A comparative study of the nitrogen nutrition of Proteaceae growing in limestone- and sandstone-derived soils of the Agulhas coast.

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Botany Honours Project

1986

ABSTRACT

The nitrogen nutrition of proteaceous species growing on limestone- and sandstone-derived soils on the Agulhas coast was compared. Soil pH was found to be highest in the calcareous soils. Nitrate levels were found to be more closely correlated to soil pH than were ammonium levels. The alkaline soils were found to have the highest nitrate concentrations. Nitrate reductase activity (NRA) was not found to be higher in the plants growing in soils of higher nitrate content.

CONTENTS

1. INTRODUCTION

Nitrate (NO_3^-) and ammonium (NH_4^+) ions are the major forms in which inorganic nitrogen is available to plants for nutrient uptake (Lee and Stewart, 1978). Plants have evolved specific adaptations to the ammonium:nitrate ratios prevailing in different environments. Calcicole plants (adapted to soils of high $CaCO_3^-$ content) and calcifuge plants (found predominantly in low nutrient, acidic soils) have been shown to have preferences for nitrate or ammonium nitrogen, respectively (Lee and Stewart, 1978). The predominance of nitrate in high pH soils is due to the favourable conditions prevailing for the oxidation of ammonium by nitrifying bacteria (Lewis, 1986). In acidic soils, inhibition of these nitrifying bacteria results in ammonium being the major form of N available for uptake by plants (Lewis, 1986).

Variations in N-assimilation strategies in plants growing on acid and alkaline soils may therefore be expected depending on the form of nitrogen available. A useful means of determining the N-uptake preference of a species is by the use of assays of nitrate reductase activity (NRA) (Runge, 1983). This substrate inducible enzyme is responsible for the initial reduction of nitrate to nitrite before it is assimilated into glutamine, via the glutamine synthetase pathway (Lewis, 1986).

Reduction of nitrate has been found to occur predominantly in the leaves when nitrate is available at high concentrations, whilst at low concentrations, reduction occurs predominantly in the roots. In this study, a comparison will be made of NRA in root and shoot tissue of proteaceous species growing on limestone- and sandstone-derived soils. These results are to be correlated with analyses of the nitrogen composition of xylem sap. An initial understanding may thereby be reached of how members of a family generally adapted to low pH soils (Rourke, 1980) may cope with nitrogen assimilation in higher pH soils where nitrate is the predominant form of N.

2. SITE DESCRIPTION

The study was carried out in the hills near Gansbaai (coordinates 34°35'S; 19°26'E) situated on the southern Cape coast. Three sites were selected for their representative nature of the area. Site 1 was situated on a limestone outcrop (cf Plate 1 and Appendix 3). Two species which were characteristic of the limestone habitat were selected for study on this site. Protea obtusifolia (Buek ex Meissner) is entirely dependent on limestone outcrops of the Alexandria Formation under natural conditions, although it can be cultivated on acid soils. Plants are frequently rooted between cracks on the limestone rocks (Rourke, 1980). Leucadendron meridianum (Williams) is the southernmost occurring member of the genus and is invariably found on limestone outcrops (Vochts, 1982).

Site 2 was situated adjacent to the limestone outcrop and supported coastal lowland fynbos in sandstone derived soil (cf Plate 2 and Appendix 3). Leucadendron salignum (Berg.) has the widest distribution range of the genus being found practically throughout the fynbos biome (Vochts, 1982). Protea repens (L.) L. is also the most widely distributed member of its genus and is found mainly in lowland fynbos vegetation. Site 2 may be regarded as intermediate between the limestone habitat and the sandstone-derived habitat.

Plate 1. Limestone outcrop supporting L. meridianum (left) and P. obtusifolia (right). The foreground shows bare limestone rock typical of site 1.

Plate 2. *Protea repens* (centre) and *L. salignum* (foreground) growing in sandstone-derived soils at site 2. The limestone outcrop can be seen in the background.

Site 3 was representative of coastal lowland fynbos (cf Plate 3 and Appendix 3). The only species studied at this site was Leucadendron salignum.

Plate 3. Locality of study site 3 situated in lowland coastal fynbos showing L. salignum in the background. (Metalasia muricatacan be seen in the foreground).

3. MATERIALS AND METHODS

How sampled?

3.1. SOIL ANALYSES

Soil samples were brought back from the field, and immediate determinations of pH, soil water content, as well as KCl extraction of soil nitrogen for nitrate and ammonium determinations, were performed.

3.1.1. pH DETERMINATIONS

5 mg of fresh, unsieved soil was shaken lightly in 50 cm^3 of distilled water. After filtering the solution through Whatman no. 1. filter paper, pH was determined using a Beckman pH meter, with a glass electrode. In addition, pH was determined by agitating 20 g of soil in 50 cm^3 of 0.01 M $CaCl_2^-$ solution, for 30 minutes (Schofield and Taylor, 1955). This method has the effect of masking the variability in the salt content of the soils from different sites (Stock, 1985). pH was then read as for the distilled H_2O extraction. Four replicates were performed for each of the three sites.

3.1.2. SOIL WATER CONTENT

Samples consisted of 8 g of 2 mm sieved fresh soil weighed out into crucibles, four replicates were used for each site. The soil was allowed to oven-dry at 105 ^{o}C for 24 hours. The wet and dry mass of each sample was determined using an Oertling balance. The % water content was then expressed for each soil sample.

After allowing the reagents to cool, absorbance was read on a Beckman spectrophotometer, at 410 nm (Cataldo et al., 1975). KNO_3 was used for determination of a standard calibration curve in the range of 0-60 µg NO_3^-. The Cataldo method for nitrate determination proved insensitive for the soils sampled. This was indicated by no colour development in any of the samples. Szechrome NAS reagent was therefore used for NO_3 determinations. According to this method, 2.5 cm^3 of Szechrome NAS reagent was added to 0.5 cm^3 of KCl extract. Colour was allowed to develop for 5 minutes, after which time absorbances were read at 570 nm. A standard curve was constructed in the range of 0-200 n molar solutions (cf Appendix 1.). The concentration of NO_3 was calculated in ug NO_3 per gram dry soil. For this purpose, 10 g of soil was oven-dried to determine the water content present in fresh soil.

