The effect of residue management on the nutrient cycle in the production of rooibos (*Aspalathus linearis*) at Nieuwoudtville, Northern Cape

by

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Thesis presented in fulfilment of the requirements for the degree of MASTER OF SCIENCE IN AGRICULURE in the Faculty of AgriSciences at Stellenbosch University

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March 2017

DECLARATION

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Date: March 2017

ABSTRACT

Rooibos (*Aspalathus linearis*) is a sensitive fynbos species with a large genetic variation, adapted to acid, nutrient poor soils, and can only be grown in certain parts of the Western- and Northern-Cape. Rooibos yields are getting poorer with increasing age of the field and the lifespan of rooibos are also just a maximum of 5 years commercially produced. A lot of medicinal/health research has been done and published on rooibos, but not much on the agricultural production aspects of rooibos. In order to increase the production and lifespan of rooibos tea further research needs to be done to better understand the rooibos plant in its cultivated environment. The role of leaf litter in fynbos, particularly in the cultivated rooibos nutrient cycle is still a grey area that could open up key management principles regarding plant water availability and plant nutrition style. The hypothesis is that the method of harvesting the rooibos seed by removing the litter layer without returning it under the plant can have a negative impact on the nutrient pools and cycle and thus lead to a shorter lifespan. The main aim was thus to look at the effect of different residue treatments on the rooibos plant nutrient cycle (uptake and nutrient pools).

Four sites across the Nieuwoudtville Bokkeveld region were selected with all the rooibos plants being ± 2 years old. Four different mulch treatments; a bare soil (leaf residue removed) treatment imitating seed harvesting (A), an added rooibos mulch (B), a natural leaf mulch (C) and an enriched rooibos mulch (D) were prepared at 4 sites. The chemical properties of soil and plant tissue from rooibos plants were tested. 5TE soil probes were used to measure the volumetric water content, EC and temperature at two soil depths of each treatment. All measurements were also duplicated using near-infrared spectroscopy (NIRS), to generate a database for future reference and to build calibrations that will be able to predict the nutrient content in the soils and plants.

It was found that soil chemical properties including P (mg kg^{-1}), Na ($cmol_c$ kg^{-1}), K ($cmol_c$ kg^{-1}), Ca ($cmol_c$ kg^{-1}), Mg ($cmol_c$ kg^{-1}), Zn (mg kg^{-1}), Mn (mg kg^{-1}), C (%) and % Na (at pH 7); and plant chemical properties including Na (%) and plant N (%), P (%), K (%), Al (mg kg^{-1}) and Fe (mg kg^{-1}) all had a significant effect of the regrowth models using multiple regression analysis. Soil P, Mg and K had the biggest positive influences on the regrowth models. During this process it was found that the N:P ratio in soil plays an important role in the uptake of N and growth. Only at treatment D, with the lowest soil N:P ratio, plant N (%) had a positive influence on the regrowth multiple regression model. Plant N and P had a moderate positive correlation (R^2=0.56).

Nutrient uptake by the rooibos plant was very high from July 2015 to September 2015. These nutrients included N, P, K, Ca, Mg, Zn, Mn, Fe and Al. From September 2015 to January 2016 however the uptake was lower for all the nutrients, but for K and Mg the uptake was higher compared to the other nutrients. The decrease in plant nutrient concentration from September 2015 to January 2016 is a result of less nutrient uptake and nutrient dilution following rapid growth of plant. There was an increase in soil exchangeable Mg and Ca from July 2015 to September 2015 due to increase in soil pH during this time. Soil exchangeable Ca (R^2=0.49) and Mg (R^2=0.61) correlated positively with pH, thus the increase in soil exchangeable Ca and Mg can be due to the increase in pH. For all the treatments there was a total decrease in soil N (significant for A and B) and plant N over the one-year period. The plant Al and Zn for all the treatments also increased over the one-year period. The increase in plant Al was not significant and the increase in plant Zn was significant for all treatments. From July 2015 to January 2016 there were differences in growth between the treatments. Treatment A resulted in the lowest estimated dry matter increase during this period and for treatment B it was the highest. The difference in estimated dry matter increase between these two treatments was also statistically significant. The estimated dry matter increase for treatment C and D was higher than treatment A but it was not significant. The mulch treatments, especially treatment B, resulted in higher P, K and Mg uptake. For all the treatments, except treatment D, the soil P decreased over the one-year period. For treatment B and D the plant P increased significantly compared to treatment A and C where the increase was not significant. The mulch treatments showed an increase in plant K, but it was only significant for treatment B over the one-year period. For all the treatments there was an increase in plant Mg, but only for treatment A the increase was not significant. All the mulch treatments also conserved more water for longer compared to treatment A. The combination of nutrient leaching from the mulch (Mg and K) and the conservation of more soil water may be the reason for the higher nutrient uptake by treatment B and thus the better growth.

From the NIRS study it was found that for these sandy soils it was only exchangeable Mg that could be predicted with good accuracy (RPD>2). The soil chemical properties pH, H (cmolc kg^{-1}), K (mg kg^{-1}), Ca (cmolc kg^{-1}), Fe (mg kg^{-1}) and C (%) models showed satisfactory predictability. For plant samples NIRS predicted P (%) and Mg (%) with good accuracy. The prediction models for N (%), K (%), Ca (%) and Na (mg kg^{-1}) were only satisfactory and for the rest it was unreliable. From these results it was thus not possible to quantitatively predict all the chemical properties in the soil and plant samples but there is potential for better calibrations in the future.

Differences in growth and vigour can also be attributed to location. The micro conditions in which a single plant grows, related to the impact of normal agricultural practices, was found to also determine the success of rooibos production. The hypothesis was supported by treatment C (the plant where natural mulch was not removed) having a higher estimated dry matter increase compared to treatment A (bare soil), but this difference was not significant. Treatment B (added mulch) however showed to have a significant impact. Rooibos production systems are far from being optimized and the amounts of unknown impacts were narrowed down by this work. This research indicates that the rooibos plant is genetically unrefined and that agricultural practices should focus more towards the natural state of fynbos nutrient availability and growth.

OPSOMMING

Rooibos (*Aspalathus linearis*) is ’n sensitiewe fynbos spesie met groot genetiese variasie, aangepas vir suur, voedingstof arme gronde en kan net verbou word in sekere gedeeltes van die Wes- en Noord-Kaap. Rooibos opbrengste raak al hoe swakker met toenemende ouderdom van die veld en die lewensduur van rooibos is ook net ’n maksimum van 5 jaar (kommersieel verbou). Baie medisinale en gesondheidsnavorsing is al gedoen en gepubliseer, maar min op die landbouproduksie aspek van rooibos. Om die produksie en lewensduur van rooibostee te verhoog en te verleng word verdere navorsing benodig om die rooibos plant in sy kommersieel verboude omgewing beter te verstaan. Die rol van ’n blaar deklaag in fynbos, veral in die rooibos voedingstof siklus, is nog steeds ’n onbekende area en kan lei tot baie belangrike bestuurs praktyke , met betrekking tot plant water beskikbaarheid en plantvoeding styl. Die hipotese is dat die manier waarop die rooibos saad geoes word deur die verwydering van die natuurlike blaar deklaag sonder om dit terug te sit onder die plant ’n negatiewe impak op die voedingstof poele en siklus het en dus lei tot laer opbrengste en ’n korter rooibos lewensduur. Die hoofdoel van die studie was dus om te kyk na die effek van verskillende rooibos blaar deklae op die rooibos plantvoedingstof siklus (opname en voedingstof poele).

Vier eksperimentele areas (± 2 jaar oud rooibostee) oor die Nieuwoudtville Bokkeveld Plato streek is gekies waar die proewe uitgevoer sou word. Vier verskillende deklaag behandelings; ’n kaal grond (A) (nabootsing van waar saad verwyder is), ’n ekstra rooibos blaar deklaag (B), ’n natuurlike blaar deklaag (C) en ’n verrykte rooibos blaar deklaag (D) is voorberei by elkeen van die uitgesoekte areas. Die chemiese eienskappe (voedingstof status) van die grond en rooibos plantweefsel is ontleed. 5TE grond sensore is gebruik om die volumetriese waterinhoud, temperatuur en EC te meet op twee dieptes, een plant van elke behandeling. Al die grond en plant monster ontledings is ook gedupliseer met behulp van NIR spektroskopie om ’n databasis vir toekomstige verwysing te genereer en om kalibrasies, wat ons in staat sal stel om die voedingstofinhoud in die grond en plante te kan voorspel.

Deur gebruikmaking van veelvuldige regressie ontledings is dit bevind dat die grond chemie, [P (mg kg^{-1}), Na ($cmol_c$ kg^{-1}), K ($cmol_c$ kg^{-1}), Ca ($cmol_c$ kg^{-1}), Mg ($cmol_c$ kg^{-1}), Zn (mg kg^{-1}), Mn (mg kg^{-1}), C (%) en % Na (by pH 7)] en plant chemiese eienskappe [Na (%) en plant N (%), P (%), K (%), Al (mg kg^{-1}) en Fe (mg kg^{-1})] almal ’n beduidende effek gehad het op die hergroei modelle. Deur hierdie proses van uitkenning t.o.v. die belangrikste voedingstowwe, is bevind dat die N:P verhouding in die grond ’n belangrike rol speel met die opname van N en ook groei. Net by behandeling D, met die laagste N:P

verhouding, het plant N (%) 'n positiewe effek gehad in die hergroei model. Plant N en P het 'n positiewe korrelasie getoon (R^2=0.56).

Voedingstof opname deur die rooibos plante was baie hoog vanaf Julie 2015 tot September 2015. Hierdie voedingstowwe sluit in N, P, K, Ca, Mg, Zn, Mn, Fe en Al. Van September 2015 na Januarie 2016 was dit laer vir al die voedingstowwe, maar vir K en Mg was die opname hoër in vergelyking met die ander voedingstowwe. Die afname in plant voedingstof konsentrasie vanaf September 2015 na Januarie 2016, is 'n gevolg van minder voedingstof opname en verdunning a.g.v. die drastiese toename in groei. Daar was 'n toename in grond uitruilbare Mg en Ca van Julie 2015 na September 2015 a.g.v. die toename in grond pH. Grond uitruilbare Ca (R^2=0.49) en Mg (R^2=0.61) het positief gekorreleer met grond pH, dus die gevolglike toename in uitruilbare Ca en Mg met toename in pH. Vir al die behandelings is daar 'n totale afname in die grond N bespeur (belangrik vir A en B) en plant N oor die tydperk van een jaar. Die plant Al en Zn vir al die behandelings het ook toegeneem oor die tydperk van een jaar. Die toename in die plant Al was nie betekenisvol nie, maar die toename in die plant Zn was betekenisvol vir alle behandelings. Vanaf Julie 2015 tot Januarie 2016 was daar verskille in groei tussen die behandelings. Behandeling A het die laagste beraamde toename in droëmassa getoon gedurende hierdie tydperk, en vir behandeling B was dit die hoogste. Die verskil in beraamde droëmassa toename tussen hierdie twee behandelings was statisties betekenisvol. Die beraamde droëmassa toename vir behandeling C en D was hoër as behandeling A, maar was nie statisties betekenisvol nie. Die deklaag behandelings, veral behandeling B, het gelei tot hoër P, K en Mg opname. Vir al die behandelings, behalwe behandeling D, het die grond P afgeneem oor die tydperk van een jaar. Vir behandeling B en D het die plant P aansienlik toegeneem in vergelyking met behandeling A en C. Slegs die deklaag behandelings het 'n toename in die plant K getoon, maar net vir behandeling B oor die tydperk van een jaar was die toename betekenisvol. Vir al die behandelings was daar 'n toename in die plant Mg, maar slegs vir behandeling A was die toename nie betekenisvol nie. Al die deklaag behandelings het ook meer water vir langer bewaar in vergelyking met behandeling A. Die kombinasie van voedingstof loging uit die deklaag (Mg en K) en die bewaring van meer grondwater m.b.v. van deklae, kan die rede wees vir die hoër voedingstof opname veral deur behandeling B en dus beter groei.

Vanuit die NIRS studie was bevind dat, vir die baie sanderige gronde, dit net uitruilbare Mg (cmolc kg^{-1}) was wat voorspel kon word met hoë akkuraatheid (RPD>2). Die modelle vir die grond chemiese eieskappe [pH, H (cmolc kg^{-1}), K (mg kg^{-1}), Ca (cmolc kg^{-1}), Fe (mg kg^{-1}) en C (%)] het bevredigende voorspelbaarheid vertoon. By die plantmonsters was dit net die P (%) en Mg (%) wat met hoë akkuraatheid voorspel kon word. Die modelle vir N (%), K (%), Ca (%) en Na (mg kg^{-1}) was net

bevredigend en vir die res onbetroubaar. Van hierdie resultate was dit dus nie moontlik om al die chemiese eienskappe van die grond en plant monsters kwantitatief akkuraat te voorspel m.b.v. NIRS nie, maar daar is potensiaal met verdere navorsing vir 'n beter kalibrasies in die toekoms.

Verskille in groeikrag kan ook toegeskryf word aan spesifieke ligging. Met die mikro omstandighede waarin ’n enkele plant groei, wat verband hou met die impak van normale landboupraktyke, is bevind dat dit ook die sukses van rooibos produksie bepaal. Die hipotese is ondersteun deur behandeling C (die plant waar natuurlike deklaag nie verwyder) wat 'n hoër geraamde droëmateriaal toename in vergelyking met behandeling A (kaal grond) getoon het, maar die verskil was nie betekenisvol nie. Behandeling B (bygevoegde deklaag) het egter tot 'n betekenisvolle toename in groei getoon. Rooibos produksiestelsels is vêr van optimaal en die hoeveelheid van onbekende impakte is verklein deur hierdie werk. Hierdie navorsing dui daarop dat die rooibos plant geneties onverfynd is en dat landboupraktyke meer moet fokus op die natuurlike toestand van fynbos voedingstof beskikbaarheid en groei.

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LIST OF ABREVIATIONS

ARC	Agricultural Research Council
NIRS	Near infrared spectroscopy
RMSEE	Root mean square error of estimation
RMSEP	Root mean square error of prediction
RPD	ratio of performance deviation
VWC	Volumetric water content

ACKNOWLEDGEMENTS

- Dr WP de Clercq, my supervisor for his guidance, encouragement and patience.
- Dr Hardie for her recommendations.
- Bertie van der Merwe for his help and assistance with start of my MSc project.
- The support and prayers from my family and friends.
- My fellow post-graduate students at the Department of Soil Science.
- All the lecturers (Dr Hoffman, Dr Clarke, Dr Rozanov and Dr van Zyl) at the Department of Soil Science of Stellenbosch for help when and where it was needed.
- The support staff (Tannie Annetjie, Matt, Nigel, Delphine and Tanya).
- Dr Hélène Nieuwoudt from the Department of Viticulture and Oenology and Institute of Wine Biotechnology for helping me with the NIR Spectroscopy (guidance and patience).
- All of the farmers, part of the Nieuwoudtville rooibos community that I visited for my research.
- The Nieuwoudtville Rooibos factory and staff for letting me use their facilities.
- The financial support from Northern Cape Department Agriculture (project and personal funding) and the National Research Foundation for personal funding (2016).

1. GENERAL INTRODUCTION

Over the past several years the global demand for rooibos (*Aspalathus linearis*) tea has increased due to the great taste as beverage and health benefits. Thanks to research it is known that rooibos tea is caffeine free, treats allergies, relieves stress, healthy for skin, ideal shield against the development of Type II diabetes etc. Rooibos farmers face challenges meeting this great demand and therefore research that focuses on the agricultural aspect of rooibos is very important. The biggest challenge the rooibos farmers face is the limited suitable and available land, because of the small area in South-Africa rooibos is adapted to grow in and environmental laws restricting farmers to establish new rooibos fields in order to protect fynbos (Pretorius, 2009). The other big challenge farmers face is the decrease of rooibos yield with an increase in age of rooibos fields. In the last few years the average rooibos yield per hectare has decreased with approximately 200 kg ha^{-1} (Smith, 2014). To overcome these challenges, solutions through research need to be found so that the rooibos demand can be met.

A lot of medicinal/health research has been done and published on rooibos, but not much on the agricultural aspects of rooibos cultivation. There are a few studies that focused on or included research on cultivated rooibos. Joubert *et al.* (1987) did a 5-month trial in a greenhouse to look at the effect of added macronutrients, nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) on the growth of rooibos seedlings. Stassen (1987) investigated the effect of different harvesting practices on some vegetative and physiological aspects. Muofhe (1997) did a thesis on the effect of N, P and Ca on N-nodulation of young rooibos plants in the field over a six-month period in a greenhouse. Louw (2006) did research on the sustainable harvesting of wild rooibos. Smith (2014) investigated the effect of long-term rooibos cultivation on soil quality and the effect of compost and foliar sprays on soil quality and plant properties. Lötter (2014) did research on the seasonal variation in carbon assimilation and nitrogen nutrition in wild and cultivated rooibos tea. Chimphango *et al* (2016) investigated the effect of organic cultivation of rooibos tea plants (*Aspalathus linearis*) on soil nutrient status in Nieuwoudtville.

This MSc study form part of a bigger research project aimed at optimising soil fertility and plant nutrient status for increased rooibos tea quality and sustainable rooibos production in the Northern Cape (de Clercq and van der Merwe, 2015). The focus of the study was to better understand the cultivated rooibos plant and its environment to be able to make recommendations that can lead to more sustainable rooibos cultivation and larger yields.

The first objective **(Chapter 4)** of this study was the first step in identifying the important nutrients, in a process where the effect of different residue management practices on the nutrient cycle are studied. In this study statistical analysis methods including principle component analysis (PCA) and multiple regression analysis were used to look at the distribution and difference between data. With the help of multiple regression analysis it was possible to identify which nutrients had a significant effect on growth in combination with the other soil nutrients.

The second objective **(Chapter 5)** of the study was to investigate the effect of different residue management practices on the nutrient cycle in the production of rooibos and also to define rooibos plant nutrient demand at different phenological stadia. When rooibos seeds are harvested the litter layer under the plant gets removed. Most of the feeding roots of the rooibos plant grow in the shallow top soil. This action of seed (litter) harvesting seems to interfere with the feeding roots of the plants, leaving them bare to the soil surface. In contrast to other fynbos plants like the renosterbos, the plant needs this litter layer. When the plant stress they drop their leaves to generate a litter layer from which they will feed again. The role of leaf litter in fynbos, particularly in the cultivated rooibos nutrient cycle is still a grey area. The top soil (30 cm depth) is thus of upmost importance when it comes to nutrient uptake and feeding of the plant. Soil samples were only taken in the top soil (rhizosphere). The residue management trials consisted of four treatments: a bare soil treatment (imitating seed harvesting), a natural leaf litter treatment, an added rooibos mulch and an added enriched rooibos mulch. Sampling for the database and residue trials was done during different times in the season. Database sampling was done during September and January, and the residue trials sampling was done during September, January and June/July at different phenological stadia: just before flowering, after flowering (prior to harvest) and during most active nutrient uptake. The objective was also to define rooibos plant nutrient demand at different phenological stadia as mentioned above. All the soil samples were chemically analysed for plant available macro- and micronutrients, total carbon and nitrogen, pH, CEC, acidity and base saturation. The plant material (harvest part) was chemically analyzed for macro- and micronutrients. The morphometry of the rooibos plant was also recorded.

The third objective **(Chapter 6)** of this study was to investigate the soil water content, temperature and EC between treatments. Data was gathered from September 2015 to June 2016. The data gathered during different phenological stadia (September, January and June/July) was then discussed. The fourth and last objective **(Chapter 7)** was to use diffuse reflectance spectroscopy to build calibrations that can predict certain plant and soil chemical properties by scanning the samples. Time and cost effective analyzing methods cannot be ignored and needs to be tested.

Researchers estimated that NIRS can save 80% of normal laboratory costs where conventional analytical methods are used. Therefore, near infrared spectroscopy (NIRS) provide a great alternative to conventional soil chemical characterization. A Bruker multi-purpose FT-NIR analyser (MPA) spectrometer was used to obtain the near-infrared spectra of the soil and plant samples.

There is currently little information on rooibos plant nutrient uptake/demand throughout the year and the effect of litter removal with seed harvesting on the rooibos nutrient cycling and growth. Research has been done on the role of the natural litter layer produced by plants in natural ecosystems such as forests and fynbos, but not in agricultural ecosystems such as rooibos. There remains thus a large volume of important research to do on soil related aspects of rooibos tea cultivation, which affect rooibos yields and sustainability.

Within the scope of this research, this thesis will focus mainly on the effect of residue management on the nutrient cycle in the production of rooibos (Aspalathus linearis) at Nieuwoudtville, Northern Cape. The hypothesis therefore entails that: a change in the natural mulching process of the rooibos plant will lead to poorer growth.

2. LITERATURE REVIEW

2.1. Introduction

Aspalathus linearis is a shrub legume endemic to the Western Cape around Clanwilliam and Wuppertal and to Northern Cape on the Bokkeveld Plateau. Very little research has been done on the production of rooibos (*Aspalathus linearis*) and how the rooibos plant and soil interact. Scientific research on rooibos in the past had a larger focus on health benefits and the ecology (Louw, 2006; Smith, 2014). The main focus of this study is to look at the effect of the natural leaf litter layer under the rooibos plant crop on the nutrient pools and cycles.

In order to make future recommendations to improve rooibos production, understanding the rooibos plant and its micro environment in cultivated conditions, is very important. Over the years the average rooibos yield per hectare saw a decrease of around 200 kg ha^{-1} (Smith, 2014). To prevent this decline in yield new and conservative cultivation methods will need to be found and implemented.

2.2. Nutrient pools and cycles

In a basic plant stand nutrients exist in many forms, or nutrient pools/compartments, within the agricultural ecosystem. A nutrient pool is the amount of a certain nutrient stored in a compartment or portion of an ecosystem (Molles, 2009). In the environment nutrients are found in the air (atmosphere), soil and water, and in living organisms such as plants and animals. In the soil the nutrients can be in solution, held on the surface of soil particles, both these forms are available for plants, or the nutrients can also be locked up in rocks, unavailable for plants (Dovey, 2012). The nutrients are stored in these pools or they move between the pools in a process known as nutrient flux. These fluxes are driven by matter and energy flows which are regulated by environmental factors. The environmental factors include temperature, water, wind, radiation, time, phenology and soil (Dovey, 2012). The rate of this flux and the size of the different pools would dictate the extent of nutrient cycling in an ecosystem (Scholes *et al.*, 2007). Thus involves nutrient cycles the storage of chemical elements in nutrient pools and the transfer/flux of nutrients between pools.

2.2.1. Nutrient transfers/fluxes

Nutrient fluxes are when nutrients move from pool to pool in an ecosystem through dynamic processes. Inputs of nutrients can be natural and anthropogenic. Natural inputs of nutrients includes photosynthesis, lightning, N-fixing by bacteria and legumes, atmospheric deposition, weathering of minerals, decomposition of plant residue and other processes(Molles, 2009). The atmosphere is a source of inorganic nutrients such as oxygen (O_2), carbon dioxide (CO_2) and water (H_2O). Lightning

and precipitation produce nitrogen, and sulphur, chloride, calcium and sodium also gets deposited by precipitation. For long-term sources of nutrients, rock weathering is one of the most important processes. This process adds nutrients to the soil in small quantities over a long period of time and includes calcium, potassium, magnesium, sodium, iron, silicon, phosphorous, aluminium and all of the micro nutrients (Pidwirny, 2006). Anthropogenic inputs would include any form of fertilizer applications.

Natural nutrient losses can occur through runoff, erosion, leaching and gaseous losses to the atmosphere. Nutrient loss through erosion is one of the biggest and most important losses in agricultural systems where cultivation leaves the soil open, bare and unprotected. These bare soils, especially the topsoil, are then susceptible to nutrient losses through wind and moving water. The topsoils have nutrient rich organic matter which stores lots of phosphorous, potassium and nitrogen. Leaching is also an important process of nutrient loss. Nutrients are then getting lost by moving with the soil solution vertically down through the soil profile into the groundwater. Nutrient losses through leaching are usually highest in disturbed ecosystems like agricultural soils. Gaseous losses occur when certain environmental conditions promote the export of nutrients in a gaseous form. This happens when the soil is wet and anaerobic. The compounds are then chemically reduced from a solid form in the soil to a gas (Pidwirny, 2006). Anthropogenic outputs/losses occur with crop removal when harvesting is taking place (Dovey, 2012).

Apart from nutrient inputs and outputs is there also internal nutrient cycling. During photosynthesis nutrients are taken up by leaves from the air and also by the roots in the soil. These nutrients are then being incorporated into living tissue. When living tissues of a plant reaches senescence and die, the nutrients returns to the soil in the form of dead organic matter. In the soil there are microbial decomposers, which transform the organic nutrients back into mineral forms through mineralization. The mineralized nutrients are then again available for uptake by plants (Pidwirny, 2006).

