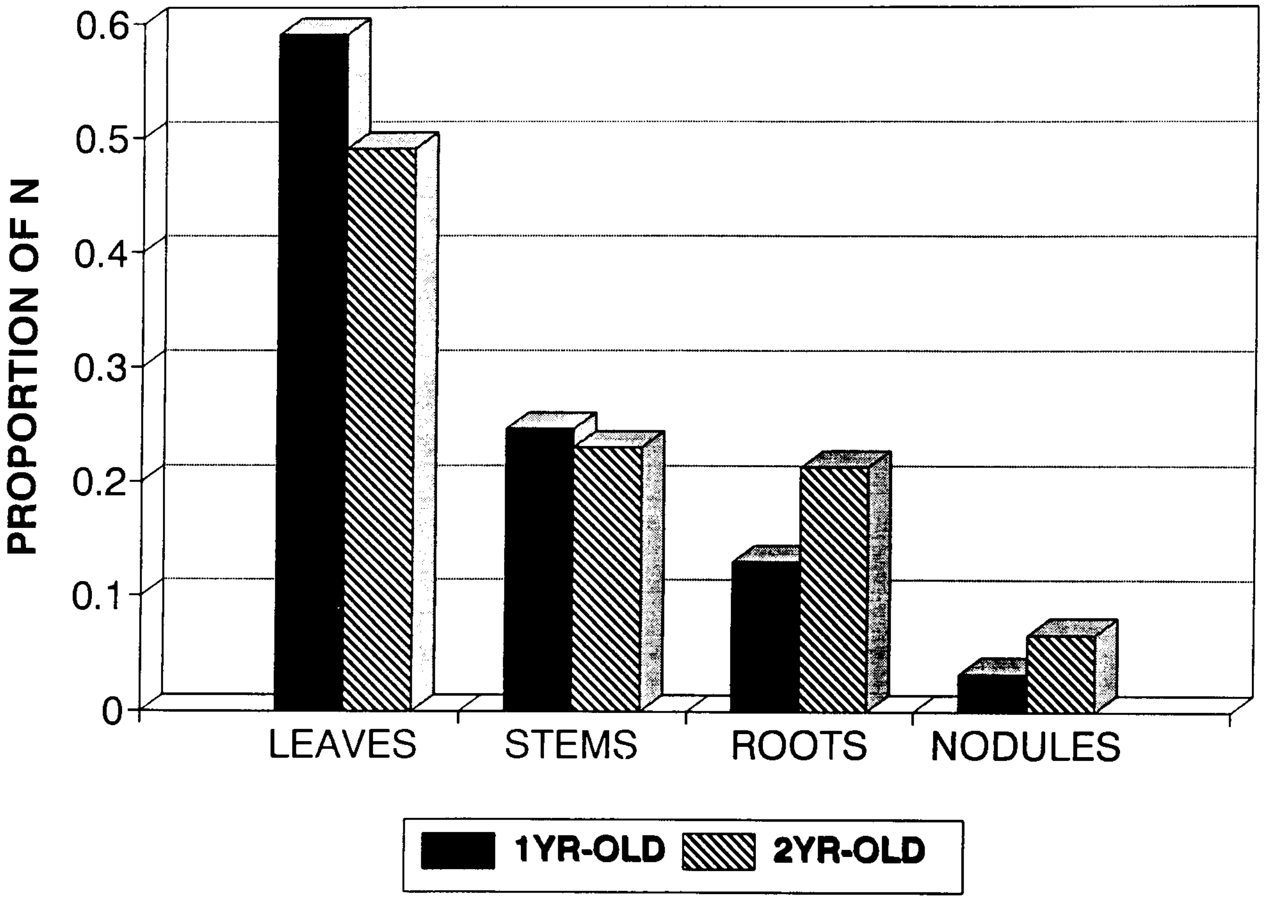

Figure 1.7 Proportional N partitioning to component plant organs by 1- & 2-year old Rooibos plants.

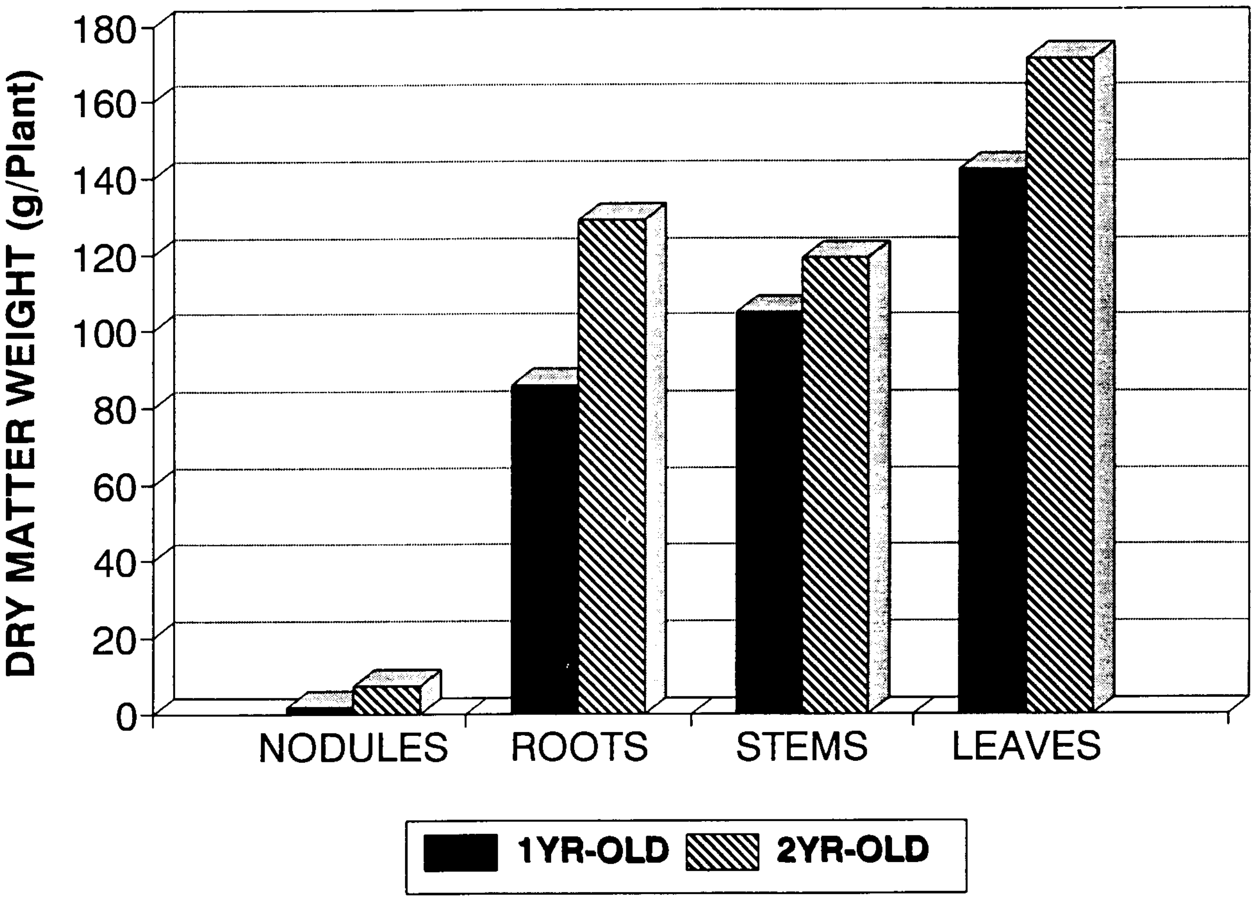

Figure 1.8 The allocation of biomass (dry matter weght) to component plant organs by 1- & 2-year old Rooibos plants.