3.1.4.2. SOIL AMMONIUM DETERMINATION

For the following procedure, three replicates were performed for each of the four samples from each site. Nessler's reagent, consisting of 143 g NaOH in 950 cm^3 H_2O, was made up to 1000 cm^3, and stirred for an hour. This solution was then allowed to stand overnight. The following day, it was filtered and then centrifuged. Following this, 2.5 cm^3 of the reagent was added to 0.5 cm^3 of the KCl and soil extract. After exactly one minute, absorbance was read at 420 nm. Samples from site 1 formed a precipitate on addition of Nessler's solution, making centrifugation of the mixture necessary for at least 45 seconds.

Owing to the delay due to centrifugation of the site 1 samples, absorbances were read after one and a half minutes. Standard curves were therefore determined for both one and one and a half minutes. This was necessary as the reagent deteriorates after one minute. Standard solutions were made using ammonium chloride, in the range of 0-500 n moles NH_4 (cf Appendix II.). Soil ammonium content was calculated in $\mu g\ NH_4^+$ per gram of dry soil, water content having been previously determined for seperate samples by oven-drying.

3.2. NITRATE REDUCTASE (NR) ASSAYS

Plant root and shoot material was collected in September, 1986, with samples being taken from three individuals of each species studied, for each of the different sites. Since the species investigated all belonged to the family Proteaceae, proteoid roots were used to distinguish material to be sampled from other neighbouring plant species. This was particularly important in the limestone soil (site 1), were roots were densely matted owing to the thin layer of soil which overlays limestone bedrock (± 15 cm). Plant material was kept in ice until NR assays could be carried out on arrival at the laboratory. Root and shoot nitrate reductase activity (NRA) were determined by an *in vitro* method after Stock and Lewis (1982), using KNO_3 as a NO_3^- source which on conversion to NO_2^- by NR gives a colorimetric reaction. Nitrate reductase was extracted from the leaves and roots by grinding 1 g of plant material in a chilled pestle and mortar.

Plate 4. Collection of plant material (L. salignum, site 3) for extraction of xylem sap.

Plate 5. Equipment used for pressure bomb method of xylem sap extraction.

The extraction medium contained 0.1 mol dm^3 phosphate buffer at pH 7.5, 1 mmol dm^{-3} EDTA and 1 mmol dm^{-3} dithiothreitol, to which was added 1.5 g of insoluble polyvinylpyrrolidone (PVP) (Loomis and Battaile, 1966). The latter was found to be contaminated with nitrite, acid washing with PVP was therefore carried out prior to useage. The extraction medium was made up to 12 cm^3 using distilled water. Acid-washed sand was used as an abbrasive medium for grinding the plant material. The extract was then squeezed through a double layer of cheese cloth. The reaction mixture for nitrate reductase consisted of 0.1 cm^3 of 1 mol dm^{-3} phosphate buffer at pH 7.5, 0.1 cm^3 of NADH (1 mg cm^3), 0.2 cm^3 of 0.1 mol dm^3 KNO_3 and 0.3 cm^3 of plant extract. The mixture was made up to a volume of 2 cm^3 using distilled water. For each plant species, the replicates and the blanks were prepared for both the root and shoot material. The samples were placed in a waterbath at 27 ^{o}C for 15 minutes. Determination of the reduction reaction was then carried out by means of 1 cm^3 of 1 % (w/v) sulphanilamine in 1.5 mol dm^3 HCl and 1 cm^3 of 0.01 % (w/v) N-(1-napthyl) ethylene-diamine hydrochloride solution. Absorbances were read at 540 nm, on a spectronic 20 absorbance spectrophotometer, after colour was allowed to develop for 5 minutes.

3.3. XYLEM SAP ANALYSES

Xylem sap extraction from shoots was carried out immediately after collection of plant material (cf Plate 4). This was done in the field by means of a pressure bomb which is normally used for the determination of water potential (cf Plate 5). The method of sap collection using a Pasteur pipette with a bulb is shown in Plate 6. Samples were placed in ice until arrival at the laboratory where they were kept frozen. Analyses were later carried out for concentrations of xylem sap nitrate, ammonium and amino acids.

3.3.1. NITRATE DETERMINATIONS

For the nitrate determinations, 2.5 cm^3 of Szechrome NAS reagent was added to 0.5 cm^3 of undiluted xylem sap. Colour was allowed to develop for 5 minutes. Absorbances were read as for the soil nitrate analyses where Szechrome was the reagent. Due to insufficient sample , replicates were not carried out. This was primarily due to the lack of time available in the field and limited freezing facilities. Results were expressed as $\mu mol\ ml^{-1}$ xylem sap.

3.3.2. AMMONIUM DETERMINATIONS

Xylem sap ammonium was determined by means of 2.5 cm^3 Nessler's reagent wich was added to a diluted sample (0.1 cm^3 sample : 0.4 cm^3 distilled water). Colour was allowed to develop for exactly one minute and absorbances were read as for the soil NH_4^+ determinations at 420 nm.

Plate 6. Collection of xylem sap using Pasteur pipette.

Three replicates were performed for the species studied at each site. The results were expressed as μmol ml^{-1} xylem sap.

