

**Investigation of soil quality (health) in commercial
production of rooibos tea (*Aspalathus linearis*) in the
Western Cape, South Africa**

by
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DECLARATION

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ABSTRACT

The global demand for rooibos tea (*Aspalathus linearis*) has steadily increased over the past five years thanks to the increased awareness of its health benefits, while rooibos tea production has decreased. If this trend continues, rooibos producers will be unable to meet the world demand. What makes rooibos a particularly challenging crop to grow is the fact that it is a sensitive fynbos species, adapted to low soil nutrient conditions, which can only be cultivated in a niche area of the Western and Northern Cape regions of South Africa. The farmers are under great pressure due to increasing production costs and environmental laws which restrict the establishment of new rooibos fields in fynbos areas. The only way for farmers to meet the demand will have to be by increasing their tea yields and quality while using the same area of land. To date, very little research has been conducted to help the producers. Rooibos farmers report that yields decline dramatically over time since the clearing of the natural fynbos vegetation. It was hypothesized that this decrease in production is most likely related to changes in soil quality (health) and ecology. Thus, the aim of this study was to examine soil (chemical, physical and microbiological properties) and plant quality parameters in cultivated rooibos fields of various ages and adjacent, rooibos stands in pristine fynbos. The results will be used to diagnose the decline in rooibos production and suggest amendment strategies to improve commercial rooibos production.

Experimental sites were selected in the Clanwilliam district, at the two oldest (Nardouwsberg and Seekoeivlei) of the six main rooibos producing areas. Virgin fynbos, young rooibos fields (2 years) and older rooibos fields (20-60 years) were selected as sampling sites. The following soil physico-chemical and biological parameters were measured; pH, total C and N, exchangeable cations and acidity, plant-available P and micronutrients, texture, bulk-density, protist counts, and total microbial biomass. The total above- and below-ground biomass, foliar nutrient content, tea quality and yield, mycorrhizae counts and N nodule counts were studied on the plants. Different remedial treatments were applied at the oldest (>60 years) cultivated site (Valkrans); the four treatments consist of two compost treatments (rooibos plant litter and rooibos + Chicken litter) and two foliar sprays (Mazinbor and Goemar).

It was found that continuous cultivation resulted in significant decreases in plant above- and below-ground biomass yields, soil C and N, and basic cations. Cultivation also led to decreases in the total microbial biomass, soil protist and root mycorrhizae counts, but an increase in extent of root N-nodulation, indicating a greater dependence on symbiotically fixed N than soil N. Soil P increased with continuous cultivation, as well as, plant foliar P content. An exponential negative correlation was found between the Bray II P in the soil and the above ground biomass ($R^2 = 0.417$) as well as with the mycorrhiza on the roots ($R^2 = 0.8134$). A positive correlation was observed between the tea quality and the mycorrhiza colonization ($R^2 = 0.4428$). An exponential negative correlation was found to exist between foliar P content and the above ground biomass yields ($R^2 = 0.6653$), as well as tea quality ($R^2 = 0.71$). It has been previously established that fynbos plants species, such as rooibos, are adapted to low P conditions and thus highly sensitive to P toxicity. Phosphorous toxicity leads to Ca, Fe and Zn unavailability within the plant, and ultimately to increased susceptibility to fungal rot and plant death. Some P toxicity symptoms (chlorosis) were found on the leaves of the rooibos plants on the site with the highest Bray II P content (18 mg kg^{-1}). A positive correlation was found between foliar B content and above ground biomass yield ($R^2 = 0.69$). A positive correlation was also found to exist between foliar Na content and plant size in the Seekoeivlei area soils ($R^2=0.4698$), potentially indicating the importance of Na in plant quality.

The compost treatments at 20 ton ha^{-1} generally increased the soil pH, CEC, Bray II P (rooibos +chicken litter only) and B, but had no effect on other soil properties. Although not statistically significant, the rooibos + chicken litter compost 2 ton ha^{-1} treatment resulted in the greatest increase in above (26.7 %) and below-ground (37.6 %) biomass, whereas, the 20 ton ha^{-1} application resulted in smaller increases in above (14.0 %) and below-ground (22.4 %) biomass. This could be attributed to possible P-toxicity, as the 20 ton ha^{-1} treatment increased soil Bray II P to above 30 mg kg^{-1} soil, whereas all the other treatments (incl. control) remained around 15 mg kg^{-1} P. The increase in soil P due to the rooibos + chicken litter compost led to a significant increase in the foliar P and resulted in a decrease in root mycorrhiza and Zn. The rooibos residue compost treatments resulted in a slight decrease in biomass yields. This could be attributed to the high C:N ratio (26:1) of the rooibos residue compost which slightly decreased foliar N. The Mazinbor foliar spray led to a slight increase

in foliar Zn, Mn and Ca, but no effect on biomass yields. The Goemar Plus foliar spray had no effect on plant properties or biomass yields.

There were several changes in the soil quality between the recently cleared pristine fynbos soils and cultivated sites. The main reason can be due to a decreased soil C resulting in decreases in exchangeable basic cations and the associated decrease in soil microbial activity. Significant increases in the soil and plant P levels were observed due to applications of phosphate by farmers. The rooibos plants showed P toxicity and reduced growth in fields with a high P content as well as a decrease in the rooibos quality. The decline in rooibos production can thus mainly be attributed to a decrease in soil C and increases in soil and plant P resulting in P toxicity. The rooibos + Chicken litter compost amendment showed an improvement in plant productions at low levels. The study was of great importance since it was established that farmers should be careful when applying a phosphate fertilizer since rooibos can be P sensitive since it is a fynbos plant. The farmers thus had a negative effect on the production of rooibos due to wrong cultivation practices used in the past. The study suggests that the addition of compost had a positive effect on the production of rooibos as long as the P levels are carefully monitored to prevent P toxicity

OPSOMMING

Die wêreld se vraag na rooibostee (*Aspalathus Linearis*) het konstant oor die afgelope vyf jaar toegeneem, omdat rooibostee geassosieer word met 'n gesonde leefstyl. Daar word egter tans 'n afwaartse neiging in tee-oeste en rooibostee-produksie waargeneem. Indien die tendens voortgaan soos tans, sal rooibostee-producente nie aan die wêreldvraag kan voldoen nie. 'n Verdere uitdaging van rooibostee-produksie is dat dit 'n sensitiewe fynbos-plant is wat aangepas is om op gronde te groei wat laag in voedingstowwe is en slegs in klein areas in die Wes- en Noord-Kaap geproduseer kan word. Faktore soos toenemende koste en omgewingsvriendelike wette stel groter uitdagings aan boere. Om sukses te behaal, is boere verplig om hul tee-opbrengs per hektaar en tee-kwaliteit te verbeter. Daar is nog min navorsing gedoen om die produsente te help. Rooibosteeboere het opgemerk dat die produksie gewoonlik drasties afneem na die eerste 5-jaar plantsiklus op nuut skoongemaakte fynbosgronde. Die hipotese is dat die afname in produksie grotendeels toegeskryf kan word aan die veranderinge in grondkwaliteit (gesondheid) en ekologie. Die doel van die studie is dus om die grond- (chemiese, fisiese en mikrobiologiese eienskappe) en plantkwaliteit parameters in gevestigde rooiboslande en aanliggende onversteurde fynbos-areas te ondersoek. Uiteindelik sal die resultate gebruik word om die afname in rooibostee-produksie te diagnoseer en verskillende regstellende behandelings voor te stel om sodoende volhoubare bestuurstrategieë te verseker om die kommersiële rooibostee-produksie te verbeter.

Eksperimentele areas (Nardouwsberg en Seekoeivlei in die Clanwilliam –distrik) is in twee van die ses hoof rooibostee-produuserende areas uitgesoek. Nuwe fynbosareas, jong rooibosteelande (2 jaar) en ouer rooibosteelande (20 – 60 jaar) is uitgekies om monsters te neem. Grondmonsters en een-jaaroue plantmonsters is in drievoud by elke area geneem. Die volgende fisies-chemiese en mikrobiologiese parameters is gemeet: pH, totale C en N, uitruilbare katione en -suurheid, plantbeskikbare P en mikro-nutriënte, tekstuur, bulkdigtheid, protiste tellings en totale mikrobiiese biomassa. Die totale bo- en ondergrondse biomassa, blaar -voedingstofinhoud, tee-kwaliteit en -opbrengs, mycorrhizae tellings en N nodule tellings van die plante is bestudeer. Verskillende behandelings vir herstel is toegepas op die oudste (>60 jaar) bewerkbare area (Vaalkrans). Die vier

behandelings behels twee komposbehandelings (rooibostee plantafval kompos en hoendermis + rooibosteestokke) en twee blaarbespuitings (Mazinbor en Goemar).

Dit is bevind dat voortdurende bewerking 'n merkbare afname in die plant se bo- en ondergrondse biomassa opbrengs, asook 'n afname in C, N en basiese katione, in die grond tot gevolg het. Bewerking het ook gelei tot die afname in die totale mikrobiële biomassa, grond protiste- en wortel mycorrhizae tellings, maar 'n toename in die N- knoppie- vorming wat dui op 'n groter afhanklikheid van simbiotiese gebonde N eerder as grond N. Grond P sowel as plant P inhoud neem toe met voortdurende bewerking. 'n Eksponensiële negatiewe korrelasie tussen die Bray II P inhoud van die grond en die bogrondse biomassa ($R^2 = 0.417$), asook met die mycorrhiza op die wortels ($R^2 = 0.8134$) is gevind. 'n Positiewe korrelasie is waargeneem tussen die teekwaliteit en die mycorrhiza kolonisasie ($R^2 = 0.4428$). Daar is ook gevind dat 'n eksponensiële negatiewe korrelasie bestaan tussen blaar P inhoud en die bogrondse biomassa opbrengs ($R^2 = 0.6653$), asook die teekwaliteit ($R^2 = 0.71$). Dit is voorheen vasgestel dat fynbos plantspesies, soos rooibostee, nie in staat is om P opname te reguleer nie. Dit lei tot toksiese P akkumulاسie en geassosieerde onbeskikbaarheid van spoorelemente in die plant en uiteindelik tot toenemende vatbaarheid vir swambesmetting en die afsterwing van die plant. Sommige P toksiese simptome (Chlorosis) is op die blare, van die rooibosplante op die steekproef area met die hoogste Bray II P inhoud (18 mg kg^{-1}), gevind. 'n Positiewe korrelasie is gevind tussen die blaar B inhoud en die bogrondse biomassa opbrengs ($R^2 = 0.69$). 'n Positiewe korrelasie is ook gevind tussen die blaar Na inhoud en die plantgrootte in die Seekoeivlei-area, wat potensieel die belangrikheid van Na in plantkwaliteit aandui.

Die kompos behandelings van 20 ton ha^{-1} het oor die algemeen die grond pH, KUK, Bray II P (slegs rooibostee-afval + hoendermis) en B laat toeneem, maar geen effek op ander grondeienskappe gehad nie. Alhoewel dit nie statisties beduidend is nie, het die rooibostee + hoendermis kompos 2 ton ha^{-1} behandeling die grootste toename in bogrondse- (26.7%) en ondergrondse (37.6%) biomassa veroorsaak, terwyl die 20 ton ha^{-1} toediening kleiner toenames in bogrondse (14.0%) en ondergrondse (22.4%) biomassa tot gevolg gehad het. Dit kon moontlik toegeskryf word aan P toksisiteit aangesien die 20 ton ha^{-1} behandeling se grond Bray II P tot bo 30 mg kg^{-1} toegeneem het, waar al die ander behandelings (insluitend kontrole) ongeveer 15 mg kg^{-1} P gebly het. Die toename in grond P as gevolg van die

rooibostee + hoendermis kompos het gelei tot 'n beduidende toename in die blaar P en het 'n afname in wortel mycorrhiza en Zn veroorsaak. Die rooibostee-kompos behandelings het in 'n effense daling in die biomassa opbrengs veroorsaak. Dit kan toegeskryf word aan die groot C:N verhouding (26:1) van die rooibostee-kompos wat verantwoordelik was vir 'n stikstof negatiewe periode en dus ook die effense daling van die blaar N inhoud. Die Mazinbor blaarbespuiting het gelei tot 'n effense toename in blaar Zn, Mn en Ca, maar 'n effense daling in biomassa opbrengs. Die Goemar Plus blaarbespuiting het geen effek op die planteienskappe nie en 'n effense afname in biomassa opbrengs is opgemerk.

Na aanleiding van die bevindinge kan daar waargeneem word dat daar wel veranderinge in die grondkwaliteit is tussen onlangs ontboste fynbosgronde en lande waar rooibostee kommersieel vir 'n aantal jare verbou is. Die hoof rede kan toegeskryf word aan die afname in die grond se C inhoud wat 'n afname in die uitruilbare basiese katione tot gevolg gehad het, sowel as 'n afname in mikrobiële aktiwiteit in die grond. Beduidende toenames in die grond sowel as blaar P inhoud is ook waargeneem en kan toegeskryf word aan toedienings van verskeie fosfaat kunsmis deur boere. Die plante begin P toksisiteit simptome toon en daar is 'n afname in die groei en kwaliteit van die tee in lande met 'n hoë P inhoud. Die afname in die rooibostee-produksie kan dus toegeskryf word aan 'n afname in die grond C en toenames in die grond- en blaar P inhoud wat P toksisiteit tot gevolg het. Die laer vlakke van rooibostee + hoendermis kompos toevoegings het 'n toename in die plantproduksie getoon in die grond tipes soos beskryf in die studie. Die studie was van belang aangesien daar vasgestel is dat rooibos boere versigtig moet wees wanneer fosfaat kunsmis gebruik word aangesien rooibos 'n fynbos plant is en dus gevoelig is vir 'n oormaat fosfaat. Boere het dus 'n effek gehad op die afname in rooibos produksie agv. verkeerdelike verbouings praktyke wat in die verlede gevolg is. Dit wil voorkom of die toediening van kompos 'n positiewe effek op die produksie van rooibos het solank die P vlakke dopgehou word om P toksisiteit te voorkom.

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1 GENERAL INTRODUCTION AND RESEARCH AIMS

The global demand for rooibos (*Aspalathus linearis*) tea has steadily increased over the past five years thanks to the increased awareness of its health benefits, while rooibos tea production has decreased. Rooibos farmers report that yields declined substantially over time since the clearing of the natural fynbos vegetation. If this trend continues, rooibos producers will be unable to meet consumer demand. What makes rooibos a particularly challenging crop to grow is the fact that it is a sensitive fynbos species, adapted to very acid, low soil nutrient conditions, which can only be cultivated in a niche area of the Western and Northern Cape regions of South Africa. Farmers are under great pressure due to increasing production costs and environmental laws which restrict the establishment of new rooibos fields in fynbos areas. The only way for farmers to meet the demand will have to be by increasing their tea yields and quality while using the same area of land.

The cause for the decline in rooibos yields since clearing of the fynbos vegetation is unknown. The only previous work on the cultivated rooibos plant properties was conducted by Strassen (1987) on a farm in Citrusdal, in order to get an estimate of the amounts of nutrients lost with each tea harvest. The effect of long-term rooibos cultivation on soil quality (health) or plant properties in comparison with natural fynbos conditions needs to be clarified. Furthermore, there is very little peer-reviewed, published information available on the effect of chemical or organic fertilizers on rooibos tea growth. Joubert et al. (1987) investigated the effect of addition of the macronutrients, nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) on rooibos seedlings growth over 5 months in a greenhouse trial. While, Muofhe (1997) investigated the effect of N, P and Ca on the growth and extent of N-nodulation of young rooibos plants in the greenhouse and in the field over a six month period. All of these studies were conducted over a short period of time (months), and none looked at tea yields or quality, which are ultimately the most important criteria for rooibos producers.

No literature could be found on the effect of micronutrients on rooibos yields or quality. The effect of long-term cultivation on the N-nodules as well as the effect of plant nutrients on the tea quality or extent of root mycorrhizal colonization needs to be clarified.

Therefore, the main aim of this study was to establish the effect of long-term rooibos cultivation on soil quality (chemical, physical and microbiological properties) (**Chapter 3**) and rooibos plant properties (**Chapter 4**). The relationship between soil quality, beneficial microorganisms (mycorrhizae and N-fixing bacteria), and rooibos growth and tea quality (**Chapter 4**) was also investigated. The reasons for the decline in rooibos production need to be identified so that amendment strategies could be devised. This study was conducted at the two oldest (Nardouwsberg and Seekoeivlei) of the six main rooibos producing areas around Clanwilliam in the Western Cape Province, South Africa. In each area, a number of sites were selected ranging from undisturbed virgin fynbos soils with wild rooibos plants, newly established rooibos fields where the fynbos was recently cleared, and old rooibos fields ranging from 20 to over 60 years of age. The following soil quality parameters were determined: bulk density, texture, pH (H₂O & KCl), total C and N, exchangeable basic cations and acidity, plant-available P and micronutrients (Cu, Zn, Fe, Mn, B), total microbial biomass, and protist numbers. The following rooibos properties were examined; the above- and below-ground biomass, macro- and micronutrients, extent of root mycorrhizal and N-nodule colonization, tea yields and quality.

The second objective was to determine the effect of selected compost and foliar sprays on soil quality and plant properties (**Chapter 5**). Once the soil and plant factors that caused the decrease in rooibos production were identified, suitable compost and foliar spray amendments were selected and applied at the oldest (> 60 years) cultivated site, Vaalkrans in December 2012. Two rooibos-litter-based composts were selected since the litter contains high amounts of the necessary nutrients needed for rooibos production and in the correct ratio. The effect of the amendments on soil quality and rooibos growth was determined 6 months after application.

Defining the effect of long-term rooibos cultivation on soil and rooibos quality is of fundamental significance, so that soil quality can be better managed and rooibos production can be increased in a sustainable manner. The ultimate aim of this research is to help rooibos farmers to keep up with the global demand while protecting their livelihood.

2 LITERATURE STUDY: AN INTRODUCTION TO SOIL HEALTH AND ROOIBOS CULTIVATION

2.1 Introduction

Most scientific research on rooibos tea focused on the health benefits of the plant and optimised tea quality through processing. Little research has been carried out on the soil quality (health) properties that influence the production of rooibos, which is the main focus of this study.

The average rooibos yield per hectare has decreased over the last five years with approximately 200 kg ha⁻¹. While only 95000 ha of land is used for continuous production of rooibos by 220 farmers (J. Brand, Rooibos Ltd., Clanwilliam, South Africa, 2013, personal communication). Usually, after 5 years, up to 48% of the rooibos plants have died (Strassen & Holtzhausen, 1990). If the soil is healthy in terms of rooibos production, it is more likely that the plants will be less susceptible to pathogens or nutrient imbalances, thus improving the longevity of the plants. It is thus of great importance to try and identify the reason(s) for the decline in the production of rooibos tea over time, in order to try and establish remediation treatments. It is important that these remediation practices will increase the production and quality of rooibos without having any negative impact on the environment, so as to ensure sustainable rooibos farming.

2.2 Soil quality (health)

2.2.1 Concept of soil quality

Soil quality (also sometimes referred to as soil health in the soil science literature) refers to the chemical, physical and microbiological features that are necessary in the soil for long-term sustainable agricultural production with a minimum environmental impact. Soil quality thus gives a picture of the overall soil functionalities. The soil quality can't be measured directly, but it can be established by studying specific soil properties (e.g., organic matter content) and by observing soil status (e.g., fertility) (Arias *et al.*, 2005). Assessing the health or quality of soil can be likened to the examination for humans where certain measurements (e.g., blood pressure and cholesterol) are taken and is used as basic indicators of system function (Larson & Pierce, 1991).

2.2.2 Soil quality chemical indicators

2.2.2.1 Soil pH

By estimating the hydrogen- ion activity, the acidity or alkalinity of the soil can be determined. The pH affects soil mineral solubility, plant nutrition availability and the activity of soil microorganisms. Acidity is usually associated with a leached soil, while an alkaline pH is usually associated with a drier climate (Arias *et al.*, 2005). The pH of soils can be altered by applying amendments, such as lime. The optimum pH of the soil will be determined by the type of plant that is grown on the soil.

2.2.2.2 Electrical conductivity (EC)

The EC of the soil-water mixture is an indicator of the amount of ions that is present in the soil solution. If too much of these salts are present, it seriously affect the growth of plants and the soil-water balance. This usually happens under conditions where soil has not been managed correctly or is located in a dry region. Electrical conductivity values between 0.0-0.8 dS m⁻¹ are appropriate for most plants (Arias *et al.*, 2005).

2.2.2.3 Cation exchange capacity (CEC)

The ability of soil to supply the plant with the main macronutrients, such as Ca, Mg and K, can be referred to as the ion exchange capacity. The CEC of the soil is the ability to reversibly retain cations. The CEC can be influenced by the pH and salt concentration. Organic matter increases the CEC, and has a positive influence especially in sandy soils as it increases the nutrient storage capacity (Arias *et al.*, 2005).

2.2.2.4 Soil organic matter (SOM)

Soil organic matter plays a major role in the functions of soil, e.g., determining soil fertility, water holding capacity and susceptibility of soil to degradation (Giller and Cadisch, 1997; Feller *et al.*, 2001). There is a direct correlation between SOM and the chemical, microbiological and physical properties of the soil. It also influences the amount of nutrients that are available for plant uptake (Arias *et al.*, 2005). Soil organic matter also influences the soil aggregate stability and the erosion extent (Mathys, 2011). Microorganisms are found more abundant in the top 10 cm of the soil than in the deeper soil layers, since the SOM is also higher in this soil layer compared to the deeper soil layers (Barnes *et al.*, 2003).

2.2.3 Soil quality physical indicators

2.2.3.1 Soil particle size distribution

The particle size density (sand, silt and clay), have an effect on the bulk density of the soil, water infiltration rate, water storage, soil aeration. A soil with higher clay content would be able to form more aggregates as apposed to a sandy soil. Soil particle size distribution is a good indication of other soil physical indicators such as bulk density, water infiltration rate and aggregate stability.

2.2.3.2 Bulk density

Bulk density is defined as the mass (oven dried soil) over the volume. It is dependent on the particle density (sand, silt & clay), the soil texture, and the packing arrangement. If the soil is tightly packed it has a high bulk density. At high bulk densities root growth, air and water movement are restricted (Arias *et al.*, 2005).

2.2.3.3 Water infiltration rate

Water infiltration rate is dependent on management strategies, as well as time. The bulk density, worm burrows, plant roots and aggregates plays an important role. Texture and the type of tillage also play an important role in the rate of water infiltration (Arias *et al.*, 2005).

2.2.3.4 Aggregate stability

Aggregates can be found if particles in the soil are bound together. Aggregates play an important role in many of the aspects that influence soil quality. For example, the movement and storage of water, soil aeration, physical protection of SOM, the prevention of erosion, root development, and microbial community activity (Arias *et al.*, 2005). Fewer aggregates are found in a sandy soil than in a clay soil.

2.2.4 Microbiological indicators of soil quality

2.2.4.1 Protists

Protists belong to the kingdom of Protista. There are 3 kinds of protists, an animal (protozoa), plant (algae) and fungus (heterotrophs, decomposers, external digestion). Protozoa plays a key role in the nitrogen and carbon cycles in the soil by regulating the decomposition rate, as well as some specific metabolic pathways. Most of the protozoa species can be found in the upper 10 cm of the soil (Couteaux & Darbyshire, 1998). Protozoa

species live primarily on bacteria, but can also eat other protozoa, soluble organic matter as well as some fungi. During assimilation of bacteria by protozoans, some nitrogen is excreted into the soil in the form of ammonia that can be taken up by plants (Ingham, 1999). Therefore, determining soil protist numbers is a good indicator of nutrient cycling in a soil, especially as they are known to play an important role in ecosystems where nutrient cycling is rapid, such as in fynbos ecosystems (Prof Alf Botha, Department of Microbiology, Stellenbosch University, 2012, Personal communication).

Each protozoon has a different method for determination, for naked amoebae and flagellates the nuclear characteristics can be observed with a light microscope or a transmission electron microscopy. For ciliates a silver staining cannot be performed and testacea can be counted and identified because of their taxonomy (Couteaux & Darbyshire, 1997). The four groups that methods can be classified into: 1) direct observation of soil suspensions, 2) soil extraction, 3) incubation of serially diluted soil suspensions with or without nutrient enrichment and lastly 4) colonisation of glass slides or chambers (Couteaux & Darbyshire, 1998). The most commonly used method is the direct one for counting active amoebae, ciliates and flagellates. This can be done by diluting the soil suspensions and then observing it under a light microscopy.

2.2.4.2 Symbiotic N-fixing bacteria

In legumes, such as rooibos, the bacteria live in small growths on the roots called nodules. Within these nodules, nitrogen fixation is carried out by the bacteria (Lindemann & Glover, 2003). This process involves the reduction of atmospheric N_2 to NH_3 . Most of the nodules will be centred on the tap root (Lindemann & Glover, 2003). When white, grey or green nodules are present, little nitrogen fixation is occurring. This can be due to an inefficient *Rhizobium* strain, poor plant nutrition, pod filling or other plant stress. If the plant is under stress, the nitrogen fixing will be reduced by the plant. It is easier and less energy consuming for the plant to absorb nitrogen from the soil than to fix it from the air (Lindemann & Glover, 2003). *Rhizobium* bacteria are one of the main nitrogen fixing bacteria and play a key role in the soil conditions that are optimum for rooibos growth, especially *Bradyrhizobium* (Muofhe & Dakora, 2000).

2.2.4.3 Mycorrhizal fungi

Mycorrhizas are present in almost every type of soil and are root fungi. They play an important role in the uptake of nutrients by plants, especially P. The mycorrhizas are usually present on or near the roots of the plants. There are different types of mycorrhiza, each having a symbiotic relationship with specific plant species. Plants that have sufficient mycorrhizal colonization are less susceptible to pathogens (Zeng, 2006) and are also more resistant to drought (Lehto, 1992). The extent of mycorrhizal rooibos root colonization can thus be used as a soil quality indicator.

2.2.4.4 Microbial Biomass

Microbial biomass plays a key role in the soil, because it converts organic matter into plant available nutrients. It also plays a key role in the storage of carbon through immobilization. It responds quickly to changes in soil conditions because of management strategies, it is thus an excellent indicator of soil quality (Mathys, 2011).

Table 2-1: Proposed Minimum Data Set of Physical, Chemical, and Biological Indicators for Screening the Condition, Quality, and Health of Soil (Doran, 1996. Modified after Doran and Parkin, 1994, and Larson and Pierce, 1994)

Indicators of soil condition	Relationship to soil condition and function (rationale as a priority measurement)	Ecologically relevant Value Si units (comparisons for evaluation)
Physical		
Texture	Retention and transport of water and chemicals; Modeling use, soil erosion and variability estimate	% Sand, silt, and clay; less eroded sites or landscape positions
Depth of soil, topsoil and rooting	Estimate of productivity potential and erosion; normalizes landscape and geographic variability	cm or m; non cultivated sites or varying landscape positions
Infiltration and soil bulk density (SBD)	Potential for leaching, productivity, and erosivity; SBD needed to adjust analyses to volumetric basis	Min/2.5 cm of water and $g\ cm^{-3}$; row and/or landscape positions
Water holding capacity (Water retention characteristics)	Related to water retention, transport, and erosivity; Available H_2O . Calculate from SBD, texture, and OM	% ($g\ cm^{-3}$), cm of available H_2O /30 cm: precipitation intensity
Chemical		
Soil organic matter (SOM) (total organic C and N)	Defines soil fertility, stability, and erosion extent; use in process models and for site, normalization	$kg\ C\ or\ N\ ha^{-1}\ -30\ cm$; non cultivated or native control
pH	Defines biological and chemical activity thresholds; essential to process modeling	Compared with upper and lower limits for plant and microbial activity
Electrical conductivity	Defines plant and microbial activity thresholds; presently lacking in most process models	$dS\ m^{-1}$; compared with upper and lower limits for plant and microbial activity
Extractable N, P, and K	Plant available nutrients and potential for N loss; productivity and environmental quality indicators	$kg\ ha^{-1}\ -30\ cm$: seasonal sufficiency levels for crop growth
Biological		
Microbial biomass C and N	Microbial catalytic potential and repository for C and N; modeling: Early warning of management, effect on SOM	$kg\ N\ or\ C\ ha^{-1}\ -30\ cm$; relative to total C & N or CO_2 produced
Potentially mineralizable N (anaerobic incubation)	Soil productivity and N supplying potential; process modeling; (surrogate indicator of biomass)	$kg\ N\ ha^{-1}\ -30\ cm/day$; relative to total C or total N contents
Soil respiration, water content, and temperature	Microbial activity measure (in some cases plants); process modeling; estimate of biomass activity	$kg\ C\ ha^{-1}\ day^{-1}$; relative microbial biomass activity, C loss vs. inputs and total C pool

2.3 Cultivation of rooibos in South Africa

2.3.1 Production area

Rooibos is a legume plant that only grows in South Africa. Rooibos is an erect or straggling, slender-stemmed bush 1.35-2 m tall (Morton, 1983). The leaves have a linear, needle like form and are about 2-6 cm long. The flowers are yellow and about 6.5 mm long. The leaves of the plant are used in the making of the beverage called rooibos tea. There are 4 naturally occurring forms: Rooi tea, Vaal tea, Swart tea and Rooibruin tea. Rooi tea is subdivided into 2 types: Nortier and Cedarberg which are similar, except Cedarberg has a broader and coarser leaf and grows wild in the Cedarberg Mountains. The plant that is commercially cultivated is the Nortier type. The native growing region of this plant is in the Western Cape, centred in the Clanwilliam district.

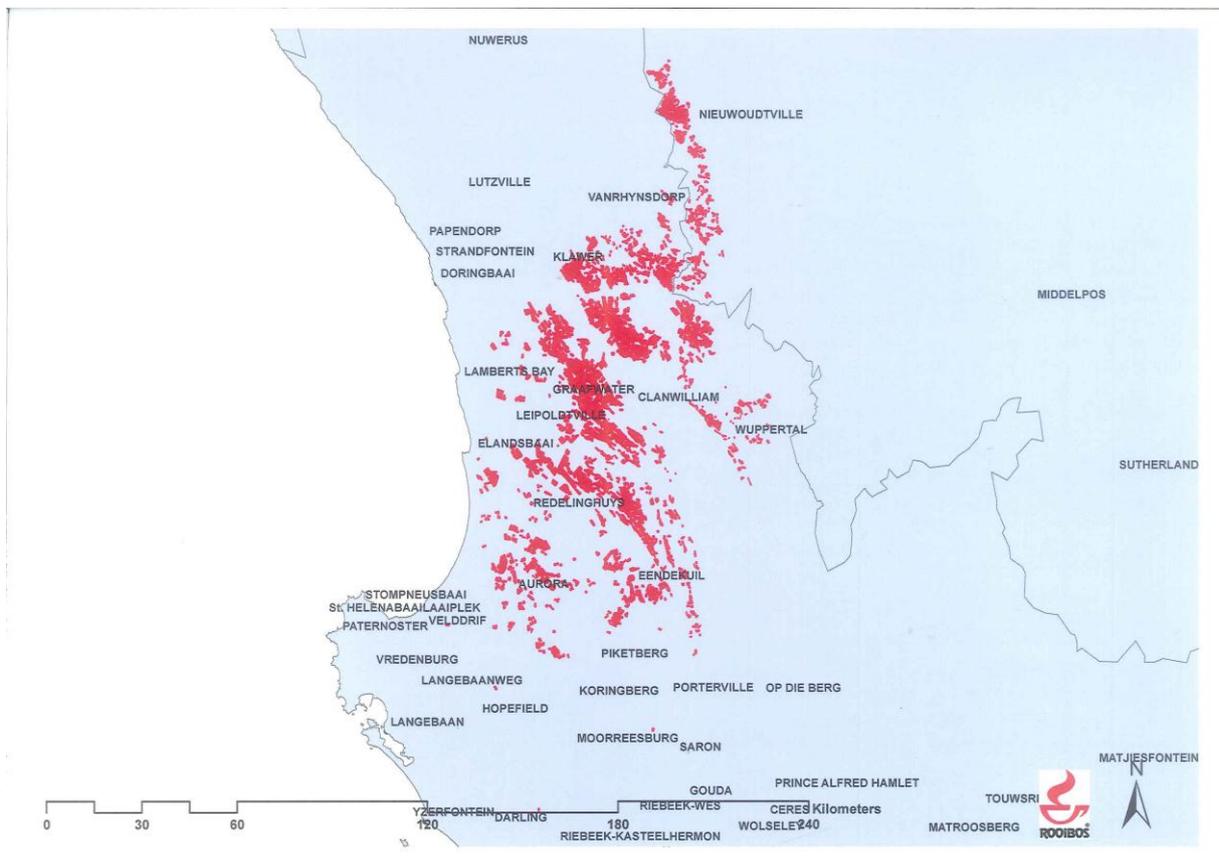


Figure 2-1: A map indicating the rooibos (*Aspalathus linearis*) distribution in the Northern and Western Cape (map was supplied by Rooibos Ltd., Clanwilliam)

Rooibos is grown in the Western- and Northern-Cape in South Africa, with the main growing area around the small town Clanwilliam (**Figure 2-1**). The rooibos region can be divided into

different areas with the 6 main areas being: Nardouwsberg/Agterpakhuis, Seekoeivlei, Vanrhynsdorp, Niewoudtville, Citrusdalberg, and Eendekuil (**Figure 2-2**).

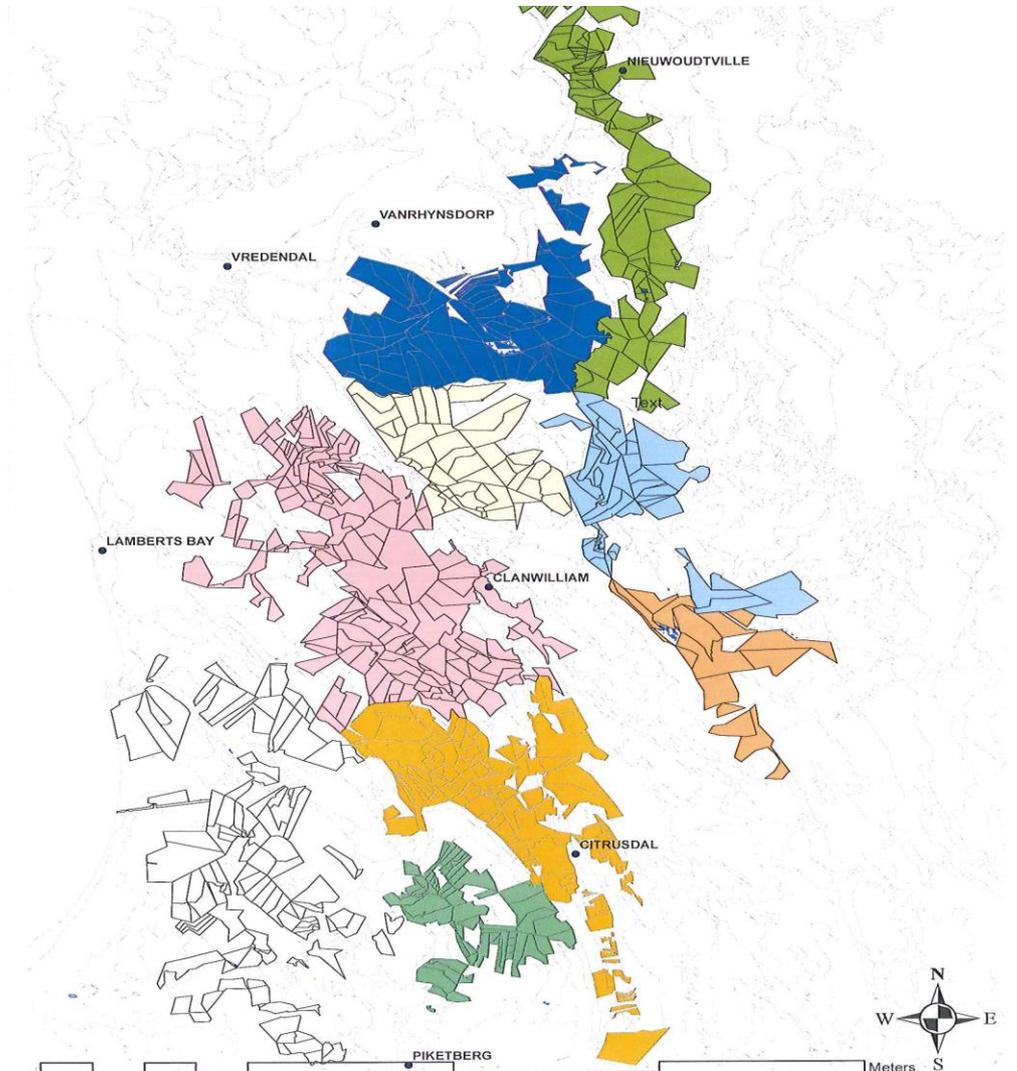


Figure 2-2: A map of the different rooibos producing areas; Eendekuil, Citrusdal, Seekoeivlei, Nardouwsberg, Agterpakhuis, Wuppertal, Vanrhynsdorp and Niewoudtville (map supplied by J brand at Rooibos Ltd., Clanwilliam)

Rooibos can only be cultivated in a relatively small geographical area in South Africa, due to very specific climatic and soil conditions to which the plant is adapted. Attempts to cultivate the plant in other countries have failed. The world demand for rooibos tea shows a steadily increase over the years whereas the supply is actually decreasing (**Figure 2-3**). Since the production area can only be increased slightly due to environmental protection laws, it is important to produce as much quality tea as possible on the land that is already in use. In

doing so the natural vegetation (especially the biodiversity), that can still be found in a large part of the rooibos area, can be protected.

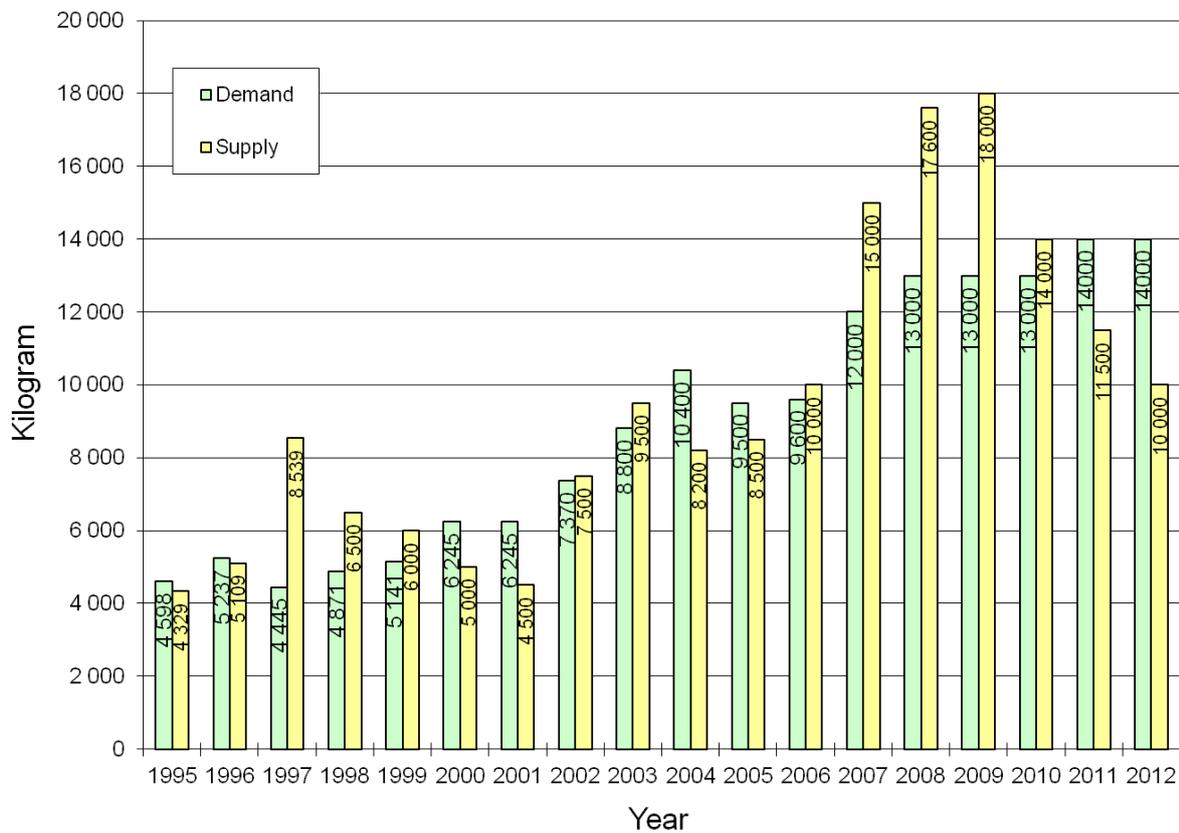


Figure 2-3: Supply and demand graph of rooibos tea in the world since 1995 (Graph supplied Rooibos Ltd., Clanwilliam)

2.3.2 Environmental conditions for rooibos production

The rainfall should be about 250-350 mm per year in winter to ensure a good harvest (J van Putten, former CEO of Rooibos Ltd., personal communication). Most of the rooibos plantations are found on soils derived from Table Mountain sandstone. Typical rooibos soils are deep sandy soils, as the rooibos taproot can grow over 2 m long. These sandy soils are also typically acidic with a pH of 4.5 – 5.5 (Nolte, 1968) and are well-drained. The soils are usually low in nutrients due to sandy texture and low organic matter content. The rooibos plant is able to take up even the smallest amount of nutrients through roots that are specially adapted for nutrient poor conditions.

Under these acidic soil conditions, other nodulating legumes and their microsymbionts are limited as they prefer near-neutral soil pH conditions and a high base status (Muofhe & Dakora, 2000). However, some strains of root bacteria isolated from rooibos roots can grow in a laboratory in a pH as low as 3 or 4 (Muofhe & Dakora, 1999). Selected legume plants, such as rooibos, have a pH raising mechanism to overcome the adverse effect of low soil pH, in order to promote symbiotic establishment and enhance nutrient acquisition (Muofhe & Dakora, 2000). It was found that the rhizosphere of rooibos shows an increase in soil pH due to the rooibos root activity and symbiotic bacterial activity. The exact mechanism is not known, but it is likely from decarboxylation of organic anions, which generates bicarbonate anions. A low pH has a negative influence on the binding of nitrogen by rhizobium bacteria, but the *Bradyrhizobium* bacteria that are present on the roots of the rooibos helps to bind the necessary nitrogen by extruding OH^- and HCO_3^- . Microbial life in the soil thus has an important role in the acquisition of nutrients by rooibos plants.

2.3.3 Cultivation practices for rooibos production

Rooibos is grown for 3-5 years before being removed. An alternative plant, such as oats or wheat, is recommended to be planted 1 or 2 years before rooibos is planted on the soil, as well as between plant cycles (Dahlgren, 1968). The reason for these rotations is to break the monoculture for at least two years in order to prevent the build-up of pathogens over time. The grain species are also used to prevent wind erosion in the intervals between rooibos cycles (Personal communication with farmers). Rooibos tea can be harvested for up to 5 years after planting where after it has to be re-established. The land is prepared using a disc or mouldboard plough to remove old rooibos plants. In some cases the rooibos are cut into smaller pieces using a “straight blade cutter” or “slasher” before the field is ploughed. The soil is ripped just before new rooibos plantation is established. Only a small amount of farmers fertilize, the most common fertilizer applied in rooibos is phosphate viz. rock phosphate. Rooibos plantations are dependent on rainwater. Pesticides are only used in small amounts to help control the following insects found on rooibos; “Bladspringer”, “Landmeter”, “Bolwurm” and “Glasvlerkmot” (Hatting, 2009).

2.3.4 Rooibos foliar elemental analyses

The foliar elemental analysis of 2- and 3-year old rooibos plants on the Citrusdal experimental farm was determined by Strassen (1987) (**Table 2-2**). Strassen (1987) found

that there were seasonal variations in the macronutrient content of the leaves. There was a decrease in N from September to January followed by an increase from February reaching a maximum in August/September. The P levels were the lowest during spring and the highest during winter. The K in the leaves increased from January/February, and reached a maximum in October. The Ca values peaked during winter, and a decrease was noted with a minimum value in April/May. The Mg concentration in the leaves decreased from September until it reached a minimum from December to April, after which it increased again. Therefore the best time to sample the leaves for analysis was found to be in February. The values for the N, P, K, Ca and Mg in the leaves of rooibos are significantly lower than those in the deciduous fruits (Strassen, 1987). This can be attributed to the fact that rooibos has a relatively high woody tissue content, which contains low concentrations of these elements. Rooibos thus needs less of these elements than deciduous fruits (Strassen, 1987).

Table 2-2: Foliar analysis form rooibos tea in Jan./Feb. (Table 5.2, Strassen, 1987)

	Elements									
	%					mg/kg				
	N	P	K	Ca	Mg	Mn	Fe	Cu	B	Zn
2-year old	1.0	0.05	0.19	0.23	0.18	77	255	6	24	17
3-year old	1.0	0.04	0.43	0.14	0.18	47	67	5	24	11

Strassen (1987) determined the amount of nutrients that were lost annually due to tea harvesting based on the assumption that 8000 plants were planted per hectare and production of 1.5 t ha⁻¹ of tea. Plant material from 750 different plants harvested at the Citrusdal experimental farm was used for the elemental analysis at harvesting. It was estimated that 27.6 kg N, 1.8 kg P, 6.9 kg K, 6.2 kg Ca, 6.2 kg Mg, 303 g Mn, 818 g Fe, 22 g Cu, 46 g B and 60 g Zn should be applied to the soil to make up for the nutrients lost by tea harvesting.

The amount of N needed may exceed the 27.6 kg N per ha, but is hard to estimate because of the rhizobium bacteria present with the ability to produce N for the plant (Strassen, 1987). According to Strassen (1987), one application of 25 kg P or 220 kg superphosphate

per ha is sufficient for a period of 10 years. An application of 10 kg K or 20 kg KCl per ha during August is recommended. Molybdenum and cobalt levels play an important role in N₂-fixation by legume plants while sulphur plays an important role in protein syntheses (Smith, 1987).

2.3.5 Effect of chemical fertilizers on rooibos plants

Phosphorus toxicity

According to Leake (1993), plants that are native to very nutrient poor soils, such as fynbos species, have adapted to take up nutrients that would otherwise be unavailable. Fynbos plants like Proteaceae are adapted to take up nutrients through the production of cluster roots, also known as proteoid roots (Hawkins *et al.*, 2008). On these cluster roots mycorrhiza can be found that help with nutrient uptake. These cluster roots are also found on rooibos (personal observation). Nutrient levels are low in the soils in the natural area where fynbos such as Proteaceae and rooibos grow naturally, especially N (2 mg kg⁻¹ NO₃⁻ and 13-27 mg kg⁻¹ NH₄⁺; (Hawkins *et al.*, 2005) and Bray II P (0.8 mg kg⁻¹ P; (Witkowski & Mitchell, 1987)). Proteaceae in South Africa are adapted to low P concentrations of between 0.8 and 8 mg kg⁻¹ P (Hawkins *et al.*, 2008).

When a higher than usual amount of P is supplied to the soil, toxicity symptoms can be observed, as these plants are very sensitive to P toxicity (Hawkins *et al.*, 2008). The symptoms include: a lack of growth, apparent iron (Fe) deficiency (interveinal chlorosis of younger leaves), red discolouration starting in the older leaves, tip necrosis in acute cases, and the root is susceptible to root fungal pathogens such as phytophthora (Leake, 1993). These symptoms have also been found on rooibos in older cultivated fields (personal observation).

Phosphate uptake by fynbos species occurs during the wet winters, whereas shoot growth occurs during summer. The P is stored in the plant until summer (Jeschke & Pate, 1995). This was confirmed in rooibos by Strassen (1987), indicating that foliar P values were lowest during spring and highest during winter. The P is stored in the stem tissue and roots, but since the capacity is limited, P can also be stored in the leaves. Phosphorus toxicity symptoms develop once the P is stored in the leaves (Shane *et al.*, 2004). The normal foliar

P content for Proteaceae in South Africa is between 0.03 and 0.08 %, whereas, values between 0.1% and 0.25% indicate toxicity (Hawkins *et al.*, 2008).

Phosphorus toxicity results in element imbalances in leaves of various fynbos plants species, which includes rooibos tea (Hawkins *et al.*, 2008). A high supply of P results in an increase in the leaf concentrations of Cl and Mn. It also results in a decrease in the total concentration of leaf Fe, as well as, bundle sheath, xylem, phloem and epidermal Fe concentrations. A decrease in total concentration of leaf Zn as well as xylem- and phloem Zn concentrations was observed. The observed toxicity symptoms can thus be explained by the following interactions within the plant: (1) The excess phosphate binds Ca in the epidermis and thus leads to necrosis; (2) the reduced Fe concentration and increase in Mn concentration leads to chlorosis and, lastly, (3) the reduced total and vascular Zn concentration leads to leaf rosetting (Hawkins *et al.*, 2008). It has been reported that increased Ca levels can intensify the P toxicity symptoms in Proteaceae species, whereas, increased N can reduce it (Nichols, 1988). It has also been reported that P toxicity symptoms can be reduced in P-sensitive plants by improving the Fe supply by applying FeSO₄ (Goodwin, 1981). Iron sulphate has an acidifying effect on the soil, so in cases where there is a chance of the pH becoming too low a magnesium carbonate could be included to help raise the pH.

Effect of macronutrients on rooibos

There is very little peer-reviewed, published information available on the effect of chemical or organic fertilizers on rooibos tea production. Most of the previous fertilizer studies on rooibos investigated the effects of additions of N, P, K, Ca and Mg in greenhouse trials on young plants (Joubert *et al.* 1987; Muofhe 1997; Muofhe & Dakora, 1999).

Joubert *et al.* (1987) performed a five month greenhouse pot trial on the effect of N, P, Ca, Mg and K on rooibos seedlings. The soil that was used during this study was a Clovelly soil form, from the Clanwilliam area. There is no indication in this study which soil horizon was used, or whether a composite soil sample was taken. The plants were planted as seeds and after five months the plants were harvested and the below and above-ground mass were measured. Different experiments were performed during this study. Experiment 1: Different amounts of P were applied to the soils in the form of Superphosphate (calcium dihydrogen phosphate), Saaifos-16 (ammoniated superphosphate), Calmafos (phosphate slag containing

Ca and Mg) and Langfos (rock phosphate containing lime). Experiment 2: Different amounts of K were applied to the soils in the form of potassium chloride and potassium sulphate. Experiment 3: Different amounts of lime were applied according to the lime requirement of the soil. Experiment 4: Different amounts of N and Mg were applied to the soil in the form of ammonium nitrate and magnesium sulphate. At a concentration of 15-20 mg kg⁻¹ Bray 2 extractable P optimum growth occurred. Growth decreased significantly if the P concentration was higher. The optimum concentration for K was found to be 60 mg kg⁻¹ Bray 2 extractable K. A lime application of 0.5 times the lime requirement was gave the best results. Optimum growth occurred at pH 5. In cases where larger amounts of K were applied, the growth was reduced. N was found to be optimum at 10 – 15 mg kg⁻¹ of soil. These results were dependant on the amount of Mg in the soil. Application of Mg to rooibos reduced Ca, N, P and K uptake (Joubert *et al.* 1987) and is thus was not recommended. The application of N did not have a significant effect on the formation of N-nodules.

Muofhe (1997) carried out greenhouse and field experiments on 2-year old rooibos plants in soils from Clanwilliam. The pH of the soils from Clanwilliam ranged between 3.8 and 5.5. An application of 5, 25 and 50 mM of N, P and Ca from NH₄NO₃, CaCl₂ and KH₂PO₄/K₂HPO₄ was added to 2-year old rooibos plants. Assuming the rooibos plant requires only an area of 0.25 m², a soil depth of 0.2 m and the soil have a bulk density of 1.55 g cm⁻³, it can be calculated that 77.5 kg of soil was used for the production of rooibos in this experiment. See Table 2-3 for approximate application rates in mg kg⁻¹ of compound per application.

Table 2-3: Indication of NH₄NO₃, CaCl₂ and KH₂PO₄/K₂HPO₄ that was applied to the soil in mg kg⁻¹ of compound per application

Treatment	5mM	25mM	50mM
Application rate (mg kg ⁻¹)			
CaCl ₂	7.16	35.8	71.6
NH ₄ NO ₃	5.16	25.82	51.6
K ₂ HPO ₄	11.23	56.19	112.3

These applications were done at 3 stages, beginning of May, June and August. The plants were then allowed to grow for 8 months and then analysed. The total biomass production of

the rooibos was increased by the application of N and P under field and greenhouse conditions. The dry matter in the shoots increased, but the roots remained the same. At high concentrations of N, there was a decline in nodulation and N₂ fixing. Calcium had a negative effect on the growth as well as the N-symbiotic performance of the rooibos in the greenhouse, as well as under field conditions. It is unclear whether the increase in biomass due to potassium phosphate application was solely due to the P or perhaps due to the K applied.

Muofhe & Dakora (1999) assessed the effects of age of rooibos plants (1-, 2- and 3-year old plants) and N fertilization on N₂ fixation in a field trial. It was found that the total N content of the plants was higher where fertilizers were applied. The symbiotic parameters also revealed a difference between plants that were fertilized and plant that were not. Two and three year old plants accumulated more biomass than plants that was one year old. The total N in the two and three year old plants was greater than those of a one year old plant. Three year old plants had a higher N content than two year old plants although the biomass of the plants was almost the same (Muofhe & Dakora, 1999). Although the pH and nutrient status of the soil were low, the symbiotic activity of rooibos plant was high (Muofhe & Dakora, 1999).

2.3.6 Rooibos tea fermentation and quality

Rooibos leaves are oxidised (referred to as fermentation in the rooibos industry) to achieve the characteristic red tea colour. The traditional process of fermentation involves the cutting the fresh plant material into small pieces, adding water, bruising by tractor, floor fermentation heaps and drying on a cement yard in thin layers by the sun (Cheney & Scholtz, 1963). No control is possible for the fermentation and drying conditions, thus resulting in a product with inconsistent quality. If drying is slow, the product can over-ferment. If it is a cold night, under fermentation can occur (Joubert, 1998). The fermentation process and the quality identification is an important part of rooibos production, since there is an international standard which the rooibos industry must meet. The rooibos producers should try and optimize their tea quality since the product price decreases with a decrease in the quality of the fermented tea.

2.3.6.1 Water

Water is an essential ingredient for the fermentation process. Although the plant material contains natural moisture, additional water is needed for the chemical process of fermentation. The amount of water needed was estimated to be 65% by mass; this relates well to the hand-moisture test that the farmers use (J van Putten, personal communication). It was found that the rooibos doesn't ferment unless the plant is bruised and a relative high temperature is present.

2.3.6.2 Aeration

Air is needed for the fermentation process (J van Putten, personal communication). In poorly aerated samples, the sweet taste of the rooibos was absent (Joubert, 1998). However the extract colour was not affected by the aeration. Poorly aerated rooibos tea smells grassy since the sweet notes necessary to balance the aroma were absent.

2.3.6.3 Temperature

The optimum temperature for rooibos fermentation was estimated to be between 38 °C and 40 °C (Joubert,1998) . Optimum temperature for the best aroma was 40 °C and 34 °C . The aroma changed from grassy to sweet at 34 °C, 36 °C and 38 °C, but lost its sweet note at 40 °C after 8 to 10 h (Joubert, 1998). The effect of temperature on the overall quality was inconclusive since no trends were found. Fermentation temperature also didn't affect the aroma, but there was an increase in aroma with an increase in temperature. Rooibos tea quality improves with an increase in fermentation temperature, but decreases with an increase in drying temperatures (Joubert & De Villiers, 1997).

2.3.6.4 Time

The taste of the tea was influenced by the fermentation time (Joubert, 1998). Tea that was fermented for 8-16 h had the lowest quality rating, whereas tea with the optimum quality had a 10-14 h fermentation period. The poor taste can be associated with insufficient oxidation and polymerization of rooibos polyphenols. Fermentation time did not affect the aroma. Optimum aroma is reached earlier in the fermentation process, with an increase in temperature. The optimum fermentation time for the best aroma was between 8h to 14h. The colour was not significantly influenced by fermentation time.

2.3.6.5 *Drying method*

Deep-layer drying of rooibos tea as opposed to thin-layer drying had no effect on the quality of the tea (Joubert, 1998). A shorter drying period was found at the deep layer drying because more of the tea was in direct contact with the air. Fermentation is thus possible at the beginning of thin layer drying, but had no noticeable effect on the quality of the tea. Sun drying is not essential for developing the properties of rooibos, such as aroma and taste of the extract as well as the leaf colour (Joubert & De Villiers, 1997).

2.3.6.6 *Rooibos tea Quality*

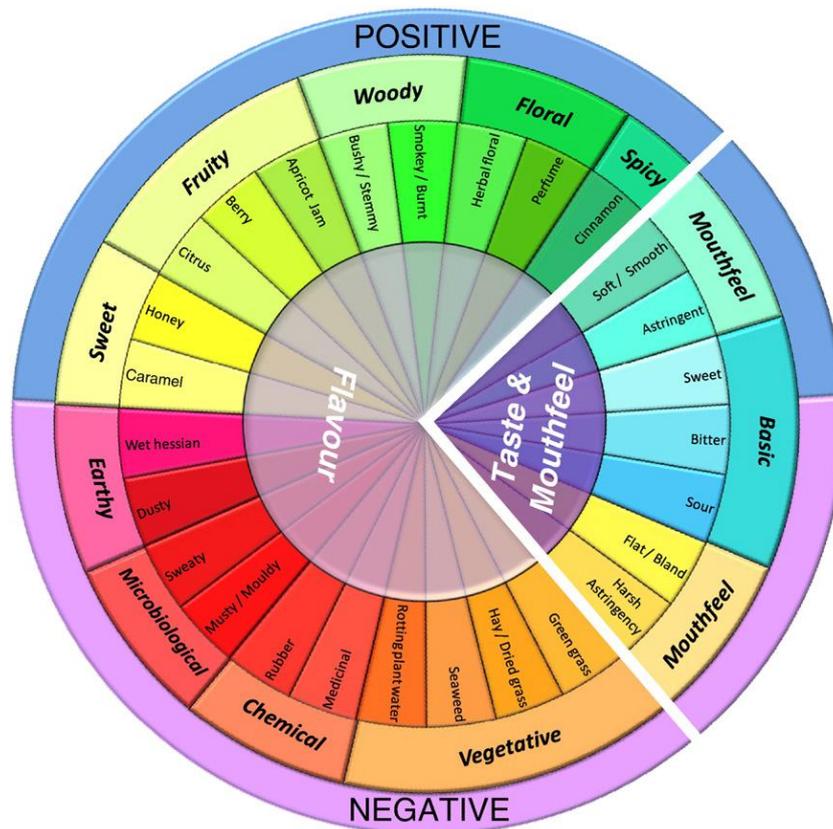


Figure 2-4: Rooibos sensory wheel comprising 27 terms that describe the sensory attributes of 69 rooibos infusions (Koch *et al.*, 2012).

The sensory wheel can be used to distinguish between a high quality and low quality tea. Low quality tea with a Grade C or D can be associated with the negative attributes and the high quality tea with a Grade A or B can be associated with the positive attributes (Figure 2-4).

2.4 Conclusions

It is important to examine the effect of long-term rooibos cultivation on soil quality parameters in order to identify the reason(s) for the decline in the production over time. The soil quality parameters will consist of chemical, biological and physical parameters that are appropriate for the highly sandy soils that rooibos is cultivated on. All of the chemical parameters mentioned in the soil quality section above will be looked at during this study. The soil physical parameters, bulk density and soil particle-size distribution will be determined, as infiltration rate, water-holding capacity and aggregate stability will be quite similar on the highly sandy, exposed soils. The microbial parameters that will be studied included total microbial biomass, protist counts, and the extent of root colonization by mycorrhizae and N-fixing bacteria. The soil quality parameters will need to be compared to the plant quality parameters; total above- and below-ground biomass, tea yields and the fermented tea quality. Tea yields and quality are the most important plant measured parameters when looking at the influence of soil quality, because this is ultimately what farmers want to achieve.

The effect of commercial rooibos cultivation on the extent of beneficial microbial colonization of rooibos plant roots has not been studied previously, thus highlighting the importance of the current proposed study. The amounts of soil nutrients found on rooibos plantations as well as in the plant over different years of cultivation have never been looked at. It is an important part of this study to try and establish typical values for each of the important soil and plant nutrients as well as the effect of these nutrients on tea yield and quality.

No field trials have been done to date on the effect of different nutrients on the rooibos plant over a long period of time. Previous studies did not evaluate the effect of fertilizers on rooibos tea yields or quality, which are ultimately the most important criteria for rooibos producers. The effect of soil treatments on microbial activity and beneficial microbes (rhizobia and mycorrhizae) in rooibos production needs clarification as it can play an important role in the amount and type of fertilizer that should be applied.

3 RESEARCH CHAPTER 3: THE EFFECT OF LONG-TERM ROOIBOS CULTIVATION ON SOIL QUALITY

3.1 Introduction

Rooibos farmers report that rooibos yields tend to decrease with an increase in the number of years that rooibos have been planted on the same field since the removal of the original fynbos vegetation. The decrease in the production may be due to a change in the soil chemical and/or physical conditions, which would in turn, affect soil ecology. Up until now, no information was available on the effect of long-term cultivation of rooibos on soil quality.

The soils used for rooibos production are usually acidic and low in nutrients due to sandy texture, low organic matter content and winter rainfall. The rooibos plant is able to take up even the smallest amount of nutrients through roots that are specially adapted for nutrient poor conditions. Studies have been done on similar types of soil in the fynbos biome, as well as, on soils where honeybush tea is produced, and it was found that the soils are extremely low in soil organic carbon, nitrogen (Hawkins *et al.*, 2005), phosphorus (Witkowski & Mitchell, 1987) and other nutrients. The effect of these soils on rooibos production has not been looked at and is of great importance to establish the effect of long-term rooibos cultivation.

Rooibos is generally planted in five year cycles in the Western Cape region. Two-thirds of the bushes are harvested annually for tea at the end of summer. A cover crop (oats or wheat) is usually planted 1 or 2 years before rooibos is planted on the soil for pathogen control. The rooibos plantations are periodically harrowed with a shallow disc to control weeds in the rooibos plantations. Rooibos farmers generally do not fertilize, except occasionally adding a source of P such as rock phosphate or superphosphate when planting the grain cover crop. One of the factors influencing the decrease in production can be related to the mining of soil nutrients, especially since the soils are extremely nutrient poor to begin with and most elements are not replenished. Examining the effect of rooibos cultivation on soil nutrient levels and chemistry over time, will help identify the most important nutrients which play a role in rooibos production and also help with remediation strategies for sustainable production.

In this chapter the effect of long-term rooibos cultivation on different soil quality parameters (chemical, physical and microbiological) will be investigated to see how it has changed since the removal of the virgin fynbos vegetation. The soil quality of virgin fynbos soils where rooibos occurs naturally will also be assessed. The physical characterisation, which includes soil texture and soil bulk density, will be determined so that the effect of periodic shallow disc harrowing to control weeds can be assessed. The chemical characterisation includes the pH, the electrical conductivity (EC), total carbon and nitrogen, exchangeable basic cations and acidity, plant-available P and trace elements, in order to note different changes in the soil chemistry due nutrient mining and the reduction of organic inputs. The microbial determinations include protist counts and total microbial biomass, since they play a major role in the nutrient cycling in soil, and are thus a good indicator of the nutrient cycle status.

3.2 Objective

The main objective of this study is to investigate the soil quality status (chemical, microbiological and physical parameters) in commercially cultivated rooibos plantations and adjacent undisturbed fynbos sites. It is anticipated that this will lead to the identification of the soil factors behind the decline in productivity of rooibos on soils that have been repeatedly cultivated.

3.3 Methods and materials

3.3.1 Site Selection

The experimental sites were chosen in the Clanwilliam district, within two of the six main rooibos producing areas. The Nardouwsberg and Seekoeivlei areas (**Figure 3-1**) were chosen for this study as they are some of the oldest rooibos producing areas. In each of these areas, cultivated sites of different ages (ranging between newly planted to 60 year old sites) were chosen with similar tillage and planting practices, where they don't routinely fertilize and the plants were of similar age (one and two-year old). Sites containing rooibos plants in undisturbed, virgin fynbos and in an abandoned rooibos plantation that had reverted back to natural vegetation were also selected (**Figure 3-2**). In all of the experimental sites, no fertilizer had been applied for at least two years before planting.

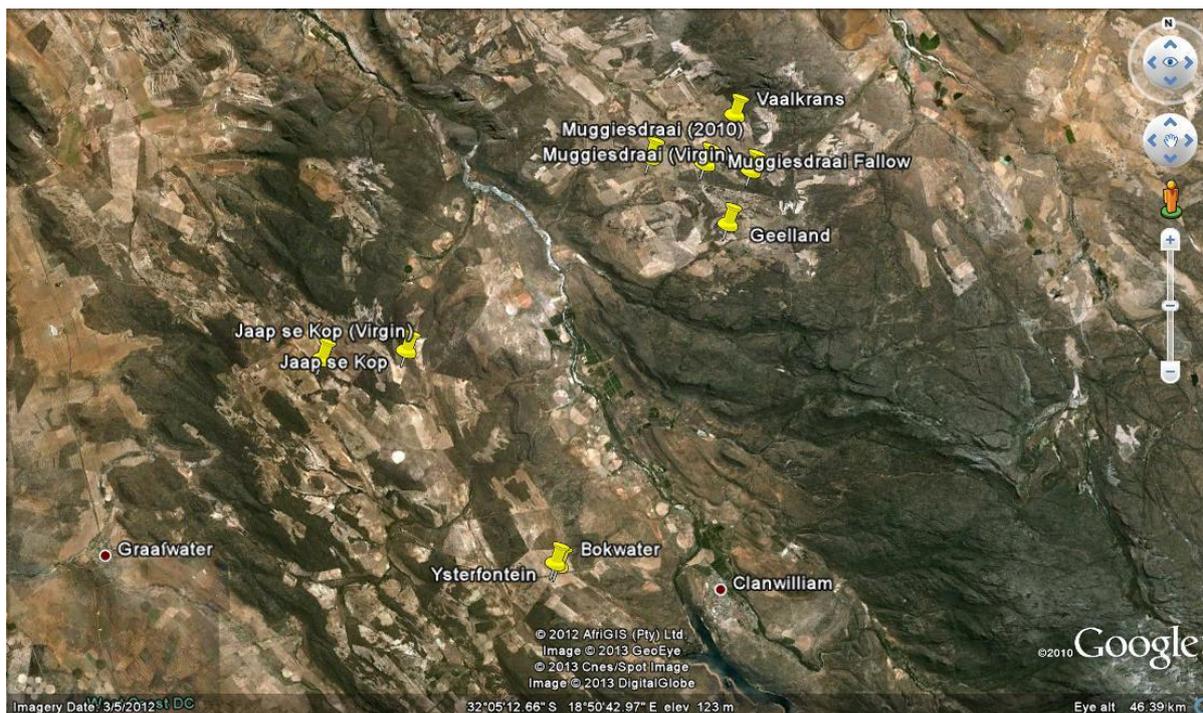


Figure 3-1: A Google earth map indicating the experimental sites near the town Clanwilliam with the Nardouwsberg area north of Clanwilliam and Seekoeivlei North-west.

In the Nardouwsberg area (**Figure 3-2**), five different experimental sites were chosen on the farms Muggiesdraai, Vaalkrans and Geelland. At Muggiesdraai there were three sites, namely a newly established rooibos field where the natural fynbos was recently cleared, a virgin fynbos sites containing wild rooibos, and a 10-year old abandoned rooibos field where the land reverted back to natural vegetation. The sites at Vaalkrans and Geelland farms were older cultivated fields, on which rooibos had been planted for 20 to 60 years.

In the Seekoeivlei area (**Figure 3-3**) four different experimental sites were chosen on the farms Ysterfontein, Bokwater and Jaap-se-kop. The Ysterfontein site was chosen on a field on which rooibos has been planted for the first time since the natural fynbos was removed. The Bokwater and Jaap-se-Kop sites were chosen as the old fields on which rooibos have been planted for 20 to 30 years. On the farm Jaap-se-Kop, there was also a virgin fynbos site, where rooibos grows naturally.

The rooibos plants at Vaalkrans, Geelland, Ysterfontein, Jaap-se-Kop and Bokwater were planted in the year 2011, therefore the plants were 1-year old at the time of sampling in June 2012. The plants at the Muggiesdraai site were planted in 2010, thus making the

plants 2-years old at the time of sampling June 2012. The age of the rooibos plants in the virgin fynbos and abandoned fallow sites were not known. The rooibos plants at the Muggiesdraai virgin fynbos site plants are “Swart tea” type, which differs from the rest of the cultivated and virgin sites which all contain the “Nortier” type.



Figure 3-2: A Google earth map indicating the 5 experimental sites in the Nardouwsberg area



Figure 3-3: A Google earth map indicating the 4 experimental sites in the Seekoeivlei area

The Bokwater site was planted more densely than the other cultivated experimental sites. The rooibos plants at this site were planted 60 cm apart within a row, and the rows were spaced 120 cm apart from each other. At the other cultivated sites the plants were planted 85 cm apart within a row, and the rows were spaced 150 cm apart from each other.