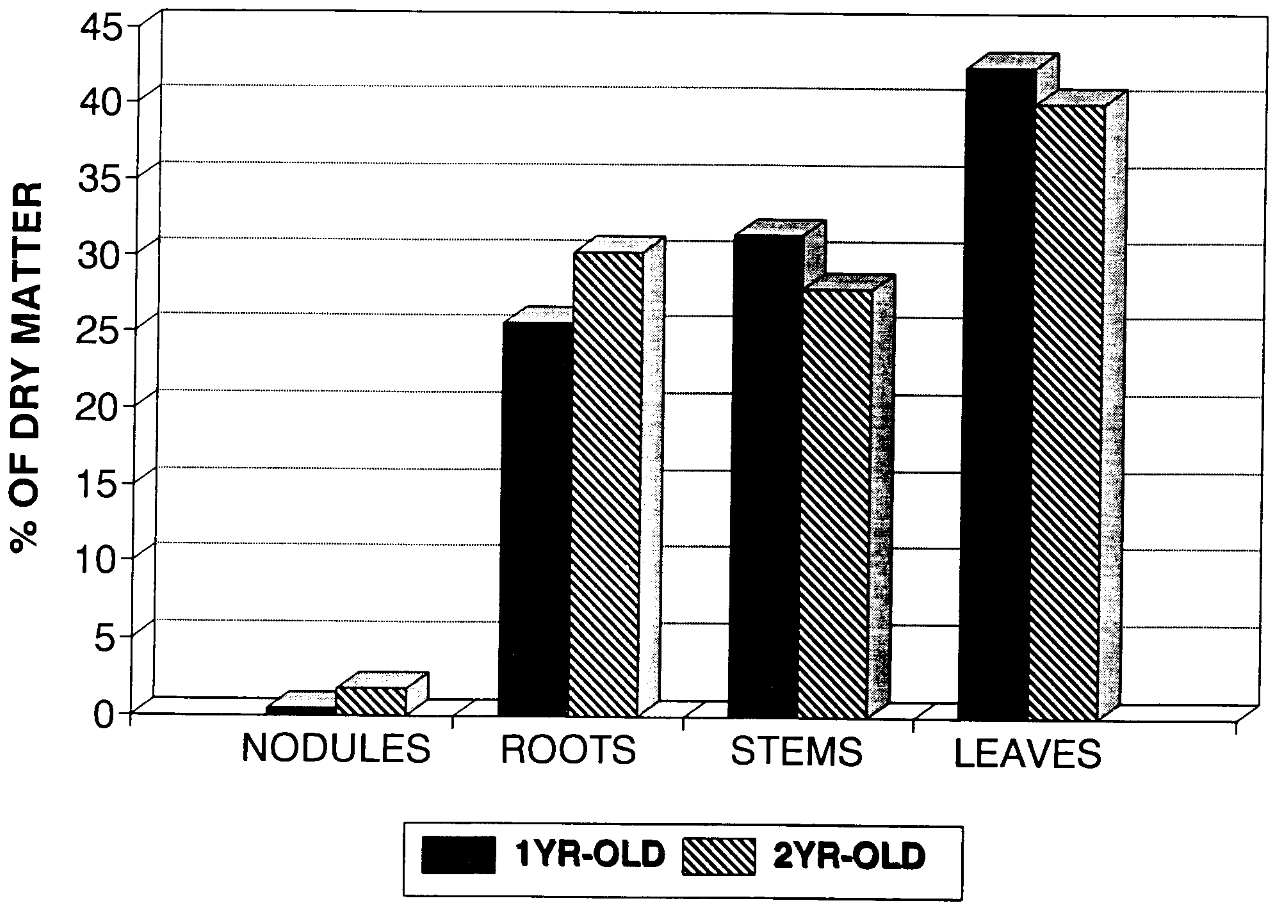

Figure 1.9 The proportional allocation of biomass (dry matter weight) to component plant organs by 1- & 2-year old Rooibos plants.

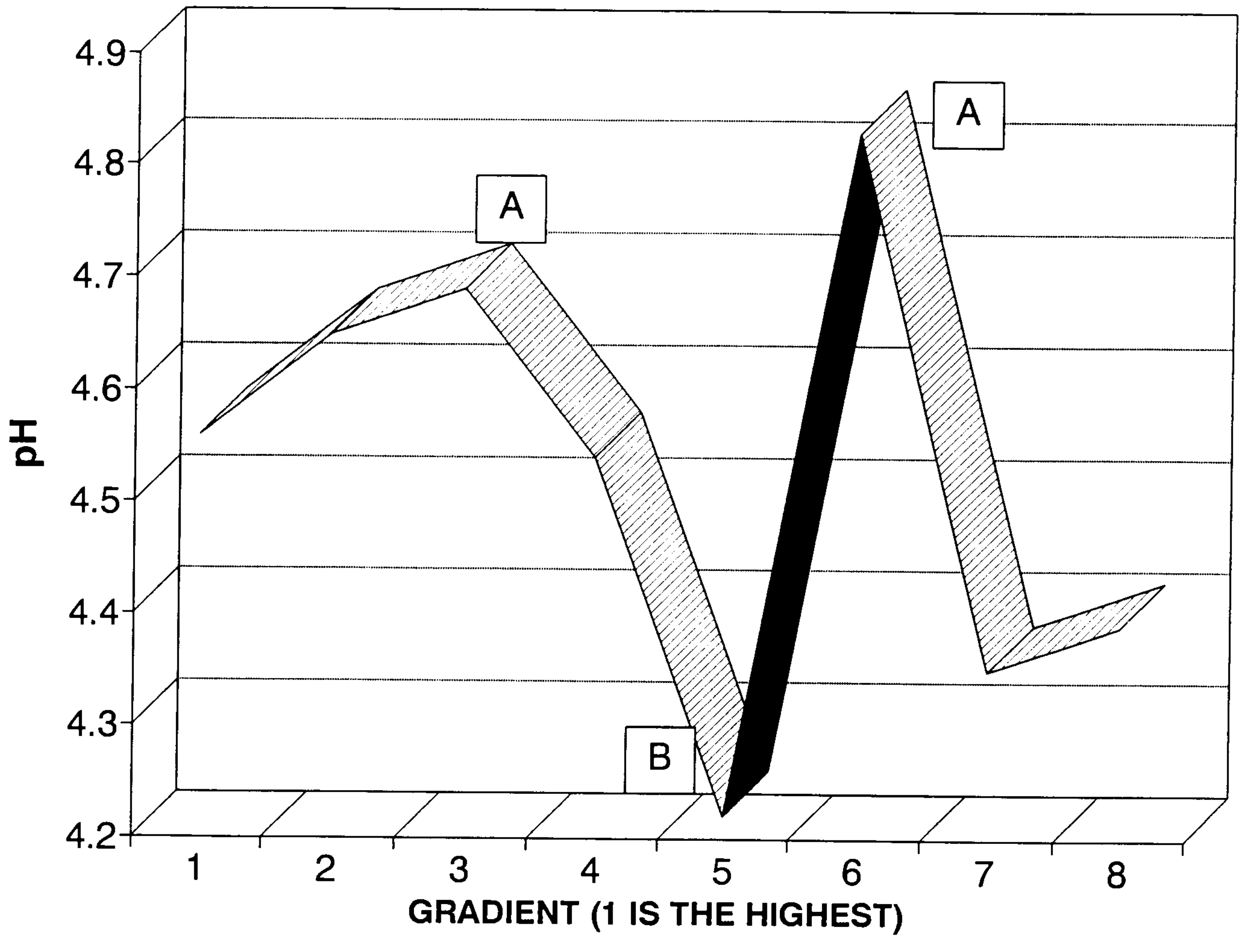

Figure 2.1 Soil pH levels along a toposequence supporting 2-year old Rooibos plants. Site 1 is at the top of the gradient, while Site 8 is at the bottom. Different letters indicate significant differences ($p < 0.05$) between values.