3.3.3. AMINO ACID DETERMINATIONS

Polyphenolics and other compounds preserved in the xylem sap of the members of the Proteaceae studied cause masking and interference in the process of amino acid analysis. It was therefore necessary to purify the samples before amino acid determinations were carried out. A Zeocarb 225 14-52 mesh particle size resin was washed in 2 N HCl solution. This was followed by washing with distilled water to remove any Cl^- ions. This was followed by thorough washing with 75 % ethanol. Resin was stored in ethanol until use. A Pasteur pipette with glass wool at the constriction was 3/4 filled with acid washed resin. An aliquot of 0.5 cm^3 of xylem sap was allowed to run through the resin column, followed by washing three times with 75 % ethanol. This process washes out the majority of contaminants. The contents of three pipettes of 1:3 ammonia:ethanol were then used to release the amino acids held by the resin. The eluate was evaporated to dryness and made up to 0.5 cm^3 using distilled water. No dilution of xylem sap was carried out prior to amino acid analysis. Determinations were made using a Beckman amino acid analyser.

4. RESULTS

4.1. SOIL pH, WATER CONTENT AND ORGANIC CONTENT

Soil pH measurements were consistently higher when distilled water was used as a solvent compared to samples mechanically shaken in $CaCl_2$ (cf Table 1). Site 1 soil had the highest pH (7.60 $\pm$ 0.02 in distilled water), whilst site 3 had the lowest (5.88 $\pm$ 0.02 in H_2O; 4.58 $\pm$ 0.01 in $CaCl_2$).

The percentage water content was found to be highest in the soil at site 1, whilst site 2 had the lowest water content (cf Table 1).

The percentage organic content of the soil followed the same trends as the % H_2O content, with site 1 having the highest percentage of soil organic material.

4.2. SOIL INORGANIC NITROGEN CONTENT

Soil nitrate was found to be present at the highest concentration in the alkaline soil of site 1 (9.95 $\pm$ 0.20 $\mu g\ NO_3^-\ g^{-1}$ dry mass). The more acid soils of site 3 had the lowest levels of soil nitrate (2.02 $\pm$ 0.60 $\mu g\ NO_3^-\ g^{-1}$ dry mass) (cf Table 2). This is in accordance with the findings of Walker and Wickramasinghe (1979) who found that soil nitrate tends to increase with increasing pH. Site 2 and site 3 had similar soil ammonium concentrations, which were significantly lower than the site 1 NH_4^+ level.

Table 1: Chemical and physical characteristics of the soil from a limestone outcrop (site 1) and two areas with sandstone-derived soils (sites 2 and 3). Each result is the mean of 3 replicates.

	Site 1	Site 2	Site 3
pH in $CaCl_2$	7.23 ± 0.02	4.89 ± 0.02	4.58 ± 0.01.
pH in distilled H_2O	7.60 ± 0.02	6.27 ± 0.01	5.88 ± 0.02
% H_2O content	8.9 ± 0.3	0.7 ± 0.1	3.4 ± 0,1
% organic content	7.2 ± 0.2	0.9 ± 0.1	2.92 ± 0.2

Table 2: Soil NH_4^+ and NO_3^- (μg N g^{-1} dry mass) from sites 1, 2 and 3. Each result is the mean of 4 replicates $\pm$ S.E. The means of these values are also shown. Soil ammonia was determined by means of Nessler's reagent whilst nitrate was determined by means of Szechrome NAS reagent.

Soil NH_4^+	μg $NH_4^+ g^{-1}$ dry mass	Site 1	Site 2	Site 3
	Sample A	8.48 $\pm$ 0.06	5.24 $\pm$ 0.04	5.48 $\pm$ 0.05
	B	8.46 $\pm$ 0.09	5.51 $\pm$ 0.04	5.46 $\pm$ 0.06
	C	8.37 $\pm$ 0.05	5.80 $\pm$ 0.12	5.86 $\pm$ 0.06
	D	8.79 $\pm$ 0.08	5.10 $\pm$ 0.04	5.32 $\pm$ 0.08
	Mean	8.52 $\pm$ 0.18	5.41 $\pm$ 0.15	5.53 $\pm$ 0.13
Soil NO_3^-	μg $NO_3^{-1} g^{-1}$ dry mass	Site 1	Site 2	Site 3
	Sample A	9.81 $\pm$ 0.60	4.85 $\pm$ 0.60	2.72 $\pm$ 0.003
	B	10.00 $\pm$ 0.80	5.64 $\pm$ 0.17	1.27 $\pm$ 0.79
	C	9.79 $\pm$ 0.31	5.13 $\pm$ 0.84	1.99 $\pm$ 0.55
	D	10.21 $\pm$ 0.87	5.59 $\pm$ 0.69	2.09 $\pm$ 0.67
	Mean	9.95 $\pm$ 0.20	5.30 $\pm$ 0.38	2.02 $\pm$ 0.60

The differences in the ammonium and nitrate concentrations of the soils at the different sites are reflected in the ratios of NH_4^+/NO_3^- shown in Table 3. The higher concentrations of NO_3^- in the alkali soils results in site 1 having the lowest NH_4^+/NO_3^- ratio (0.85 ± 0.002), despite the fact that ammonium concentrations were also greatest at this site. Site 3 showed the highest NH_4^+/NO_3^- ratio (2.95 ± 0.49). Site 2 may be regarded as having an intermediate NH_4^+/NO_3^- ratio.

4.3. FORMS OF NITROGEN PRESENT IN THE PLANT

The definite trend in NH_4^+/NO_3^- ratios found in the soil (i.e. a decrease from site 1 to site 3) was not reflected in the xylem sap NH_4^+/NO_3^- ratios of plant species growing at those sites (cf Table 4). What is clear is that the xylem sap was transporting far greater concentrations of ammonium than nitrate (at least an order of magnitude in all the individuals studied).

Figure 1 shows that NH_4^+ appears to be the major compound being used for transport of nitrogen in all species.

P. obtusifolia (97.7 %) and *L. meridianum* (83.4 %), growing on alkaline soils (site 1), showed a greater proportion of NH_4 in the xylem sap than those species growing in acid soils(*P. repens* (80.8 %) and *L. salignum* (74.6 % at site 2 and 78.8 % at site 3).

Nitrate concentrations in the xylem sap were consistently low in all species.