2.2.2. Nutrient pools in the soil

In the soil nutrients exists in many different forms and pools that range from readily available, soluble forms, to weakly bound and strongly bound/precipitated forms. The soluble nutrients in solution are in the appropriate ionic form for immediate uptake, but are also susceptible for leaching out of the soil root zone with downward moving water or can be lost in runoff (Peter *et al.*, 1999; Pidwirny, 2006). Exchangeable cations are a pool for short-term storage of nutrients that replenishes the soil solution with nutrient ions. The nutrients can either be in organic or inorganic forms that are transformed into plant available nutrients through various weathering reactions with chemical and

biochemical agents. The exchangeable cations are held by the negative charge on soil organic matter and clay particles (Hodges, 2010). This limits cation losses in the root zone from leaching out. Soil organic matter is a very important source of nutrients that gets slowly released as it decomposes. Soil minerals are also sources of nutrients and range from soluble types (sulphates and chlorides) to insoluble forms (apatite, feldspars, mica)(Peter *et al.*, 1999).

2.2.2.1. Carbon

In the terrestrial carbon cycle the carbon exists as organic or inorganic carbon. The carbon pool in the atmosphere is small and is stored as carbon dioxide (CO_2), carbon monoxide (CO) and methane (CH_4). The soil is the largest pool of carbon in terrestrial ecosystems (Wang *et al.*, 2010). Inorganic carbon pool of the soil is stored in sedimentary rocks such as dolomite ($CaMg(CO_3)_2$) and limestone ($CaCO_3$). The inorganic carbon from the atmosphere (CO_2) gets incorporated into the plant biomass through photosynthesis. When this plant biomass, including root biomass and litter fall, decomposes with the help of microbes such as bacteria and fungi, it forms part of the soil organic matter. Soil organic carbon forms a major part of the carbon pool in the near surface horizon. The soil organic carbon and the amount of soil organic matter are directly related to each other. Sometimes soil organic carbon is how organic matter in the soil is measured. The input of carbon to the soil can be directly through growth and death of plant roots, and indirectly through carbon transfer to soil microbes from roots. Certain plants have symbiotic associations between their roots and specialized fungi (mycorrhizae) in the soil. The plant roots provide energy to the fungi in the form of carbon compounds in return for limiting nutrients such as phosphorus from the fungi (Todd and Schulte, 2012). Decomposition of biomass in the soil by microbes leads to CO_2 loss through microbial respiration, while some of the carbon stays in the soil with the formation of humus. Humus, a small carbon pool in the soil, is the product of biomass decomposition through microbes that is resistant for further breakdown and highly recalcitrant. Other carbon losses in the soil can occur through erosion and leaching of dissolved carbon.

2.2.2.2. Nitrogen

Nitrogen in the soil is mostly present in the organic, rather than the inorganic form and large quantities are required for plant growth. It is the most common, most important and often the first limiting nutrient for agricultural crops (Peter *et al.*, 1999). The movement of N from the atmosphere, through the plant then soil and back in to the atmosphere are called the nitrogen cycle. This cycle consists of nine important processes: uptake by plant from soil, mineralization, nitrification, immobilization, N_2 fixation, denitrification, volatilization and leaching. Only inorganic N (NH_4^+ and NO_3^-) forms can be taken up by plants. Mineralization is the biological breakdown of organic-N to

release NH_4^+ available for plant uptake. Ammonium can get quickly converted to NO_3^- through a process called nitrification by bacteria (nitrosomonas and nitrobacter) in the soil which is an oxidation process. Immobilization is the absorption of inorganic N forms by plants and microbes into organic forms like proteins and amino acids (Hodges, 2010). Another process that contributes to the N pool is N fixation. This process transforms N_2 from the atmosphere into plant available forms by symbiotic and non-symbiotic fixation; and also lightning. Symbiotic N fixation takes place through micro-organisms which grow in association with a host plant. Non-symbiotic N fixation is carried out by blue-green algae and free-living bacteria in the soil. In the atmosphere the heat of the lightning can form nitrate, which then can make its way to the soil through rainfall. Nitrogen losses from the N pool occur through the volatilization, leaching of inorganic N, plant uptake and denitrification. Volatilization refers to the loss of ammonia (NH_3) as a gas to the atmosphere. This usually occurs in soils that have added ammonia fertilizers and with a high pH (Jones and Jacobsen, 2001). At a high pH NH_4 gets more easily converted to NH_3. Nitrate is very mobile in the soil and is therefore very easy to get lost through leaching out of the soil profile into the groundwater. Denitrification is the process whereby nitrate (NO_3^-) is lost to the atmosphere as nitrogen gas (N_2) during anaerobic conditions. This process is favoured especially in warm, moist soils (Jones and Jacobsen, 2001).

2.2.2.3. Phosphorous

Phosphorous is an anion that has low mobility and availability in the soil. This anion reacts strongly with both solid and solution phases of the soil. Mobility in the soil is thus very limited except for organic soils and sandy white bleached soils with very low CEC's (Hodges, 2010). Losses of P can occur through leaching, erosion and crop removal. The primary source of phosphorous (P) in soil and eventually soil organic matter is the weathering of minerals in parent rock material. In the phosphorous cycle there is no gaseous component, therefore losses of P only be replenished by P released from primary minerals like apatite (Lajtha and Schlesinger, 1988). In the soil, phosphorous pools exist in many different forms. There are the plant available inorganic forms called orthophosphates (HPO_4^{-2} or $H_2PO_4^-$) in the soil solution, the slowly available sorbed P (sorbed to Fe/Al oxides), mineral P forms ($CaHPO_4$, $AlPO_4$, $FePO_4$) that is relatively insoluble and organic P (microbial, plant detritus and humus). Both inorganic and organic pools have labile P tied up with non-labile compounds. Organic P is not available for plant uptake until the phosphorous is released by mineralization after the decomposition of organic materials. The mineralization process, as with nitrogen, is carried out by microbes in the soil and is affected by the same factors such as soil moisture content, organic material composition, pH and oxygen concentration. Low moisture effects crop development and it also limits the movement of P, whereas excess moisture limits P uptake. Organic material with a low P content would favour immobilization, and with a high P content

mineralization. The dominant form of P (HPO_4^{2-} or $H_2PO_4^-$) is determined by the pH of the soil. When the soil pH values are greater than 7.0 the predominant form is HPO_4^{2-}, while with the soil pH between 4.3 and 7.0 the $H_2PO_4^{1-}$ is the predominant form. In most soils the phosphorus availability for plants is highest between soil pH of 6 and 7 (Hodges, 2010). Soil pH also influences the availability of P. When the pH values are high (alkaline soils) Ca and Mg phosphates develop and aluminium and iron phosphates develop with low pH (acidic soils). The surface horizons usually has a larger P content then the subsoil due to greater biological activity, accumulation of organic material, and the sorption of added P (Sharpley, 1995). In the soil solution the amount of P is usually low and needs a constant replenishment from the organic and inorganic labile pools. The total P content of the soil is less important than the rate at which labile P is converted to plant available soluble P. Phosphorus fluxes between the solid and soluble phase can occur through precipitation and dissolution of P compounds, and also through sorption and desorption of P ions on the functional groups of sesquioxide surfaces (Dovey, 2012).

2.2.2.4. Base cations (K, Ca and Mg)

Base cations (K, Ca and Mg) don't have any organic forms and only exist in their cationic form. They are predominantly held on the soil exchange complex. The ability and capacity on which the soil can hold and bind these cations depends on the cation exchange capacity (CEC). Cation exchange capacity is an ingrained property of soils that defines the total sum of exchangeable cations that can be absorbed at a specific pH. The CEC of a specific soil is depended on the clay percentage, type of clay, pH and amount of organic matter (humus). Low CEC soil is more susceptible for base cation deficiencies. Base cations enter terrestrial ecosystems through weathering (mineral dissolution), and atmospheric deposition and is one of the only long-term "net" sources. Other sources includes throughfall leaching, organic matter mineralization, root exudation and soil desorption that forms part of the base cation cycle (Ouimet and Duchesne, 2005). Primary minerals are the original source of base cations. When mineral weather is taking place there is an increase of the availability of base cations. The base cations are first in the non-exchangeable, slowly available form, then they enter the exchangeable pool and after some time the soil solution from where they can be absorbed by plants in their ionic form (K^+, Ca^{2+} and Mg^{2+})(Brady and Weil, 1999). The rate at which mineral weathering is taking place are regulated by the soil mineral composition, climate (temperature and moisture), soil depth, CO_2 and acidity. Base cations are usually lost from an ecosystem through erosion, harvesting of biomass and leaching. In soils that is acidic or/and low in clay and organic matter (low buffering ability) leaching can cause big base cation losses.

2.3. Nutrient pools in biomass

Harvesting results in direct nutrient losses because of biomass removal and indirect losses through accelerated runoff, leaching and soil erosion after harvest. The amount of nutrients lost varies with the amount of biomass removed (Dovey, 2012).

The total litter accumulation in a site is the product of in situ litter production, litter deposition from outside, litter destruction (physical and biotic agents), and litter removal. The litter accumulated can be reduced by decomposition, chemical and physical degradation, and heterotrophic consumption. Litter production is mainly an episodic process in desert ecosystems because drought and hot winds initiate a pulse of leaf mortality (Facelli and Picket, 1991). It is also a very important pathway for nutrient return to the soil, especially for N and P. Certain plant species becomes dormant to evade the drought and when the dry season begins it produces a pulse of leaf abscission. When leaf senescence is taking place, a portion of the nutrients in the senescent leaves gets reabsorbed into younger new leaves. Usually plants that grow in soils with low nutrient availability produce litter with low nutrient concentrations, where a bigger proportion of the nutrients get reabsorbed. The reason for the production of a poor quality litter may be that schlerophyllous plants have adapted to the nutrient poor soils by the preservation of nutrients in the plant (Mitchell *et al.*, 1986).

2.4. Rooibos

2.4.1. Rooibos history

More than 300 years ago in the mountainous Cederburg region of the Western Cape (South Africa) the indigenous Khoisan tribe was the first to discover that one can use the wild rooibos plant to brew a tasty tea. The Khoisan then already used basic processing methods, the same methods that are in use today: first the cutting and bruising of the rooibos leaves and stems, then the “sweating” of the tea heaps and finally the drying of tea in the sun. It was in 1904 that Benjamin Ginsberg met the descendants of the Khoi and saw the marketing potential and economic value of the cultivation of rooibos tea. He was a descendant of the Popoff-family that was one of the biggest distributers of Eastern tea in Europe. In 1930 rooibos tea was first commercially produced by a Clanwilliam doctor Le Fras Nortier on the farm Klein Kliphuis. Later on in the early 1940’s Henry Charles Ginsberg, Benjamin Ginsberg’s son, established the first big scale rooibos plantations. From 1954 the “Rooibos Tea Marketing Board” was in control of the quality and marketing of rooibos to expand the market and stabilize the industry. In 2005 a non-profit company, The South African Rooibos Council, was set up to manage the interests of the Rooibos industry (Joubert, 2011).

2.4.2. Production area

The distribution of the rooibos plant is very limited. The natural and cultivated region of rooibos stretches from Nieuwoudtville, in the Northen Cape, to Piketberg, Western Cape, all along the Cederberg mountain range as can be seen in Figure 1. Wild rooibos only flourishes between 450m above sea level and 900m below sea level in a mediterranean-type climate, where cultivated rooibos is grown over a bigger geographical range (Lötter *et al.*, 2014). Attempts to grow it in other regions in South Africa and in other countries were unsuccessful. There are 6 main areas where rooibos is cultivated: Nardouwsberg/Agterpakhuis, Seekoeivlei, Vanrhynsdorp, Nieuwoudtville, Citrusdalberg en Eendekuil (Smith, 2014).

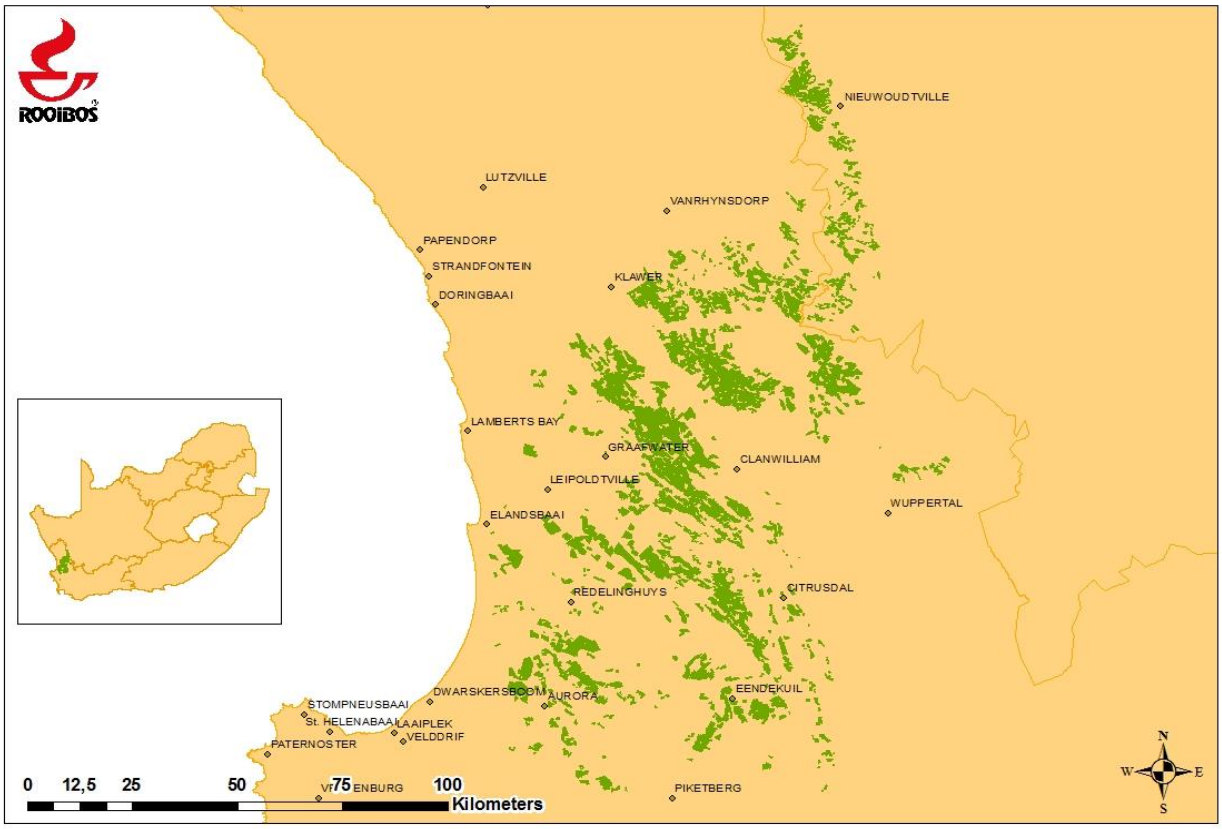

Figure 1: Map indicating the extent of rooibos (*Aspalathus linearis*) cultivation (map was supplied by Rooibos Ltd., Clanwilliam).

2.4.3. Environmental conditions and adaptations

The optimum precipitation annually is between 380 to 635mm, which mainly takes place in winter, with some showers in early summer and late autumn (Morton, 1983). The rooibos plant grows in nutrient-poor, deep, well-drained, sandy and acidic (4.5-5.5 pH) soils (Morton, 1983) that are derived from Table Mountain sandstone. Rooibos is an evergreen leguminous sclerophyllous fynbos plant and they are hard, tough and stiff, and this is an adaptation of fynbos to stresses such as infertile soils and seasonal water deficits. The rooibos plant also has other adaptations to survive in

these dry climate and nutrient poor soils. The rooibos plant have a strong taproot that can be found 2 m or deeper in the soil with the main purpose of taking up water especially in the dry seasons when the water is deep in soil profile. There is also a fine root system just under the soil surface with rhizobium nodules, cluster roots and mycorrhizal roots. The rhizobia in the nodules fix atmospheric nitrogen (symbiotic N fixation). The rooibos plant has the ability to fix well over 100 kg N/ha annually (Sprent *et al.*, 2009). The fine (feeding) roots also develop cluster roots (Hawkins *et al.*, 2011) to help with the uptake of nutrients, and mycorrhizal roots for enhanced uptake of phosphorus (Chimphango *et al.*, 2016).

In these same conditions the growth of other nodulating legumes with their microsymbionts is limited (Muofhe and Dakora, 2000). It was found that some strains of the root nodule bacteria that were isolated from the rooibos plant could survive and grow in a pH of 3 or 4 in the laboratory (Muofhe and Dakora, 1999). Some of the mechanisms that certain legume plants use to increase the rhizosphere pH include the excretion of OH^- and HCO^-_3 during NO^-_3 assimilation and the decarboxilation of organic acids. The mechanism of pH elevation by the rooibos plant use is still unclear, but Muofhe and Dakora (2000) observed that the decarboxylation of organic acids led to a rhizosphere pH increase. Another important mechanism (adaptation) of plants growing in Mediterranean ecosystems is nutrient re-absorption. Nutrients of senescing leaves are then mobilised and transported to other/new plant tissues (Lötter *et al.*, 2014).

The rooibos branches are about 60 cm long with a red-brown colour. Mature plants are adapted (needle shaped leaves) to hot summers and cold winters, but when the plant is young it is sensitive to frost. The needle-like leaves are 2-6 cm long and the flowers are in a “pea” form, yellow and exists in short clusters, 6.5 mm long (Morton, 1983).

2.4.4. Cultivation practices for rooibos

There are four main types of naturally occurring rooibos forms: the “Rooi”-, “Vaal”-, “Swart”- and “Rooibruin” Tea. “Rooi Tea” can be subdivided into 2 types: namely the “Nortier” and “Cedarberg” type. The “Nortier” type is the selected and improved type specifically for cultivation and the making of rooibos tea. The “Cedarberg” type has a coarser and broader leave and only grows wild around Clanwilliam in the Cedarberg Mountains(Morton, 1983).The cultivated “Nortier”type is thought to originate from a wild type (reseeder) that was found in the southern Cederberg, Pakhuis area during the 1930’s, but clear records are not available (Hawkins *et al.*, 2011). Thus is the entire commercial industry still depended on one *Aspalathus linearis* selection that was made around 80 years ago. Growth rate, seed production and taste of the rooibos dictated the selection (Lötter *et al.*, 2014). Rooibos is an evergreen perennial crop that is grown for between 3-5 years before it is removed

from the land. Seeds are usually sown from February to March or August and early September (Nieuwoudtville) and seedlings are planted during May to July (with winter rain) or in September (Nieuwoudtville) if rain was enough. After 8 to 12 months they are cut (topped) to force branching and then 2 years from planting they can be harvested (Morton, 1983) for the first time with the use of sickles or other, more expensive, machinery. Harvesting takes place between December and April during summer and early autumn when the top part of the bush is cut to a height of 30-45 cm from the soil. In the second year of harvesting it is important to harvest slightly higher than the previous year so that new growth from the wood of previous season is possible. After each cycle of 3-5 years it is recommended to plant grain crops such as oats or wheat (Dahlgren, 1968). This is necessary to prevent pathogens from reaching high population numbers/density and also to help maintain soil fertility, soil organic matter and soil structure.

The obtain rooibos seeds, needed to raise seedlings of or to sow directly in the soil, is a hard and time consuming process that demands hours of manual labour. After the flowers of the rooibos plant dies it drops its seeds to the surface of the soil and/or mix with the litter layer (senescent leaves of plant) under the plant. With rooibos seed harvesting, this litter layer and a small amount of topsoil is removed from the soil by hand. With the help of water, a pan and a sieve the sand and organic material are then separated from the rooibos seed (personal communication with farmers).

2.4.5. Nutrient status of rooibos plants and rooibos cultivated fields

The research of (Stassen, 1987) showed that there is seasonal variation in the amount of macronutrients in the harvested part, stem and roots of the rooibos plant. The harvest part, stem and roots followed the same pattern throughout the year. The N % reached its maximum in August/September and its minimum in January/February. The two main periods of N increases is from February to May and from July to October. The P % reached its maximum in September/October and its minimum in January to April. There was an increase in P % from April/May to September/October. The K % peaked in October/November and reached its minimum December/January. The two main increases of K % were from February to May and from June to October. The Ca % peaked in August/September and reached its minimum in April. The two main increases were during April to June and July to August/Sept. The Mg % reached a maximum in September and a minimum April. The main Mg % increase was during April to September (Stassen, 1987).

Lötter *et al* (2014) did research on the the seasonal variation in the nitrogen nutrition and carbon assimilation in wild and cultivated rooibos. Research of (Lötter *et al.*, 2014) showed that the N concentration in rooibos leaves and stems was highest during the late winter (September) and

lowest in the summer (February). This corresponds with the results of Stassen (1987). The soil N was also higher in winter then in the summer, thus showed similar pattern to plant N. This pattern observed in rooibos coincide with other studies on schlerophyllous shrubs in Mediterranean climate regions (Lötter *et al.*, 2014). The rooibos plants had a higher C:N ratio in the summer, and this is an indication that the plants is developing schlerophylly. Lötter *et al.* (2014) also recorded that the lowest photosynthetic activity is during summer (Feb) and highest in winter. The lower photosynthetic activity in the summer is a result of less stomatal conductance due to low water availability. Plants in the Mediterranean region, such as rooibos, take up nutrients during winter (rainy season), before any growth aboveground, and these minerals are stored in the old evergreen leaves. With growth nutrient re-absorption takes place where the nutrients are then moved from the senescing leaves to other/new plant tissue (Mooney and Rundel, 1979; Lötter *et al.*, 2014).

Smith (2014) found that there is a significant decrease in plant biomass, soil basic cations, C and N with continuous cultivation. There was also a decrease in the microbial biomass, root mycorrhizae and protist counts. The decrease in soil carbon, lead to the decrease in basic cations and total microbial biomass and also soil protist counts. This decrease in soil carbon is the result of less total amount of organic biomass that is returned to the soil each year, because two-thirds of the plant gets harvested each year and rooibos monoculture is practiced (Smith, 2014).

Chimphango *et al* (2016) did research on the effect of organic cultivation of rooibos tea plants on the soil nutrient status in Nieuwoudtville. It was found that organic cultivation of rooibos at Nieuwoudtville was sustainable, maintaining soil nutrition. The decreasing pattern of soil nutrient concentration with increasing age of cultivated plots over a five-year period relative to uncultivated plots was not seen. This maintenance of the soil nutrient status over a rooibos production period of five years might have been the result of good soil management practices associated with practice methods for sustainable rooibos production by Pretorius (2008) (Chimphango *et al.*, 2016).

2.5. The role of mulching

Mulch is a layer of plant material applied to the soil surface. In this study the mulch represent the leaf litter that the rooibos plants shed each year.

The direct and indirect effects of litter on the environment are both chemical and physical. Litter holds mineral nutrients and also reduces leaching with the help of decomposers that temporary immobilize the limiting nutrients. The decomposition rate is strongly affected by the plant quality and nutrient status (C:N) (Aerts and Chapin, 1999). Litter decomposition returns nutrients to the soil and plays an important part in the plant nutrient cycle (Mitchell *et al.*, 1986). Nutrient leaching from

the senescent leaves also adds nutrients to the soil. Magnesium and potassium are very mobile and leach easily from fresh litter, whereas N, P and Ca leach at a much slower rate (Aerts and Chapin, 1999). The environmental conditions and physicochemical characters of the litter regulate the rate of nutrient release from the organic matter. The length of residence of nutrients in the litter varies greatly with the type of litter and may greatly affect the nutrient dynamics (Facelli and Picket, 1991).

Light and rain gets intercepted by the litter and it also effects the transfer of water and heat between the atmosphere and soil. Litter intercepts solar radiation and therefore insulates the soil temperature from air temperature. The litter also affects the water exchange rate between soil and atmosphere, especially in desert conditions where the litter increases water availability mainly due to reduced evaporation. Water evaporation gets directly affected by litter through the increase in resistance to water vapour diffusion and indirectly by reduced soil temperature. During rainfall litter prevents disaggregation by reducing the impact of raindrops on the soil and this result in better infiltration (Facelli and Picket, 1991).

The main advantages of mulches are not only soil moisture conservation and nutrient supply for plants, but also for soil organisms (Jodaugiene *et al.*, 2010). Surface mulching also sustain soil microbial biomass and activity affective in highly sandy soils (Tu *et al.*, 2006).

2.6. NIR Spectroscopy for soil and plant chemical characterization

To use our base resources more efficient and also to preserve it for future generations we need to get a better understanding of the soil as a complete system. Large amounts of accurate soil data, chemical and physical, is necessary to sensibly manage our base resources (Rossel *et al.*, 2006). Traditional assessment (conventional methods) of soil quality, including routine soil chemical and physical analysis in the laboratory, is to date still a very expensive method. This makes traditional assessment of soil quality only possible for those who can afford it.