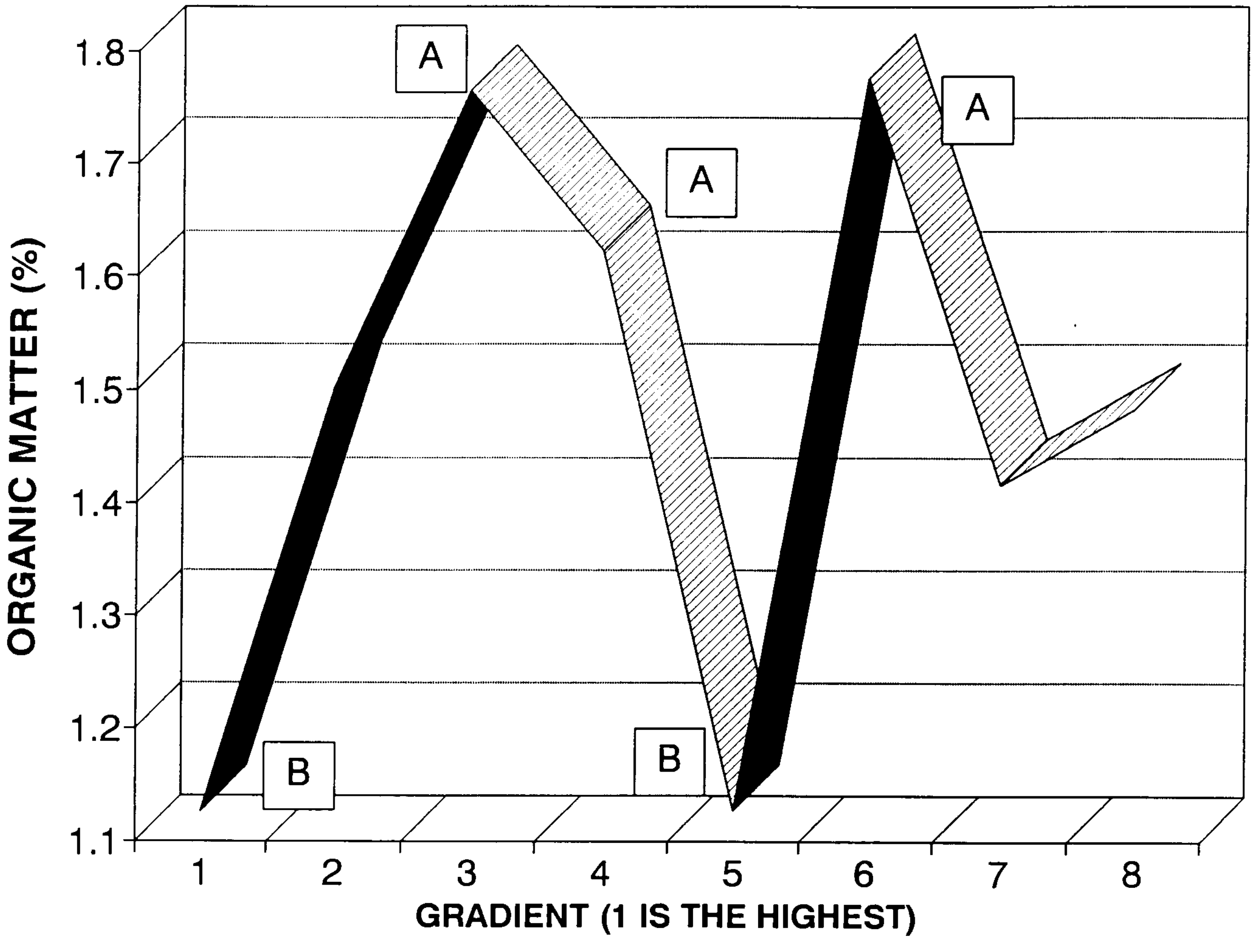

Figure 2.2 Soil organic matter levels along a toposequence supporting 2-year old Rooibos plants. Site 1 is at the top of the gradient, while Site 8 is at the bottom. Different letters indicate significant differences ($p<0.05$) between values.