Prior to site selection, the soil depth was tested with a 2 m iron rod. It was tested in the row as well as between the rows, to see whether there were any depth differences due to impermeable layers. The soil depth was tested at 10 different places within a proposed site and an average depth was taken for the site in and between the rows. No sites were selected unless the average depth of the soil was at least 50 cm deep. The three replicate sites within a rooibos field were then randomly selected. A 10 x 10 m square area was selected and ensured that it contained a minimum of 55 plants. In the cases of the virgin sites and the fallow site, only one plant in each replica was sampled because the rooibos plants are scattered amongst the other fynbos vegetation. The GPS coordinates of each site were taken in the centre of the site (**Table 3-1**).

It was later discovered that the Ysterfontein (Y1) Site 1 (**Table 3-1**) was located on a “heuweltjie” (ancient termite mound), which means there was previous termite activity on this particular part of the field which affects soil properties, including slightly higher clay content and an accumulation of soil nutrients. It was therefore decided to omit this “heuweltjie” site from the study as the soil properties were significantly different from the rest of the cultivated sites. The effect of “heuweltjies” on rooibos tea yields and quality should be further examined in a future study.

Table 3-1: Site coordinates and plant number for each experimental site

	Experimental site	Coordinates
Nardouwsberg	MV1	S 32°00 967' E18°54 958'
	MV2	S 32°00 962' E18°54 949'
	MV3	S 32°07 233' E18°54 540'
	M1	S 32°00 784' E18°52 155'
	M2	S 32°00 753' E18°52 040'
	M3	S 32°00 786' E18°51 722'
	G1	S 32°02 315' E18°54 054'
	G2	S 32°02 264' E18°53 984'
	G3	S 32°02 415' E18°53 848'
	V1	S 31°59 871' E18°54 277'
	V2	S 31°59 823' E18°54 244'
	V3	S 31°59 783' E18°54 215'
	MF1	S 32°00 952' E18°53 365'
	MF2	S 32°00 949' E18°53 363'
	MF3	S 32°00 949' E18°53 363'
Seekoeivlei	JV1	S 32°05 161' E18°42 471'
	JV2	S 32°05 164' E18°42 482'
	JV3	S 32°05 164' E18°42 498'
	Y1	S 32°10 277' E18°48 725'
	Y2	S 32°10 288' E18°48 716'
	Y3	S 32°10 264' E18°48 737'
	B1	S 32°10 277' E18°48 791'
	B2	S 32°10 295' E18°48 789'
	B3	S 32°10 306' E18°48 803'
	J1	S 32°05 161' E18°42 471'
	J2	S 32°05 164' E18°42 482'
	J3	S 32°05 164' E18°42 498'

Nardouwsberg sites (Sites are in an approximate 10 km radius)

1. Muggiesdraai Virgin (MV) fynbos: On the farm “Muggiesdraai” where no cultivation has ever been performed. The rooibos plants in this area grow naturally and thus the age of the bushes are unknown.
2. Muggiesdraai (M) New rooibos Plantation: On the farm “Muggiesdraai” where rooibos was cultivated for 2 years since the removal of natural fynbos vegetation in 2010.
3. Vaalkrans (V) Older rooibos Plantation: On the farm “Vaalkrans” where rooibos has been cultivated for more than 60 years.
4. Geelland (G) Older rooibos Plantation: On the farm “Geelland” where rooibos has been cultivated for more than 20 years
5. Muggiesdraai Fallow (MR) Abandoned rooibos Plantation: On the farm “Muggiesdraai” where the field was left alone to return to its original fynbos since 2000

Seekoeivlei sites (Sites are in an approximate 20 km radius)

1. Jaap-se-Kop Virgin (JV) fynbos: On the farm “Jaap-se-Kop” where no cultivation has ever been performed. The rooibos plants in this area grow naturally and thus the age of the bushes are unknown.
2. Ysterfontein (Y) New rooibos Plantation: On the farm “Ysterfontein” where rooibos was cultivated for 2 years since the removal of natural fynbos vegetation.
3. Bokwater (B) Older rooibos Plantation: On the farm “Bokwater” where rooibos has been cultivated for more than 20 years.
4. Jaap-se-Kop (J) Older rooibos Plantation: On the farm “Jaap-se-Kop” where rooibos has been cultivated for more than 30 years.

3.3.2 Soil Sampling

There were two soil sampling depths, i.e., a topsoil and subsoil sample at 0-20 cm and 20-40 cm, respectively. These depths were selected due to the periodic shallow disc harrowing that is used to control weeds in the rooibos plantations which lead to substantial mixing of the topsoil to a depth of 20 cm. Soil sampling was performed in June 2012 after the first winter rainfall. It was decided to sample at this time of the year as the soil microbes would be most active due to soil moisture and the rooibos plants would be actively taking up nutrients at this time (Strassen, 1987). The soils at each sampling site were also classified and described according the South African Soil Classification system (Soil Classification working group, 1991).

3.3.2.1 Bulk density

Soil bulk density was determined 0-20 cm and 20-40 cm in triplicate at each 10 x 10 m site using the undisturbed core method (Blake & Hartge, 1986). The bulk density samples were taken in the same holes that were dug when sampling the rooibos plants (See **Chapter 4**). The samples were taken in the row, in which the rooibos was planted. The mass of the soil cores were determined in the laboratory after it was oven-dried at 60 °C for 48 hours. The total soil volume was determined by taking the measurements off the ring that was used during core sampling.

3.3.2.2 Chemical analysis

Six soil samples were taken at each sampling depth (0-20 and 20-40 cm), using a hand auger and bulked in the field to make a composite soil sample at each of the three 10 x 10 m sites. The six samples were taken next to the rooibos bush on the west or south side of the bush in the row; depending in which direction the rooibos plants were planted.

The virgin fynbos and fallow sites were sampled differently from the cultivated sites due to the isolated occurrence of rooibos bushes in the natural vegetation. In these cases, six soil samples were taken at each sampling depth (0-20 and 20-40 cm) around a single rooibos bush. The six samples were bulked in the field to make a composite sample for analysis. Prior to analysis the soil samples were air-dried for 48 hours and sieved through a 2 mm sieve.

3.3.2.3 Microbial analysis

Only the topsoil (0-20 cm) was sampled for the microbial determinations. The sample was taken with a hand auger that was sterilized with 99% ethanol and bulked in the field to make a composite soil sample at each of the three 10 x 10 m sites. The soil was placed in a sampling bag in a cooler box, and then stored in the refrigerator until it was analysed. The protist count was analysed within 10 days after the soil was sampled, because after that the microorganisms return to their cyst phase and can't be studied under the microscope. The total microbial biomass was determined on soil within a month after sampling.

3.3.3 Soil Analyses

3.3.3.1 Chemical analyses

Soil pH

The pH of the soil was determined in both water and 1 M KCl using a 1:2.5 suspension ratio on a mass basis (Rowell, 1994).

Soil Electrical Conductivity (EC)

The EC was determined in a 1:5 soil to water suspension ratio on a mass basis (Rhoades, 1996). This method was selected due to the sandy texture of the soil, which makes it difficult to assess when the saturation point has been reached using the saturated paste method.

Total carbon and nitrogen

The soil sample was ball-milled to a fine powder prior to C and N determination. The total C and N content of the soil was determined using a dry combustion method (Eurovector EA Elemental Analyzer, EuroVector Instruments & Software, Miland Italy).

Inorganic NO_3^- and NH_4^+

The inorganic nitrate and ammonium was determined using 2M KCl Colorimetric methods (Page, Miller, & Keeney, 1982).

Exchangeable basic cations and acidity

The exchangeable basic cation (Ca, Mg, K, Na) content of the soils was determined using 1M NH₄OAc (pH 7.0) method (Thomas, 1982). The exchangeable acidity of the soils was determined using a 1M KCl extraction method (Thomas, 1982).

Effective cation exchange capacity (ECEC)

The effective cation exchange capacity (ECEC) of the soil was calculated by adding the 1M NH₄OAc extracted exchangeable basic cations content and the 1M KCl extracted exchangeable acidity

Plant-available phosphorus

Plant-available P was determined using the Bray 2 extraction method (SSSA, 1990) which is suited for soil samples from the winter-rainfall region of the Western Cape.

Sulfur

Total sulphur was determined using the acid digestion method to oxidize total sulphur to sulfate (Page, Miller, & Keeney, 1982).

Plant-available micronutrients

Plant-available micronutrients (Cu, Zn, Mn and Fe) were determined using the DPTA-extraction method (SSSA, 1990). Plant-available B was determined using the hot-water extraction method (SSSA, 1990).

Particle size analysis

The pipette method was used to determine the sand, silt and clay content of the soils (Gee & Bauder, 1986).

Citrate bicarbonate dithionate (CBD)

The CBD extractable Fe, Al and Mn: dithionite-citrate-bicarbonate content of the soils was determined using 0.3M sodium citrate and 1M NaHCO₃ method (Mehra & Jackson, 1960).

3.3.3.2 Soil microbial determinations

Protist counts

Protist counts were performed using the MPN method as described by Woomer (1994). Sixty grams of top soil was taken and diluted by adding 240 mL tap water. The suspension was shaken well and left to settle for 24 hours. The soil was then filtered off and the extract was autoclaved at 121 °C for 20 min. A 0.5 mL aliquot of the extract was placed into the different wells as food for the protists.

A dilution series was made by adding 1 g of soil to 9 mL of physiological saline solution (PSS). The PSS was made up by adding 6.8 g of NaCl to 1 L of water. Then 1 mL of the previous soil-PSS extraction solution was diluted with another 9 mL of PSS. Each test tube was placed on a vortex mixer, before the 1 mL was transferred. The test tube was sterilized by burning the rim of the test tube with a Bunsen burner before each and after each transfer. This process was repeated up to a dilution of 10^{-6} . The PSS solution was also autoclaved for 20 min at 121 °C. After this the different solutions were placed into the various wells which were replicated four times. That gave a total of 24 wells that were filled. Yeast (30 μmol) was also placed in the wells as a nutrient for the protists. The plates were then stored in a dark place for 4 to 5 days for the protists to populate (Woomer, 1994). An excel spreadsheet was used to convert the plate patterns to the MPN of total protists (Briones & Reichardt, 1999).

Total microbial Biomass

Total soil microbial biomass was determined using the method described by Islam and Weil (1998). A calibration curve was established using sucrose stock solution and making a dilution series of 0, 10, 20, 40, 80, 200 and 400 mg C L⁻¹. A 5 mL aliquot of each of the dilutions was taken and 1 mL of 0.17M K₂Cr₂O₇ and 5 mL of concentrated sulphuric acid 18M were added to it. The solutions were microwaved at 500 J m L⁻¹ and the volume adjusted to 30 mL. The absorbance was measured at 590 nm using a UV-Vis spectrophotometer.

Ten grams of oven-dried-equivalent field moist soil was placed in a 50 mL centrifuge tube and adjusted to 80 % water-filled porosity (WFP) by adding 2.5 mL distilled water. The tube was closed with a pin-hole cap and microwaved at 400 J g⁻¹. A second set of soil samples were prepared but not microwaved. A 25 mL aliquot of 0.5M K₂SO₄ was added and shaken horizontally for 60 min at 250 rpm. It was centrifuged for 5 min at 500 rpm and the solution was filtered using a 40 Whatman filter paper.

A 5 ml aliquot of each of the filtered extracts and a 1 mL of 0.17M $K_2Cr_2O_7$, 5 mL of concentrated sulphuric acid (0.18M) were added. The solution was micro waved at $500 J ml^{-1}$ and the volume was adjusted to 30 ml. The absorption were measured at 590nm using the spectrophotometer (Islam & Weil, 1998).

3.3.4 Statistical analysis

The statistical analyses were performed using SAS enterprise Guide 5.1. The data was tested for significant statistical differences with a 95% confidence interval between all the different experimental sites in both the Nardouwsberg as well as the Seekoeivlei area using the Tukey standardized t-test. The correlation between the plant and fermented tea quality parameters and the different soil (chemical, physical and microbiological) and plant nutrient parameters were done using Marlow's CP test. The statistical data is presented in **Appendix D**.

3.4 Results and discussion

3.4.1 Soil classification

The following predominant soil forms were found in the Nardouwsberg and Seekoeivlei sampling sites: Fernwood, Cartref and Constansia.

Fernwood, Rosa (Figure 3-4)

Orthic A horizon – 0-200mm deep, Dry colour 10YR 7/3, Wet colour 10YR 7/6

E-Horizon – 200mm-1500mm deep, Dry colour 10YR 7/4, Wet Colour 10YR 6/8. Yellow

Unspecified Horizon - >1500mm

Constansia, Potberg (Figure 3-5)

Orthic A horizon – 0-200mm deep, Dry Colour 10YR7/4

Yellow-brown apedal horizon – 200-1100mm deep, Dry Colour 10YR 6/6, Wet colour 10YR 6/8

E Horizon - >1100mm



Figure 3-4: Fernwood soil form, Rosa soil family, on the Ysterfontein experimental site in the Seekoeivlei area



Figure 3-5: Constansia soil form, Potberg soil family, on the Muggiesdraai experimental site in the Nardouwsberg area

Cartref, Witzenberg (transition Wasbank) (Figure 3-6)

Orthic A horizon - 0-200mm, Dry Colour 10YR7/4

E – Horizon – 200-600mm deep, dry colour 10YR 7/4, Yellow

Litocutanic B horizon - >600mm, Rock colour 10YR 4/8, Relic plinthic rock



Figure 3-6: Cartref soil form, Witzenberg soil family on the Vaalkrans experimental site in the Nardouwsberg area with the Relic plinthic rock on the right

Table 3-2: The soil form, family, profile depth and restrictions for each of the experimental sites

Experimental site	Soil Form	Soil Family	Profile depth	Restriction
MV1	Cartref	2200 Witzenberg	1000mm	“Kaing klip”/ Relic plinthic
MV2	Cartref	2200 Witzenberg	1000mm	“Kaing klip”/ Relic plinthic
MV3	Fernwood	1110 Penicuik	>1500mm	
M1	Fernwood	2210 Rosa	>1500mm	
M2	Constansia	1100 Potberg	>1500mm	
M3	Fernwood	2210 Rosa	>1500mm	
G1	Cartref	2200 Witzenberg	600mm	“Kaing klip”/ Relic plinthic
G2	Cartref	2200 Witzenberg	500mm	“Kaing klip”/ Relic plinthic
G3	Cartref	2200 Witzenberg	600mm	“Kaing klip”/ Relic plinthic
V1	Cartref	2200 Witzenberg	600mm	“Kaing klip”/ Relic plinthic
V2	Cartref	2200 Witzenberg	600mm	“Kaing klip”/ Relic plinthic
V3	Fernwood	2210 Rosa	>1500mm	
MR1	Cartref	2200 Witzenberg	600mm	“Kaing klip”/ Relic plinthic
MR2	Cartref	2200 Witzenberg	600mm	“Kaing klip”/ Relic plinthic
MR3	Cartref	2200 Witzenberg	600mm	“Kaing klip”/ Relic plinthic
JV1	Cartref	2200 Witzenberg	900mm	“Kaing klip”/ Relic plinthic
JV2	Fernwood	2210 Rosa	>1500mm	
JV3	Fernwood	2210 Rosa	>1500mm	
Y1	Fernwood	2210 Rosa	>1500mm	“Heuweltjie”
Y2	Fernwood	2210 Rosa	>1500mm	
Y3	Fernwood	2210 Rosa	>1500mm	
B1	Fernwood	2210 Rosa	>1500mm	
B2	Fernwood	2210 Rosa	>1500mm	
B3	Fernwood	2210 Rosa	>1500mm	
J1	Fernwood	2210 Rosa	>1500mm	
J2	Fernwood	2210 Rosa	>1500mm	
J3	Fernwood	2210 Rosa	>1500mm	Plough bank (30 cm)

In the Nardouwsberg area, most of the experimental sites’ soils contain an iron-cemented (plinthic) rocky layer that is commonly referred to as “kaing klip”. These plinthic rocks have a red/yellow colour (**Figure 3-6**). Farmers report that the rooibos produced on the soils

containing a plinthic layer is of a better quality. Plinthic layers typically form due to periodic water-logging (fluctuating water table) and were formed under different climatic and soil conditions from the present and are therefore classified as relic plinthic rock (Soil Classification working group, 1991). The Cartref forms can thus also be described as transition Wasbank forms, which consist of an Orthic A horizon on an E horizon on a hard plinthic B horizon. It is possible that water can be stored in the litocutanic B horizon due to its impermeability. Most of the taproots grew to a depth of 1.5 m, but some were found at a depth of 2.5 m while sampling. Most roots ended in the litocutanic layer and it was impossible to follow the roots any further (**Figure 3-7**). These long tap roots are most likely what keep the plants growing during the hot, dry summer months.



Figure 3-7: A rooibos taproot with a length of 1.5 m (Right). A taproot entering the litocutanic layer (relic plinthic) at depth of 900 mm (Left).

Muggiesdraai Virgin Sites 1 and 2 (MV1 and MV2) also contain a plinthic layer at about 1 m depth. All of the Geelland sites (G1-G3) and Vaalkrans site 1 and 2 (V1 and V2) contain a plinthic layer at about 600 mm.

In the Seekoeivlei area, the most common soil form was a Fernwood, which means that deep sandy soils were found in this area, with only one exception in the Jaap-se-Kop virgin experimental site which also had a litocutanic B on a depth of 900mm and is thus classified as a Cartref.

3.4.2 Soil Chemical and physical determinations

3.4.2.1 Particle size distribution and soil bulk density

All of the experimental sites had high sand contents above 90% at both 0-20 cm and the 20-40 cm depths, and they were predominantly classed as coarse sands (**Table 3-3**). The soils had very low clay contents with the clay values between 1.6 and 3.2 %. Therefore, the water holding and nutrient holding capacity of the soil are low, and the soils nutrients can easily be leached in these soils.

In the topsoil (0-20 cm) there was no significant statistical difference ($P = 0.0416$) in the bulk density between sites, whereas, at 20-40 cm there was a significant difference ($P = <0.0001$) between the different experimental sites, especially between the virgin and older sites in the Seekoeivlei area. The soils in the Nardouwsberg area (Valkrans, Geelland and Muggiesdraai) generally had a higher bulk density than the soils in the Seekoeivlei area (Ysterfontein, Jaap-se-Kop and Bokwater) (**Figure 3-8**). The difference in the bulk densities between the two areas can be attributed to the differences in soil texture (**Table 3-3**). The Nardouwsberg soils contain more coarse sand than the Seekoeivlei soils (**Table 3-3**), which can lead to a higher bulk density since the Nardouwsberg area had a higher overall bulk density than the Seekoeivlei area (**Figure 3-8**). The mean bulk densities ranged between 1.44 to 1.68 g cm⁻³ (**Figure 3-8**). These values correlate well to the 1.6 g cm⁻³ that is generally reported for sandy soils, as sandy soils have a low volume of pores (Hillel, 1998). Root growth will only be constricted once the bulk density of a sandy soil exceeds 1.8 g cm⁻³ (USDA, 2008), therefore no root restriction was found and cannot be the reason for the decrease in tea yields. The bulk density in the topsoil 0-20 cm was lower than the bulk density in the 20-40 cm, except in the case of the Jaap-se-Kop virgin site (**Figure 3-8**). This can be attributed to higher organic matter content in topsoils at all sites, and also to periodic tillage at cultivated sites which loosens the topsoil but results in compaction in the subsoil.

Table 3-3: Soil particle size distribution (0-20 cm and 20-40 depths) at all sites.

	Sites	Clay	Silt			Sand						Texture class	
			Fine Silt	Coarse silt	Total	Very fine Sand	Fine Sand	Medium sand	Coarse Sand	Very Coarse Sand	Total		
0-20cm	<i>Nardouwsberg Sites</i>												
	Muggiesdraai Virgin	1.9	1.0	3.9	4.9	5.8	20.0	31.4	24.8	11.1	93.1	Sand (Coarse)	
	Muggiesdraai (3 Y)	2.9	1.3	4.7	5.9	8.1	20.9	27.2	22.3	12.8	91.2	Sand (Coarse)	
	Geelland (20 Y)	1.9	1.6	3.5	5.2	5.5	14.0	25.6	37.7	10.1	92.9	Sand (Coarse)	
	Vaalkrans (60 Y)	2.9	0.5	3.9	4.4	4.5	15.0	25.6	33.2	14.5	92.8	Sand (Coarse)	
	Muggiesdraai Fallow (10 Y)	1.8	1.0	2.4	3.4	5.7	22.8	44.8	19.0	2.5	94.8	Sand (Coarse)	
	<i>Seekoeivlei Sites</i>												
	Jaap-se-Kop Virgin	1.8	0.5	3.5	4.0	9.9	31.2	34.3	17.4	1.5	94.2	Sand (Medium)	
	Ysterfontein (2 Y)	2.5	1.8	3.6	5.4	7.3	27.2	35.3	15.7	6.6	92.2	Sand (Coarse)	
	Bokwater (20 Y)	2.1	1.4	5.4	6.8	8.0	27.0	33.9	15.8	6.4	91.1	Sand (Coarse)	
Jaap-se-Kop (30 Y)	2.2	0.1	3.0	3.1	7.0	28.5	35.5	16.2	6.0	94.7	Sand (Coarse)		
20-40cm	<i>Nardouwsberg Sites</i>												
	Muggiesdraai Virgin	2.3	0.9	2.6	3.5	5.5	21.1	32.0	24.8	10.7	94.1	Sand (Coarse)	
	Muggiesdraai (3 Y)	3.2	1.5	3.5	4.9	9.7	22.6	28.3	20.6	10.6	91.9	Sand (Coarse)	
	Geelland (20 Y)	2.6	1.6	4.7	6.4	5.9	14.3	24.7	35.8	10.4	91.1	Sand (Coarse)	
	Vaalkrans (60 Y)	3.2	1.7	5.7	7.4	7.5	18.7	24.7	27.4	11.1	89.4	Sand (Coarse)	
	Muggiesdraai Fallow (10 Y)	2.1	0.7	2.9	3.5	6.6	22.6	42.9	19.4	2.9	94.4	Sand (Coarse)	
	<i>Seekoeivlei Sites</i>												
	Jaap-se-Kop Virgin	1.6	1.0	2.5	3.5	9.5	32.1	34.9	16.9	1.4	94.9	Sand (Medium)	
	Ysterfontein (2 Y)	2.6	2.1	4.1	6.2	7.7	27.5	34.0	15.4	6.7	91.3	Sand (Coarse)	
	Bokwater (20 Y)	2.3	1.0	3.6	4.6	7.0	28.5	35.5	16.2	6.0	93.1	Sand (Coarse)	
Jaap-se-Kop (30 Y)	2.1	0.7	3.3	4.0	6.7	25.7	40.8	16.7	4.0	93.9	Sand (Coarse)		

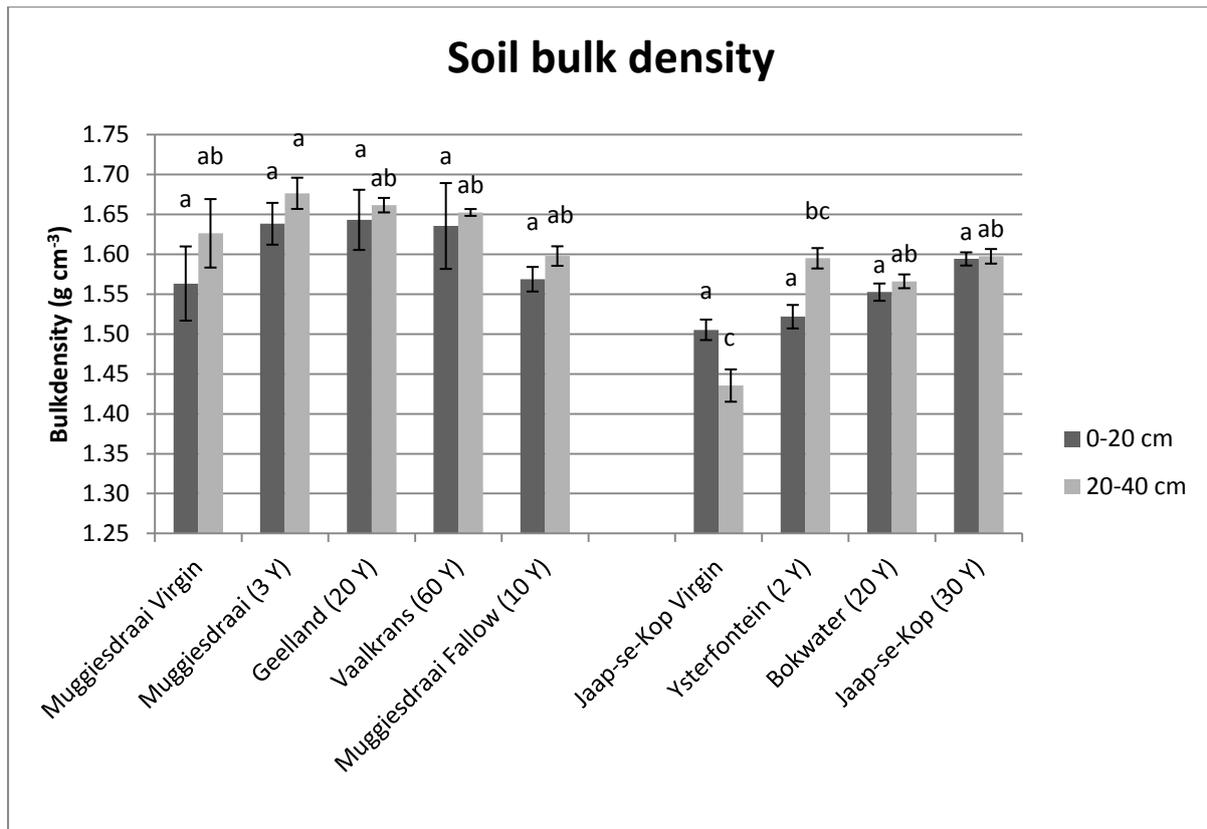


Figure 3-8: Soil bulk density at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth.

It is not clear why the Jaap-se-Kop virgin site had a lower bulk density in the subsoil, but it could be attributable to the dense rooting systems in the subsoil. The bulk densities in the Nardouwsberg and Seekoeivlei areas were lower in the virgin and fallow sites than in the cultivated sites (**Figure 3-8**), which can also be attributed to slightly higher carbon contents at these sites (**Figure 3-12**), as well as the lack of tillage and traffic. It appears that in the Nardouwsberg area, the fallow soil's bulk density returned to values similar to that of the undisturbed virgin soil (**Figure 3-8**).

There appeared to be an increase in the soil bulk density with the increase in the number of years of rooibos cultivation in the Seekoeivlei area, most notably in the subsoil, but this trend was not evident in the Nardouwsberg area (**Figure 3-8**). The increase in soil bulk density with continuous rooibos cultivation can be attributed to the degree of soil compaction due to tillage and the second reason is due to the removal of soil organic matter thus changing the soil structure. The type of tillage that was used also had an effect on the

bulk density. The farms Vaalkrans, Geelland, Muggiesdraai and Jaap-se-Kop used the same method of tillage, i.e., they were prepared by using a mouldboard plough (200 mm) the year before planting and a ripper (600 mm) just before planting. At Bokwater, the soil was prepared by using a disc plough the year before planting and a ripper (600 mm) was used just before planting. The Ysterfontein site was prepared by using a disc plough two years before planting, a mouldboard plough was used in the year before planting (200 mm) and the soil was ripped (600 mm) just before planting. Higher bulk densities were found at the experimental sites, Vaalkrans, Geelland, Muggiesdraai and Jaap-se-Kop, where a mouldboard plough was used (Figure 3-8).

3.4.2.2 Citrate bicarbonate dithionate (CBD) extractable Fe, Mn and Al oxides

CBD extractable Al in the soil

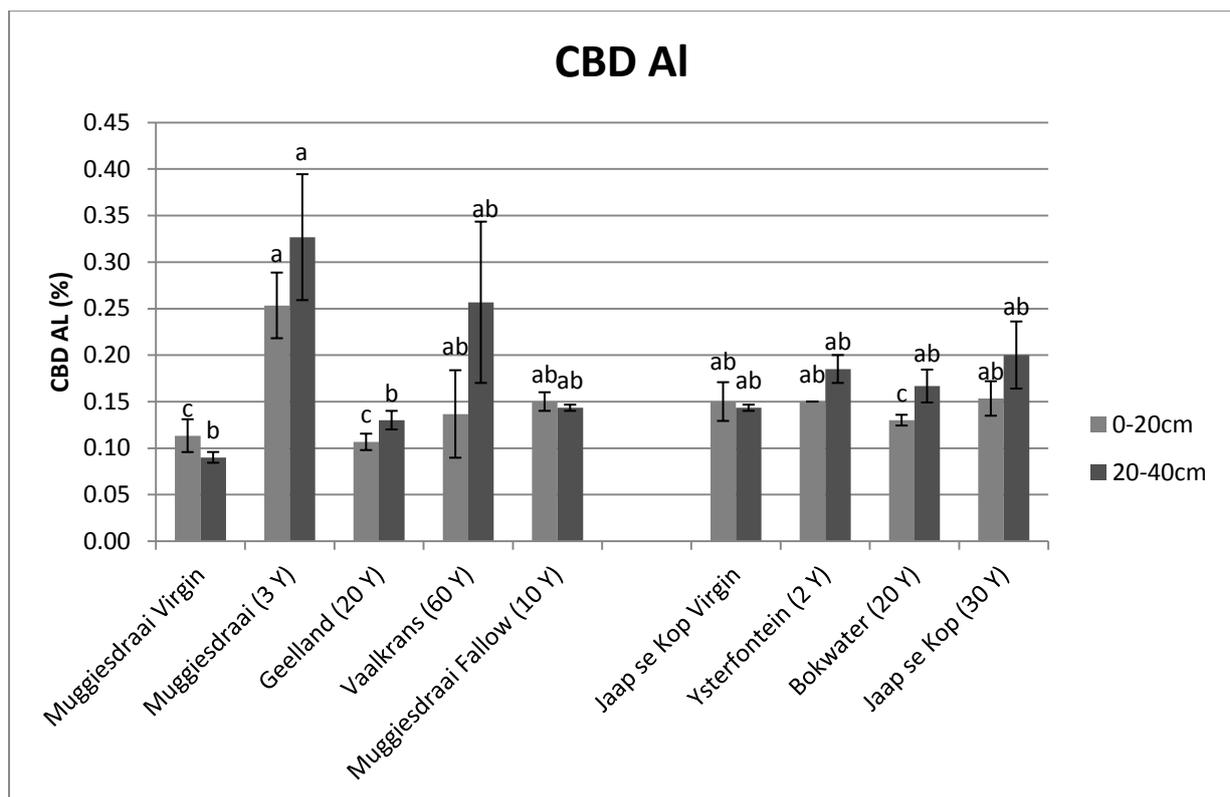


Figure 3-9: CBD extractable Al at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth.

There are significant statistical differences in the CBD Al content between the different experimental sites in the 0-20 cm (P = 0.0195) and 20-40 cm (P = 0.0162) layer. Higher CBD

Al levels were found in the Vaalkrans and Muggiesdraai experimental sites (**Figure 3-9**), this is most likely due to the fact that the clay content is higher in these specific sites (**Table 3-3**) resulting in higher dissolution of Al.

CBD extractable Fe in the soil

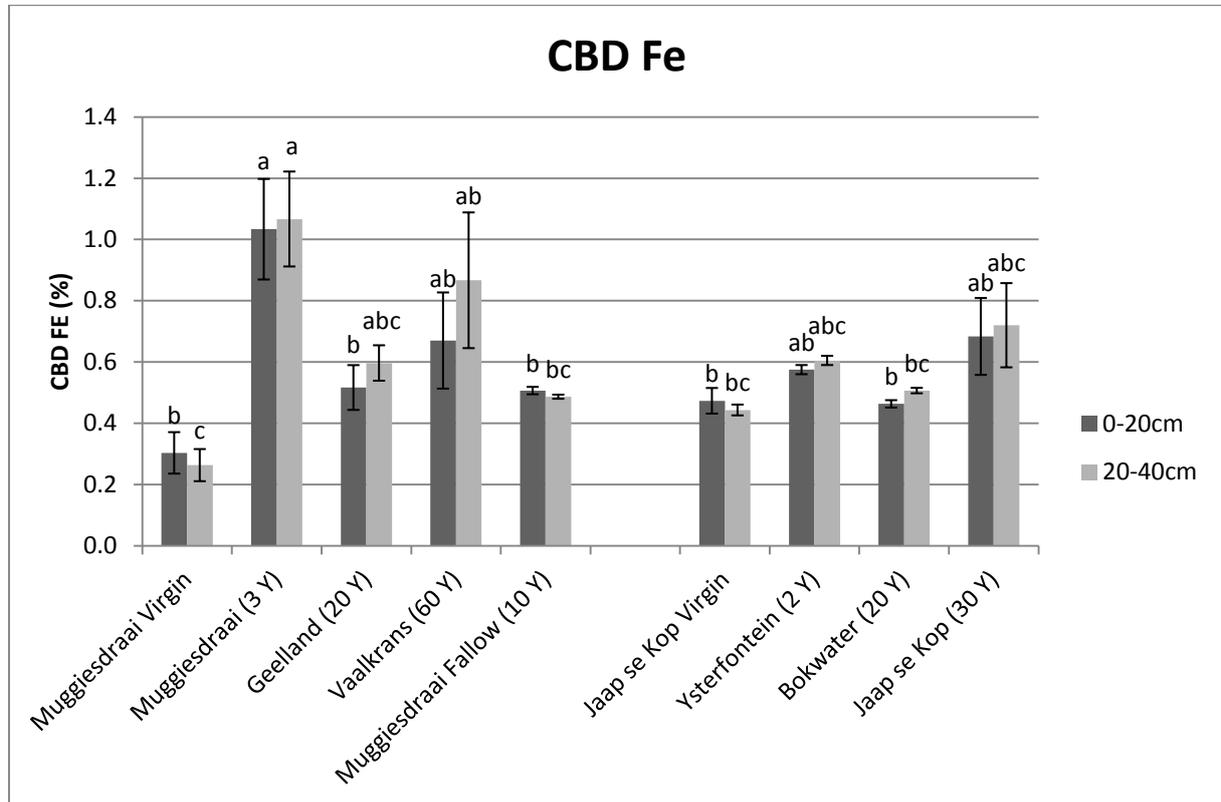


Figure 3-10: CBD extractable Fe at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth.

Significant statistical differences were noted in the CBD Fe content of the different experimental sites in the 0-20 cm ($P = 0.0052$) and 20-40 cm ($P = 0.0053$) layer. Higher mineral iron levels were found in the Muggiesdraai, Geelland and Vaalkrans experimental sites, due to the relic plinthic layer found in these sites (**Figure 3-6**).

CBD extractable Mn in the soil

There were no significant statistical differences in the CBD extractable Mn content between the different experimental sites in the 0-20 cm ($P = 0.0348$) and 20-40 cm ($P = 0.4665$) layer.

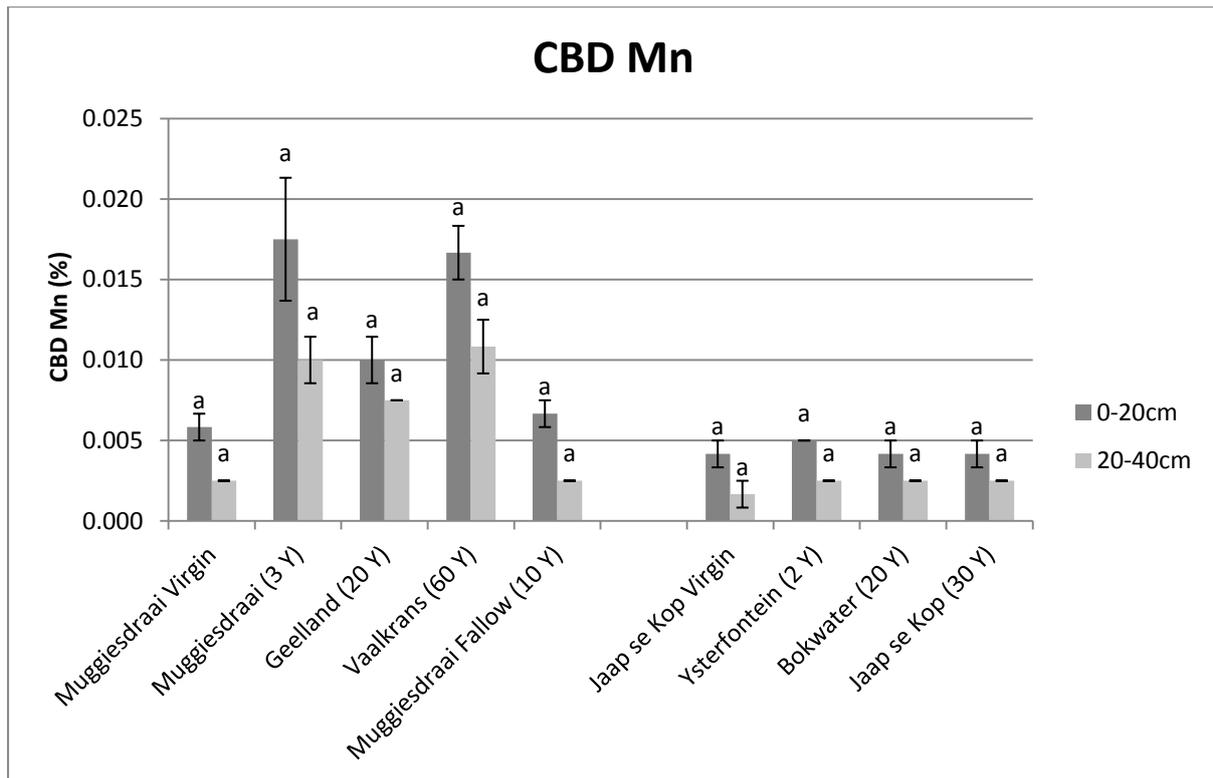


Figure 3-11: CBD extractable Mn at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

3.4.2.3 Total Carbon

The soil carbon content of the soils is extremely low, ranging between 0.06 - 0.3 % (**Figure 3-12**). The soil carbon differs significantly in the 0-20 cm ($P = 0.0008$) and 20-40 cm ($P < 0.0001$) layers, and a definite downwards trend can be seen in the soil carbon with an increase in the number of years that the field have been used for the cultivation of rooibos tea (**Figure 3-12**). The decrease in the carbon is likely due to the fact that less organic matter is returned to the soil annually, because almost two thirds of the rooibos plants are harvested each year. Another factor that plays a role in the low carbon values is the sun, since the cultivated fields is continuously tilled the organic matter is more exposed to the sun and decomposition rates increase dramatically. Sandy soils also do not stabilize humus as effectively as clay rich soils. There is also an indication that the field will return to its natural state in the case where the Muggiesdraai fallow have been left for 10 years (**Figure 3-12**).

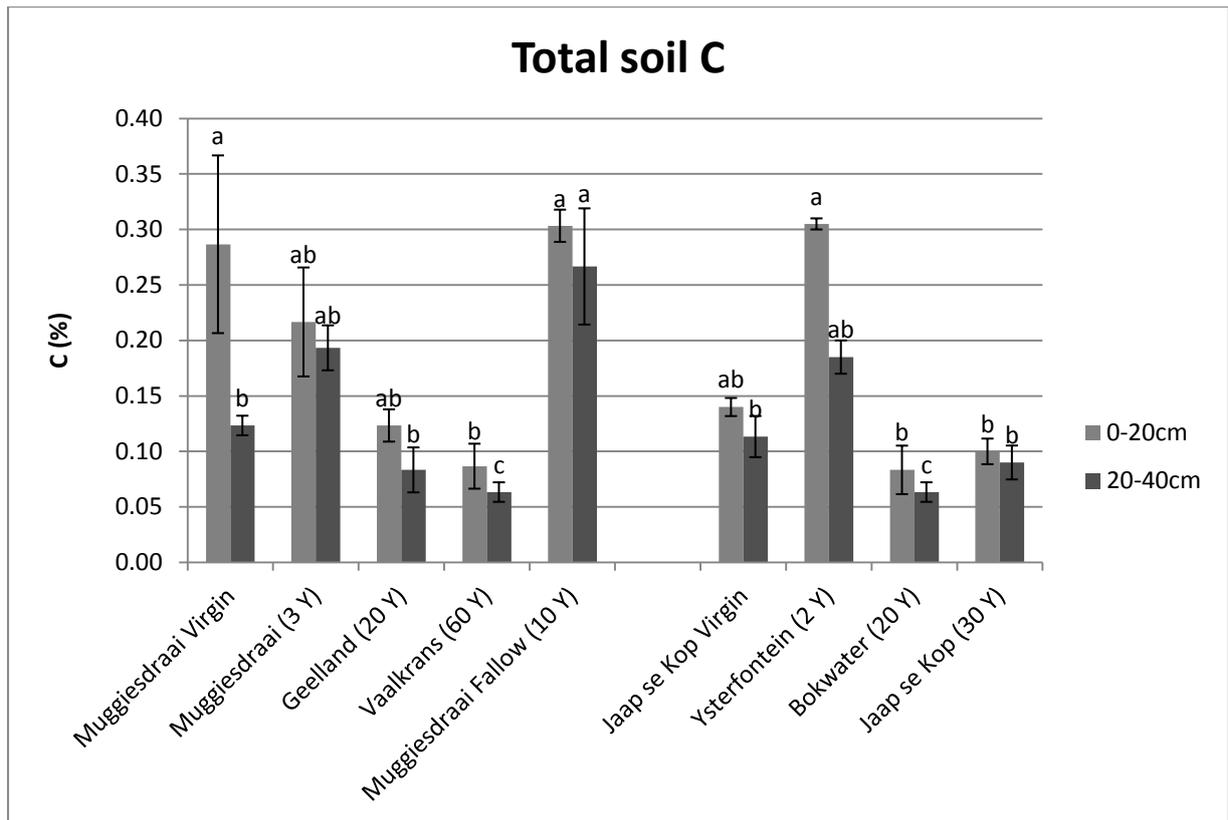


Figure 3-12: Total soil carbon (%) at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The recently cleared sites (Muggiesdraai and Ysterfontein), as well as the Muggiesdraai fallow site, have a higher C:N Ratio in the 0-20 cm layer (>15) (**Figure 3-13**), this is most likely due to the fact that a lot of undecomposed organic matter was applied to the soil during the preparation of the fields resulting in higher soil carbon levels (**Figure 3-12**). The organic matter is thus still in the process of breaking down. Low inorganic nitrogen levels was found in the Muggiesdraai and Ysterfontein experimental sites (<0.02 %) (**Figure 3-27**), thus also resulting in the higher C:N ratio and slower breakdown of organic matter. Another reason for the wide C:N ratios could be that the CN analyzer becomes less accurate at low N levels.

The virgin and older sites (Muggiesdraai virgin, Geelland, Vaalkrans, Jaap-se-Kop Virgin, Bokwater and Jaap-se-kop) had a C:N ratio of below 11:1 (**Figure 3-13**), this can be due to the fact that the organic matter is broken down into humus. These low values correlates well with the C:N values (5.2:1) found on sandy fynbos soils near Malmesbury (Van der

Merwe, 2009). The CN ratio's in the older sites are not optimum for microorganisms, as they have a C:N ratio of 8:1 in their bodies and require this from the environment to maintain the ratio in their bodies (Service, 2011). The amount of carbon to nitrogen is thus the best in the recently cultivated fields, where the natural fynbos have been removed and in the fallow site, where the field have been left to return to its natural habitat since higher soil carbon levels and lower nitrogen levels were found in these sites and resulted in a slower breakdown process of the organic matter.

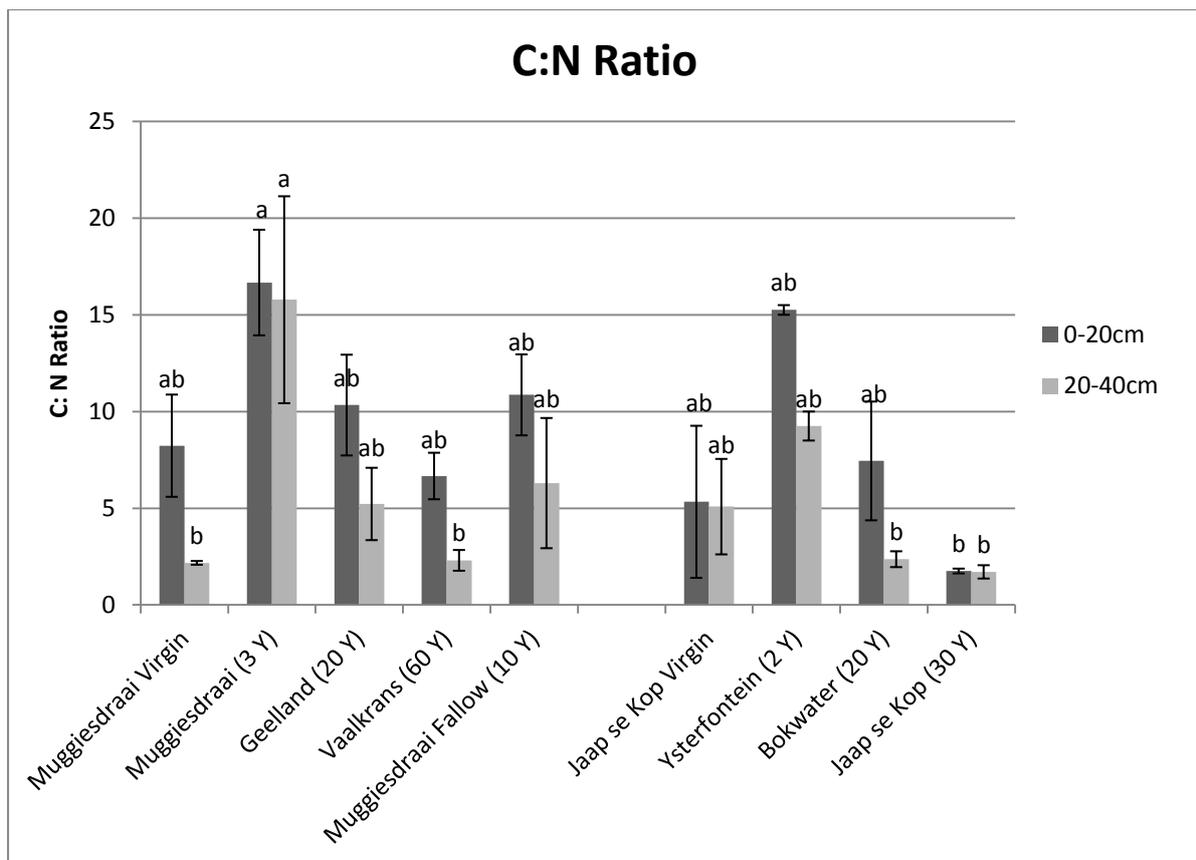


Figure 3-13: C:N Ratio at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

3.4.2.4 Soil pH

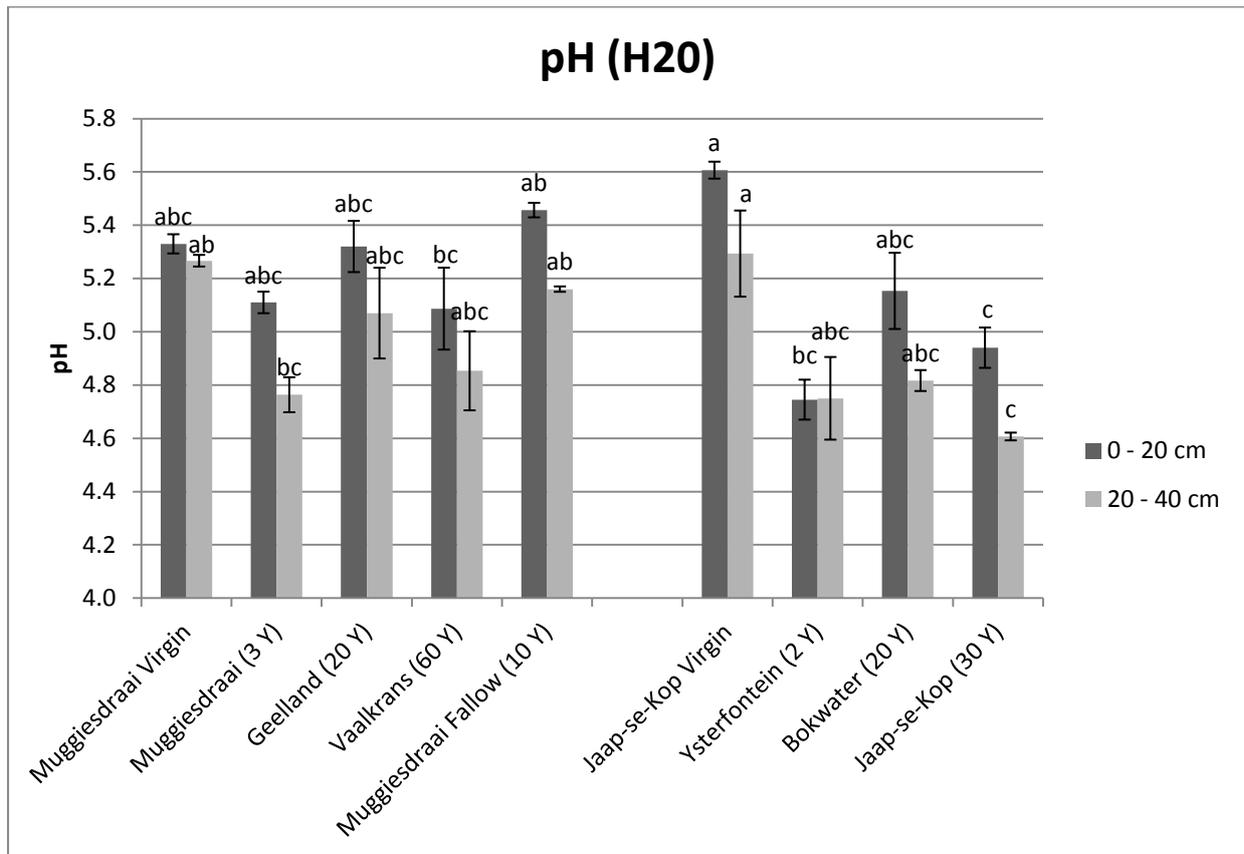


Figure 3-14: Soil pH (H₂O) at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The soil pH (H₂O) ranged between 4.5 and 5.5 at the experimental sites (**Figure 3-14**). The pH (H₂O) values at the experimental sites differed significantly in the 0-20 cm ($P = 0.0027$) and the 20-40 cm ($P = 0.0025$) layers. The pH values of the 0-20 cm layer are slightly higher than the pH in the 20-40 cm layer. The higher pH in the top soil is attributed to the roots taking up all the basic cations and transferring it to the leaves and when the plant dies, it is returned to the top soil. The soil pH did not differ between experimental sites in the Nardouwsberg area. There is also no decrease or increase in the pH as the number of years under rooibos production increase. In the Seekoeivlei area the pH between the Jaap-se-Kop Virgin site and the oldest cultivated Jaap-se-Kop site on which rooibos have been planted for 30 years differ significantly. The pH is lower in the older site, which is most likely due to the fact that some of the basic cations have leached out of these soils. Another reason for the decrease in the pH can be due to the loss of organic matter since the soil carbon decreased

with an increase in the age of the field (**Figure 3-12**) which leads to a decrease in the basic cations and the CEC. The pH values correlate well with the values described by J van Putten (personal communication) as well as with the pH found in honeybush tea soils (Joubert *et al.*, 2007).

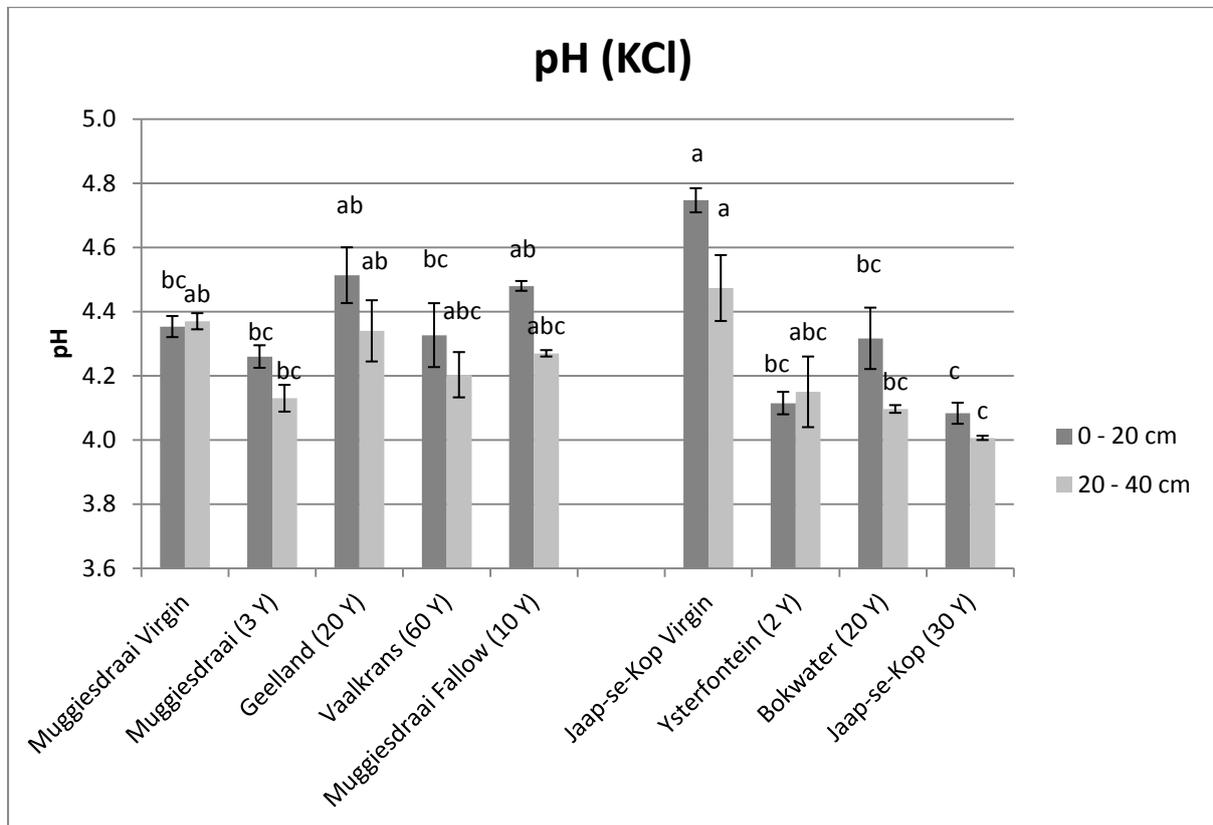


Figure 3-15: Soil pH (KCl) at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The pH (H₂O) and pH (KCl) differs with about 1 pH unit (**Figure 3-14** and **Figure 3-15**), which means that soils contain considerable amounts of exchangeable acidity despite the low carbon (**Figure 3-12**) and clay contents (**Table 3-3**). The pH (KCl) at experimental sites differs statically significantly in the 0-20 cm (P = 0.0002) and the 20-40 cm (P = 0.0018) layers.

3.4.2.5 Electrical conductivity (EC)

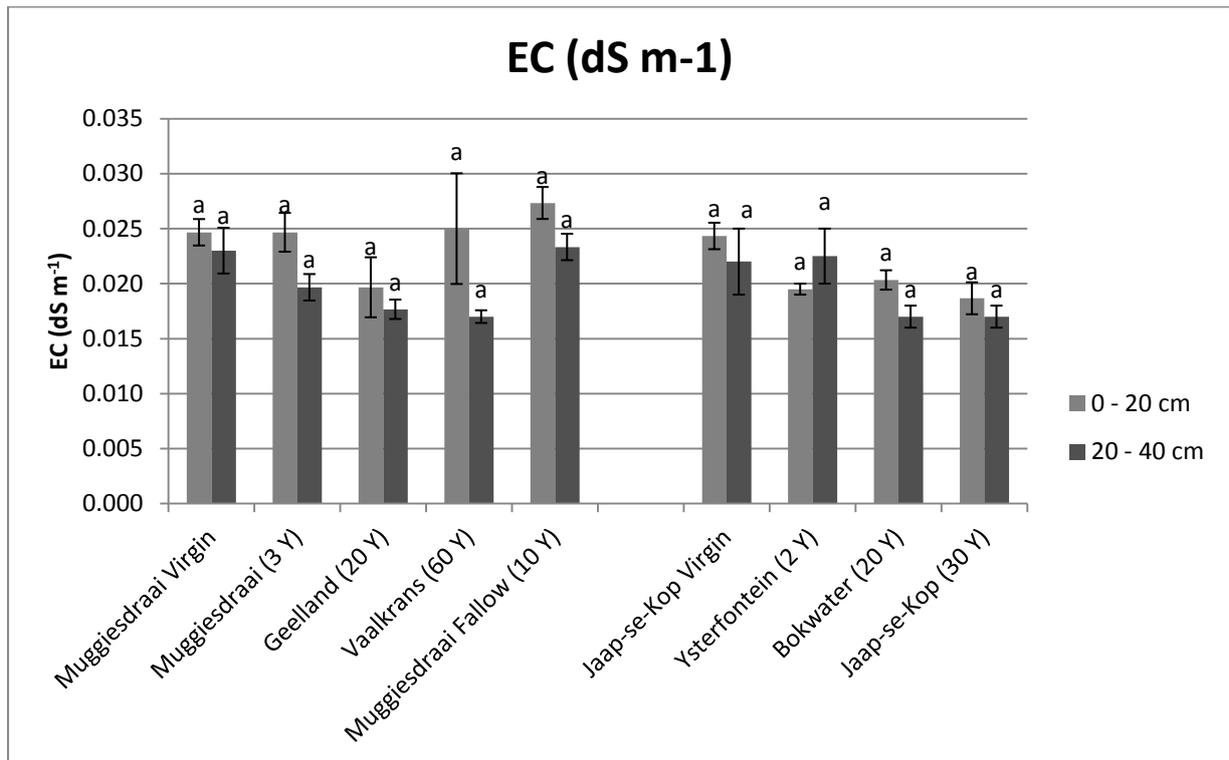


Figure 3-16: Electrical conductivity (EC) (dS m⁻¹) at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The electrical conductivity (EC) values of all the sites are two orders of magnitude lower than the threshold value of 4 dS m⁻¹ for saline soils, indicating that they are highly leached soils (**Figure 3-16**). The EC values between the different experimental sites don't differ significantly between the each other in the 0-20 cm (P = 0.4768) and 20-40 cm (P = 0.4673) layers, but a definite trend can be seen; there is a decrease in the EC with an increase in the number of years the field have been used for the cultivation of rooibos. The EC correlates well with the lower pH values (**Figure 3-14** & **Figure 3-15**), indicating that the soil has become more leached.

3.4.2.6 Exchangeable basic cations and acidity

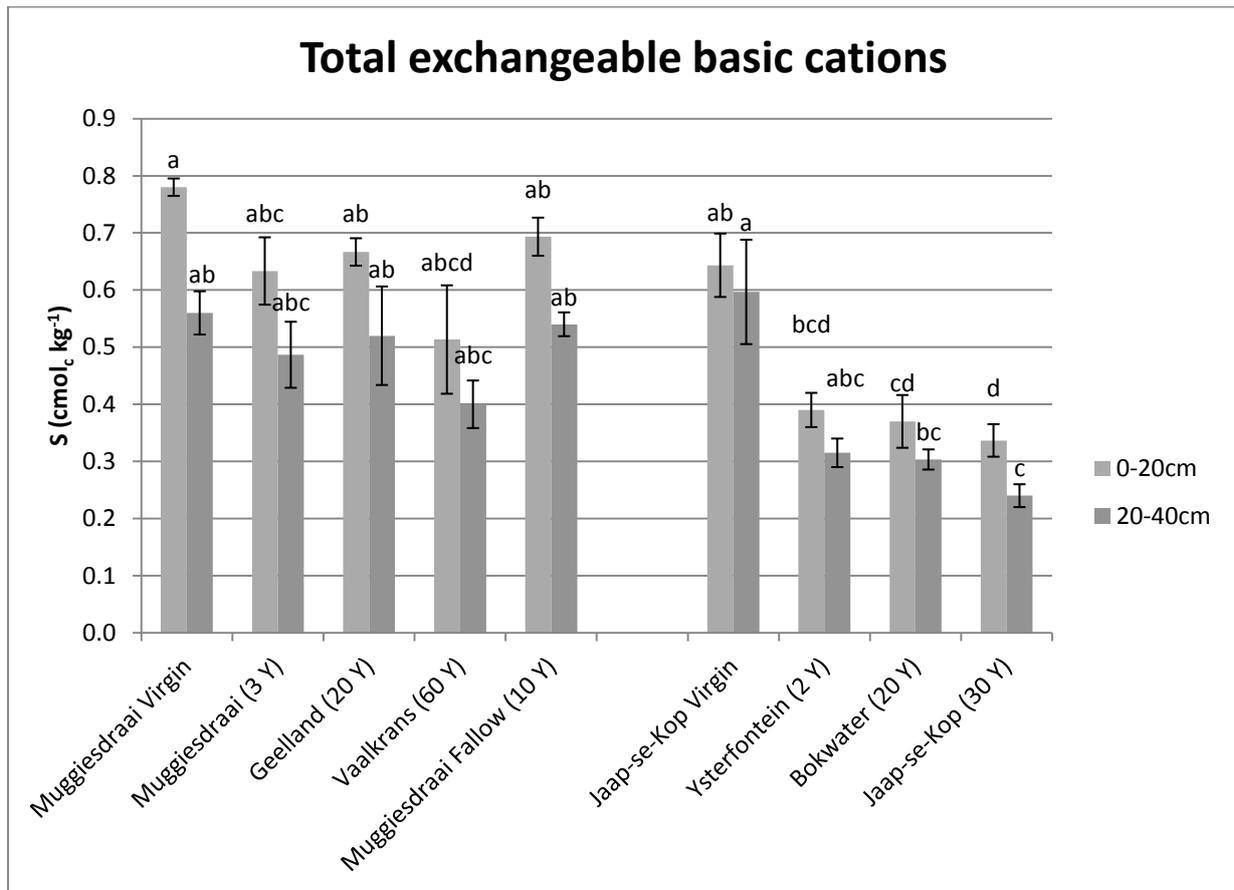


Figure 3-17: Total exchangeable basic cations at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

There is a decrease in the exchangeable basic cations with an increase in the number of years that the soil has been used for rooibos cultivation (**Figure 3-17**). There is also a decrease in the total exchangeable basic cations with an increase in the depth of the soil (**Figure 3-17**). The fallow site appears to have a similar exchangeable cation status as that of the virgin sites. The decrease in the total exchangeable basic cations can be due to the fact that the soil organic carbon decreased (**Figure 3-12**), resulting in a decrease in the cation exchange capacity (CEC). Another reason for the decrease in total exchangeable basic cations can be due to nutrient mining. Farmers harvest the rooibos each year resulting in a loss of basic cations but they don't replenish the basic cations. The older sites are more leached since the plants have been removed; this can also be seen in the extremely high resistance values of the soils (**Appendix A**). There is a significant difference in the total

exchangeable cations between the different experimental sites in both the 0-20 cm ($P = 0.0003$) and the 20-40 cm ($P = 0.0029$) layer.

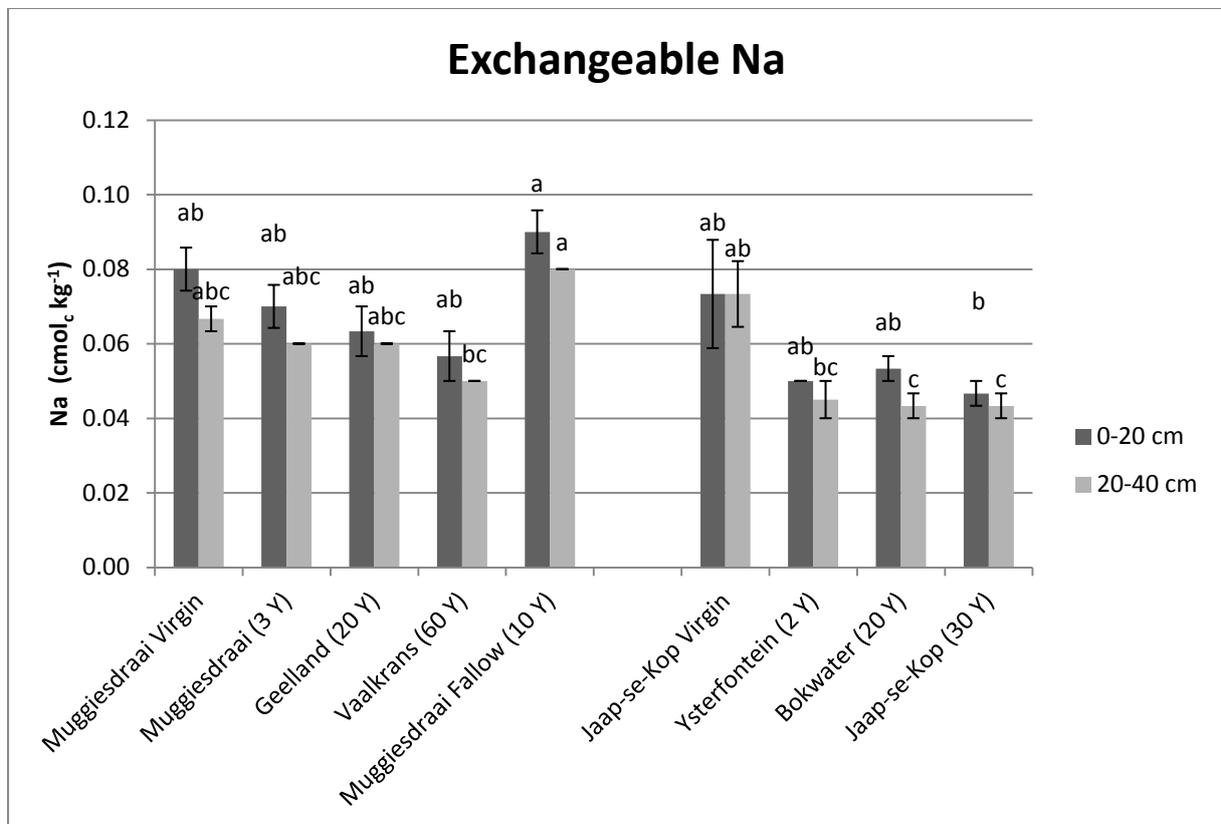


Figure 3-18: Exchangeable Na in the soil at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown ($n = 3$). The letters of significance differ between the experimental sites as well as the depth

The virgin and fallow soils tend to have higher exchangeable Na contents than those in the older cultivated sites like the Jaap-se-Kop, Bokwater and Vaalkrans sites (**Figure 3-18**). There is a significant difference in the exchangeable Na between experiment sites in both the 0-20 cm ($P = 0.0211$) and the 20-40 cm ($P = 0.0003$) layer. The same downwards trend was noticed as was noticed in the total exchangeable cations (**Figure 3-17**), exchangeable Ca (**Figure 3-20**) and exchangeable Mg (**Figure 3-21**). The main reason for the decrease can possibly be contributed to soil mining and loss of organic matter resulting in a lower CEC (**Figure 3-23**) for holding nutrients in the soil.

The average sodium values found in the different experimental sites correlates well with the values found in honeybush tea by (Joubert, et al., 2007). The average sodium values found

in the different experimental sites were 0.04-0.09 cmol kg^{-1} (**Figure 3-18**) while average values of 0.09 cmol kg^{-1} were found by Joubert et al., 2007 on a sandy soil in the Western Cape.