In the last 40 years diffuse reflectance spectroscopy (near- and mid- infrared) has been extensively developed, measuring the composition of cereals, fruit, vegetables, meat etc. It was initially developed during the early 1970s and since then it has developed to become a robust and fast analytical method (Zornoza *et al.*, 2008). Research on diffuse reflectance spectroscopy to use in soil and leaf analysis experienced a boom in the last 10 years. In this research numerous investigations have been done on measuring soil and plant properties in different configurations (Bellon-Maurel and McBratney, 2011). Other spectroscopic techniques includes nuclear magnetic resonance (NMR), mass spectroscopy (MS), vis (VIS), near infrared (NIR) and mid infrared (MIR) spectroscopy (Rossel *et*

al., 2006). Near infrared reflectance spectroscopy was first used in soil science in the 1960s (Nduwamungu *et al.*, 2009).

Diffuse reflectance spectroscopy is one of the easy-to-use and low-cost alternative techniques for soil quality assessment and thus makes it very attractive for agricultural use (Nduwamungu *et al.*, 2009; Bellon-Maurel and McBratney, 2011). The other advantages of using near infrared diffuse reflectance spectroscopy (NIRS) are: the technique is relatively simple to use, non-destructive, no hazardous chemicals are used, highly reproducible, sample preparation is easy and many soil properties can be inferred from a single spectrum (Thomsen *et al.*, 2012; Wetterlind *et al.*, 2013).

NIRS use the wavelength range of 700 – 2500 nm (roughly 12800-4000 cm^{-1}) When a sample is scanned, the radiation of chemical bounds such as C-H, O-H, N-H, C-O, S-H, CH2, and C-C are absorbed, in accordance to the concentration of compound, in the NIR region (Zornoza *et al.*, 2008; Nduwamungu *et al.*, 2009). The radiation causes the vibration, either bending or stretching, of molecular bonds to various degrees and they absorb the light (Stenberg *et al.*, 2010). Individual components of the soil matrix are not linearly related to NIR absorption (Russel, 2011). In the NIR region minerals in the soil have different spectral fingerprints because of combinations of OH, carbonate groups and sulphate and because of strong overtone absorption. Though, the macro and micro elements in the soil matrix are associated with forms of hydroxides, oxides and other compounds or organic matter fractions (Cozzolino and Moròn, 2003). Soil physical properties like surface structure, water films on the soil surface and particle size can influence the diffuse reflectance of the soil samples. A spectrum has a lack of specificity and consists of lots of highly correlated neighbouring wavelengths. For this reason multivariate calibration techniques was used to correlate the spectra with the soil properties that needs to be predicted (Wetterlind *et al.*, 2013).

2.7. Conclusions

There is still a lot of research needed to better understand the rooibos plant and its environment from an agricultural perspective. This study will focus on the role of the litter layer under the plant formed by the senescent leaves of the rooibos and what effect it has on the topsoil and the rooibos plant. This has never been studied before. Nutrients in a natural rooibos ecosystem get cycled, whereas in rooibos monoculture, nutrients are removed and lost with harvesting. Because fertilizing rooibos is not yet an economical and common practice for most farmers, nutrient mining may be the reason for the decrease in yields in older fields. During nutrient cycling in the micro environment of a plant there are nutrient losses and gains. Rooibos leaf litter is part of the nutrient cycle and the effect it has on the soil nutrient and water status in the top soil of the rooibos plant micro

environment is important for developing cultivation and management practices that can lead to more sustainable rooibos production.

The use of NIR spectroscopy is a relative new method to predict and store soil and leave chemical data. There has been successful results in previous studies and therefore have the potential to be a new and cheaper alternative to the conventional analysis in the laboratory.

3. STUDY AREA: SITE DESCRIPTION AND, MATERIALS AND METHODS

3.1. Introduction

Nieuwoudtville is situated in the Northern Cape just east from the VanRhyn's Pass, which takes you up from the coastal plain to the Bokkeveld Plateau. Nieuwoudtville is famous for its vegetation diversity and is known as the "Bulb Capital of World", and attracts visitors from all over the world to witness the flower spectacle from August to September. The primary agricultural activities at the Nieuwoudtville area include wheat, sheep and rooibos farming (O'Farrell *et al.*, 2007; Solomon, 2015). A small piece of land is also used for potato farming.

The Nieuwoudtville rooibos production area falls within the Greater Cederberg Biodiversity Corridor (GCBC) where the main focus is of protecting threatened habitats, application of biodiversity guidelines, improving farming practices and exposure of the industry to social and economic opportunities. In order to fulfil these aims, with the help of the rooibos industry the "Biodiversity Best Practice Guidelines for Rooibos Production" was initiated by the SA Rooibos council and CapeNature (Pretorius, 2009). Next to Nieuwoudtville there is a new factory (Nieuwoudtville Rooibos Pty Ltd), which does everything from the fine cutting of rooibos to making the end rooibos tea product. There is also the Heiveld Co-operative, founded in 2001 and has tea courts 50 km south of Nieuwoudtville in the Suid-Bokkeveld. Heiveld sells organically certified rooibos to the expanding Fair Trade market. For lots of farmers, especially the small scale farmers in this area, rooibos is their main income and plays an important role in their economic activities.

3.2. Study area

3.2.1. Physical location

The research was carried out in the Northern Cape rooibos district which forms part of the Bokkeveld Plateau region. The Bokkeveld Plateau is situated in the northwest Cape, about 350 km north of Cape Town near Nieuwoudtville, at an altitude of 750 m above sea level. The area south of Nieuwoudtville is known as the Suid-Bokkeveld and north of Nieuwoudtville is the Noord-Bokkeveld where the Bokkeveld Mountains are found. The lower escarpments to the north lead to the Namaqauland and to the south the Cederberg. Figure 3-1 illustrates the study area on the Bokkeveld Plateau.

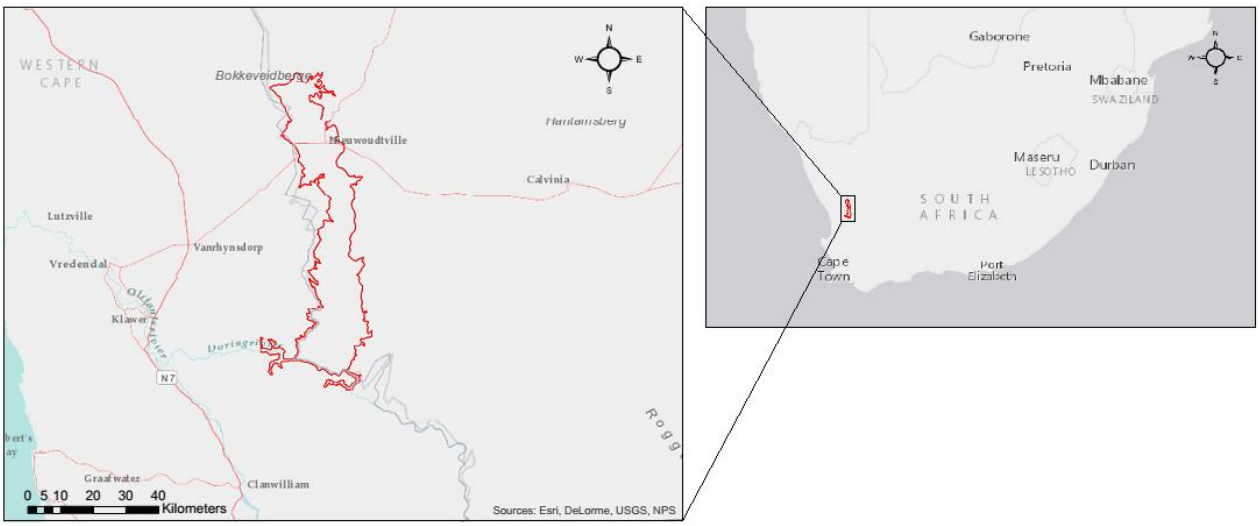

Figure 3-1: The rooibos study area, Nieuwoudtville, Northern Cape

3.2.2. Climate

The Bokkeveld Plateau region falls in the winter-rainfall region. Most of the rainfall occur during the winter months May to July, with occasional thunder showers in the spring and summer months. The winter rainfall is mostly frontal and orographic. At the edge of the escarpment in the west the mean annual rainfall varies between 500 and 650 mm, and declines quickly to around 350 mm at Nieuwoudtville (Manning and Goldblatt, 1997). The higher north of the plateau receives more rain than the low-lying south (Louw, 2006).

At Nieuwoudtville the temperature is highest during February, reaching an average maximum of 31.8°C, and lowest in July with an average minimum of 4.8°C (O'Farrell et al., 2007). The averaged climate data for Nieuwoudtville (2009-2014) can be seen in Table 3-1.

Table 3-1: Average climate data for Nieuwoudtville from 2009-2014

Months	P (mm)	T (°C)		RH (%)		Total ETo (mm)	Total Radiation (MJ/m2)	Wind Speed (m/s)
		Min	Max	Min	Max			
January	13.4	12.5	31.0	24.1	87.6	204.0	29.7	3.4
February	13.4	13.3	32.0	21.9	86.1	169.6	26.7	3.0
March	9.6	12.0	30.5	22.3	84.9	153.6	21.9	2.6
April	20.7	9.5	27.4	22.8	82.1	106.9	16.5	2.4
May	66.6	6.4	22.0	33.6	91.0	67.2	11.8	2.3
June	69.4	4.8	19.4	34.5	87.5	39.8	10.2	2.5
July	41.8	3.7	19.5	33.0	87.0	48.9	12.6	2.4
August	64.0	4.0	19.0	35.7	92.4	63.2	13.4	2.6
September	38.2	4.8	21.3	30.0	92.0	100.0	19.3	2.6
October	25.2	7.8	23.4	32.0	90.6	117.7	23.2	3.1
November	32.7	8.9	26.6	25.2	88.3	168.5	28.4	3.3
December	37.1	11.6	28.6	27.2	89.3	191.9	29.5	3.3

3.2.3. Geology

The geology of the Bokkeveld Plateau forms part of the Cape Supergroup, which gave rise to quite different soil types and a great variety in vegetation. Shale beds of the Malmesbury System is found lower down in the stratigraphy and can be seen driving up the VanRhyn's Pass. A thin band of hard sandstone beds are found above the shale beds and is part of the Cape System. On top of the sandstone lies Dwyka Tillite, part of the Karoo System. This Dwyka Tillite is exposed from north to south for about 60 km along a narrow band and was formed about 300 million years ago when the whole Karoo area was covered by a massive glacier (Manning and Goldblatt, 1997).

From east to west the underlying rock formations are as follows: Table Mountain Sandstone, Dwyka Tillite, and dolerite (Solomon, 2015). The geology of the Bokkeveld Plateau is shown in Figure 3-2**,** where the Skurweberg-, Rietvlei-, Dolerite- and Gydo Formations (part of the Table Mountain Group) and the Dwyka Group are found. The rooibos fields are found only on the Skurweberg and Rietvlei Formations. The Skurweberg- and Rietvlei Formations are part of the Table Mountain Group. The Skurweberg Formation consists of thick-bedded sandstone with thin beds of small-pebble conglomerate and the Rietvlei Formation consists of thin-bedded feldspathic sandtone. The Gydo formation is part of the Bokkeveld Group. All these groups and formations are part of the Cape Supergroup.

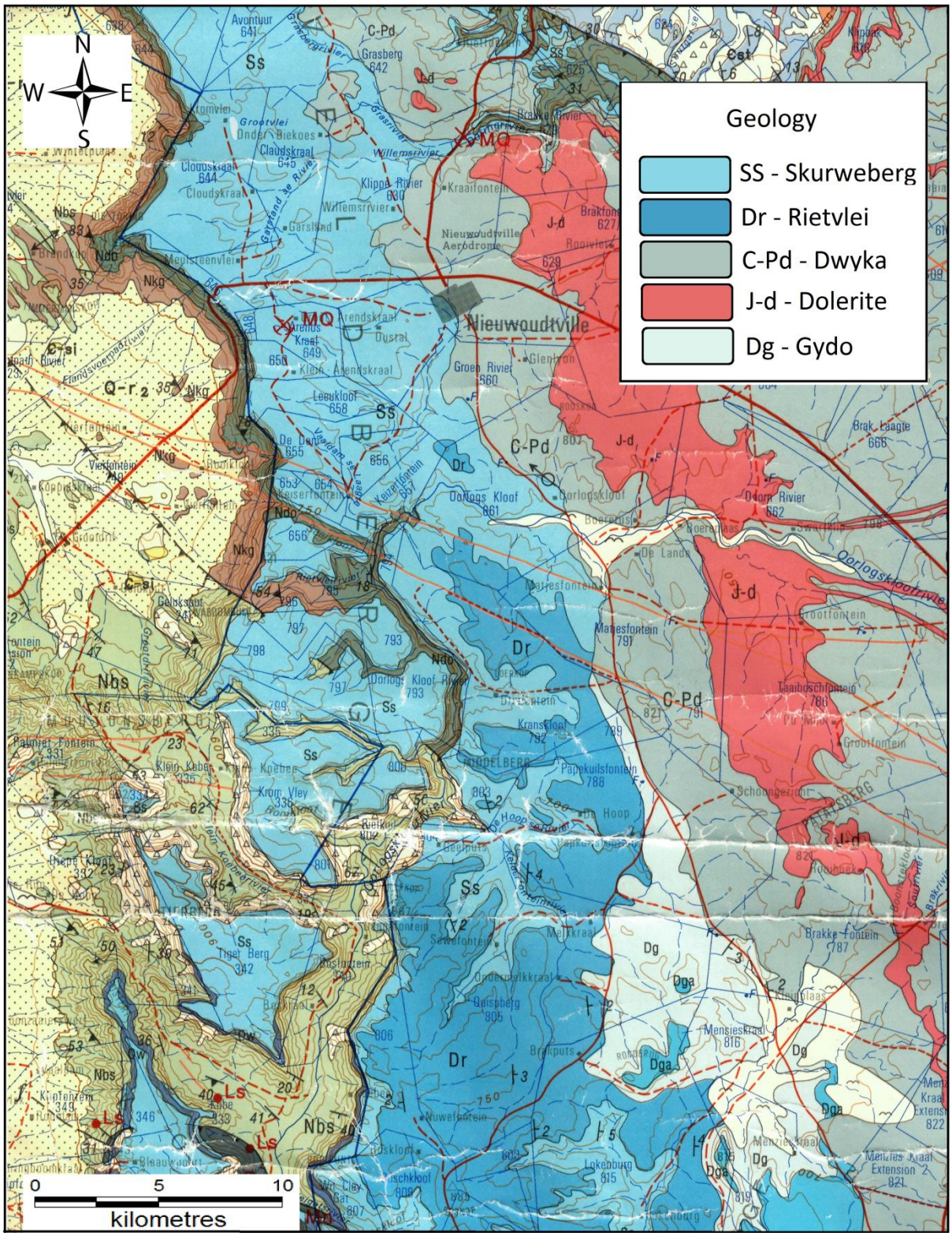

Figure 3-2: Geology of the Bokkeveld Plateau (Geo Map 3118 Calvinia).

3.2.4. Soils

The lots of different soil types found in the Bokkeveld Plateau resulted from bedrock weathering through erosion. The Cape Sandstone (Table Mountain Sandstone) gave rise to the coarse-grained oligotrophic sandy soils, which are the main soils used for the cultivation of rooibos and potatoes.

These soils are well drained and therefore also fairly acidic and nutrient poor. Soils derived from the Dwyka Tillite (Dwyka-derived shales) contain poorly drained, hard and stony clayey soils, which are slightly acidic and may become waterlogged in the wet season (Manning and Goldblatt, 1997; O'Farrell et al., 2007). Chemical changes that occur during the wet season gave these soils a yellow or grey colour and is known by the locals as *vaalgrond, which* is used as natural field rangelands, pastures and also wheat and oat croplands. All of the study sites however, are located near the edge of the plateau (escarpment) on Table Mountain Sandstone.

The Bokkeveld Plateau falls within 14 different land types and each land type has its own combination of terrain type, soil pattern and microclimate. Out of all the land types, Clovelly, Mispah, Avalon, Longlands, Kroonstad, Estcourt, Swartland, Glenrosa, Cartref and Constantia are the most dominant soil forms (Sobszyk *et al*, 1989)

3.2.5. Vegetation

The four main vegetation types that occur on the Bokkeveld Plateau include Cape Fynbos (on Table Mountain Sandstone, Renosterveld on Dwyka Tillite) with a higher rainfall (>300), Karoo vegetation (on Dwyka Tillite) and Karoo (on dolerite derived soils) (O'Farrell *et al.*, 2007; Lemons *et al.*, 2012). The fine grained and rich soils derived from shale, dolerites, granites, mudstones, limestones and silcretes supports renosterveld vegetation. Fynbos predominantly occurs on leached, acidic, sandy nutrient-poor soils (Solomon, 2015). The dolerite derived soils supports the succulent Karoo vegetation. The renosterveld to fynbos transition is soil induced, but the transition from renosterveld to succulent Karoo is most likely climate controlled (Solomon, 2015).

3.2.6. Integrated GIS monitoring framework for potential rooibos habitat units ("terroirs")

De Clercq and van der Merwe (2015) developed an integrated GIS monitoring framework to determine potential rooibos habitat units or "terroirs" in the Nieuwoudtville rooibos areas. The tools used to develop this framework included Excel, Google Earth, GIS software (ArcMap and QGIS) and remote sensing software (Erdas Imagine).

Excel was used to create a non-spatial database, which simplified the storing and analysing of field data (e.g. environmental parameters and rooibos plants) and this formed the first part of the framework. This database was used as a tool for data manipulation, preparation of field data for GIS analysis and interpretation of GIS spatial data. Spatial databases and spatial raster layers were created with ArcMap 10.1 and QGIS. Rooibos growth and preference definitions were formulated for rooibos conditions with the help of personal communication with rooibos tea farmers. This was then linked to GIS spatial entities such as Land Type and SRTM90m Digital Elevation Model (DEM), which

was used for spatial analysis, storage and mapping purposes. For the development of terrain morphological units, the 90m DEM needed to be refined. The 2m-contour GIS layer derived from the SRTM 90m played a big part in the spatial analysis process for the development of terrain morphological units (crest, convex mid slope, concave foot slope and drainage line or valley bottom). Terrain morphological units are important because it can be linked to soil forming factors or habitat conditions, for example clay %, texture, soil depth, etc.

To help understand how the integrated GIS monitoring framework was designed, a schematic representation can be seen in Figure 3-3. Figure 3-3 also shows all the parameters (e.g. altitude, aspect, slope, depth, clay etc.) used in developing potential rooibos habitat units. The final step in the framework in developing the habitat units involved integration of all the derived layer classes that is relevant (altitude, slope, aspect, soil depth and clay forming potential). The final map for potential rooibos habitat units can be seen in Figure 3-4.

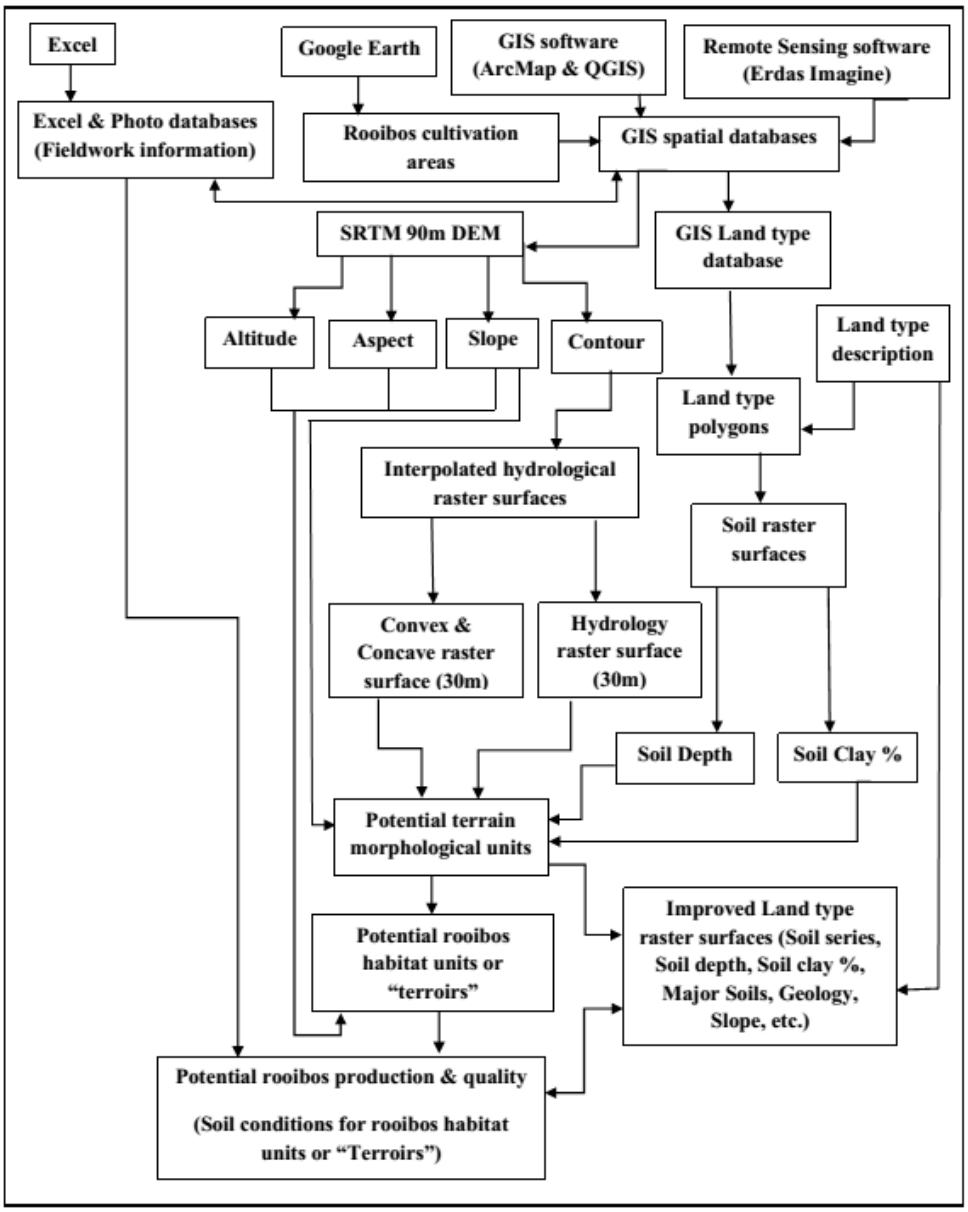

Figure 3-3: Schematic representation of the GIS framework describing the methodology for creating rooibos habitat units (de Clercq and van der Merwe, 2015)

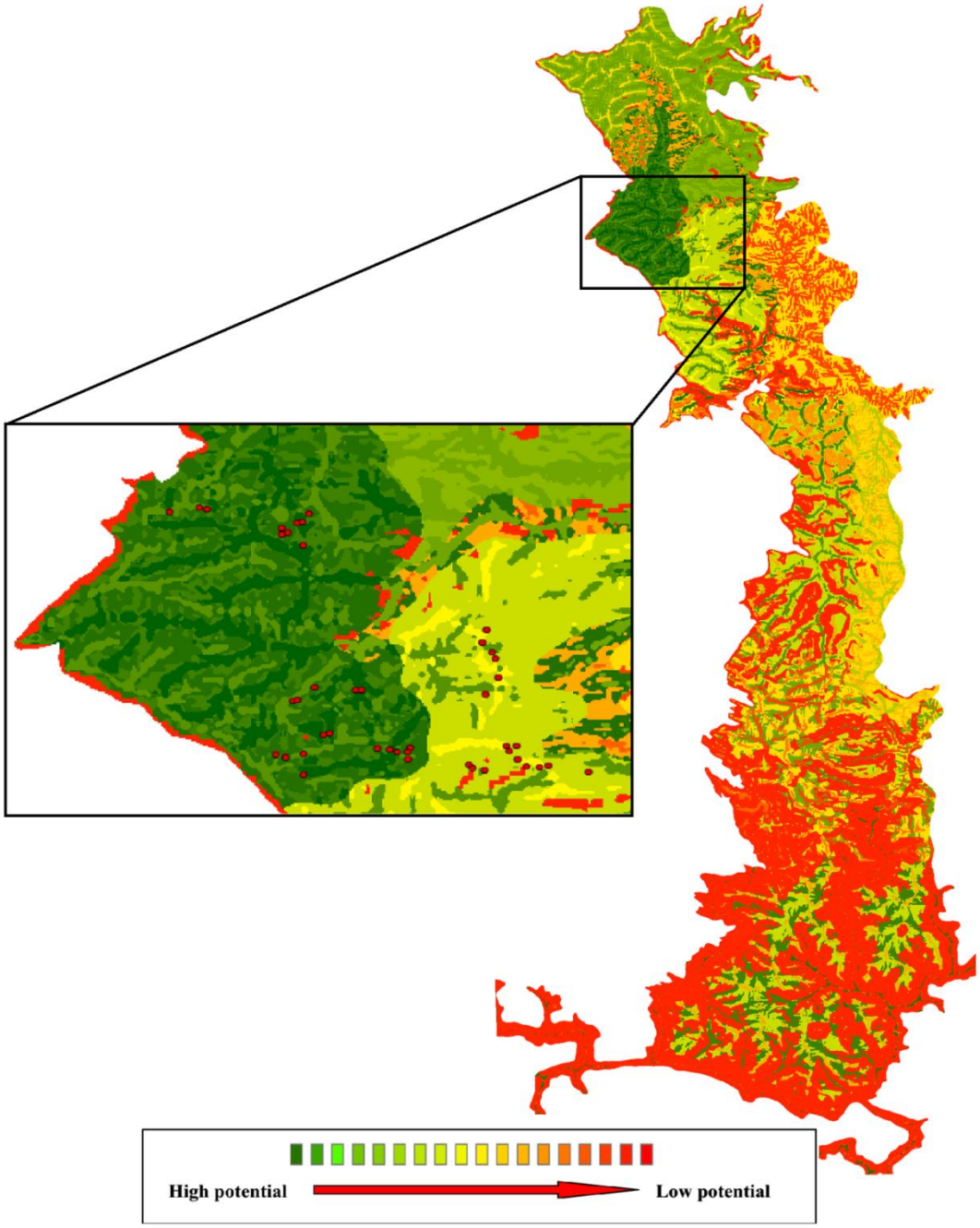

Figure 3-4: Potential rooibos habitat units in the Northern Cape Bokkeveld region (adjusted from de Clercq and van der Merwe)

Figure 3-4 illustrates the variation and location of the potential rooibos habitat units in the Nieuwoudtville rooibos area. The main map (1:600000) on the right illustrate the different habitat units over the Noord- and Suid-Bokkeveld, while the small map (1:62500) on the left shows the database sampling sites (red dots) for a specific area. The high to very high rooibos production potential (very deep sandy soils with wetter and cooler conditions) are represented by the dark

green colours. The low rooibos production potential (shallow clayey, very steep and drier conditions) areas are represented by the orange to red colours.