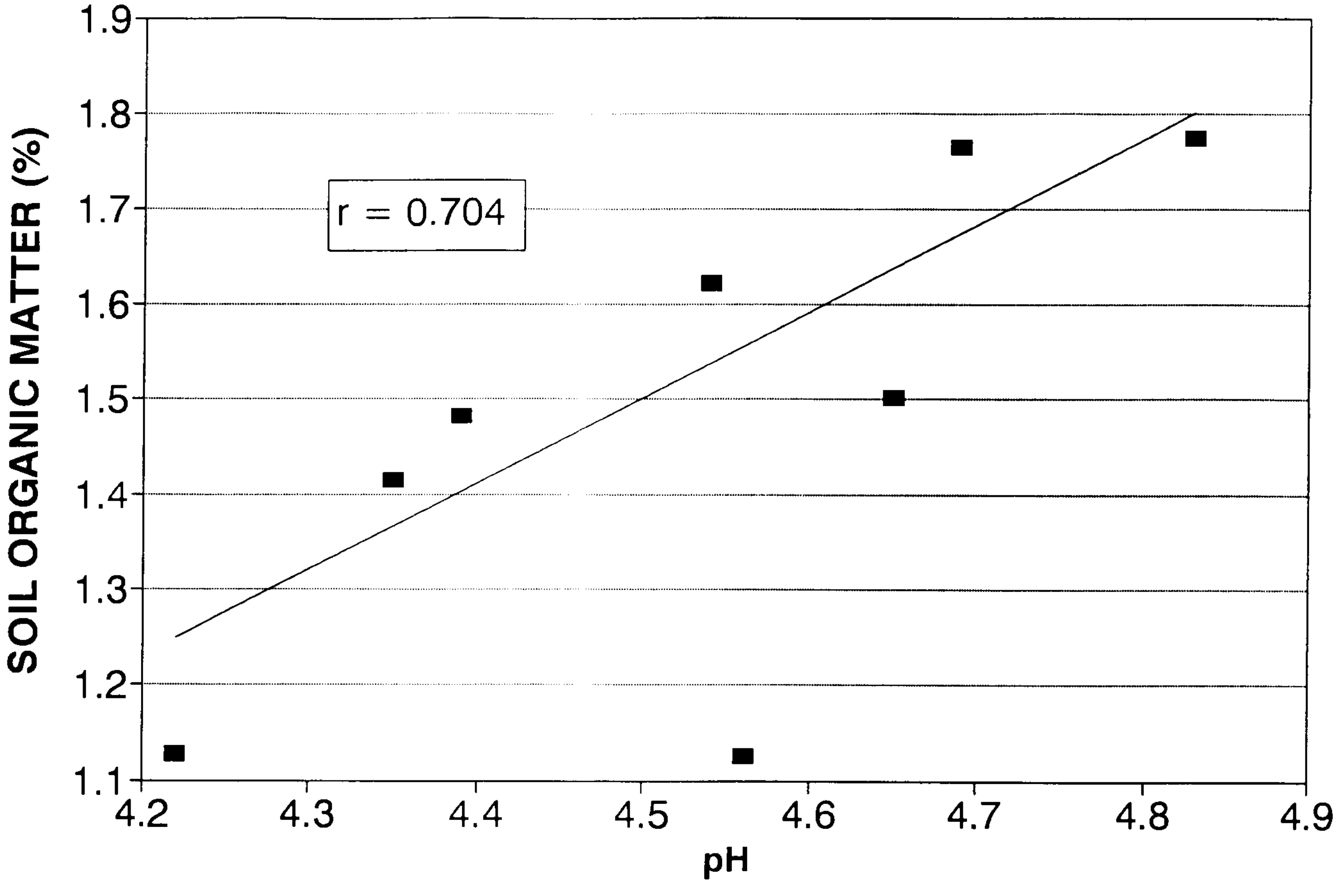

Figure 2.3 **Plot of soil organic matter vs. number of plants along a toposequence, showing a significant ($p<0.05$) positive correlation.**

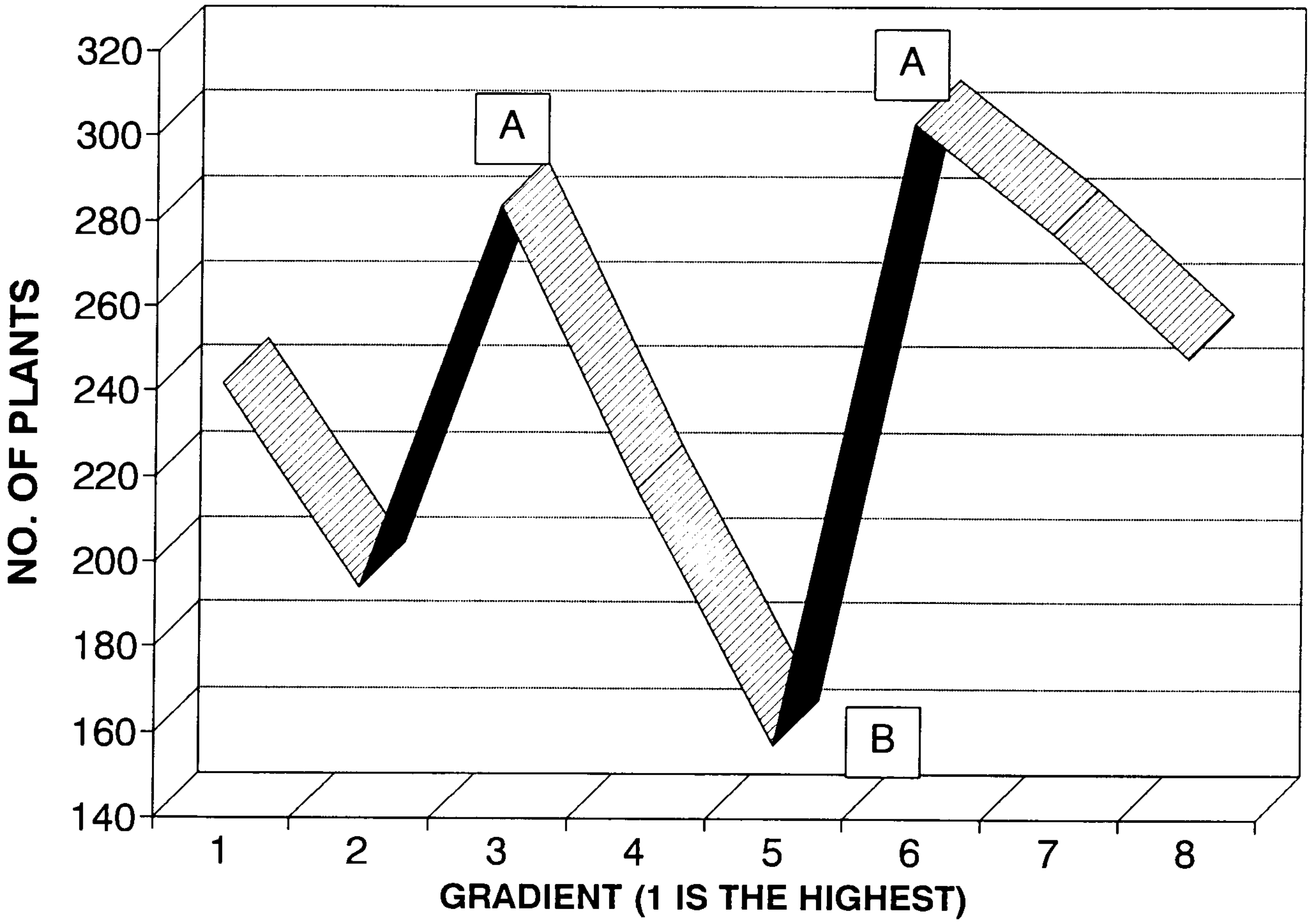

Figure 2.4 The number of plants along a toposequence supporting 2-year old Rooibos plants. Site 1 is at the top of the gradient, while Site 8 is at the bottom. Different letters indicate significant differences ($p < 0.05$) between values.