Table 3: Soil NH_4^+/NO_3^- ratios from three soil sites, site 1 being alkaline whilst sites 2 and 3 are acid. Each result is the mean of 4 replicates ± S.E.

	Site 1	Site 2	Site 3
Soil NH_4^+/NO_3^- ratio	0.85 ± 0.002	1.02 ± 0.05	2.95 ± 0.49

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Table 4: Xylem sap inorganic N concentrations (μmol N ml^{-1}) of plant species from alkaline (site 1) soil and acid (sites 2 & 3) soil. NH_4^+/NO_3^- ratios are also shown. Each of the NH_4^+ samples are the mean of 3 replicates; there were no replicates for NO_3^- samples. The NH_4^+/NO_3^- ratio is also shown.

	μmol NH_4^+ ml^{-1}	μmol NO_3^- ml^{-1}	$\mu NH_4^+/NO_3^-$
P. obtusifolia (site 1)	1.601	0.046	35.0
L. meridianum (site 2)	0.408	0.025	16.3
P. repens (site 2)	1.708	0.032	53.4
L. salignum (site 2)	0.643	0.031	20.7
L. salignum (site 3)	0.602	0.014	43.0

Table 5: Absolute concentration of N in xylem sap of each of the forms of N (mol ml^{-1}) of plants grown in alkaline (site 1) and acid soils (sites 2 & 3). Total N concentration is also shown.

	NH_4^+	NO_3^-	Amino acids	Amides	Total N
P. obtusifolia (site 1)	1.601	0.046	0.031	0.006	1.684
L. meridianum (site 1)	0.408	0.025	0.033	0.023	0.489
P. repens (site 2)	1.708	0.032	0.705	0.044	2.492
L. salignum (site 2)	0.643	0.031	0.105	0.037	0.861
L. salignum (site 3)	0.602	0.014	0.127	0.082	0.825

There was no clear relationship between the amount of nitrate in the xylem sap and the site at which the species was growing. Despite the presence of some nitrate in the xylem sap, the only NRA recorded was barely detectable in trace amounts, in the P. obtusifolia leaf material. No nitrate reductase activity was recorded in any root material.

Assimilated nitrogen (in the form of amino acids and amides) formed a greater proportion of the xylem-transported nitrogen in species growing on acid soils (site 2 and site 3), with the majority of this component consisting of amino acids.

Table 5 shows that the total nitrogen in the xylem sap tended to be higher in the Protea species. P. repens displayed the greatest capacity for transport of N in the xylem sap, followed by P. obtusifolia. L. salignum had similar xylem sap N-levels at both sites where it was found i.e. sites 2 and 3.

The very low amino acid concentrations in all samples can be seen in Table 6. Site 1 samples had consistently lower levels of amino acids in the xylem sap than samples from sites 2 and 3. Asparagine was absent from both species (P. obtusifolia and P. repens) growing in alkaline soil (site 1), but was present in all samples from acid soils. Cystine and methionine were absent from all samples analysed, whilst only a trace of proline and arginine was recorded, both of these being present in P. repens. All amino acids from valine onwards as shown in Table 6. were absent or present in trace amounts in samples taken at site 1.