3.2.7. Rooibos plant and soil nutrient status

Research for the rooibos database continued and all the existing sites with rooibos was visited again in September 2015 and January 2016 in order to take soil samples for chemical analysis and to document the rooibos plant morphology. Data was thus gathered at the following times: September 2014, January 2015, September 2015 and January 2016. Soil samples were taken at two depths, i.e., topsoil at 2 cm, and subsoil at 2-20 cm, respectively. Soil samples were only taken at existing rooibos fields. If a rooibos field was cleared (ploughed) and replaced by another crop after September 2014, sampling for that specific field did not continue. The number of soil samples taken at these intervals varied and were as follows; September 2014: 152; January 2015: 152; September 2015: 120 and January 2016: 104. Figure 3-5 and Figure 3-6 show the average concentration of rooibos plant and soil nutrients for the Nieuwoudtville area. These graphs were prepared to show trends and change over time. This sampling and results impacted on the further interpretation of this work and formed the regional framework for this study. It is presented here as an introduction to the study on plant and soil nutrient interaction and the results portray a geographically averaged summary for the region. The results also provide a longer term perspective to the study and therefore generate credibility to the more detailed results of the mulch study. The methods of all the chemical analysis for these results was the same as the methods explained later in this **Chapter 3**.

Plant nutrients

The differences in nutrient content from January to September (3 months) and from 2014/2015 to 2015/2016 (1 year) for both September and January respectively were studied.

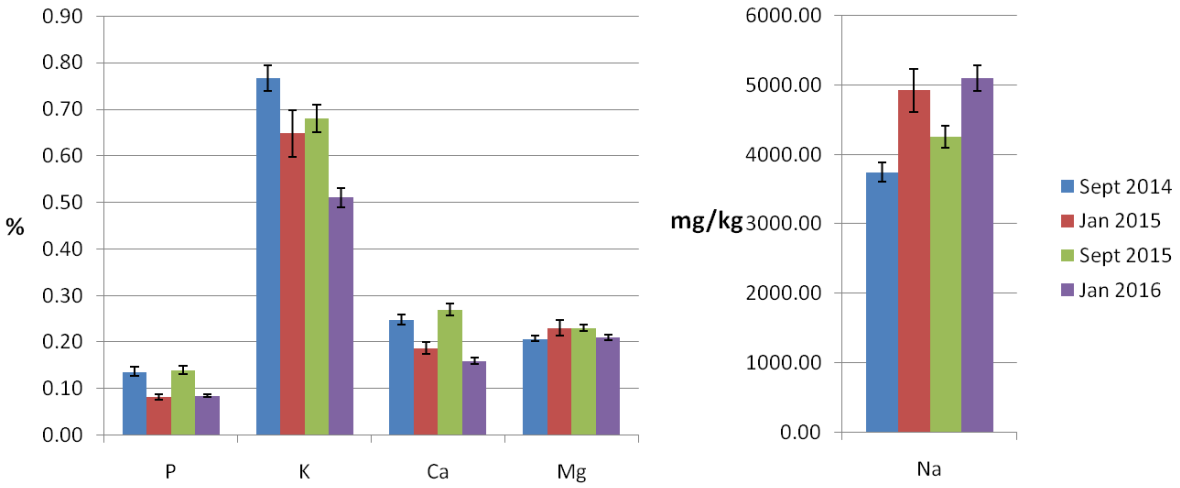

Figure 3-5: Plant P, K, Ca and Mg concentration in rooibos plant material (left).The concentration of Na in rooibos plant material (right) at Nieuwoudtville.

The concentration of the elements in Figure 3-5 (left) show all, except for Mg, the same trend; a higher concentration in September than January. From September to January the element concentrations decreased, which could be a "diluting" effect because of nutrient translocation from the old growth to the new growth. Only Mg showed little change throughout the study period. Over one year there is a decrease in K concentration from September 2014 and January 2015 to September 2015 and January 2016. The other nutrients P, Ca and Mg did not show much variation in concentration over the study period. The increase in the concentration of Na from September to January can be seen in Figure 3-5 (right). However, there was only a very small increase over a one-year period (Sept. 2014 to Sept. 2015 and Jan. 2015 to Jan. 2016)in plant Na.

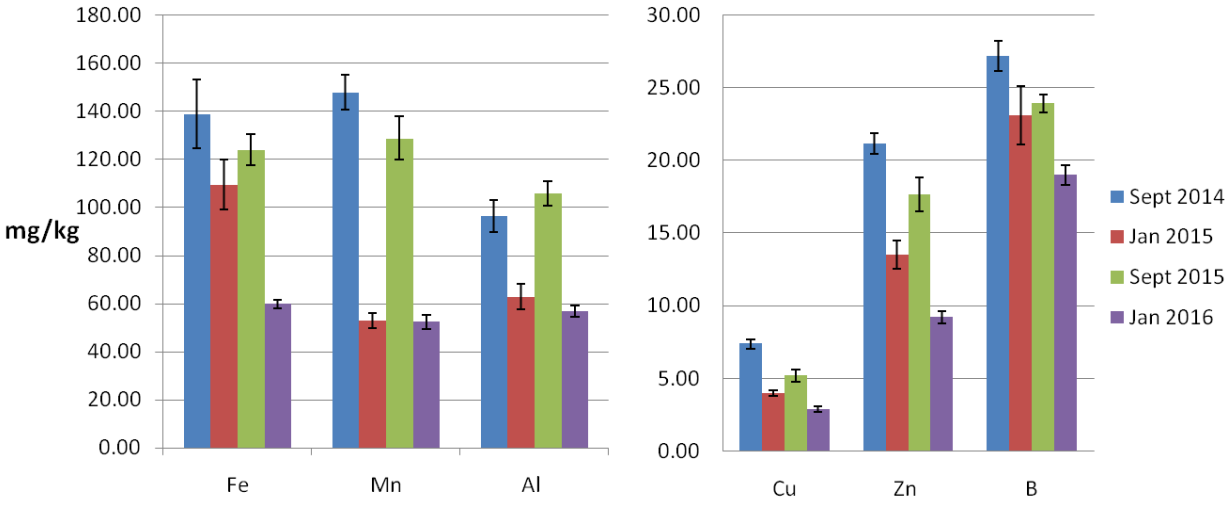

Figure 3-6: The concentration of Fe, Mn, Al (left); and Cu, Zn and B (right) of rooibos plant material at Nieuwoudtville.

All the nutrients in Figure 3-6 (left and right) show the same trend with higher values in September and lower in January. There was a big decrease in the concentration of Fe, Cu, Zn and B, over a one-year period while Mn and Al did not show a decrease.

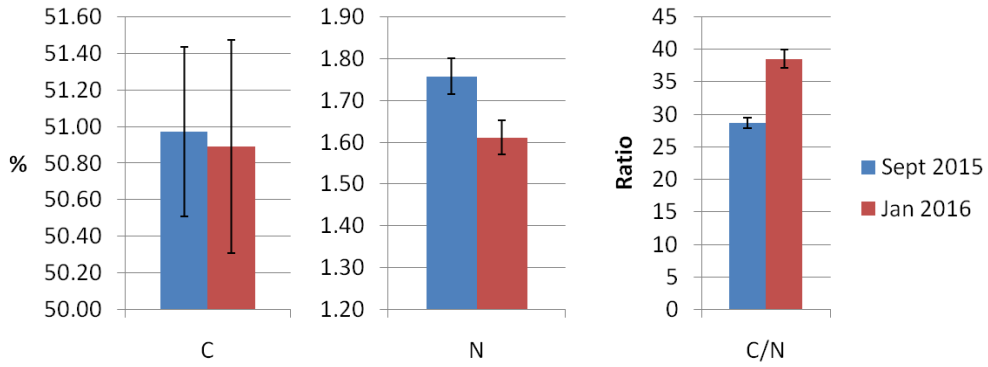

Figure 3-7: Total C, N and C:N ratio in the rooibos plant material at Nieuwoudtville.

There was a very small to no change in the total C content of the plant, while the total N content decreased (Figure 3-7). The higher N content in September 2015 resulted in the lower C:N ratio.

Soil nutrients

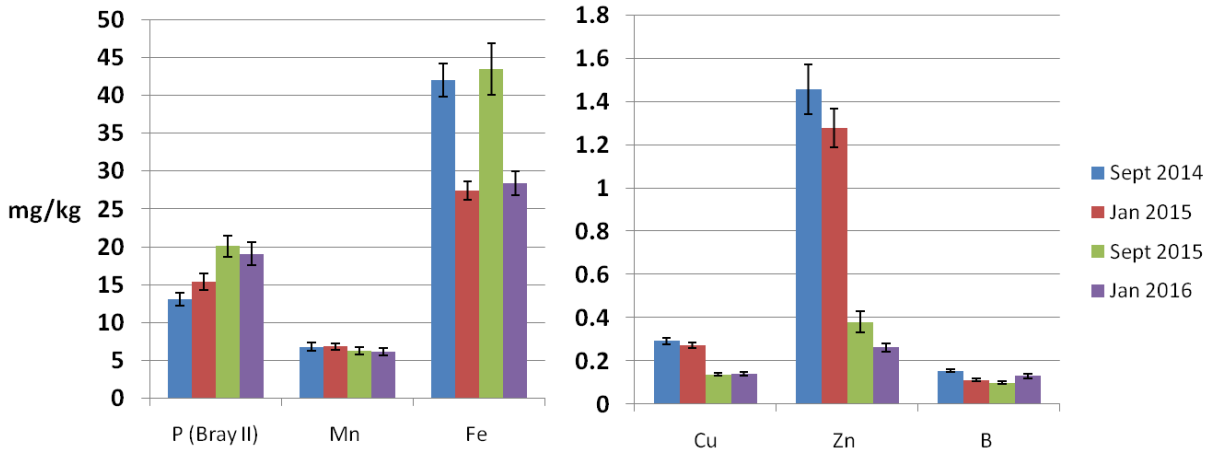

Figure 3-8: Plant available P, Mn and Fe (left); and Cu, Zn and B (right) in the soil of rooibos cultivated fields at Nieuwoudtville

Figure 3-8 (left and right) show that there was very little variation in the concentration of plant available Mn and B in the soil throughout the study period. There was a big increase in P (Bray II) from the start to the end of the study period. During September 2014 and 2015 the concentration of Fe in the soil was much higher than January 2015 and 2016, but over the one-year period Fe levels were the same. Cu and Zn showed big decreases throughout the study period.

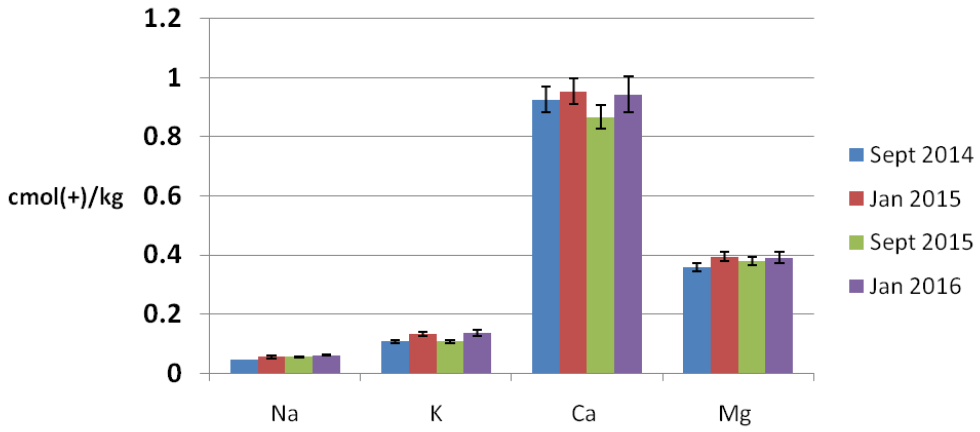

Figure 3-9: Exchangeable base cations in the soil of rooibos cultivated fields at Nieuwoudtville

The exchangeable base cations did not show a significant variation throughout the study period. Na and K showed a very small increase while Ca and Mg showed a small decrease over a one year period (Figure 3-9).

The increase in the soil P (Bray II) did not lead to an increase in plant P levels. While there was a small increase soil exchangeable K, there was actually a big decrease in plant K. The small variation in the soil exchangeable Ca during the study period, corresponded with the Ca concentration in the plant, which did not show big decreases or increases over a one-year period. The soil exchangeable Mg also corresponds with the Mg concentration in the plant that showed little variation throughout the study period. Both the soil exchangeable Na and the plant Na levels showed an increase. Although there was small change in the concentration of Fe in the soil, there was a big decrease in plant Fe over a one-year period. The concentration of Cu, Zn and B, all decreased in both the soil and plant over the study period.

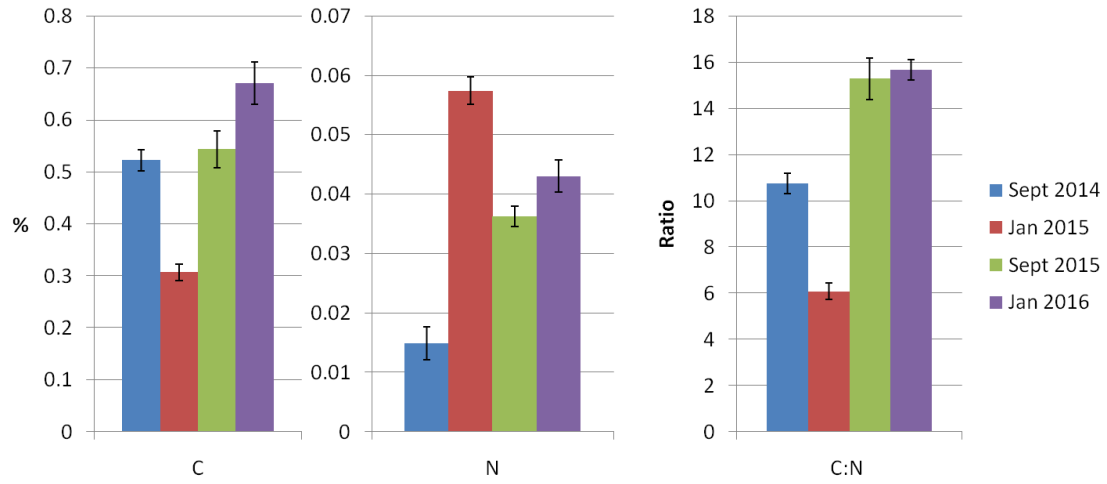

Figure 3-10: Total C, N and C:N ratio of the soil of rooibos cultivated fields at Nieuwoudtville.

There was a general increase in the total soil C. The total soil N was lower in September compared to January, but from September 2014 to 2015 there was an increase and from January 2015 to 2016 there was a decrease (Figure 3-10). The C:N ratio was much lower in January 2015 compared to the other sampling times.

3.3. The mulch trial site description

3.3.1. Field location and description

Four experimental sites for the rooibos trials were selected in the Nieuwoudtville area, one in the North-Bokkeveld and three in the South-Bokkeveld. All four sites are located in existing rooibos farms. Two of these sites were not part of the rooibos fields monitored for the database. The rooibos fields were chosen according rooibos age and the plants in the field according to growth. The age of the rooibos had to be at least 2 years for the natural leaf litter treatment to work and there had to be enough growth to sample plant material in July 2015. In the North-Bokkeveld the one experimental site was located on the farm Cloudskraal. The other three experimental sites in the South-Bokkeveld were located on the farms Oorlogskloof, Grootlaagt en De Dammetjies (Figure 3-11).

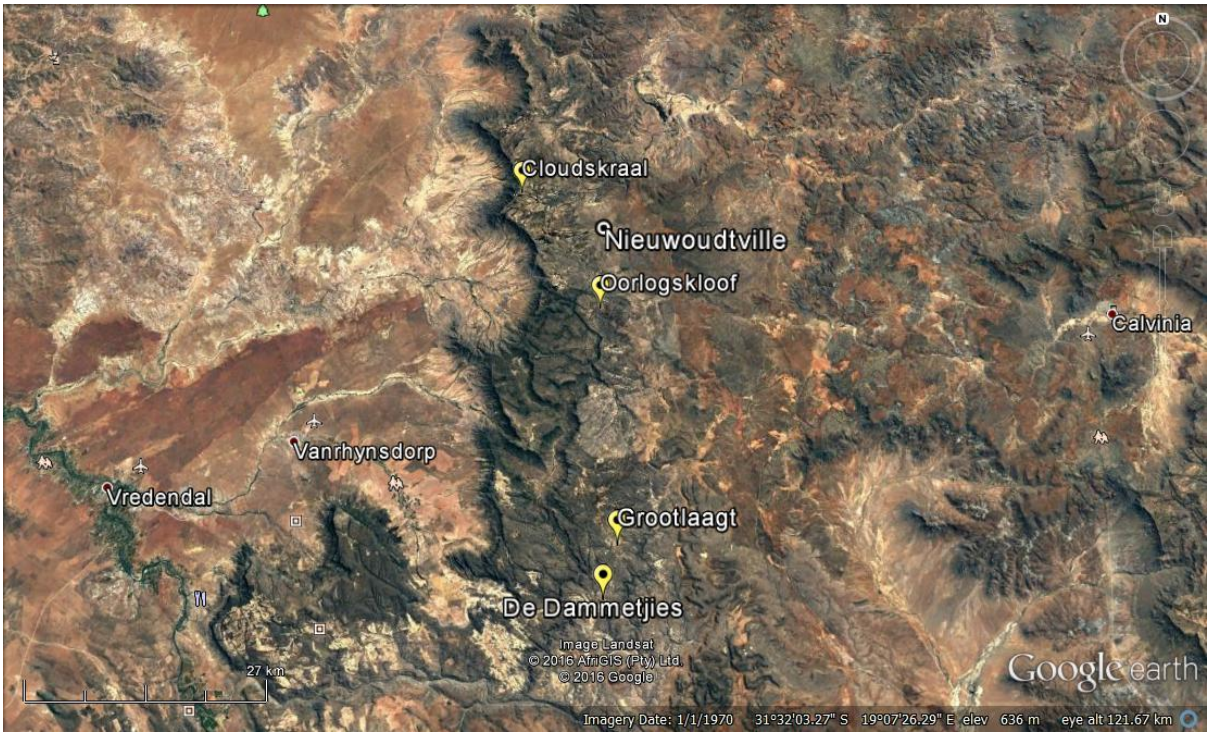

Figure 3-11: A Google Earth map of where the four experimental sites are located.

The rooibos field chosen as experimental site on Cloudskraal was on its second rooibos crop cycle (second time that rooibos cultivation took place on this specific field). This rooibos field had 2 year old (September 2015) rooibos plants. The rooibos cultivation cycle consisted of 1 year oats, 1 year lupines and 5 years' rooibos. Soil cultivation consisted of rip and offset disc ploughing. Fertilizer was only used for lupines. MAP (Mono-ammonium Phosphate) fertilizer was used at a rate of 100kg/ha. The spacing in the row between plants was small with an average in-row plant spacing of 30 cm and a spacing of 100 cm between rows.

The rooibos plants on the Oorlogskloof experimental site were 2 years (September 2015) old. The rooibos field were on its third rooibos crop cycle. After the second rooibos cultivation the field was fallowed for one year before rooibos was sowed again. Soil cultivation consisted of only offset disc ploughing. No fertilizer was used in rooibos cultivation. In-row plant spacing average was 40 cm and the spacing between rows was 50 cm.

On the Grootlaagt experimental site, the rooibos plants were 2 years old (September 2015). The rooibos field was on its sixth rooibos crop cycle. The land was fallow only for 1 year before rooibos seeds were sown. No fertilizer was used in rooibos cultivation. The average plant spacing at Grootlaagt was above 1 m in and between rows.

The experimental site of De Dammetjies was located in a rooibos field on its fourth rooibos crop cycle. The rooibos plants were 2 years (September 2015) old. No fertilizer was ever used on this

field. This rooibos field was left fallow for two years after each crop cycle. No other crops were cultivated on this land in the past, thus had no other crop history than rooibos. Soil cultivation consisted of rip only. Hoeing with spades in the rooibos plant rows was also a practise performed by the farmer in order to manage weeds. The average in-row plant spacing at De Dammetjies was 25 cm and the between row plant spacing was 170 cm.

At all four farms rooibos seeds were used and not rooibos cuttings. Only De Dammetjies used untreated (organic) seeds. During our trials, the rooibos at all the farms were harvested between December 2014 and January 2016, except for Grootlaagt.

3.3.2. Soil classification and texture

Only one soil pit was made per treatment site because the plants chosen for the treatments were closely grouped together, except at Cloudskraal where two groups were distanced at about 350 m. At Cloudskraal a profile pit (1.5 m) was dug by an excavator at site 1 and another profile pit was dug by hand at site 2. At Oorlogskloof, Grootlaagt and De Dammetjies, soil pits were dug by hand to a depth where the soil was too hard to dig deeper. A soil auger was then used to get soil samples from deeper horizons, to help classify the soils. At each of the four sites the soils were classified according to the South African Soil Classification system (Group and Macvicar, 1991). Particle size analysis for the 0-5 cm and 5-30 cm depths were done with the hydrometer method (3 sand fraction) using sodium hexametaphosphate (Soil Classifcation Working Group, 1991) at Elsenburg.

The profile pits for each of the trial sites are shown in Figure 3-12 and Figure 3-13, and in Table 3-2 the texture results are shown. It was not possible to dig through the very gravelly E horizon at the site 2 of Cloudskraal. Because site 1 and 2 were in the same field and rooibos habitat unit, it was concluded that the classification of the two soils were to be the same.