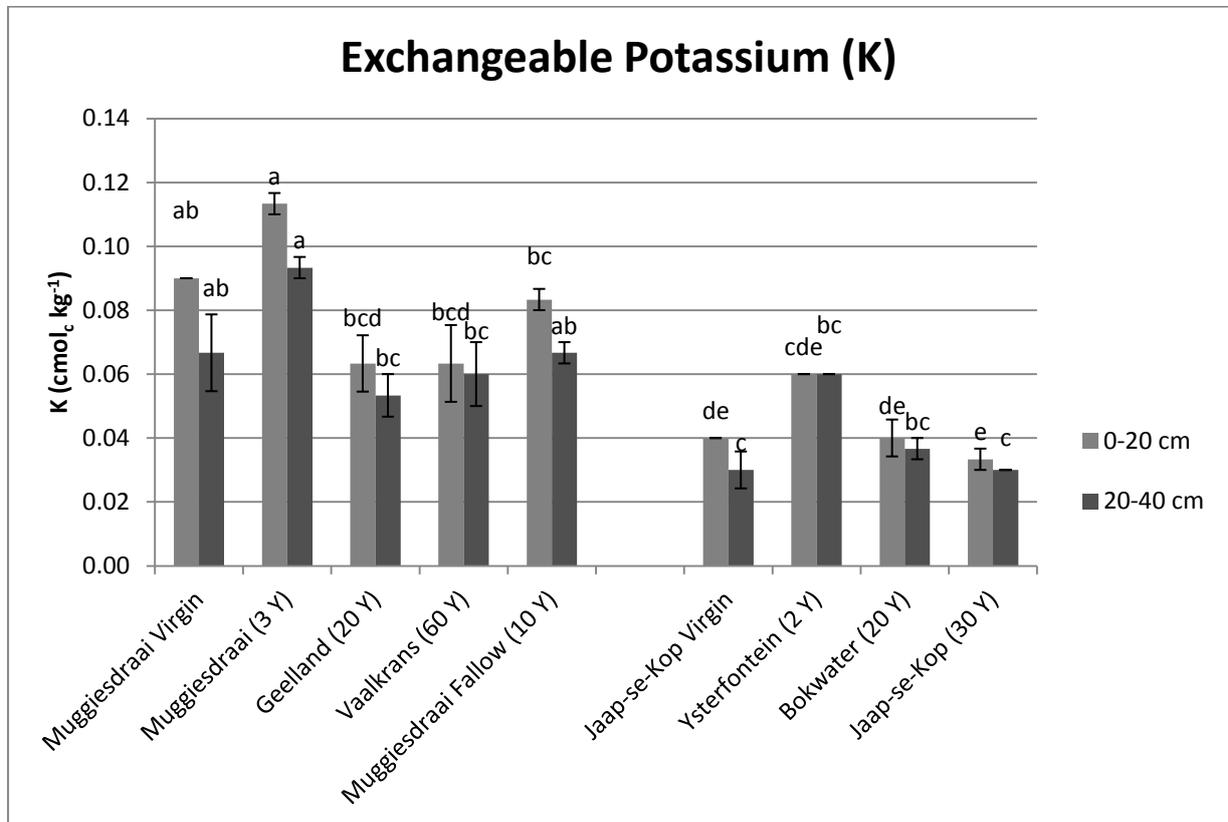


Figure 3-19: Exchangeable Potassium (K) in the soil at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The exchangeable K contents of the Nardouwsberg soils are slightly higher than those in the Seekoeivlei area (**Figure 3-19**). This can possibly be related to different parent material. A slight increase in the K content can be noticed in the recently cleared and planted sites. This can possibly be due to the increase in plant material returned to the soils resulting in an initial increase before the K content stabilizes again. No fertilizers were applied to the Ysterfontein and Muggiesdraai experimental sites. The soils on which rooibos has been planted for the first time appear to have a higher K content than the soils on which rooibos have been planted for a number of years. The Muggiesdraai and Ysterfontein have a higher K value than the Geelland, Vaalkrans, Bokwater and Jaap-se-Kop areas in both the 0-20 cm and the 20-40 cm. The K in the Jaap-se-kop virgin site is relative low. Potassium is known to

leach easily in sandy soils with a low pH and CEC since Al would exchange with the K. The K in the soil solution will thus leach out of the profile, as is the case for the soils studied.

A significant difference in the exchangeable K content between the different experimental sites in both the 0-20 cm ($P < 0.0001$) and the 20-40 cm ($P < 0.0001$) layer was observed. The average exchangeable K values found in the different experimental sites are similar to the values found in honeybush tea soils by (Joubert *et al.*, 2007). The average K values found in the different experimental sites were 0.02-0.12 cmol kg^{-1} (**Figure 3-19**), whereas, average values of 0.06 cmol kg^{-1} were found by Joubert *et al.*, 2007.

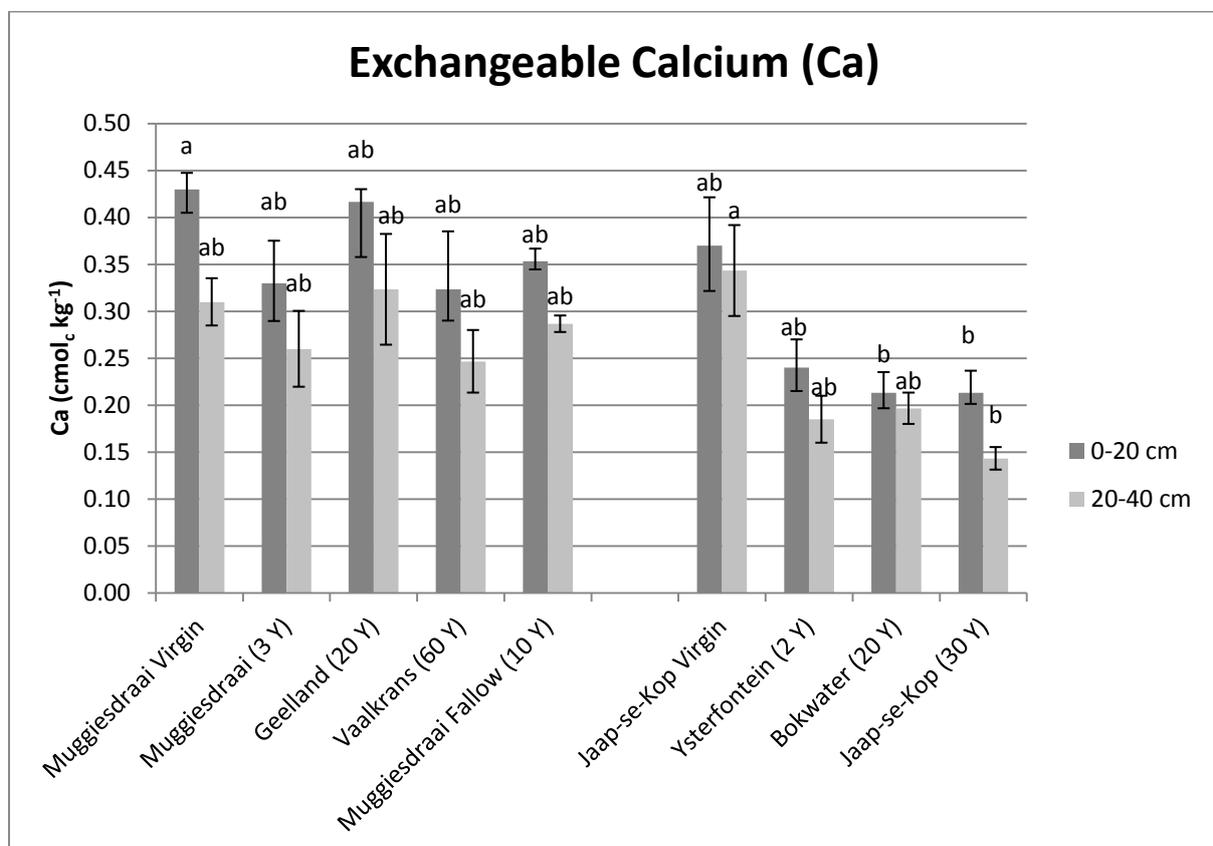


Figure 3-20: Exchangeable Calcium (Ca) in the soil at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown ($n = 3$). The letters of significance differ between the experimental sites as well as the depth

The Nardouwsberg and the Seekoeivlei area did not differ significantly in terms of Ca content. The Ca content in the Seekoeivlei area decreased with an increase in number of years that rooibos have been planted on the soils (**Figure 3-20**). There is a significant statistical difference in the exchangeable Ca between the different experimental sites in both the 0-20 cm ($P = 0.0159$) and the 20-40 cm ($P = 0.0279$) layer. The average Ca values

found in the different experimental sites were 0.2-0.5 cmol kg^{-1} (**Figure 3-20**) while average values of 0.45 -1.00 cmol kg^{-1} were found by Joubert *et al.*, 2007 for soil on which honeybush was planted.

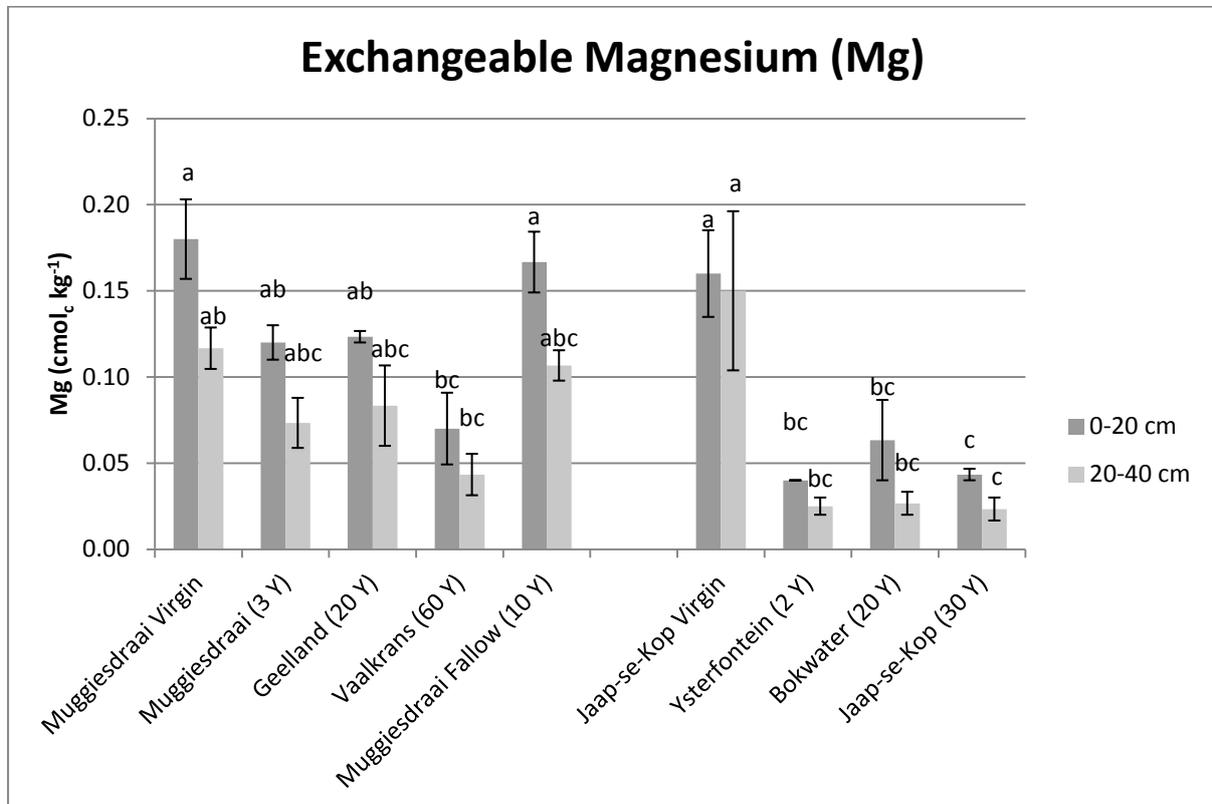


Figure 3-21: Exchangeable Magnesium (Mg) in the soil at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The virgin and fallow site had a higher average Mg content in comparison to the rest of the sites (**Figure 3-21**). The same trends were found as in the exchangeable basic cations (**Figure 3-17**) and exchangeable Na (**Figure 3-18**). The Nardouwsberg area also has a higher average Mg content than the Seekoeivlei area. Significant differences were found in the exchangeable Mg between the different experimental sites in both the 0-20 cm ($P < 0.0001$) and the 20-40 cm ($P = 0.0013$) layer (**Figure 3-21**). The average Mg values found in the different experimental sites correlates well with that of honeybush tea by (Joubert *et al.*, 2007). The average Mg values found in the different experimental sites were 0-0.2 cmol kg^{-1} (**Figure 3-21**) while average values of 0.16 cmol kg^{-1} were reported by Joubert *et al.*, 2007.

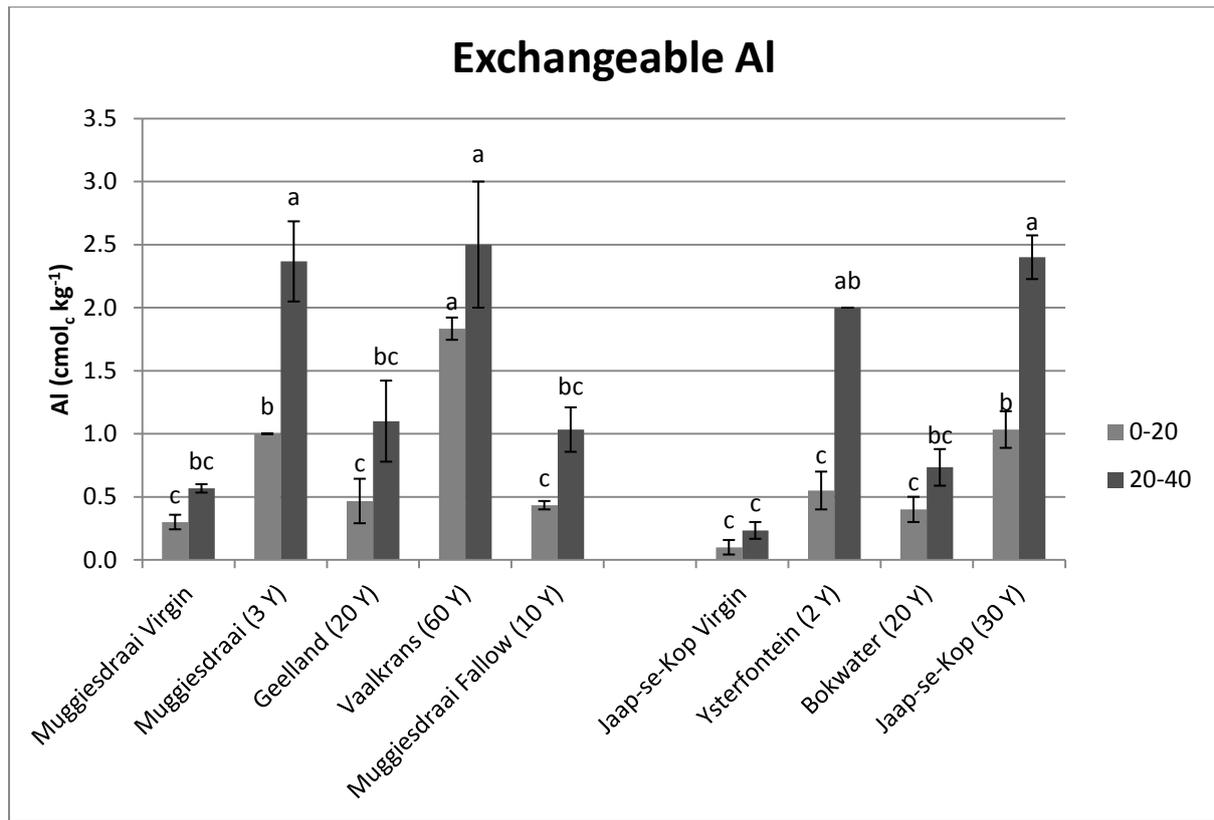


Figure 3-22: Exchangeable Aluminium Al (cmol_c kg⁻¹) at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

In both the Nardouwsberg, as well as the Seekoeivlei area, the exchangeable Al tended to increase with an increase in the age of the experimental site (**Figure 3-22**). A slight negative correlation was observed between the exchangeable Al and the pH (KCl) in both the 0-20 cm ($R^2=0.0612$) and the 20-40 cm ($R^2=0.5018$) layer (data not shown). The exchangeable Al increased on the exchange sites with a decrease in the pH since the basic cations is replaced from the exchange sites and leached out of the soil. The Al³⁺ hydrolysis reactions buffer the soil pH in the 4.5-5 range (McBride, 1994) and would occur in these soils since the pH (KCl) varies between 4 and 5 (**Figure 3-14** & **Figure 3-15**). Clays are dissolved at these pH's releasing more Al. Significant differences were observed between the different experimental sites in both the 0-20 cm ($P < 0.001$) as well as the 20-40 cm layer ($P < 0.001$). The biggest differences occur in the Muggiesdraai, Vaalkrans, Ysterfontein and Jaap-se-Kop experimental sites (**Figure 3-22**).

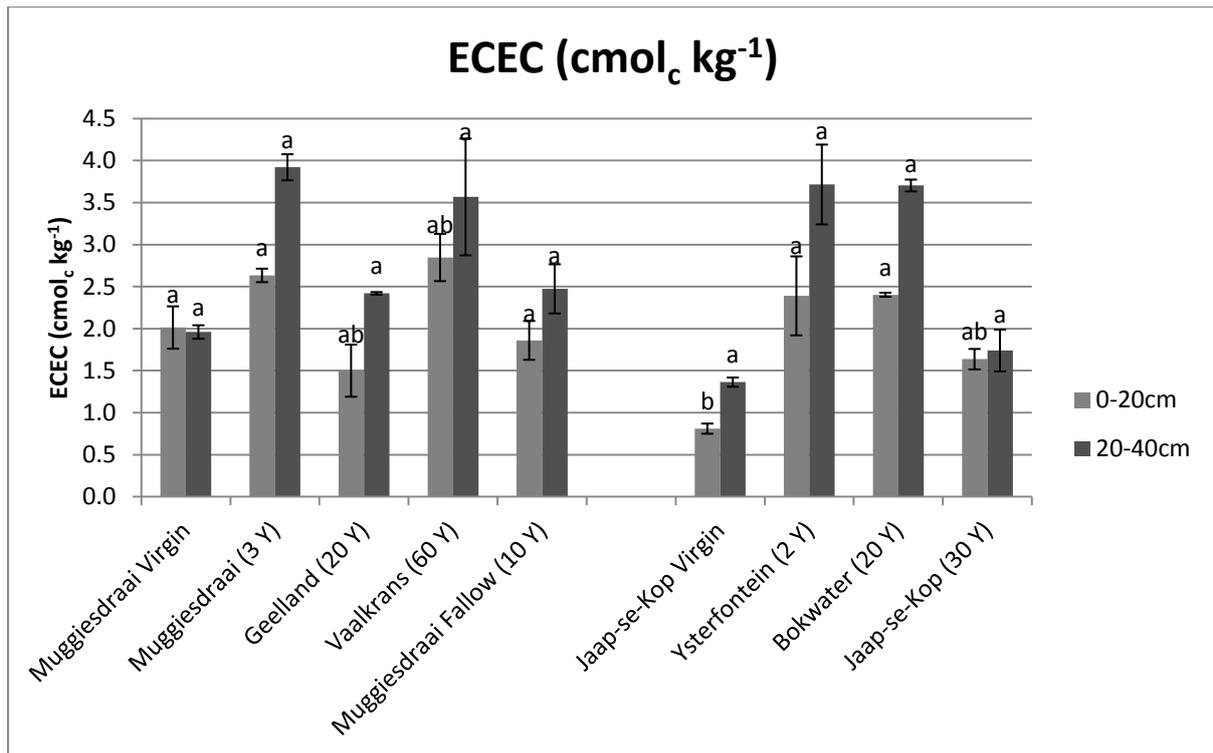


Figure 3-23: Effective cation exchange capacity (ECEC) (cmol_c kg⁻¹) at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The ECEC values in all the experimental sites are extremely low, with values ranging from 0.6 to 4.0 cmol_c kg⁻¹. These low values can be expected in these soils. All of the experimental sites had very low clay content (**Table 3-3**) and a high sand content. The total soil carbon was also extremely low (**Figure 3-12**), with values of less than 0.1 %. Since the clay and organic matter content is low, there are less exchange sites and this resulted in low CEC values. The decrease in the ECEC related to the decrease in soil carbon. Similar to the soil carbon, the ECEC decreased with an increase in the age of the experimental sites (**Figure 3-23**). Significant differences in the ECEC was observed between the different experimental sites in the 0-20 cm (P = 0.0008) layer, but not in the 20-40 cm (P = 0.4453) layer.

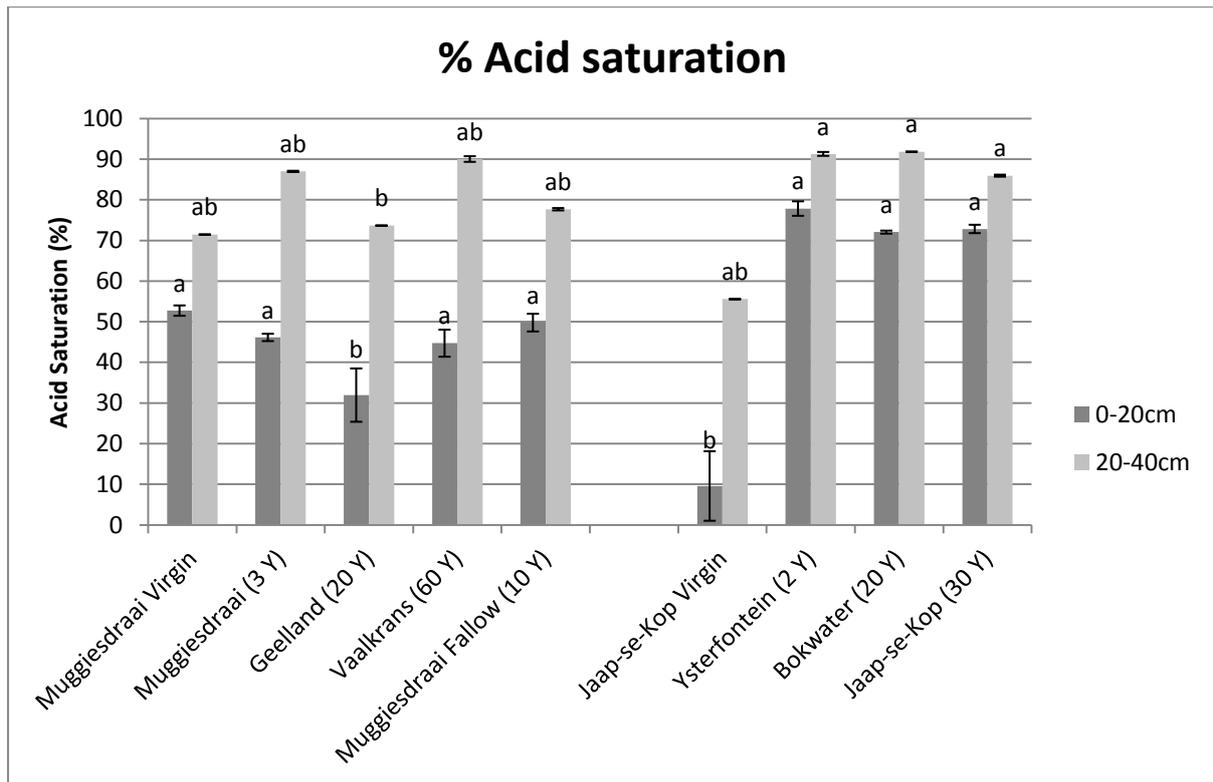


Figure 3-24: Percentage Acid saturation at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

There was not much variation in the % acid saturation in Nardouwsberg area virgin and cultivated soils. However, in the Seekoeivlei area, there appeared to be a large increase in acid saturation from the virgin site to the cultivated sites (**Figure 3-24**). The reason for the high acid saturation is due to a loss of carbon and basic cations through “mining” without addition of litter or plant material to the soil. The higher acid saturation in the Seekoeivlei area can be ascribed to the fact that the basic cations were leached from the soil more easily than in the Nardouwsberg area. The reason for this being that the Nardouwsberg area has a lower rainfall than the Seekoeivlei area and a relic plinthic layer to prevent the water from draining from the soil easily. There is a significant difference in acid saturation between the different experimental sites in both the 0-20 cm ($P = 0.0003$) and 20-40 cm ($P = 0.0019$) layers.

3.4.2.7 Macronutrients (N, P, S)

Nitrogen (N)

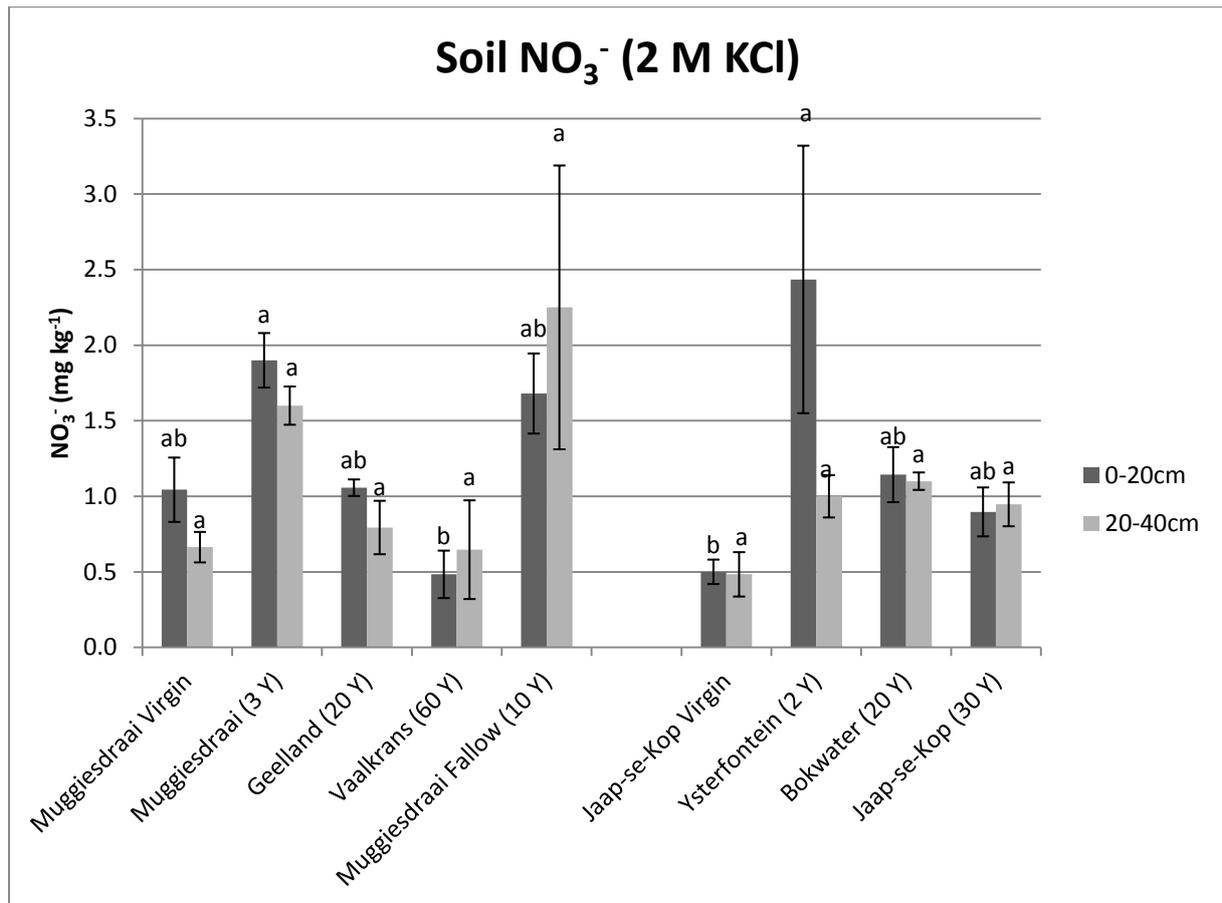


Figure 3-25: Nitrate in the soil at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

No significant trend was observed between the amount of nitrate (NO₃⁻) and the age of the experimental site (**Figure 3-25**). The average values found in the different experimental sites differs between 0.5 - 2.5 mg kg⁻¹ and correlates well with the 2 mg kg⁻¹ for typical levels natural fynbos areas (Hawkins *et al.*, 2005). The amount of NO₃⁻ in the soil is an indication of the amount present in the soil during analysis. The NO₃⁻ levels in the soil change constantly. There was a significant difference in the 0-20 cm layer in between experimental sites (P = 0.0054), but no significant differences in the 20-40 cm layer (P = 0.0761). No definite trends were observed in both the Nardouwsberg and Seekoeivlei area.

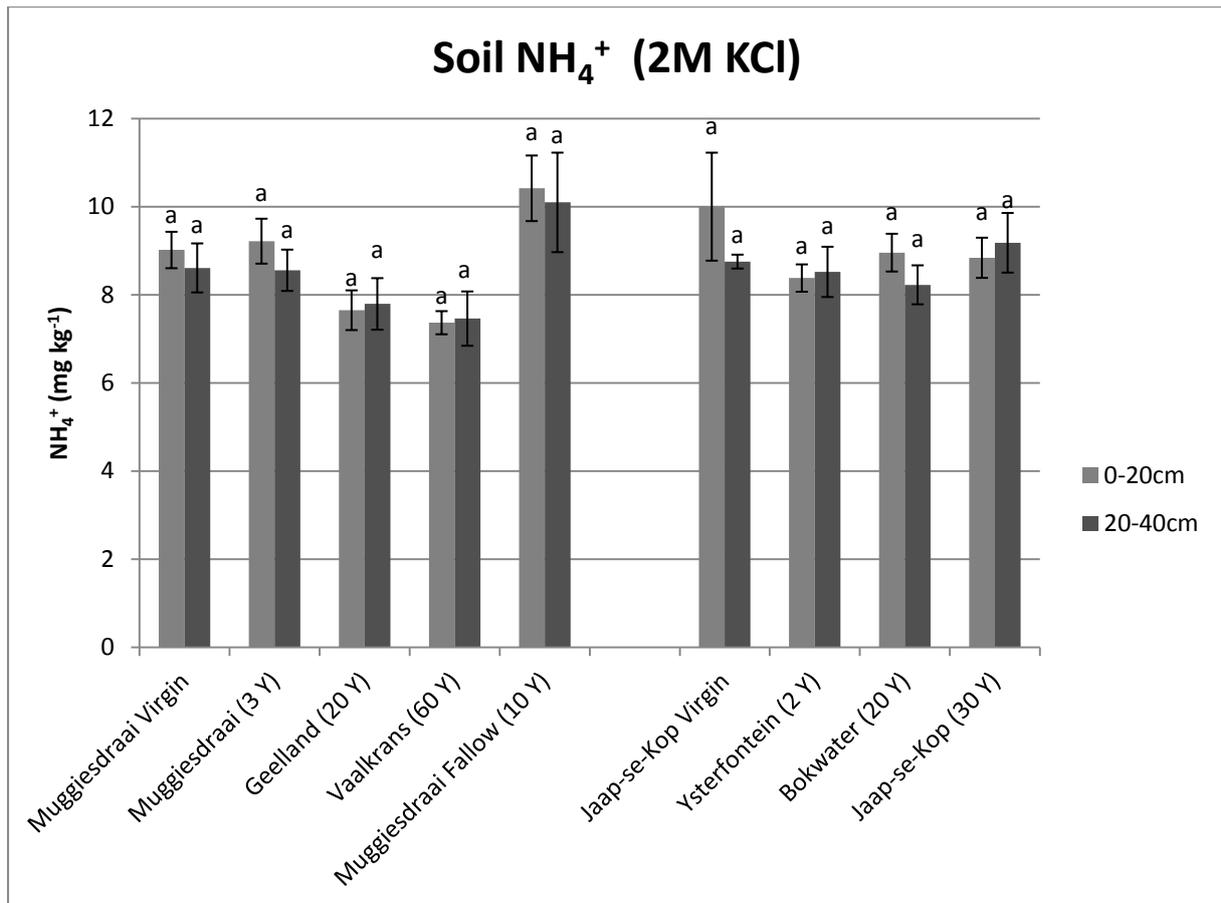


Figure 3-26: Ammonium in the soil at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

No significant trends were observed between the amount of ammonium (NH₄⁺) and the age of the experimental site. The average values found in the different experimental sites differed between 7 - 10 mg kg⁻¹ and correlated well with the 13-27 mg kg⁻¹ found by (Hawkins, et al., 2005) for typical levels natural fynbos areas. It appears that the ammonium found in the rooibos soils were less than the ammonium found in a typical natural fynbos areas. The amount of ammonium in the soil is only an indication of the amount present in the soil during the analysis. There are also no significant differences in the soil NH₄⁺ between experimental sites in the 0-20 cm (P = 0.0768) and 20-40 cm (P = 0.1478) layer.

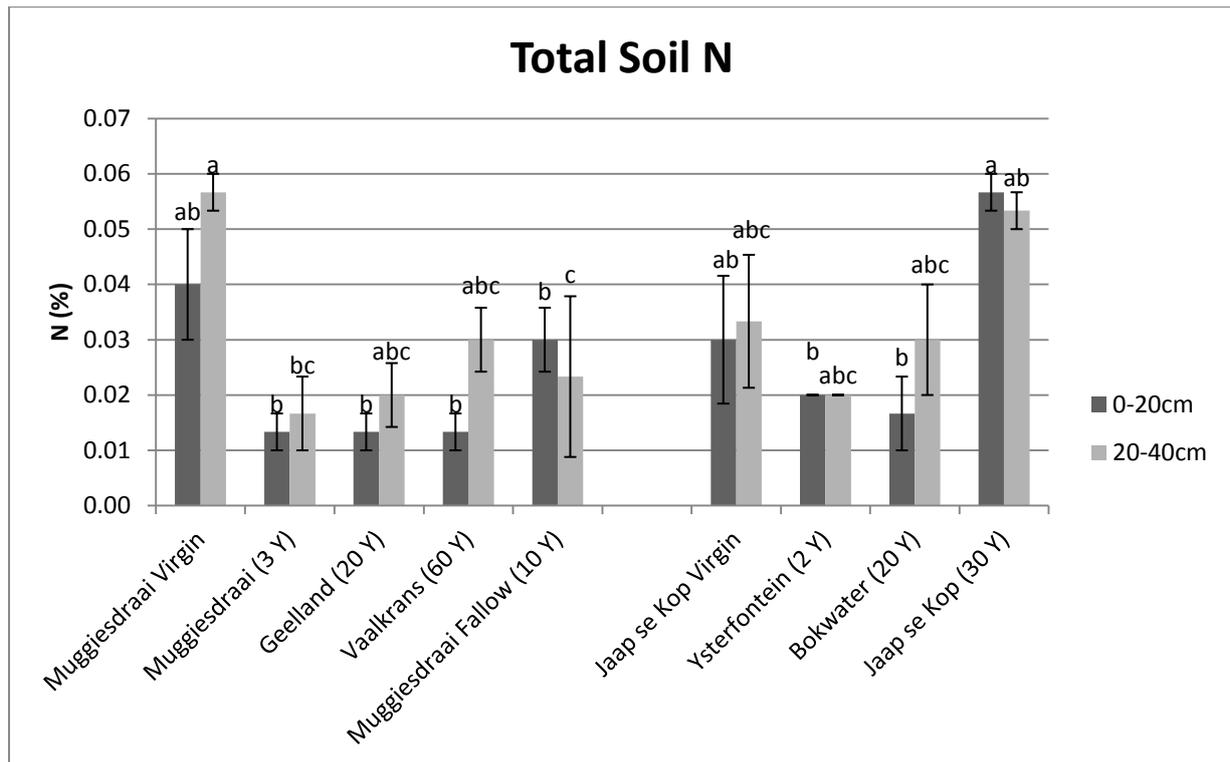


Figure 3-27: Total Soil N at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

There is a significant difference in total N between the different experimental sites in both the 0-20 cm ($P = 0.0020$) and the 20-40 cm ($P = 0.0298$) layer and big differences were noted in the Nardouwsberg (Muggiesdraai virgin) and Seekoeivlei (Jaap-se-Kop) experimental sites (**Figure 3-27**). As Jaap-se-Kop (30 Y) did not receive any N fertilizer and the rotation crop did not consist of legumes, the higher N content can possibly be due to the fact that the rooibos increased the N during the previous cycle since a high N-nodule count was found in this specific site (will be described in **chapter 4**) and the rotation crop (oats) was unable to reduce the high N levels in the soil.

Plant available phosphorus (Bray II)

Significant differences in Bray II P between the different experimental sites in both the 0-20 cm ($P < 0.0001$) and the 20-40 cm ($P < 0.0001$) layer were observed (**Figure 3-28**).

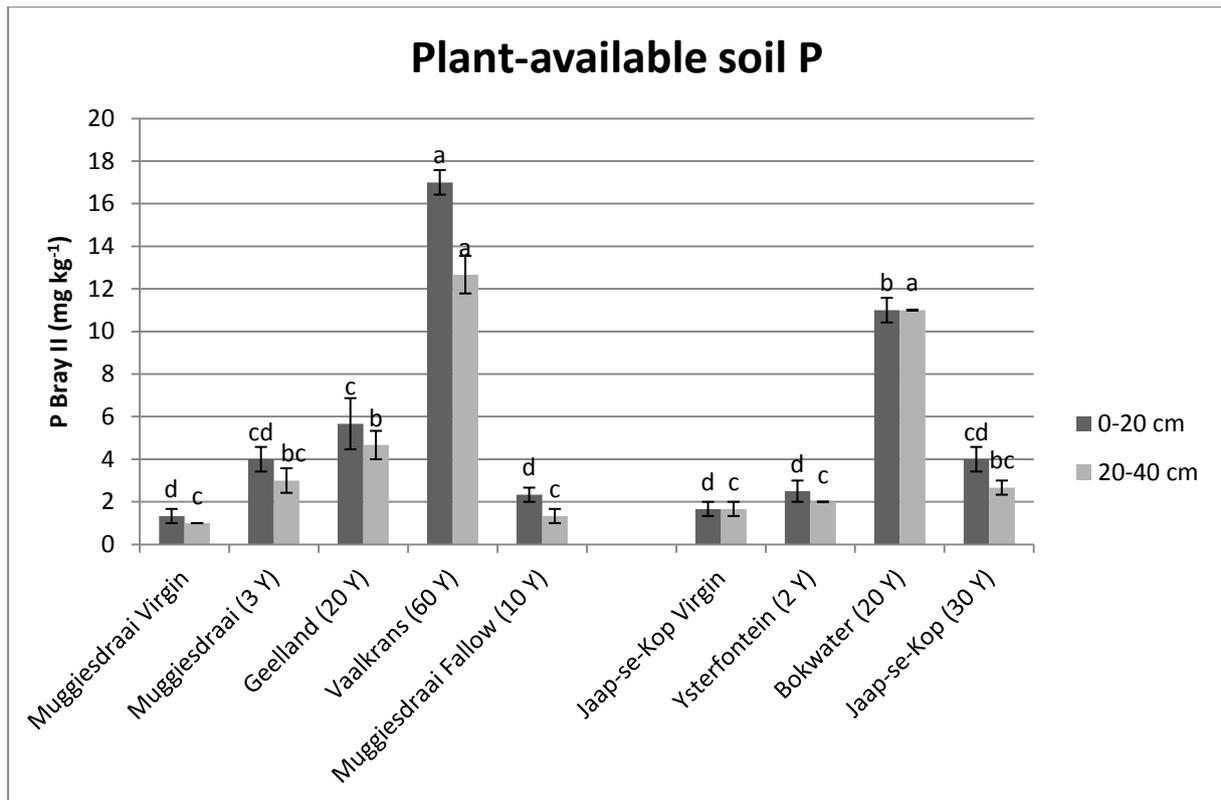


Figure 3-28: Plant-available P Bray II at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The virgin sites, as well as the sites that have been planted for the first time since the natural vegetation have been removed, had a very low plant-available P (below 2 mg kg⁻¹). Most of the P values are very low (6 mg kg⁻¹ or less). These values correlate well with the 0.8-8 mg kg⁻¹ Bray II found in nutrient low natural fynbos soils (Witkowski & Mitchell, 1987). The values are lower than the 11 mg kg⁻¹ (Bray II) values found by in Honeybush soils (Joubert *et al.*, 2007). The highest P values were found in the Vaalkrans and Bokwater sites. The high P value in the Vaalkrans site is attributed to the rock phosphate applied in 2007. The Bokwater site also received P fertilizer in previous years. A definite trend was observed in both the Nardouwsberg and Seekoeivlei area namely P in the soil increases with an increase in the age of the experimental site. The reason for the increase is due to the fact that the farmers were advised (by fertilizer salespersons) in the past to apply P to increase the plant production (personal communication). Phosphate is a highly reactive element forming highly insoluble precipitates with Fe, Al and Ca, and persists for decades in the soil resulting in high P levels (Marschner, 1995).

The Bray II P values are significantly lower than the threshold value of 25 mg kg⁻¹ P known for healthy crop development. The rooibos plants are adapted to growing in acidic, low P soils. Soil P is most soluble (plant-available) at a pH between 5.5 and 6.5. The pH of these soils are well below that, but Muofhe & Dakora (2000) indicated that the pH in the rooibos rhizosphere increases due to the rooibos root activity and symbiotic bacterial activity, which could also enhance P availability. The rooibos have cluster that helps the rooibos plant with the uptake of P in these low phosphorus soils (Lambers *et. al.*, 2006). Therefore P toxicity can easily be experienced by fynbos plants since it is adapted to survive in these low P soils (Leake, 1993) and excess P is available in older cultivated sites.

Sulphur (S)

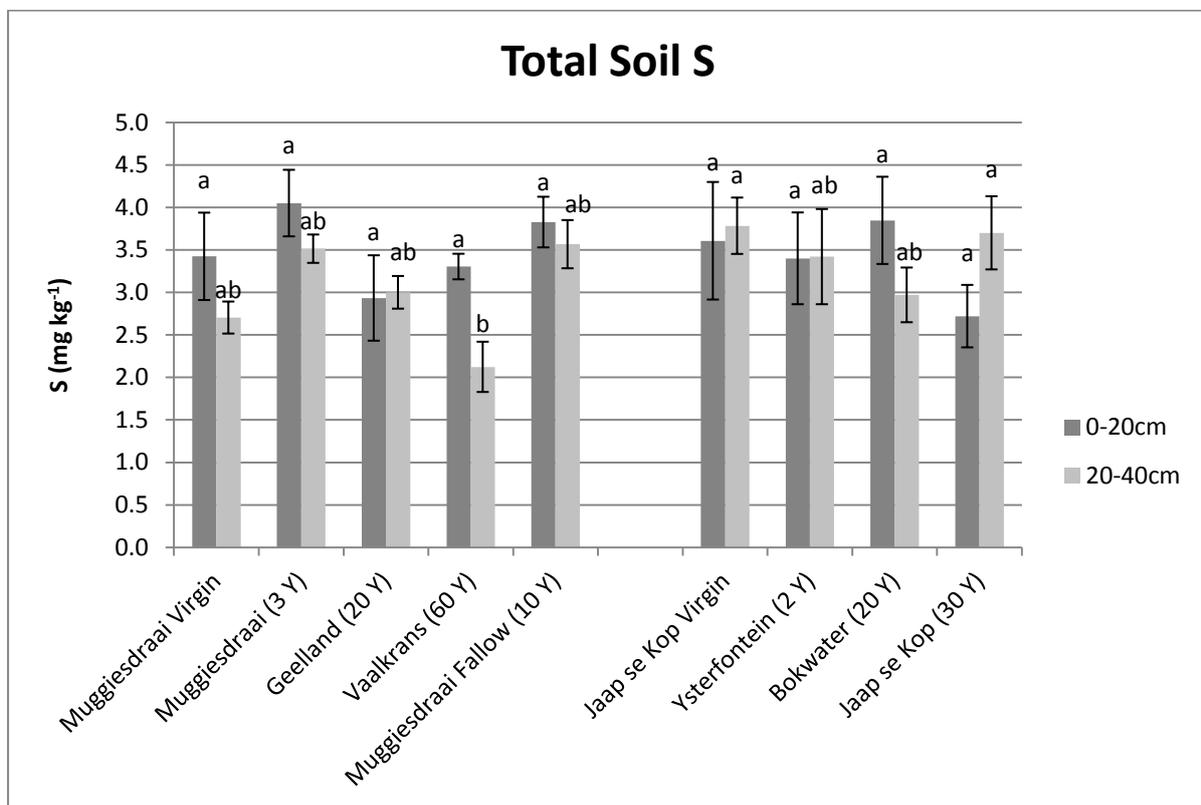


Figure 3-29: Total soil S at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

There are no significant statistical differences in S between the different experimental sites in the 0-20 cm (P = 0.5576) layer, but differences were found in the 20-40 cm (P = 0.0152)

layer (Figure 3-29). The S content in the soils ranged between 2 and 4 mg kg⁻¹. The subsoil S content at the oldest cultivated site, Vaalkrans, was lower than the other sites.

3.4.2.8 Micro nutrients (Cu, Zn, Fe, Mn, B)

Copper (Cu)

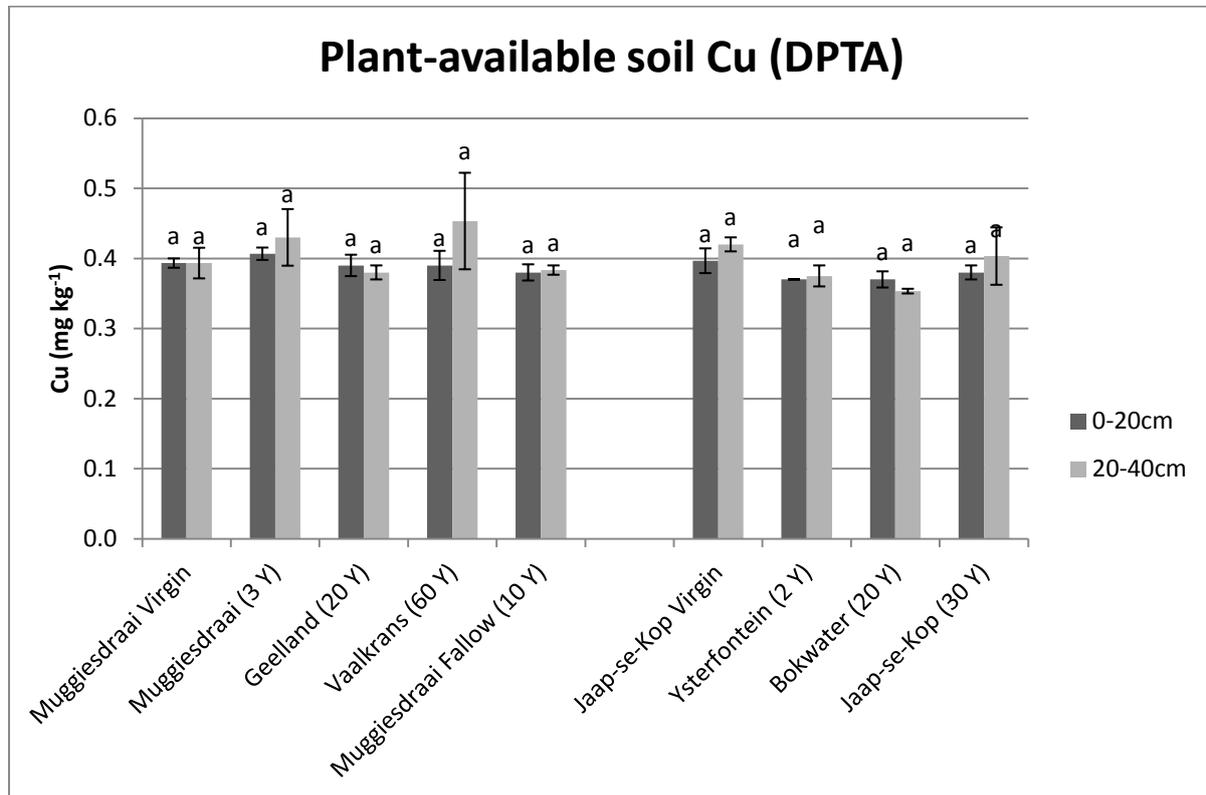


Figure 3-30: Plant-available soil Cu contents at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

There were no significant differences in the plant available Cu content between experimental sites in both the 0-20 cm (P = 0.8699) and 20-40 cm (P = 0.6662) layers. The Cu found at the different experimental sites (0.3-0.5 mg kg⁻¹) was slightly higher than the 0.1 mg kg⁻¹ reported by Joubert *et al.*, 2007 for honeybush soils.

Zinc (Zn)

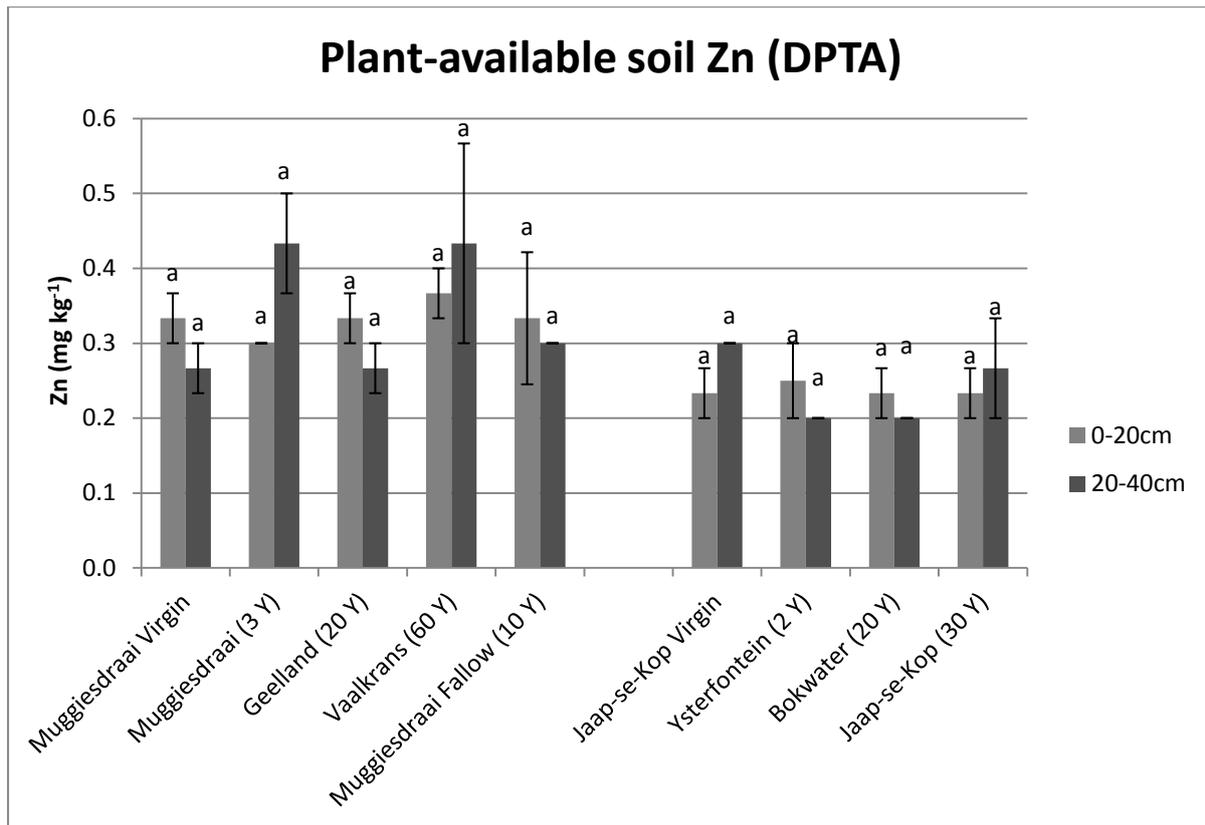


Figure 3-31: Plant-available soil Zn contents at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The average Zn contents found at the different experimental sites were extremely low (**Figure 3-31**) in comparison to the values found in honeybush tea soils (Joubert *et al.*, 2007). The average Zn values found in the different experimental sites ranged between 0.2-0.5 mg kg⁻¹ (**Figure 3-31**) while average values of 10.3 mg kg⁻¹ were reported by Joubert *et al.*, 2007. The reason for this can be that the soils taken from the flood plains of the Berg River have a Zn deposit from a Zn source in the mountains.

The Zn in the Seekoeivlei area doesn't differ much between experimental sites (**Figure 3-31**). There are also no significant differences in the Zn content between the different experimental sites in the 0-20 cm (P = 0.2401) and 20-40 cm (P = 0.1224) layers. In the Nardouwsberg area, bigger differences were observed between the different experimental sites. It also appears that soils in the Nardouwsberg area have a higher Zn content than the Seekoeivlei area.

Iron (Fe)

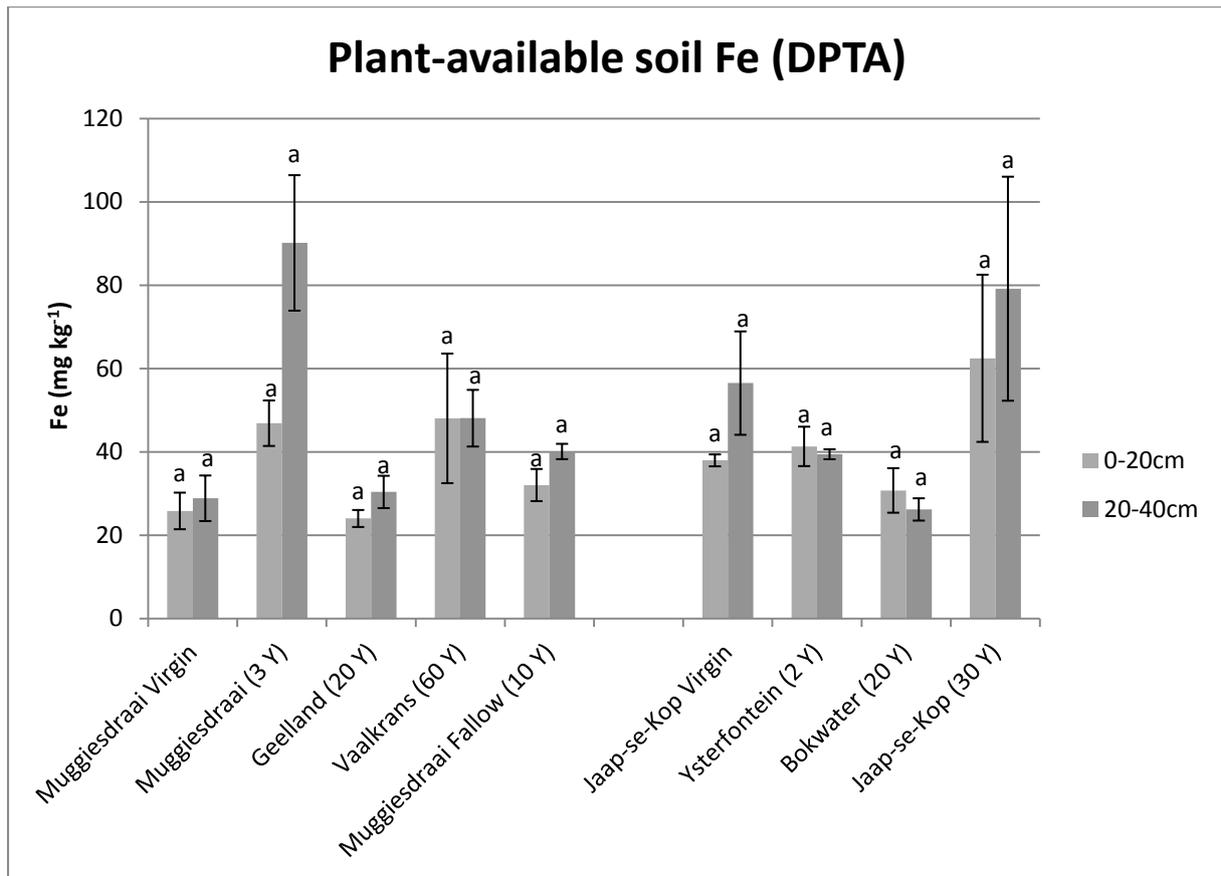


Figure 3-32: Plant-available soil Fe contents at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

No significant differences in plant-available Fe were observed between experimental sites in the 0-20 cm ($P = 0.1722$) and 20-40 cm ($P = 0.1510$) layer (**Figure 3-32**). A weak correlation was observed between the CBD-extractable Fe in the 0-20 cm ($R^2=0.4125$) and 20-40 cm ($R^2=0.6408$) layer (data not shown). The plant available Fe content was higher in soils with a higher CBD extractable Fe content in the soil (**Figure 3-10**) therefore the correlation found was rather with the CBD extractable Fe in the soil than with the age of the experimental site. The amount of plant available Fe in the soil appears to be more dependent on the area, rather than the age of the site.

Manganese (Mn)

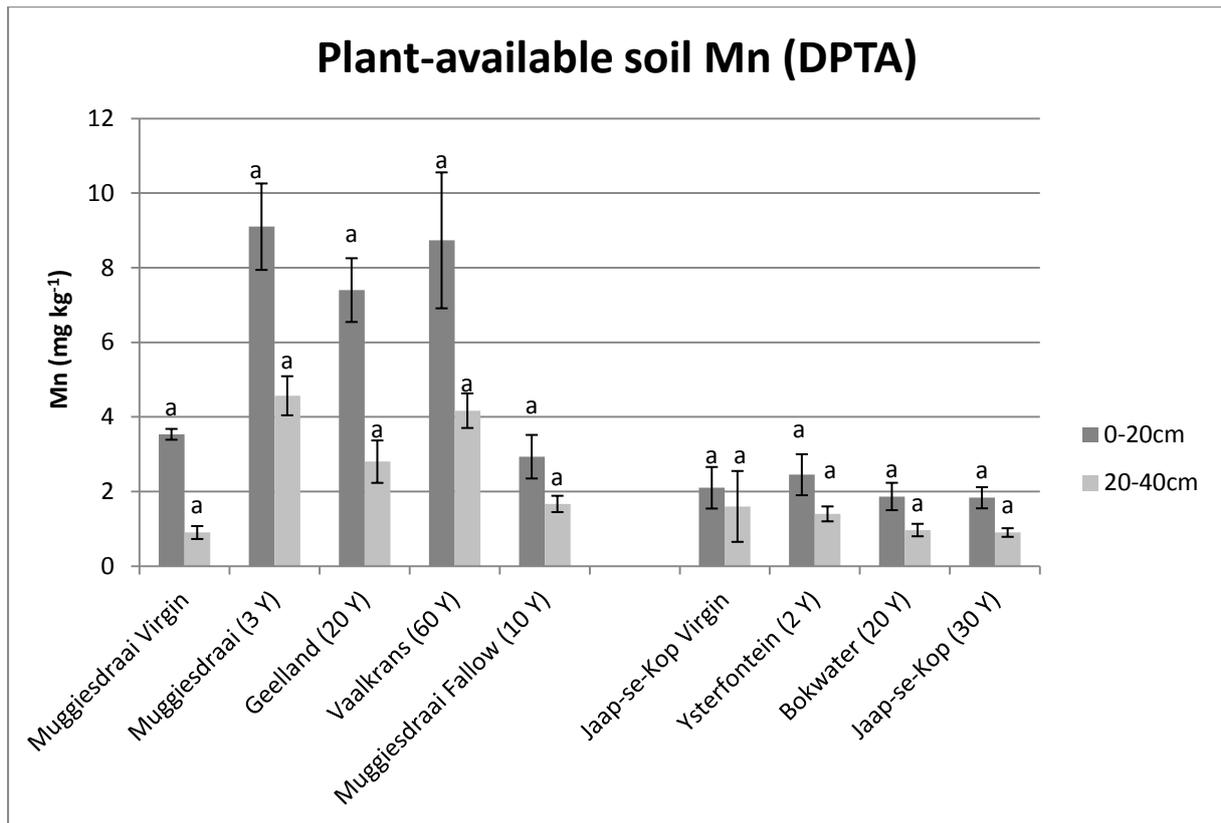


Figure 3-33: Plant-available soil Mn contents at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The average plant-available Mn contents determined in the experimental sites (**Figure 3-33**) are similar to the values reported for honeybush tea soils (Joubert *et al.*, 2007). The average manganese values observed in the experimental sites ranged between 0.1 and 9.1 mg kg⁻¹ (**Figure 3-33**) while average values of 1.3 mg kg⁻¹ were reported by Joubert *et al.*, 2007.

The soil Mn content is on average higher in the Nardouwsberg area than in the Seekoeivlei area (**Figure 3-33**). The virgin sites as well as the fallow site have a low Mn content. The amount of Mn in the soil appears to be dependent on the area, rather than the age of the site. A strong correlation was found between the CBD Mn oxides (**Figure 3-11**) and the plant available Mn in both the 0-20 cm ($R^2=0.7293$) and the 20-40 cm ($R^2=0.7751$) layer (data not shown). The plant available Mn increased with an increase in the Mn oxides in the soil. There were no significant statistical differences in the plant available Mn content between the different experimental sites in the 0-20 cm ($P = 0.0909$) and 20-40 cm ($P = 0.3464$) layer.

No significant statistical differences in the plant available Mn content were noted between the different experimental sites in the 0-20 cm ($P = 0.0348$) and 20-40 cm ($P = 0.4665$) layer.

Boron (B)

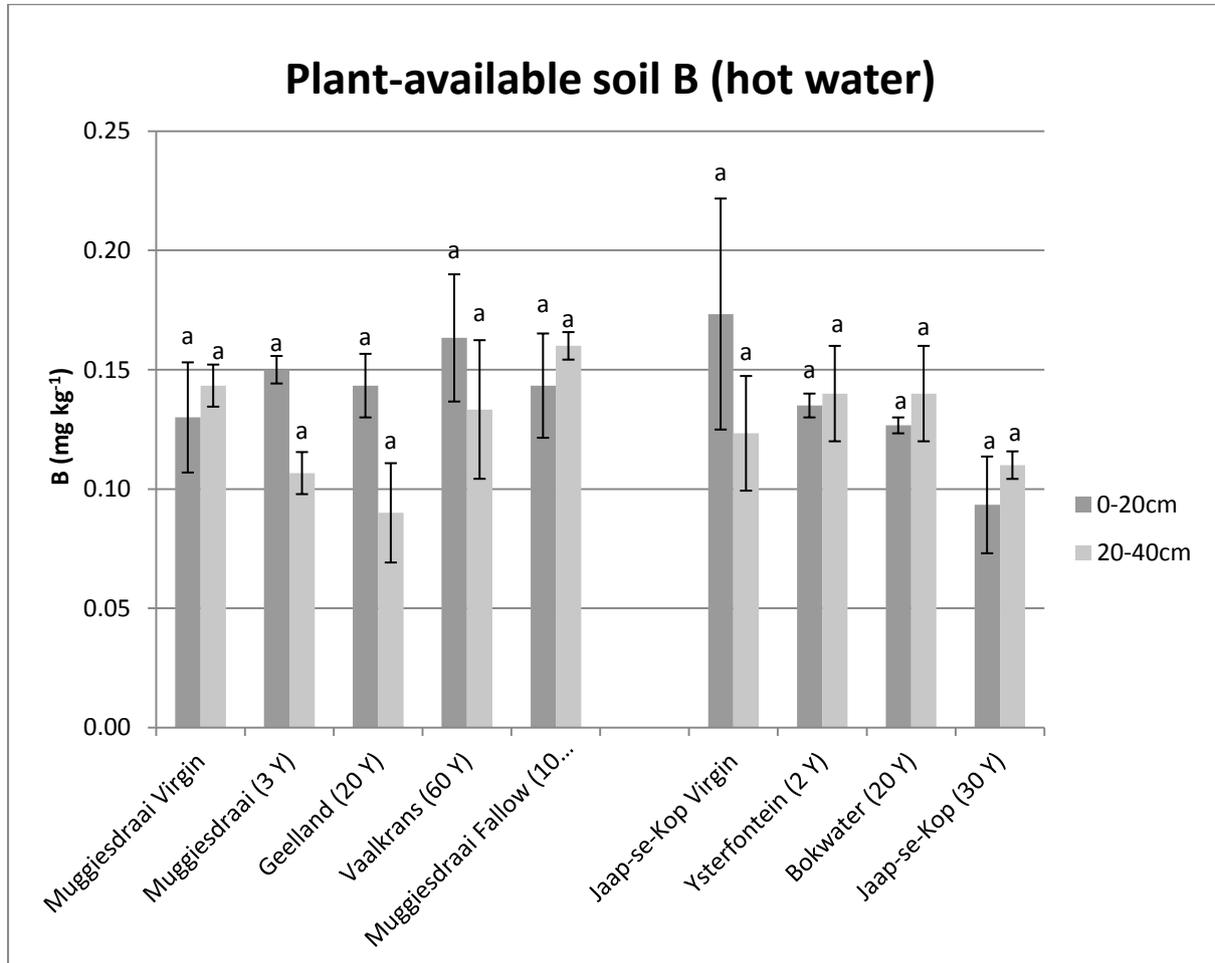


Figure 3-34: Plant available B in the soil at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

There were no significant statistical differences in the B content between the different experimental sites in the 0-20 cm ($P = 0.3195$) and 20-40 cm ($P = 0.1668$) layers. The average boron values observed in the current study (0.05-0.2 mg kg⁻¹) (**Figure 3-34**) are similar to the average values (0.2 mg kg⁻¹) that were found in honeybush tea soils by (Joubert *et al.*, 2007).

3.4.3 Soil microbial determinations

3.4.3.1 Protist counts

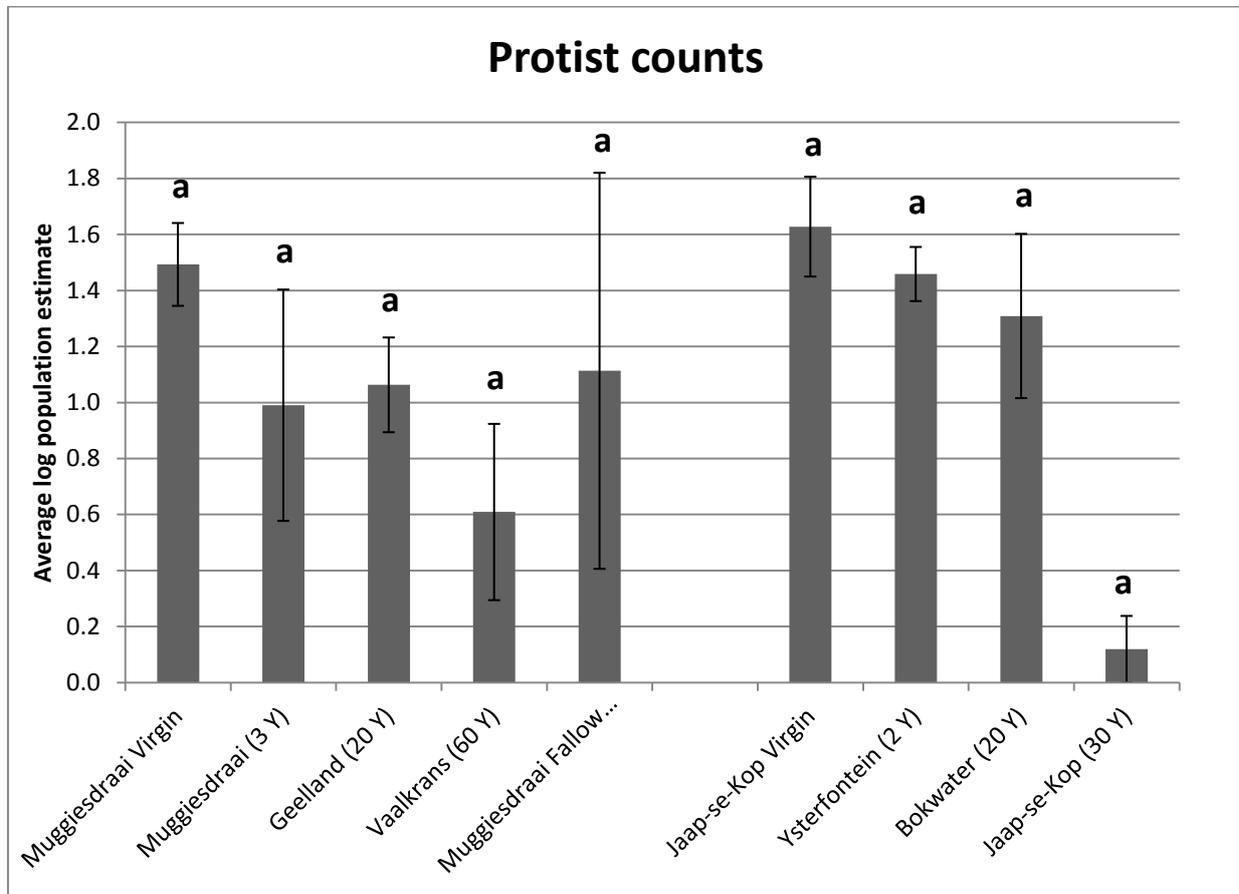


Figure 3-35: Protist population at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The protist numbers in the soil can be used as an indicator for organic matter breakdown, and is thus an indicator of the extent of nutrient cycling occurring in the soil (Couteaux & Darbyshire, 1997). The protist counts were on average higher in the virgin soils than in the cultivated soils (**Figure 3-35**), however, the differences are not significant ($P = 0.0793$). Ysterfontein that was planted for the first time after the natural fynbos was removed had a much higher protist population than Vaalkrans and Jaap-se-Kop that was planted for more than 20 years. The virgin soils (Muggiesdraai and Ysterfontein) have a high protist population. This might be attributed to the fact that there is more organic material in the soil and therefore more biomass that is delivered back to the soil resulting in a higher content than those in the fields that have only rooibos plants. The standard error in the

Muggiesdraai and Muggiesdraai Fallow soils is very high. In both cases a protist hot spot was possibly sampled in one of the 3 replicas, which resulted in a high standard error. A definite trend can be seen in both the Nardouwsberg and the Seekoeivlei area. There is a decrease in the protist numbers in the soil, with an increase in the number of years that the field have been used for the cultivation of rooibos. The same trend was observed for soil carbon (Figure 3-12).