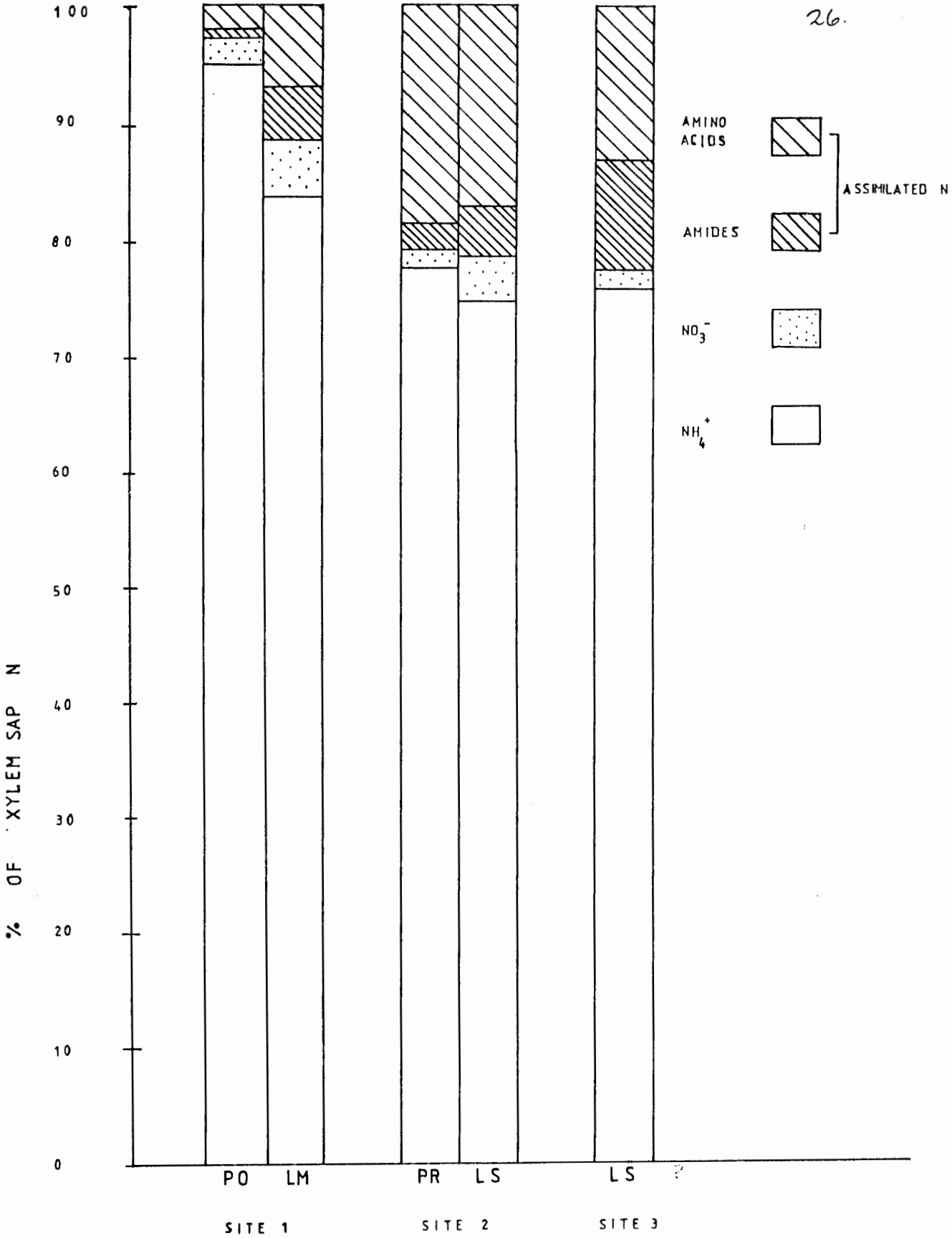

Figure 1. Percentage composition of the different nitrogen components of the xylem sap in proteaceous species on alkaline (site 1) and acid (sites 2 and 3) soils.

Table 6: Concentrations of amino acids of plants growing in alkaline soil (site 1) and acid soils (sites 2 & 3).

	Site 1		Site 2		Site 3
	LM ✱	PO +	LS x	PR ✱	LS ✱
Aspartic acid	0.003	0.003	0.010	0.029	0.014
Threonine	0.013	+	0.008	0.017	0.006
Serine	0.013	0.010	0.044	0.094	0.030
Asparagine	-	-	0.007	0.010	0.036
Glutamic acid	0.001	+	0.007	0.010	0.027
Glutamine	0.023	0.006	0.024	0.037	0.046
Proline	-	-	-	+	-
Glycine	0.006	0.010	0.032	0.051	0.020
Alanine	0.004	0.006	0.019	0.033	0.002
Valine	-	-	0.003	0.009	0.003
Cyst ine	-	-	-	-	-
Methionine	-	-	-	-	-
Isoleucine	-	+	0.002	0.007	0.002
Leucine	-	+	0.002	0.005	0.002
Tyrosine	-	-	0.002	0.005	0.001
Phenylalanine	-	-	0.001	0.004	0.001
Lysine	+	-	0.003	0.012	0.002
Histodine	-	-	0.003	0.016	+
Arginine	-	-	-	+	-
Y-amino-butyric acid	-	+	0.002	+	0.002

LM - L. meridianum
PO - P. obtusifolia
LS - L. salignum
PR - P. repens

+ no replicates
✱ mean of 2 readings
x mean of 3 readings
+ indicates a trace is present
- indicates no amino acid detected

5. DISCUSSION

pH may be considered as one of the most important factors contributing to the chemical characteristics of soils, perhaps mainly through its influence on nutrient availability Kinzel (1983). The large difference in pH, found in this study, for soils underlain by limestone (site 1) and those derived from sandstone (site 2 and site 3) may therefore contribute to the differences in species composition apparent between limestone and sandstone-derived soils. Site 2 may have had a higher pH than site 3 due to its close proximity to the limestone outcrop.

The pH values measured in this project are in accordance with previous findings that soils overlying limestones are neutral to slightly alkaline, whilst pH is acidic over siliceous rock (Kinzel, 1983). Climatic differences have been found to further influence soil pH, the effect of high rainfall being especially marked on quartzite soils where leaching of nutrients (and subsequent replacement by H^+ ions on the cation exchange sites) occurs.

The decrease in pH when extraction was performed using $CaCl_2$ compared with pH read in distilled water is in accordance with the findings of Stock (1985).

It has been suggested that extraction by means of salts leads to a reduction in pH due to the release of H^+ ions held loosely by cation exchange sites (Kinzel, 1985), resulting in unrealistically low pH values.

However, this method is frequently used by other workers, and have been be used in this study for comparative purposes. However, the absolute values of pH are perhaps not as important in this study as the large difference which exists between limestone-derived and sandstone-derived soil types.

Besides pH, percentage water content and percentage organic content will also affect nutrient availability to the plants. Percentage water content was found to be directly related to percentage organic matter content (cf Table 1), both factors being higher in the site 1 soil than in the soils at site 2 and site 3. This suggests that organic content is playing an important role in determining the water holding capacity of the soil. A further reason for the high percentage water content in the site 1 soil is that drainage would be poor in the shallow soils underlain by limestone bedrock. Furthermore, the dense mat of roots found at this site, may have contributed to increasing the water holding capacity of the soil.

The trend of decrease in pH from site 1 to site 3 appeared to be independent of the ammonium content of the soils. Site 2 and site 3 had similar ammonium levels although site 3 had a lower pH (cf Table 3).

However, soil pH was found to be closely related to the soil nitrate concentration, low pH possibly inhibiting the process of nitrification especially at site 3.

The ammonium to nitrate ratios were found to be inversely related to the soil pH (cf Table 3). Thus, pH may be seen to be an important factor in determining the relative proportions of ammonium and nitrate at the different sites studied.

NH_4^+:NO_3^- ratios found in the xylem sap were far higher than those measured in the soil. An explanation for this could be that nitrate was reduced to NH_4^+ in the roots by means of nitrate reductase. If this is the case, it would suggest that the *in vitro* assay procedure used to determine NRA was insufficiently sensitive to give positive results. However, the trace of NRA measured in leaf material in *P. obtusifolia* indicates that the reagents used were able to indicate nitrite derived from the reduction of nitrate. The use of PVP would have overcome the effect of compounds masking the reduction process. Lewis and Stock (1978) have shown that nitrate can be used as a N-source in the nutrition of proteaceous plants, but they were unable to show NRA due to the presence of polyphenolics in the plant material which interfered with the assay. Low, but significant levels of NR activity were found by Stock and Lewis (1982) in root and shoot material of *P. repens*, where PVP was used to bind with polyphenolic compounds in the extraction medium. This indicates that PVP is effective in binding with polyphenol compounds.