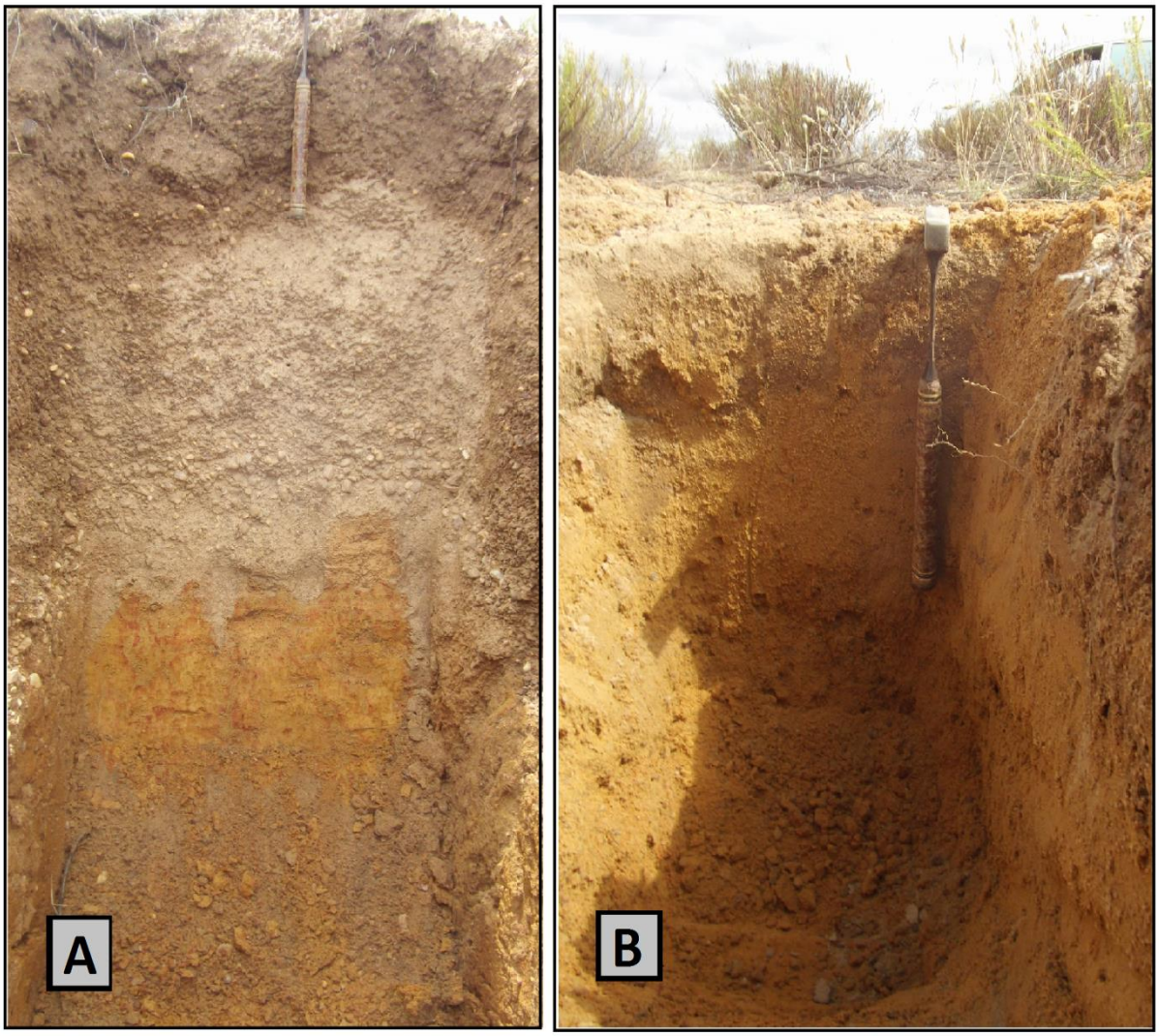

Figure 3-12: Soil profile pits at Cloudskraal, site 1 (A: Cartref) and Oorlogskloof (B: Clovelly)

The soil at Cloudskraal, Figure 3-12 (A), was very difficult to classify because of its very old nature. The A horizon was classified as an orthic A with a depth of 250 mm. The next horizon was classified as an E horizon (yellow in moist state) with a depth of 250 mm to 700 mm. The A and E horizons were very gravelly, containing rounded iron nodules, which is a stable form, originating from the weathering of the old surface material and explains why they are still found in this area (Clarke and Ellis, University Stellenbosch, Soil Science Department, 2016, personal communication). The B horizon was classified as a lithocutanic B horizon, which consisted of relic plinthite. The soil at Cloudskraal therefore was classified as a Cartref 2200 (Witzenburg) with an effective depth of 700 mm. The relic plinthite was very hard and can be restrictive to root growth. At Oorlogskloof, Figure 3-12 (B), the soil was classified as a Clovelly 2100 (Buckland). The orthic A horizon reached a depth of 150 mm and the B horizon a depth of 500 mm. The B horizon was classified as a yellow-

brown apedal, which had a dry colour of 10YR 6/6 and wet colour of 10YR 4/6. Below the B horizon the soil consisted of hard gravel only, which was too difficult to dig through.

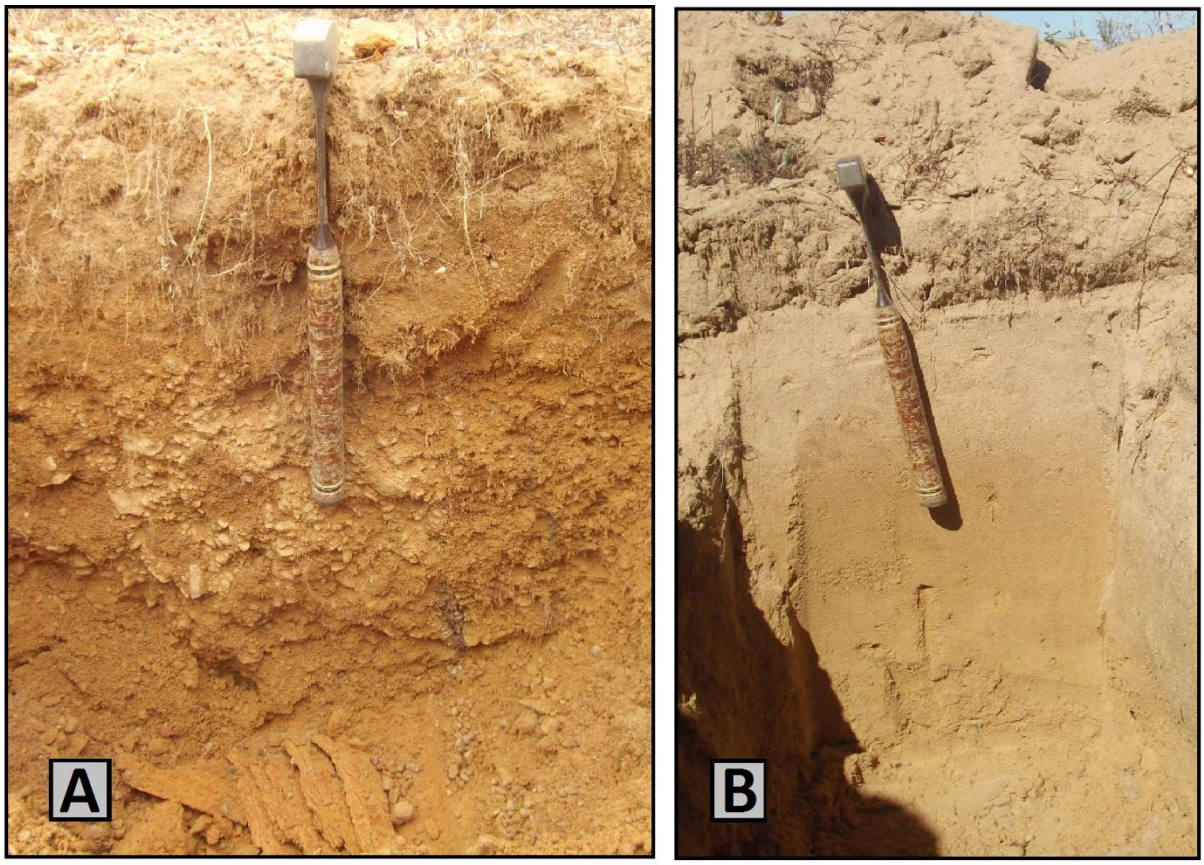

Figure 3-13: Soil profile pits at Grootlaagt (A: Wasbank) and De Dammetjies (B: Clovelly)

The soil profile pit for Grootlaagt can be seen in Figure 3-13. The soil was classified as a Wasbank 2000 (Lynedoch), with a "yellow" E in the moist state. The A horizon reached a depth of 250 mm and the gravelly E horizon a depth of 550 mm. A plinthic B horizon qualified as hard because it was again not possible to dig through. At De Dammetjies, Figure 3-13 (B), the soil was classified as a Clovelly 2100 (Buckland). The yellow-brown apedal B horizon had a dry colour of 10YR 7/6, with a colour of 10YR 5/6 in the wet state. This profile had an effective depth of 650 mm. The unspecified material under the B horizon consisted of relic plinthite (rock, which restricts root growth).

According to Smith (2014), farmers reported that rooibos produced on soils that contains a plinthic layer is of better quality. Plinthic layers form due to a fluctuating water tables (periodic water saturation) that leads to the reduction, mobilization and then the migration and precipitation of iron as nodules, mottles and concretions (Fey, 2005). The soils at these sites are not very deep, although the growth of the rooibos was good. At 700 mm the soil at the Cloudskraal site was the deepest, with 550 mm at Grootlaagt as the shallowest. Lots of other factors can add to better growth, but it is

also possible that these plinthic layers can store water that benefits the plant, which the deep sandy soils cannot do.

The soil textures for each of trial sites are given in Table 3-2. All the soils at the trial sites had very high sand contents of above 90% with Cloudskraal having the lowest sand content of 92% and Grootlaagt and De Dammetjies the highest with 96% at both depths. The gravel content was the highest at the Cloudskraal site 1 and Grootlaagt with contents above 20% at a 0-5 cm depth and above 15% in the 5-30 cm depth. De Dammetjies had none to very little (1%) gravel.

Table 3-2: Soil particle size analysis for the trial sites at the 0-5 cm and 5-30 cm depth

				Sand					
Depth	Sites	Clay	Silt	Coarse sand	Medium sand	Fine sand	Total	Gravel	Texture class
0-5 cm	Cloudskraal site 1	4	4	47	18	27	92	23	Sand (Coarse)
	Cloudskraal site 2	4	4	42	23	27	92	11	Sand (Coarse)
	Oorlogkloof	4	2	58	15	21	94	12	Sand (Coarse)
	Grootlaagt	2	2	49	29	18	96	22	Sand (Coarse)
	De Dammetjies	2	2	47	33	16	96	0	Sand (Coarse)
5-30 cm	Cloudskraal site 1	4	4	39	23	30	92	16	Sand (Coarse)
	Cloudskraal site 2	4	4	43	25	24	92	9	Sand (Coarse)
	Oorlogkloof	4	2	63	15	16	94	6	Sand (Coarse)
	Grootlaagt	2	2	51	19	26	96	17	Sand (Coarse)
	De Dammetjies	2	2	55	25	16	96	1	Sand (Coarse)

3.3.3. Potential rooibos habitat units (terroirs) of trial sites

The trial sites were also located on the GIS monitoring framework developed for potential rooibos habitat units. The data linked with the habitat units of the sites, were then compared with the data got from the field. This data includes aspect, slope, terrain, soil depth and % clay.

The potential rooibos habitat units or “terroirs” map were used to indicate differences between the trial sites. The aspect, slope, terrain, clay % and soil depth of the field and GIS framework for each of the trail sites were compared. The geology, slope derivatives and land type information were also considered in the GIS framework (Table 3-3). All these different factors together played a role in the suitability and potential of a specific land or field to grow rooibos.

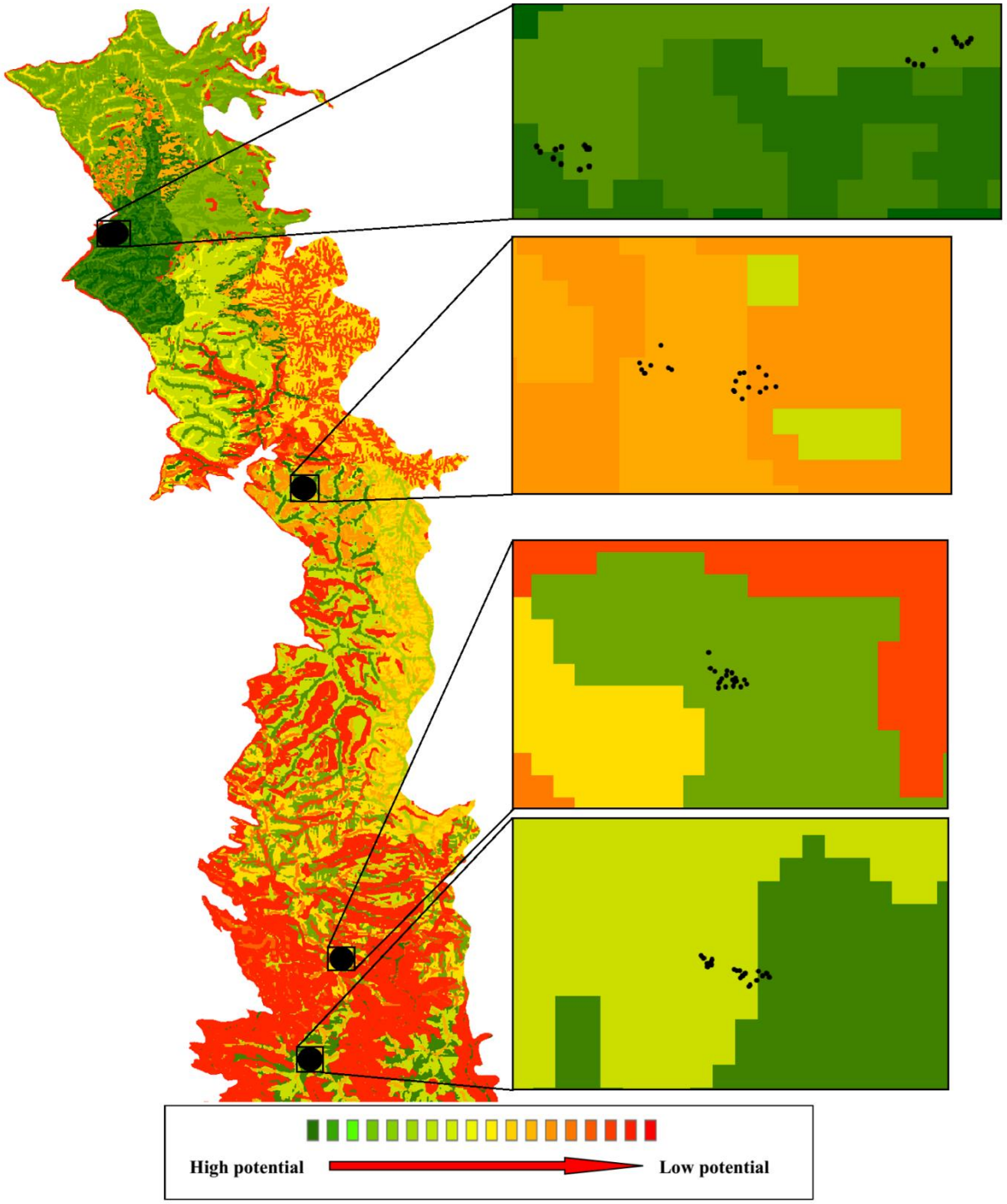

Figure 3-14: Potential rooibos habitat units for each of the trial sites (adjusted from de Clercq and van der Merwe, 2015). From top to bottom these sites are at the following farms; Cloudskraal, Oorlogskloof, Grootlaagt and De Dammetjies.

The differences in potential habitat units for the four trial sites can be seen in Figure 3-14. The trial site at Cloudskraal is situated in a habitat unit with a high to very high potential for rooibos and the Oorlogskloof trial site is situated in a habitat unit with a medium to low potential for rooibos. Figure 3-14 shows that to the south of the Bokkeveld on the potential rooibos habitat unit map, the space or area to grow rooibos gets a lot smaller. The trial site at Grootlaagt is situated in a habitat unit with

high potential, whereas the trial site in De Dammetjies in a habitat unit with a medium to high potential to grow big and healthy rooibos. There is thus variation in the potential rooibos habitat units between the four trial sites.

In Table 3-3, the GIS framework for potential rooibos habitat unit data and also the data obtained directly from the field are given next to each other. Other data such as land type, geology, slope description and potential habitat unit ("terroir") are also given. It can be seen in Table 3-3 that the data from the field observations and from the GIS framework (database), compare favourably. However, at Grootlaagt the soil depth and clay did not compare favourably.

Table 3-3: Field data and the data from the GIS framework for potential rooibos habitat units

	Cloudskraal		Oorlogskloof		Grootlaagt		De Dammetjies	
	Field	**GIS framework**	**Field**	**GIS framework**	**Field**	**GIS framework**	**Field**	**GIS framework**
Aspect	SE (cool)	S (cool)	W (warm)	NW (warm)	NE (cool)	N (cool)	NW (warm)	NW (warm)
Terrain	3-2	3-2	3-1	1	3-2	3-2	3-1	3-1
Slope	3	1-8	3	1-3	2	1-8	0	8-35
Clay (A)	4	3	3	3	2	13	2	3
Depth	70cm	75cm	50cm	35cm	55cm	80cm	65cm	50cm
Land type	Ca135		Hb106		Bb58		Ai64	
Geology	Quarzitic sandstone, shale and tillite of the TM group		Quarzitic sandstone on the TM group		Mainly sandstone of the Vryheid Formation with dolerite and alluvium		Quarzitic sandstone, shale and tillite of the TM group	
Slope description	Flat to medium steep		Flat to medium steep		Flat to medium steep		Flat to medium steep	
Potential "terroir"	High to very high sand/deep		Very sandy/ medium - low shallow		Medium to high loamy sand/very deep		Very sandy/ medium - medium deep	

3.4. Research materials and methods

3.4.1. Experimental Setup

The following treatments were selected for the study:

Treatment 1: Bare soil treatment (A)

Treatment 2: Added rooibos mulch (litter) (B)

Treatment 3: Natural leaf mulch (C)

Treatment 4: Added enriched rooibos mulch (D)

Treatment A (Figure 3-15 A) is duplicating the harvesting of the rooibos seed. The litter layer under the plant and a thin layer of topsoil were removed (± 15 cm radius). For treatment 2 and 4 rooibos litter was obtained from the factory and made into a pile and mixed thoroughly. Three random samples were taken from the pile and were chemically analysed. These samples represented the chemical properties of the rooibos litter being used for the mulch trials in treatment 2 (B) and 4 (D). The rooibos litter was then weighed of in bags of 200 g each before it was applied. The old mulches of the plants were removed and each one got precisely 200 g of rooibos mulch. When applied at a radius of ± 15 cm the thickness of the mulch layer were ± 1 cm. The 200 g of mulch was not excessive and was more or less the same amount of mulches the bigger plants had. By removing the old mulch and adding a specific amount (200 g) allowed me to have control over the amount of mulch under the plant whereas with treatment C it was not possible. In treatment C (Figure 3-16 C) the plant was left as it was with no addition or reduction to the natural mulch layer. Treatment 3 (C) can also be seen as the control of the study. The amount of litter for treatment C varied among plants and the specific amount was unknown.

Figure 3-15: Images of treatment A (bare soil) and B (added rooibos mulch). A wire grid block is 8 × 8 cm.

For treatment 4 (Figure 3-16 D), the added mulch was enriched with a fertilizer and the fertilizer chosen was the MAP (monoammonium phosphate) of Aquasol. It is a water soluble fertilizer with a composition of 260g P kg^{-1} and 120g N kg^{-1}. This fertilizer can also be used as a foliar spray. The nitrogen is in the ammonium form and is more resistant to leaching, and a slower release form of nitrogen. When the mulch was applied to the plants in treatment D, it was mixed (enriched) with 10g of the MAP fertilizer powder. The application rate would thus be 95kg ha^{-1} of MAP fertilizer (N: 11.4 kg ha^{-1} and P: 24.7 kg ha^{-1}) Application was done in September 2015.

Figure 3-16: Images of treatment C (natural mulch, undisturbed) and D (added enriched mulch). A wire grid block is 8 × 8 cm.

The average Bray II P values in most of these soils were significantly lower than the threshold value of 25 mg kg^{-1} P known for healthy crop development. The mobility of P in soils is very limited, except in organic and also sandy white bleached soils with very low CEC's (Hodges, 2010). Currently in rooibos cultivation fertilizer is only added to the soil before or with planting. Some farmers also use foliar sprays to add fertilizer during the rooibos 5-year cycle. Smith (2014) did a study on the effect of foliar sprays on the leave nutrient content and on the above- and below-ground biomass and according to him it seems foliar sprays are not compatible with rooibos plants. This study led to the opportunity to look at a different fertilizer method. The aim of adding fertilizer to the mulch was to see if it was possible for the mulch to slowly release the fertilizer into the soil (rhizosphere of the plant) throughout the season, thus aiding as another method of applying fertilizer to the rooibos field during the rooibos 5-year cycle. At each farm five plants were chosen randomly for each treatment. There were thus 20 plants per site (four sites).

In Table3-4 the chemical characteristics of the natural mulch and applied mulch are shown. In January 2016 the natural mulch had a lower N, P, K, Zn and Mn concentration than the added mulch. The reason for this is that the rooibos rests obtained at the factory was that of new growth and thus contained higher concentrations of these nutrients. Both the mulches had a higher C:N ratio higher than 24:1. This means that both mulches would decompose slow and result in a temporary N deficit, with N immobilization being dominant. The C:N ratio for the natural mulch was much higher that could thus lead to more N immobilization.

Table 3-4: Chemical characteristics of the added and natural mulches.

Time of year	September 2015	January 2016	
Rooibos mulch type	Added mulch	Added mulch	Natural mulch
C:N	31.97	31.58	41.85
N (%)	1.75	1.55	1.13
C (%)	55.86	47.92	46.20
P (%)	0.09	0.07	0.03
K (%)	0.57	0.32	0.22
Ca (%)	0.37	0.32	0.29
Mg (%)	0.28	0.23	0.21
Na (mg kg^{-1})	5556.67	4451.00	4720.00
Fe (mg kg^{-1})	614.53	568.79	2086.13
Cu (mg kg^{-1})	3.91	3.54	2.23
Zn (mg kg^{-1})	23.29	10.56	3.73
Mn (mg kg^{-1})	197.73	165.20	138.15
B (mg kg^{-1})	38.73	33.79	35.81
Al (mg kg^{-1})	190.00	343.75	667.50

3.4.2. Soil and Plant Sampling

Soil samples were taken in two different depths within the rhizosphere of the rooibos plants. The two topsoil samples were taken at a depth of 0-5 cm and 5-20 cm respectively. A small auger was used to lower the impact of sampling in the rhizosphere. The main feeder roots of the rooibos plant occur in these shallow depths (study area), and therefore soil samples were taken at these shallow depths. Plant samples were taken from the top part of the plant (new growth) and consisted of shoots and leaves. The plant samples were dried the same day it was taken to prevent oxidation to influence the chemical analysis.

Both soil and plant sampling were done in specific phenological stadia of the plant; just before flowering (September), prior to harvest after flowering and leaf drop (January) and during most active nutrient uptake (June/July). The morphometry of the plants were also recorded at each time samples were taken, and these parameters included plant height (cm), width (cm), leaf size (cm), length of regrowth, weight of dry leaves (g), weight of dry wood (g), weight of dry leaves and wood in total (g) and subjective ratio between green leaves vs woody parts (wet).

3.4.3. Chemical analysis

Soil samples taken in July 2015 and September 2015 were too small to do all the necessary chemical analysis. The soil samples for each depth of each treatment at one farm, five samples in total, were then bulked to get one big sample. We also had to consider the fact that repetitive sampling would

have impacted on the soil volume and root activity in the field that we intended to study. This led to having 8 samples from one farm instead of 40. In January 2016 and June 2016 bigger samples were taken, thus no bulking of soil samples. Soil samples under a rooibos plant were only taken when the plant was still alive. Soil samples were air dried and sieved through a 2 mm sieve before it was sent to Bemlab (Pty) Ltd for chemical analysis.

After sampling of the plant material, the samples were oven dried at 70 °C for 24 hours (Temminghoff and Houba, 2004) at the Nieuwoudtville rooibos factory. The IKA A 10 mill was then used to mill the plant material to a fine powder The dry milled plant samples were then stored in air tight plastic sample holders before being analysed. The chemical analysis of the plant material was done at Elsenburg.

3.4.3.1. Soil Analysis

Soil pH

The soil pH in 1 M KCl was determined using a 1:2.5 suspension ratio (Jones, 1999).

Resistance (Ohm)

A saturated soil paste was made with the soil sample and de-ionised water. The electrical resistance measured in the soil paste is inversely proportional to the salt concentration (Jones, 1999).

Total carbon and Nitrogen

For C and N determination the soil samples first had to be ball-milled to a fine powder. The Eurovector Elemental Analyzer^{TM} (dry combustion method) was used to determine the total C and N.

Exchangeable basic cations

The exchangeable cations (K, Ca, Mg and Na) were extracted using 1M ammonium acetate (NH_4OAc) at pH 7, and determined by inductively coupled plasma (ICP) (Jones, 1999).

Exchangeable acidity (H^+)

The K_2PO_4 potassium sulphate extraction method was used (Jones, 1999). This extraction method gives the exchangeable acidity of the soil at pH 7.

Cation saturation (K, Ca, Mg and Na)

Cation saturations were calculated for the soils at pH 7.

Plant-available phosphorus

Plant-available P was extracted using the Bray 2 method and determined using ICP. The Bray 2 extraction method is suitable for soil samples from the winter-rainfall region of the Western Cape (Jones, 1999).

Plant-available micronutrients (Cu, Zn, Mn, B and Fe)

Plant-available micronutrients were extracted using Disodium EDTA and was determined using ICP (Jones, 1999).

Soluble Sulphur(S)

Water Soluble Sulphate, from a 2:1 water extract, is analysed either by ICP-OES (Jones, 1999).

Texture

Particle size analysis was done with the hydrometer method (3 fraction) using sodium hexametaphosphate (Committee, 1990).