3.4.3.2 Total microbial biomass

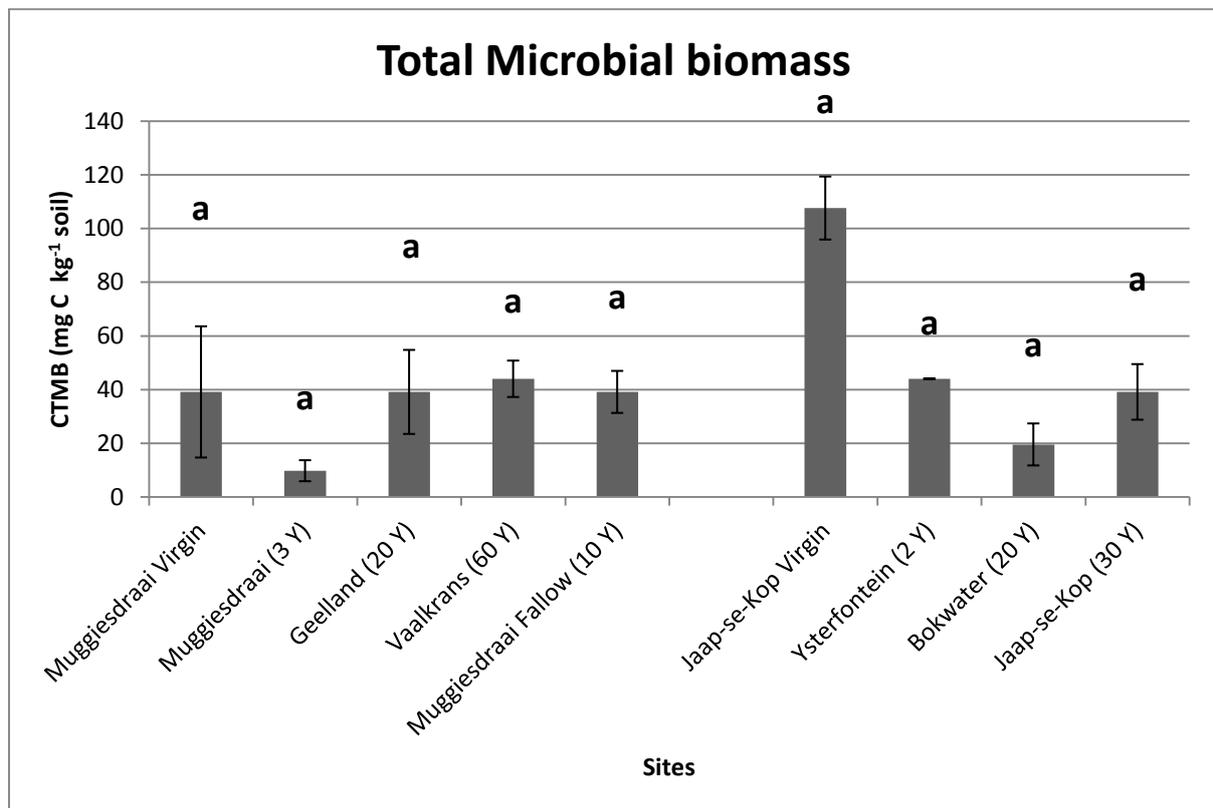


Figure 3-36: Total microbial biomass at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

As could be expected, the total microbial biomass C was higher on the virgin soils than on the cultivated sites (Figure 3-36) in the Seekoeivlei area. The Ysterfontein site on which rooibos have been planted for the first time since the natural vegetation was removed also had a higher microbial biomass with an amount of about 100 mg C kg⁻¹ soil. It also has a very small standard error. The older sites such as Vaalkrans, Geelland, Jaap-se-Kop and Bokwater have small values, lower than 60 mg C kg⁻¹ soil. The reason why the microbial biomass is higher at the virgin sites and the Ysterfontein site is probably due to the fact that more

biomass is returned annually to the virgin sites but not to the older site, thus increasing the microbes in the soil. The reason for the low microbial biomass value in the Muggiesdraai site is unclear. There is a definite downwards trend in the experimental sites with an increase in the number of years that the field have been used for the production of rooibos tea. There is no significant statistical differences between the different experimental sites ($P = 0.0942$) due to the big standard errors.

3.5 Conclusions

Fernwood soil forms were found mainly in the Seekoeivlei area, whereas, the Cartref soil forms were found more in the Nardouwsberg area which is located at a higher altitude above sea level and in a more mountainous area. The lithocutanic layer in the Cartref soil form is a relic plinthic rock, and these soils can thus be described as a transition Wasbank. These relic plinthic rocks were formed under different climatic conditions and are now part of the weathering mother material. A higher CBD Iron content was noticed in the sites situated on a relic plinthic layer since it contains more Fe. Soils in both areas were predominantly classified texturally as course sands, containing between 1-3 % clay.

The Nardouwsberg area soils generally had a higher coarse sand content and higher bulk density values than the Seekoeivlei area. An increase in the subsoil bulk density was observed in the Seekoeivlei area with an increase in the age of the experimental site which could be attributed to compaction due to tillage practices. However, none of the bulk densities exceeded 1.68 which lies below the threshold value for compaction in sandy soils of 1.8 g cm^{-3} ; therefore no root restriction was found and cannot be the reason for the decrease in tea yields.

The soil carbon contents were very low (0.1-0.3 %), which is typical for sandy fynbos soils. The soil carbon decreased with an increase in the number of years the field have been used for the cultivation of rooibos tea. This was attributed to the fact that in the rooibos fields the soil surface is exposed to the sun and that there is a decrease in the total amount of organic biomass that is returned to the soil annually since about two-thirds the plant is harvested.

The decrease in organic matter had a number of effects on the soil physical, chemical and microbiological properties. The chemical properties are greatly affected, since the CEC

decreased with a decrease in the organic matter. The soil microbiological properties are negatively affected since the main energy source is being depleted thus resulting in a negative effect on the soil nutrient cycles. A decrease was noted in both the protist populations as well as the total microbial biomass, with an increase in the number of years a certain field have been used for rooibos production. These trends seen in the protist populations as well as the total microbial biomass correlates well with the decrease in the soil organic carbon.

There was a definite decrease in the exchangeable basic cations with an increase in the number of years that the field have been used for rooibos cultivation, with Na, Ca and Mg showing the downwards trend. The K did not show a definite downwards trend. A high acid saturation of up to 90 % was found in some of the experimental sites, due to losses in the exchangeable basic cations through nutrient mining and leaching. These results correlate well with the low pH values found in both H₂O (4.5 – 5.5) and KCl (4.0-4.8). The ECEC also showed a decrease with an increase in the number of years, and is most likely due to the depletion of the organic matter.

A pronounced increase in the P content of the soil was observed in both the 0-20 cm and 20-40 cm layer with an increase in the age of the fields is due to the fact that farmers were encouraged to apply P to the soils. In the oldest cultivated sites the Bray II P content of the soil increased up to 18 mg kg⁻¹ as opposed to the 2 mg kg⁻¹ P content in the virgin fynbos areas where rooibos grew wild. The effect of the higher P content on the plant properties will be looked at in detail in **Chapter 4**.

Continuous cultivation resulted in several changes in soil quality, most significantly: decreased soil C, exchangeable basic cations, soil microbial activity but an increase in plant-available P. The decline in rooibos yields with long-term cultivation can be attributed to a decrease in the soil organic carbon, and concomitant decline in microbial nutrient cycling and CEC. In the next chapter the effects of this change in soil quality parameters on the plant properties will be studied, as well as the effect on the quality of the fermented rooibos tea.

4 RESEARCH CHAPTER 4: THE EFFECT OF LONG-TERM ROOIBOS CULTIVATION ON PLANT PROPERTIES

4.1 Introduction

Little research has been done on the effect of essential plant nutrients on rooibos yields (Strassen, 1987) and the N-nodules on the roots (Muofhe, 1997), while, no work has been done on the effect of nutrients on rooibos tea quality and root mycorrhizal colonization.

Muofhe (1997) indicated that in the presence of high concentrations of N, two year old rooibos plants showed a decline in nodulation and N₂ fixing. Calcium had a negative effect on the growth as well as the N-symbiotic performance of rooibos. Muofhe and Dakora (1999) studied the effects of rooibos plant age on N₂ fixation in the field. Symbiotic parameters revealed a difference between plants that was fertilized and plant that was not (Muofhe & Dakora, 1999). The total N in the 2- and 3-year old plants was greater than those of a 1 year old plant. Plants with an age of 3 years had a higher N content than 2 year old plants but the biomass of the plants was almost the same (Muofhe & Dakora, 1999).

Strassen (1987) determined the amount of nutrients that were lost annually due to tea harvesting based on the assumption that 8000 plants were planted per Ha and produce 1.5 t ha⁻¹ of tea. Plant material of 750 different plants was used from the Citrusdal experimental farm for the analysis of different elements in the plant during different harvesting practices he determined that 27.6 kg N, 1.8 kg P, 6.9 kg K, 6.2 kg Ca, 6.2 kg Mg, 303 g Mn, 818 g Fe, 22 g Cu, 46 g B and 60 g Zn should be applied to the soil to make up for the nutrients lost by tea harvesting.

The amount of N needed can exceed 27.6 kg N per ha, but is difficult to estimate as the rhizobium bacteria has the ability to produce N for the plant (Strassen, 1987). According to Strassen (1987), one application of 25 kg P or 220 kg superphosphate per ha is sufficient for a period of 10 years. An application of 10 kg K or 20 kg KCl per ha is recommended during August. Molybdenum and cobalt levels play an important role in N₂-fixation by legume plants (Smith, 1987). Sulphur also plays an important role in protein syntheses (Smith, 1982). Leaf analyses of two year and three year old plants indicated that only the K and the Fe contents differed significantly.

In this chapter the effect of the soil changes mentioned in the previous chapter on the plant properties will be studied, including macro- and micronutrient uptake, plant size and tea quality. Investigation of the effect of soil nutrient deficiencies or toxicities on plant properties will help with the identification of the plant nutrients that play an important role in rooibos production and thus creating guidelines that may help to increase the sustainability of rooibos production.

4.2 Objective

The main objective is to examine the effect of long-term rooibos cultivation on plant properties (plant size, plant nutrients, root colonization by beneficial microbes, tea yield and fermented tea quality), and to examine the relationship between soil quality and plant quality. This will be done to identify plant factors influencing the production and quality of rooibos tea.

4.3 Methods and materials

4.3.1 Plant sampling

4.3.1.1 Total above- and below-ground biomass

The rooibos plants were destructively sampled in June 2012. This entailed excavating the whole bush with as many roots attached as possible. The above- and below-ground biomasses were then separated at the soil level. The biomass samples were cut into smaller pieces and dried over a period of 2 weeks and the mass was determined. At each of the cultivated 10 x 10 m sites five rooibos bushes were sampled. At the virgin and fallow sites only one bush was sampled at each of the three replicate sites, as wild rooibos plants are relatively scarce and sampling was destructive.

4.3.1.2 Root sampling for microbial colonisation

During the root biomass sampling, the parts of the roots with the most active growth were taken for determining the extent of mycorrhizal colonization. The younger, actively growing roots or roots with a “cluster” on it (**Figure 4-1**) were sampled for mycorrhizal colonization determination. The fresh roots were immediately placed in a 250 mL autoclaved bottle with 50 % ethanol and stored at room temperature to be analysed at a later stage in the lab. The roots can be stored in ethanol for a long time before it has to be analyzed. The tips of the roots were taken from five different bushes to ensure that it is a composite sample.



Figure 4-1: Cluster roots at the Ysterfontein experimental site in the Seekoeivlei area

4.3.2 Plant analyses

Total above-ground biomass nutrients

According to Strassen (1987) February is the best time for foliar sampling since the foliar concentration of elements are at their threshold values, but the samples in this project were taken in June in order to compare the foliar samples with the soil samples. Ten grams of the dried and chopped above-ground biomass plant samples from each site were taken and finely ground, and then total macro- and micronutrients were determined using Kjeldahl method (N), and acid digestion and ICP-MS (P, Ca, Mg, K, Na, Fe, B, Zn, Mn, Cu, Al & S).

Tea yield

The rooibos tea yield was calculated by weighing the total amount of dry tea produced at the end of the first year (Sept. 2012) and in the beginning of the second year (Feb. 2013)

and multiplying it by the area that it covered in order to work out the yield per hectare. The plants were harvested in the traditional way using sickles (**Figure 4-2**).



Figure 4-2 : Rooibos harvested in the 10x10 m experimental site on the farm Ysterfontein in (Feb, 2013).

Mycorrhiza counts

The ethanol-preserved roots were washed with water to remove sand particles. The roots were then cleared by autoclaving for 15 min in a 250 mL bottle filled with 10 % KOH. After the roots were autoclaved it was washed again with water (Brundrett, 1994a. The roots were then cut into 1 cm pieces, placed in a bottle, and Chlorazol black E (CBE) was added until the roots were completely covered with the solution. The bottles with roots were placed inside the oven at 90° C for one hour. This process was done to stain the roots. After 1 hour the bottles were removed from the oven, the CBE solution was filtered off, and the roots were washed again with distilled water. The roots were placed in a bottle and 50% glycerol was added to destain for 10 days at room temperature. Ten 1 cm root pieces were placed on a slide were studied under a microscope at the 40x magnification in order to do the mycorrhiza count (**Figure 4-3**) (Brundrett, Melville, & Peterson, 1994b).



Figure 4-3: Root samples placed on plates to be studied under microscope for Mycorrhiza colonization

N-nodule counts

The roots of each of the five bushes that were dug out per site were studied. The tap roots, as well as, the side-roots were looked at, as the N-nodules can be found in the top part of the soil (0-20 cm) (**Figure 4-4** and **Figure 4-5**). At each site, the percentage of bushes with nodules on the tap root was calculated. The number of side-roots with nodulation were counted and divided into 3 groups, namely: 0-9, 10-20 and above 20 roots with nodules. A numeric value was assigned to each of these groups: 0-9 was given a 2; 10-20 was given a 3 and above 20 was given a 5. The percentage were the worked out. At the end the percentage of each of the side roots as well as the tap root was used to work out an overall percentage.



Figure 4-4: Root samples at the Muggiesdraai experimental site (M1)



Figure 4-5: Muggiesdraai experimental site (M1) (Nodules on the tap and side roots)

4.3.3 Tea Quality

The rooibos was harvested in September 2012 and February 2013. Only the cultivated sites (Muggiesdraai, Vaalkrans, Geelland, Ysterfontein, Bokwater and Jaap-se-kop) were harvested. The rooibos tea was chopped into 3 mm pieces after harvesting. Afterwards it was then placed in small heaps (**Figure 4-6**) and water was added to saturate it to about 60 % by weighing the tea and according to its weight water was added (250 mL per kg rooibos tea) to help with the fermentation process. The wet heaps were then bruised with a hammer to start the fermentation process and left for 10 hours overnight to ferment. The next day, the fermented heaps were spread out to dry. The quality and yield of the dry tea was determined by sending it to Rooibos Ltd in Clanwilliam. An independent panel judged the different aspects and thus resulting in the determination of the different sensory qualities of the fermented tea. The panel consists out of 3 – 4 members and each of the members has experience in judging the overall fermented tea quality for at least 4 years.



Figure 4-6: Rooibos heaps busy fermenting for the different experimental sites

The quality of the tea was determined by a panel at Rooibos Ltd in Clanwilliam looking at 4 sensory aspects; the dry colour, the wet colour, the extract colour and the taste. The dry colour was determined by placing 12 g of the sieved sample into a Petri-dish. The Petri-dish was placed under a light and the dry colour was observed and evaluated with a mark out of 10. The wet colours, extract colour en taste was determined by weighing 5.8 g of the sieved sample and placing it into a cup and adding 280 – 300 ml of boil distilled water. It was left for 5 min. Afterwards the wet tea leaves was placed on a separate glass holder and the extract was left in the cup. The wet colour was evaluated under the light by giving it a mark out of 10. The extract colour was evaluated under the light and given a point out of 10. The extract was tasted and evaluated by giving it a mark out of 10. (Figure 4-7)



Figure 4-7: Determining the rooibos tea quality (Dry colour, Wet colour, Extract colour and taste) of the Ysterfontein experimental sites.

4.3.4 Statistical analysis

The statistical analysis was performed using SAS enterprise guide 5.1. The data was tested for significant statistical differences with a 95% confidence interval between all the different

experimental sites in both the Nardouwsberg as well as the Seekoeivlei area using the Tukey standardized t-test. The correlation between the plant and fermented tea quality parameters and the different soil (chemical, physical and microbiological) and plant nutrient parameters were done using Marlow's CP test. The statistical data are presented in (Appendix D).

4.4 Results and discussion

4.4.1 Plant analysis

4.4.1.1 Total above and below-ground biomass yield

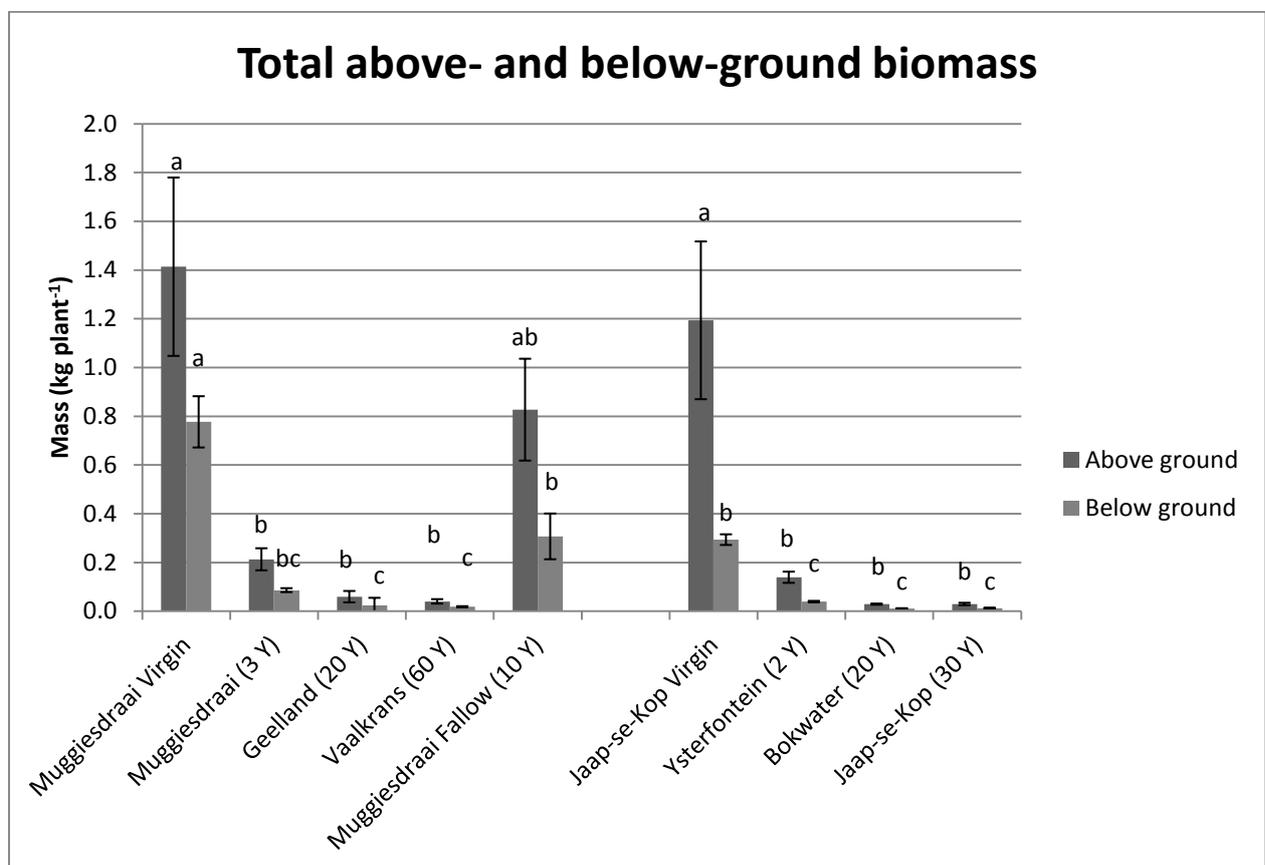


Figure 4-8: Total above and below-ground biomass at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The rooibos plants at the cultivated sites were much smaller than the wild plants (<0.2 kg), which is most likely due to the fact that the wild plants were older (**Figure 4-8**). The wild rooibos plants also had less leaves but more stems and twigs (personal observation). In the case of the Muggiesdraai site (M1, M2 and M3); 2-year old plants were sampled not 1-year

old plants like the rest of the cultivated sites e.g. Vaalkrans, Geelland, Ysterfontein, Jaap-se-Kop and Bokwater. The different experimental sites for the above- and below-ground biomass differ significantly for both the above-ground biomass ($P = 0.002$) and below ground biomass ($P < 0.001$). The recently cleared fynbos sites, Ysterfontein and Muggiesdraai, also showed a higher biomass than the rest of the cultivated sites.

A definite decrease in the biomass could be noted with an increase in the number of years the field have been used for the production of rooibos tea (**Figure 4-8**). The decrease in the plant size can likely be due to the changes in the soil quality (soil organic carbon, microbial cycling, accumulation of P, and depletion of certain nutrients) described in **Chapter 3**.

A positive correlation was noted between the soil carbon in the 0-20 cm layer and the above-ground biomass of the rooibos plants in the cultivated sites ($R^2 = 0.5843$) (**Figure 4-9**). The increase in soil organic matter had a positive effect on plant growth (**Figure 4-9**) possibly by improving the structure of the soil, the water holding capacity, cation exchange capacity and is a source of nutrients (**Chapter 3**).

A negative correlation was found between soil Bray II extractable P and plant above-ground biomass (**Figure 4-10**). An exponential decrease in the growth with an increase in Bray II extractable P was found in both the 0-20 cm ($R^2 = 0.417$) (**Figure 4-10**) and the 20-40 cm ($R^2 = 0.449$) (**Appendix B**) layers for all experimental sites. A weaker exponential correlation was found between the above-ground biomass and Bray II P in the 0-20 cm layer for the cultivated sites ($R^2 = 0.1819$) (**Figure 4-11**). Joubert *et al.* (1987) found that optimum growth of rooibos seedlings under greenhouse conditions was achieved at a soil concentration of 15 – 20 mg Bray II extractable P per kg. The seedling growth significantly decreased if the P concentration became too high ($>30 \text{ mg kg}^{-1}$). The amount of Bray II P found in the soils during this study was significantly lower ($2\text{-}18 \text{ mg kg}^{-1}$) than the amount of Bray II P applied during the pot trials of Joubert *et al.* (1987).

4.4.1.2 Correlations between plant analysis and soil quality

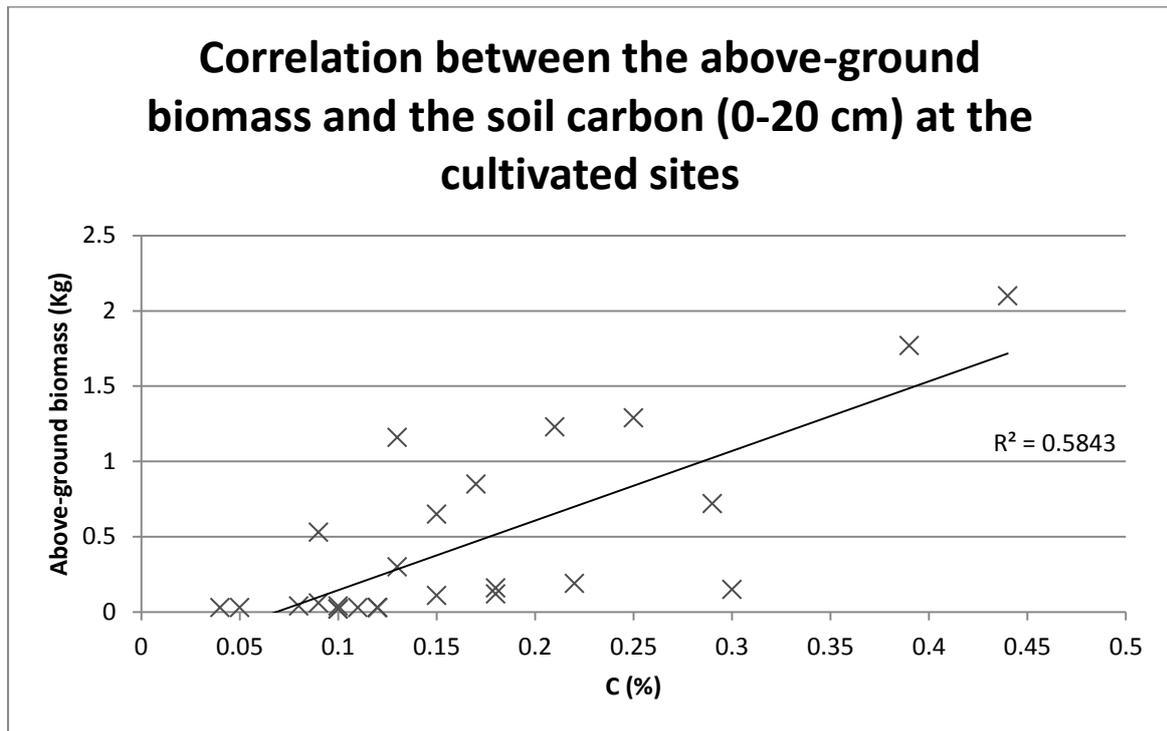


Figure 4-9: Correlation between the above-ground biomass and the % soil carbon in the 0-20 cm layer at the cultivated sites

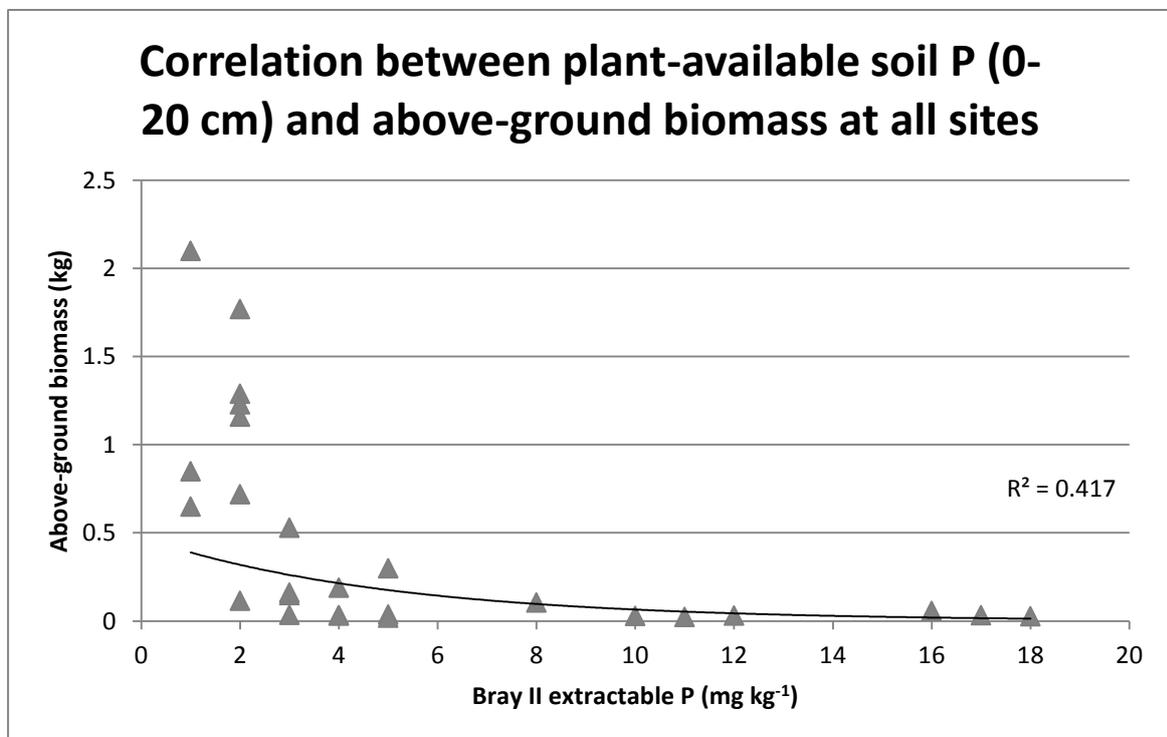


Figure 4-10: Correlation between plant-available P in the (0-20 cm) layer and the above-ground biomass at all experimental sites

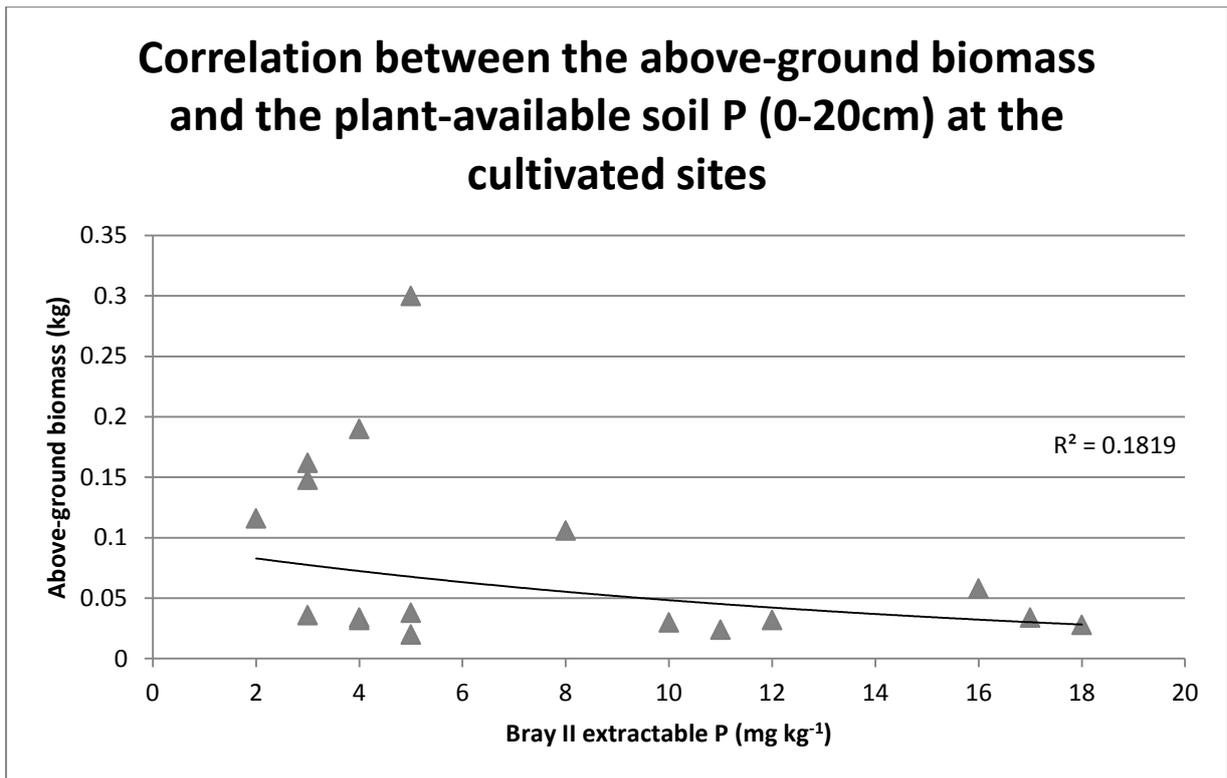


Figure 4-11: Correlation between the above-ground biomass and the plant available P (0-20 cm) at the cultivated sites

4.4.2 Macronutrients

4.4.2.1 Nitrogen (N)

N-nodule count

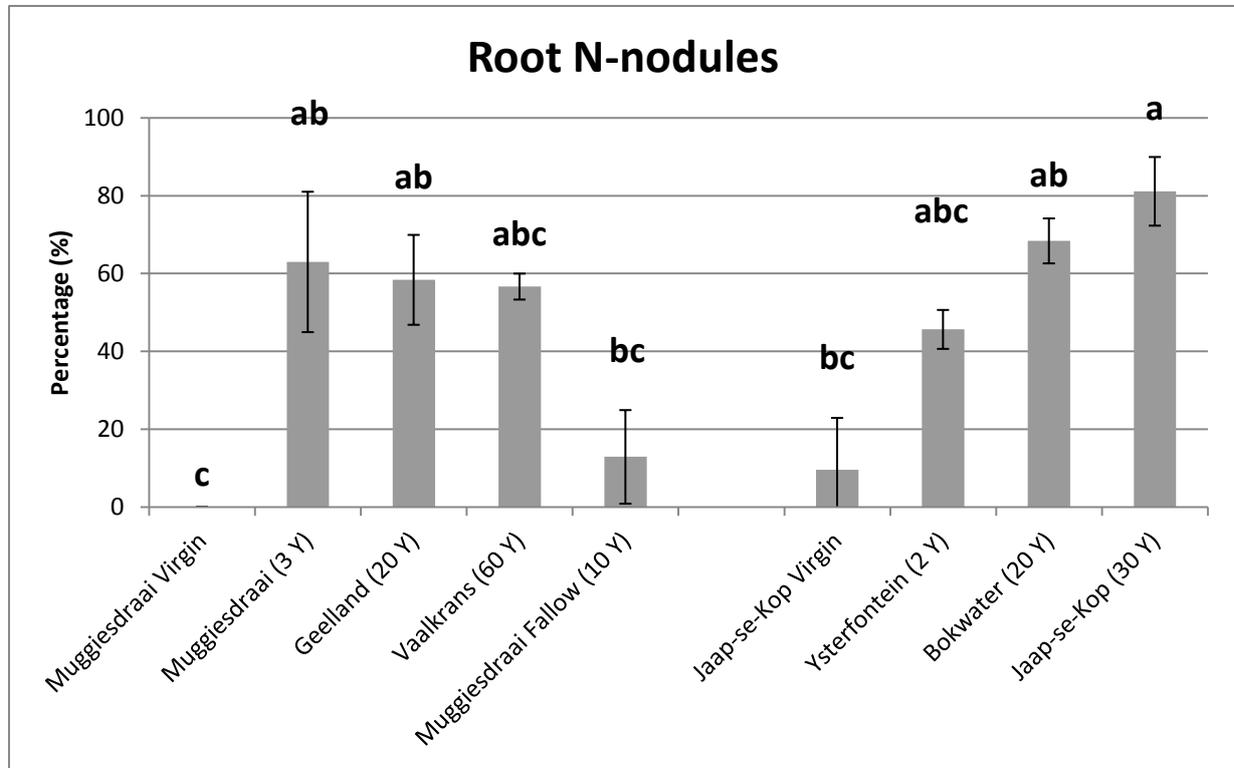


Figure 4-12: N Nodule count on the roots of the rooibos at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

In the Seekoeivlei area, a significant increase in the extent of N-nodulation in the roots was found with an increase in the age of the cultivated site (**Figure 4-12**). This trend was not evident at the Nardouwsberg area. However, it is clear that plants in the cultivated sites contained more N-nodules than the wild plants. The cultivated plants were thus more dependent on symbiotically-fixed N rather than the N in the soil. This is most likely due to a decrease in plant-available N in the soil as a result of decreased inputs of organic matter in the cultivated sites (**Figure 3-27**). There is a significant difference in the N-nodules on the roots between the different experimental sites ($P = 0.0018$) (**Figure 4-12**).

During this study it was not determined whether the N-nodules were active or not. These results are only an indication whether there were N-nodules present on the roots. It could

be that some of the plants have N-nodules on the roots from previous years, but isn't actively binding nitrogen.

Foliar Nitrogen

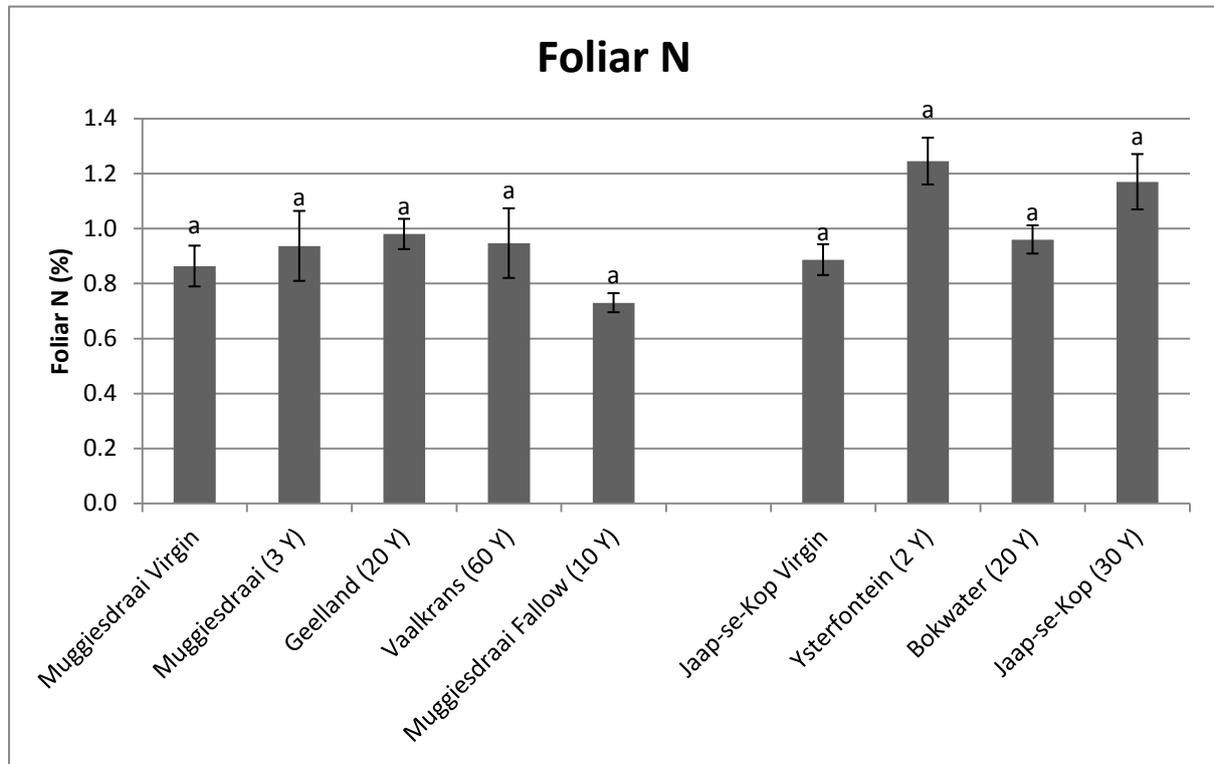


Figure 4-13: Foliar nitrogen (N) contents at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar N content of the 1-year old plants ranged between 0.9 and 1.2 %, with an average of 1.04 % (**Figure 4-13**). This value correlates well with the 1.0 % foliar N found in rooibos plants on a Citrusdal experimental farm sampled in Jan/Feb (Strassen, 1987). There were no significant difference in the N content of the leaves between the different experimental sites ($P = 0.1939$). The foliar N content in the Seekoeivlei area was higher than in the Nardouwsberg area, this can most likely be attributed to a higher combination of nitrogen in the soil (**Figure 3-27**) and higher N-nodules on the roots in the Seekoeivlei area (**Figure 4-12**). The foliar N content of the Ysterfontein and Jaap-se-Kop sites were higher; this could be attributed to the high soil N content of the Jaap-se-Kop site (**Figure 3-27**), as well as, higher number of N-nodules on the roots at the Ysterfontein site (**Figure 4-12**). In the Jaap-se-Kop a higher N-nodule count was found and the high N content in this site can be due to

symbiotically bound nitrogen (**Figure 4-12**). The small differences in the foliar N content between experimental sites is an indication that the rooibos was sufficiently applied with N at all the sites, since they are adapted to symbolically bind its own N during periods of N deficiency. Nitrogen is responsible for various processes in the plant, e.g. photosynthesis, growth, reproduction and maintaining the genetic identity of the plant since it is an important part of the DNA. It is also fundamental as building blocks for amino acids and proteins in plants, (Havlin, et al., 1999). No correlation was found between the soil inorganic N (NO_3^- , NH_4^+) content or the total soil N content and the N content of the leaves (**Data not shown**).

4.4.2.2 Phosphorus (P)

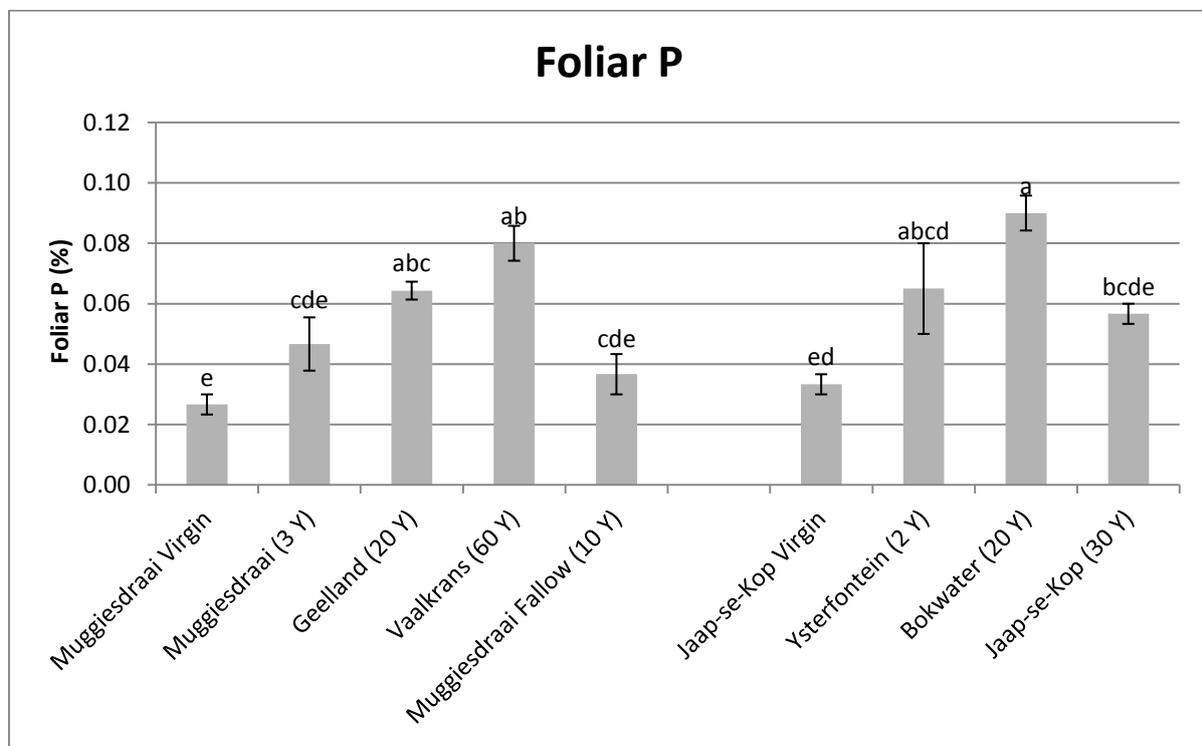


Figure 4-14: Foliar P content at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The percentage of P in the leaves of 1-year old plants ranged between 0.06 and 0.09 %, with an average of 0.07 % (**Figure 4-14**). This is slightly higher than the 0.05 % P previously reported on 1-year old plants on a Citrusdal experimental farm (Strassen, 1987). Rooibos on the virgin soils have a lower P content (<0.04 %). The norm for natural, healthy Proteaceae in South Africa is 0.03 - 0.08 % (Hawkins *et al.*, 2008). There is a definite trend between the

age of the cultivated site and the amount of P in the leaves in both the Nardouwsberg and Seekoeivlei area. An increase in the P content in the leaves was noticed with an increase in the age of the cultivated site, which also correlates to higher soil P (**Figure 3-28**) in the older sites. According to Hawkins *et al.* (2008), Proteaceae starts to show signs of P toxicity (chlorosis, necrosis, rosetting) when the percentage P in the leaves exceeds 0.1 %. The observed P toxicity symptoms was attributed to the following: (1) the excess PO_4^{3-} binds Ca in the epidermis and thus leads to necrosis, (2) the reduced iron concentration and increase in Mn concentration leads to chlorosis, and (3) the reduced total and vascular Zn concentrations lead to leaf rosetting. These symptoms were observed on some of the rooibos plants at the older cultivated sites, Vaalkrans and Bokwater(**Figure 4-15**), that had the highest P content in the soil (**Figure 3-28**) and leaves (**Figure 4-14**), even though the foliar P levels in the leaves are below the 0.1% Proteaceae P toxicity value. There is a significant difference in the P content in the leaves between the different experimental sites ($P < 0.0001$). Phosphorus is important for root growth, and in particular energy storage and transfer in plants (Havlin *et al.*, 1999). We can assume that root development is extremely important during the first year of rooibos growth, especially deep tap root development, as it is essential for survival in the hot and dry summer months. Phosphorus is thus especially important during the first year of the plant, but if the P levels in the soils are too high the plant starts to show P-toxicity symptoms. This can be one of the reasons why that the cultivated rooibos plants live only three to four years, while in the past, they used to last up to eight years (personal communication with farmers).

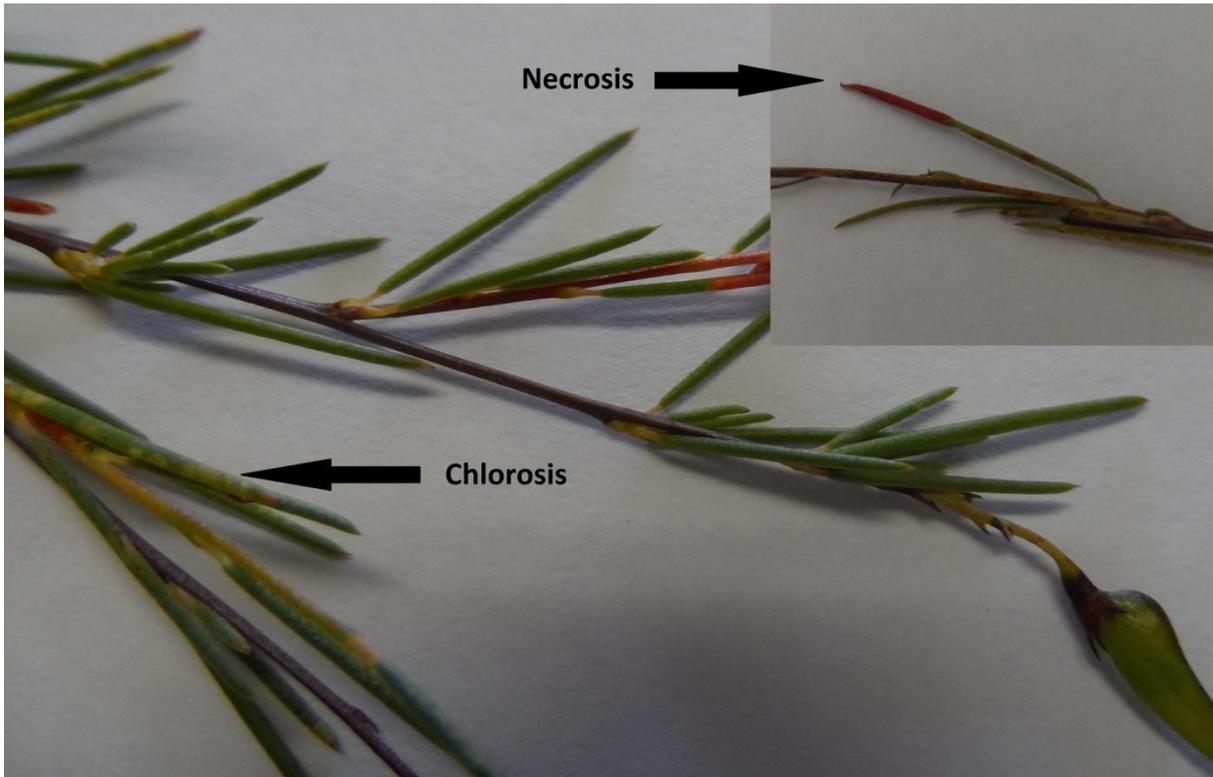


Figure 4-15: Roobos leaves showing signs of P toxicity (Chlorosis) on the Vaalkrans experimental site in the Nardouwsberg area

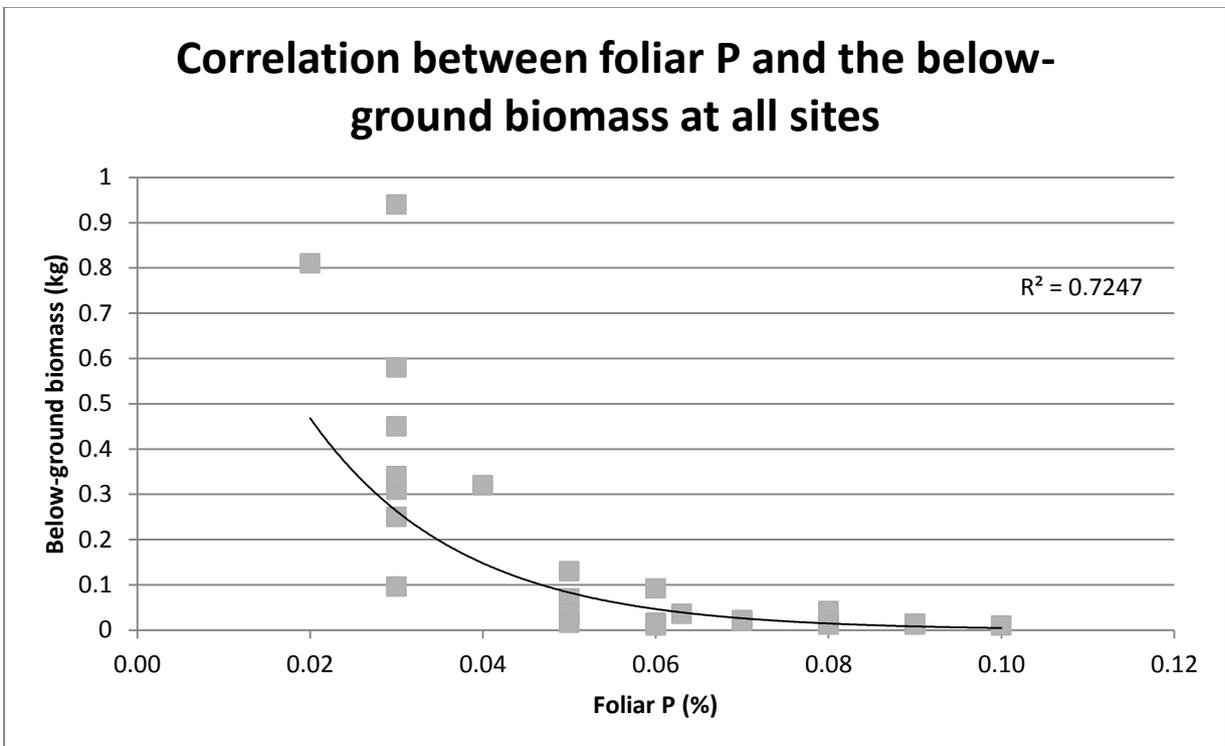


Figure 4-16: Correlation between the foliar P and below-ground biomass at all sites.

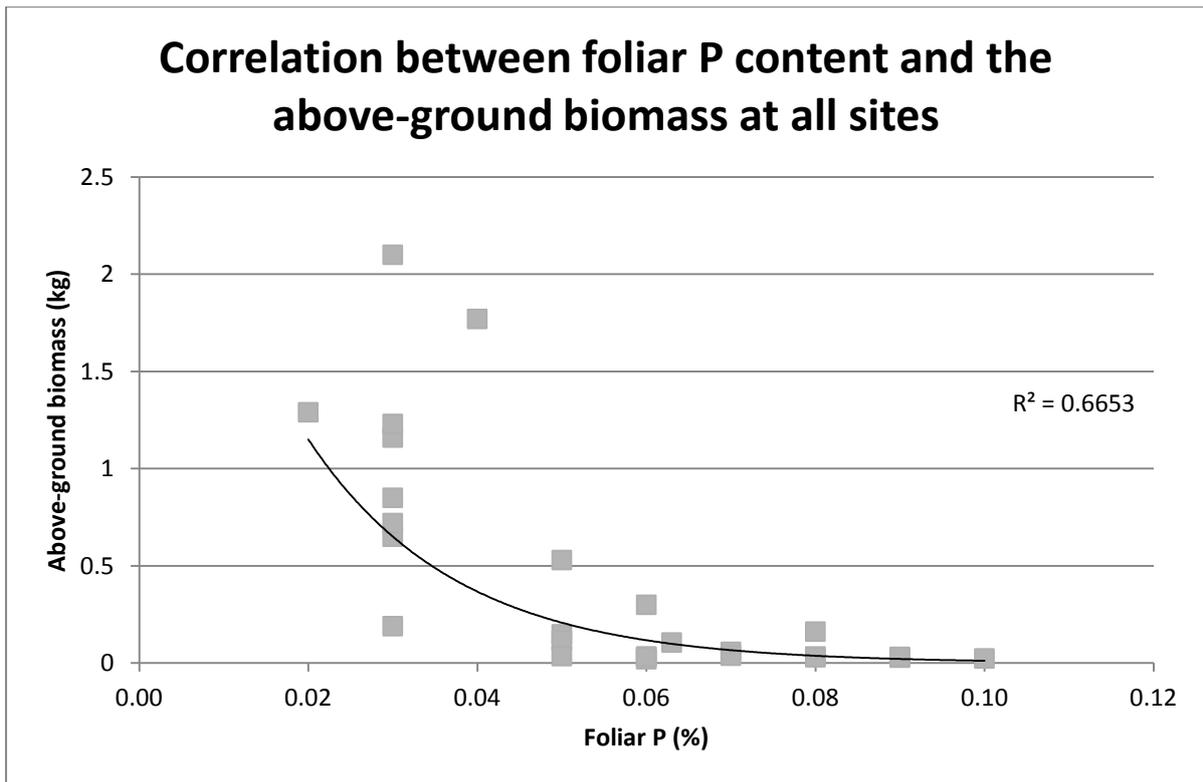


Figure 4-17: Correlation between the foliar P and the total above-ground biomass at all sites

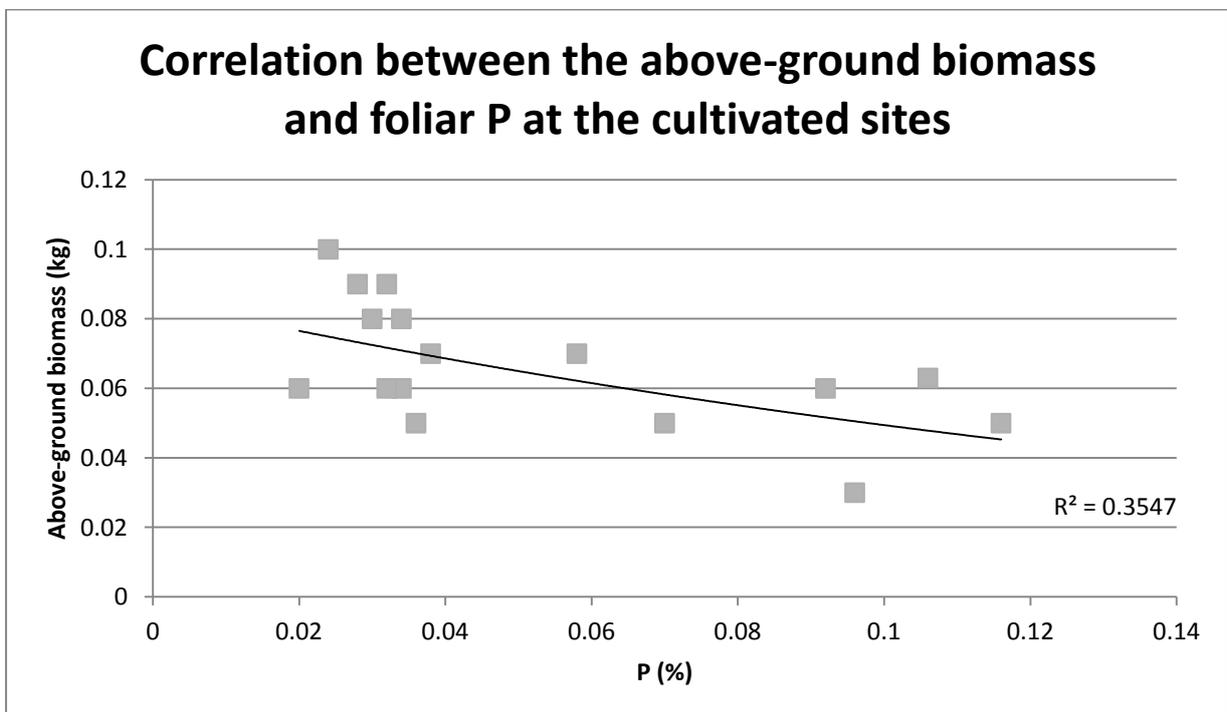


Figure 4-18: Correlation between the above-ground biomass and the foliar P at the cultivated sites

A negative correlation was found between the below- and above-ground biomass and the foliar P content at all the sites (Figure 4-16 and Figure 4-17), as well as, at the cultivated

sites only (**Figure 4-18**). The below-ground biomass decreases exponentially with an increase in P ($R^2 = 0.7247$) as well as the above-ground biomass ($R^2 = 0.5329$), see **Figure 4-16** and **Figure 4-17**. The above-ground biomass decreased exponentially with an increase in the foliar P in only the cultivated sites ($R^2=0.3547$) (**Figure 4-18**). It appears that the decrease in the above- and below-ground biomass yields with increasing age of the cultivated site can be due to P toxicity, as the strongest negative correlations with biomass yields are soil and foliar P content.

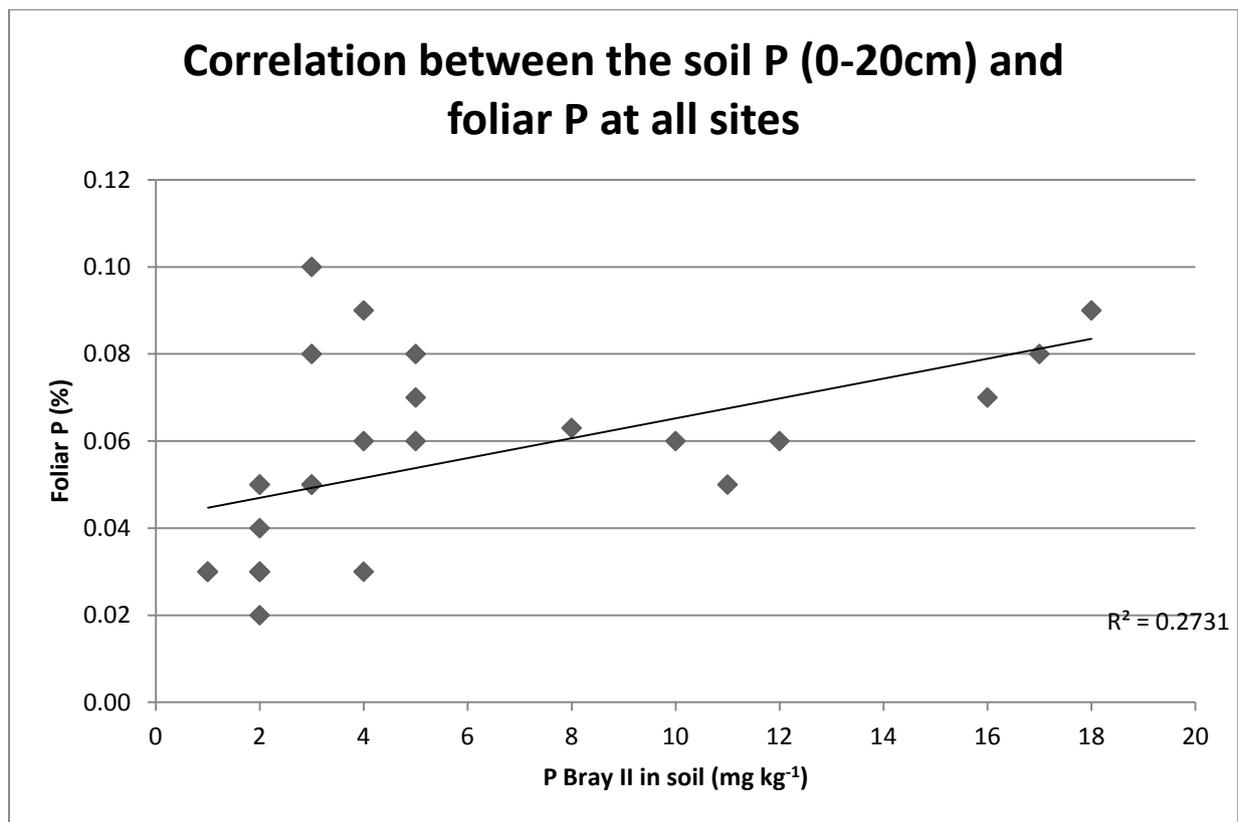


Figure 4-19: Correlation between the soil P (0-20cm) and foliar P at all sites

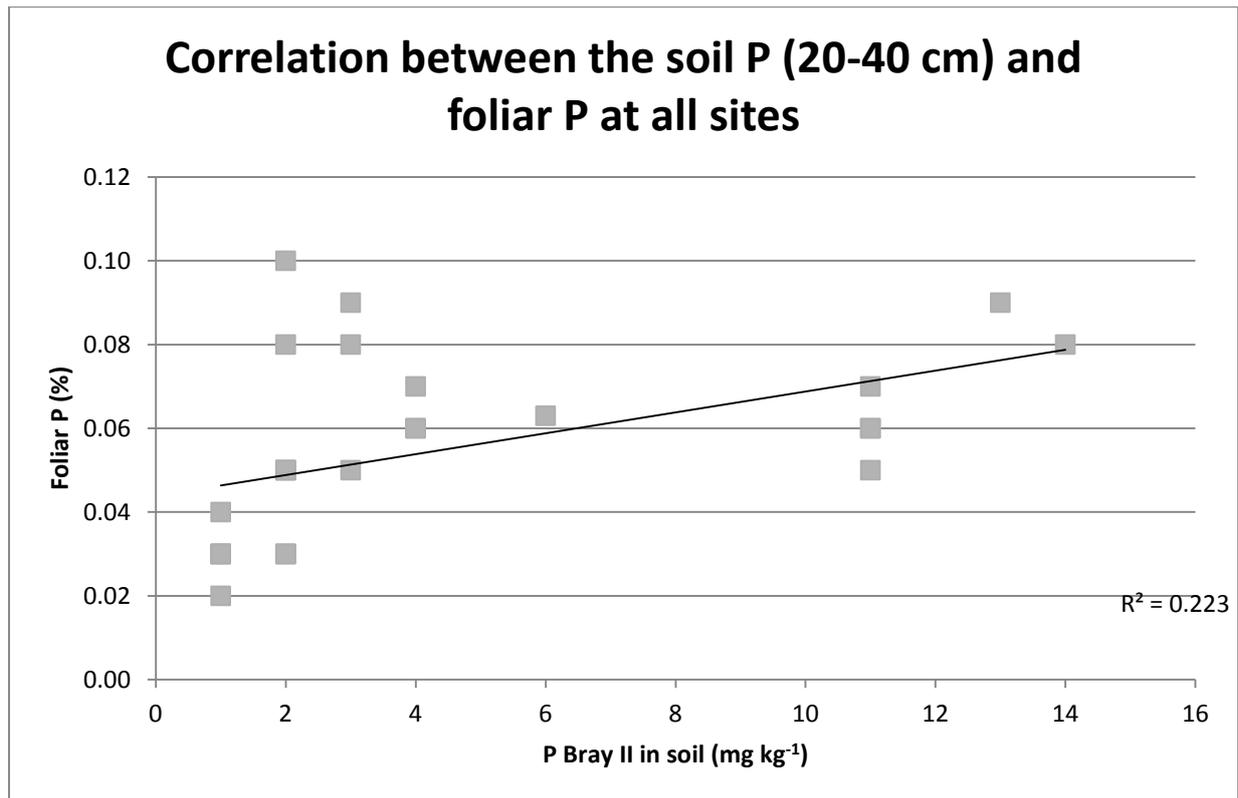


Figure 4-20: Correlation between the soil P (20-40 cm) and foliar P at all sites

There is a weak positive correlation between the soil P and foliar P contents (**Figure 4-20**). This weak correlation might be because the mycorrhiza fungi also have a significant enhancing effect on plant P uptake, helping the plant to acquire sufficient P under very low soil P conditions. The mycorrhiza fungi were found to colonize roots more actively in the newly planted sites with lower soil P contents (**Figure 4-21**). The extent of mycorrhizal root colonization on plants from the virgin and fallow sites was not determined because the cluster roots that were used for the determination were not present or could not be clearly distinguished from other plants during sampling in a natural field with a lot of roots present.

There was a significant difference in the mycorrhiza fungi present on the roots ($P < 0.0001$) between the sites on which rooibos have been planted for the first time and the older sites. The mycorrhizal colonization was much higher per root sample at the younger sites (Ysterfontein and Muggiesdraai) (**Figure 4-22**), than on the older sites (**Figure 4-23**).

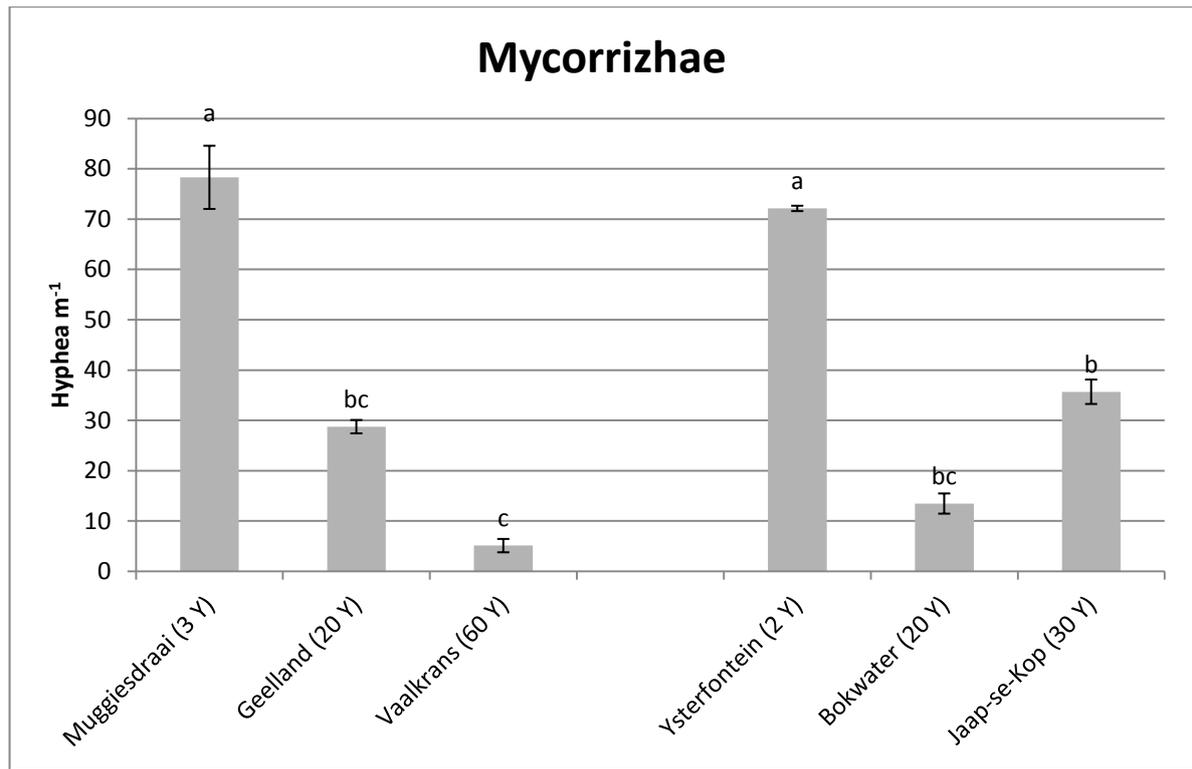


Figure 4-21: Root Mycorrhiza colonization for the cultivated Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The mycorrhizal colonization decreases exponentially with an increase in the soil P in both the 0-20 cm ($R^2 = 0.8134$) (Figure 4-24) as well as the 20-40 cm layer ($R^2 = 0.7224$) (Appendix B). This can be attributed to sufficient plant-available P present in the soil, thus the rooibos plant doesn't need to expend energy to promote or maintain mycorrhizal root colonization to help with the uptake of P from the soil. There was a weak positive correlation between the mycorrhiza on the roots and the amount of P in the leaves ($R^2 = 0.1915$) (Figure 4-25). The amount of P in the leaves is thus due to the combined effect of plant available P in the soil and the mycorrhiza on the roots. Rooibos plants take up nutrients in the winter when the soil is wet, and store it in the plant until spring when it starts to grow actively (Strassen, 1987). The P taken up by the plant will firstly be stored in the stem of the plant, before it is stored in the leaves (Jeschke & Pate, 1995). Once the amount of P taken up by the plant exceeds the amount of P that can be stored in the stems, the plant starts to store the P in the leaves and P-toxicity symptoms can be noted. The rooibos plant is adapted to take up P in soils with very low P levels. It is therefore unnecessary to add large amounts of P to the soil. Moderate soil P levels can result in a P

toxicity as seen in older plantations where farmers have been adding P to the soil for a number of years.