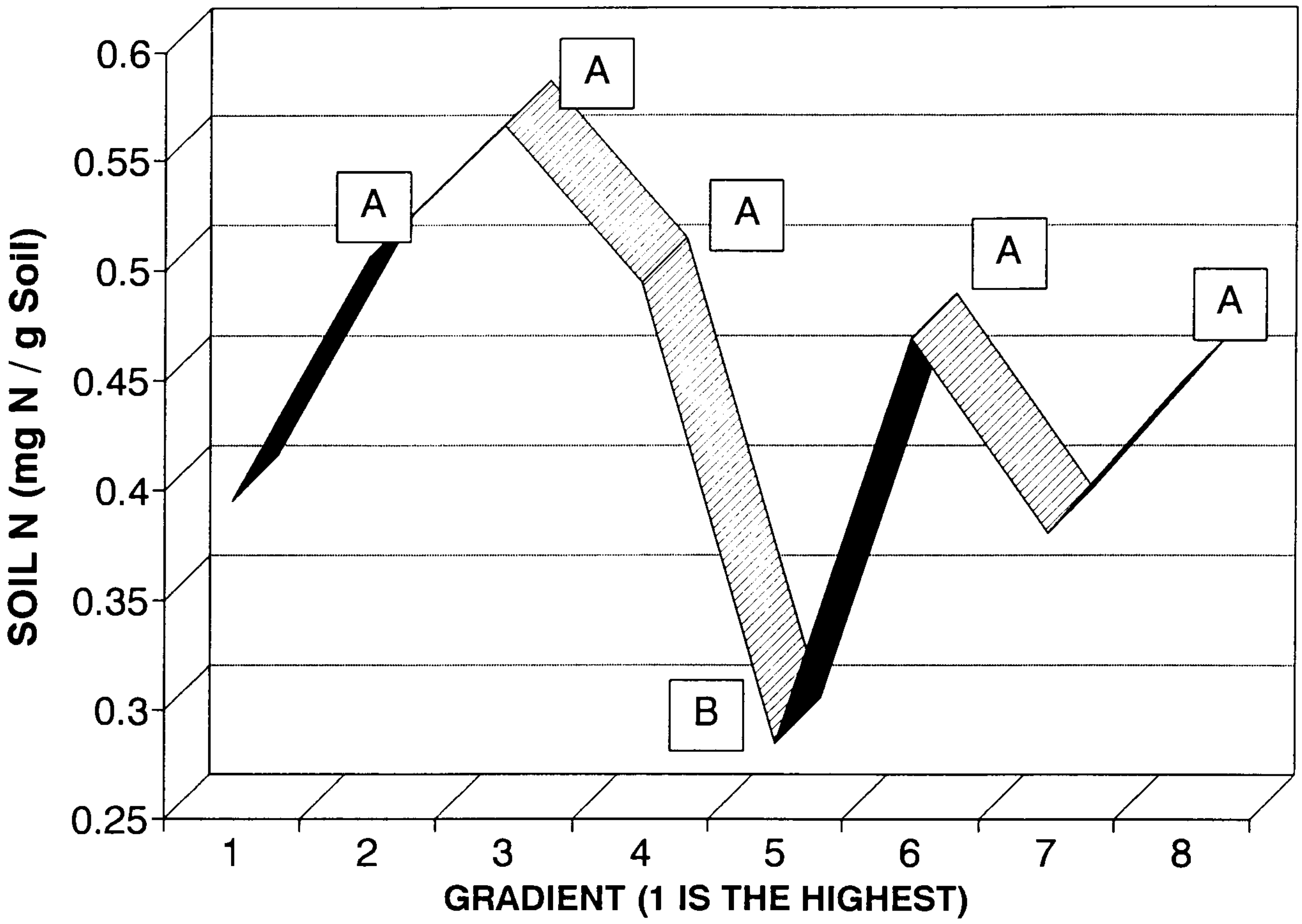

Figure 2.5 Soil N levels along a toposequence supporting 2-year old Rooibos plants. Site 1 is at the top of the gradient, while Site 8 is at the bottom. Different letters indicate significant differences ($p < 0.05$) between values.

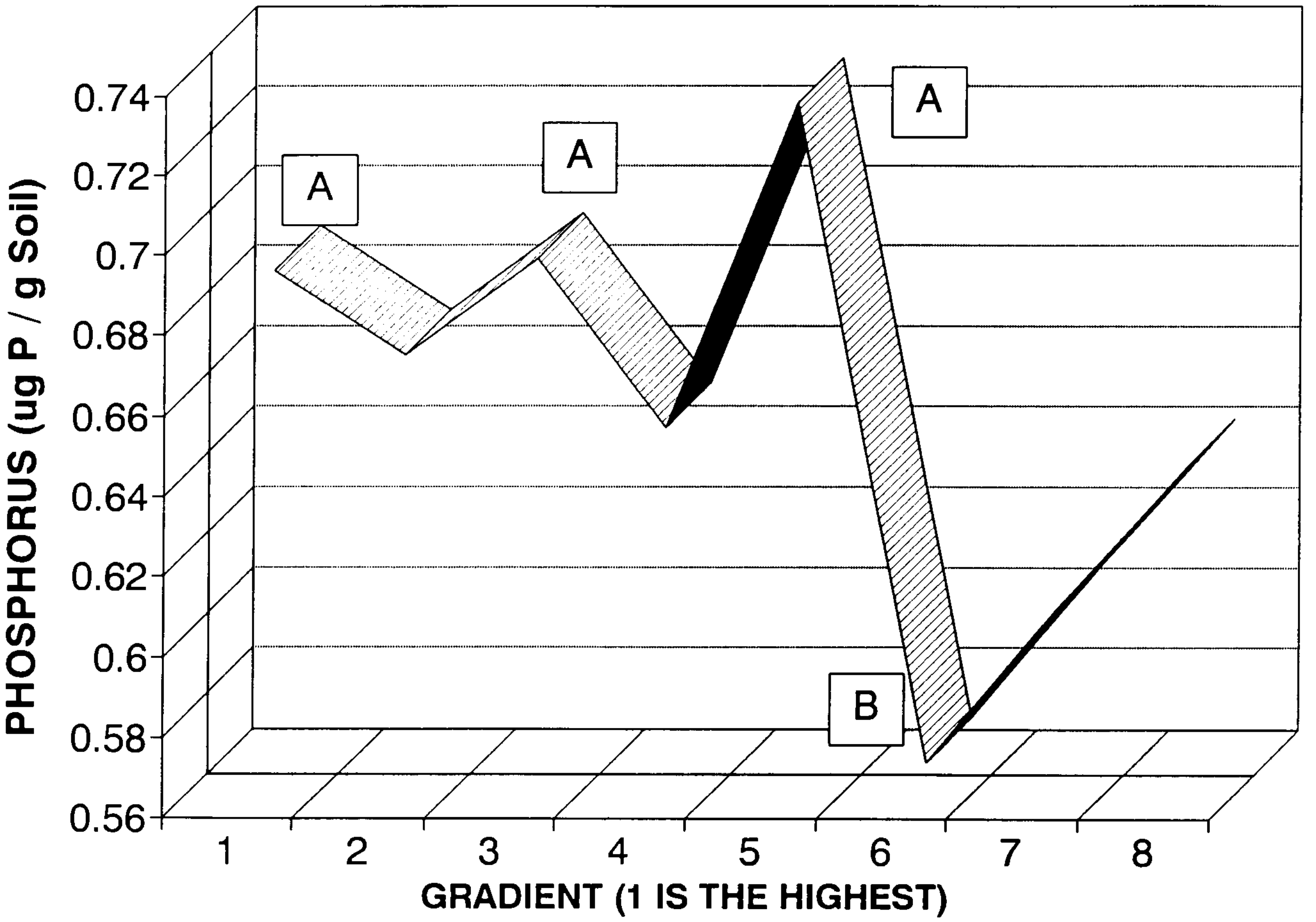

Figure 2.6 Soil P levels along a toposequence supporting 2-year old Rooibos plants. Site 1 is at the top of the gradient, while Site 8 is at the bottom. Different letters indicate significant differences ($p<0.05$) between values.