An alternative explanation for the high levels of NH_4^+ in the xylem sap, compared with NO_3^-, for plants growing at sites 1 and 2, is that ammonium uptake was taking place more rapidly than nitrate uptake. This would result from the fact that ammonium uptake is an active and passive process whilst nitrate uptake is a purely active process, requiring permease enzymes for the transfer of ions from the soil medium into the root. Nitrate uptake is therefore a rate-limited process depending on the number of uptake sites available. The ammonium ions have been shown to have the capacity to outcompete nitrate ions at the site of uptake (Haynes and Goh , 1978). Stock (1985) found higher rates of uptake for the ammonium than for nitrate ions in P. repens grown in a medium where these ions were present in equal quantities. This confers support to the argument that ammonium uptake is a more efficient process.

The preferential uptake of NH_4^+ at the expense of NO_3^- in the alkaline soil may further be explained by the fact that NH_4^+ is at its maximum availability in the higher pH range (Haynes and Goh , 1978). The plants growing in the alkaline soil may therefore not be able to take advantage of a balanced NH_4^+/NO_3^- nutrition which would overcome the necessity for internal pH regulation. Since nitrate and ammonium have opposite charges, their uptake differentially influences the internal pH balance. When NH_4^+ is absorbed H^+ ions are excreted into the root environment which causes a reduction of pH in the rhizosphere (Kinzel, 1983).

This may significantly reduce the availability of macronutrients which are most available in the neutral pH range, whilst heavy metals become more available possibly resulting in heavy metal toxicity or the chelation of anions e.g. of PO_4^- by Fe, thus rendering P unavailable for uptake (Kinzel, 1983).

pH balance is maintained after uptake of NO_3^- by means of the synthesis of organic acids within the cells or else by the excretion of HCO_3^- ions into the soil medium, thereby increasing pH in the rhizosphere. This process of anion excretion enhances cation absorption, but inhibits anion uptake. The reverse is true for NH_4^+ uptake (Haynes and Goh , 1978). Uptake of a combination of these two ions overcomes the need for balancing of internal pH and therefore is an energy saving process (Gutschick, 1981); it also prevents the development of an unfavourable rhizosphere pH.

The high percentage of NH_4^+ in the xylem sap suggests that ammonium is not being assimilated in the root. This is contrary to findings in other studies, in which ammonium has been found to be assimilated primarily in the root (Haynes and Goh , 1978; Lee and Stewart, 1978). A major reason suggested for root assimilation is that concentrations of ammonium, in the leaf, greater or equal to 2 mM may uncouple the process of photosynthesis (Lewis, 1986).

However, in this study xylem sap NH_4^+ concentration never exceeded 1.708 umol NH_4 per ml and therefore was not sufficient to uncouple the photosynthetic process. It may be suggested that shoot assimilation of NH_4^+ under conditions where soil nitrogen is a limiting factor is an energy saving mechanism. Root assimilation of NH_4^+ necessitates the translocation of carbon skeletons to the root in the phloem. Carbon skeletons resulting from the fixation of carbon in the dark phase of photosynthesis may be used directly in the assimilation of ammonium if assimilation is occuring in the leaves (Gutschick, 1981).

Pate (1980) has suggested that ammonium found in xylem exudates may be a result of post harvest breakdown of solutes. Consequently, the findings of high NH_4^+ levels in the xylem sap in this study should perhaps be interpreted with caution.

Assimilated nitrogen was found to be consistently higher in the xylem sap of species growing in acid soils (cf Fig. 1. and Table 6.). The majority of this portion of xylem N consisted of amino acids, with amides making up the remainder. A higher proportion of amides and amino acids in the site 2 and site 3 samples may be a result of a higher proportion of assimilation in the roots, when compared to site 1 samples. However, it may be possible that higher assimilated N levels in the acid soils are the result of recycling of N from storage pools within the plant.

This may be due to lower soil nutrient status and therefore a greater necessity for efficient nutrient usage (Stock, 1985). Stock (1985) showed that internal cycling of N was an important means by which nutrients could be conserved in soils of low nutrient status. For this reason, Pate (1980) has suggested that in soils of poor nutrient status, xylem sap components may be a poor indication of the uptake processes taking place.

It may be interesting to note that the Protea spp. had higher levels of xylem sap N than did the Leucadendron spp. (cf Table 5) at site 1 and site 2. This observation is difficult to explain in terms of the available evidence, but it may be a function of their larger size and possible greater uptake of nutrient.

6. CONCLUSIONS

pH was found to strongly influence the levels of nitrate in the soil, the highest levels being recorded in limestone-derived soils. This implies that the nitrifying bacteria are most active in this soil type.

However, the high soil nitrate levels were not reflected in the nitrogen nutrition of Proteaceae growing in the Agulhas region. Although assimilated N-levels are proportionaly higher in the plants growing in acid soils, levels of nitrate uptake were similar in both soil types. Preferential uptake of ammonium at higher soil pH may primarily have been due to its greater availability in this pH range. Ammonium would have outcompeted nitrate ions at the uptake sites.

According to the definition of a calcicole plant, these species are not specifically adapted to soils of high pH in terms of their nitrate uptake abilities. Evidence for this comes from the fact that nitrate reductase activity (NRA) was not recorded in any significant amounts in the plants on limestone - derived soil.

The differential distribution of plant species in limestone- and sandstone-derived soils is therefore probably not directly related to their nitrogen nutrition. Several other factors such as tolerance to high $CaCO_3$ levels may be important in the success of plant species in the calcareous habitat.

ACKNOWLEDGEMENTS

I should like to thank Professor Lewis for his supervision and interest in this project, Pete Jurgens for the amino acid analyses and Axel von Zelewski for his assistance in the field and in the laboratory.