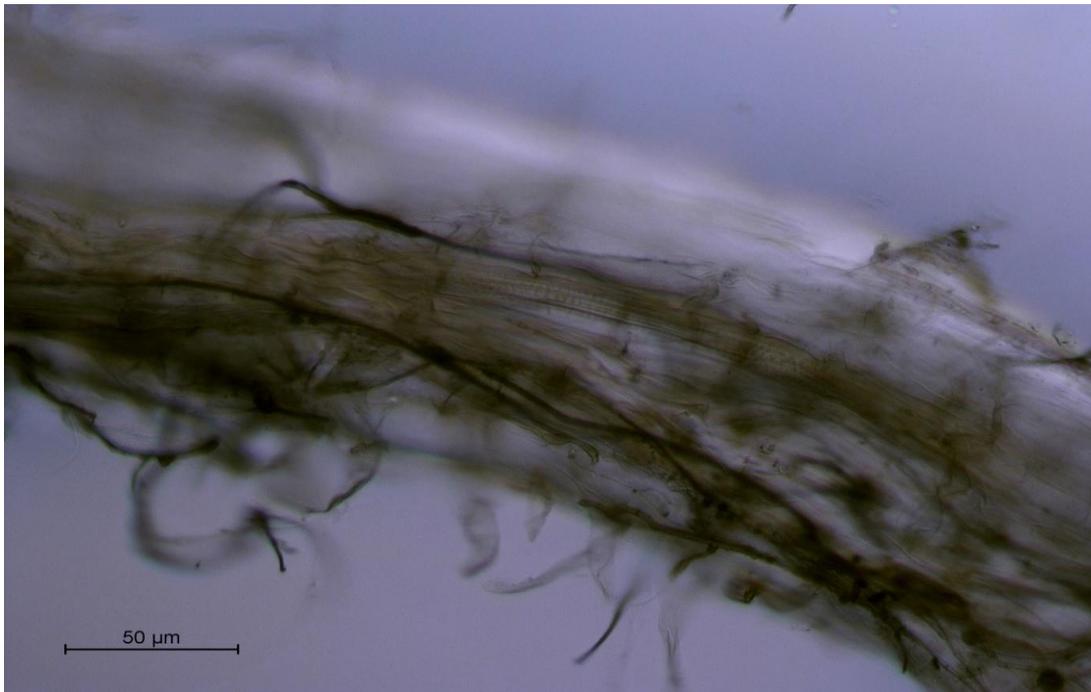


Figure 4-22: Mycorrhiza hyphae colonization on the roots at the Ysterfontein experimental sites in the Seekoeivlei area



Figure 4-23: Mycorrhiza hyphae colonization on the roots at the Vaalkrans experimental sites in the Nardouwsberg area

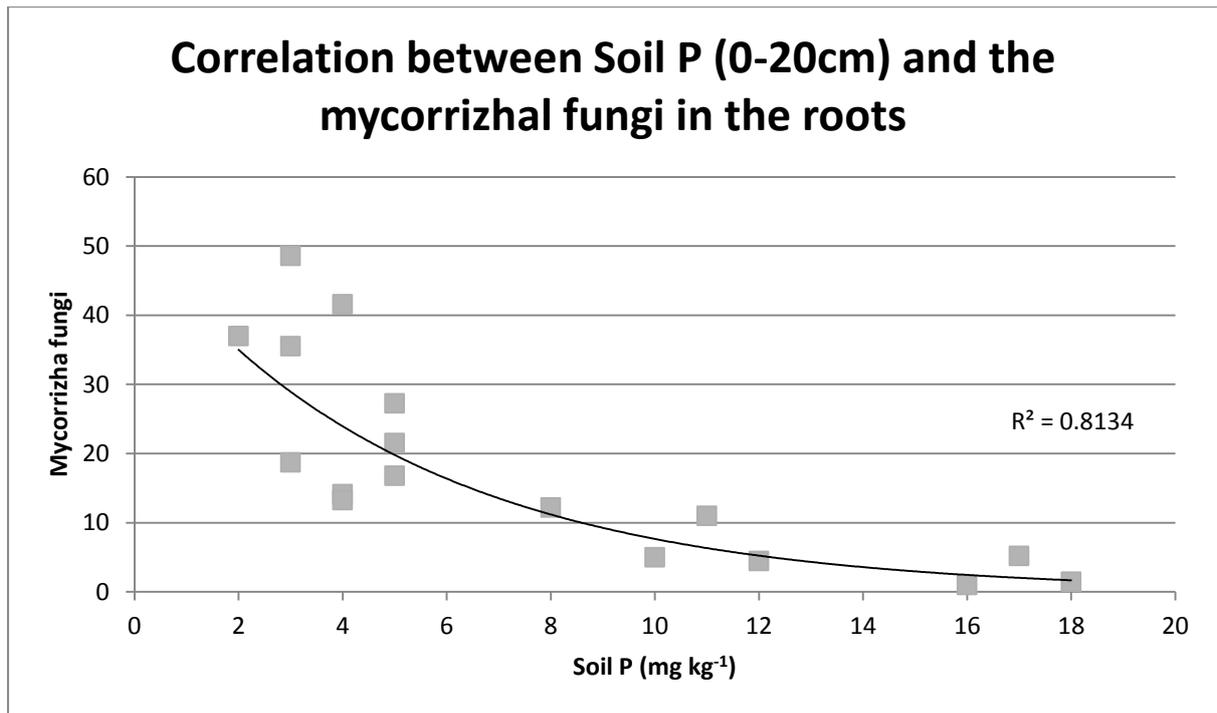


Figure 4-24: Correlation between Soil P (0-20cm) and the mycorrhiza fungi in the roots

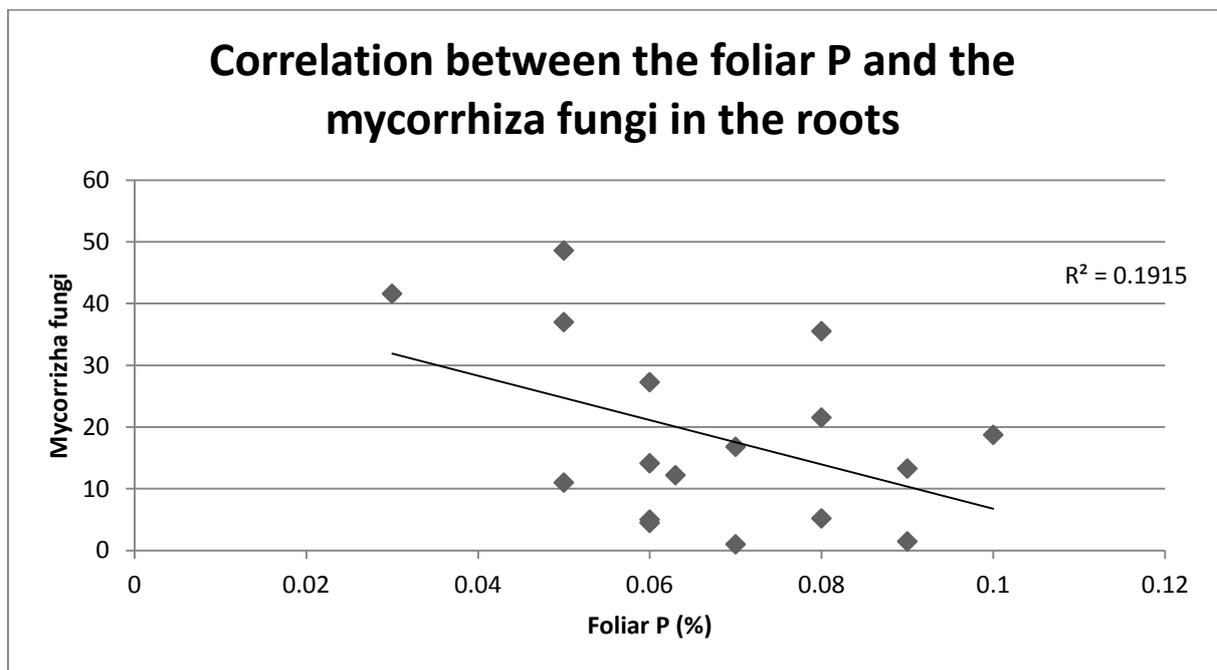


Figure 4-25: Correlation between the foliar P and the mycorrhiza fungi in the roots

4.4.2.3 Potassium (K)

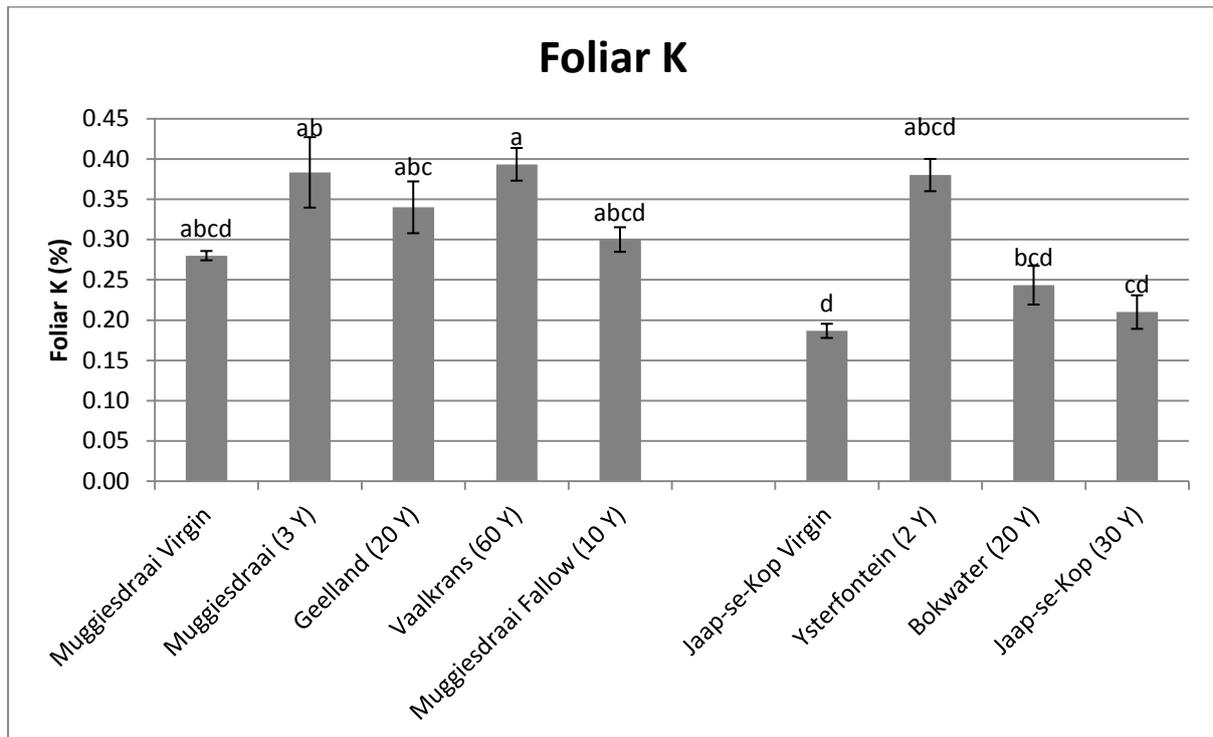


Figure 4-26: Foliar K at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar K content of the 1-year old plants ranged between 0.21 and 0.39 %, with an average of 0.30 % (Figure 4-26). This value is slightly higher than the 0.19 % found by (Strassen, 1987). The Nardouwsberg sites generally had a higher K foliar content than the Seekoeivlei area, except for the Ysterfontein site. This could be due to differences in the clay mineralogy. The reason for the higher foliar K content at the Ysterfontein experimental site can be due to the fact that the soil K was higher at this site (Figure 3-19). The foliar K contents of the older wild plants did not differ significantly from the 1- and 2-year old plants. There is a significant statistical difference in the foliar K content between the different experimental sites ($P = 0.018$). Potassium has several important roles in the plant; it plays a role in the movement of N in the plant, it is important for maintaining the osmotic potential and ionic relations. It also controls the cuticular layer thickness and opening and closing of stomata of plants which in turn affects drought-, cold- and pest resistance of the plants (Havlin *et al.*, 1999). The high soil and foliar K levels at the Ysterfontein site could be one of the contributing factors to the good growth in this particular site.

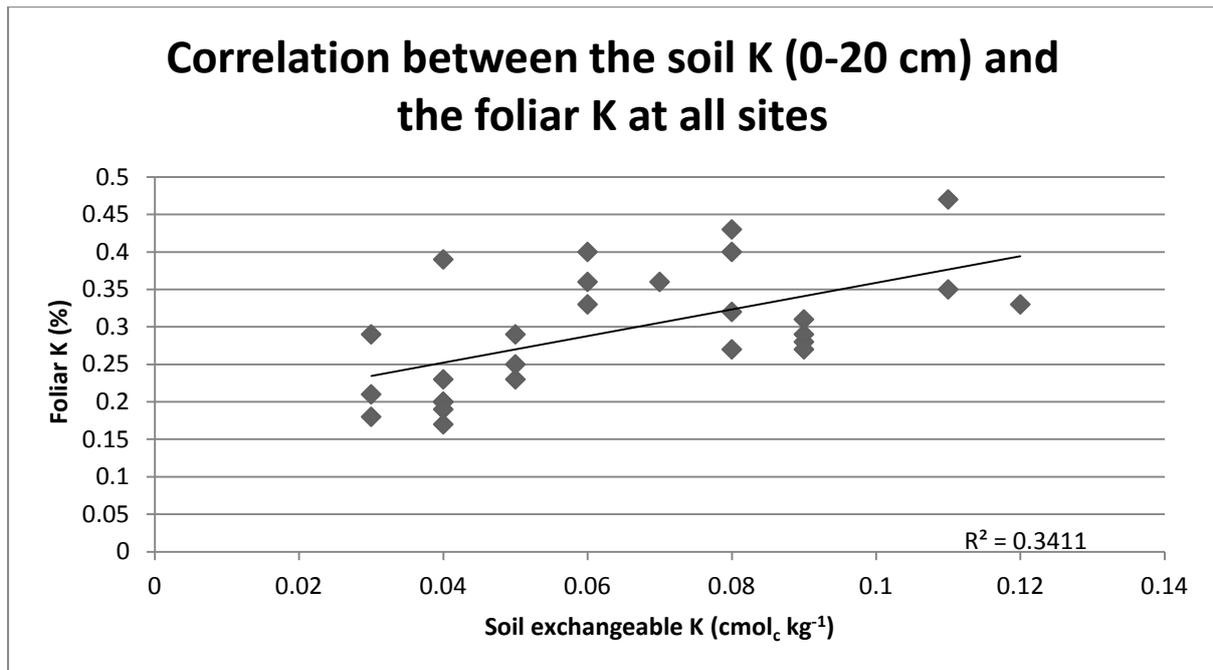


Figure 4-27: Correlation between soil K (0-20 cm) and foliar K at all sites

There was a weak positive correlation between the soil K in both the 0-20 cm layer ($R^2 = 0.3411$) (Figure 4-27) as well as the 20-40 cm layer ($R^2 = 0.4551$) (Appendix B) and foliar K levels. The K appears to have no positive or negative effect on the growth of the plant since no correlation was found between the K in the soil or leaves and the above-ground biomass (data not shown).

4.4.2.4 Calcium (Ca)

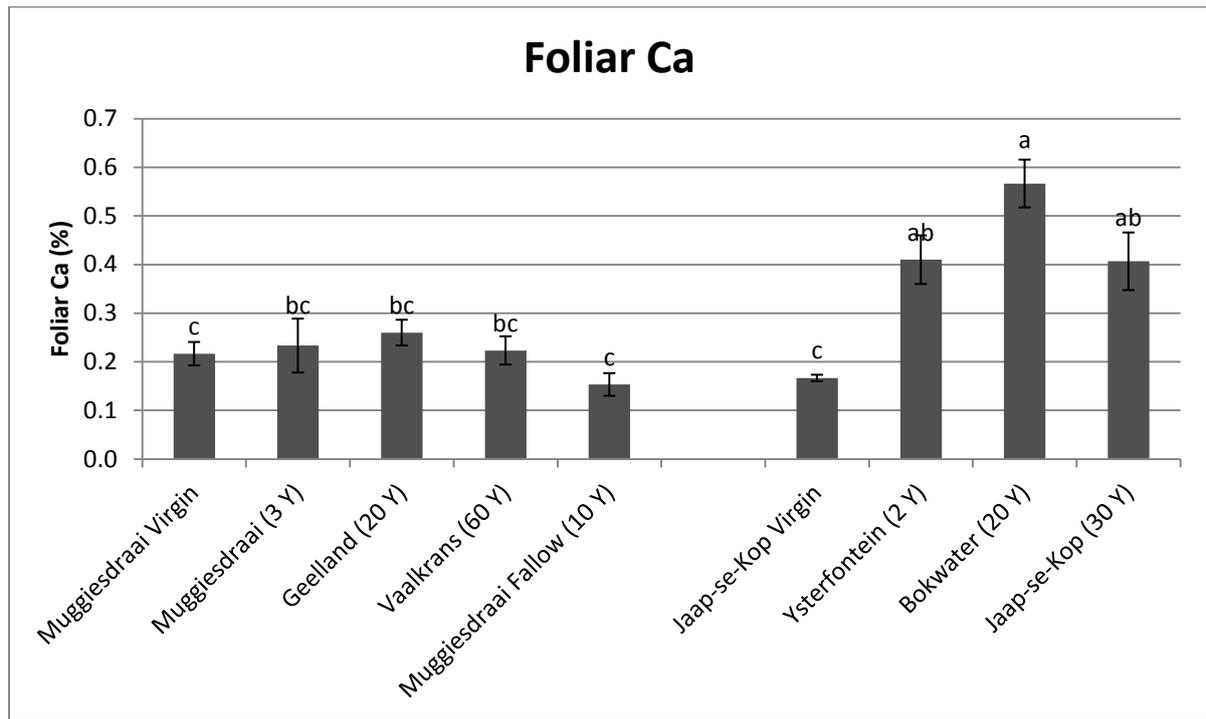


Figure 4-28: Foliar Ca at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites as well as the depth

The foliar Ca content of the 1-year old plants was between 0.22 and 0.57 %, with an average of 0.37 % (**Figure 4-28**). These values were higher than the 0.23 % reported by Strassen (1987). The Nardouwsberg area foliar Ca contents (0.15 - 0.26 %) were generally lower than the Seekoeivlei area (> 0.4 %), the reason for this is unclear since higher exchangeable Ca content in the soil was observed in the Seekoeivlei area (**Figure 3-20**). No foliar applications were added to the Seekoeivlei site that could have resulted in the higher Ca content. The older plants in the virgin sites, had very low levels of Ca (<0.2 %) which could be due to a dilution factor as the plants are more woody. There is a significant statistical difference in the Ca content of the leaves between the different experimental sites ($P < 0.0001$). Calcium is important for cell growth and elongation, and the integrity of cell walls (Havlin *et al.*, 1999). There was a weak negative correlation between the Ca in the soil in both the 0-20 cm layer ($R^2 = 0.3189$) (**Figure 4-29**) as well as the 20-40 cm layer ($R^2 = 0.3361$) (**Appendix B**) and the foliar Ca content. This negative relationship is quite an unexpected as the Ca in the leaves should increase with an increase in soil Ca, since there is more Ca available for uptake by the plant. A moderate positive correlation was found between the P in the leaves and

the Ca content of the leaves ($R^2 = 0.4346$) (data not shown); the plant is thus trying to overcome the binding of the Ca in the epidermis. Even with the increase in the Ca, necrosis was still observed on the leaf tips (**Figure 4-15**).

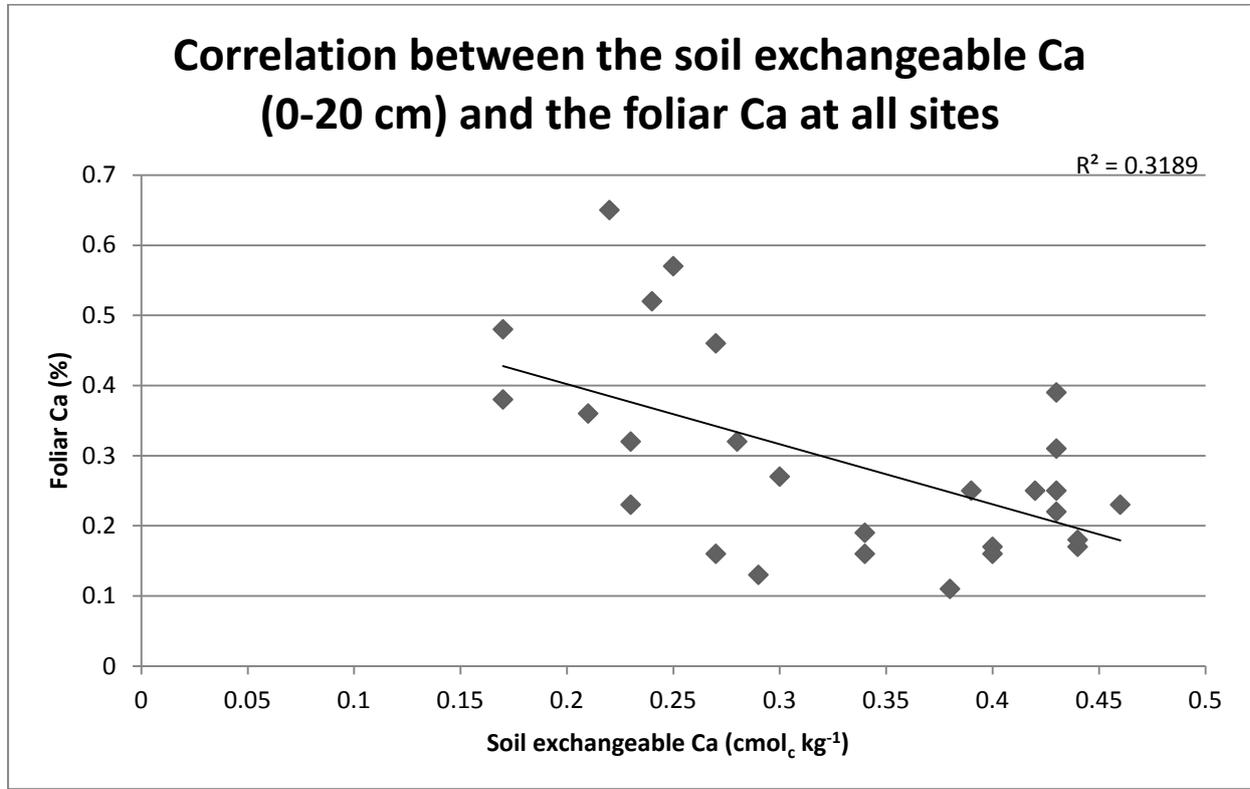


Figure 4-29: Correlation between the soil exchangeable Ca (0-20 cm) and the foliar Ca at all sites

4.4.2.5 Magnesium (Mg)

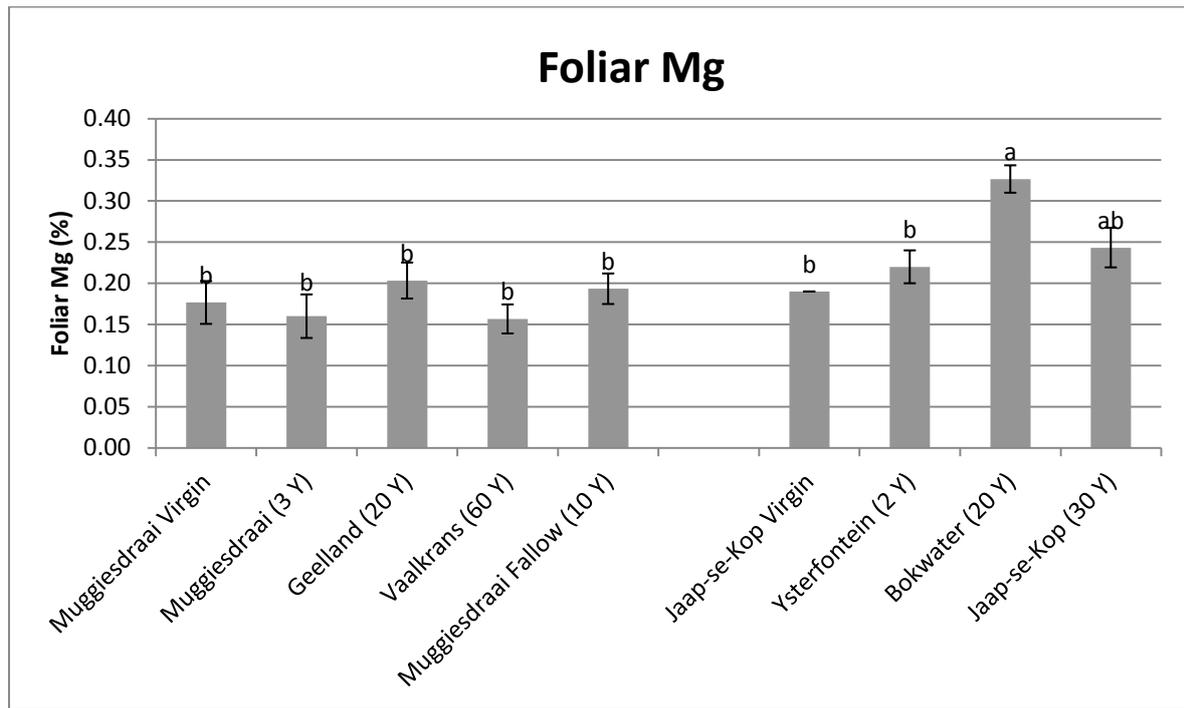


Figure 4-30: Foliar Mg at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar Mg content ranged between 0.16 and 0.33 %, with an average of 0.22 % (**Figure 4-30**). This is similar to the 0.18 % Mg found by Strassen (1987). The foliar Mg content of the older rooibos plants on the virgin sites didn't differ from the Nardouwsberg area, with values between 0.15 and 0.20 %. The foliar Mg content did not differ significantly at the various sites, except for the Bokwater experimental site differs from the rest ($P = 0.0024$). In the Nardouwsberg, as well as, the Seekoeivlei area, there is no significant correlation between the above-ground biomass and the foliar Mg content (data not shown). No correlation was found between the Mg in the soil and the foliar Mg content (data not shown). Magnesium is important for photosynthesis and plays an important role in the translocation of phosphorus (Havlin, et al., 1999). The Mg plays an important role in the transfer of P in the plant; it might be the reason for the high Mg content in the Seekoeivlei area. The Bokwater had a high P content (**Figure 4-14**) and Mg is necessary to move the P in the plant from the roots to the twigs and finally into the leaves in the case of excess P. The Mg content increased with an increase of P in the Seekoeivlei area ($R^2=0.5582$) (data not shown), however this trend was not observed for the Nardouwsberg area.

4.4.2.6 Sulphur (S)

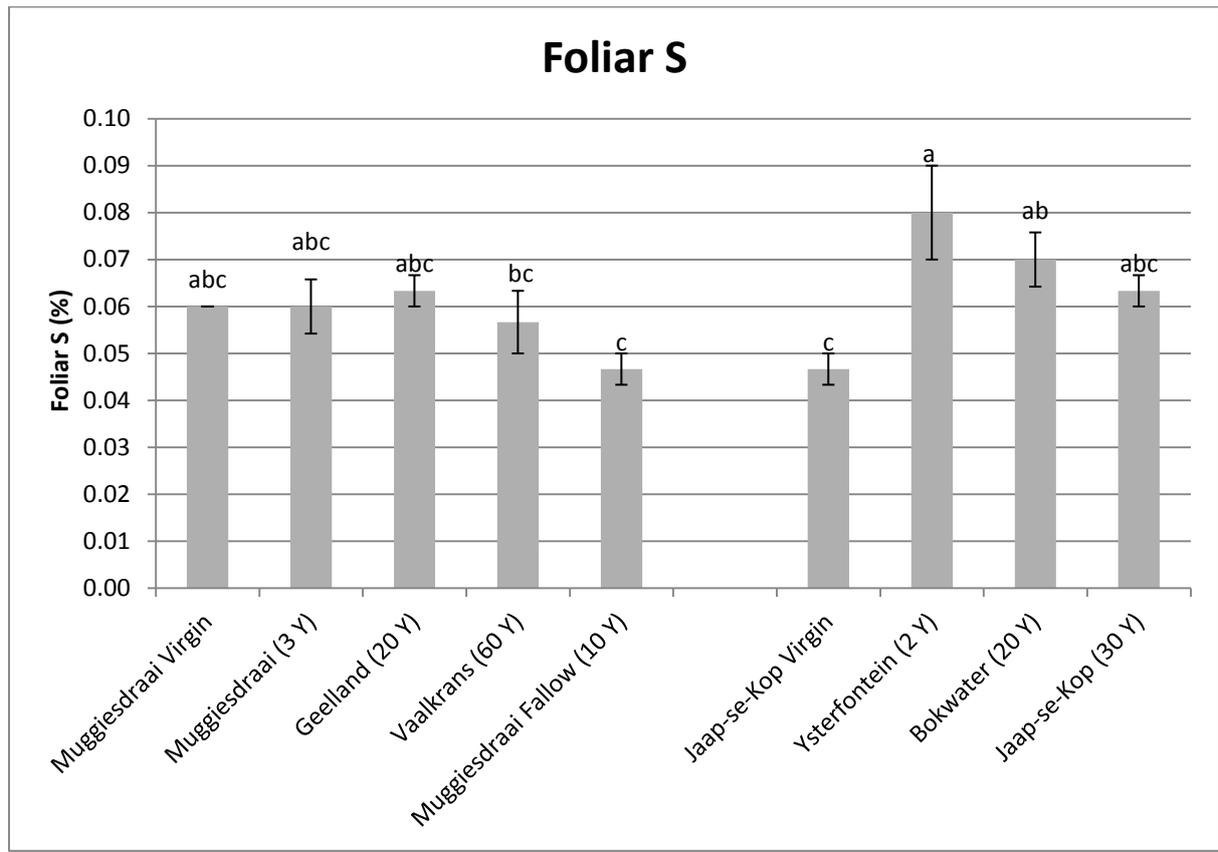


Figure 4-31: Foliar S at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar S content ranged between 0.05 and 0.08 %, with an average of 0.07 % (**Figure 4-31**). The older plants at the Muggiesdraai Fallow and Jaap-se-Kop virgin sites contained lower S contents, which might be due to a dilution factor as the plants were woodier. There is a significant difference in the S content of the leaves between the different experimental sites ($P < 0.0024$). No correlation was found between the above-ground biomass and the foliar S content, as well as, between the soil S content and the foliar S content (data not shown). Sulphur is important for the forming of chlorophyll and plays a role in the amount of nodules found on the roots of legumes (Havlin, et al., 1999). No correlation was found between the foliar S content and the N-nodule count on the roots (data not shown).

4.4.3 Micronutrients (Fe, Cu, Zn, Mn and B)

Table 4-1: Foliar micronutrient contents (Fe, Cu, Zn, Mn and B) at Nadouwsberg and Seekoeivlei sites.

Site	Fe	Cu	Zn	Mn	B
	mg kg ⁻¹				
<i>Nardouwsberg</i>					
Muggiesdraai Virgin	215.10 a	3.65 a	24.79 a	45.16 a	19.79 a
Muggiesdraai (3 Y)	269.00 a	2.49 a	14.20 a	134.02 a	38.27 a
Geelland (20 Y)	205.30 a	2.99 a	17.80 a	173.07 a	33.69 a
Vaalkrans (60 Y)	196.40 a	3.44 a	18.16 a	141.73 a	28.35 a
Muggiesdraai Fallow (10 Y)	377.47 a	2.46 a	17.24 a	88.69 a	25.52 a
<i>Seekoeivlei</i>					
Jaap-se-Kop Virgin	249.30 a	2.60 a	21.48 a	45.91 a	20.39 a
Ysterfontein (2 Y)	145.00 a	4.01 a	15.30 a	125.25 a	83.82 a
Bokwater (20 Y)	144.07 a	3.13 a	17.21 a	215.87 a	34.76 a
Jaap-se-Kop (30 Y)	168.77 a	4.85 a	12.80 a	156.43 a	25.56 a

Note: The letters of significance differ between the experimental sites as well as between the different micro nutrients

The foliar Fe content ranged between 100 and 200 mg kg⁻¹, with an average of 170 mg kg⁻¹ (Table 4-1). According to the Pais & Jones, (2000) the foliar Fe content falls in the category of sufficient or normal (100-500 mg kg⁻¹). The foliar Fe contents of the present study are lower than the value of 255 mg kg⁻¹ reported by Strassen (1987). The older plants generally had a higher Fe content than the younger plants (Table 4-1). The virgin sites as well as the rehabilitated site with the older plants have a higher Fe content (>200 mg kg⁻¹). This can be attributed to a dilution factor as the plants are woodier. The Muggiesdraai site with the 2-year old plants also had higher Fe content. It would thus seem that the plants take up more

Fe over the years. There is no significant difference in the Fe content of the leaves between the different experimental sites ($P = 0.1062$). No correlation was found between the above-ground biomass and the foliar Fe content, or the soil Fe content and the foliar Fe content (data not shown). Iron plays a role in the oxidation-reduction reaction in the plant and a role in the forming of chlorophyll. Iron is also found in certain enzymes and proteins (Havlin *et al.*, 1999). Unlike the study by Hawkins *et al.* (2008) in proteacea, a decrease in the Fe content of the leaves was not noted with an increase in the P content. A weak negative correlation were found between the P and Fe content ($R^2 = 0.2272$) (data not shown).

The foliar Cu content at all sites ranged between 2-5 mg kg⁻¹, with an average of 3.76 mg kg⁻¹ (**Table 4-1**). According to the Pais & Jones, (2000), the typical Cu deficiency levels in crop leaves are between 2 and 5 mg kg⁻¹. No deficiency symptoms were observed on the experimental sites. The foliar Cu contents of the present study are lower than the 6 mg kg⁻¹ reported by Strassen (1987). There were no significant statistical differences in the foliar Cu contents between the different experimental sites ($P = 0.0579$). The Cu can be found in the seeds and growing parts of the plant and is important for respiration (Havlin *et al.*, 1999). Most of the rooibos plantations are harvested before flowering so the Cu is not necessary in that aspect. There was not a significant correlation between the foliar Cu content and the above-ground biomass for all the experimental sites $R^2 = 0.0079$ (**Appendix B**) as well as in the Seekoeivlei area (data not shown). There was a negative correlation between the foliar Cu content and the above-ground biomass in the Nardouwsberg area ($R^2 = 0.8224$) (**Figure 4-32**). No correlation was found between the soil Cu content and the foliar Cu content (**data not shown**).

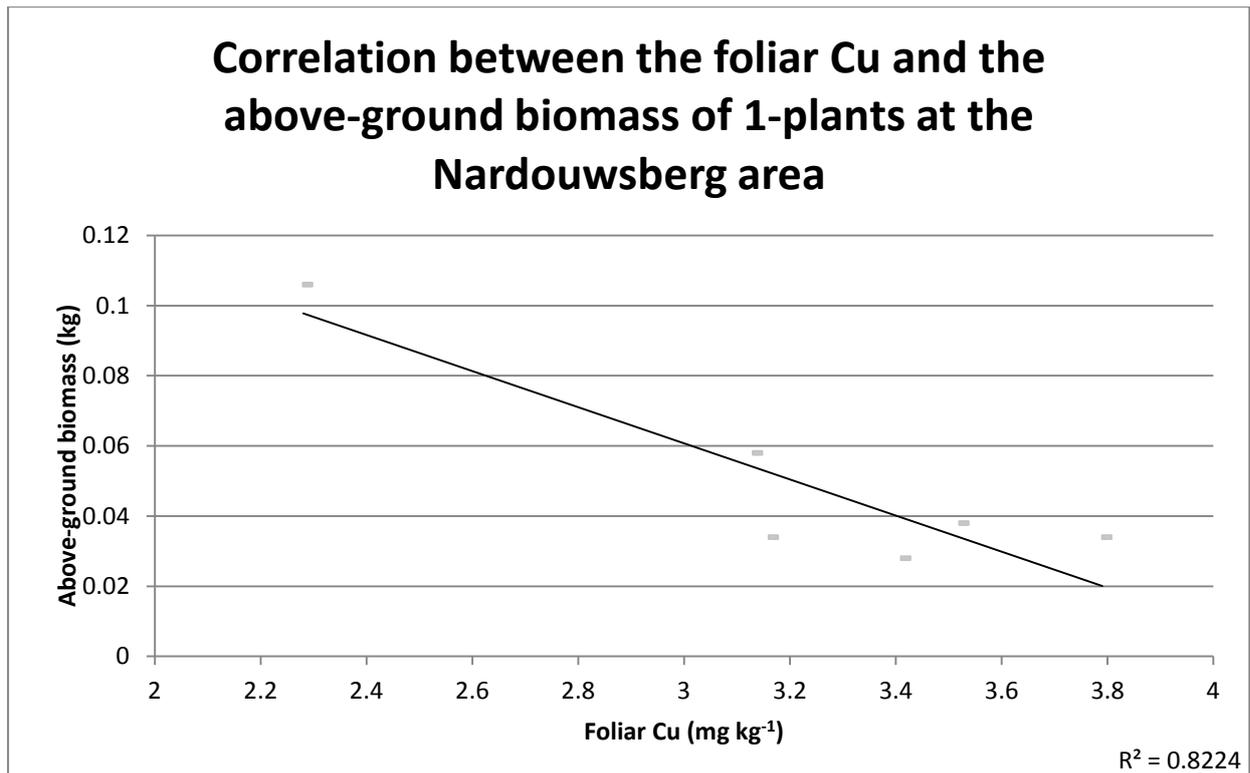


Figure 4-32: Correlation between the foliar Cu and the above-ground biomass of 1-plants at the Nardouwsberg area

The percentage of Zn in the leaf analysis for the one year old plants was between 10 and 20 mg/kg, with an average of 16.28 mg kg⁻¹ (**Table 4-1**). The deficiency levels of Zn in the leaves falls between 1 and 20 mg kg⁻¹ (Pais & Jones, 2000). This value correlates well with the 17 mg kg⁻¹ found on the Citrusdal experimental farm in Jan/Feb (Strassen, 1987). The Zn values in the one year old plants did not differ significantly. The values in the virgin soils were higher, but have a very big standard error and thus not conclusive. The rehabilitated plants Zn values relate well to those of the second year old plants in the Muggiesdraai experimental site. There is no significant statistical difference in the Zn content of the leaves between the different experimental sites ($P = 0.9581$). No correlation was found between the above-ground biomass and the Zn content of the leaves as well as between the soil Zn content and the foliar Zn content (data not shown). Zinc plays an important role in the activation of enzymes and regulates the pH in the cell fluids. It also plays an important role in the forming of the chlorophyll and growth hormones (Havlin *et al.*, 1999). No correlation between the Zn and the P were found (data not shown), leaf resetting would thus not be expected on these experimental sites, and were also not found.

The percentage of Mn in the leaf analysis for the one year old plants was between 120 and 220 mg kg⁻¹, with an average of 161.83 mg kg⁻¹ (**Table 4-1**). According to the Pais & Jones, (2000) the value falls under the category of sufficient or normal, because the value falls between the 20 to 300 mg kg⁻¹ values. The value of the Mn was higher than the value of 77 mg/kg found on the Citrusdal experimental farm in Jan/Feb (Strassen, 1987). The Mn content of the plants in the virgin site is significantly lower than the plants in the sites with the one year old plants (**Table 4-1**). Ysterfontein have a lower value than the rest of the sites. The Mn can thus have a negative effect on the above-ground biomass of the plant. There was an increase in the Mn content of the leaves with an increase in the age of the site, but appears to have a bell shape and starts to decrease later on (**Table 4-1**). There was a significant statistical difference in the Mn content of the leaves between the different experimental sites ($P = 0.0102$) especially between the virgin as well as fallow sites and the older cultivated sites. The reason for the lower foliar Mn content in the virgin and fallow sites can be attributed to a dilution factor as the plants are woodier in those sites than in the cultivated sites. No correlation was found between the above-ground biomass and the Mn content of the leaves as well as between the soil Mn content and the foliar Mn content (data not shown). Mn plays an important role in the photosynthesis as well as in the oxidation-reduction processes in the plant e.g. reducing nitrate to amine. Iron also improves the uptake of nitrate and metabolism of iron in the plant activity (Havlin *et al.*, 1999). A positive correlation was found between the P content of the leaves and the Mn content of the leaves ($R^2=0.5544$) (data not shown); thus leading to chlorosis see **Figure 4-15**.

The percentage of B in the leaf analysis for the one year old plants was between 25 and 80 mg kg⁻¹, with an average of 40.06 mg kg⁻¹ (**Table 4-1**). These values fall into the category of sufficient or normal, because it lies between 10-200 mg kg⁻¹ (Pais & Jones, 2000). However, it was higher than the value of 24 mg kg⁻¹ reported for Citrusdal experimental farm in Jan/Feb (Strassen, 1987). The virgin sites with the older plants had a lower boron value (20 mg kg⁻¹). The Muggiesdraai which is an older site with two year old plants had a higher B value than the rest of the sites, except the Ysterfontein site. The Ysterfontein site is significantly higher in B than the rest of the sites. It is about double. No definite trends can be seen in both the Nardouwsberg as well as the Seekoeivlei area (**Table 4-1**). The Ysterfontein sites have a significantly higher B content than the rest of the experimental

sites (**Table 4-1**). The Ysterfontein sites (Y2 and Y1) were near Y1 that was situated on top of an old termite activity. This can be the reason for the higher B content in the Ysterfontein experimental site. No correlation was found between the Boron in the soil and the boron in the leaves. There is a significant statistical difference in the B content of the leaves between the different experimental sites ($P < 0.0001$). Boron plays a role in the forming of pectin and lignin. It plays a role in the transfer of carbohydrates and phosphate ions over the membranes in the plant. It is also plays a role in the absorption of certain cations (Havlin *et al.*, 1999).

No correlation was found between the foliar B content and the above-ground biomass at all the experimental sites (data not shown). There is a strong positive correlation between the foliar B content of the 1-year old plants and their above-ground biomass ($R^2 = 0.8873$) (**Figure 4-33**). This positive correlation was seen in both the test areas; Nardouwsberg ($R^2 = 0.7644$) and the Seekoeivlei ($R^2 = 0.952$).

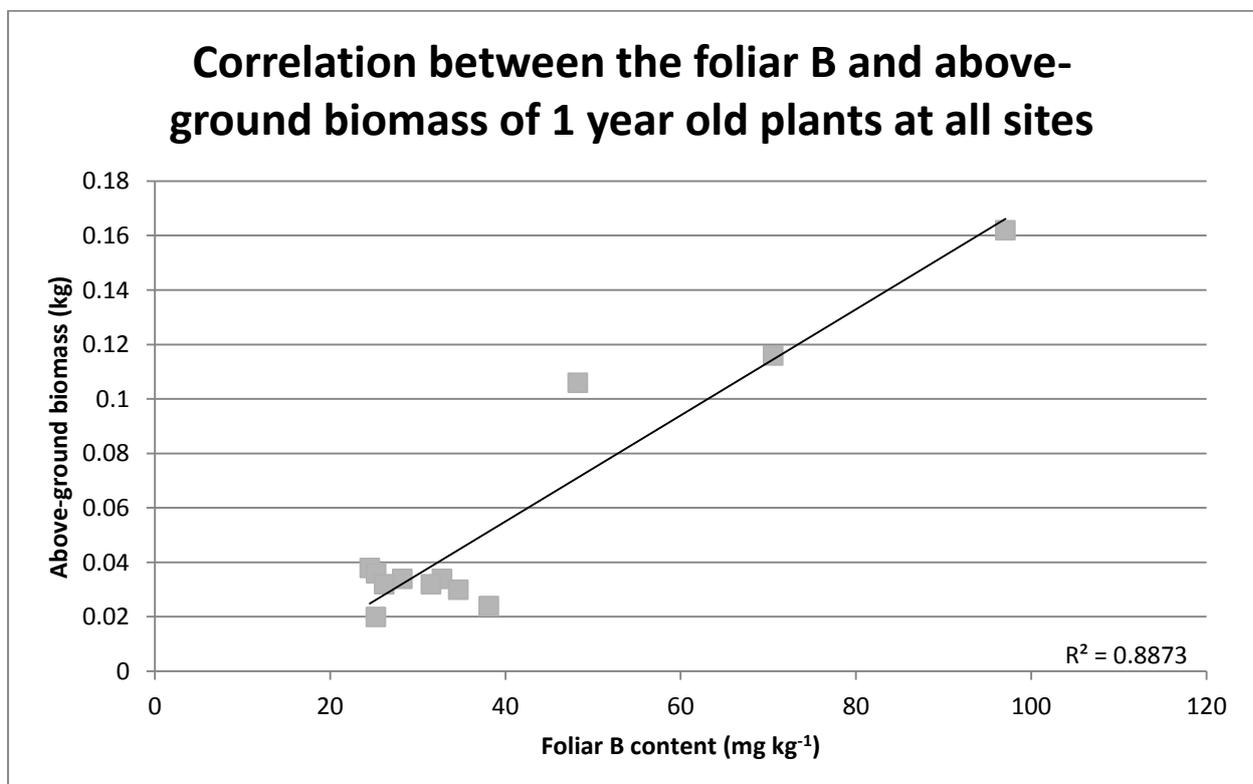


Figure 4-33: Correlation between the foliar B and above-ground biomass of 1-year old plants at all sites

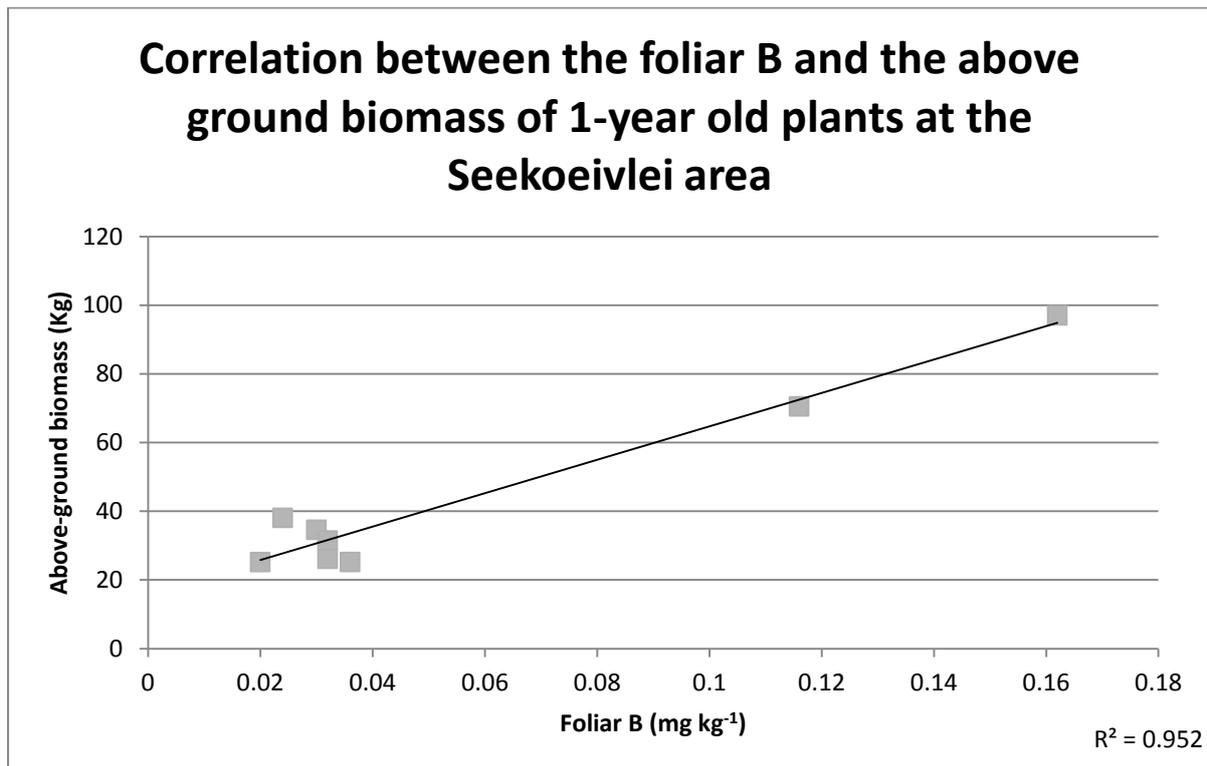


Figure 4-34: Correlation between the foliar B and the above-ground biomass of 1-year old plants at the Seekoeivlei area

4.4.4 Other elements

4.4.4.1 Sodium (Na)

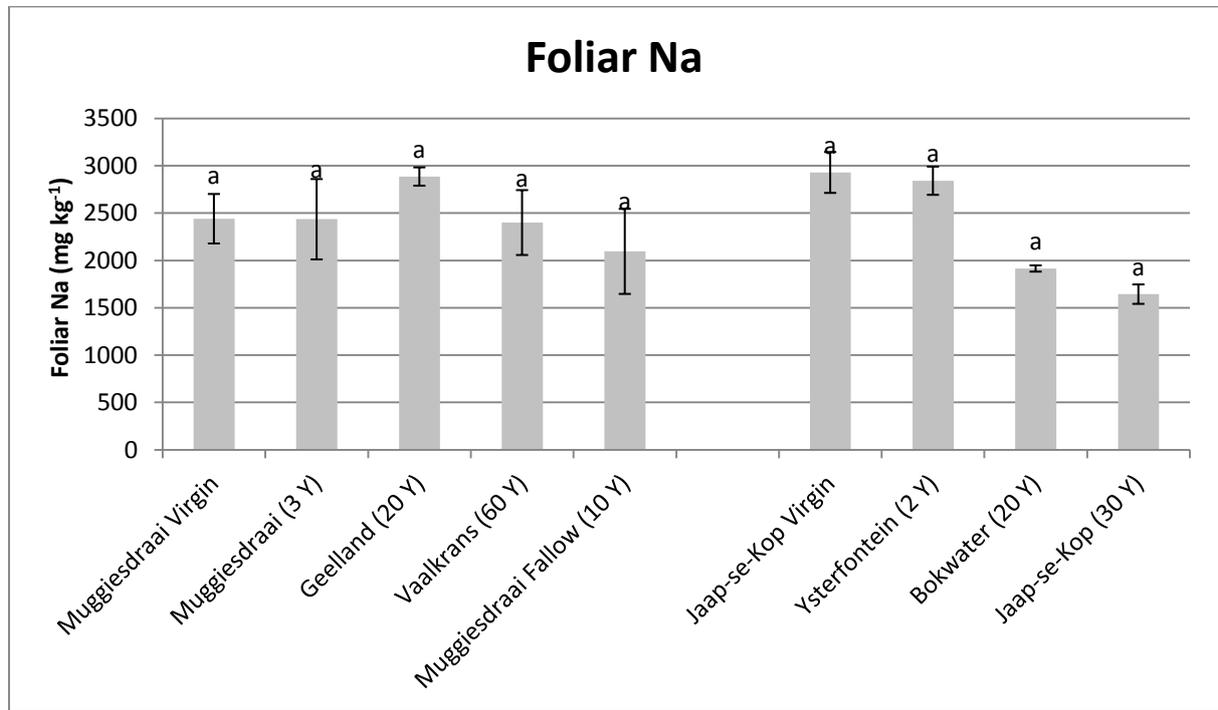


Figure 4-35: Foliar Na at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar Na content ranged between 1600 and 2900 mg kg⁻¹, with an average of 2346 mg kg⁻¹ (Figure 4-35). There was decrease in the Na content of the leaves with an increase in the age of the cultivated site in the Seekoeivlei area, while no noticeable trend can be seen at the Nardouwsberg area (Figure 4-35). There is no significant difference in the Na content of the leaves between the different experimental sites (P = 0.0747).

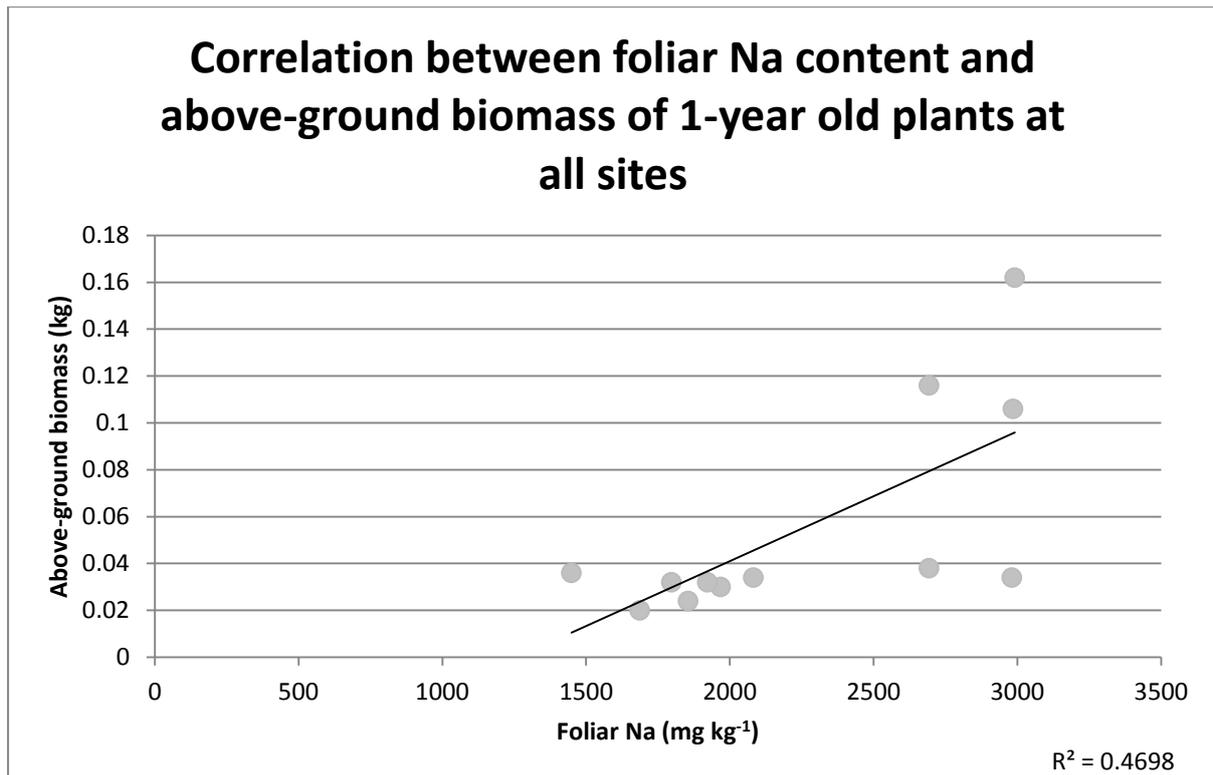


Figure 4-36: Correlation between foliar Na content and above-ground biomass of 1-year old plants at all sites

There was a moderate positive correlation between the foliar Na content and the above-ground biomass of the 1-year old plants ($R^2 = 0.4698$) (**Figure 4-36**). There was a positive correlation between the Na in the leaves and the above-ground growth on one year old plants in the Seekoivlei area ($R^2 = 0.4518$) (**Appendix B**). No correlation was found between the Na content of the soil and the Na content of the leaves (data not shown).

4.4.5 Tea yield and quality

4.4.5.1 Tea yield

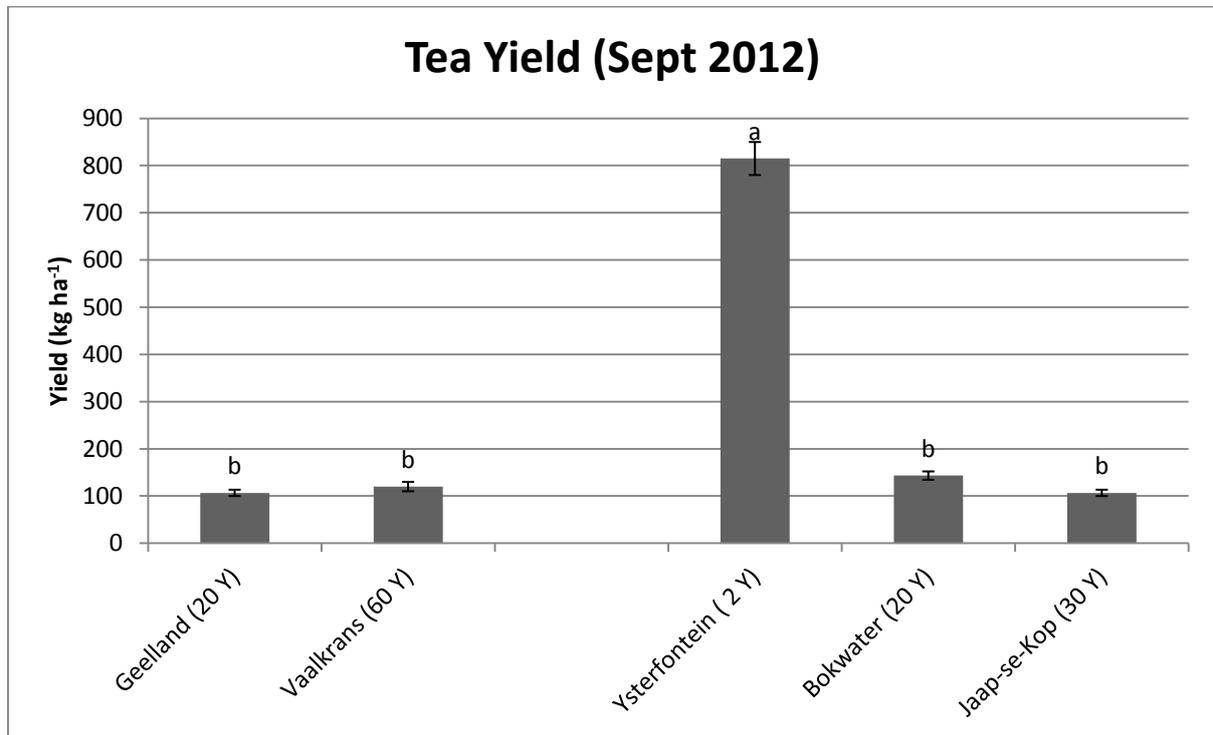


Figure 4-37: September tea yield for rooibos planted in 2011 ("Toptee") at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

A definite decrease was noticed with an increase in the age of the cultivated sites in the Seekoeivlei area. In the Seekoeivlei a drastic decrease was noticed between the Ysterfontein site that was recently cleared and the older cultivated sites (**Figure 4-37**). Not all of the sites were harvested, only the sites that were planted in 2011 (1-year old plants), and only the tip of the plant to insure that the plant forms a bush. The Muggiesdraai site was harvested in 2013 see (**Figure 4-38**), but not in 2012 (**Figure 4-37**). There was a significant difference in the "toptee" yields between the different experimental sites ($P < 0.0001$) in 2012, with Ysterfontein producing a higher yield than the other sites (**Figure 4-37**).

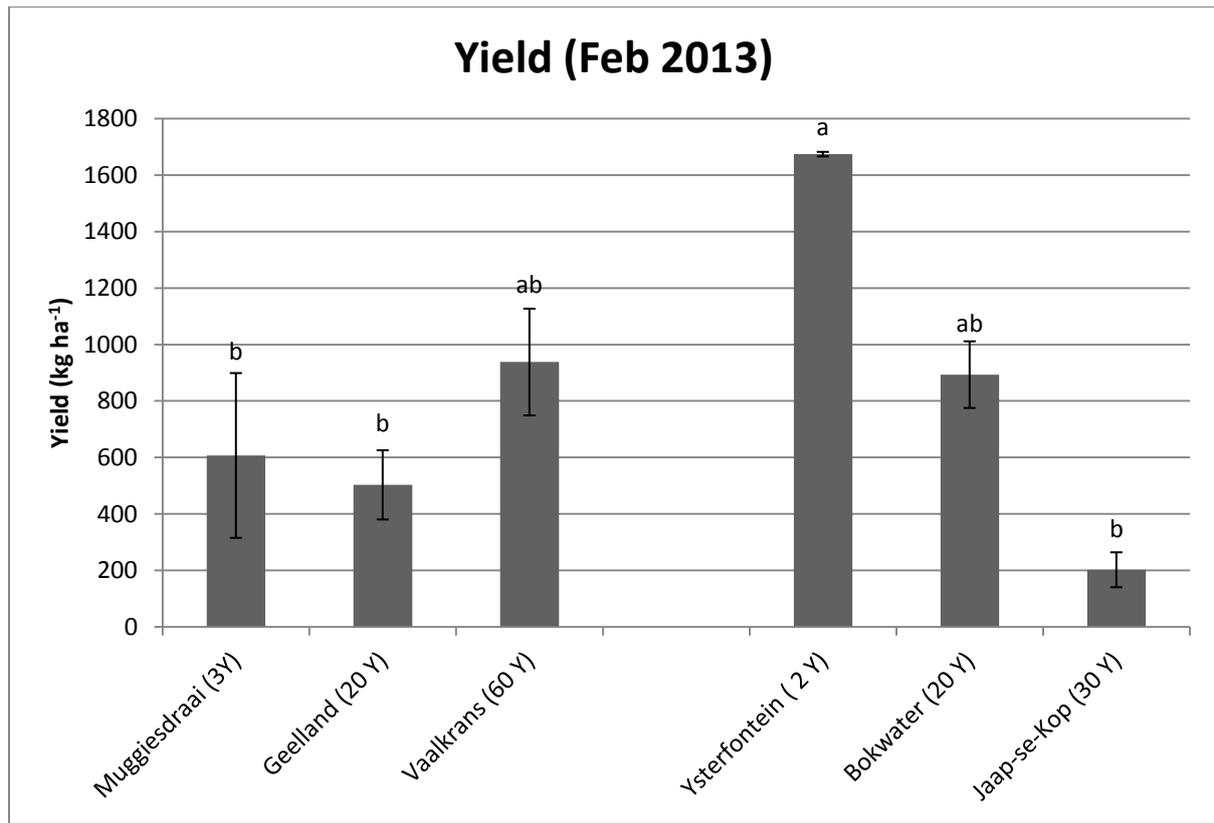


Figure 4-38: Rooibos tea yields harvested in February 2013 at the cultivated Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

A definite decrease in rooibos yield was noted with an increase in the age of the cultivated field in the Seekoeivlei area (**Figure 4-38**). There was a significant difference in the February yields between the different experimental sites ($P = 0.0059$). No trend was noted in the Nardouwsberg area. The Muggiesdraai site had a high standard error (**Figure 4-38**), as a result of the fact that there was a serious dieback at one of the M3 experimental sites in the Muggiesdraai experimental site. The yields in the virgin and fallow experimental sites could not be determined since only one plant was sampled per site.

4.4.5.2 Tea Quality

A significant difference was noticed in the dry colour between the Vaalkrans and the other sites ($P = 0.0044$), in both the Nardouwsberg, as well as Seekoeivlei area. No definite upwards or downward trends was noticeable between the different ages of the fields and the dry colour of the fermented rooibos tea (**Table 4-2**).

Table 4-2: Dry colour, wet colour, extract colour and taste quality of the rooibos tea harvested from the 1-year old plants at cultivated sites (Oct. 2012).

Sites	Wet Colour (%)	Dry Colour (%)	Extract Colour (%)	Taste (%)
Geelland (20 Y)	60 _a	60 _a	57 _a	60 _a
Vaalkrans (60 Y)	57 _a	40 _b	53	27 _b
Ysterfontein (2 Y)	55 _a	60 _a	60 _a	60 _a
Bokwater (20 Y)	60 _a	53 _a	53 _a	60 _a
Jaap-se-Kop (30 Y)	57 _a	57 _a	60 _a	60 _a

Note: The letters of significance differ between the experimental sites as well as between the different sensory aspects (wet-, dry-, extract-colour and taste)

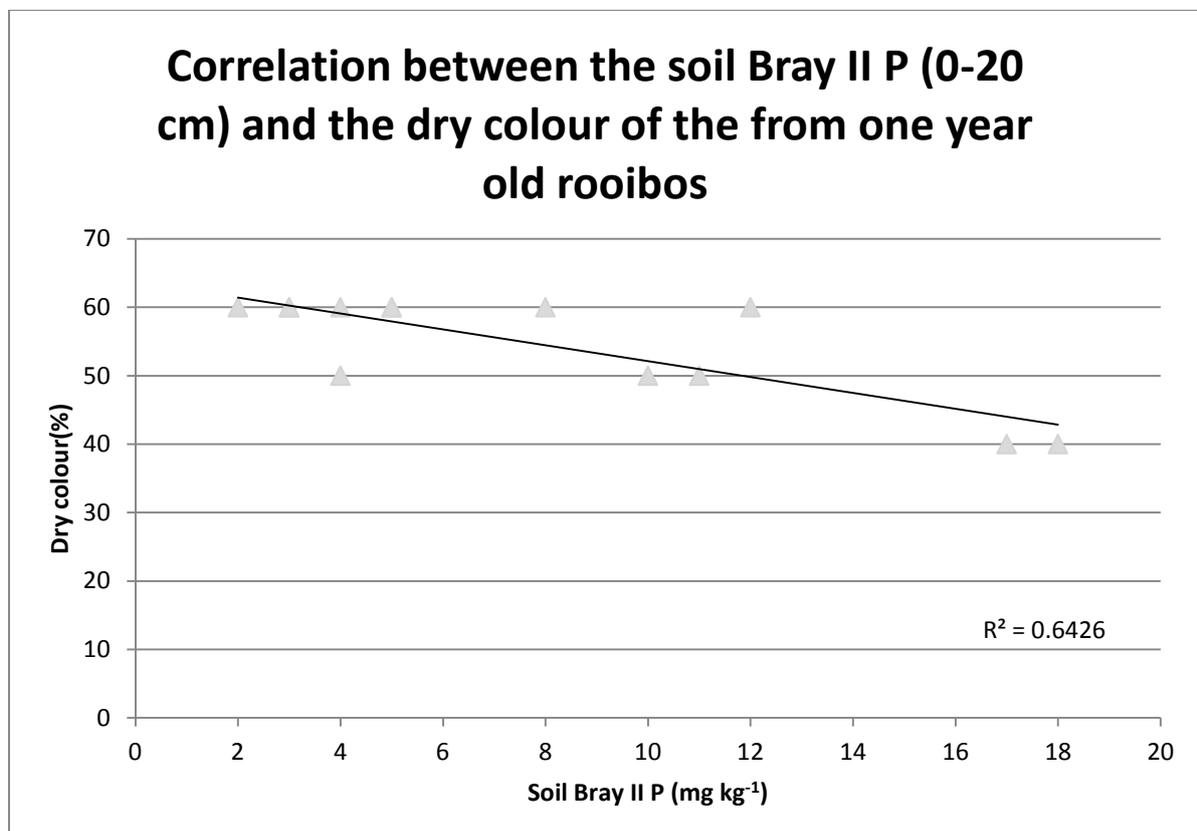


Figure 4-39: Correlation between the soil Bray II P (0-20 cm) and the dry colour of the rooibos tea of 1-year old plants

There was a negative correlation between the dry colour of fermented rooibos tea and the amount of plant-available P in the soil, in both the 0-20 cm ($R^2 = 0.6426$) (**Figure 4-39**) and the 20-40 cm soil layers ($R^2 = 0.4872$) (**Appendix B**). The reason for this correlation was most likely due to excess P in the soil and plant and giving rooibos the ability to bind

nutrients that enhance colour formation during the fermentation process, such as Fe, Zn and Ca. A decrease in the quality of the fermented rooibos tea has been observed in 4 of the 6 main rooibos producing areas (i.e., Nardouwsberg, Agterpakhuis, Seekoeivlei and Gifberg) by farmers that applied luxurious amounts of P fertilizer (personal communication).

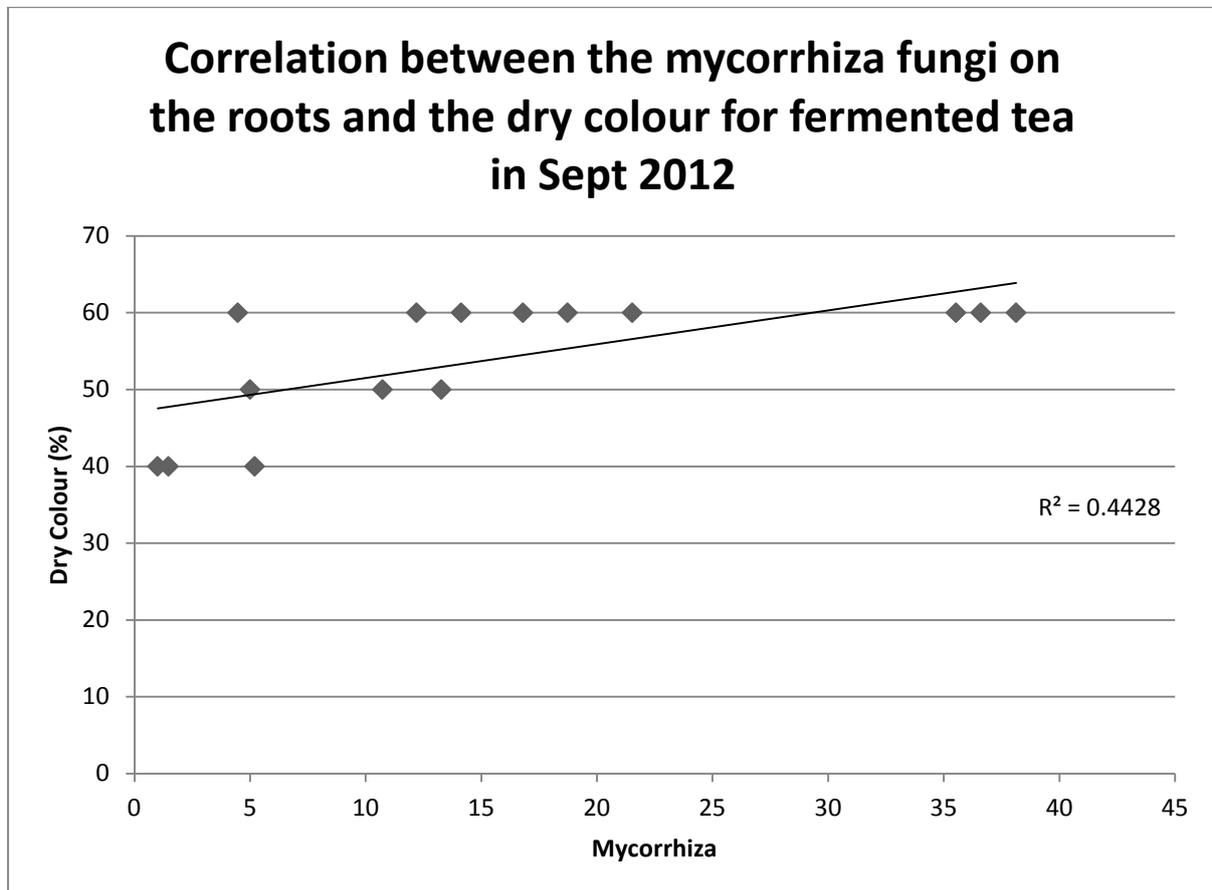


Figure 4-40: Correlation between the mycorrhiza fungi on the roots and the dry colour for fermented tea in Sept 2012

A positive correlation was found between the mycorrhiza on the roots of the plants and the dry colour of the fermented rooibos tea ($R^2 = 0.4428$) (Figure 4-40). The dry colour increases with an increase in the mycorrhiza hyphae on the roots. A high mycorrhiza count on the roots, can help the plant to be less susceptible to pathogens and drought ((Zeng, 2006); (Lehto, 1992)). The increase in dry colour can thus relate to the fact that the plant has sufficient water and nutrients and are overall healthier thereby indicating that the amount of mycorrhiza fungi on the root can be an indicator of plant health. There was a significant statistical correlation between the mycorrhiza on the roots and the dry colour of the fermented tea ($P = 0.0068$).