3.4.3.2. Plant Analysis

The plant material was analysed for % P, K, Ca, Mg and mg kg^{-1} Na, Fe, Cu, Zn, Mn, B and Al using dry cremation and inductively coupled plasma (ICP). The % C and N was determined using the Eurovector EA Elemental Analyzer (dry combustion method).

3.4.4. Method of statistical analysis (Chapter 4)

Before the effect of different residue treatments on the rooibos nutrient cycle and pools were investigated in **Chapter 5**, a systematic approach and interpretation of analytical results was needed to identify the interaction between results. The same applied to the complexity of interactions in soils, and therefore multivariate analysis was performed. To look at groupings and categorical differences between all the data points (data point = soil and plant analysis; and growth for specific time) principle component analysis was performed on the raw data using SIMCA™ 14 software. SIMCA™ (Version 14, 2015, MKS Umetrics, Sweden) was also used to build a correlation matrix. Multiple regression analysis was performed on the data using Statistica™ (Version 13, 2015, Dell software, Tulsa) software. Multiple regression models were built for each growth property (dependant variable) with the soil and plant analysis as independent variables. These regression models were first done on the raw data. After looking at all the data and models one growth property was identified, which was then used as the main growth property in multiple regression analysis, where data was transformed as needed.

3.4.5. Soil volumetric water content, temperature and EC trial (Chapter 6)

3.4.5.1. Bulk density

Soil bulk density was determined at all four plants where the probes were installed. An undisturbed core sample (Klute, 1986) was taken at the 0-5 cm depth and the 15-20 cm depth, same depth as the probes, with a volumetric soil sampler. The soil samples were then oven dried for 48 hours at 70°C. The known volume off the ring that was used to take the core sample and the dry weight of the soil was then used to calculate the bulk density of the soil at each depth for each of the four plants. Core samples for the bulk density were taken just before the probes were installed.

3.4.5.2. Weather station installation

A weather station was installed near the rooibos field to continuously measure precipitation and air temperature at an hourly interval.

3.4.5.3. Probe calibration and installation

Soil varies in bulk density, mineralogy, texture and salinity and the generic mineral calibration for the ECH_2O sensors (including 5TE) results in ± 3-4% accuracy for most soils. A soil specific calibration however can increase the accuracy to ± 1-2% (Cobos and Chambers, 2010). Thus for best possible accuracy a soil specific calibration was needed. First a bulk soil sample was collected at the sites where the 5TE probes were installed (at the 0-5 cm top soil and at 15 cm depths). The preparation of the bulk soil sample included air drying and also the removal of large rocks and other objects that could complicate the calibration process. Calibration containers were then used to pack soil at the approximate field bulk density. Six calibration containers were used, each with a different volume of water (Table 3-5). The 5TE probes were inserted in each container and 3 readings (raw data) of each container were taken. The mean of the 3 readings were used. Soil cores were then taken from each container with a volumetric soil sampler and weighed (wet weight). These soil samples were then oven dried for 48 hours at 70°C and weighed after (dry weight). With this data the volumetric water content (VWC) could be calculated.

Table 3-5: Data gathered for probe calibration

Sample no	Raw VWC (cm^3/cm^3)	Mass of drying container (g)	Sample volume (cm^3)	Mass of wet soil + container (g)	Mass of dry soil + container (g)	Mass and volume of water (cm^3)	Dry soil mass (g)	Soil bulk density ($g/cm^{3)}$	VWC (cm^3/cm^3)
1	0.032	123.66	98.17	291.62	290.95	0.67	167.29	1.7040	0.006824564
2	0.075	139.92	98.17	290.43	284.22	6.21	144.30	1.4698	0.063254541
3	0.131	79.28	98.17	249.51	236.8	12.71	157.52	1.6045	0.129462997
4	0.160	86.14	98.17	257.89	246.16	11.73	160.02	1.6300	0.119480799
5	0.239	90.19	98.17	281.79	254.73	27.06	164.54	1.6760	0.275630897
6	0.278	89.99	98.17	295.00	266.11	28.89	176.12	1.7939	0.294271124

To find a calibration function a scatter plot was prepared, with the sensor output on the X-axis, and the calculated VWC on the Y-axis (Figure 3-17). A trendline was then used to construct a calibration function (linear equation).

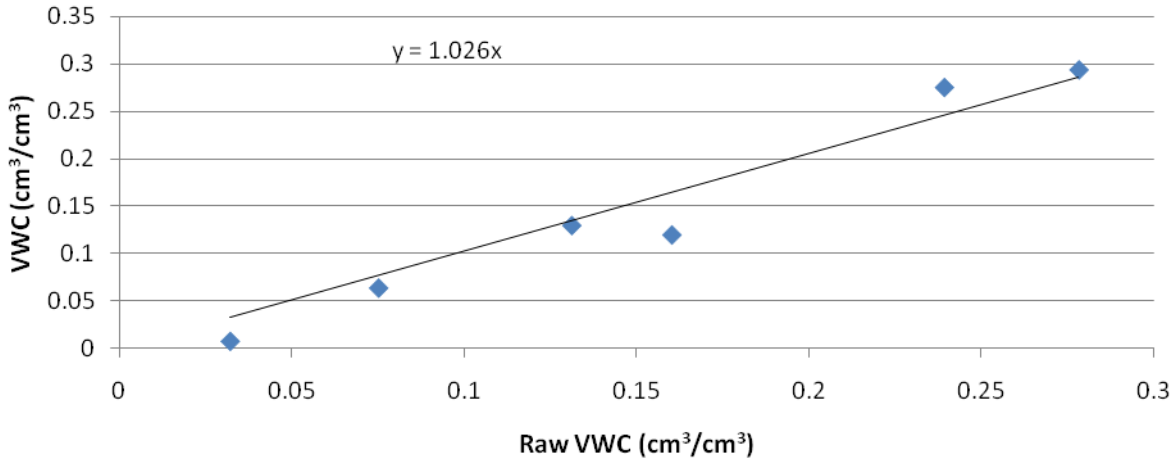

Figure 3-17: Plot of calibration data with linear equation

The calibration function, y = 1.026*X*, can now be applied to the raw data downloaded from data-logger (Decagon Em 50). The equation can then be set as: VWC = 1.026*raw data.

It was decided to install the probes at Cloudskraal because the soil was not too gravelly compared to Oorlogskloof; and access to the farm from Nieuwoudtville was easy and fast so the loggers could be checked up on more frequently. Decagon 5TE soil moisture probes was used to log the volumetric water content (VWC), temperature and EC of the soil at the 5 cm and 20 cm depth (Figure 3-18) and ECH_2O logger was used to log the data. One plant of each treatment (Treatment A, B, C and D) got probes and the plants chosen were in close proximity.

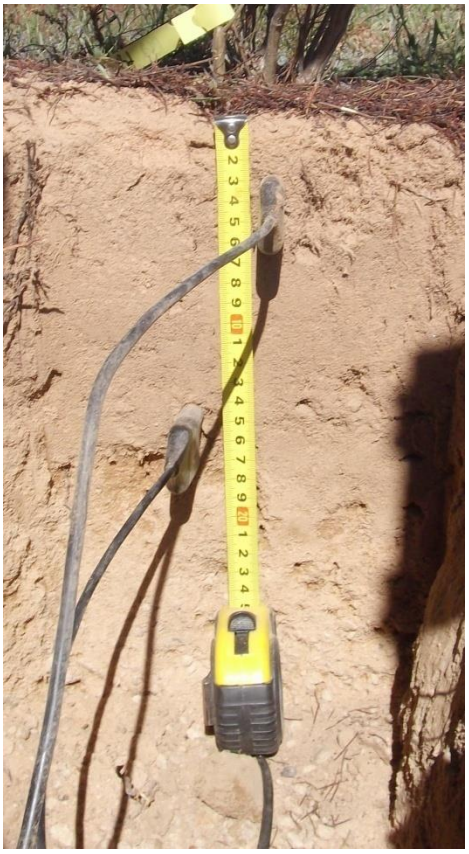

Figure 3-18: Installation of probes in soil to a depth of 5 cm and 20 cm

3.4.6. Near infra-red spectroscopy (Chapter 7)

The soil samples used for deriving the NIR calibration, were the samples taken in January 2015 for the database study (de Clercq and van der Merwe, 2015). The plant samples used were the first samples taken in July 2015 for the trials. NIRS analysis was performed using a Bruker multi-purpose FT-NIR analyser (MPA) spectrometer and OPUS 7.2 software. Fourier-transform NIR (FT-NIR) has the advantage of higher scan speed, wavelength accuracy, has a HeNe laser that allows self-calibrating, and wavelength and simplified mechanics (Magwaza *et al.*, 2013). Spectral data was obtained within the wave range of 12500-3800 cm^{-1} (equivalent of 800-2777.7 nm) of both the plant and soil samples using the integrating sphere (IS) of the instrument. The use of the IS is recommended for heterogeneous samples, such as soil and plant material, which are used to measure the diffuse reflectance of the highly scattering solid media (Magwaza *et al.*, 2013). The NIR standard procedure for soils, according to Mashimbe (2013), was used to scan the soil samples. The sieved soil sample was placed in the aluminium cup (IS), which has a fully NIR transparent membrane, with a surface area (50 mm width), at the bottom. The soil sample was then scanned at a resolution of 8 cm^{-1} for a sample scan time of 128 scans and the absorbance of each sample was saved (Mashimbye, 2013). For the scanning of the plant samples the procedure of Galvez-Sola *et al.*, (2015) was followed. The

plant material was scanned at a resolution of 8 cm^{-1} for a sample scan time of 64 scans. Because of the heterogeneity of the soil and plant samples, three spectra of each sample were obtained.

3.5. Conclusions

Only a thin stretch of land along the plateau on the Table Mountain Sandstone is suitable for rooibos production in the Northern Cape. Nieuwoudtville had an average yearly rainfall of 432 mm for the past 5 years (2009-2014) which is between the optimum of 380 to 635 mm for rooibos. Not only do weather and soil physical and chemical properties determine the growth, health and longevity of rooibos plants but also other abiotic factors such as terrain, aspect and slope. Together these factors form a unique habitat and when applied to build a GIS framework for potential rooibos habitat units. It is then clear that in the North- and South Bokkeveld, there is a great variety in habitats that can result in a great variety of rooibos health, growth and quality outcomes. The rooibos plant is adapted to thrive in its natural habitat and when used in monoculture, the plant faces new challenges.

During the surveys of September 2014 and January 2015 it was not only noted that in a single field a lot of variation occurred in growth and mortality, but also that neighbouring plants, with the same habitat, indicated differences in growth and mortality. It was also noted in-field that where the farmers harvested seeds, the plants appeared to be in a poorer condition compared to the others. This led to the hypotheses that when the litter layer under the plant was removed for seed harvesting, it negatively affected the health and longevity of the rooibos plant and that the action induced a change in the nutrient support to the rooibos plant. A NIR study was initiated as it was noted during the setup of the trial layout that a large amount of samples was needed and analysed were very expensive. NIR has the potential to replace expensive traditional lab analysis, which could be of great advantage to rooibos farmers in order to perform quick and less expensive soil and plant chemical analysis.

Also worth noting is that with this study, the opportunity was identified to learn something more about the nutrient utilisation habits of the rooibos plant, by looking at how it responds to changes in the litter layer below the plant.

4. STATISTICAL ANALYSIS USING PRINCIPLE COMPONENT (PCA) AND MULTIPLE REGRESSION ANALYSIS

4.1. Introduction

When working with a large data sets, which includes five categorical variables and 41 continuous variables where one or more variable can impact the other, methods were needed to mine the important and significant interactions from the complex dataset. The interacting of nutrients (synergetic and antagonistic) in a nutrient cycle, and its influence on growth, is highly complex and needs to be identified with the help of statistical analysis. This chapter is the first step in identifying the important nutrients, in a process where the effect of different residue management practices on the nutrient cycle were studied.

4.2. Descriptive statistics, PCA score plot and correlation matrices for whole trial database.

In Table 4-1 the descriptive statistics for the growth properties are shown. In Table 4-3 and Table 4-2 the descriptive statistics for the soil and plant chemical properties are shown.

Table 4-1: Descriptive statistics for plant growth properties

Growth properties	Valid N	Mean	Median	Minimum	Maximum	Std.Dev.
Average Leaf Size (mm)	564	2.06	2.02	1.00	3.78	0.33
Average Regrowth (cm)	440	26.38	23.00	3.00	70.50	11.72
Leaves vs Wood	482	3.09	3.00	1.00	5.00	0.64

Table 4-2: Descriptive statistics for all plant chemical data

Plant properties	Valid N	Mean	Median	Minimum	Maximum	Std.Dev.
Plant C (%)	564	51.52	52.00	42.03	58.39	2.38
Plant N (%)	564	1.49	1.45	0.00	2.52	0.36
Plant P (%)	564	0.07	0.06	0.01	0.22	0.04
Plant K (%)	564	0.44	0.41	0.15	0.81	0.15
Plant Ca (%)	564	0.16	0.16	0.05	0.40	0.06
Plant Mg (%)	564	0.20	0.20	0.05	0.38	0.05
Plant Na (mg kg^{-1})	564	4676.16	4564.50	190.00	12130.00	2091.94
Plant Fe (mg kg^{-1})	564	105.88	97.01	46.54	480.20	46.40
Plant Cu (mg kg^{-1})	564	3.40	2.97	0.87	9.00	1.71
Plant Zn (mg kg^{-1})	564	8.08	7.48	2.22	20.90	3.52
Plant Mn (mg kg^{-1})	564	80.46	71.56	13.10	311.40	47.53
Plant B (mg kg^{-1})	564	23.41	23.14	9.37	43.12	7.50
Plant Al (mg kg^{-1})	564	81.28	74.50	33.00	190.00	29.18

Table 4-3: Descriptive statistics for all soil chemical data

Soil properties	Valid N	Mean	Median	Minimum	Maximum	Std.Dev.
Soil pH (KCl)	564	4.65	4.60	4.00	5.80	0.34
Soil Resistance (Ohm)	564	5325.85	5070.00	470.00	14550.00	2760.30
Log® soil resistance	564	3.66	3.71	2.67	4.16	0.27
Soil H+ (cmol kg^{-1})	564	0.43	0.42	0.20	0.94	0.11
Soil P (mg kg^{-1})	564	21.21	11.26	2.00	391.25	41.84
Soil K (mg kg^{-1})	564	38.41	33.60	15.64	119.06	16.14
Soil Na ($cmol_c$ kg^{-1})	564	0.08	0.07	0.03	0.76	0.05
Soil K ($cmol_c$ kg^{-1})	564	0.10	0.09	0.04	0.30	0.04
Soil Ca ($cmol_c$ kg^{-1})	564	0.56	0.56	0.28	1.40	0.15
Soil Mg ($cmol_c$ kg^{-1})	564	0.34	0.32	0.15	0.85	0.09
Soil Cu (mg kg^{-1})	564	0.11	0.10	0.00	0.30	0.05
Soil Zn (mg kg^{-1})	564	0.20	0.20	0.10	3.00	0.17
Soil Mn (mg kg^{-1})	564	4.19	4.00	0.40	13.30	1.94
Soil B (mg kg^{-1})	564	0.12	0.12	0.03	0.37	0.06
Soil Fe (mg kg^{-1})	564	27.98	25.26	2.72	152.00	13.41
Soil C (%)	560	0.55	0.54	0.16	3.08	0.22
Soil N (%)	561	0.03	0.03	0.00	0.20	0.01
Soil % Na (pH 7)	564	5.43	4.72	1.94	40.07	2.77
Soil % K (pH 7)	564	6.36	6.06	3.24	13.74	1.88
Soil % (pH 7)	564	36.82	36.05	18.40	50.64	5.85
Soil % Mg (pH 7)	564	22.34	22.07	13.06	33.41	3.20
T-Value ($cmol_c$ kg^{-1})	564	1.52	1.50	0.89	3.02	0.27
Soil Soluble S	564	8.21	8.78	4.33	19.40	2.43

The 8 principle components accounted for 71% of the variance. The PCA t-scores for component 1 and 2, illustrated in Figure 4-1 **,** covered the maximum variation in the x-space with R2X values of 0.203 and 0.149. The PCA score plots are used to interpret relations among data points while loading plots are used for interpreting relations among variables. In the following PCA score plots, the data points that are near each other on the graph, are very similar and should correlate well, while data points far from each other are different and will have a low correlation coefficient. These PCA score plots thus show the variation between data points.

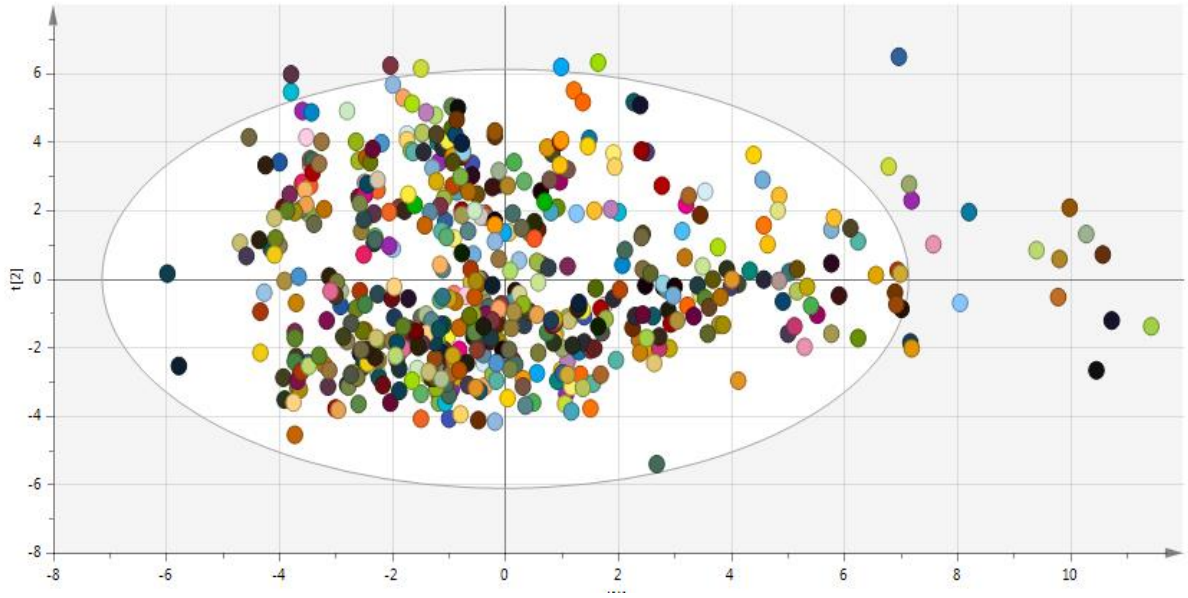

Figure 4-1: PCA score plot showing each sample taken over the study period/course.

The PCA score plot in Figure 4-1 show the variance of data for each sample. From this result it was noted that the scatter plot showed no clear groupings of data points. It was also noted that there is a small division of data on the X axis. This PCA score also clearly indicates differences between data points of different sampling times and soil depths

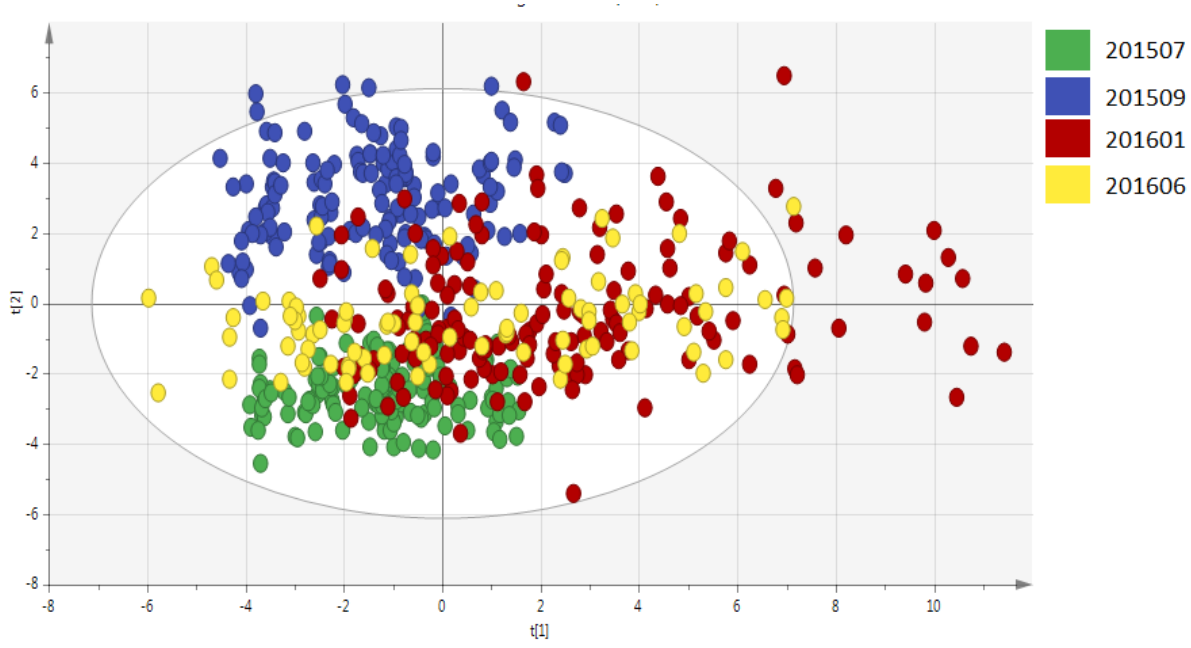

Figure 4-2: PCA score plot showing each sample taken over study period and coloured according to time of sampling.

In the PCA score scatter plot it can be seen that there is a definite difference in data points taken at different times of the year (Figure 4-2). Samples taken in July (green) differed a lot from the samples taken in September (blue). Sample data of January (red) and June (yellow) and not too different

from each other. This PCA thus shows that there is a big change in the soil and plant chemistry; and also growth from July to September.

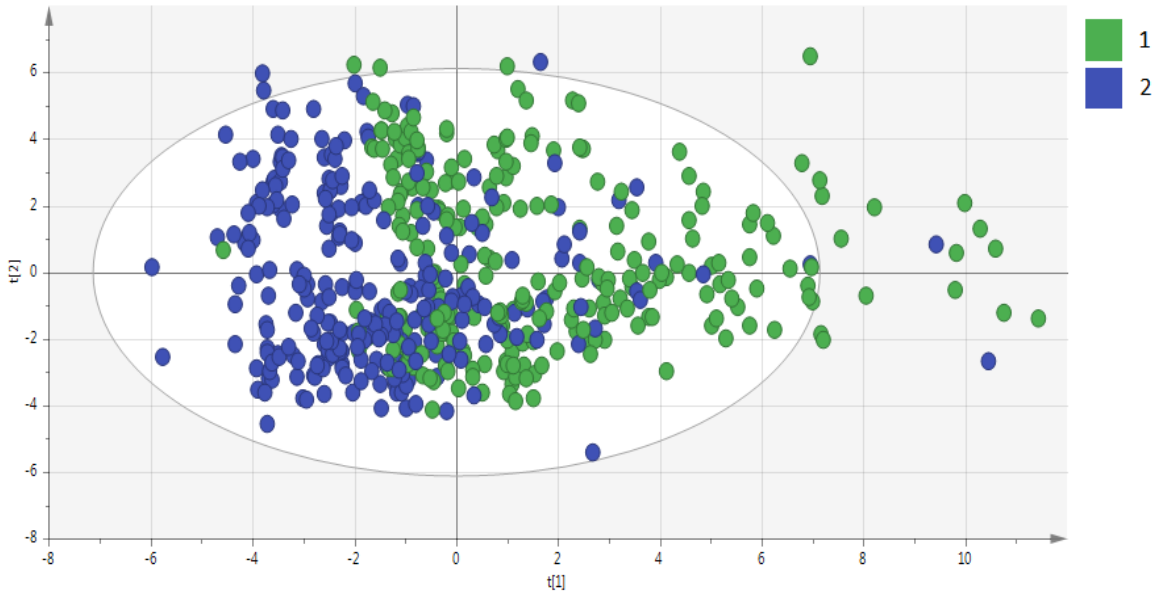

Figure 4-3: PCA score plot showing each sample taken over study period and coloured according to depth. (1: 0-5 cm and 2: 5-20 cm).

The PCA scatter plot shows that throughout the four times samples were taken over one year that there was a difference in data when looking at the two depths at which soil samples were taken (Figure 4-3). Even before looking at the chemistry of the soils it is already visible from Figure 4-3 that there is a definite difference in the chemistry of the two depths.