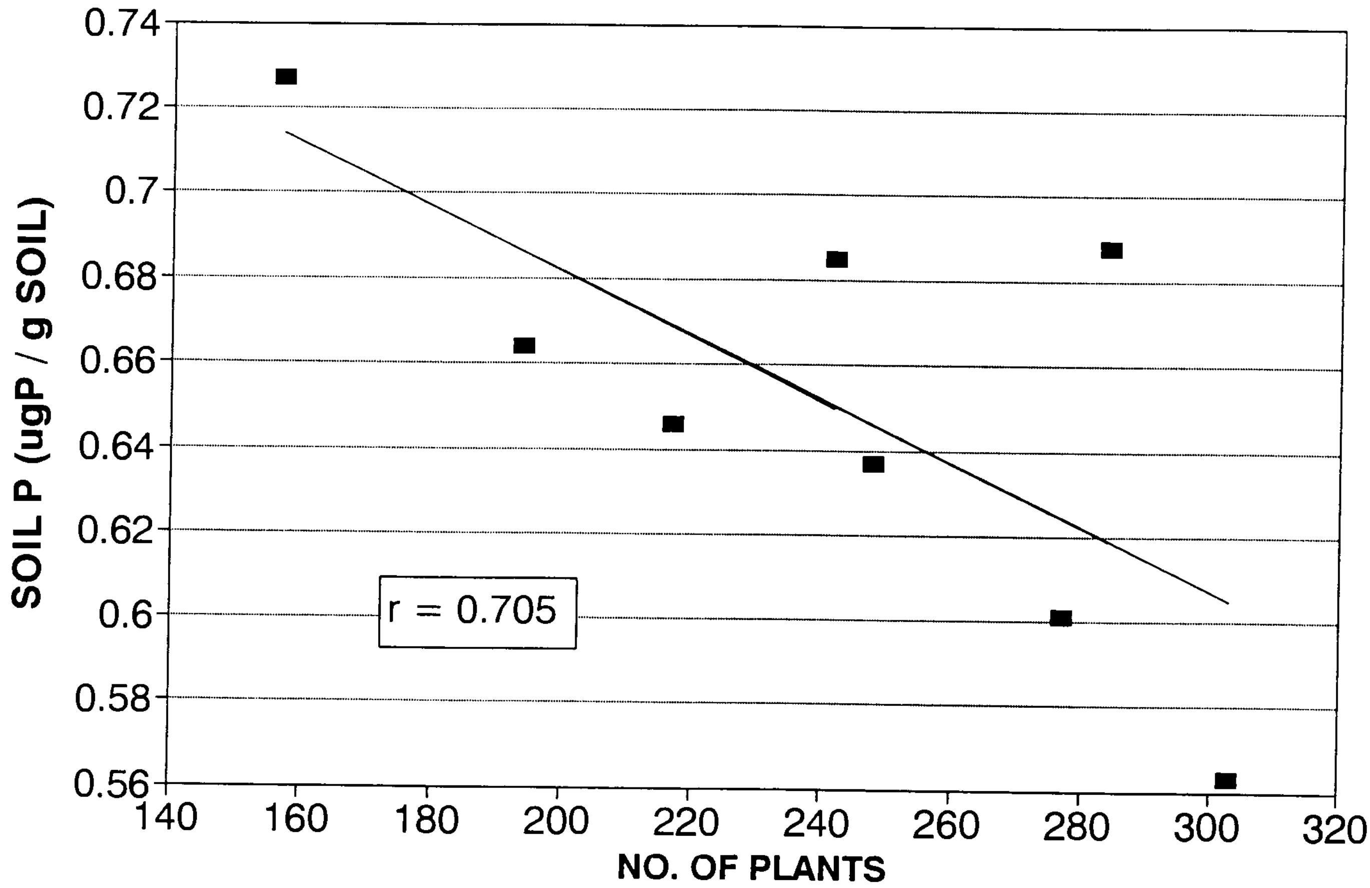

Figure 2.7 The plot of Soil P vs. the number of plants along a toposequence, showing a significant ($p<0.05$) negative correlation.

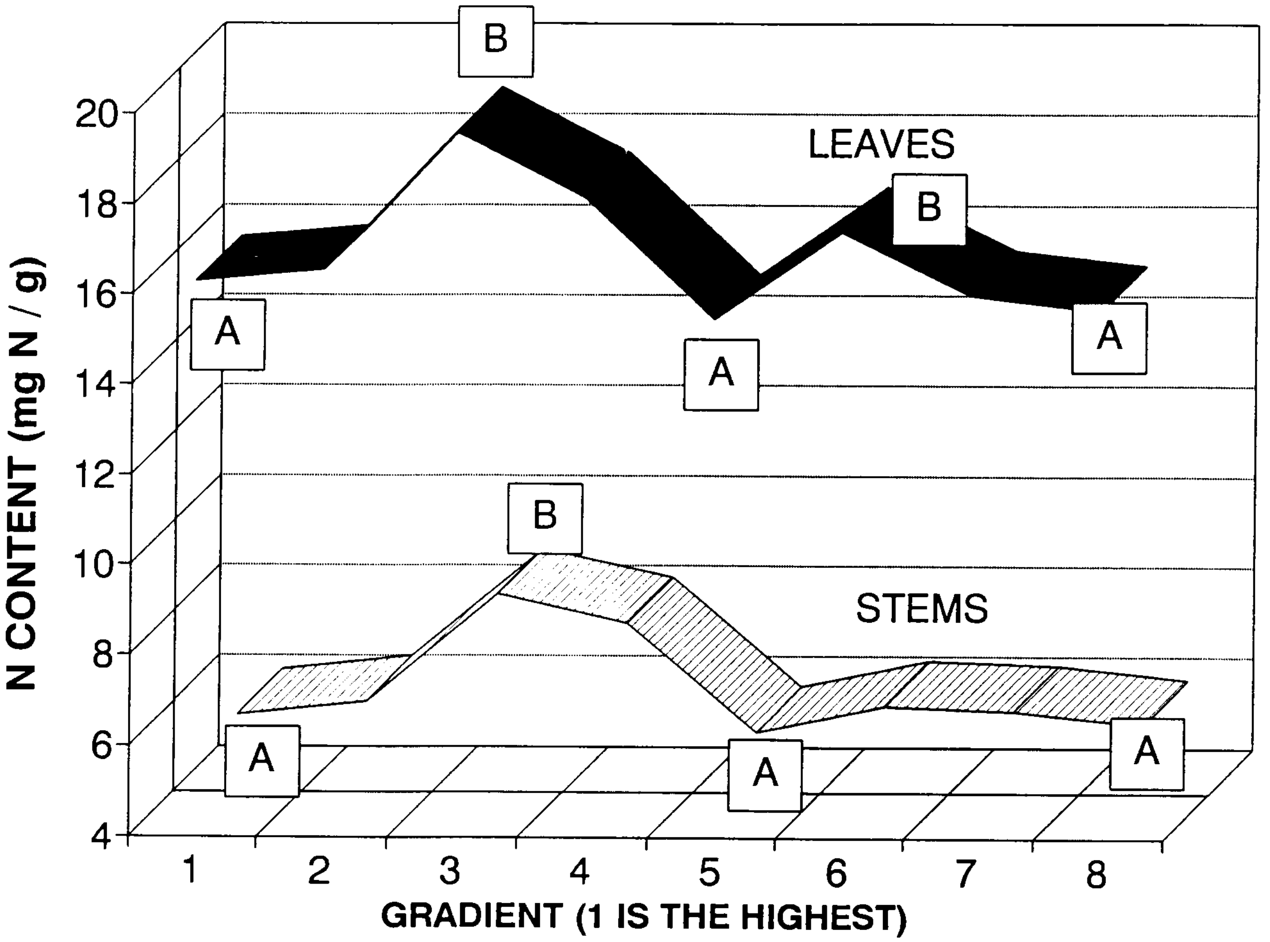

Figure 2.8 Leaf and stem N contents of 2-year old Rooibos plants along a toposequence. Different letters indicate significant differences ($p < 0.05$) between values.