No significant differences or trends in the wet colour (**A significant** difference was noticed in the dry colour between the Vaalkrans and the other sites ($P = 0.0044$), in both the Nardouwsberg, as well as Seekoeivlei area. No definite upwards or downward trends was noticeable between the different ages of the fields and the dry colour of the fermented rooibos tea (**Table 4-2**).

Table 4-2) and extract colour (**A significant** difference was noticed in the dry colour between the Vaalkrans and the other sites ($P = 0.0044$), in both the Nardouwsberg, as well as Seekoeivlei area. No definite upwards or downward trends was noticeable between the different ages of the fields and the dry colour of the fermented rooibos tea (**Table 4-2**).

Table 4-2) can be noticed between the different sites, in both the Nardouwsberg and Seekoeivlei area ($P = 0.6004$) and ($P = 0.2155$). A significant difference was observed in the taste between the Vaalkrans and other sites, in both the Nardouwsberg, as well as Seekoeivlei area ($P < 0.0001$). There is also no definite trends was noticeable between the different ages of the fields and the taste of the fermented Rooibos tea. The low value for the Vaalkrans area was due to the fact that the rooibos tea developed a bad taste during the fermentation process due to external factors (motor oil patch on the store floor).

The rooibos tea produced in February 2013 was unfortunately under-fermented and the normal sensory qualification techniques could not be used. This can be the reason why the results did not show the same correlations as the results in 2012. No significant trends or correlations were found in the dry colour, wet colour, extract colour and taste (**Appendix B**). There were significant differences in dry colour, wet colour, extract colour and taste between the different experimental sites in both the Nardouwsberg and Seekoeivlei area ($P = 0.0052$), ($P = 0.0014$), ($P = 0.0201$) and ($P < 0.0001$).

4.5 Conclusions

The rooibos above- and below-ground biomass yields decreased with an increase in the age of the field used for rooibos cultivation. The decrease in the above- and below-ground biomass can be attributed to several factors. Negative correlations were found between the above-ground biomass and the following soil parameters; total soil carbon and the Bray II P content of the soil. The decrease in soil carbon had a couple of influences on the soil parameters viz. the exchangeable basic cations, protists and total microbial biomass

decreased with a decrease in soil carbon and it appears the combination of these parameters had a negative effect on the above-ground biomass ($R^2=0.5843$).

An exponential decrease in the above-and below-ground biomass was observed with an increase in the P content of the soil ($R^2=0.417$) and plant ($R^2=0.665$), the reason for this being that rooibos is adapted to low P conditions and is highly sensitive to P toxicity. The same increasing trend seen in soil P content with increasing age of the cultivated field, was observed in the foliar P content. The foliar P content at the older cultivated sites approaches the critical value where fynbos plants start to show toxicity symptoms ($> 0.1 \text{ mg kg}^{-1}$). The rooibos in the oldest cultivated fields showed some of the P toxicity symptoms described on Proteaceae viz. necrosis and chlorosis. A positive correlation was found between the foliar Mn and P content ($R^2=0.5544$), while a weak negative correlation was found between the foliar Fe and P content ($R^2=0.2272$), which correlates with the P toxicity symptoms in fynbos described by Hawkins et al (2007). A moderate positive correlation was found between the P in the leaves and the Ca content of the leaves ($R^2 = 0.4346$); the reason can be that the plant is trying to overcome the binding of the Ca in the epidermis. Even with the increase in the Ca, necrosis was still observed on the leaf tips. The increase in foliar Mn and decrease in foliar Fe most likely resulted in the chlorosis that was found on the leaves in the Vaalkrans experimental site, that contained the highest soil and foliar P content. It is thus likely, that P toxicity is one of the main reasons for the decrease in the yields of the rooibos. A negative correlation was also found between the soil P content and the dry colour of the fermented rooibos tea ($R^2=0.6426$). The dry colour forms part of the sensory quality test and the decrease in dry colour had a negative influence on the quality and farmers get paid less for their final product.

The higher P content in the soil had a negative influence on the mycorrhiza colonization of the rooibos plant. An exponential decrease in the mycorrhiza fungi on the roots was noted with an increase in the P content of the soil ($R^2=0.8134$). Mycorrhiza are known to help the plant to be more drought resistant and pathogen resistant, the decrease in the mycorrhiza can be one of the reasons for the fact that the rooibos plant cycle decreased from eight years to five years before the plant has to be removed and new rooibos must be planted. A positive correlation was found between the mycorrhiza fungi on the roots and the dry colour of the fermented rooibos tea ($R^2=0.4428$). The decrease in mycorrhiza showed a

decrease in the dry colour of the fermented rooibos tea. The rooibos plants showed P toxicity and reduced growth in fields with a high P content. The high P content had a negative effect on the mycorrhiza colonization and the dry colour of the fermented rooibos tea.

In the Seekoeivlei area an increase in the N-nodules was found with an increase in the age of the cultivated field. It appears that the plants in continuously cultivated fields showed a greater dependence on N-fixation. During this study however it was not determined whether the N-nodules on the roots was active or not, only how many was present. No significant differences were noted in the N content of the leaves between the different experimental sites and it would appear that the plants had sufficient N, whether by N uptake from the soil or from symbiotically fixed N.

A negative correlation was noted between the foliar Cu content and the above-ground biomass in the Nardouwsberg area ($R^2=0.8224$), but not in the Seekoeivlei area. While a positive correlation was found between the foliar B content and the above-ground biomass for one 1-year old plants ($R^2=0.952$). The other micro nutrients Zn, Fe and Mn showed no positive or negative correlation with the above-ground biomass. A positive correlation was found between the Na in the leaves of the 1-year old plants and the above-ground biomass ($R^2=0.4698$); it would appear that Na could be important for rooibos growth in the first year.

The precise critical threshold value for soil and foliar P for rooibos should be determined in future studies to know exactly when the addition of P will have a negative effect on the yields and quality. The effect of molybdenum in the soil and plant should be studied since it plays an important role in photosynthesis process as well as in the process of forming N-nodules in legume plants. The nutrient values in different ages of the plant should be studied to see whether different nutrients is needed in different years of cultivation and also to see whether other nutrients is necessary in the next year.

5 RESEARCH CHAPTER 5: THE EFFECT OF COMPOSTS AND FOLIAR SPRAYS ON ROOIBOS TEA PRODUCTION AND QUALITY

5.1 Introduction

Most of the previous fertilizer studies on rooibos investigated the effects of additions of macronutrients N, P, K, Ca and Mg in greenhouse trials on young plants (Joubert *et al.*, 1987; Muofhe, 1997; Muofhe & Dakora, 1999). The results reported by Joubert *et al.*, 1987 showed at a concentration of 15-20 mg kg⁻¹ Bray II extractable P optimum growth was found. Growth decreased significantly if the P concentration was higher. The optimum concentration of K was found at 60 mg kg⁻¹ Bray II extractable K. The optimum N application was found to be at 10 – 15 mg N kg⁻¹ of soil. These results were dependant on the amount of Mg in the soil. Application of Mg to the rooibos plants reduced Ca, N, P and K uptake (Joubert *et al.* 1987) and is thus was not recommended. No published studies could be found on the application of rooibos plant litter compost to commercially produced rooibos tea.

Several changes were observed in both the soil quality and plant quality in the previous chapters. Certain elements show decreases, for an example the % soil C. The decrease in carbon also had an effect on the decrease in the CEC, since there were less exchange sites for the basic cations to bind to. The microbial activity also decreased with a decrease in the carbon in the soil. Many of the changes in the soil quality can thus relate to a decrease in the soil carbon. Therefore, two different composts were applied to the older experimental sites in the Nardouwsberg area on the farm Vaalkrans since the soils have been depleted of most nutrients. Correlations were found between micronutrients (Cu and B) in the plant and plant quality. Therefore, two micronutrients foliar sprays, were thus applied to try and improve the above-ground yield and the fermented tea dry colour.

5.2 Objectives

The soil factors for the decline in growth were identified in the previous chapters. Therefore, the aim of this study was firstly to evaluate the addition of locally-produced rooibos compost on soil health and plant quality. The second aim of the study was to evaluate the effect of foliar sprays to supplement nutrient deficiencies.

5.3 Methods and materials

Two compost treatments and two foliar spray treatments, each at two different application rates, were applied to 2-year old rooibos plants at the oldest cultivated site, Vaalkrans in December 2012 (**Figure 5-1**). The different treatments were: 1) a compost made from rooibos twigs and chicken litter (RR+CL), 2) a compost made only from rooibos twigs and leaves (RR), 3) a Mazinbor foliar spray containing the trace elements, manganese, zinc and boron (**Table 5-1**), 4) a Goëmar Plus 86 foliar spray (**Table 5-2**) and 5) a control with no composts or foliar spray. The chemical and physical characteristics of the composts (**Table 5-3**), and chemical analysis of the groundwater used to make up the foliar sprays (**Table 5-4**), were determined by Bemlab Ltd, Somerset West.

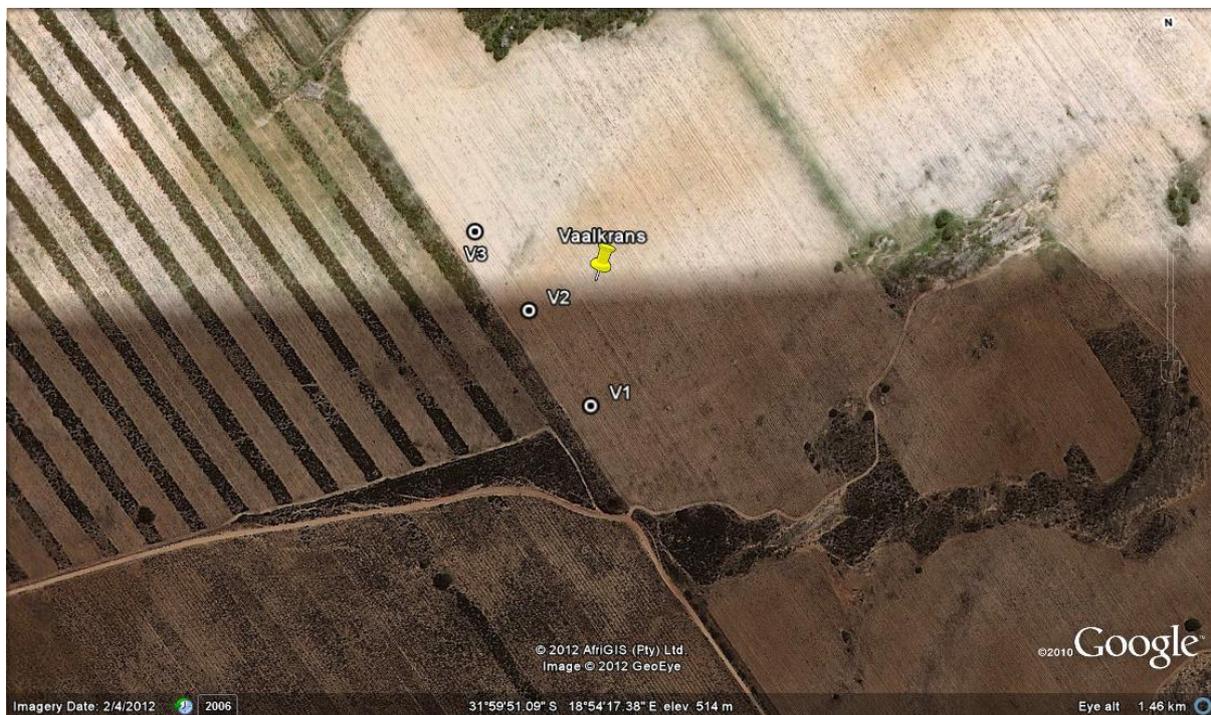


Figure 5-1: Google earth image of the 3 experimental sites on Vaalkrans

The 10 x 10 m sampling sites were divided into 12 equal blocks where the treatments were applied (**Figure 5-2**). The composts were applied at 2 ton ha⁻¹, and a 20 ton ha⁻¹. The compost were applied on the soil surface and dug into a depth of 20 cm using a spade (**Figure 5-3**). The Marzinbor foliar spray was applied at 2 l ha⁻¹ (ratio: 4.5 ml Mazinbor to 3 l of water) and at 10 l ha⁻¹ (ratio: 6 ml Mazinbor to 3 l of water), while the Goëmar foliar spray was applied at 2 l ha⁻¹ (ratio: 7.5 ml Goëmar to 3 l of water) and 4 l ha⁻¹ (ratio: 15 ml Goëmar to 3 l of water), according to recommendations given by the representative who supplied

the foliar sprays. The Marzinbor and Goëmar foliar sprays were applied twice within a seven day interval, the first application was on 10 December 2012 and the second application was on 17 December 2012. The compost were applied on 10 December 2012 along with the first foliar spray application.

The soil and biomass sampling was performed in June 2013, six months after the application of the treatments. The soil chemical and microbiological parameters (Total C & N, pH, EC, exchangeable cations, plant-available micro nutrients, total microbial biomass), as well as, the plant parameters (total above- and below-ground biomass, N-nodules, mycorrhiza, macro and micro nutrients) were determined as described in **Chapter 3 and Chapter 4**. The tea was harvested (for tea yield and tea quality determination) on the 20 Feb. 2013, only three months after the application of the treatments, this was done because of the tight schedule of the MSc study. The tea quality was analysed as previously described in **Chapter 3**, the only difference was in the classification of fermented rooibos quality at Rooibos Ltd. The fermented tea was under fermented since small amounts of tea were used in the fermenting process, so the normal procedures for obtaining the quality could not be used as described in **Chapter 4**. A new system was thus designed for the classification in the treatments. The tea was placed in a line and compared to each other by a panel consisting of three Rooibos Ltd employees and given a mark out of five to calculate the percentage of each of the different sensory aspects.

Table 5-1: Mazinbor chemical composition (Ag-Chem, 2013)

Product	Formulation	Reg. no	Compilation (g l ⁻¹)			
			Ca	Mn	Zn	B
Mazinbor	Suspension Conc.	K7542	133	45	90	91

Table 5-2: Goëmar BM 86 derived from urea, magnesium sulphate, boric acid, sodium molybdate (Agrimar, 2012)

Product	Formulation	Reg. no	Compilation (%)					
			N	Mg	S	B	Mo	Na
Goëmar BM 86	Foliar Nutritional	GA14	5.0	2.4	3.2	2.0	0.02	0.6

Table 5-3: Characteristics of the rooibos + chicken litter and rooibos residues composts

Compost	pH	Resis- tance (ohm)	Water %	Density kg/m ³	N %	P %	K %	Ca %	Mg %	Na %	Mn mg/kg	Fe mg/kg	Cu mg/kg	Zn mg/kg	B mg/kg	C %	Ash %
Chicken litter + rooibos	6.45	90	46.05	552.55	1.82	0.65	0.73	1.045	0.38	0.14	186.42	2156.40	29.58	205.09	30.44	22.15	55.85
Rooibos plant litter	5.20	125	17.45	115.25	1.54	0.05	0.37	0.25	0.21	0.20	98.36	2600.29	3.66	64.55	64.55	41.24	6.13

Table 5-4: Analysis of the water used in the foliar sprays

Water	pH	EC mS/m	Na	K	Ca	Mg	Fe	Cl	CO ₃	HCO ₃	SO ₄	B	Mn	Cu	Zn	P	NH ₄ -N	NO ₃ -N	F	TDS
Muggiesdraai	5.7	168	156.8	8.4	22.6	68.2	0.04	513.7	16.8	43	0.11	0.10	0.06	0.32	0	0.05	2.78	0	1072	

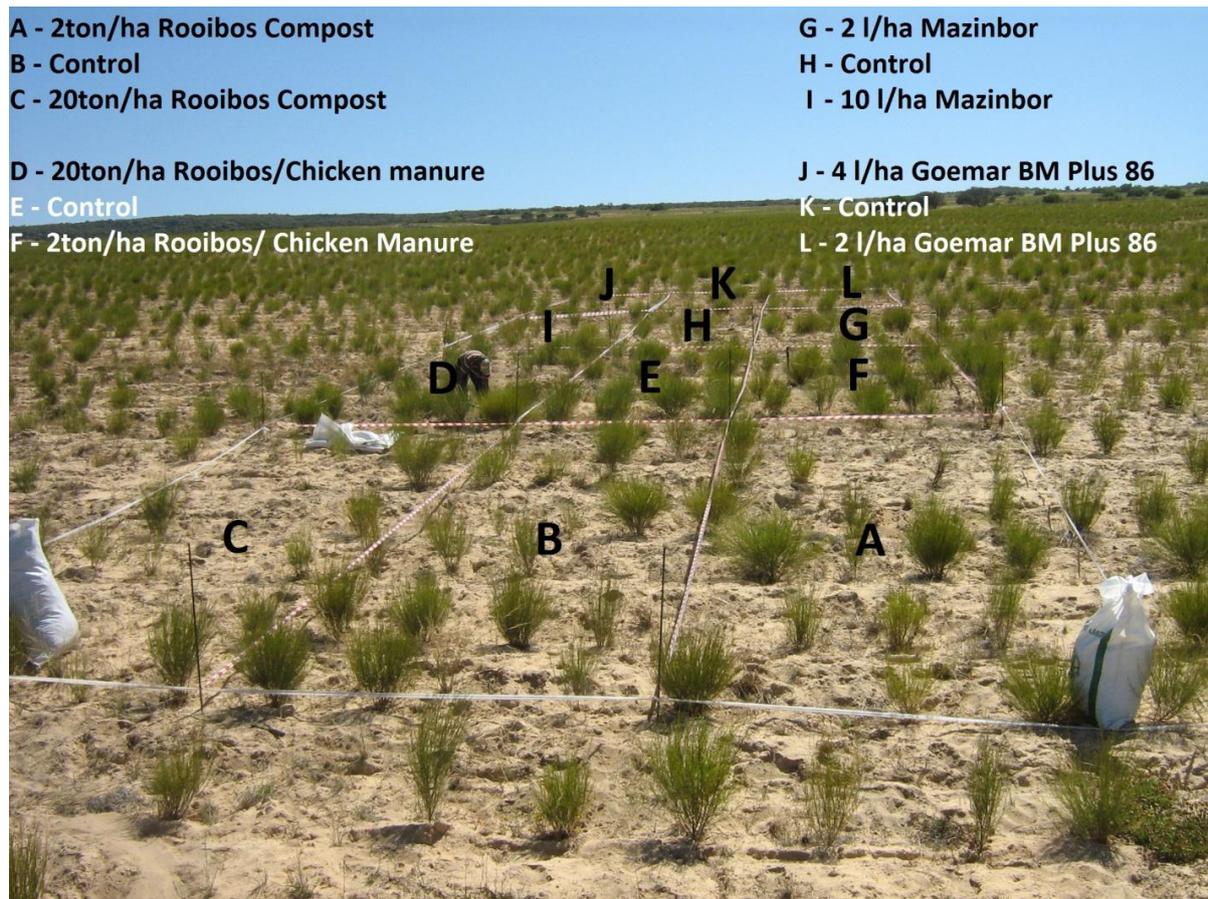


Figure 5-2 : Experimental design for the different treatments

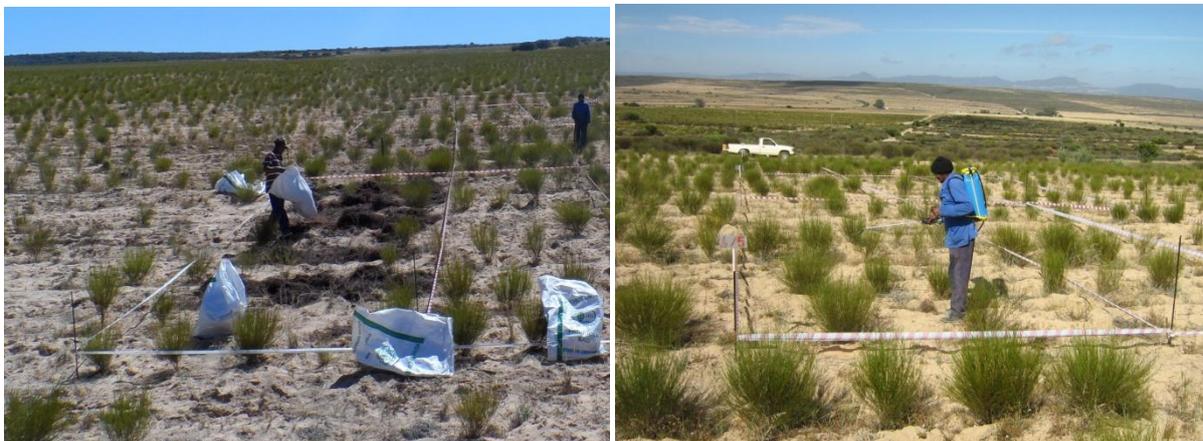


Figure 5-3 : Rooibos compost (20 ton ha^{-1}) application to one of the experimental sites (Left). Foliar applications to one of the experimental sites (Right)

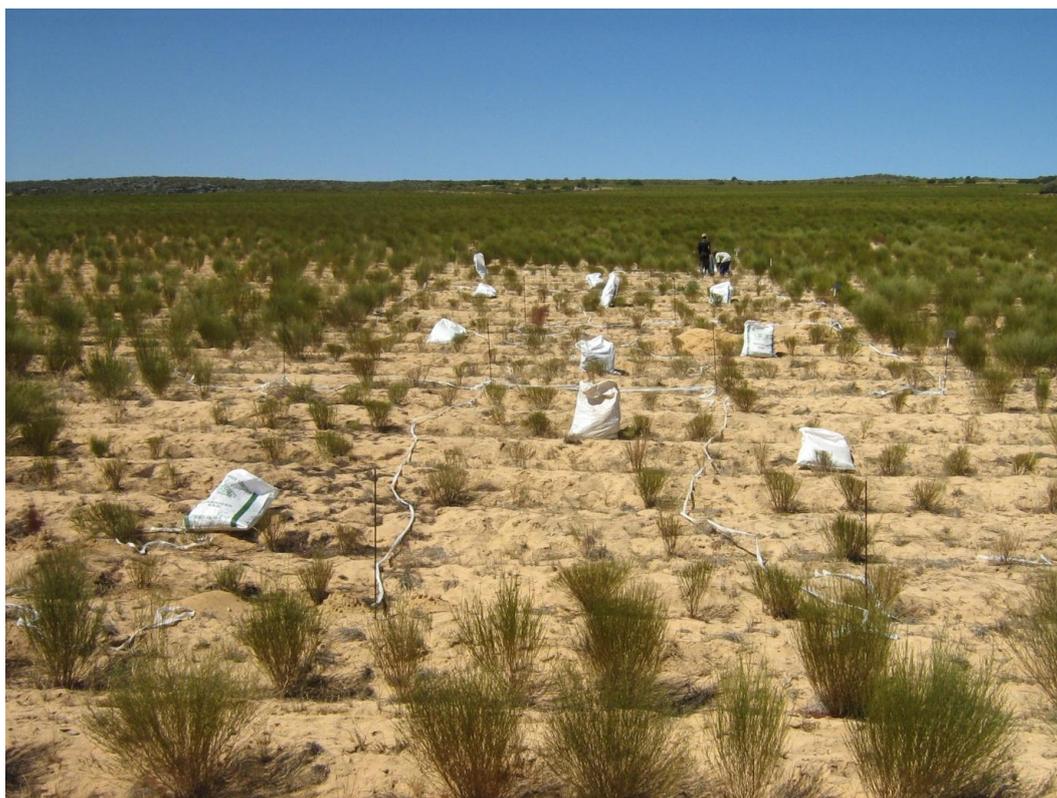


Figure 5-4: Harvesting of rooibos tea on the 20 Feb 2013 for the determination of quality and yield

5.4 Results and discussion

5.4.1 Soil Analysis

Only the control and compost treatment's soil data will be presented, as the foliar sprays were applied directly to the plants.

5.4.1.1 Soil Carbon

No significant changes in soil C were found due to the addition of the compost treatments to the soil (**Figure 5-5**). A slight increase in the 0-20 cm soil C was found in the rooibos plant litter compost treatments. Higher increases were expected since compost was applied directly in the 0-20 cm layer. A slight increase in 20-40 cm soil C was found in rooibos plant litter compost treatments and the 2 t ha⁻¹ rooibos + chicken litter compost treatment. This could be due to an increase in root biomass at this depth due to the compost application and the mixing of organic matter in the soil by insects. No significant differences in the soil carbon was noted between the different treatments for both the 0-20 cm (P= 0.4451) and the 20-40 cm (P = 0.1876) layers.

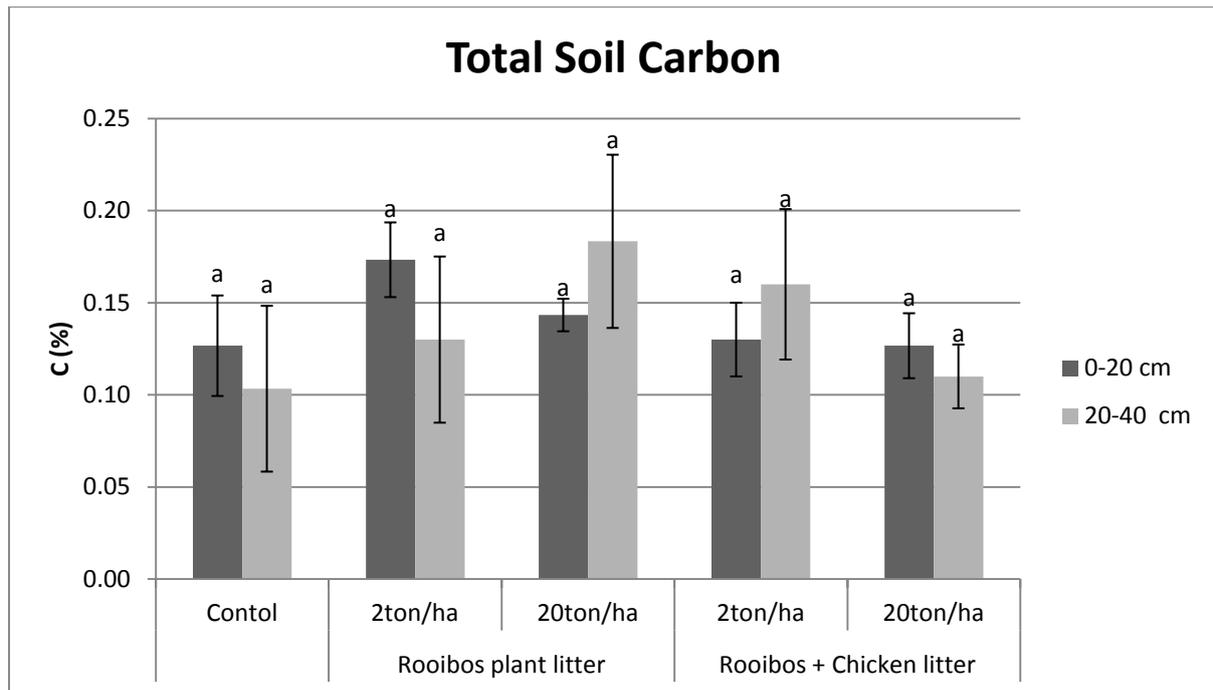


Figure 5-5: Total soil Carbon values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the

5.4.1.2 Soil pH

The pH values measured in water and 1M KCl of the control and compost treated soils (**Figure 5-6 & Figure 5-7**) are similar to the previous pH values reported in **Chapter 3 (Figure 3-14 & Figure 3-15)**. No significant differences in the pH in water was noted between the different treatments for both the 0-20 cm ($P = 0.0752$) and the 20-40 cm ($P = 0.4320$) layers. However, there was a slight increase in the soil pH (H_2O) in the 20 t ha⁻¹ compost treatments (**Figure 5-6**). No significant differences in the pH (KCl) was noted between the different treatments for both the 0-20 cm ($P = 0.1427$) and the 20-40 cm ($P = 0.4503$) layers. The same trend was seen in the pH (KCl) values (**Figure 5-7**); an increase in the pH (KCl) was noticed in the 20 ton ha⁻¹ treatment for the rooibos and rooibos + chicken litter compost treatments. The reason for the increase in the pH in the 20 ton ha⁻¹ rooibos and chicken litter compost treatment was due to the high pH of the compost (6.45) (**Table 5-3**) in comparison to the soil pH (**Figure 5-6**). The increase in the soil pH of the 20 ton ha⁻¹ rooibos compost treatment may be due to plant response since the pH (H_2O) of the compost (5.2) (**Table 5-3**) was lower than the pH of the soil (5.5-5.8) (**Figure 5-6**).

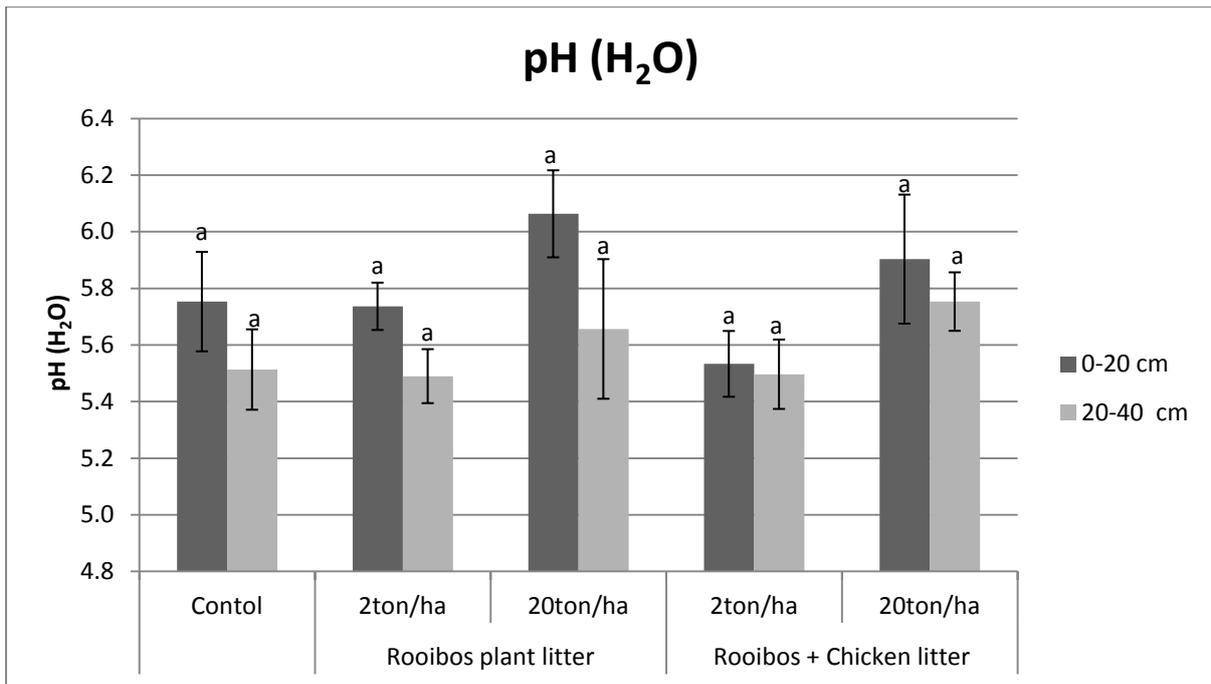


Figure 5-6: pH (H₂O) values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

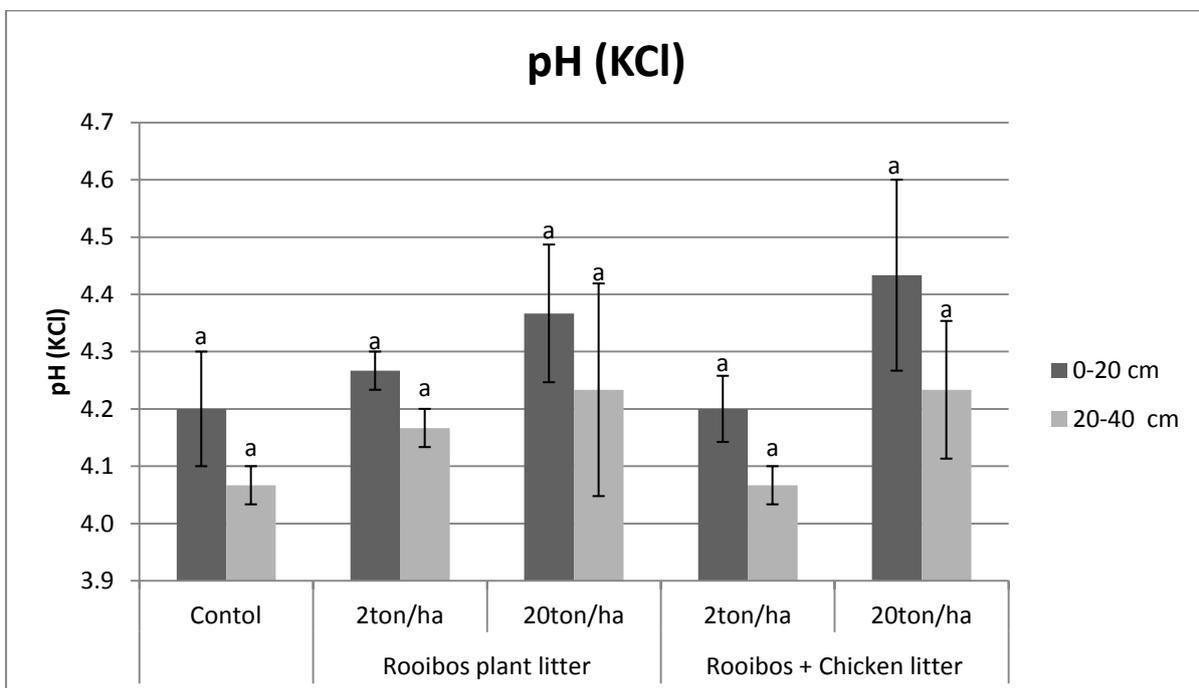


Figure 5-7: pH (KCl) values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

5.4.1.3 Electrical Conductivity (EC)

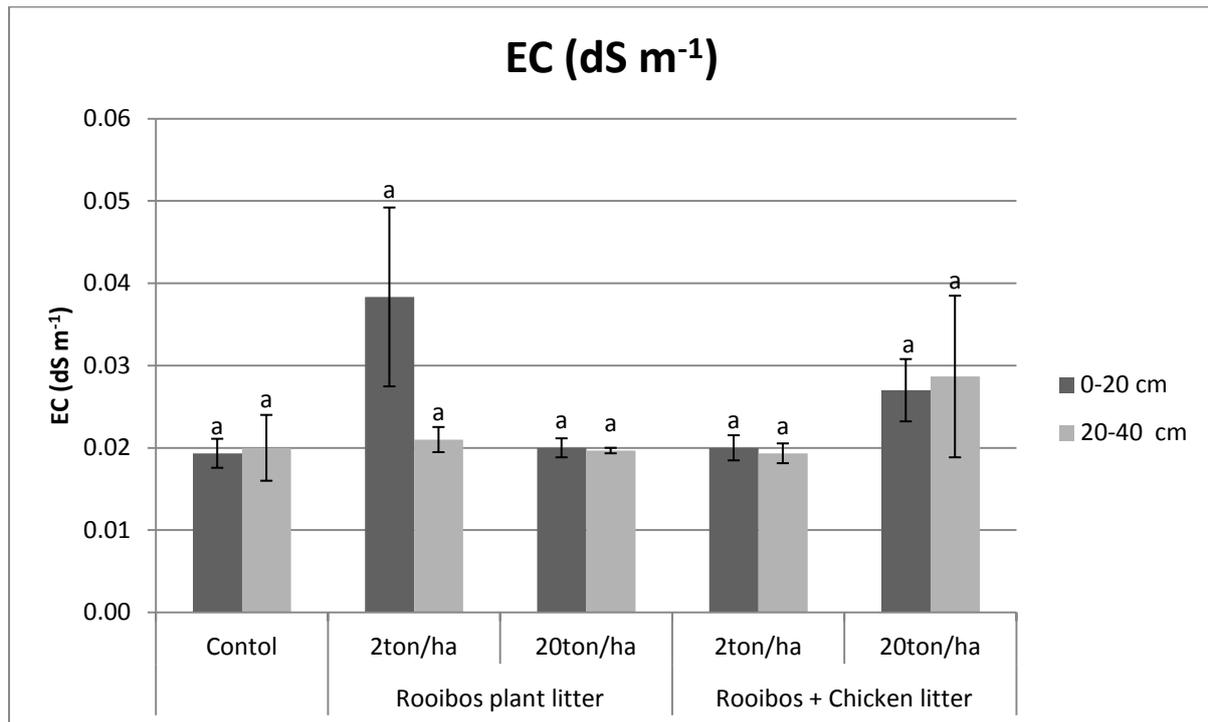


Figure 5-8: Electrical conductivity values of the control and compost treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown ($n = 3$). The letters of significance differ between the treatments as well as the depth

An increase in the EC was noticed in the 0-20 cm layer in the rooibos residues compost treatment, as well as, a slight increase in the 0-20 cm and 20-40 cm layer in the rooibos and chicken litter compost treatment (**Figure 5-8**). No significant statistical differences in the EC was noted between the different treatments for both the 0-20 cm ($P = 0.1944$) and the 20-40 cm ($P = 0.4662$) layers.

5.4.1.4 Total microbial biomass

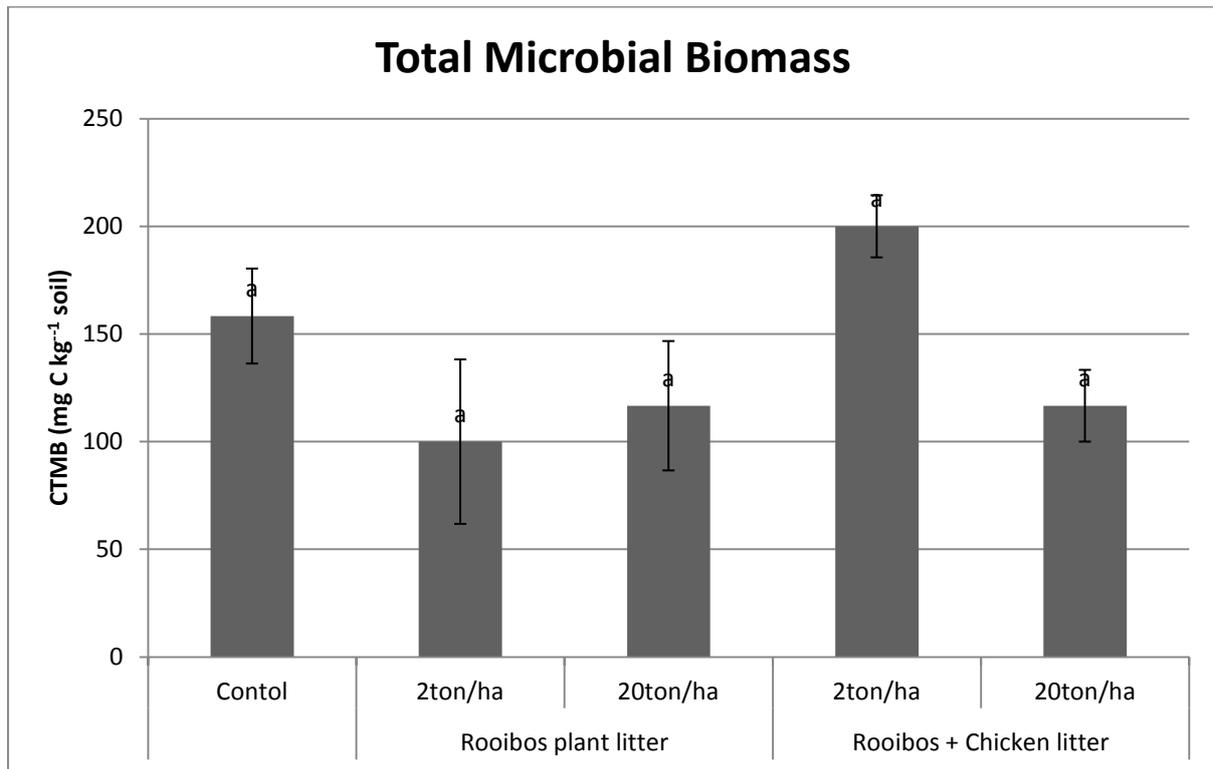


Figure 5-9: Total microbial biomass of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown ($n = 3$). The letters of significance differ between the experimental sites

The total microbial biomass values correlates with the values found in Chapter 3 (Figure 3-36). The 2 ton ha⁻¹ rooibos + chicken litter compost treatment had a higher total microbial biomass while a decrease in the microbial biomass was noticed in the rooibos plant litter compost (Figure 5-9). No significant differences in the total microbial biomass were noted between the different treatments ($P = 0.5195$).

5.4.1.5 Exchangeable basic cations

Total Basic Cations

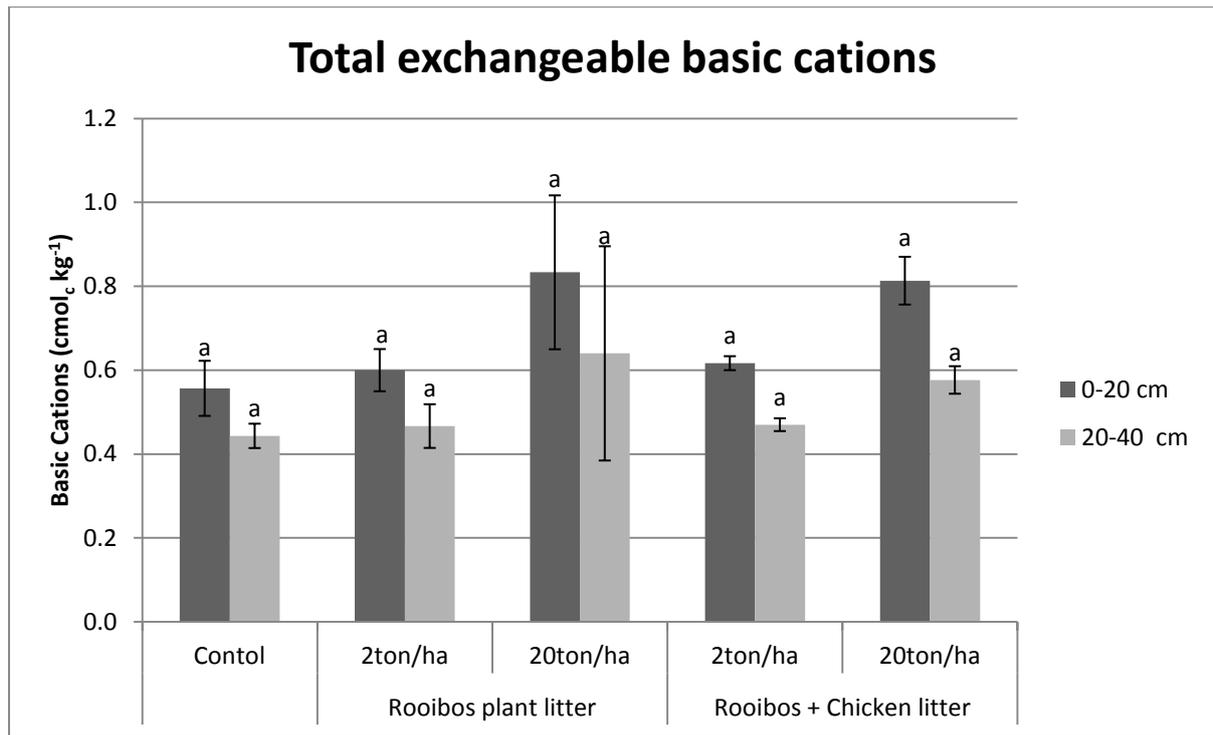


Figure 5-10: Total exchangeable basic cations values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

A slight increase in the total exchangeable basic cations was noted in treatments where compost was applied (**Figure 5-10**) although no significant differences occurred in the 0-20 cm ($P = 0.1620$) and 20-40 cm ($P = 0.7089$) layers.. This was due to the fact that the Ca (**Figure 5-11**), K (**Figure 5-12**) and Mg (**Figure 5-13**) showed the same trend. This was possibly due to an increase in organic matter and the compost was relative high in exchangeable cations especially the Ca, Mg and K (**Table 5-3**).

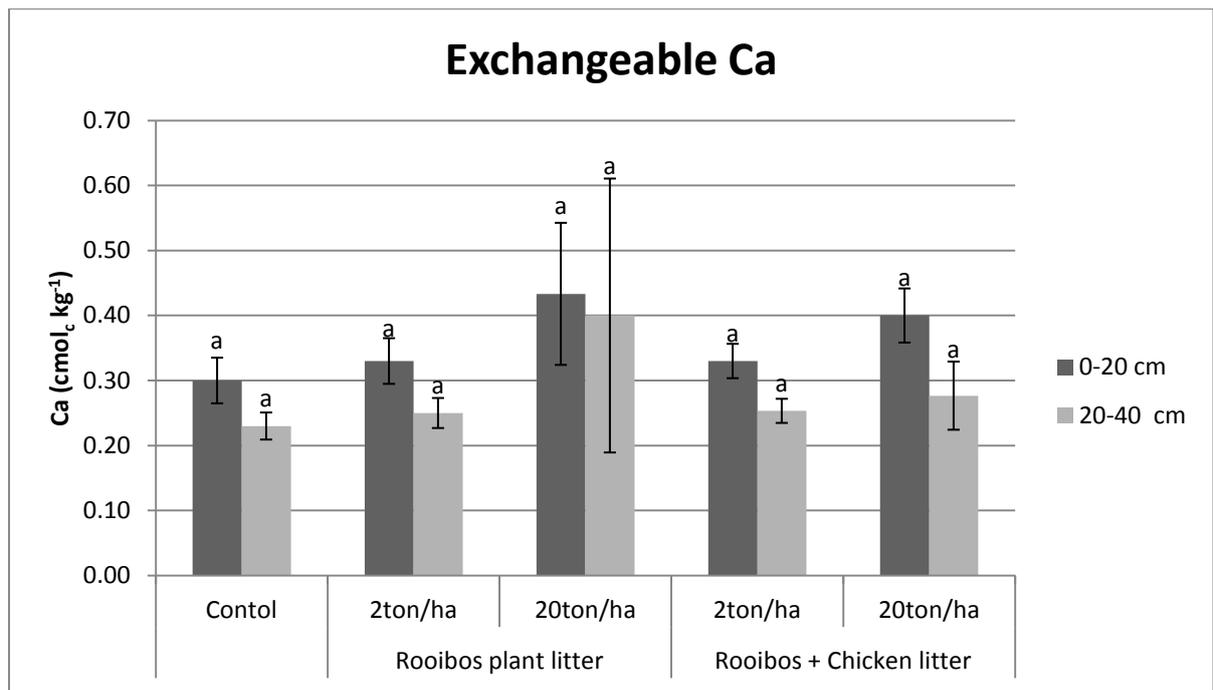


Figure 5-11: Soil exchangeable Ca values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

The values correlate well with the exchangeable Ca content of the soil in Chapter 3 (**Figure 3-20**). A slight increase was observed for both compost treatments (**Figure 5-11**) although no significant differences occurred in both the 0-20 cm (P = 0.3819) and 20-40 cm layers (P = 0.7114).

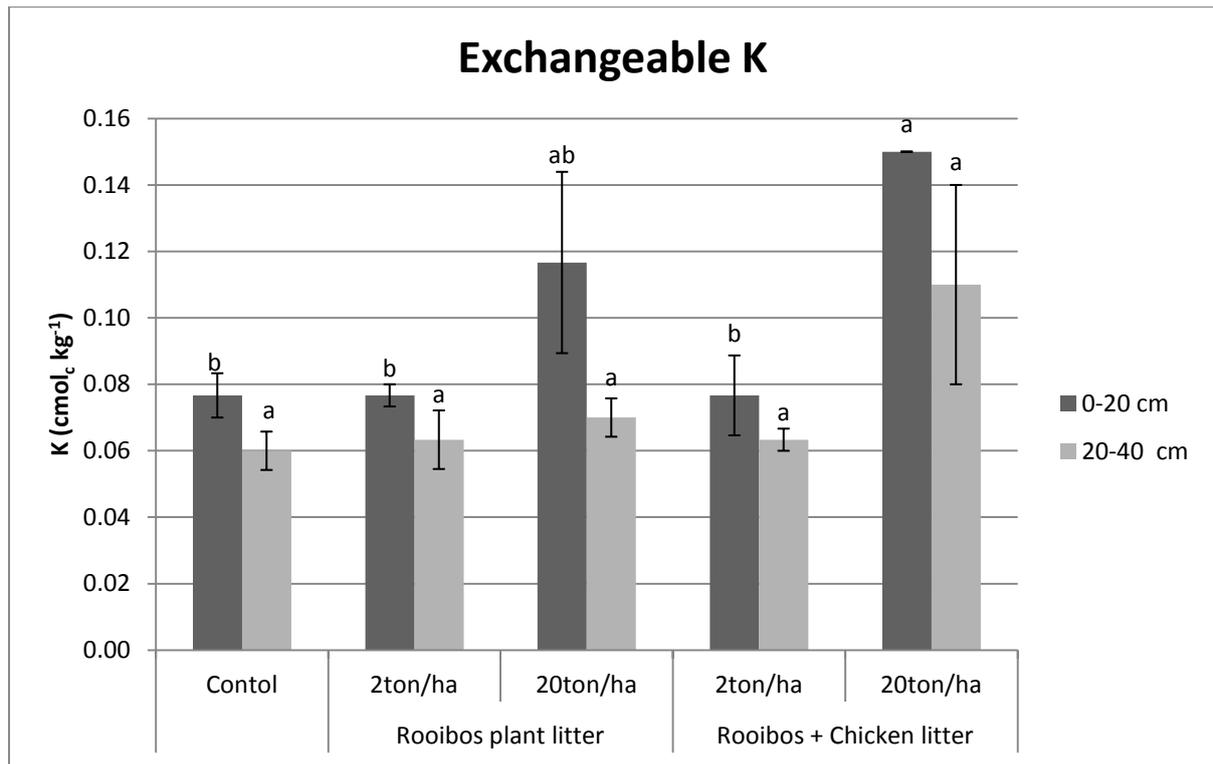


Figure 5-12: Soil exchangeable K values of the control and compost treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

The values correlate well with the exchangeable K content of the soil in Chapter 3 (**Figure 3-19**). The same trend was noticed in the compost treatments for the exchangeable K as in the exchangeable Ca. An increase was noticed with an increase in the compost for both treatments (**Figure 5-13**). No significant difference in K was found in the 20-40 cm ($P = 0.1827$), but in the 0-20 cm layers ($P = 0.0242$) differences was noticed.

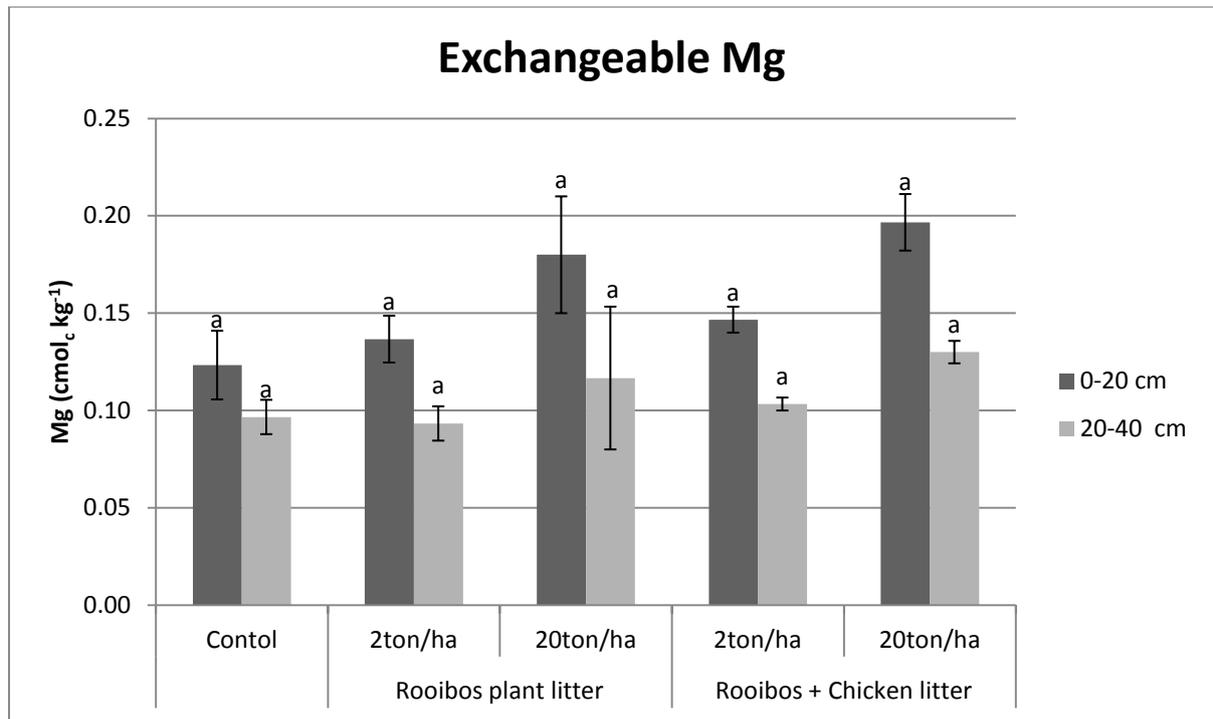


Figure 5-13: Soil exchangeable Mg values of the control and compost treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

The values correlate well with the exchangeable Mg content of the soil in Chapter 3 (**Figure 3-21**). The same trend was noticed in the exchangeable Mg as in the exchangeable Ca. An increase in the exchangeable Mg content was noticed with an increase in the compost for both treatments (**Figure 5-13**) although no statistical significant difference in the Mg were found between the different treatments in both the 0-20 cm ($P = 0.0521$) and 20-40 cm layers ($P = 0.5198$). The increase in the Ca, K and Mg was due to an increase in the Ca, K and Mg content of the two compost treatments as well as a increase in the exchange sites (**Figure 5-15**); both the composts had a relative high Ca (1.045 RR+CL & 0.25 % RR), K (0.725 RR+CL & 0.37 % RR) and Mg content (0.38 RR+CL & 0.21 % RR) (**Table 5-3**) in comparison to the soil thus resulted in the increase.

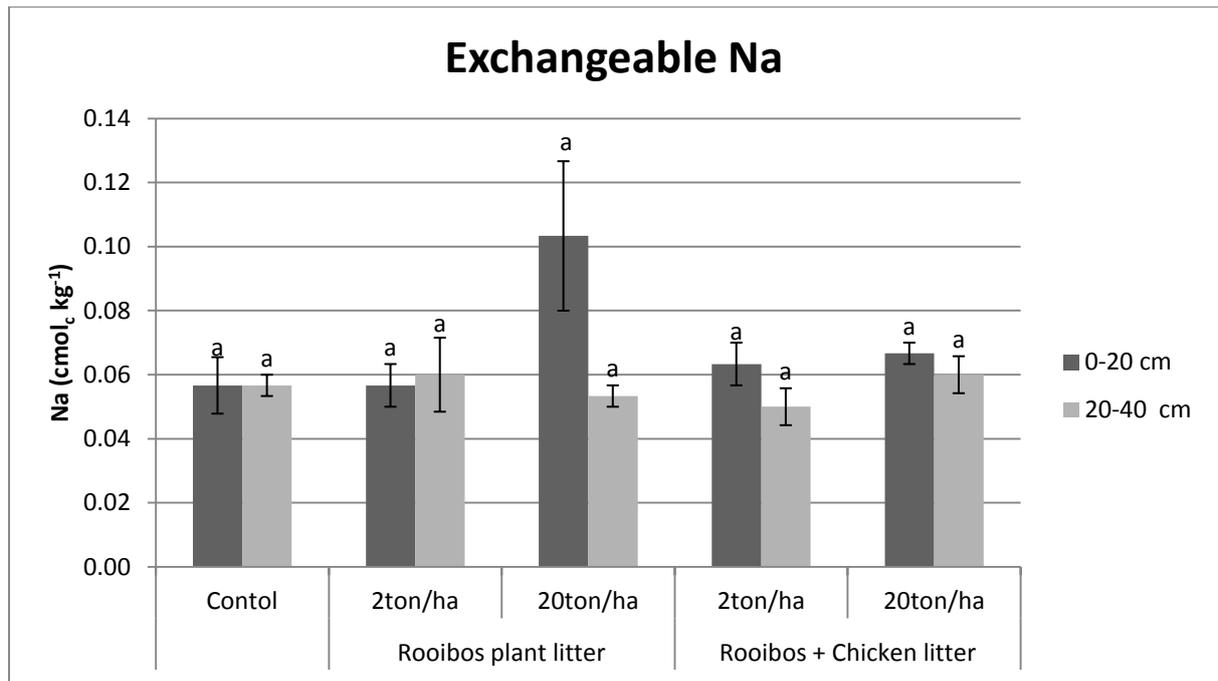


Figure 5-14: Soil exchangeable Na values of the control and compost treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

No significant difference in the Na were found between the different treatments in both the 0-20 cm ($P = 0.5044$) and 20-40 cm layers ($P = 0.7078$). The high Na content found in the 0-20 cm layer of the 20 ton ha⁻¹ rooibos compost treatment (**Figure 5-14**) was possibly due to the fact that the compost had a high Na content (0.20 %) (**Table 5-3**).

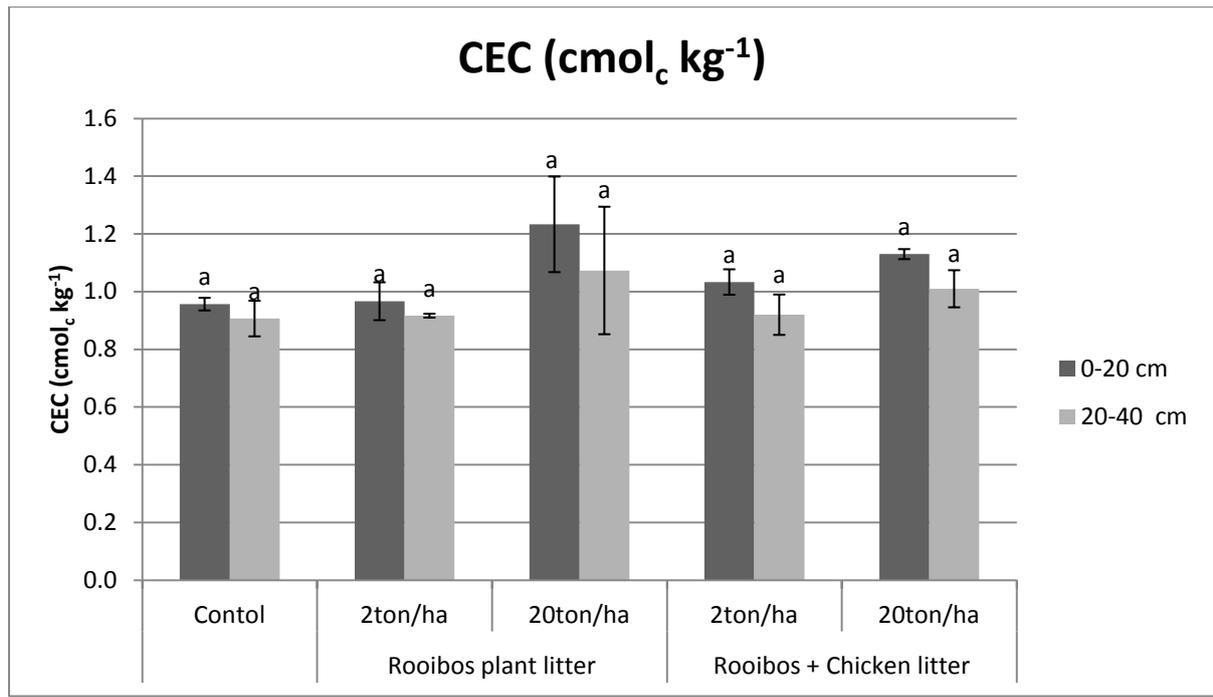


Figure 5-15: Cation exchange capacity (CEC) values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

A slight increase was noticed in the 20 ton ha⁻¹ treatment for both compost applications (**Figure 5-15**), although no significant differences in the CEC was noted between the different treatments for both the 0-20 cm (P= 0.2249) and the 20-40 cm (P = 0.7737) layers. The slight increase in the CEC was attributed to the slight increase inorganic carbon (**Figure 5-5**).

5.4.1.6 Macro nutrients (N & P)

Nitrogen

The total soil N values measured in the control and compost treated soils (**Figure 5-16**) are similar to the previous total soil N values reported in Chapter 3 (**Figure 3-27**). However, there was a slight increase in the total soil N in the 20 t ha⁻¹ compost treatments (**Figure 5-16**). This can be attributed to a relative high N content (1.54 % & 1.82 %) content of the compost applied (**Table 5-3**). No significant difference in the total N were found between the different treatments in both the 0-20 cm (P = 0.7880) and 20-40 cm layers (P = 0.6789).

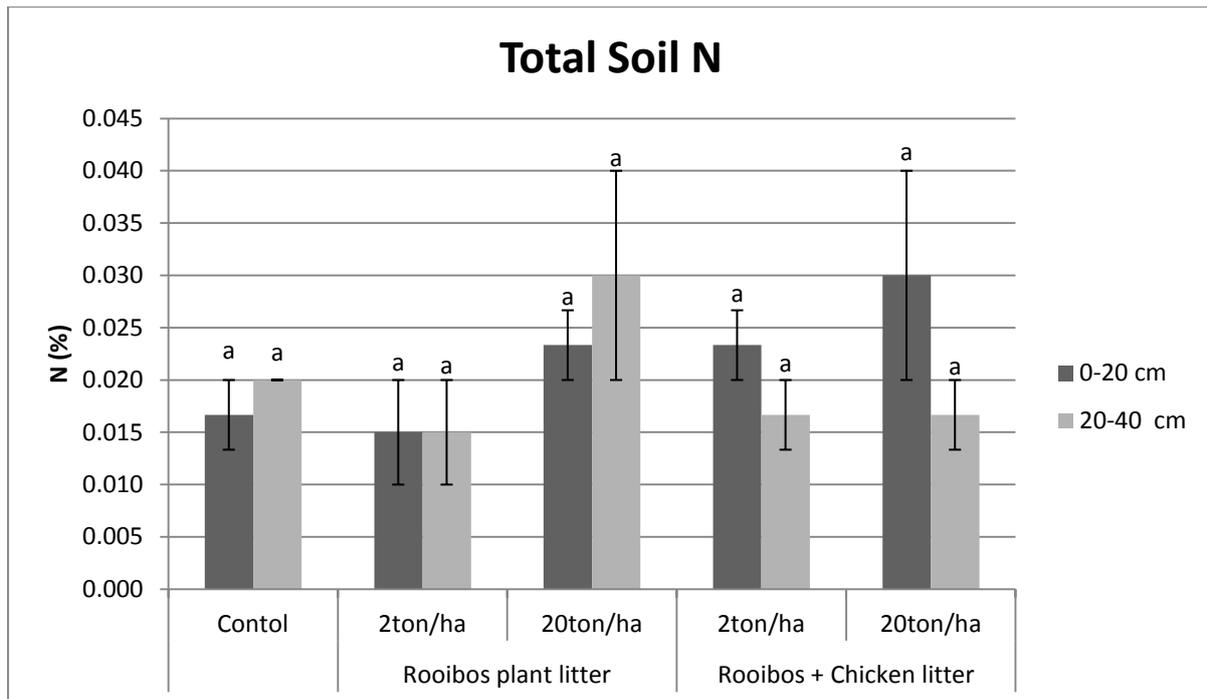


Figure 5-16: Total soil N values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

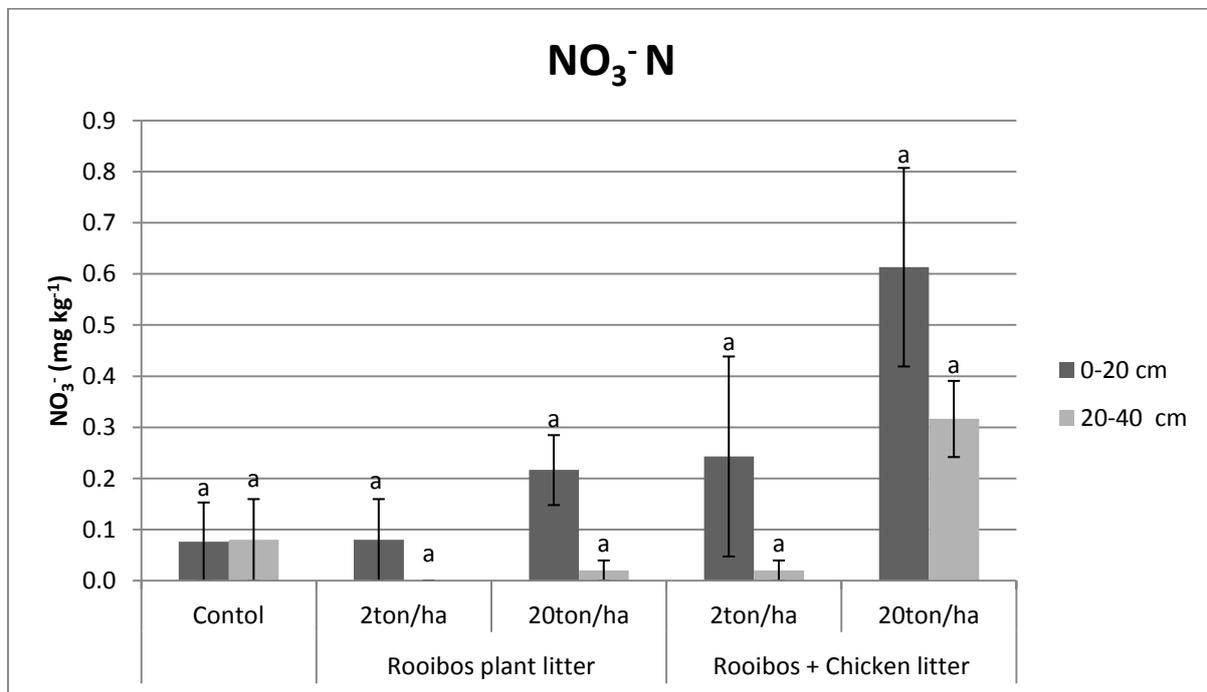


Figure 5-17: Soil NO₃⁻ values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

A increase in the soil NO_3^- was noted in the compost treatments (**Figure 5-17**). The reason for this increase in nitrate can be due to the fact that both composts had a relative high N content (1.54 % RR+CL & 1.82 % RR) (**Table 5-3**). It is also an indication of litter breakdown due to microbial activity. The nitrate increased with an increase in the amount of compost and the type of compost. No significant difference in the NO_3^- were found between the different treatments in both the 0-20 cm ($P = 0.5687$) and 20-40 cm layers ($P = 0.6079$). The NH_4^+ did not show any differences (**Appendix C**)

Plant available Bray II P

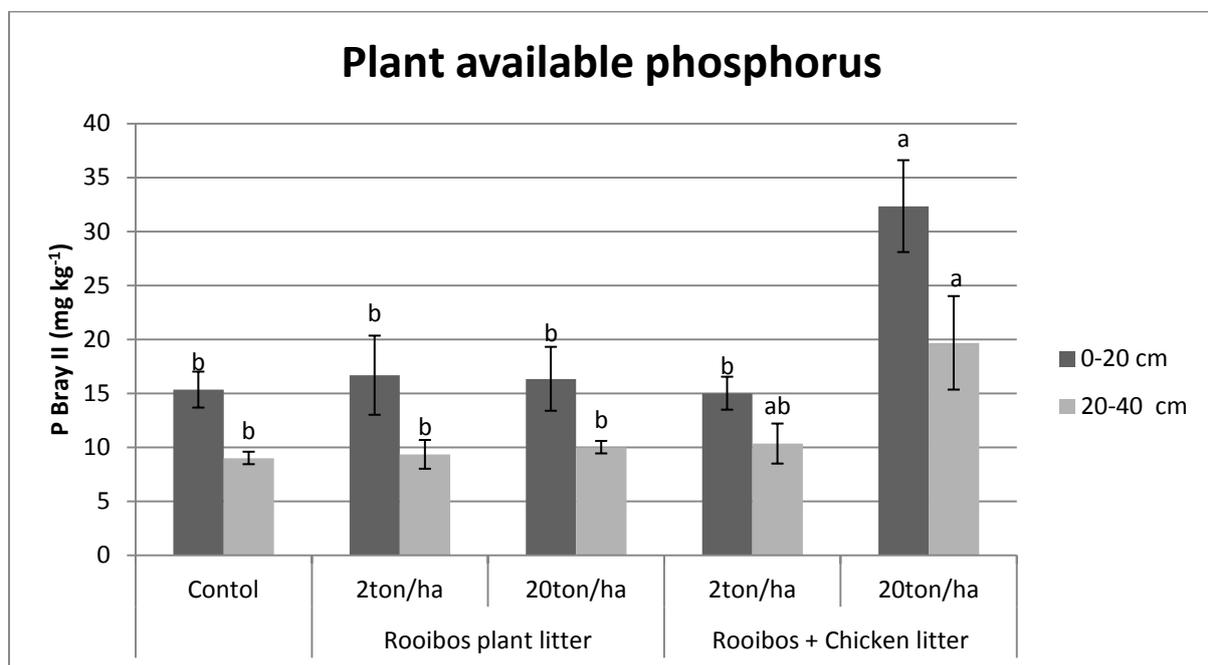


Figure 5-18: Plant available soil Phosphorus (P) values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

The soil Bray II P content of the 20 ton ha⁻¹ treatment was significantly higher than the rest of the treatments (**Figure 5-18**). A significant statistical difference was noted in the soil Bray II P content between the different treatments in both the 0-20 cm ($P = 0.0005$) and 20-40 cm ($P = 0.0215$) layers. The reason for the increase in the P is due to the application of chicken litter compost that had a high P content (0.65 %) (**Table 5-3**). The Bray II P value of the soil surpasses the 30 mg kg⁻¹ threshold value for rooibos seedlings toxicity that was found by Joubert, 1987.