The PCA loading plot (Figure 4-4) is a visual expression of variables and its relation to each other, which ones are positively or negatively correlated, or not correlated at all. The variables near the 0 of p1 and p2 are not well explained by the model. Variables that are close together have a high positive correlation, while variables on the opposite side of the origin will have a negative correlation. For example in Figure 4-4 at the top can be seen that Plant P, Zn, Cu and Ca are close together, meaning they are highly positively correlated. This can be supported by the correlation coefficients in Table 4-7, which show that Zn, Cu and Ca are strongly related to P with correlation coefficients of 0.83, 0.68 and 0.65 respectively. It is also evident that Soil resistance (log) and soil K opposes soil Na, meaning they are strongly negatively correlated with correlation coefficients of -0.65 and -0.70 respectively (Table 4-4).

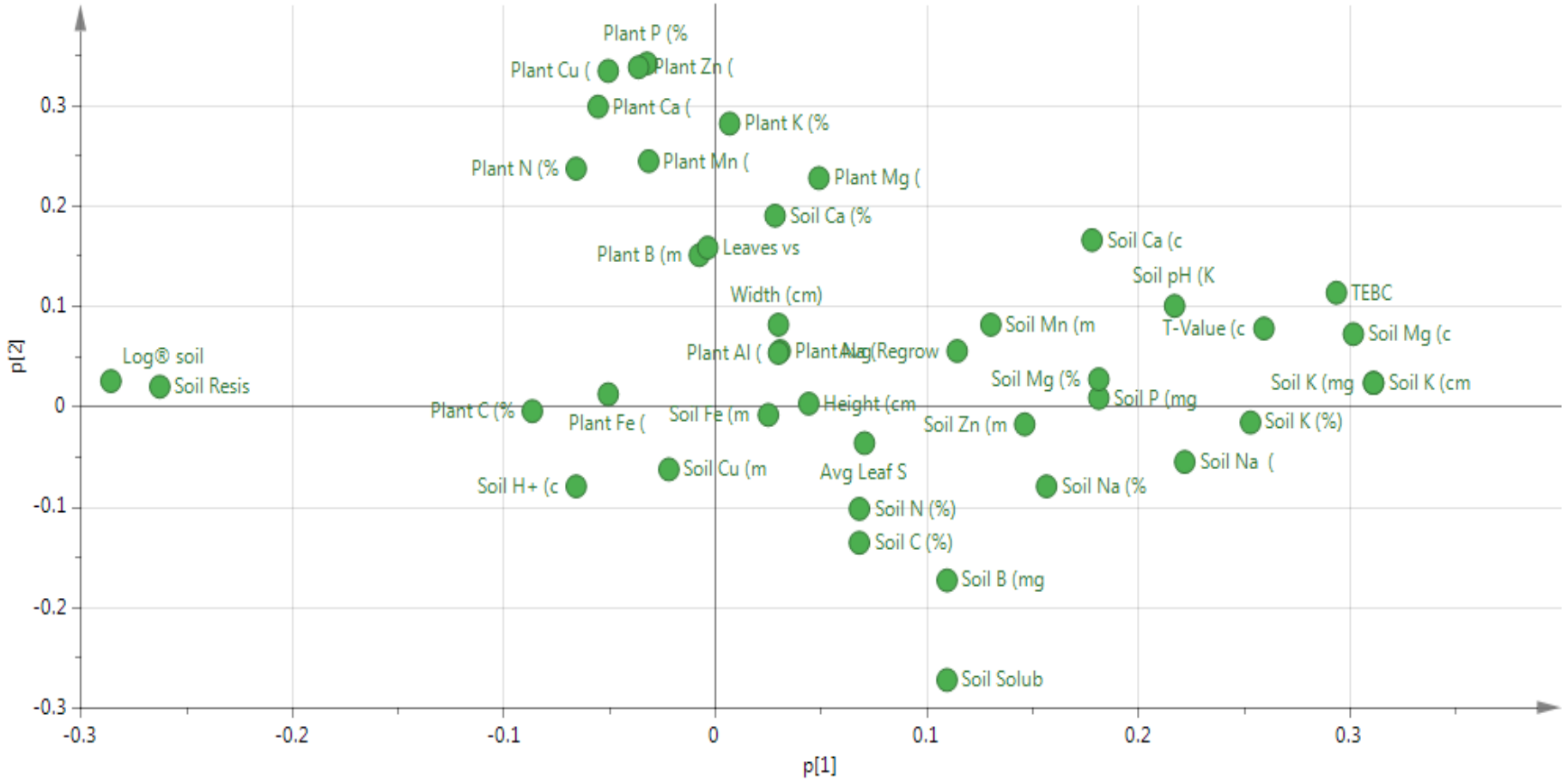

Figure 4-4: PCA loadings plot showing each variable including soil and plant chemical analysis and growth properties

The correlation matrixes of Table 4-4 to Table 4-10 show the one to one correlations between chemical and growth properties that will be discussed. Some of these correlation matrixes was too big to insert as one and was thus broken down to two or more matrixes. In Table 4-4 it can be seen that pH has a moderately strong correlation with exchangeable K, Ca and Mg. These correlations make sense because soil pH affects the amount of exchangeable cations in the soil. These results are similar to the positive correlations found in the international study of Sillanpää (1982). Higher pH values is associated with higher CEC and thus basic cations (Abreu Jr *et al.*, 2003). The pH values only ranged between 4.00 and 5.8, which can be the reason why the correlations are not that strong. Exchangeable acidity (H^+) and pH had a moderately strong negative correlation (Table 4-4), which was also to be expected because the higher the acidity the lower the pH ($-\log_{10}[H^+]$). Na, K and Ca had a moderately strong negative correlation with soil resistance (Table 4-4). Soil resistivity is normally influenced by temperature, moisture and dissolved salts. The negative correlation thus meant that the increase in exchangeable Na, K and Ca (salts) lead to lower soil resistance at specific soil temperature and soil moisture content.

A correlation was found between soil P and exchangeable K in the soil, also Mn and exchangeable K (Table 4-4), but no literature could be found to explain the soil P and K correlation. K and Mn have a direct synergistic relationship (Malvi, 2011), which can be the reason for the weak positive correlation. This relationship can be the reason for the positive correlation between K and Mn. Exchangeable Ca and Mg had the highest correlation with the T value of the soil, which showed that Ca and Mg had the biggest influence or contribution to change of the T-value. Calcium and Mg are positively correlated with pH (Table 4-5), and therefore the change in pH over the year can be the reason for the correlation between Ca and Mg. Ca and Mg are generally not as water soluble as Na and K, thus the moderately strong correlation between resistance and Na and K respectively.

Table 4-4: Correlation matrix 1 for soil properties vs. soil properties.

	pH (KCl)	Resis (Ohm)	Log® resis	H+ (cmol kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Na ($cmol_c$ kg^{-1})	K ($cmol_c$ kg^{-1})	Ca ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Fe (mg kg^{-1})	Soluble S
pH (KCl)	1	-0.38	-0.41	-0.64	0.17	0.52	0.32	0.52	0.49	0.61	-0.07	0.29	0.20	0.09	-0.19	-0.05
Resis (Ohm)		1	0.94	0.12	-0.34	-0.58	-0.61	-0.58	-0.23	-0.58	0.19	-0.21	-0.09	-0.14	-0.12	-0.33
Log® resis			1	0.11	-0.51	-0.65	-0.70	-0.65	-0.27	-0.63	0.16	-0.25	-0.12	-0.15	-0.09	-0.37
H+ (cmol kg^{-1})				1	0.19	-0.12	-0.16	-0.12	-0.09	-0.15	0.26	-0.04	0.13	0.16	0.35	0.22
P (mg kg^{-1})					1	0.53	0.30	0.53	0.22	0.42	0.01	0.27	0.19	0.03	0.06	0.18
K (mg kg^{-1})						1	0.47	1.00	0.40	0.78	-0.02	0.42	0.50	0.32	0.07	0.20
Na ($cmol_c$ kg^{-1})							1	0.47	0.09	0.41	-0.15	0.13	-0.02	0.08	0.10	0.24
K ($cmol_c$ kg^{-1})								1	0.40	0.78	-0.02	0.42	0.50	0.31	0.07	0.20
Ca ($cmol_c$ kg^{-1})									1	0.63	0.02	0.13	0.41	0.09	0.08	0.03
Mg ($cmol_c$ kg^{-1})										1	-0.05	0.38	0.37	0.26	0.07	0.14
Cu (mg kg^{-1})											1	0.10	0.25	0.24	0.04	0.25

Table 4-5: Correlation matrix 2 for soil properties vs. soil properties.

	C (%)	N (%)	Na (%)pH 7	K (%)pH 7	Ca (%)pH 7	Mg (%)pH 7	T-Value (cmolc kg^{-1})
pH (KCl)	0.07	-0.03	0.23	0.48	0.46	0.63	0.36
Resis (Ohm)	-0.07	-0.08	-0.55	-0.50	0.10	-0.41	-0.47
Log® resis	-0.04	-0.07	-0.60	-0.54	0.10	-0.38	-0.54
H+ (cmol kg^{-1})	0.12	0.10	-0.26	-0.31	-0.42	-0.60	0.26
P (mg kg^{-1})	0.01	0.06	0.16	0.40	-0.10	0.09	0.47
K (mg kg^{-1})	0.14	0.08	0.29	0.89	-0.02	0.45	0.67
Na ($cmol_c$ kg^{-1})	0.03	0.03	0.94	0.39	-0.22	0.22	0.37
K ($cmol_c$ kg^{-1})	0.15	0.07	0.29	0.89	-0.02	0.45	0.67
Ca ($cmol_c$ kg^{-1})	0.06	-0.02	-0.16	0.05	0.79	0.05	0.81
Mg ($cmol_c$ kg^{-1})	0.16	0.05	0.19	0.53	0.20	0.65	0.81
Cu (mg kg^{-1})	0.22	0.06	-0.20	-0.07	-0.04	-0.17	0.07

Calcium and Mg are not as water soluble as Na and K, thus the weaker negative correlation with resistance. No other strong correlations were found in Table 4-6 between the rest of the soil nutrients. In the study of Kumar and Babel (2011) a weak positive correlation was also found between soil Zn and Mn like in Table 4-6. A weak positive correlation was found between soil C and the T-value, and a moderate weak correlation was found between soil Mn and the T-value. A moderate strong correlation between B and soluble S was also found.

Table 4-6: Correlation matrix 3 for soil properties vs. soil properties.

	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Fe (mg kg^{-1})	C (%)	N (%)	Na (%)pH 7	K (%)pH 7	Ca (%)pH 7	Mg (%)pH 7	T-Value (cmol kg^{-1})	Soluble S
Zn (mg kg^{-1})	1	0.34	0.16	0.04	0.24	0.04	0.04	0.38	-0.05	0.29	0.27	0.08
Mn (mg kg^{-1})		1	0.22	0.10	0.18	0.04	-0.19	0.37	0.19	0.01	0.47	-0.02
B (mg kg^{-1})			1	0.08	0.27	0.07	-0.01	0.26	-0.13	0.12	0.26	0.50
Fe (mg kg^{-1})				1	-0.01	0.00	0.04	-0.03	-0.13	-0.20	0.24	0.09
C (%)					1	0.33	-0.02	0.11	-0.05	0.09	0.16	0.31
N (%)						1	0.01	0.06	-0.08	0.00	0.06	0.13
Na (%) pH 7							1	0.33	-0.33	0.22	0.08	0.17
K (%) pH 7								1	-0.20	0.54	0.28	0.14
Ca (%) pH 7									1	-0.02	0.30	-0.19
Mg (%) pH 7										1	0.11	-0.04
T-Value ($cmol_c$ kg^{-1})											1	0.22
Soluble S												1

Only for treatment D, macronutrients N and P were added as fertilizer and this can be the reason why not so many negative correlations were seen among macronutrients, because nutrients such as K, Ca and Mg were not added. When imbalanced nutrient additions are made, it may result in imbalances in the soil, which may have a negative impact or interaction on the uptake of other nutrients (Fageria, 2001). Because of the sandy nature of the soils, the nutrient content was low

compared to other studies and there were also limited variation. Throughout the season the growth performance or photosynthesis rate thus also influences the uptake of nutrients by the plant. This uptake of nutrients in natural nutrient poor soils can thus be the reason for the lots of positive correlations among plant nutrients. A lot of weak to strong correlations were found among plant properties and is shown in Table 4-7. The moderately strong positive correlation found between plant N and P in this study had also been reported by other studies, where N leads to increased uptake of P (Fageria, 2001; Maistry, 2014). Demand for N by plants can also be associated with increased supply of P through root production and higher investment in N-carrier enzymes (Maistry, 2014). Some studies also reported that under certain concentrations (varies among plants) of ions in nutrient solution, Ca can stimulate the absorption of P and K (Fageria, 2001), which can be the reason for the positive correlation between them in this study. In the study of Smith (2014) a moderate positive correlation (R^2=0.44) between the P and Ca content of the leaves were also found. Smith (2014) also found a positive correlation between plant P and Mg (R^2=0.56) and this result agrees with the correlation in Table 4-7. Mg is needed by the plant to transport P from the roots to the leaves, which can be the reason for the positive correlation (Havlin *et al.*, 2005). Ca, Mg, Na and K have antagonistic effects (they compete for absorption by the plant) if one is supplied in excess because of its chemical similarities (Fageria, 2001). Not one of these elements were supplied and thus no antagonistic effect was seen. Only Na and Ca had a very weak negative correlation.

From the correlation matrix in Table 4-7 it can also be seen that the some of the macro nutrients have strong correlations with some of the micro nutrients and the same for micro nutrients among each other. It was hard to find literature that could help explain these correlations, but for Mn it was found that it's interaction with other nutrients in the soil varied among concentration levels and plants. In this study Mn correlated positively and moderately strongly with P, Ca, Cu and Zn which, except for Zn, is the same results found in a study on rice plants (Fageria, 2001), where increased Mn had a synergetic effect on the uptake of P, Ca, Cu and Zn. Magnesium facilitates the uptake of P and thus the weak positive correlation. The moderate strong correlations of Ca and Zn with P, is strange. Phosphorus is antagonistic with Ca and Zn because of the very string bonds they form which prevents uptake by plant.

Table 4-7: Correlation matrix of plant properties vs. plant properties.

	C (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})
C (%)	1	0.06	0.04	0.04	0.07	-0.20	-0.15	0.12	-0.08	0.05	0.04	-0.17	-0.12
N (%)		1	0.56	0.52	0.51	0.25	0.19	-0.01	0.51	0.51	0.38	0.15	-0.10
P (%)			1	0.70	0.65	0.38	0.15	-0.08	0.68	0.83	0.60	0.10	-0.05
K (%)				1	0.45	0.22	0.29	-0.16	0.56	0.67	0.34	0.11	-0.16
Ca (%)					1	0.44	-0.10	0.16	0.63	0.62	0.66	0.15	0.14
Mg (%)						1	0.02	-0.02	0.44	0.35	0.40	0.42	0.21
Na (mg kg^{-1})							1	-0.36	0.14	0.12	-0.08	0.09	-0.36
Fe (mg kg^{-1})								1	0.04	-0.06	-0.02	0.01	0.55
Cu (mg kg^{-1})									1	0.72	0.53	0.45	0.08
Zn (mg kg^{-1})										1	0.62	0.20	0.03
Mn (mg kg^{-1})											1	0.16	-0.02
B (mg kg^{-1})												1	0.28
Al (mg kg^{-1})													1

No strong correlations between soil chemical properties and plant chemical properties were found except with soil soluble S (Table 4-8 and Table 4-9). In the international study of (Sillanpää, 1982), it was also found that the correlations between pH and plant macronutrients such as N, P, K, Ca and Mg was very weak, which is the same in this study. Soil pH also had no correlation with the micronutrient contents of the plant. Weakly negative correlations were found between the plant nutrients Cu and Zn, with soil nutrient B. High B supplementation have been found to reduce uptake of Zn, Fe and Mn, and increased uptake of Cu (Fageria *et al.*, 2002), but in this study the correlation with Cu was negative.

Table 4-8: Correlation matrix 1 for soil vs. plant properties

	Plant												
Soil	C (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})
pH (KCl)	-0.10	-0.02	0.00	0.05	0.04	0.14	0.08	0.03	0.15	0.04	-0.04	0.20	0.08
Resis (Ohm)	0.31	0.15	0.15	-0.05	0.20	-0.19	-0.13	0.18	0.08	0.12	0.13	-0.15	-0.17
Log® resis	0.35	0.18	0.16	-0.01	0.22	-0.21	-0.14	0.19	0.10	0.14	0.13	-0.15	-0.13
H+ (cmol kg^{-1})	0.08	0.08	0.05	0.06	0.01	-0.20	-0.06	-0.02	-0.22	0.03	0.09	-0.45	-0.12
P (mg kg^{-1})	-0.17	-0.01	0.03	0.03	-0.07	0.16	0.06	-0.10	-0.07	-0.03	0.02	-0.04	-0.02
K (mg kg^{-1})	-0.12	-0.14	0.03	0.11	-0.06	0.12	0.05	-0.18	-0.11	0.00	0.01	-0.12	-0.01
Na (cmol$_c$ kg^{-1})	-0.18	-0.14	-0.21	-0.09	-0.20	0.11	0.11	-0.08	-0.13	-0.19	-0.08	0.16	0.14
K (cmol$_c$ kg^{-1})	-0.11	-0.14	0.03	0.11	-0.06	0.12	0.05	-0.18	-0.11	0.00	0.01	-0.12	-0.01
Ca (cmol$_c$ kg^{-1})	-0.20	0.07	0.28	0.14	0.23	0.21	-0.01	0.11	0.15	0.20	0.08	-0.08	0.18
Mg (cmol$_c$ kg^{-1})	-0.12	-0.07	0.04	0.15	-0.03	0.14	0.06	-0.03	-0.03	0.06	-0.01	0.00	0.11
Cu (mg kg^{-1})	-0.02	0.07	-0.02	-0.12	0.03	-0.19	-0.07	-0.08	-0.08	-0.03	0.11	-0.23	-0.24
Zn (mg kg^{-1})	0.00	-0.07	-0.01	-0.01	-0.06	-0.02	-0.08	-0.10	-0.15	-0.04	-0.01	-0.14	0.00
Mn (mg kg^{-1})	0.04	0.02	0.27	0.07	0.24	0.07	-0.18	-0.16	0.04	0.24	0.31	-0.25	-0.10
B (mg kg^{-1})	-0.06	-0.19	-0.30	-0.24	-0.24	-0.24	-0.08	-0.07	-0.41	-0.34	-0.17	-0.35	-0.18

Soil soluble S had a moderate strong correlation with plant P, K, Ca, Cu and Zn. Weak correlations were found with plant N and Mn. In a study on barley it was found that there was a decrease in plant nitrate with an increase in soil labile sulphur (Matula, 2004). This agrees with the weak negative correlation (R^2=0.36) of soil soluble S with plant N. It was also found in this study that soil soluble S had moderate negative correlation with plant P, K, Ca, Cu and Zn.

Table 4-9: Correlation matrix 2 for soil vs. plant properties

	Plant												
Soil	C (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Al (mg kg^{-1})
Fe (mg kg^{-1})	0.07	0.07	0.04	0.13	0.02	0.01	-0.07	0.01	-0.11	0.08	0.03	-0.19	0.01
C (%)	0.00	-0.09	-0.18	-0.19	-0.09	-0.30	-0.09	0.10	-0.29	-0.21	-0.10	-0.31	0.01
N (%)	0.02	-0.08	-0.14	-0.05	-0.07	-0.10	0.01	0.00	-0.16	-0.08	-0.07	-0.14	-0.01
Na (%)pH 7	-0.14	-0.15	-0.27	-0.13	-0.24	0.09	0.12	-0.08	-0.11	-0.23	-0.12	0.26	0.13
K (%)pH 7	-0.05	-0.18	-0.06	0.05	-0.12	0.10	0.04	-0.23	-0.11	-0.06	-0.02	-0.02	-0.07
Ca (%)pH 7	-0.15	0.12	0.29	0.06	0.28	0.20	-0.03	0.18	0.30	0.23	0.05	0.10	0.18
Mg (%)pH 7	0.02	-0.10	-0.14	0.06	-0.15	0.07	0.08	-0.05	0.04	-0.04	-0.09	0.28	0.05
T-Value (cmol kg^{-1})	-0.17	0.00	0.16	0.15	0.08	0.12	0.02	0.00	-0.05	0.11	0.07	-0.22	0.12
Soluble S	-0.27	-0.36	-0.58	-0.49	-0.47	-0.24	-0.04	-0.05	-0.54	-0.59	-0.31	-0.28	-0.11

The study of Smith (2014) found that soil and plant Na had a positive effect on the aboveground biomass. This agrees with the positive correlations in this study (Table 4-10). Other significant positive correlations of Smith (2014) that agree with the results in Table 4-10 include plant K (R^2=0.31) and soil K (R^2=0.27). Soil C, N, Ca, Zn, Mn, B, Fe and soluble S showed no to very weak correlations with average leaf size and regrowth, while plant N, Ca, Mg, Fe, Cu, Zn, Mn, B and Al also showed no to very weak correlations.

Table 4-10: Correlation matrix for soil and plant properties vs. growth properties.

Soil variables	Avg Leaf Size (mm)	Avg Regrowth (cm)	Leaves vs Wood
pH (KCl)	-0.06	-0.01	0.19
Soil Resis (Ohm)	-0.10	-0.35	-0.08
Log® resis	-0.05	-0.34	-0.05
Soil H+ (cmol kg^{-1})	0.03	0.18	-0.34
Soil P (mg kg^{-1})	-0.10	0.09	-0.04
Soil K (mg kg^{-1})	-0.02	0.27	-0.06
Soil Na ($cmol_c$ kg^{-1})	0.11	0.30	0.14
Soil K ($cmol_c$ kg^{-1})	-0.02	0.27	-0.06
Soil Ca ($cmol_c$ kg^{-1})	-0.02	0.10	0.04
Soil Mg ($cmol_c$ kg^{-1})	-0.05	0.30	0.05
Soil Cu (mg kg^{-1})	-0.02	-0.13	-0.34
Soil Zn (mg kg^{-1})	-0.01	-0.04	-0.07
Soil Mn (mg kg^{-1})	-0.07	-0.06	-0.31
Soil B (mg kg^{-1})	0.09	-0.01	-0.39
Soil Fe (mg kg^{-1})	0.00	0.10	-0.02
Soil C (%)	0.00	-0.01	-0.24
Soil N (%)	-0.05	0.09	-0.06
Soil Na (%)pH 7	0.15	0.20	0.21
Soil K (%)pH 7	0.05	0.15	-0.03
Soil Ca (%)pH 7	-0.26	-0.14	0.17
Soil Mg (%)pH 7	0.05	0.10	0.20
T-Value (cmol kg^{-1})	-0.15	0.30	-0.09
Soil Soluble S	0.05	0.00	-0.49

Plant variables	Avg Leaf Size (mm)	Avg Regrowth (cm)	Leaves vs Wood
Plant C (%)	0.06	-0.05	0.07
Plant N (%)	-0.07	-0.01	0.11
Plant P (%)	-0.16	0.17	0.20
Plant K (%)	-0.05	0.31	0.32
Plant Ca (%)	-0.08	-0.03	0.16
Plant Mg (%)	-0.06	0.01	0.25
Plant Na (mg kg^{-1})	0.01	0.27	0.07
Plant Fe (mg kg^{-1})	-0.15	-0.10	0.16
Plant Cu (mg kg^{-1})	-0.07	0.04	0.29
Plant Zn (mg kg^{-1})	-0.12	0.13	0.20
Plant Mn (mg kg^{-1})	-0.03	0.00	-0.04
Plant B (mg kg^{-1})	-0.04	-0.04	0.38
Plant Al (mg kg^{-1})	-0.11	0.10	0.33

4.3. Multiple regression analysis on raw data

The correlation matrix was used to look at linear relationships between variables, which is helpful to see if one variable influence another, directly or indirectly. But, to better understand the interaction between soil and plant chemical properties and their impacting on plant growth, multiple regression analysis was needed. The use of multiple regression analysis helps to examine how multiple independent variables (soil and plant chemical properties) are related to one dependent variable. These regression models, if suitable, can then be used to predict the independent variable (growth properties).

Multiple regression models were made not just for all the data together but also for each of the two soil depths and for each of the residue management treatments (A, B, C and D) that were used in this study to look at how the models varied from the original all data model.

Each model in Table 4-11 was statistically significant ($p<0.001$), only the average leaf length models for treatment A and B were not as significant ($p<0.05$). The difference between the models for both depths can be as a result of the difference in nutrient contents and at what depth the plant absorbed (uptake) more nutrients. When looking at the different treatments the models for growth properties average length of regrowth and subjective leaf to wood ratio were better than that of average leaf length. The difference in model R^2 among the treatments can because of the impact the treatments have on nutrient content in the soil and difference in plant nutrient uptake. When looking at models for all three growth properties treatment D had the best. The reason treatment D (added enriched (MAP) mulch) had such good models can be because of the added nutrients with fertilizer which enhanced the impact it would have on growth.

Table 4-11: Summative assessment of growth indicators, representing the predictive significance of each model supported by the database.