Soil Phosphorus Content

As with soil nitrogen and organic matter, the soil phosphorus content was highest in the 2-year Rooibos field, and this level was significantly greater ($p<0.05$) than the 1-year level (Figure 1.4). Soil P correlated positively with soil pH ($p<0.025$), soil organic matter ($p<0.01$) and soil N ($p<0.01$).

Effects of Topography on Soil Characteristics

Soil pH

The soil pH of Site 5 was significantly lower ($p<0.05$) than the pH of Sites 3 & 6 (Figure 2.1). Since the soil pH values of Sites 6, 7 & 8 were not significantly different ($p<0.05$) from those of Sites 1, 2, 3 & 4, it can be concluded that there was no significant trend in soil pH down the soil catena.

Soil Organic Matter

The soil organic matter contents at Sites 1 & 5 were significantly lower ($p<0.05$) than those at Sites 3, 4, & 6 (Figure 2.2). Regression analyses showed a positive correlation ($p<0.05$) between soil organic matter and soil pH (Figure 2.3).

Number of Plants

The number of plants at Site 3 was significantly lower ($p<0.05$) than at Sites 3 & 6 (Figure 2.4). There was a positive correlation ($p<0.05$) between the number of plants along the transect and soil organic matter (Table 2).

Soil Nitrogen Content

The soil nitrogen content at Site 5 was significantly lower ($p<0.05$) than that of Sites 2, 3, 4, 6 & 8 (Figure 2.5). Soil N correlated positively ($p<0.025$) with leaf and stem N, soil pH and organic matter.

Soil Phosphorus Content

Soil phosphorus showed a trend along the toposequence that was the inverse of the general pattern found for the other parameters. The Site 6 phosphorus content was signicantly lower ($p<0.05$) than that of Sites 1, 3 & 5 (Figure 2.6), and there was a significant negative correlation ($p<0.05$) between soil P and the number of plants along the transect (Figure 2.7).

Estimates of Soil N Uptake Vs. Symbiotic N

Total N

The 2-year old plants had a significantly higher ($p<0.05$) N content than the 1-year olds (Figure 1.6).

Soil N Uptake

The N content of the reference plants in the 2-year field was double that of the 1-year old reference plants. Thus, it can be inferred that the 2-year old Rooibos plants were deriving twice as much N from the soil as the 1-year Rooibos plants.

Symbiotic N

The N-difference method showed that N derived from fixation was higher in the 2- than in the 1-year old Rooibos plants. However, this difference proved not to be significant ($p>0.05$), most likely due to the small sample sizes ($n=3$ & $n=4$).

Nitrogen Allocation

The increase in nitrogen content from the 1- to 2-year Rooibos plants resulted in a significantly greater ($p<0.05$) proportional N content in the roots and nodules of the 2-year compared to the 1-year plants (Figure 1.7).

Biomass Allocation

The average dry matter weights of all 2-year old Rooibos plant organs were greater than those of the 1-year olds (Figure 1.8). In terms of proportional allocation of dry matter weight, the 2-year old Rooibos plants invested relatively more carbon to roots and nodules than did the 1-year olds (Figure 1.9).

Effects of Age on Leaf and Stem N Contents

The leaf N content of the 2-year old Rooibos plants was significantly greater ($p<0.05$) than the 1-year old plants (Figure 1.5), while there were no significant differences in the stem N contents over the three years. Both leaf and stem N correlated positively ($p<0.01$) with soil pH, organic matter, and soil N.

Effects of Topography on Leaf and Stem N

Leaf and stem N contents were significantly higher ($p<0.05$) at Site 3 than at Sites 1, 2, 5, & 8. Leaf N along the toposequence correlated positively ($p<0.05$) with soil pH, organic matter, and soil N, while Stem N only correlated with soil organic matter (Table 2).

Discussion:

Effects of Cropping on Soil Characteristics

Soil pH, soil organic matter, soil N and soil P were all significantly lower ($p<0.05$) in the 1-year old Rooibos field compared to the 2-year old field. The soil of the 1-year field had a pH of 4.14, a level of acidity that most plants cannot tolerate, due to the rapid increase in aluminum toxicity below pH 5.0 (Mayz de Manzi & Cartwright 1984). The organic matter content (0.78%) was also low in relation to known ranges of 0.1% in desert soils to over 50% in Histisols (Barber 1984).

The significant increase in soil organic matter content from the 1- to the 2-year field probably came from inputs by the Rooibos plants and the previous oats crop, sown one year prior to Rooibos cultivation. This would suggest a slow rate of organic matter decomposition during the first year.

Since decomposition rates are highest in warm moist soils, the dry summers and cold, wet winters of the Clanwilliam district would not be conducive to rapid decomposition. In addition, the high acidity of the soil in the first year would have hindered the mineralization and nitrification processes in the soil (Gahoonia & Nielsen 1992), thus slowing down the decomposition of plant debris even further. Also, because the previous crop (oats) was not fertilized with nitrogen, and because the soils in the Clanwilliam area are generally low in N, the oats residue would have had a high C:N ratio, thereby reducing rates of decomposition (Poorter *et al.* 1992).

In terms of pH, the low organic matter content of the 1-year soil may have promoted acidification through increased leaching of the exchangeable base-forming cations Ca^{2+}, Mg^{2+}, K^+, and Na^+. Also, organic matter can bind to soluble aluminum in an unexchangeable form (Brady 1990). So, if organic matter content of a soil is low, most of the aluminum remains in the soil solution as aluminum ions, which contribute to greater soil acidity through their tendency to hydrolyze and produce H^+ ions:

$$Al^{3+} + H_2O \longrightarrow Al(OH)^{2+} + H^+$$

Since organic matter is the primary source of soil nitrogen, it is not surprising that the organic matter content correlated positively ($p<0.01$) with soil nitrogen over the three years. A surprising result, though, was the significant ($p<0.05$) rise in soil phosphorus content from the first to the second year, followed by a decline in the third year. Considering that 300 kg. ha^{-1} of phosphate fertilizer was applied to both oats crops prior to the Rooibos, one would have expected to see the highest phosphorus levels in the 1-year soil. However, the oats crop probably took up a considerable amount of the added phosphorus, which was only released back to the soil as the oats residue slowly decomposed. The phosphorus so released got taken up by the Rooibos plants for growth and establishment of symbiosis, as P is very important for nodulation and N_2 fixation (Robson *et al.* 1981, Israel 1987). This hypothesis is borne out by the positive correlation ($p<0.01$) found between soil P and soil organic matter levels.

Whereas soil organic matter decreased slightly but not significantly ($p>0.05$) after the second year, soil pH increased ($p>0.05$), indicating that the increase in soil organic matter was not the only cause of the increase in soil pH. Recent evidence (L.M. Muofhe, pers. com.) shows that Rooibos roots can reduce soil acidity. This is in sharp contrast to reports on other plants which decrease rhizosphere pH in order to increase the mobility of calcium phosphate, as exemplified by rape plant roots (Gahoonia & Nielsen 1992, Hoffland *et al.* 1989).

Soil organic matter and phosphorus contents both showed an insignificant decline after the second year, while soil N decreased at a significant rate ($p<0.05$). These three soil parameters all correlated positively ($p<0.025$) over the three years, showing that nitrogen and phosphorus came primarily from organic matter. However, the declines observed might have been due to exhaustion of N and P by Rooibos plants and complete decomposition of oats residue. Rooibos itself is sclerophyllous, and its leaves are harvested each year for tea, so there would have been very little organic matter contribution to the soil.