5.4.1.7 Micro nutrients (Cu, Fe, Mn, Zn & B)

The plant-available Cu, Fe and Mn in the soils were not affected by the treatments and no significant statistical differences were found (**Appendix C**).

Zinc

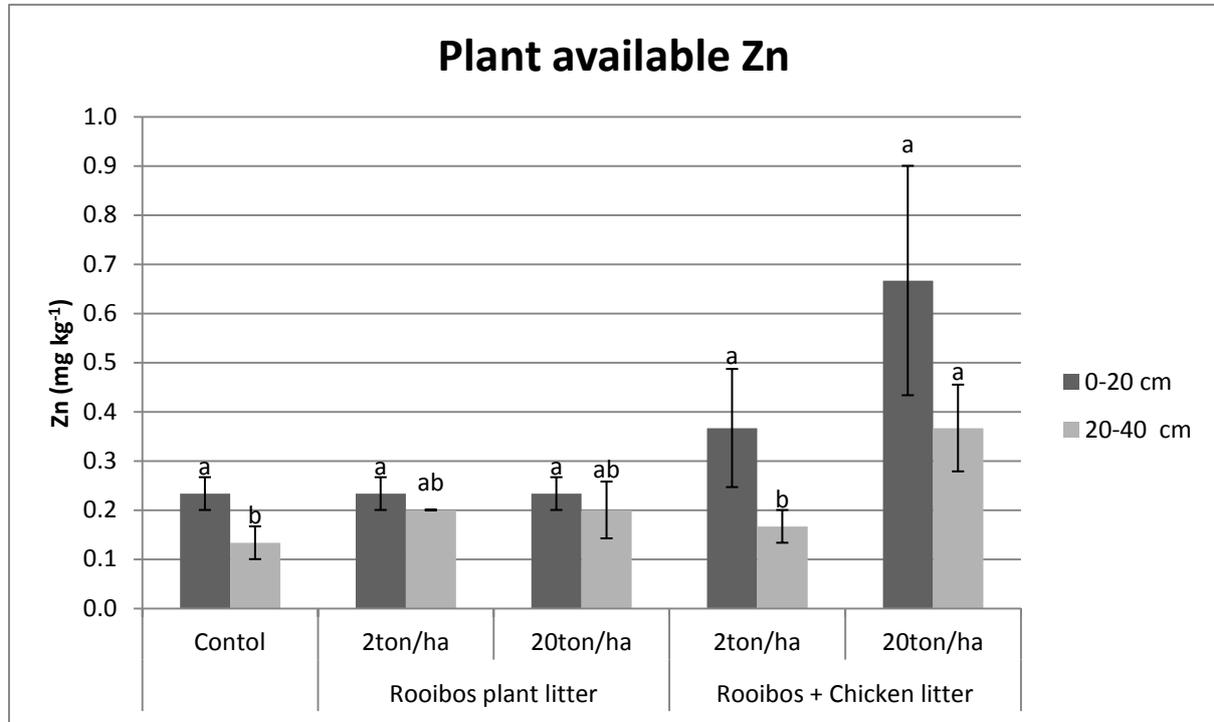


Figure 5-19: Plant available soil Zn values of the control and compost treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

The rooibos compost did not show any trends, but a increase was noticed in the Zn content of the soil in the rooibos + Chicken litter compost (**Figure 5-19**) although no significant difference in the Zn were found between the different treatments in the 0-20 cm ($P = 0.0959$), but in the 20-40 cm layers ($P = 0.0252$) differences were noted. The reason for the increase in the Zn content can be attributed to the high Zn content of the rooibos + chicken litter compost ($205.09 \text{ mg kg}^{-1}$) (**Table 5-3**).

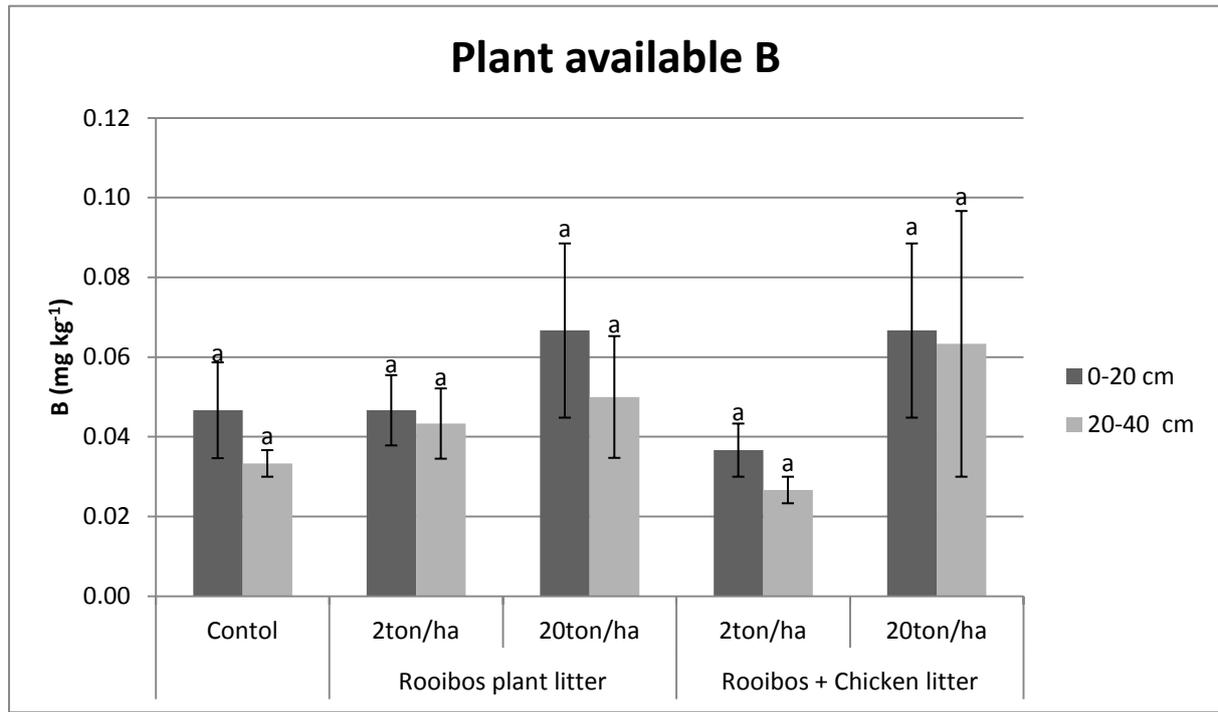
Boron

Figure 5-20: Plant available soil B for values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

No significant difference in the B were found between the different treatments in both the 0-20 cm ($P = 0.5935$) and 20-40 cm layers ($P = 0.5384$). A slight increase was noticed in the B content in both the 20 ton ha⁻¹ compost treatments (**Figure 5-20**). This was due to the relatively high B content of the rooibos (64.55 mg kg⁻¹) and rooibos + chicken litter compost (30.44 mg kg⁻¹) (**Table 5-3**).

5.4.2 Plant analyses

5.4.2.1 Total above- and below-ground biomass yield

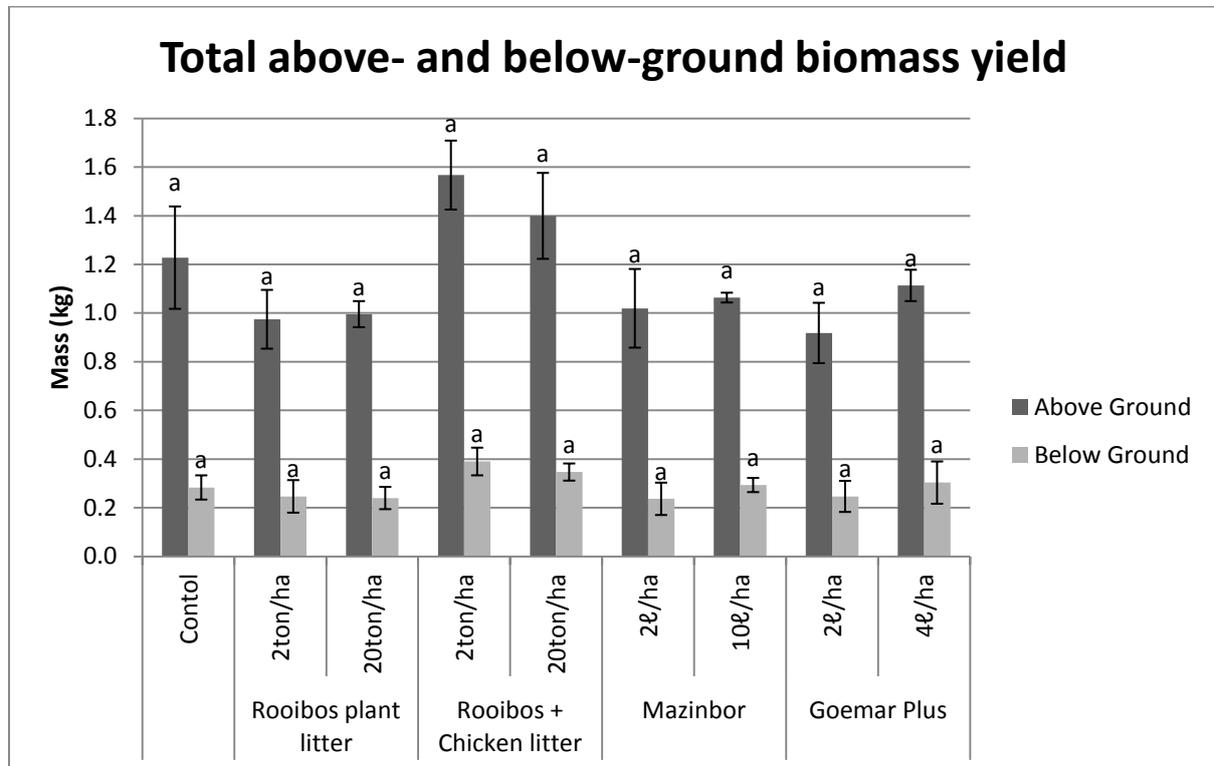


Figure 5-21: Total above- and below-ground biomass yield of the control, compost and foliar spray treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown ($n = 3$). The letters of significance differ between the treatments as well as the above- and below-ground biomass

Only the rooibos + chicken litter compost tended to show an increase the above- and below-ground biomass (**Figure 5-21**). The 2 ton ha^{-1} rooibos + chicken litter compost treatment resulted in the greatest increase in above (26.7 %) and below-ground (37.6 %) biomass, whereas, the 20 ton ha^{-1} application resulted in much smaller increases in above (14.0 %) and below-ground (22.4 %) biomass. Higher root density was observed in the rooibos + chicken litter compost treatments (visual observation), with higher P levels in the soil (**Figure 5-18**). It appears that the 2 ton ha^{-1} rooibos + chicken litter compost had a better effect on the above-ground biomass in comparison to the 20 ton ha^{-1} in the rooibos + chicken litter compost treatment. This is probably due to an excess of P in the soil in the 20 ton ha^{-1} rooibos + chicken litter compost (**Figure 5-18**), which resulted in a negative effect on the above-ground biomass as was found in Chapter 4 (**Figure 4-10**). In the rooibos plant

litter compost a decrease in the above-ground biomass was noted, this can possibly be due to a nitrogen negative period that occurred after the rooibos compost, that had a wide C:N ratio (26:1) (**Table 5-3**) was applied. The foliar applications (Mazinbor and Goëmar) had no effect on the above- and below-ground biomass (**Figure 5-21**). No significant differences was noted between the different treatments for the above- ($P = 0.1455$) and below- ($P = 0.5883$) ground biomass. A slight increase in the below ground biomass in the rooibos + chicken litter compost with a higher P content was noticed (**Figure 5-21**). This was possibly due to the higher P content, since P is important for root development (Havlin *et al.*, 1999), thus increasing the below ground biomass. The same trend was seen in the below ground biomass as in the above-ground biomass. No significant trend was noticed in the foliar treatments.

5.4.2.2 Nitrogen

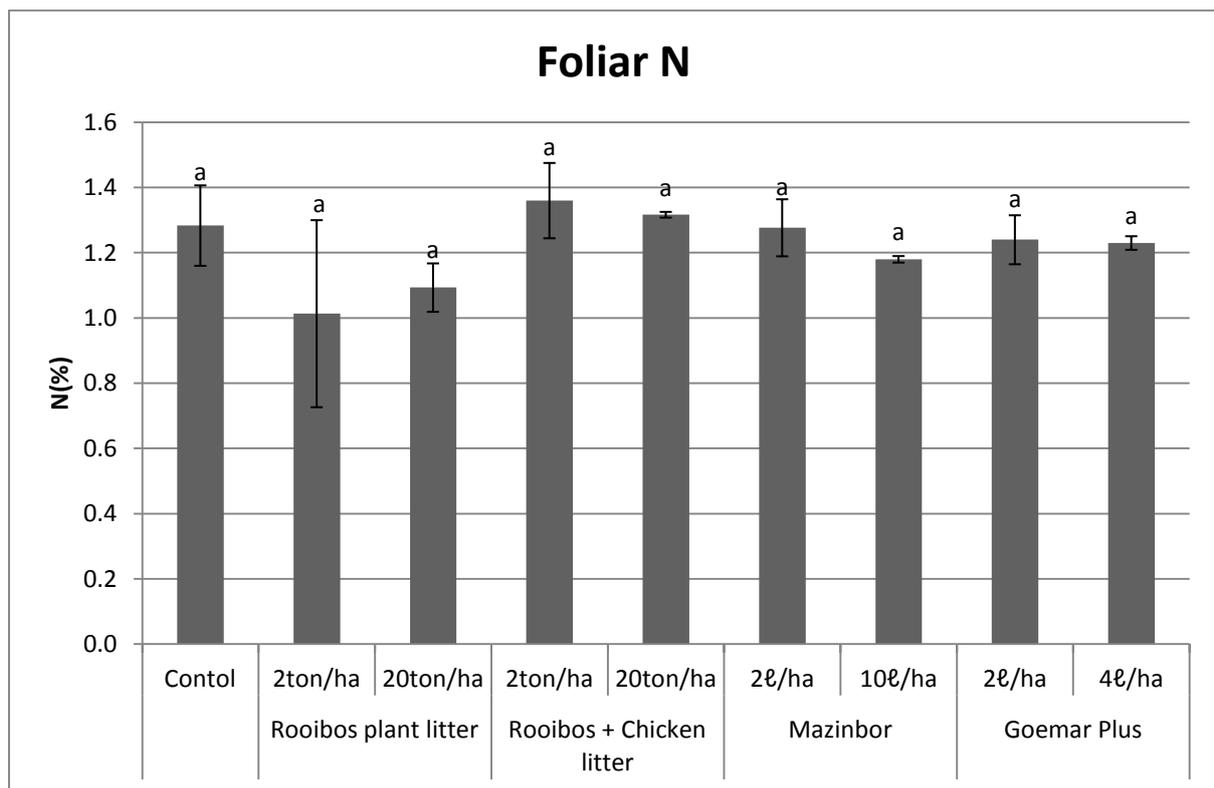


Figure 5-22: Foliar N contents of the control, compost and foliar spray treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar N content did not increase from the first to the second year after planting and N content remained between 0.8 and 1.4 % (**Figure 4-13 & Figure 5-22**). The foliar N of the

rooibos did not differ significantly between treatments (**Figure 5-22**). A slight decrease in the nitrogen was noted at the rooibos plant litter treatments (**Figure 5-22**). This was most likely due to the fact that there was a N negative period in the soil, since only dry organic matter was applied with a wide C:N ratio (**Table 5-3**). The N in the Goëmar treatments did not increase the foliar N levels; even though the Goëmar had a nitrogen content of 5 % (**Table 5-2**). The lack in increase can be attributed to only small amounts of nutrients taken up by the leaves and that the solution sprayed was extremely diluted. No significant differences in the foliar N were observed between treatments ($P = 0.6703$).

N-Nodules

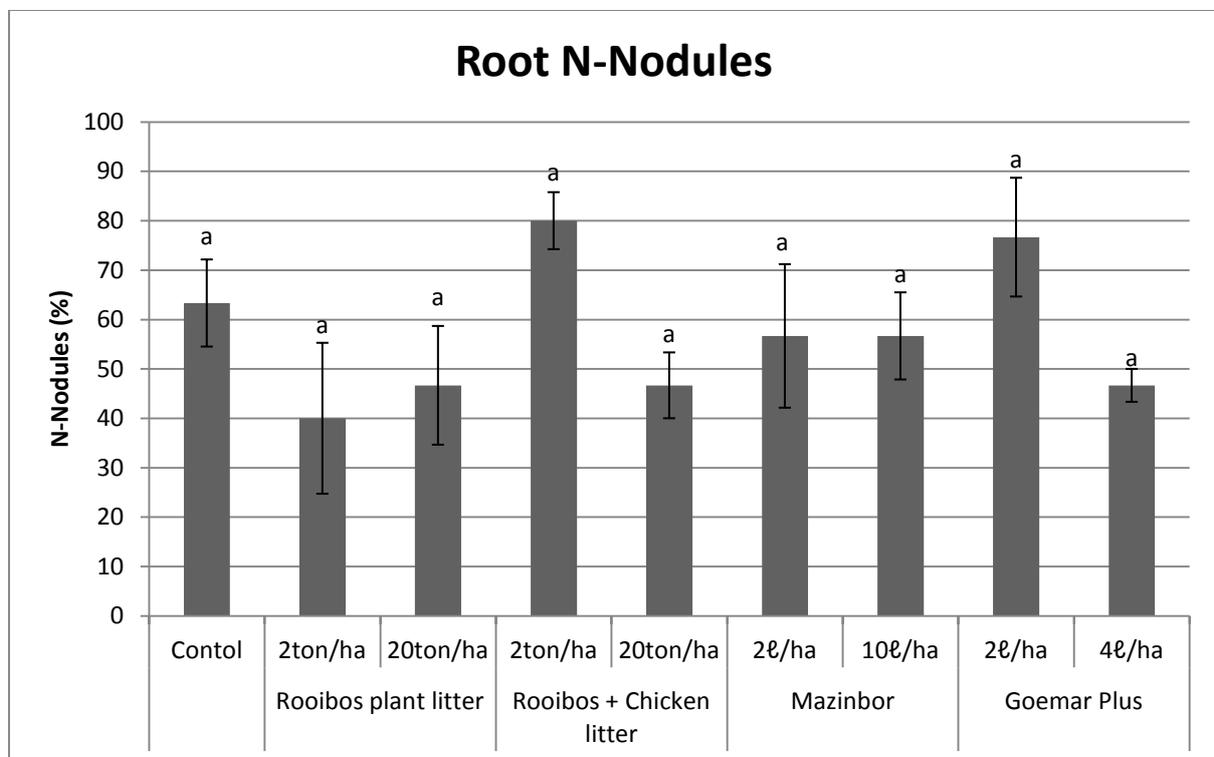


Figure 5-23: N-Nodules on the roots in the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown ($n = 3$). The letters of significance differ between the treatments

No significant differences was noted between the different treatments for the N-Nodules on the roots ($P = 0.1428$). The foliar N in the plants (**Figure 5-22**) of all treatments was sufficient. The plants therefore did not need to produce extra N-nodules, indicating sufficient levels of N in the soil. Any extra N applied in the form of compost or foliar sprays were, therefore unnecessary.

5.4.2.3 Phosphorus

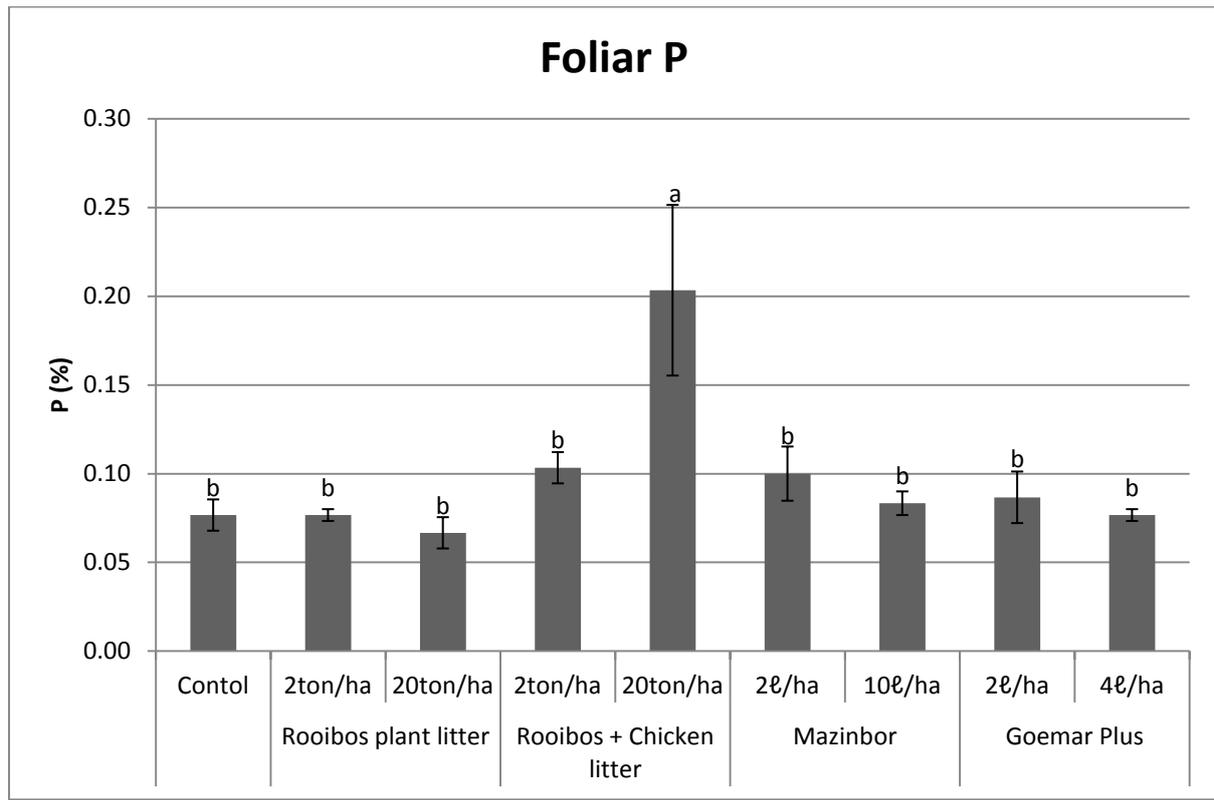


Figure 5-24: Foliar P contents of the control, compost and foliar spray treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

A significant increase in the foliar P content of the 2 ton ha⁻¹ rooibos + chicken treatment was observed (**Figure 5-24**). The reason for the increase in the foliar P content can be attributed to the increase in the Bray II soil P content (**Figure 5-18**). The foliar P content in the 2 ton ha⁻¹ rooibos +chicken litter compost was above the 0.1 % threshold value for fynbos plants (Hawkins *et al.*, 2008). The 20 ton ha⁻¹ rooibos and chicken litter was well above the 0.1 % threshold value, and P toxicity symptoms would be expected in these sites as well as a decrease in the above-ground biomass from the 2 to 20 ton ha⁻¹ as was found (**Figure 5-21**). A significant difference was noted in the plant P content between the different treatments (P = 0.0018). These high soil and foliar P contents will have a negative effect on the growth of the rooibos as found in Chapter 4 (**Figure 4-10 & Figure 4-11**). The high P content (30 mg kg⁻¹) resulted in high foliar P content (**Figure 5-24**) and a decrease in the above-ground biomass, after an initial increase in the biomass at the 2 ton ha⁻¹

treatment (**Figure 5-21**). These results confirm that was found about the effect of too much P on the growth and P toxicity.

Mycorrhiza

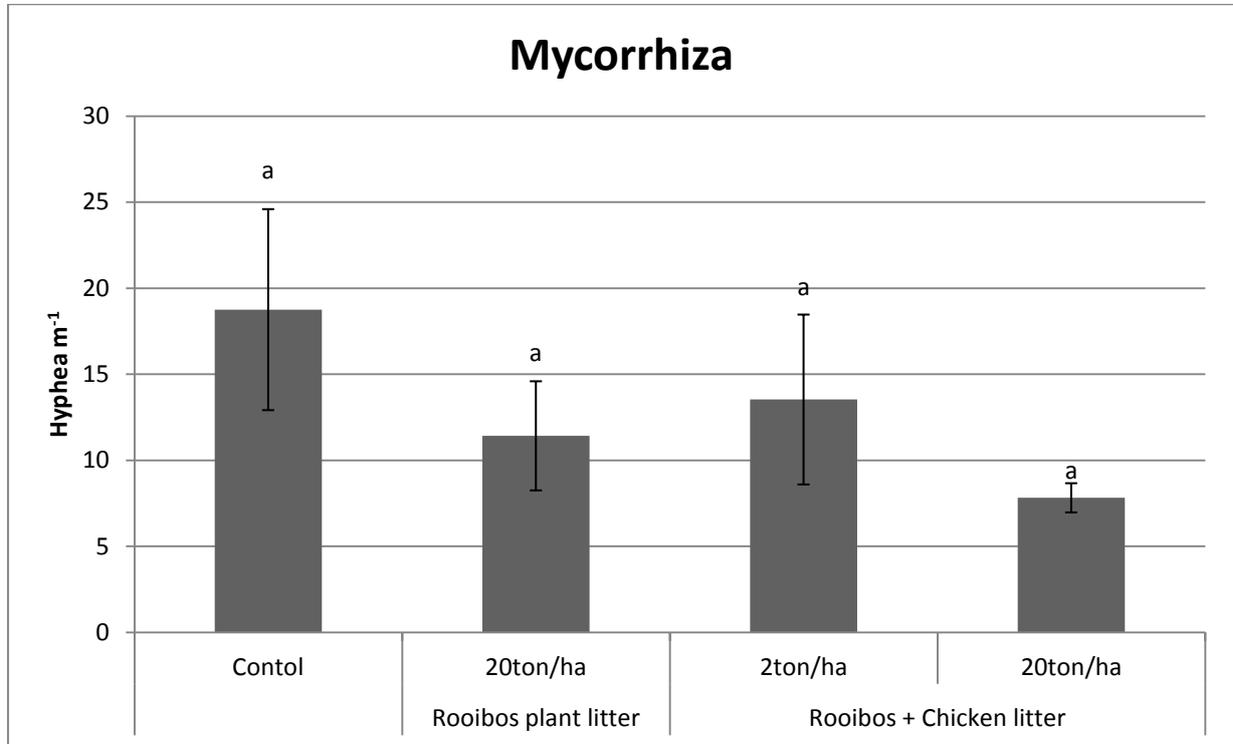


Figure 5-25: Mycorrhiza on the roots in the control and compost treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments

A downwards trend was noted in the mycorrhiza on the roots of the plants in the rooibos + chicken litter treatment (**Figure 5-25**) even though no significant differences was noted between the different treatments for the Mycorrhiza colonization in the roots ($P = 0.3687$). The reason for the decrease can be attributed to the increase in the Bray II P content of the soil (**Figure 5-18**), since a chicken litter compost was applied that had a high P content (**Table 5-3**). These results confirm the results found in **Chapter 4** about the correlation between the soil P content and the mycorrhiza on the roots (**Figure 4-24**). The addition of P fertilizers on these soils with an already high P content had a further negative effect on the growth as well as the symbiotically micro organisms.

5.4.2.4 Potassium

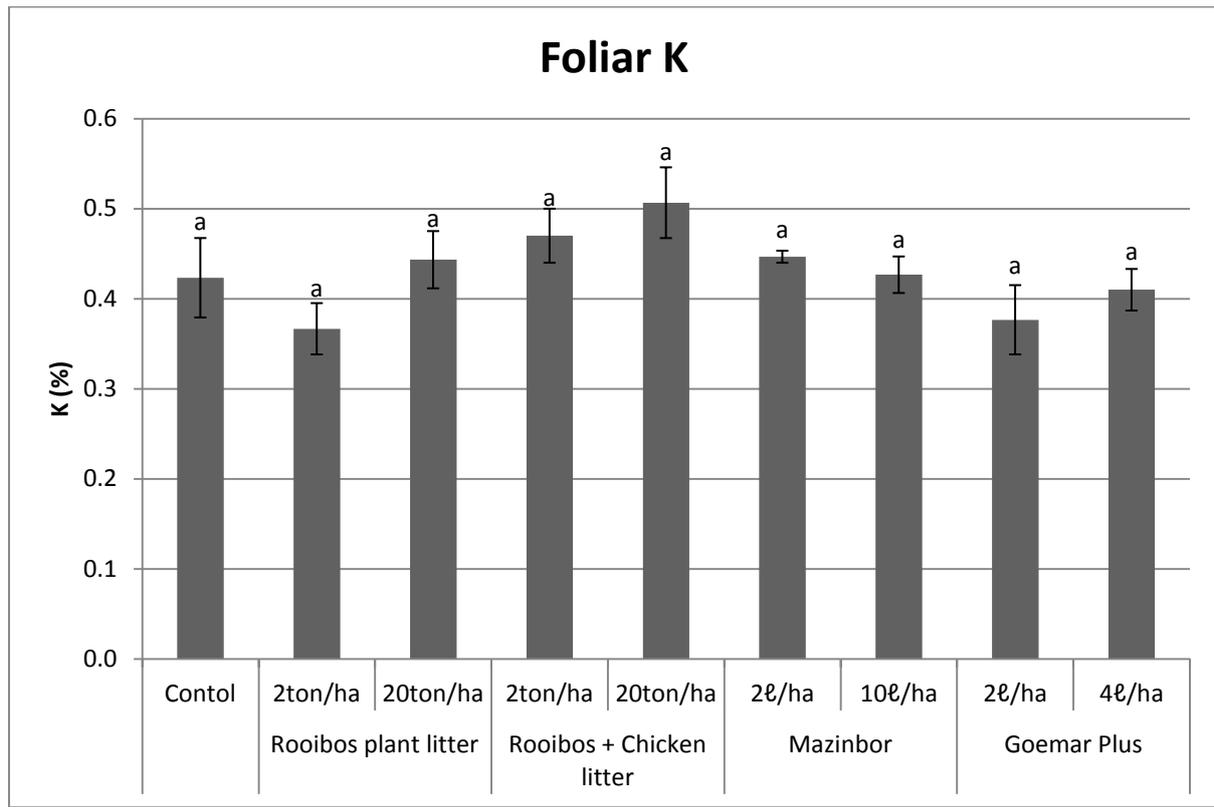


Figure 5-26: Foliar K contents of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The only slight increase was noted in the compost treatments, especially in the 20 ton ha⁻¹ roibos + Chicken litter compost treatment (**Figure 5-26**). The reason for this is probably due to the fact that the compost had a high K content (0.725 %) (**Table 5-3**) and thereby increased the soil K (**Figure 5-12**). No K was present in the Mazinbor and Goëmar foliar mixtures, and no increase in the foliar K was detected. No significant difference in the foliar K were noted between the different treatments (P = 0.1108).

5.4.2.5 Calcium

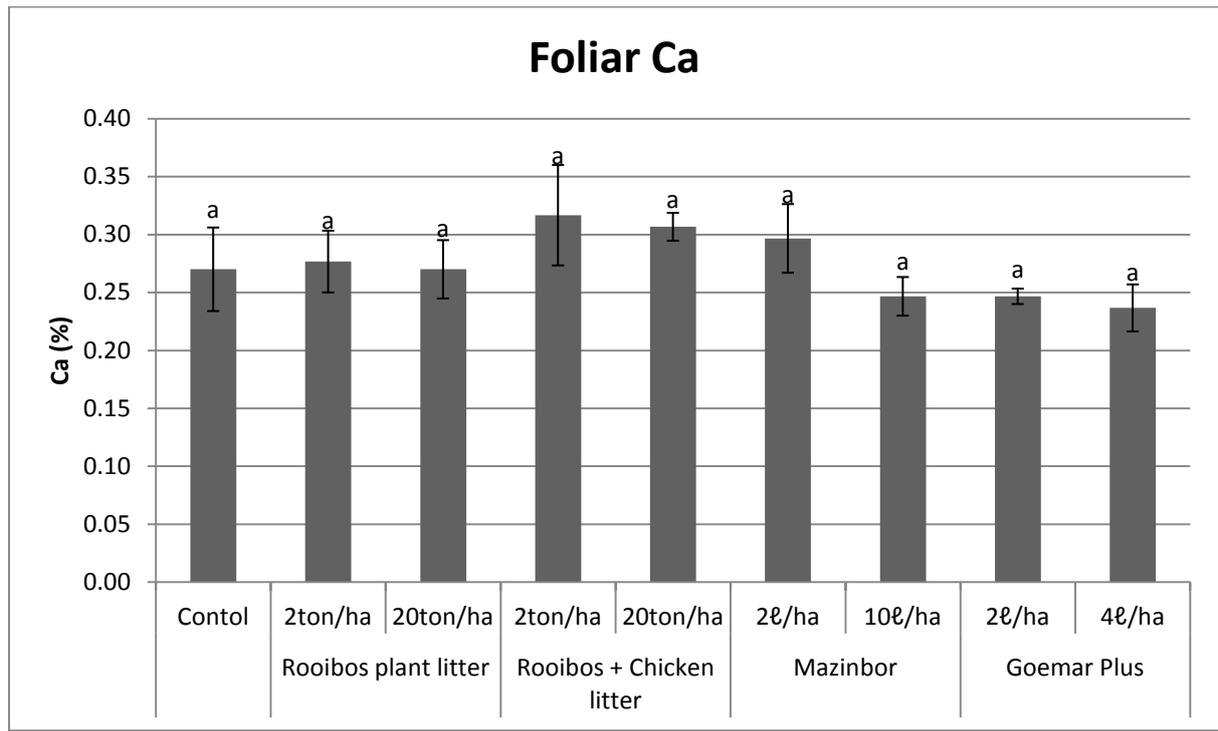


Figure 5-27: Foliar Ca contents of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar Ca content did not show an increase from the first to second year after planting (0.2 - 0.3 %) (**Figure 4-28 & Figure 5-27**). A slight increase can be noted in the rooibos + Chicken litter compost as well as the 2 l ha⁻¹ Mazinbor treatment (**Figure 5-27**) although it was not significant statistically different (P = 0.4160). The rooibos + chicken litter compost had a high Ca content (0.52 cmol_c kg⁻¹) (**Appendix C**), and thereby increased the Ca content of the soil. The Mazinbor mixture had a Ca content of 133 g l⁻¹ (**Table 5-1**) thereby increased the Ca content of the plant in the 2 l ha⁻¹ treatment. The reason for the lack of Ca content increase in the 20 l ha⁻¹ is not clear.

5.4.2.6 Magnesium

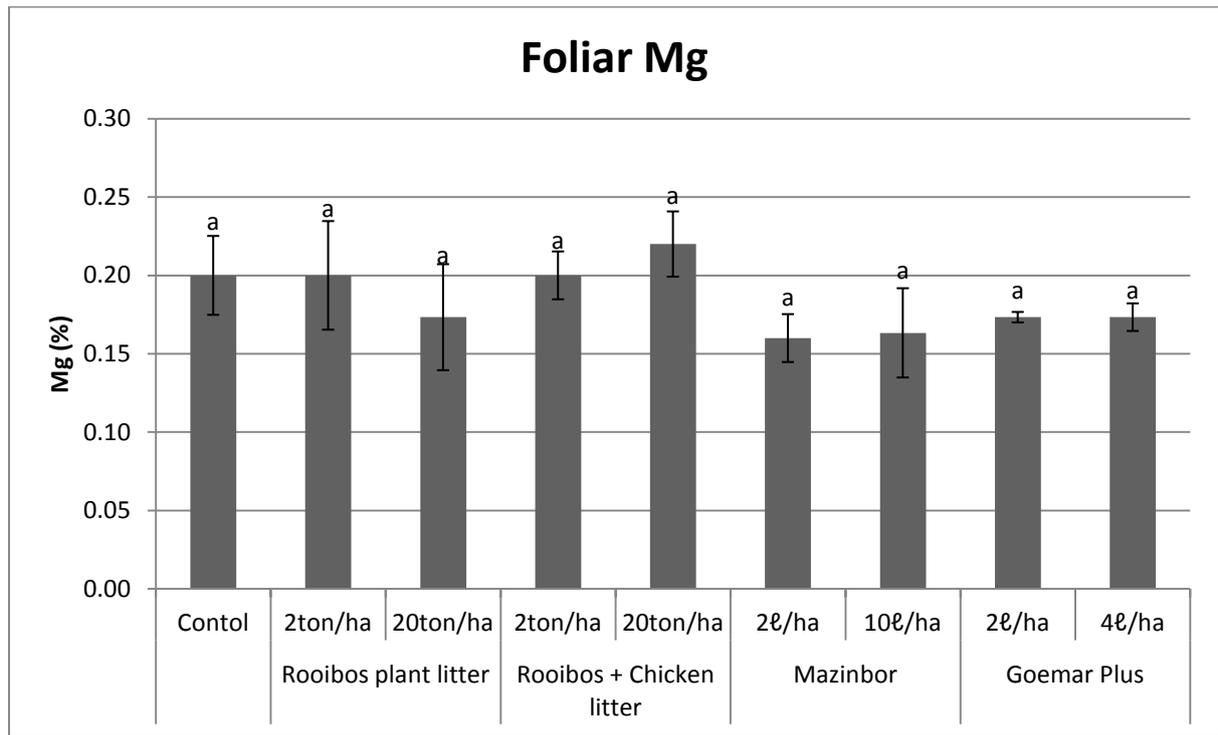


Figure 5-28: Foliar Mg contents of the control, compost and foliar spray treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

No difference in the Mg foliar content was noticed between the first and second year after planting (**Figure 4-30** & **Figure 5-28**). No trends were observed in any of the treatments (**Figure 5-28**). No significant difference in the foliar Mg contents were noted between the different treatments ($P = 0.4739$). No increase in the foliar Mg content was observed in the Goëmar application, even though the foliar spray had an Mg content of 2.4 % (**Table 5-2**).

5.4.2.7 Sulphur

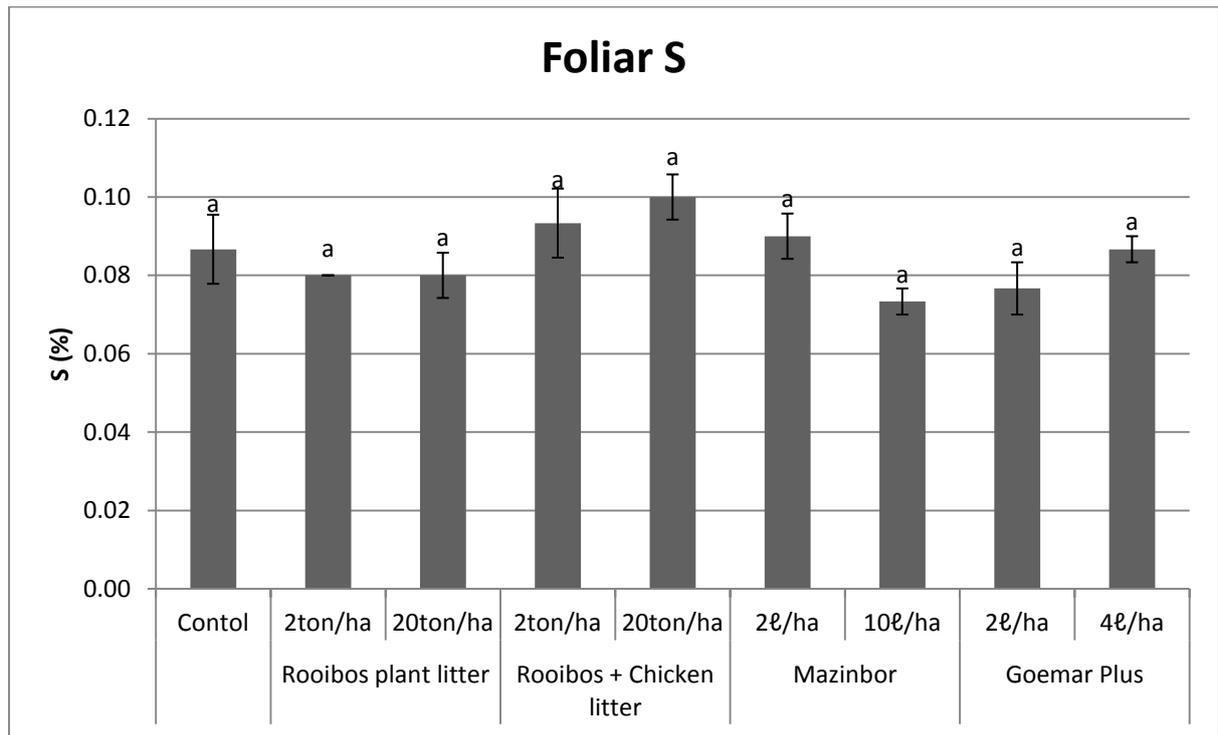


Figure 5-29: Foliar S contents of the control, compost and foliar spray treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar S content increased from the first (0.05 %) (**Figure 4-31**) to second (0.07-0.1 %) (**Figure 5-29**) year after planting. No trends were observed between the different treatments even in the Goëmar foliar treatment although it had a sulphur content of 3.2 % (**Table 5-2**). The rooibos + chicken litter compost showed a slight increase in the S content in both the 2 ton ha⁻¹ and 20 ton ha⁻¹. The S content of the compost and soil were not analyzed so no conclusive conclusions can be drawn. No significant statistical difference in the foliar S were noted between the different treatments (P = 0.0798).

5.4.2.8 Zinc

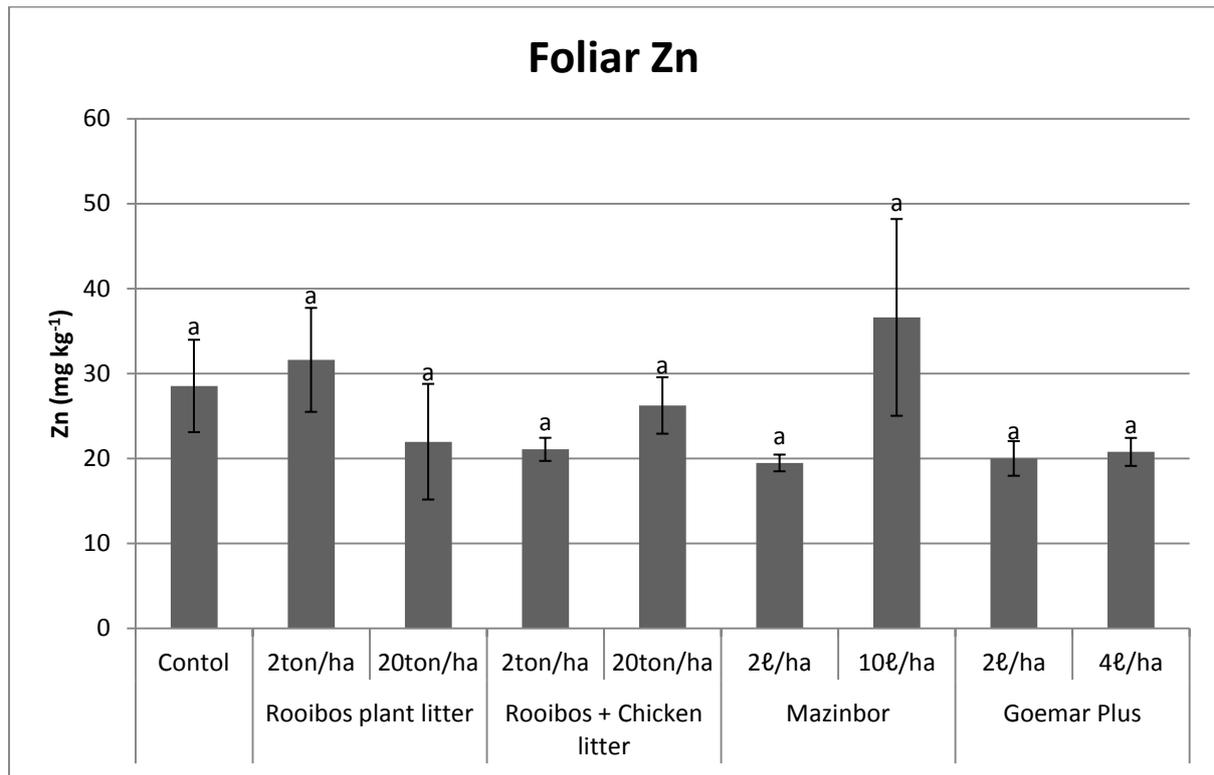


Figure 5-30: Foliar Zn contents of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

No significant differences in the foliar Zn content were observed between the first and second year after planting (**Table 4-1 & Figure 5-30**). Trends were noticed in the Zn content (even though it was not significant statistically different ($P = 0.2695$)), a slightly higher Zn content was noticed in the 10 l ha^{-1} Mazinbor treatment (**Figure 5-30**) as was expected since the foliar spray contains Zn in its mixture (90 g l^{-1}) (**Table 5-1**). A slight decrease in the 2 ton ha^{-1} and 20 ton ha^{-1} rooibos + chicken litter compost treatments was noticed. The increase in the soil (**Figure 5-18**) and plant P (**Figure 5-24**) in the rooibos + chicken litter compost treatments led to lower Zn availability, as Zn and phosphate are highly antagonistic. The decrease in the Zn content can indicate P toxicity in fynbos (Hawkins *et al.*, 2008).

5.4.2.9 Boron

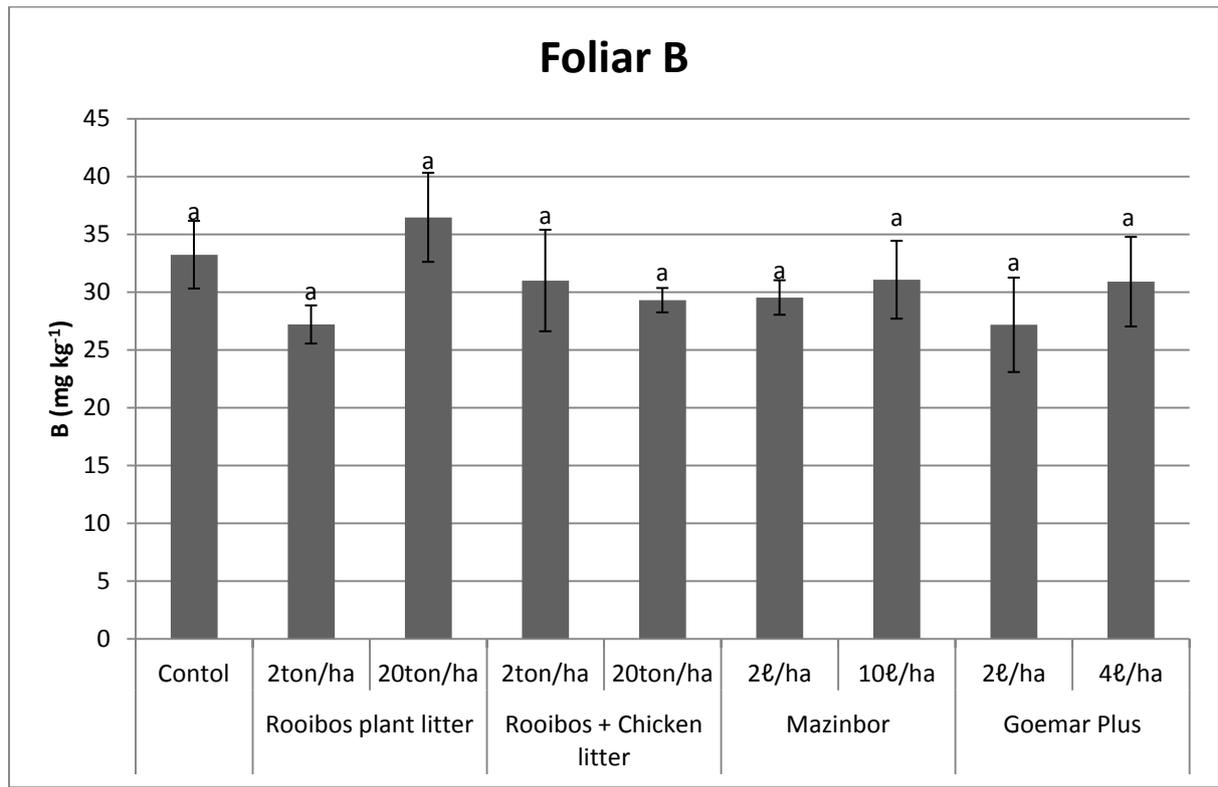


Figure 5-31: Foliar B contents of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

No significant differences ($P=0.6344$) in the foliar B content was noted between the first and second year after planting (**Table 4-1 & Figure 5-31**). No trends were noticed in the foliar B content for the compost and foliar treatments (**Figure 5-31**). The Mazinbor and Goëmar foliar sprays did not have an effect on the foliar B content even though B were applied to the leaves in both the Mazinbor (91 g l^{-1}) and Goëmar (2 %) foliar sprays (**Table 5-1 & Table 5-2**).

5.4.2.10 Sodium

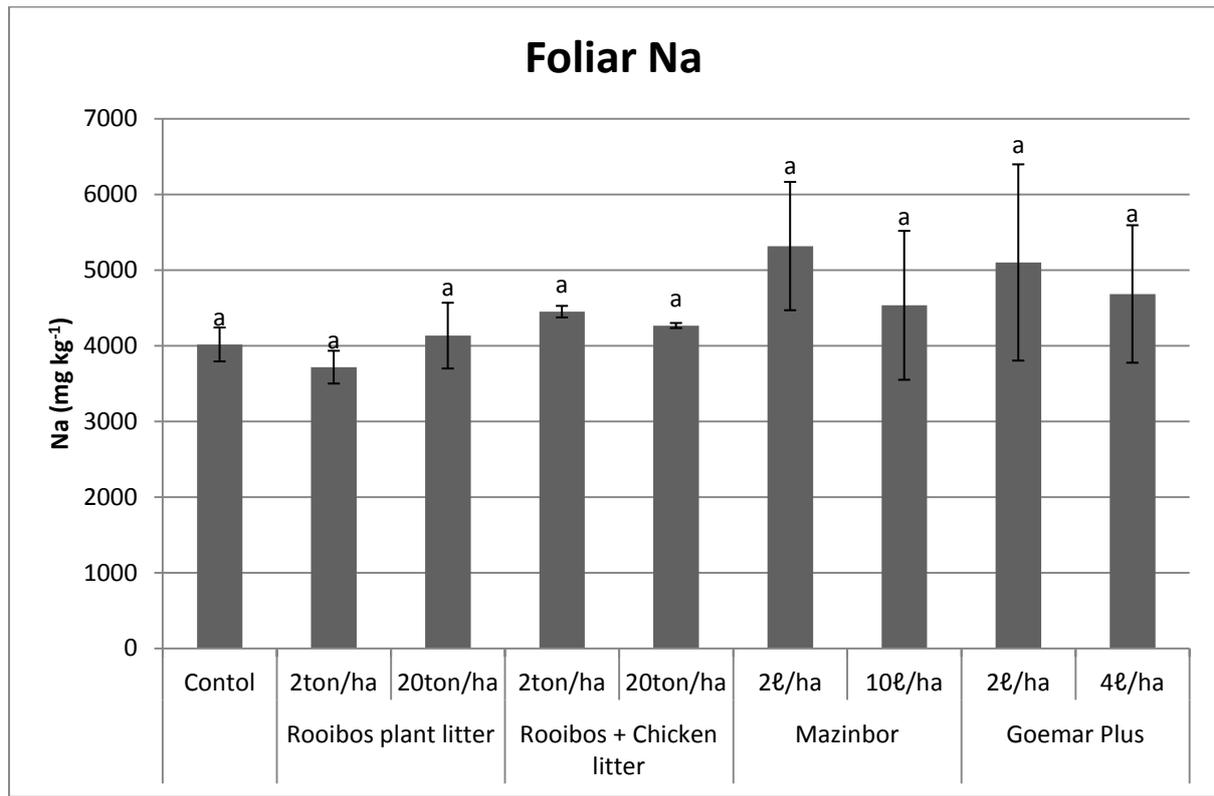


Figure 5-32: Foliar Na contents of the control, compost and foliar spray treatments at the oldest (Vaalkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

The foliar Na content increased from the first year (± 2500 mg kg⁻¹) (Figure 4-35) to the second year (4000 -6000 mg kg⁻¹) (Figure 5-32). It appears that the plant took up more Na in its second year. No definite trends were noticed for the different treatments especially the composts trails (Figure 5-32). The experimental sites treated with foliar sprays show a slight increase in the foliar Na content as well as higher standard error (Figure 5-32), the reason for the increase in the foliar Na content can be due to the fact that the foliar applications was diluted using a water with high Na content (156.8 mg l⁻¹) (Table 5-4). The Mazinbor did not have Na content, while the Goëmar did have a low Na percentage (0.6 %) (Table 5-2). No significant difference in the foliar Na were found between the different treatments (P = 0.6165).

5.4.3 Tea Yield and Quality

5.4.3.1 Tea yield

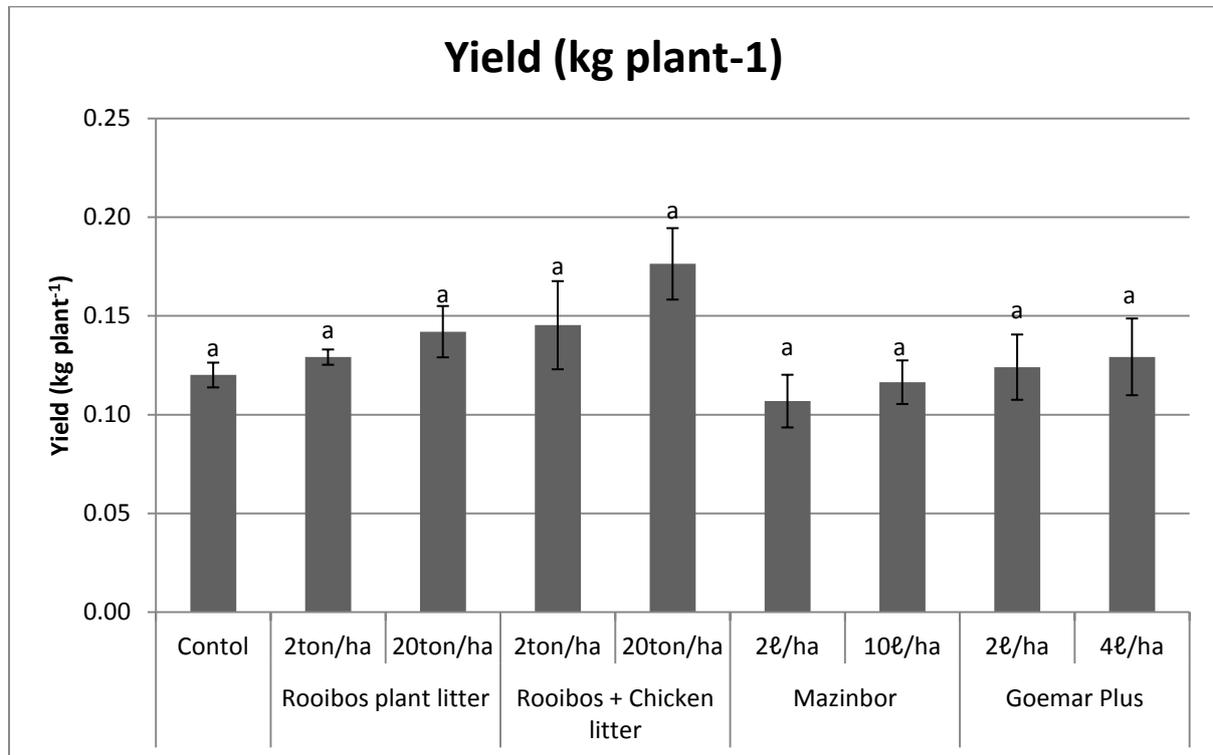


Figure 5-33: Tea yields (after 3 months) of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

No significant differences in the yields were noted between the different treatments for the plant yields ($P = 0.1119$). The tea yields tended to increase slightly with an increase in the amount of compost and the type of compost (**Figure 5-33**). A possible reason for the slight increases that were observed can be due to the fact that the compost didn't have time to fully decompose. The rooibos was harvested only 3 months after the compost was applied, during the summer time. Thus the soil was most likely not moist enough for significant decomposition to occur. The full effect of the compost will most likely only be seen the following year (2014). The foliar sprays had no apparent effect on the tea yields (**Figure 5-33**).

5.4.3.2 Tea quality

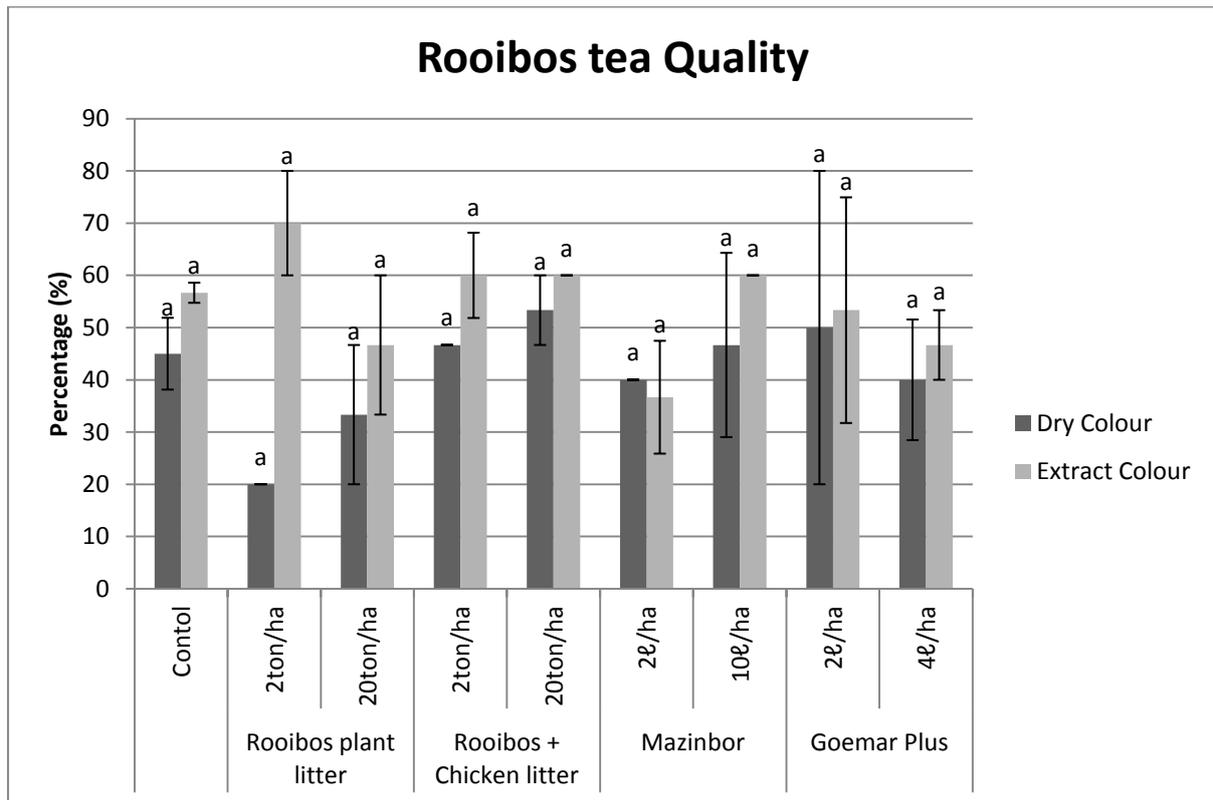


Figure 5-34: Dry- and Extract-colour for the compost and foliar application on the oldest experimental (Vaalkrans) site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the dry colour and extract colour.

Only the Dry colour and Extract colour was taken into account to evaluate the tea quality, since the wet colour had the same results as the dry colour and in all of the treatments the tea was under fermented. A decrease in the dry colour of the fermented tea was noticed in the rooibos plant litter treatment, the reason for the decrease can most likely be attributed to N-deficiency in the leaves (**Figure 5-22**). There was a nitrogen negative period in the soil since only dry organic matter was applied with a wide C:N ratio (**Table 5-3**). No significant trends were noticed between the different treatments (Rooibos plant litter, Rooibos + Chicken litter, Mazinbor or Goemar) and extract colour (**Figure 5-34**) No significant statistical differences can be noted between the different treatments for both the dry colour ($P = 0.7693$) and the extract colour ($P = 0.4641$).

5.5 Conclusion

The 20 t ha⁻¹ **rooibos + chicken litter compost** treatment had a noticeable effect on the soil pH, EC, total microbial biomass, exchangeable basic cations, total soil N, soil NO₃⁻ and Bray II soil P as well as the soil micronutrients (Zn and B), but had no noticeable effect on the soil C and micro nutrients (Cu, Fe and Mn). A slight increase was noticed in the pH (H₂O) and pH (KCl) in the 20 ton ha⁻¹ treatment. The EC in the 20 ton ha⁻¹ treatment showed a slight increase. The total microbial biomass was slightly higher in the 2 ton ha⁻¹ treatment and the exchangeable basic cations (Ca, Mg, K and Na) increased especially in the 20 ton ha⁻¹ treatment. The exchangeable K had a significant higher content in the 0-20 cm layer; this was due to the high basic cation content of the compost especially the K. However, there was a slight increase in the total soil N in the 20 t ha⁻¹ compost treatments. A definite increasing trend was noticed in the soil NO₃⁻ with an increase in the rooibos + chicken litter compost. The soil Zn increased with an increase in the rooibos +chicken litter compost, due to the high Zn content of the chicken litter used in the compost. The increase in the soil and plant P in the rooibos + chicken litter compost treatments led to lower Zn availability. The decrease in the Zn content can indicate P toxicity in fynbos as was expected to be the reason for the decrease in the 20 ton ha⁻¹ rooibos + chicken litter compost treatment. The soil B content did not increase in the 2 ton ha⁻¹ treatment, but a slightly higher B content was noticed in the 20 ton ha⁻¹ treatment.

Although not statistically significant, the rooibos + chicken litter compost 2 ton ha⁻¹ treatment resulted in the greatest increase in above (26.7 %) and below-ground (37.6 %) biomass, whereas, the 20 ton ha⁻¹ application resulted in much smaller increases in above (14.0 %) and below-ground (22.4 %) biomass. One of the main reasons for this could possibly be due to P-toxicity. The chicken litter had no effect on the P levels in the soil and plant in the 2 ton ha⁻¹ treatment but a significant increase was noted in the 20 ton ha⁻¹. The increase in the soil P led to an increase in the plant P and resulted in a decrease in the Mycorrhiza colonization on the roots. The same results were found as in **chapter 2 and 3**, thus confirming the results found in those chapters. A slight increase in the plant N, K, Ca and S was noticed in both the 2 and 20 ton ha⁻¹ experimental sites, but no trends were found in the Mg, Mn, B and Na. The plant Zn content showed a decrease, even though the soil Zn content showed an increase with the compost application.

The 20 t ha⁻¹ **rooibos plant litter compost** treatment had a noticeable effect on the pH, EC, total microbial biomass as well as the exchangeable basic cations but had no noticeable effect on the soil C, soil N, Bray II P and micro nutrients (Cu, Zn, Fe, Mn and B). A slight increase was noticed in the pH (H₂O) and pH (KCl) in the 20 ton ha⁻¹ treatment. The EC in the 2 ton ha⁻¹ treatment showed an increase. The total microbial biomass decreased in both the 2 and 20 ton ha⁻¹ treatments and the exchangeable basic cations (Ca, Mg, K and Na) increased especially in the 20 ton ha⁻¹ treatment the same as in the rooibos + chicken litter compost. The sodium had a significant higher content in the 0-20 cm layer; this was due to the high basic cation content of the compost especially the Na. A slight decrease was noticed in the above- and below-ground biomass that can possibly be due to a nitrogen negative period, since the compost applied had low nitrogen content. This also resulted in a lower foliar N content no significant trends or differences were noticed in any of the other foliar nutrient content.

The **Mazinbor** application led to a slight increase in the Zn, Mn and Ca but did not have an effect on the foliar B content. The increase was seen to a greater extent in the 2 l ha⁻¹ as appose to the 10 l ha⁻¹. The application of the trace elements (Mazinbor) had no noticeable effect on the above- and below-ground biomass.

The **Goëmar** did not have any noticeable effect in the 6 month period after the foliar application. None of the elements (N, Mg, S, B and Na) found in the Goëmar mixture was found in higher concentrations in the leaves. The Mo content of the plants were not determined before the application or after the application, so no conclusion can be drawn about the effect the Mo would have on the plant. It seems that the foliar spray is not compatible with rooibos plants, and the Goëmar foliar spray was not taken up by the rooibos plant leaves or the leaves with the higher concentrations was harvested in Feb 2013 resulting in normal levels when the plant was analyzed in Jun 2013.

A slight decrease in the dry colour of the fermented rooibos tea was noticed in the rooibos plant litter compost treatment, this can most likely be attributed to the N negative period in the foliar N content. The rooibos + chicken litter treatment had no effect on the dry or wet colour of the fermented rooibos tea after 3 months, although, it might have affected it after the wet season when significant nutrient uptake would have occurred. An increase in the

tea yields were noticed in both compost treatments in both the 2 and 20 ton ha⁻¹ treatments although it was not statistically significant. The foliar sprays had no noticeable effect on the yield, dry colour or wet colour that was determined 3 months after application. The yields, dry colour and wet colour of the compost and foliar sprays should be repeated since it was determined after just 3 months. Clearer results might have been found if the compost had sufficient time to decompose during the wet season and when rooibos takes up nutrients.

The rooibos + chicken litter compost resulted in an initial increase in the above- and below-ground biomass at the 2 ton ha⁻¹ treatment, but showed a much smaller increase at the 20 ton ha⁻¹ treatment. This is likely a result of the significant increase in the soil P due to the high P content of the compost, and resulted in an increase in the foliar P and a decrease in the foliar Zn. The results of this compost study support our previous year's findings about the negative effect of excessive soil P on rooibos growth. Thus high P composts, such as those enriched with chicken litter, should only be applied in relatively small amounts on rooibos plants so as to avoid P toxicity. Furthermore, the P in the soil and in the rooibos leaves should be monitored seasonally so that P toxicity is avoided. Phosphorus is important for root development in rooibos plants, but rooibos is not adapted for normal to high crop soil P levels, which can quickly lead to P toxicity symptoms and eventually plant death. The rooibos residues compost resulted in a slight decrease in the above-ground biomass yields, and this can be attributed to a decrease in foliar N content due to the wide C:N ratio of the compost. It is suggested that N fertilizers be applied with the rooibos residue compost. A positive aspect of the rooibos residues compost is that it is low in P, and therefore it is easier to enrich the soil C while controlling soil P levels with this type of compost than composts enriched in high P animal wastes. It also contains the elements that the rooibos plants need in the correct ratios. The Mazinbor and Goemar foliar sprays had no noticeable effect on the yields or above- and below-ground biomass. The Mazinbor and Goëmar foliar sprays should be repeated during the other seasons (Spring, Winter and Autumn) to see whether the plant is more susceptible for nutrient uptake through the leaves.

6 GENERAL CONCLUSIONS AND FUTURE RESEARCH PROSPECTS

Roibos farmers reported that roibos production decreased dramatically after the first five years after the removal of the indigenous fynbos vegetation. Little or no information was available on the soil quality status of soils used for roibos production. Furthermore no studies had been carried out on the effect of soil quality on roibos production.