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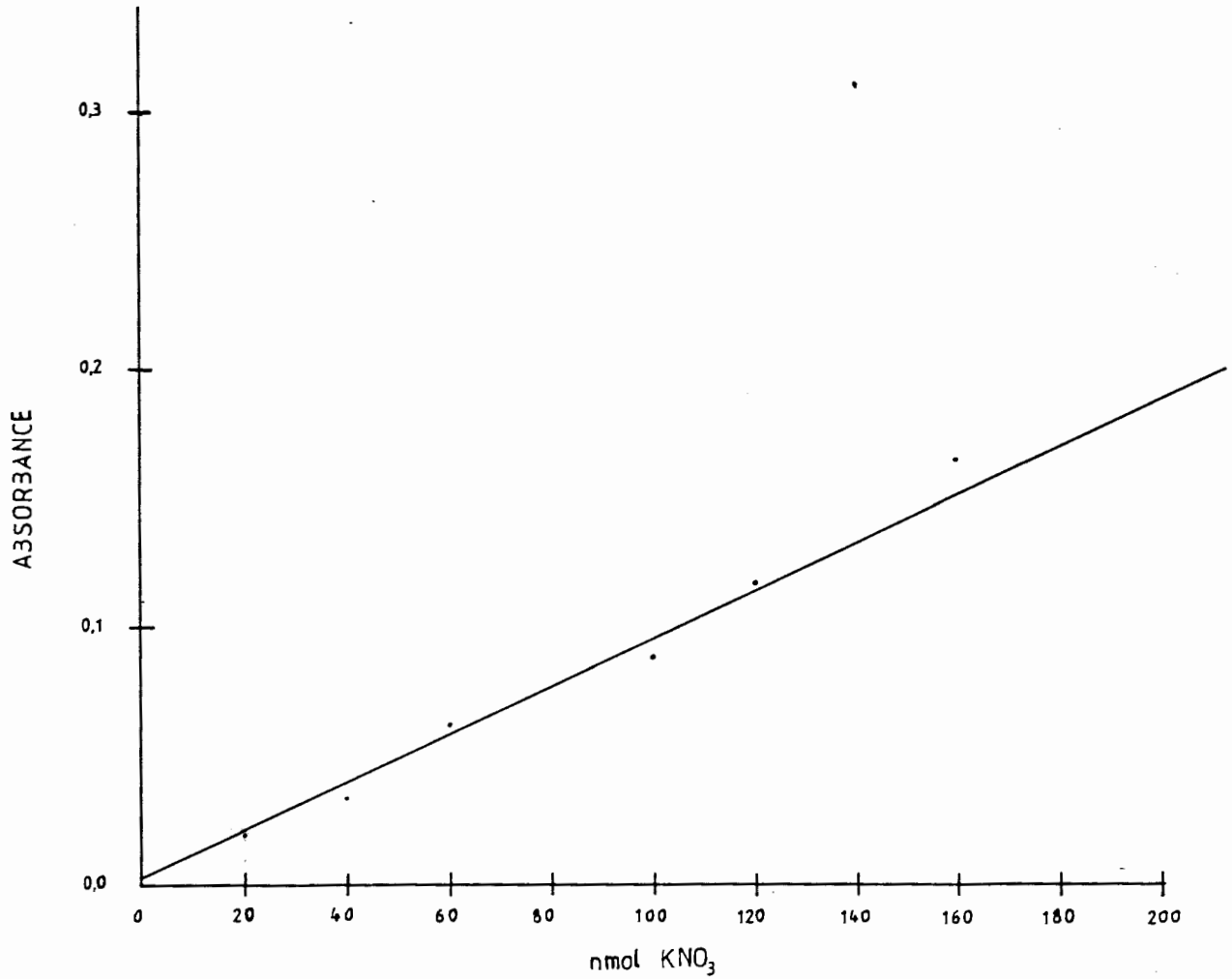
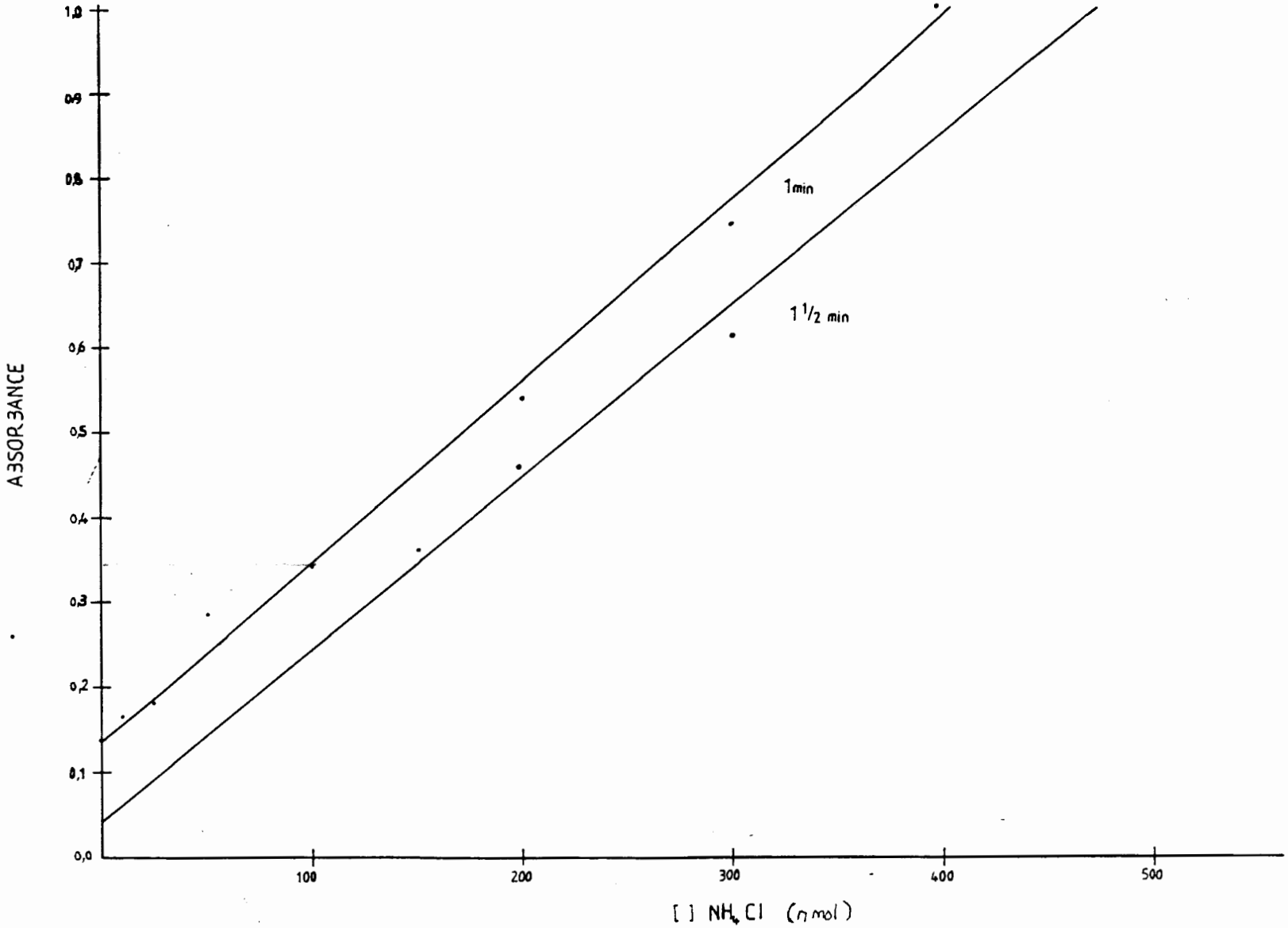
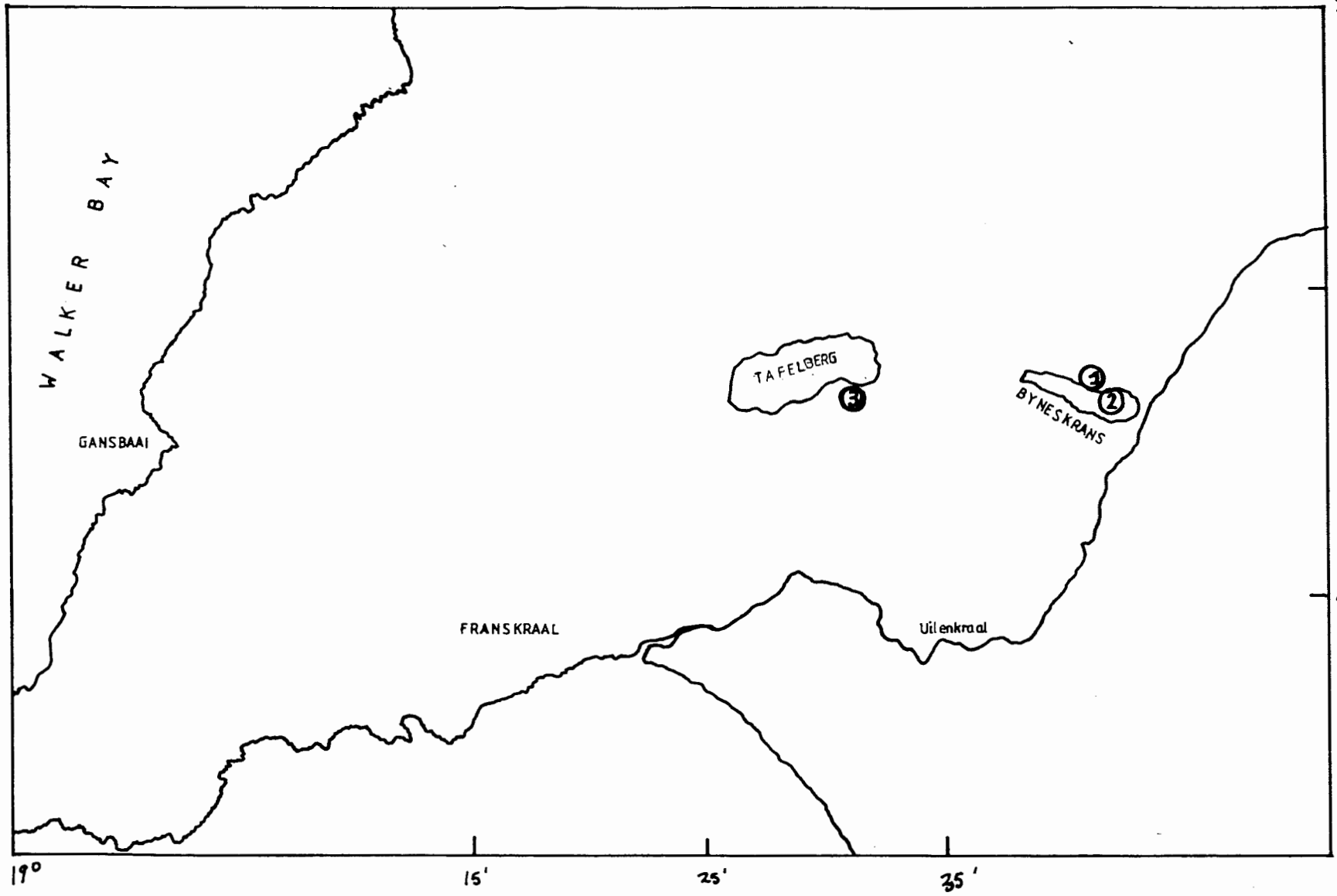


Appendix 1. Standard curve for Szechrome used in the determination of nitrate levels in soil samples and xylem sap.



Appendix 2. Standard curve for Nessler's reagent used in xylem sap and soil nitrate analyses.



Appendix 2. Map of the Agulhas region showing the location of study sites used in this project.