Phosphorus Nutrition: a factor in early senescence of cultivated Rooibos?

There have been reports (W. Nel, pers. com.) that Rooibos plants established in natural fynbos communities adjacent to Rooibos fields outlive the cultivated plants. The reason is commonly believed to be a fungal infection that intensifies with plant maturity. From the data obtained in this study, it seems that soil P contents may decline after the second year, leading to a phosphorus deficiency by the fourth year. By this stage, there have been no fertilizer P inputs to the soil for more than four years, whereas considerable quantities of phosphorus have been lost, not only in the three Rooibos harvests, but also in the two oats crops. Furthermore, the organic matter originating from the oats residue will be exhausted by the fourth year, thus bringing to an end a major source of soil phosphorus.

Therefore, although the early onset of death in cultivated Rooibos plants is directly attributable to fungal attack, it is possible that P deficiencies may be undermining the health of the mature plants, rendering them prone to infection. This hypothesis can be tested by applying phosphate fertilizer annually throughout the life-span of a Rooibos crop, and comparing the degree of fungal infection with that of a control field that has not been fertilized since the Rooibos was planted.

Effects of Plant Age and Cropping Length on N Nutrition

Total N was significantly greater ($p<0.05$) in 2-year old Rooibos plants compared to 1-year old plants (Figure 1.6). These increased levels of N would have come from symbiotic fixation and soil N uptake, both of which were greater in the 2-year old plants. This increase in total nitrogen content from the 1- to 2-year Rooibos plants resulted in a significantly greater ($p<0.05$) nitrogen content in the leaves, roots and nodules of the 2-year plants compared to the 1-year plants (Figures 1.5 & 1.7).

These changes in total N content may be the result of depressed symbiotic N fixation in the 1-year soil due to the low pH, since acidity adversely affects nodule development and functioning in the bradyhizobium-legume symbiosis (Paulino *et al.* 1987). In fact, pH affects legume yields through reduction in nodule number (Wood *et al.* 1984) and nitrogenase activity (Mayz de Manzi & Cartwright 1984). In addition, low N uptake by roots due to low soil nitrogen content in the 1-year soil may also have contributed to the lower leaf and stem N contents of the 1-year plants. Evidence for these two hypotheses lies in the fact that nitrogen derived from symbiotic N fixation and from soil N uptake is lower in the 1-year old plants than in 2-year olds (Figure 1.6). This is further supported by the fact that stem and leaf N contents both correlated positively ($p<0.01$) with soil pH and soil N content.

The higher nitrogen contents in the 2-year plants is also attributable to effective symbiosis manifested by the significant ($p<0.05$) increase in root and nodule dry matter weight by the second year (Figure 1.8). Furthermore, there was proportionally more nitrogen in the roots and nodules of the 2-year olds (Figure 1.7). Thus, the 2-year old plants not only had a greater capacity to fix more nitrogen than the 1-year plants, but also to take up more N from the soil.

Effects of Toposequence on Soil and Plant Characteristics

The soil and plant parameters studied along the 400m transect down the 2-year old Rooibos field showed very large ranges. For example, there were twice as many plants at Site 6 than at the adjacent Site 5 (Figure 2.5), while the soil N content at Site 3 was roughly double that at Site 5 (Figure 2.4). Since none of these parameters showed either a general increase or decrease down the gradient, it appears that factors other than topography were responsible for the observed trends.

Statistical analyses revealed a number of recurring patterns in the data. Soil pH, soil organic matter, soil nitrogen, and the number of plants were all significantly lower ($p<0.05$) at Site 5 than at Sites 3 & 6. However, the soil phosphorus content was significantly higher at Site 5 than at Site 6 (Figure 2.6). In fact, the soil P content correlated negatively ($p<0.05$) with the number of plants (Figure 2.7). In addition, the soil organic matter contents at Sites 3 & 5 were significantly ($p<0.05$) lower than at Sites 1 & 6. However, if the main source of the organic matter was the decomposition of the oats residue, one would expect to find a uniform distribution of organic matter throughout the field. In an attempt to piece together these seemingly conflicting results, two hypotheses are presented:

Hypothesis 1: Site 5 was the site of an ancient termitarium, or "heuweltjie", which are common in the Clanwilliam district. Since termites have very efficient digestive processes, termite deposits commonly have a lower organic matter content than undisturbed topsoil. In addition, these deposits are comprised largely of low organic subsoil material brought to the surface as the mounds were being formed (Brady 1990). Crop growth in soil in areas where termite mounds have existed is often poor, not only because of low nutrient contents but also because of the greater compactness of some of the mound material, the particles of which were cemented together by the termites as the mound was constructed (Brady 1990).

Because of greater compactness and low nutrient content of heuweltjie-derived soil at Site 5, the growth of the oats crops may have been hindered. Thus, there would have been lower organic matter inputs to the soil, resulting in increased soil acidity. Low pH, in turn, may have reduced nodulation and nitrogenase activity (Paulino *et al.* 1987), thereby undermining the health of the successive Rooibos crop, and resulting in fewer plants. Thus, less soil P would have been taken up, accounting for the negative correlation ($p<0.05$) between these two soil parameters.

Hypothesis 2: A fungal infection may have been more prevalent at some sites (eg. Site 5) than at others, severely affecting the survival of the Rooibos plants at the juvenile stage. The resultant low Rooibos densities at these sites would have meant less organic matter production through leaf-fall and root death. Low organic matter levels would lead to increased soil acidity, further undermining Rooibos survival and growth. Ultimately, these lower plant numbers would result in less soil P uptake, hence the negative correlation ($p<0.05$) between the number of plants and soil P.

To test which, if either, of the above-mentioned hypotheses is correct, a few field and laboratory experiments need be performed. If the degree of soil compactness and soil particle cementation at Site 5 is greater than at the other sites, then support will have been provided for the heuweltjie hypothesis. Evidence for increased fungal attack at Site 5, however, can be found with the aid of a microscope, either by looking at tissue from Rooibos plants, or directly at the soil. Alternatively, by growing Rooibos in the laboratory in soils from the different sites, the degree of fungal infection in the plant organs will give an indication of the relative concentrations of fungi in the soils of those sites.

CONCLUSION

This study has served to shed light on the interaction between Rooibos and its environment in the cultivated state. Significant differences in the soil and plant characteristics studied were found to occur over both space and time. It is proposed that the interaction between soil organic matter and soil pH not only has a significant influence on the other soil parameters, but also on the N nutrition of Rooibos. Since a large proportion of this organic matter is derived from previous crops, cropping history plays a major role in the cultivation of Rooibos. The nitrogen status of Rooibos plants has proved its value as an effective index of plant response to different environmental conditions. In addition, the crucial role of soil phosphorus in the health of Rooibos plants has been confirmed, and it is hypothesized that premature death of cultivated plants may be enhanced by soil P deficiencies. Finally, in the event that fungal infection is the cause of the patches of low productivity in Rooibos fields, a major challenge will be to explain the successful establishment of fungi at these sites. If the factors that render certain patches of soil prone to fungal attack could be countered, Rooibos tea yields would be considerably improved.

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