Therefore, the main objective of this study was to investigate the effect of long-term roibos cultivation on soil quality status (chemical, microbiological and physical parameters) in comparison to undisturbed fynbos sites where roibos grows naturally. It was anticipated that this will lead to the identification of the soil factors behind the decline in productivity of roibos on soils that have been repeatedly cultivated. Another objective was to examine the relationship between soil quality and plant quality (plant size, the plant nutrients, the tea yield and fermented tea quality). This was done to identify plant factors influencing the production and quality of roibos tea. The extent of roibos root colonization by beneficial plant microorganisms, rhizobium N-nodules and mycorrhiza fungi, was also examined and was related to the plant and soil quality. Once the soil factors for the decline in growth were identified, alternative management strategies and treatments were investigated to enhance soil quality and roibos productivity. This included evaluating the addition of locally-produced roibos compost on soil health and plant quality, as well as, supplementing any additional deficient nutrients in the form of foliar sprays.

It was found that continuous cultivation resulted in significant decreases in plant above- and below-ground biomass yields, soil C and N, and basic cations. Cultivation also led to decreases in the total microbial biomass, soil protist and root mycorrhizae counts. A positive correlation was found between the above-ground biomass and the soil carbon in the 0-20 cm layer ($R^2=0.5843$). There is also a decrease in the total amount of organic biomass that is returned to the soil annually, since about two-thirds of the plant is harvested annually, and monoculture is practiced. This leads to a decrease in the soil carbon, leading to a decrease in the basic cations, as well as, decreases in the total microbial biomass and soil protist counts.

Soil P increased with continuous cultivation, as well as, plant foliar P content. An exponential negative correlation was found between the Bray II P in the soil and the above-

ground biomass ($R^2 = 0.417$). An exponential negative correlation was found to exist between foliar P content and the above-ground biomass yields ($R^2 = 0.6653$), as well as tea quality ($R^2 = 0.71$). This supports previous studies that indicated that fynbos plant species, such as rooibos, are adapted to low P conditions and thus highly sensitive to P toxicity, leading to associated Ca, Fe and Zn unavailability within the plant, and ultimately to increased susceptibility to fungal rot and plant death. Certain P toxicity symptoms (chlorosis) were found on the leaves of the rooibos plants on the site with the highest Bray II P content (18 mg kg^{-1}).

A high P content in the soil had a negative influence on the mycorrhiza colonization of the rooibos plant. An exponential decrease in the mycorrhiza fungi on the roots was noted with an increase in the P content of the soil in both the 0-20 cm ($R^2 = 0.8134$) as well as the 20-40 cm layer ($R^2 = 0.7224$). Mycorrhiza fungi are known to help plants be more drought and pathogen resistant. Thus, the observed decrease in the mycorrhizal root colonization can be one of the contributing reasons why rooibos planting cycles have decreased from eight to five years. A decrease in mycorrhiza was also correlated with a decrease in dry colour intensity of the fermented rooibos tea.

In the Seekoeivlei area an increase in the N-nodules was found with an increase in the age of the cultivated field, indicating a greater dependence on symbiotically fixed N rather than soil N. However it would appear that the plants had sufficient N, whether through N uptake from the soil or from symbiotically bound N, as there were no differences in the foliar N content between sites. A weak positive correlation was found between foliar B content and above-ground biomass yield ($R^2 = 0.69$). A weak positive correlation was also found to exist between foliar Na content and plant size in the Seekoeivlei area soils ($R^2=0.47$). A negative correlation was observed between the above-ground biomass and the foliar Cu content of 1 year old plants in the Nardouwsberg area ($R^2=0.82$).

The increase in soil and plant P resulted in a decline in the above- and below-ground biomass, and fermented tea quality, most likely due to P toxicity. Therefore, it can be concluded that the main reason for the decrease in the production and tea quality can be related to the decrease in the soil carbon, as well as the increase in the soil and plant P that results in P toxicity.

Although not statistically significant, the 2 ton ha⁻¹ rooibos + chicken litter compost treatment resulted in the greatest increase in above (26.7 %) and below-ground (37.6 %) biomass, whereas, the 20 ton ha⁻¹ application resulted in much smaller increases in above- (14.0 %) and below-ground (22.4 %) biomass. This is possibly a result of the significant increase in the soil P due to the high P content of the compost, and resulted in an increase in the leaf P and a decrease in the leaf Zn. The results of the compost study confirmed the negative effect of excessive soil P on rooibos growth. Thus high P composts, such as those enriched with chicken litter, should only be applied in relatively low amounts to avoid P toxicity. Furthermore, the P in the soil and in the rooibos leaves should be monitored seasonally so that P toxicity is avoided. Phosphorus is important for root development in rooibos plants, but rooibos is not adapted to high soil P levels, which can quickly lead to P toxicity symptoms and eventually plant death. Thus, it can be concluded that rooibos yields can be increased with rooibos + chicken litter compost, as long as the P levels are not significantly elevated and are monitored. The rooibos residues compost resulted in a slight decrease in the above-ground biomass yields, and this can be attributed to a decrease in foliar N content due to the high C:N ratio (26:1) of the compost. It is suggested that N fertilizers be applied with the rooibos residue compost. A positive aspect of the rooibos residues compost is that it is low in P, and therefore it is easier to enrich the soil C while controlling soil P levels with this type of compost than composts enriched in high P animal wastes. It also contains the elements that the rooibos plants need in the correct ratios. The Mazinbor and Goemar foliar sprays had no noticeable effect on the quality, yields or above- and below-ground biomass.

Future research prospects

The precise P toxicity threshold value (soil and plant) for rooibos should be established in future studies to determine exactly when the addition of P will have a negative effect on the yields and quality. It is likely that the soil P critical values will vary with soil type, as P availability is affected by sesquioxides, organic matter and soil pH. The nutrient contents in plants of different ages should be determined to see whether different nutrients are needed in different years of cultivation. The nutrient levels in very healthy and very sick (dying) bushes should also be compared in order to establish rooibos leaf norms. This could prove

quite difficult, as rooibos is grown from seed, and is therefore genetically very heterogeneous.

Long-term compost or other slow-release fertilizers trials, incorporating mulching practices, should be further investigated. Application of N fertilizers and iron sulphate to remediate P toxicity symptoms on rooibos fields already containing high levels of soil P and showing toxicity symptoms also needs to be evaluated.

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8 APPENDICES

Appendix A. Research Chapter 3 supplementary data

Resistance

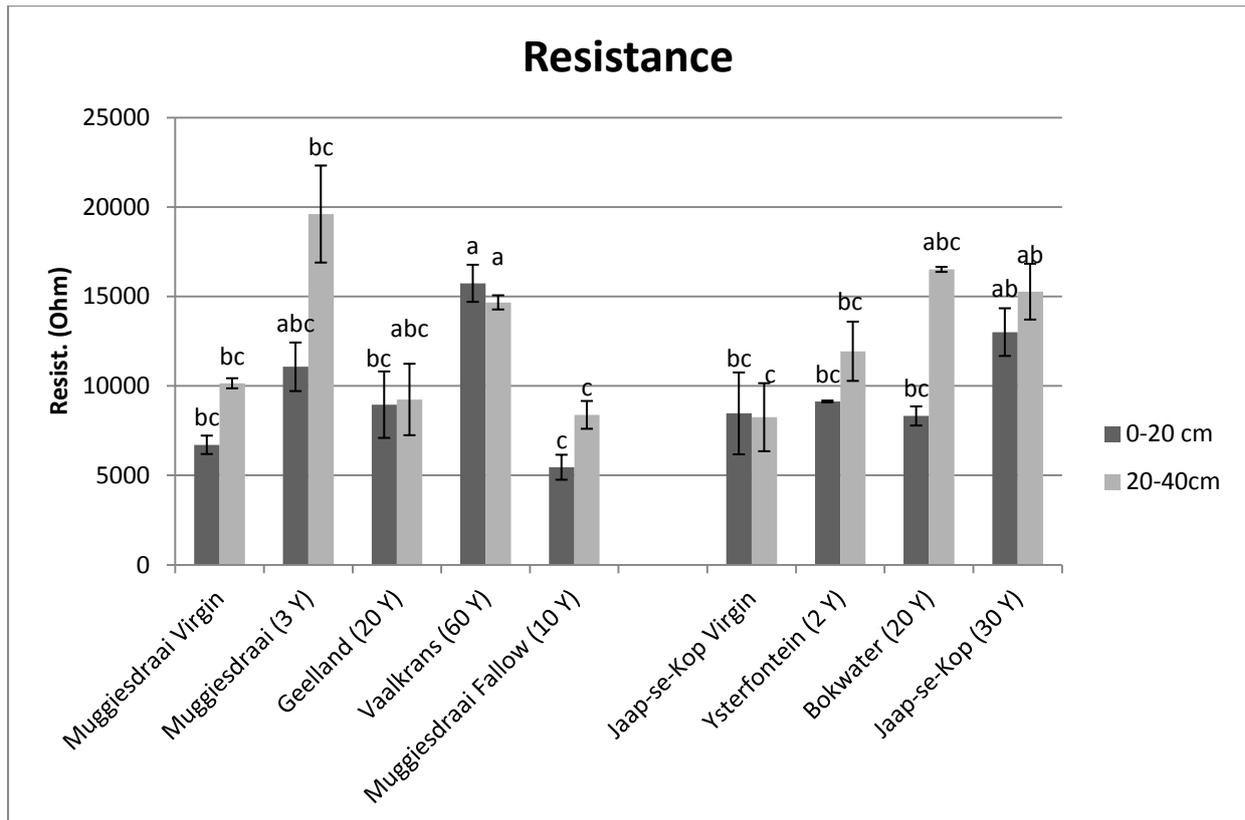


Figure A-81: Resistance at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

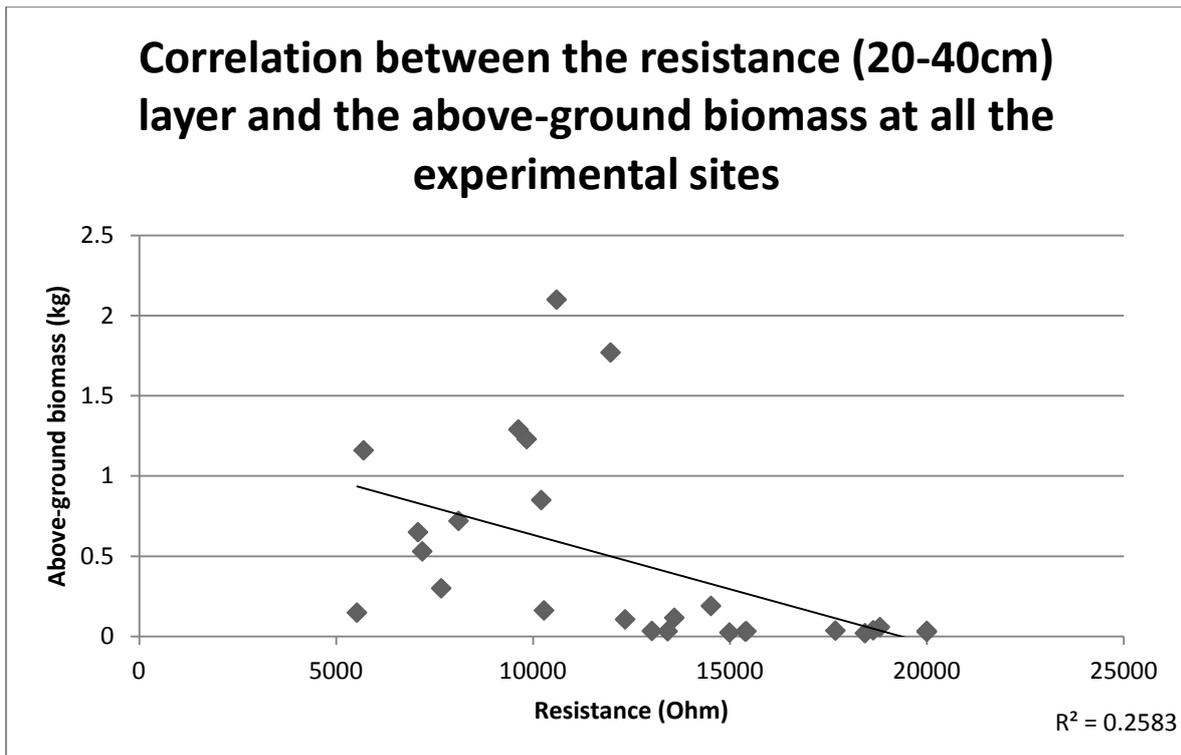


Figure A-82: Correlation between the resistance in the 20-40cm layer and the above-ground biomass at all the experimental sites

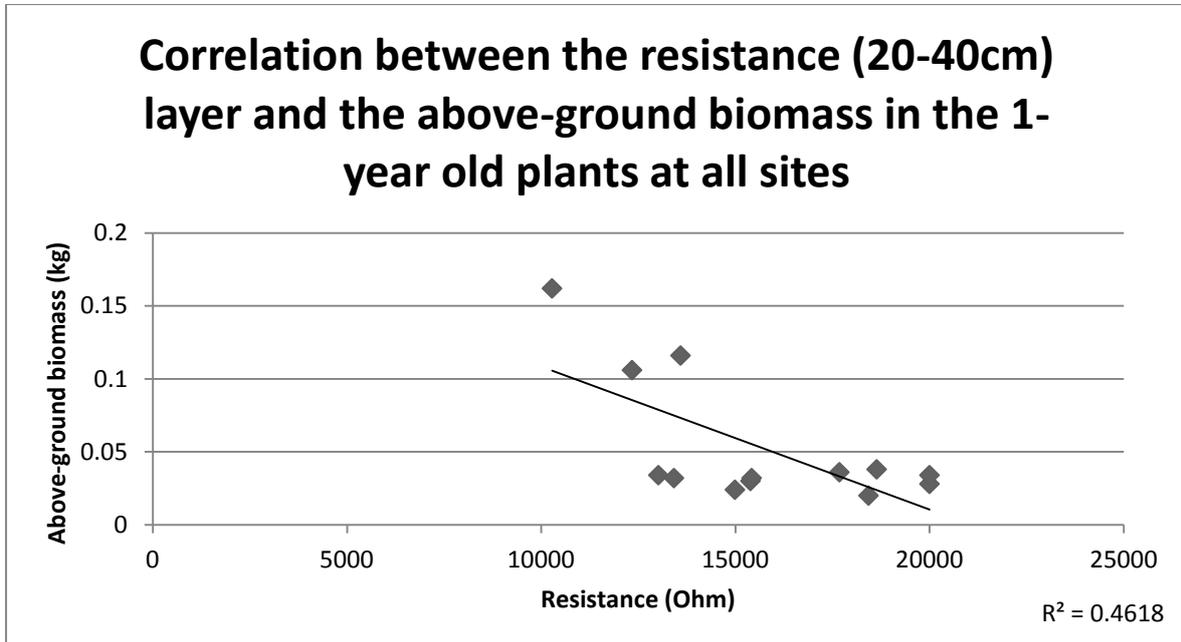


Figure A-3: Correlation between the resistance (20-40cm) layer and the above-ground biomass in the 1-year old plants at all sites

Appendix B. Research Chapter 4 supplementary data

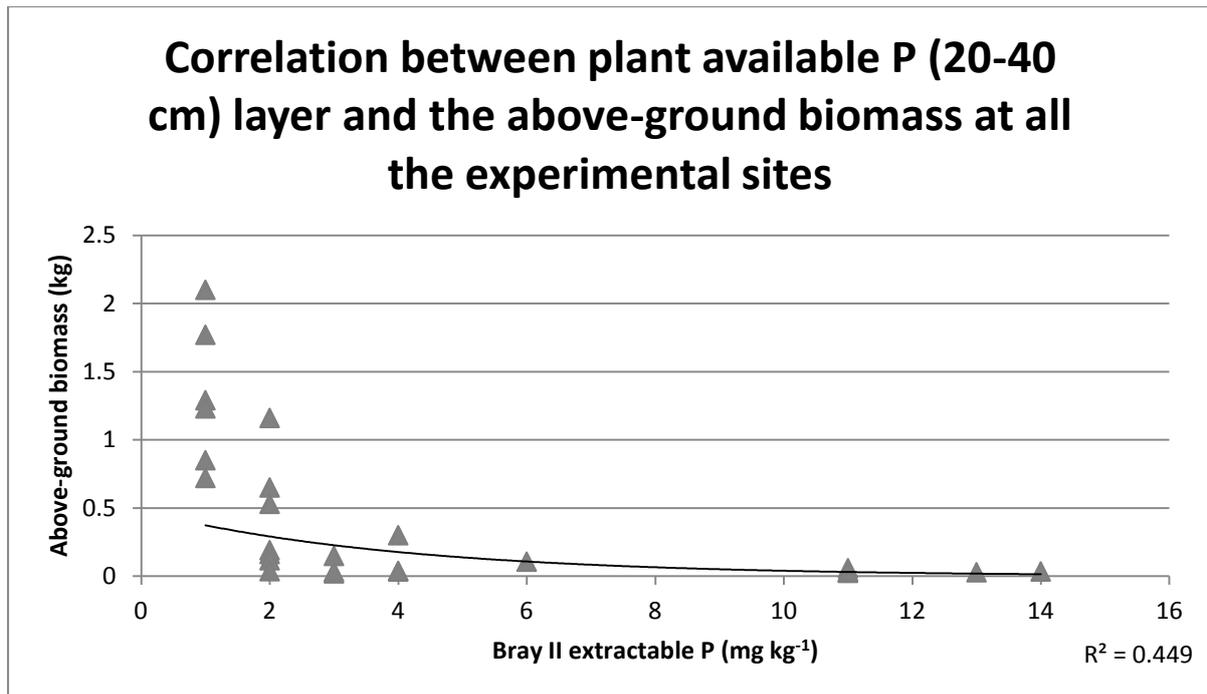


Figure B-1: Correlation between plant available P in the (20-40 cm) layer and the above-ground biomass at all the experimental sites

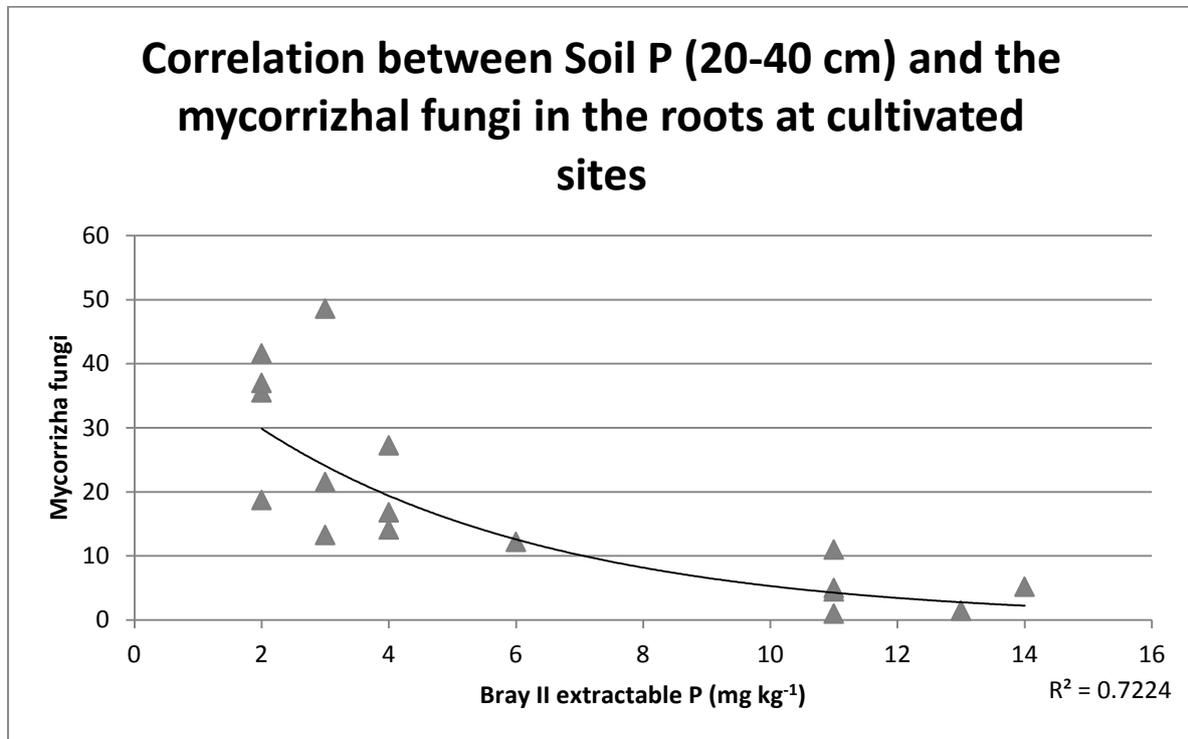


Figure B-2: Correlation between Soil P (20-40 cm) and the mycorrhiza fungi in the roots at cultivated sites

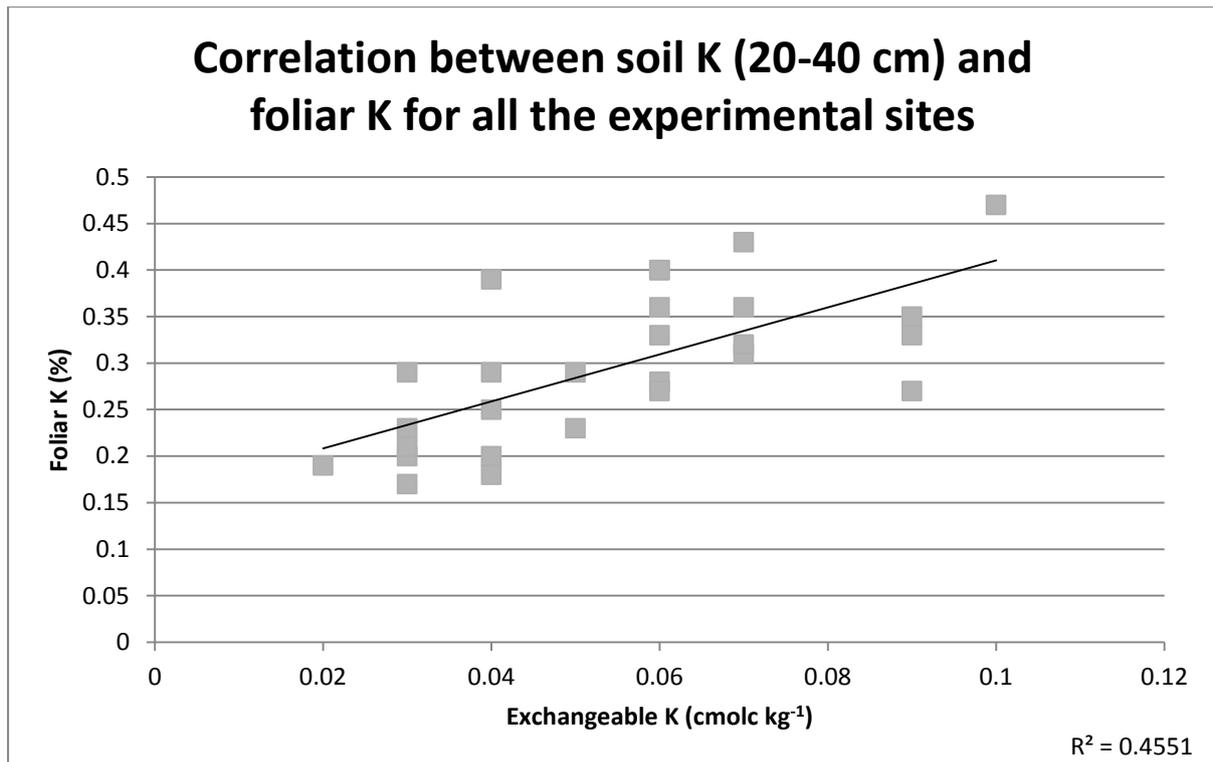


Figure B-3: Correlation between soil K (20-40 cm) and foliar K at all the experimental sites

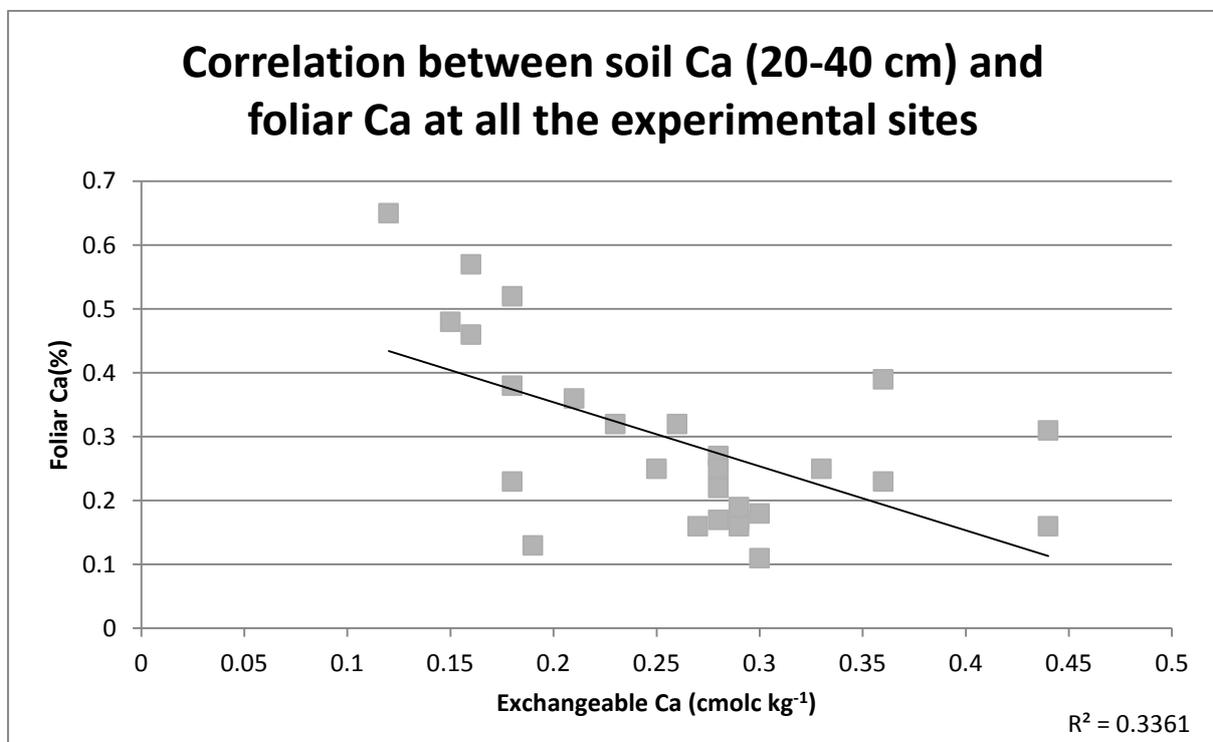


Figure B-4: Correlation between soil Ca (20-40 cm) and foliar Ca at all the experimental sites

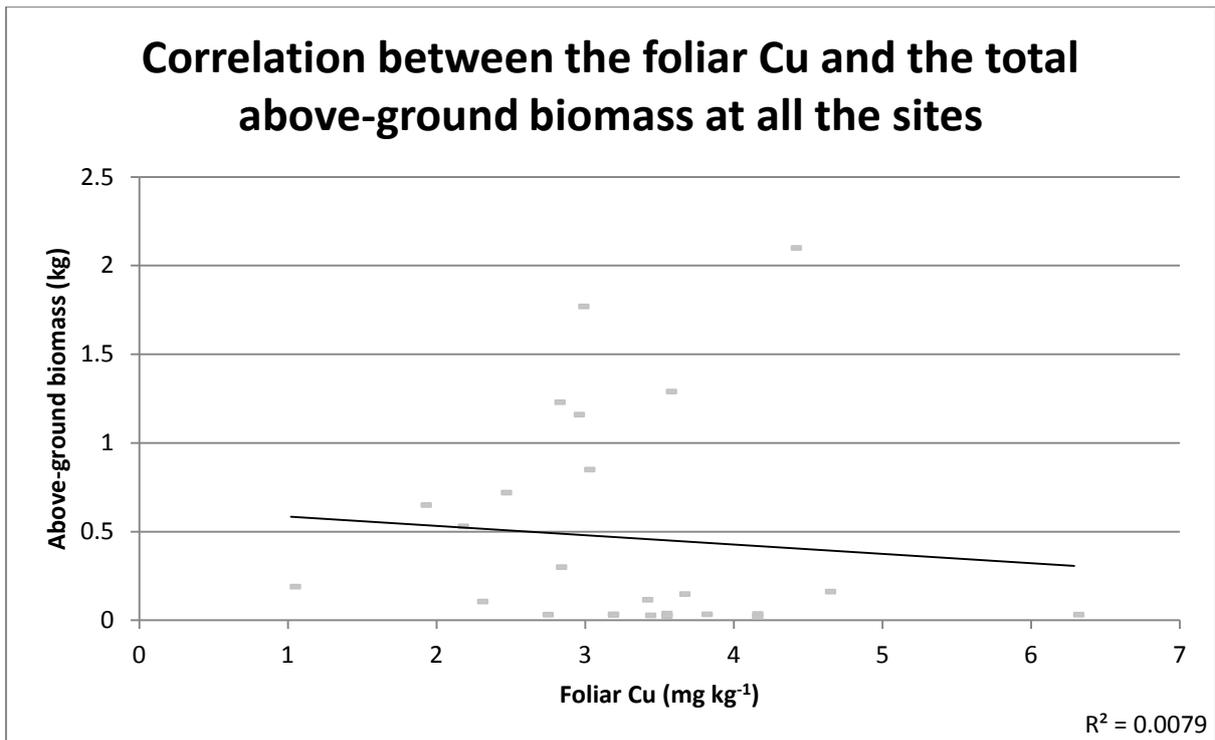


Figure B-5: Correlation between the foliar Cu and the total above-ground biomass at all experimental sites

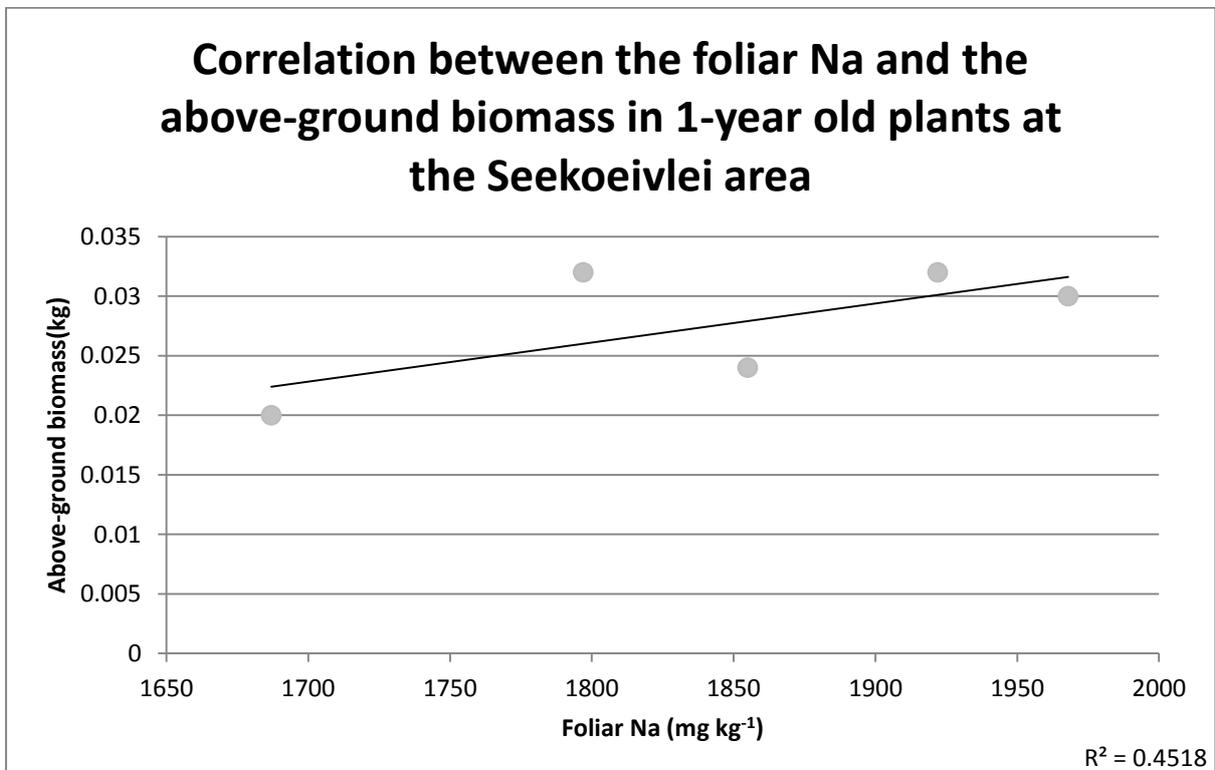


Figure B-6: Correlation between the foliar Na and the above-ground biomass in 1-year old plants at the Seekoeivlei area

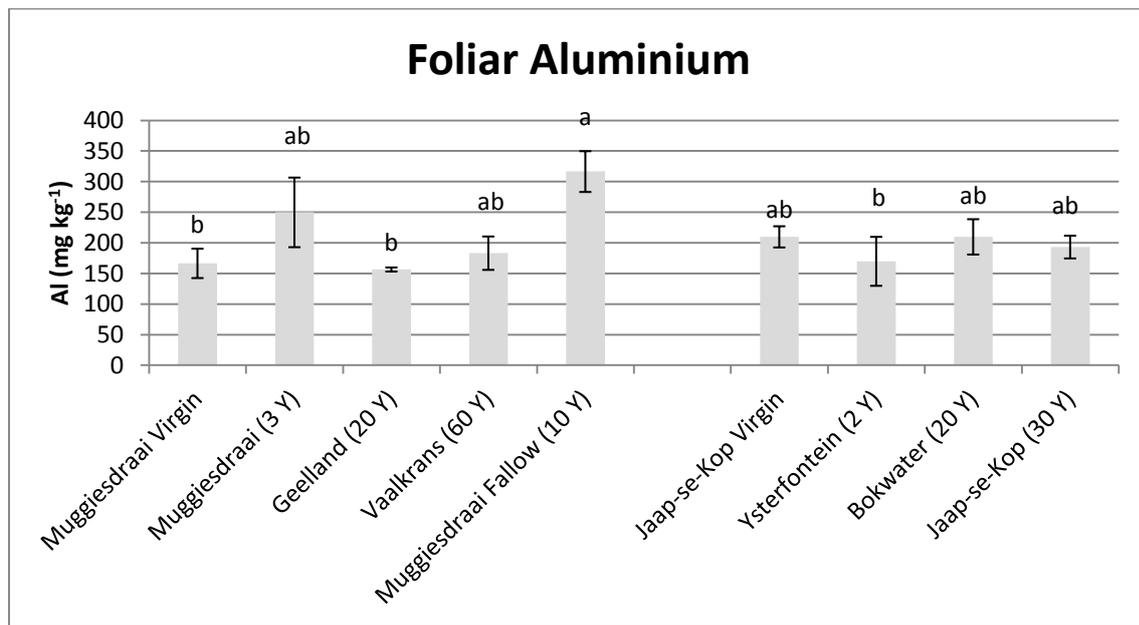


Figure B-7: Foliar Aluminium at the Nardouwsberg (left) and Seekoeivlei (right) sites. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

Table 8B-1: Dry colour, wet colour, extract colour and taste quality of the rooibos tea harvested from all sites (Feb 2013).

Sites	Wet Colour (%)	Dry Colour (%)	Extract Colour (%)	Taste (%)
Muggiesdraai Virgin	0 b	0 b	25 b	0 c
Muggiesdraai (3 Y)	37a	37 ab	53 ab	47 ab
Geelland (20 Y)	43 a	43 a	63 a	53 a
Vaalkrans (60 Y)	48 a	45 a	53 ab	30 b
Muggiesdraai Fallow (10 Y)	20 ab	20 ab	50 ab	40 ab
Jaap-se-Kop Virgin	20 ab	20 ab	60 a	45 ab
Ysterfontein (2 Y)	55 a	55 a	65 a	50 ab
Bokwater (20 Y)	53 a	53 a	60 a	43 ab
Jaap-se-Kop (30 Y)	30 ab	30 ab	50 ab	30 b

Note: The letters of significance differ between the experimental sites as well as between the different sensory aspects (wet-, dry-, extract-colour and taste)

Appendix C. Research Chapter 5 supplementary data

Soil bulk density

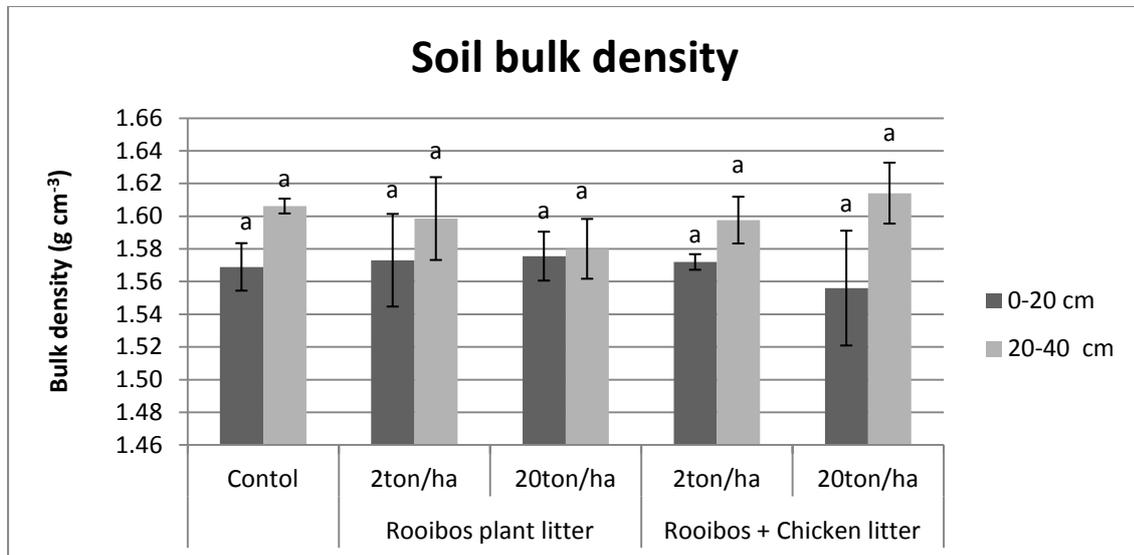


Figure C-1: Soil Bulk density values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

Resistance

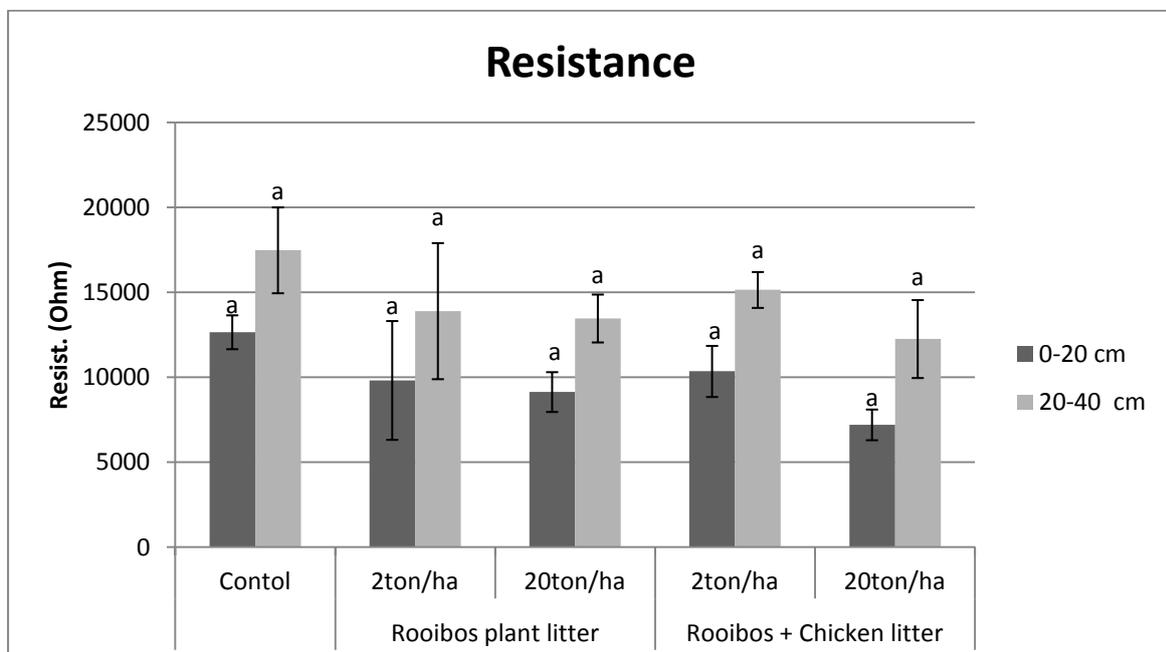


Figure C-2: Resistance values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

Soil Nutrients

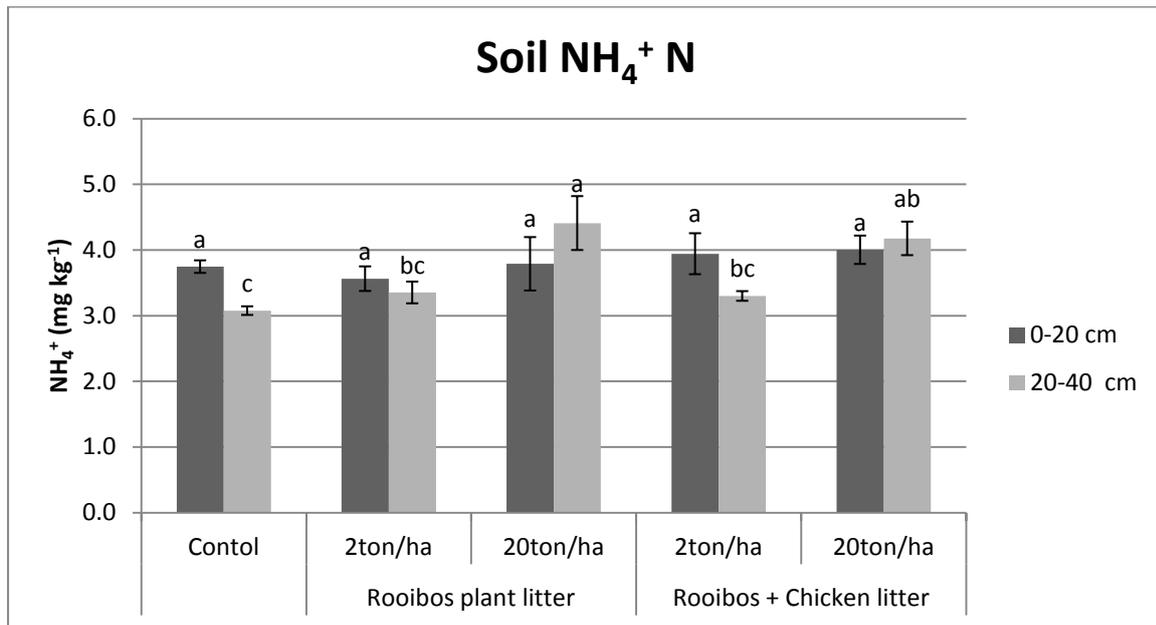


Figure C-3: Soil Ammonium (NH₄⁺) values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

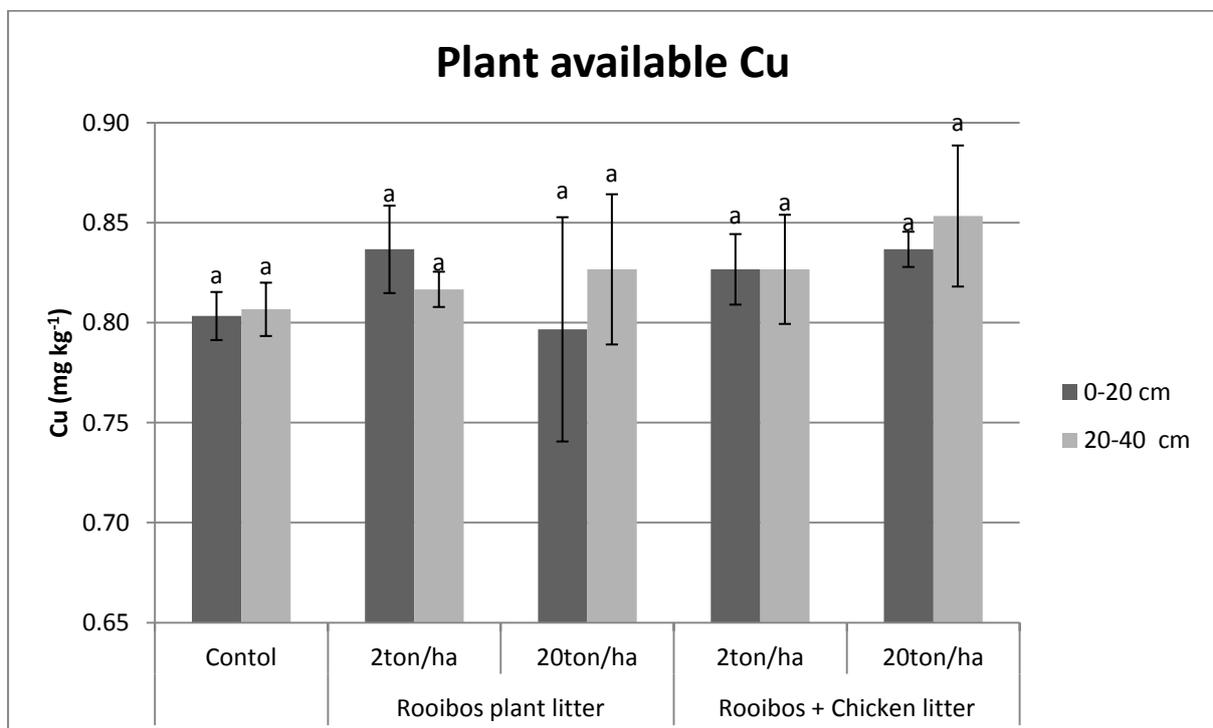


Figure 8C-4: Plant available Copper (Cu) values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

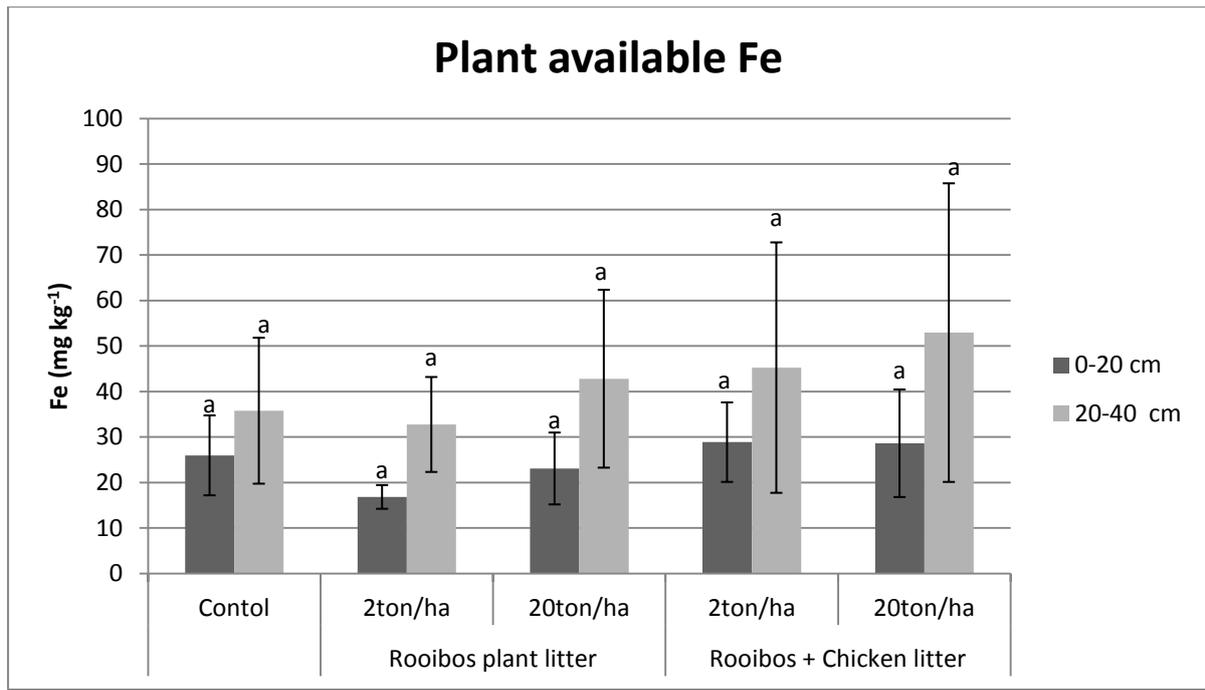


Figure 8C-5: Plant available Iron (Fe) values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

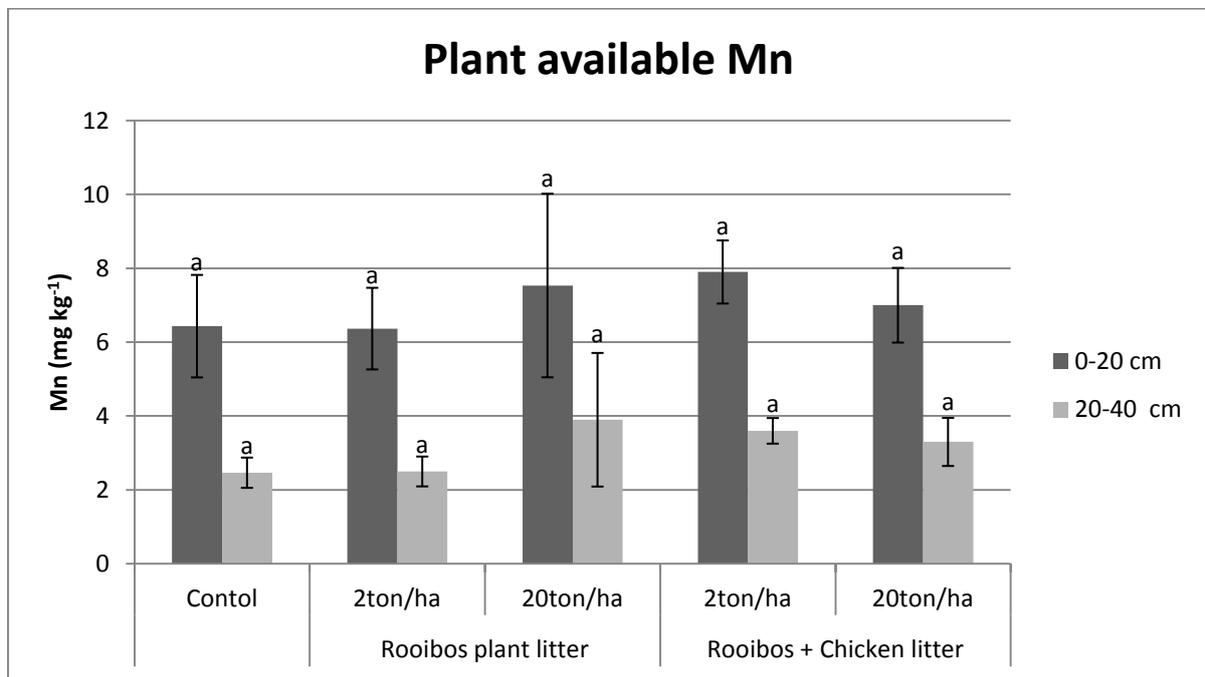


Figure C-6: Plant available Manganese (Mn) for values of the control and compost treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the treatments as well as the depth

Plant Nutrients

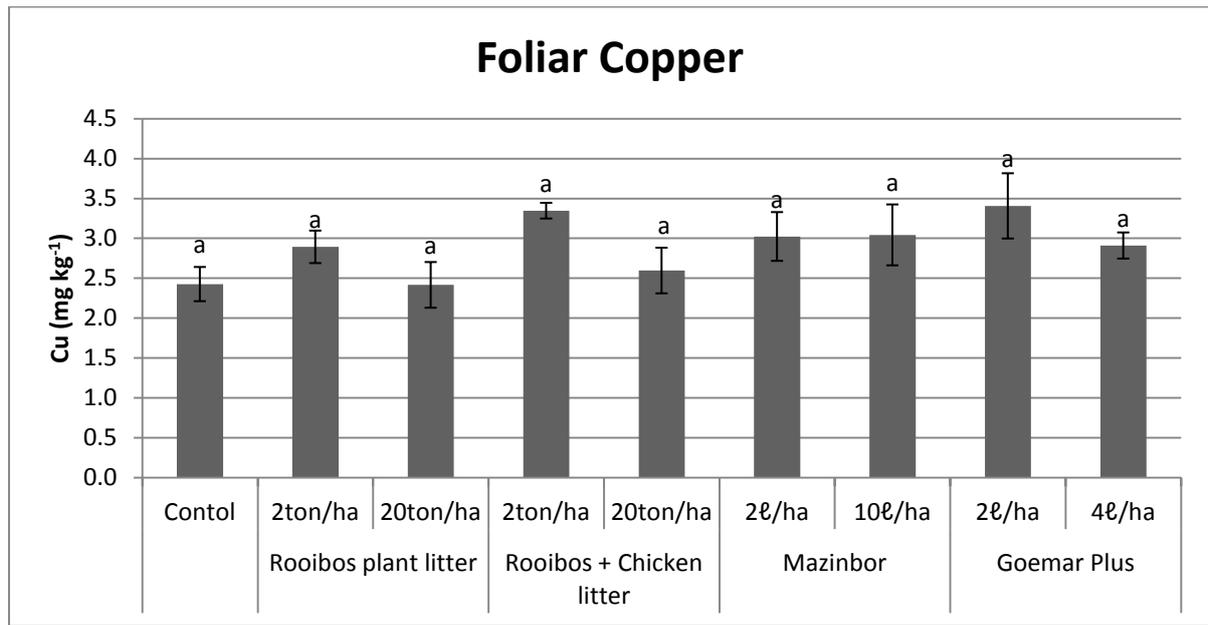


Figure 8C-7: Foliar Copper (Cu) contents of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

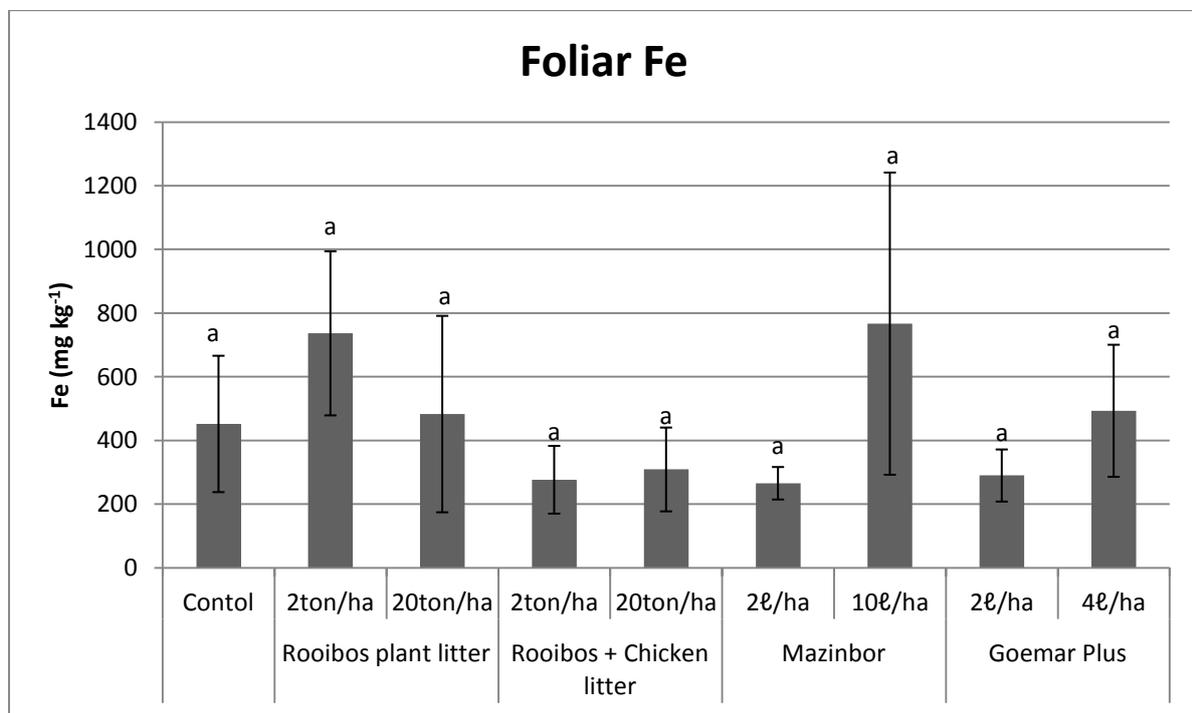


Figure 8C-8: Foliar Iron (Fe) contents of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

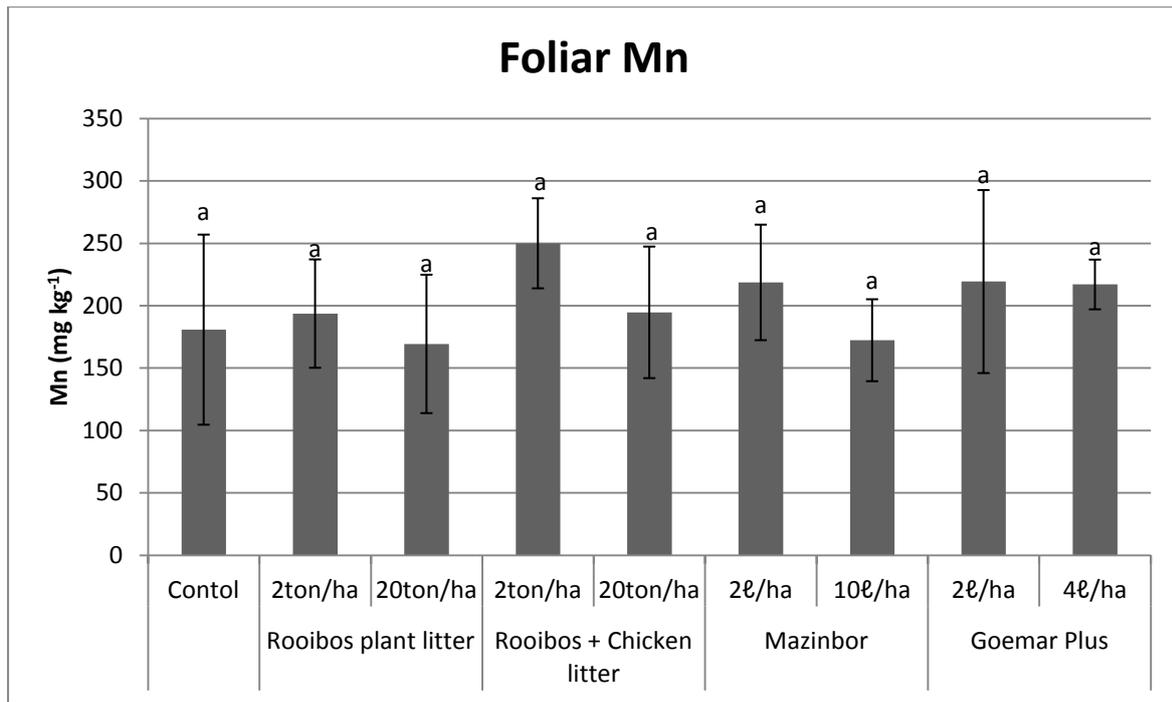


Figure 8C-9: Foliar Manganese (Mn) contents of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

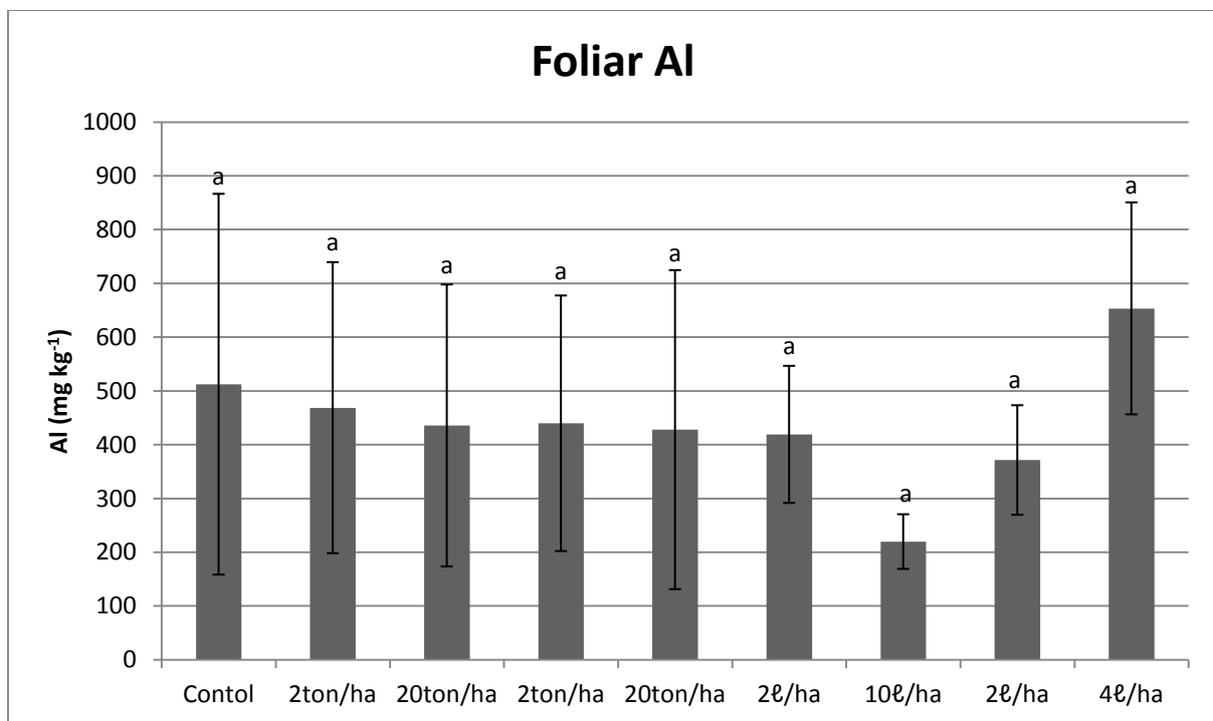


Figure C-10: Foliar Aluminium (Al) contents of the control, compost and foliar spray treatments at the oldest (Valkrans) experimental site. The standard error bars for each mean of each field site are shown (n = 3). The letters of significance differ between the experimental sites

Appendix D. Statistical Data

Table D-1: Correlation between the above-ground biomass and the different soil and plant factors influencing it at all of the experimental sites

	P - Value	Effect
Bulk density (20-40 cm)	P = 0.0851	
Below ground biomass	P < 0.001	+ effect
Nodules	P = 0.0557	
pH H ₂ O (20-40 cm)	P = 0.0034	- Effect
pH KCl (0-20 cm)	P = 0.0020	+ effect
EC (20-40 cm)	P 0.0393	+ effect
Foliar P	P = 0.0434	- Effect
Foliar Ca	P = 0.0098	+ effect
Foliar Na	P = 0.0051	+ effect
Foliar Fe	P = 0.0264	+ effect
Foliar Mn	P = 0.0309	- Effect
Foliar B	P = 0.0226	- Effect
Soil Ca (0-20 cm)	P = 0.0445	+ effect
Soil Mg (0-20 cm)	P = 0.0843	
Soil Mn (0-20 cm)	P = 0.0016	- Effect
Soil Mn (20-40 cm)	P = 0.0153	- Effect
Soil N (20-40 cm)	P = 0.1342	
Soil C (0-20 cm)	P < 0.0001	+ effect
Soil Na (20-40 cm)	P < 0.001	+ effect
Soil CBD Al (0-20 cm)	P = 0.0200	+ effect
Soil CBD Fe (20-40 cm)	P = 0.0006	- Effect

Table D-2: Correlation between the above-ground biomass and the different soil and plant factors influencing it at the experimental sites with 1-year old plants

	P - Value	Effect
Bulk density (0-20 cm)	P = 0.0345	- Effect
Protist	P = 0.0067	- Effect
Below ground biomass	P < 0.0001	+ effect
pH H ₂ O (0-20 cm)	P = 0.0350	+ effect
pH KCl (0-20 cm)	P = 0.0809	
pH KCl (20-40 cm)	P = 0.0027	- Effect
EC (20-40 cm)	P = 0.0009	+ effect
Foliar NH ₄ Nitrogen	P = 0.0840	
Foliar P	P = 0.0075	- Effect
Foliar K	P = 0.0006	+ effect
Foliar Ca	P = 0.0100	+ effect
Foliar Na	P = 0.0597	
Foliar Fe	P = 0.1431	
Foliar Cu	P = 0.1259	
Foliar Zn	P = 0.0571	
Foliar Mn	P = 0.0429	- Effect
Foliar B	P = 0.0002	+ effect
Foliar Al	P = 0.0189	- Effect
Soil C (0-20 cm)	P = 0.0233	+ effect
Soil C (20-40 cm)	P = 0.0142	+ effect
Soil B (0-20 cm)	P = 0.8656	
Soil P (20-40 cm)	P = 0.0647	
Soil K (20-40 cm)	P = 0.0129	+ effect
Soil Fe (20-40 cm)	P = 0.1934	
Soil CBD Al (20-40 cm)	P = 0.5967	

Table D-3: Correlation between the dry colour influencing the quality and the different soil and plant factors influencing it at the experimental sites with 1-year old plants

	P - Value	Effect
Protist	P = 0.0917	
Foliar P	P = 0.0400	- Effect
Foliar K	P = 0.1815	
Foliar Ca	P = 0.3483	
Foliar Na	P = 0.0470	+ effect
Foliar B	P = 0.6461	
Soil resistance (0-20 cm)	P = 0.5472	
EC (0-20 cm)	P = 0.0851	
Soil H ⁺ (0-20 cm)	P = 0.1010	
Soil C (0-20 cm)	P = 0.0910	
Soil P Bray II (0-20 cm)	P = 0.0049	- Effect
Soil P Bray II (20-40 cm)	P = 0.1070	
Soil Na (0-20 cm)	P = 0.1418	
Soil K (0-20 cm)	P = 0.0553	
Soil Mn (0-20 cm)	P = 0.7640	
Soil Fe (0-20 cm)	P = 0.8809	
Soil Cu (20-40 cm)	P = 0.1070	
Soil Zn (20-40 cm)	P = 0.1559	
Soil B (20-40 cm)	P = 0.0215	- Effect
Soil CBD Mn (0-20 cm)	P = 0.0116	- Effect

Table D-4: Correlation between the wet colour influencing the quality and the different soil and plant factors influencing it at the experimental sites with 1-year old plants

	P - Value	Effect
Protist	P = 0.2215	
Foliar NH ₄ nitrogen	P = 0.0172	+ effect
Foliar Mn	P = 0.1000	
Soil Na (0-20 cm)	P = 0.4842	
Soil B (0-20 cm)	P = 0.2664	
EC (0-20 cm)	P = 0.6817	
Soil Resistance (20-40 cm)	P = 0.5865	
Soil S (0-20 cm)	P = 0.5652	
Soil Zn (20-40 cm)	P = 0.0307	+ effect
Soil Mn (20-40 cm)	P = 0.0648	
Soil Fe (20-40 cm)	P = 0.0518	
Soil CBD Al (20-40 cm)	P = 0.0592	

Table D-5: Correlation between the extract colour influencing the quality and the different soil and plant factors influencing it at the experimental sites with 1-year old plants

	P - Value	Effect
Protist	P = 0.0235	+ effect
Foliar P	P = 0.2978	
Foliar Zn	P = 0.3489	
Foliar Mn	P = 0.2562	
EC (20-40 cm)	P = 0.5442	
Soil C (0-20 cm)	P = 0.4763	
Soil Bray II P (0-20 cm)	P = 0.1790	
Soil Bray II P (20-40 cm)	P = 0.2910	
Soil Cu (0-20 cm)	P = 0.2005	
Soil Zn (20-40 cm)	P = 0.0483	- Effect
Soil CBD Al (0-20 cm)	P = 0.0101	- Effect
Soil CBD Mn (0-20 cm)	P = 0.0580	
Soil CBD Al (20-40 cm)	P = 0.1019	

Table D-6: Correlation between the taste influencing the quality and the different factors soil and plant influencing it at the experimental sites with 1-year old plants

	P - Value	Effect
Bulk density (20-40 cm)	P = 0.0833	
Foliar P	P = 0.0135	- Effect
Foliar K	P = 0.0763	
Foliar Mg	P = 0.0086	+ effect
Foliar Na	P = 0.0105	+ effect
Foliar Fe	P = 0.2104	
EC (0-20 cm)	P = 0.1365	
Soil Resistance (0-20 cm)	P = 0.0441	- Effect
Soil Resistance (20-40 cm)	P = 0.2531	
Soil Bray II P (0-20 cm)	P = 0.0074	- Effect
Soil Bray II P (20-40 cm)	P = 0.1844	
Soil K (0-20 cm)	P = 0.3690	
Soil Mn (0-20 cm)	P = 0.2716	
Soil Mn (20-40 cm)	P = 0.0010	- Effect
Soil Fe (0-20 cm)	P = 0.7917	
Soil Fe (20-40 cm)	P = 0.0076	- Effect
Soil Cu (20-40 cm)	P = 0.0326	+ effect
Soil B (20-40 cm)	P = 0.0744	
Soil S (20-40 cm)	P = 0.0107	+ effect
Soil CBD Fe (0-20 cm)	P = 0.0789	
Soil CBD Al (0-20 cm)	P = 0.0291	- Effect
Soil CBD Al (20-40 cm)	P = 0.0175	- Effect
Soil CBD Mn (20-40 cm)	P = 0.0002	- Effect