	Growth properties	R^2	Adjusted R^2	Std. Error of estimate	P
All data	Average leaf length	0.63	0.61	1.01	<0.001
	Average length of regrowth	0.46	0.41	9.00	<0.001
	Subjective leaf to wood ratio	0.51	0.48	0.47	<0.001
0-5 cm	Average leaf length	0.59	0.54	1.01	<0.001
	Average length of regrowth	0.50	0.40	9.08	<0.001
	Subjective leaf to wood ratio	0.74	0.54	0.46	<0.001
5-20 cm	Average leaf length	0.75	0.72	0.85	<0.001
	Average length of regrowth	0.52	0.43	8.83	<0.001
	Subjective leaf to wood ratio	0.52	0.44	0.48	<0.001
A	Average leaf length	0.43	0.23	0.23	<0.05
	Average length of regrowth	0.73	0.61	7.00	<0.001
	Subjective leaf to wood ratio	0.66	0.52	0.46	<0.001
B	Average leaf length	0.37	0.16	0.33	<0.05
	Average length of regrowth	0.82	0.73	6.47	<0.001
	Subjective leaf to wood ratio	0.68	0.55	0.41	<0.001
C	Average leaf length	0.52	0.37	0.87	<0.001
	Average length of regrowth	0.68	0.54	7.38	<0.001
	Subjective leaf to wood ratio	0.75	0.65	0.41	<0.001
D	Average leaf length	0.71	0.51	0.24	<0.001
	Average length of regrowth	0.70	0.57	8.22	<0.001
	Subjective leaf to wood ratio	0.77	0.68	0.34	<0.001

All the soil and plant properties that had a significant and near significant contribution to the regression model for each growth property are listed in Table 4-12 to Table 4-18.

4.3.1. All data

In the model for average leaf length (R^2=0.63), soil resistance (Ohm), log of soil resistance, soil Na ($cmol_c\ kg^{-1}$) and Na (%) had the most significant (p<0.001) effect. Soil Na ($cmol_c\ kg^{-1}$) had the biggest positive contribution (b=87.65) to the model. This agrees with the correlation in Table 4-10 (R^2=0.60). Soil Ca ($cmol_c\ kg^{-1}$) had the largest negative contribution (b=--5.63) to the model.

In the model for average length of regrowth (R^2=0.46) soil P (mg kg^{-1}), K (mg kg^{-1}), Ca (mg kg^{-1}), Mn (mg kg^{-1}) and Ca (%) had the most significant (p<0.001) effect. The biggest positive contribution to the model were by soil K ($cmol_c\ kg^{-1}$) (b=333), Mg ($cmol_c\ kg^{-1}$) (b=129.83) and plant P (b=85.11). Soil Ca (mg kg^{-1}) (b=-110.089) had the biggest negative contribution to the model.

For the subjective leaf to wood ratio model (R^2=0.51) only two soil and two plant nutrients had a significant effect. Plant Al (mg kg^{-1}) had the lowest significance (P=0.011). Plant K (%) made the biggest positive contribution (b=1.24) and soil soluble S the biggest negative contribution (b=-0.115).

Table 4-12: Multiple regression results for model of each growth property when using all the data.

Growth properties	Properties	B	Std.Err. of b*	t	p Value
Average leaf length	Soil Resistance (Ohm)	0.000	0.000	-3.535	0.000
	Log® soil resistance	4.975	0.746	6.670	0.000
	Soil Na ($cmol_c\ kg^{-1}$)	87.654	5.549	15.797	0.000
	Soil Ca ($cmol_c\ kg^{-1}$)	-5.633	2.232	-2.524	0.012
	Soil Na (%)	-1.042	0.100	-10.395	0.000
	Plant Mg (%)	2.981	1.264	2.358	0.019
	Plant Fe ($mg\ kg^{-1}$)	-0.003	0.001	-2.145	0.032
	Plant Cu ($mg\ kg^{-1}$)	0.092	0.049	1.869	0.062
Average length of regrowth	Soil pH (KCl)	-6.071	2.938	-2.066	0.039
	Soil H+ ($cmol\ kg^{-1}$)	35.922	17.950	2.001	0.046
	Soil P ($mg\ kg^{-1}$)	-0.074	0.020	-3.758	0.000
	Soil K ($cmol_c\ kg^{-1}$)	332.899	93.050	3.578	0.000
	Soil Ca ($cmol_c\ kg^{-1}$)	-110.089	24.038	-4.580	0.000
	Soil Mg ($cmol_c\ kg^{-1}$)	129.830	47.920	2.709	0.007
	Soil Zn ($mg\ kg^{-1}$)	-21.149	6.327	-3.343	0.001
	Soil Mn ($mg\ kg^{-1}$)	-1.747	0.386	-4.528	0.000
	Soil C (%)	9.096	4.733	1.922	0.055
	Soil K (%)	-3.399	1.648	-2.062	0.040
	Soil Ca (%)	1.962	0.475	4.126	0.000
	Plant C (%)	0.577	0.289	1.998	0.046
	Plant P (%)	85.106	28.086	3.030	0.003
	Plant K (%)	17.264	5.295	3.261	0.001
	Plant Zn ($mg\ kg^{-1}$)	-0.746	0.308	-2.417	0.016
	Plant Al ($mg\ kg^{-1}$)	0.083	0.030	2.777	0.006
Subjective leaf to wood ratio	Soil Mn ($mg\ kg^{-1}$)	-0.095	0.018	-5.167	0.000
	Soil Soluble S	-0.115	0.017	-6.695	0.000
	Plant K (%)	1.240	0.266	4.667	0.000
	Plant Al ($mg\ kg^{-1}$)	0.003	0.001	2.568	0.011

4.3.2. Soil depth (5 cm)

In the model for average length of regrowth, there was only four soil and plant chemical properties that had a statistical significance ($p<0.05$). Plant K (%) had the biggest positive influence ($b=1.24$) in the model.

Soil Mn ($mg\ kg^{-1}$) had the most significant ($p<0.001$) effect on the average length of regrowth model. Soil K ($cmol_c\ kg^{-1}$) had the biggest positive influence ($b=359.97$) in the model and Soil Ca ($cmol_c\ kg^{-1}$) the biggest negative influence. Soil P ($mg\ kg^{-1}$) and plant Zn ($mg\ kg^{-1}$) almost had a significant ($p<0.05$) effect in the model.

Only three chemical properties had a significant effect in the subjective leaf to wood ratio, with plant K (%) having the only positive influence (b=1.32).

Table 4-13: Multiple regression results for model of each growth properties when using data just from top soil profile 0-5 cm.

Parameters	Properties	B	Std.Err. of b*	t	p Value
Average leaf length	Soil Mn (mg kg^{-1})	-0.095	0.018	-5.167	0.000
	Soil Soluble S	-0.115	0.017	-6.695	0.000
	Plant K (%)	1.240	0.266	4.667	0.000
	Plant Al (mg kg^{-1})	0.003	0.001	2.568	0.011
Average length of regrowth	Soil P (mg kg^{-1})	-0.049	0.026	-1.911	0.058
	Soil K ($cmol_c$ kg^{-1})	359.973	161.017	2.236	0.027
	Soil Ca ($cmol_c$ kg^{-1})	-99.805	34.743	-2.873	0.005
	Soil Cu (mg kg^{-1})	39.419	18.212	2.164	0.032
	Soil Zn (mg kg^{-1})	-31.247	9.310	-3.356	0.001
	Soil Mn (mg kg^{-1})	-1.952	0.539	-3.619	0.000
	Plant P (%)	111.741	41.706	2.679	0.008
	Plant K (%)	18.510	8.066	2.295	0.023
	Plant Mg (%)	-38.096	19.093	-1.995	0.047
	Plant Zn (mg kg^{-1})	-0.896	0.456	-1.962	0.051
	Plant Al (mg kg^{-1})	0.114	0.044	2.577	0.011
Subjective leaf to wood ratio	Soil Mn (mg kg^{-1})	-0.078	0.025	-3.066	0.002
	Soil Soluble S	-0.116	0.026	-4.401	0.000
	Plant K (%)	1.318	0.407	3.240	0.001

4.3.3. Soil depth (5-20 cm)

In the average leaf length model (R^2=52) both the Na chemical properties had the highest significance (p<0.001). Plant Mg (%) and plant Fe (mg kg^{-1}) was near having a statistical significant effect. Soil Na ($cmol_c$ kg^{-1}) had a very big positive influence (b=70.07) on the model compared to the other significant properties in the model.

In the average length of regrowth model (R^2=75) it was Soil H^+ (cmol kg^{-1}), B (%) and Ca (%) that had the most significant effect in the model. The biggest positive influence in the model were made by Soil H^+ (cmol kg^{-1}) (b=130.27) and Soil Mg (cmol kg^{-1}) (b=171.13), but soil Mg (p=0.042) had low significance.

Soil Mn (mg kg^{-1}) and soluble S had the most significant (p<0.001) effect in the model. Soil K made the biggest positive influence (b=25.42) in the model.

Table 4-14: Multiple regression results for model of each growth parameter when using data just from topsoil profile from 5-20 cm.

Growth properties	Properties	B	Std.Err. of b*	t	p Value
Average leaf length	Log® soil resistance	3.349	1.101	3.041	0.003
	Soil Na ($cmol_c$ kg^{-1})	70.072	6.837	10.249	0.000
	Soil Fe (mg kg^{-1})	0.013	0.005	2.511	0.013
	Soil Na (%)	-0.717	0.119	-6.045	0.000
	Plant Mg (%)	2.841	1.598	1.778	0.077
	Plant Fe (mg kg^{-1})	-0.003	0.002	-1.823	0.070
Average length of regrowth	Soil H^+ (cmol kg^{-1})	130.268	32.359	4.026	0.000
	Soil P (mg kg^{-1})	-0.199	0.061	-3.246	0.001
	Soil Ca ($cmol_c$ kg^{-1})	-128.753	45.223	-2.847	0.005
	Soil Mg ($cmol_c$ kg^{-1})	171.126	83.439	2.051	0.042
	Soil Mn (mg kg^{-1})	-2.083	0.781	-2.668	0.008
	Soil B (mg kg^{-1})	-68.717	18.117	-3.793	0.000
	Soil C (%)	22.986	7.353	3.126	0.002
	Soil Ca (%)	2.891	0.808	3.580	0.000
	Plant K (%)	18.884	7.792	2.424	0.016
Subjective leaf to wood ratio	Soil K ($cmol_c$ kg^{-1})	25.422	10.227	2.486	0.014
	Soil Mn (mg kg^{-1})	-0.153	0.039	-3.947	0.000
	Soil Fe (mg kg^{-1})	0.006	0.003	1.775	0.077
	Soil K (%)	-0.382	0.170	-2.242	0.026
	Soil Soluble S	-0.114	0.029	-3.909	0.000
	Plant K (%)	1.001	0.407	2.460	0.015

4.3.4. Treatment A (bare soil)

Plant Mg (%) and Fe (%) had the most significant effect in the average leaf length model (R^2=0.43), with Mg (%) having the biggest negative contribution (b=-1.67) in the model. Log resistance (ohm) of soil had the only positive contribution (b=0.769).

For the average length of regrowth model (R^2=0.73) soil Mn (mg kg^{-1}), soil C (%), soluble S and plant Mn (%) had the most significant (p<0.01) effect in the model with plant C (%) the almost significant (p=0.059). The biggest positive contribution to the model were by log soil resistance (b=33.57), soil C (%) (b=37.48) and plant K (%) (b=12.98). Soil Mn (mg kg^{-1}) had the biggest negative contribution to the model (b=-4.05).

Both the significant chemical properties had a negative contribution to the model for subjective leaf to wood ratio (R^2=0.66).

Table 4-15: Multiple regression results for model of each growth parameter when using just data from treatment A.

Growth properties	Properties	B	Std.Err. of b*	t	p Value
Average leaf length	Log® soil resistance	0.769	0.364	2.111	0.037
	Soil Mg (%)	-0.081	0.043	-1.888	0.062
	Plant Mg (%)	-1.764	0.667	-2.647	0.009
	Plant Fe (mg kg^{-1})	-0.004	0.001	-2.818	0.006
Average length of regrowth	Log® soil resistance	33.573	16.164	2.077	0.041
	Soil Mn (mg kg^{-1})	-4.046	1.036	-3.906	0.000
	Soil C (%)	37.477	11.900	3.149	0.002
	Soil Soluble S	-3.280	1.103	-2.974	0.004
	Plant C (%)	1.156	0.603	1.918	0.059
	Plant K (%)	30.844	12.980	2.376	0.020
	Plant Cu (mg kg^{-1})	-2.822	1.114	-2.534	0.013
	Plant Mn (mg kg^{-1})	0.081	0.028	2.855	0.006
Subjective leaf to wood ratio	Soil Mn (mg kg^{-1})	-0.159	0.053	-3.016	0.003
	Soil Soluble S	-0.206	0.058	-3.540	0.001

4.3.5. Treatment B (added mulch)

Only three chemical properties were of significance in the average leaf length model (R^2=0.37) and all had a negative contribution. Plant K (%) had the most significant (p<0.01) effect in the model and the biggest contribution (b=-1.16).

In the average length of regrowth model (R^2=0.82) soil P, Mn (mg kg^{-1}) and Na (%) had the most significant effect in the model (p<0.005). Plant Zn almost had significance with a p value of 0.06. Soil

Mg ($cmol_c\ kg^{-1}$) made the biggest positive contribution to the model (b=425), while the soil Na ($cmol_c\ kg^{-1}$) the biggest negative contribution (b=-866.44).

In the subjective leaf to wood ratio model (R^2=0.68) soil soluble S and plant B (mg kg^{-1}) had the most significant effect in the model (p=0.001). Soil Zn (mg kg^{-1}), plant Na (mg kg^{-1}) and plant Fe (mg kg^{-1}) almost had statistical significant impact in the model with p values between 0.5 and 0.8. The significant chemical properties all made a negative contribution in the model, with soil Mn making the biggest contribution (b=-0.15).

Table 4-16: Multiple regression results for model of each growth parameter when using data just from treatment B.

Growth properties	Properties	B	Std.Err. of b*	t	p Value
Average leaf length	Soil Soluble S	-0.058	0.029	-2.013	0.047
	Plant K (%)	-1.157	0.392	-2.951	0.004
	Plant Al (mg kg^{-1})	-0.006	0.002	-2.525	0.013
Average length of regrowth	Soil H+ (cmol kg^{-1})	152.654	79.765	1.914	0.060
	Soil P (mg kg^{-1})	2.620	0.548	4.777	0.000
	Soil Na ($cmol_c\ kg^{-1}$)	-866.438	316.691	-2.736	0.008
	Soil Mg ($cmol_c\ kg^{-1}$)	425.509	181.679	2.342	0.022
	Soil Cu (mg kg^{-1})	-84.299	45.750	-1.843	0.070
	Soil Mn (mg kg^{-1})	-8.039	1.009	-7.964	0.000
	Soil B (mg kg^{-1})	-65.814	22.864	-2.879	0.005
	Soil Fe (mg kg^{-1})	0.236	0.108	2.190	0.032
	Soil Na (%)	18.089	5.124	3.530	0.001
	Plant Cu (mg kg^{-1})	3.498	1.388	2.520	0.014
	Plant Zn (mg kg^{-1})	-1.370	0.720	-1.902	0.061
	Plant Mn (mg kg^{-1})	0.100	0.036	2.763	0.007
Subjective leaf to wood ratio	Soil Zn (mg kg^{-1})	0.593	0.320	1.852	0.068
	Soil Mn (mg kg^{-1})	-0.145	0.052	-2.799	0.006
	Soil Soluble S	-0.151	0.044	-3.423	0.001
	Plant Na (mg kg^{-1})	0.000	0.000	-1.792	0.077
	Plant Fe (mg kg^{-1})	0.005	0.003	1.916	0.059
	Plant B (mg kg^{-1})	-0.040	0.011	-3.622	0.001

4.3.6. Treatment C (natural mulch)

In the average leaf length model (R^2=0.52) it was plant Mg (%) that had the most significant (p=0.001) effect and also made the biggest contribution in model (b=9.5). Log resistance (ohm) also had a big influence (b=5.8) on the model. Soil resistance (ohm) and plant Na (%) was close to having a significant effect in the model. Soil Fe (mg kg^{-1}) had the highest significant effect in the model.

Soil K ($cmol_c$ kg^{-1}), Ca ($cmol_c$ kg^{-1}), Ca (%) and plant P(%) had the most significant (p<0.001) effect in the average length of regrowth model (R^2=0.68), while log resistance (ohm) (p=0.021) and plant B (%) (p=0.027) had the least significant effect. Biggest positive contribution in model was made by plant K ($cmol_c$ kg^{-1}) (b=1030.96) and soil Mg ($cmol_c$ kg^{-1}) (b=420.52). The biggest negative contribution was made by soil Ca ($cmol_c$ kg^{-1}) (b=-460.37).

Plant K (%) had the most significant (p<0.001) effect in the subjective leaf to wood ratio model (R^2=0.75) and plant Al (mg kg^{-1}) the least significant (p=0.026). Plant K also gad the biggest positive contribution (b=-2.49) in the model and soil Ca ($cmol_c$ kg^{-1}) had the biggest negative contribution (-8.74).

Table 4-17: Multiple regression results for model of each growth parameter when using data just from treatment C.

Growth properties	Properties	B	Std.Err. of b*	t	p Value
Average leaf length	Soil Resistance (Ohm)	0.000	0.000	-1.951	0.054
	Log® soil resistance	5.808	2.013	2.885	0.005
	Soil Fe (mg kg^{-1})	0.019	0.007	2.790	0.006
	Soil Soluble S	0.219	0.074	2.962	0.004
	Plant Mg (%)	9.503	2.445	3.886	0.000
	Plant Na (mg kg^{-1})	0.000	0.000	1.857	0.066
Average length of regrowth	Soil pH (KCl)	-16.984	6.317	-2.689	0.009
	Soil Resistance (Ohm)	-0.005	0.002	-2.769	0.007
	Log® soil resistance	57.246	24.257	2.360	0.021
	Soil K ($cmol_c$ kg^{-1})	1030.975	246.845	4.177	0.000
	Soil Ca ($cmol_c$ kg^{-1})	-460.373	95.932	-4.799	0.000
	Soil Mg ($cmol_c$ kg^{-1})	420.519	128.776	3.265	0.002
	Soil Zn (mg kg^{-1})	-81.015	24.586	-3.295	0.001
	Soil Fe (mg kg^{-1})	0.139	0.077	1.793	0.077
	Soil C (%)	35.561	10.107	3.518	0.001
	Soil K (%)	-11.641	3.790	-3.072	0.003
	Soil Ca (%)	6.172	1.431	4.312	0.000
	Soil Mg (%)	-5.245	1.991	-2.635	0.010
	Soil Soluble S	-2.064	0.817	-2.526	0.014
	Plant N (%)	-11.235	3.917	-2.868	0.005
	Plant P (%)	231.122	61.315	3.769	0.000
	Plant B (mg kg^{-1})	-0.391	0.173	-2.258	0.027
Subjective leaf to wood ratio	Soil Ca ($cmol_c$ kg^{-1})	-8.743	4.459	-1.961	0.053
	Soil Ca (%)	0.140	0.068	2.078	0.041
	Soil Soluble S	-0.146	0.045	-3.260	0.002
	Plant N (%)	-0.619	0.209	-2.962	0.004
	Plant P (%)	-7.140	3.141	-2.273	0.026
	Plant K (%)	2.485	0.557	4.458	0.000
	Plant Mn (mg kg^{-1})	-0.004	0.002	-2.940	0.004
	Plant Al (mg kg^{-1})	0.008	0.004	2.135	0.036

4.3.7. Treatment D (added enriched mulch)

Soil Na ($cmol_c$ kg^{-1}) and Na (%) had most significant ($p<0.001$) effect on the average leaf length model ($R^2=0.88$); and soil Fe (mg kg^{-1}) had the least significant ($p=0.016$). Soil Mg ($cmol_c$ kg^{-1}), K (%) and plant Na (%) almost had a significant effect in the model. Soil Na ($cmol_c$ kg^{-1}) made the biggest positive contribution ($b=142.36$) and Soil K ($cmol_c$ kg^{-1}) the biggest negative contribution to the model.

For the average leaf length model all the chemical properties listed in Table 4-18 log resistance (ohm) (p=0.053) was almost significant while the rest were very significant. Soil H^+ ($cmol_c$ kg^{-1}) (exchangeable acidity) had the biggest positive contribution (b=124.76) and soil N (%) had the biggest negative contribution (b=-373.74).

In the model for subjective leaf to wood ratio plant Mn (%) had the most significant (p=0.008) effect, and the biggest positive contribution to the model were made by plant P (%) (b=5.50). The biggest negative contribution in the model were made by soil B (mg kg^{-1}) (b=-3.23).

Table 4-18: Multiple regression results for model of each growth parameter when using data just from treatment D.

Growth properties	Properties	B	Std.Err. of b*	t	p Value
Average leaf length	Soil P (mg kg^{-1})	7.001	2.941	2.381	0.019
	Soil Na ($cmol_c$ kg^{-1})	-13.961	5.359	-2.605	0.010
	Soil Na (%)	0.256	0.090	2.827	0.006
	Soil K (%)	0.058	0.029	2.000	0.048
	Plant N (%)	-5.373	1.552	-3.463	0.001
	Plant K (%)	2.066	0.908	2.275	0.025
	Plant Mg (%)	0.000	0.000	-2.031	0.045
	Plant Na (mg kg^{-1})	-0.002	0.001	-3.290	0.001
	Plant Cu (mg kg^{-1})	0.034	0.017	2.061	0.042
	Plant Mn (mg kg^{-1})	-0.010	0.005	-1.968	0.052
Average length of regrowth	Soil pH (KCl)	-25.955	8.378	-3.098	0.003
	Log® soil resistance	-49.814	25.315	-1.968	0.053
	Soil H+ (cmol kg^{-1})	124.762	39.390	3.167	0.002
	Soil P (mg kg^{-1})	-0.136	0.047	-2.886	0.005
	Soil Cu (mg kg^{-1})	-55.929	21.190	-2.639	0.010
	Soil Zn (mg kg^{-1})	43.057	13.614	3.163	0.002
	Soil Mn (mg kg^{-1})	-3.565	1.155	-3.088	0.003
	Soil N (%)	-373.737	123.331	-3.030	0.003
	Plant N (%)	7.030	3.169	2.218	0.030
Subjective leaf to wood ratio	Soil Mn (mg kg^{-1})	-0.103	0.041	-2.523	0.013
	Soil B (mg kg^{-1})	-3.233	1.242	-2.604	0.011
	Soil Soluble S	-0.054	0.026	-2.070	0.041
	Plant P (%)	5.504	2.638	2.087	0.040
	Plant K (%)	1.618	0.535	3.025	0.003
	Plant Fe (mg kg^{-1})	0.002	0.001	2.502	0.014
	Plant Mn (mg kg^{-1})	-0.004	0.001	-2.723	0.008

From all of this data it was possible to see interactions between variables and its influence on each other and on growth. With the help of the multiple regression models it was also possible to see the

working of all the soil and plant chemical properties together in order to see which chemical properties had a statistical significant effect on the model and how big (positive or negative) the contribution/influence of each significant property in the model was. The data however were raw and possible data transformations were needed to better the models. When reflecting on the performance of the models it was noted that the growth property average length regrowth had a consistent performance. It was decided that only multiple regression models for average length of regrowth would be built. Multiple regression analysis was only done on average length of regrowth with new transformed (if necessary) soil and plant chemical data.

4.4. Multiple regression analysis on data with transformed variables

Before next multiple regression analysis were done the normal distribution and skewness of the each soil and plant chemical property was looked at using Statistica 13 software. Acceptable skewness limits of ±2 (Trochim and Donnelly, 2001; Field, 2013; Gravetter and Wallnau, 2016) were used. Data transformation methods were used as suggested by (Howell, 2012) and (Tabachnick *et al.*, 2001) as seen in Table 4-19. In Appendix A the normal distribution for the skew data compared to the transformed data can be seen.

Table 4-19: Descriptive statistics for skew data. (K = largest score +1; C = smallest score +1)

Property	Mean	Min	Max	Std.Dev.	Skewness	Transformation method
Soil P (mg kg^{-1})	21.21	2.00	391.25	41.84	4.82	NEWX = LOG10(X)
Soil Na ($cmol_c$ kg^{-1})	0.08	0.03	0.76	0.05	5.61	NEWX = LOG10(C+X)
Soil Zn (mg kg^{-1})	0.20	0.10	3.00	0.17	9.62	NEWX = LOG10(X)
Soil Fe (mg kg^{-1})	27.98	2.72	152.00	13.41	2.81	NEWX = LOG10(X)
Soil C (%)	0.55	0.16	3.08	0.22	3.19	NEWX = LOG10(C+X)
Soil N (%)	0.03	0.00	0.20	0.01	3.62	NEWX = LOG10(X)
Soil Na (%)	5.43	1.94	40.07	2.77	4.49	NEWX = LOG10(X)
Plant Fe (mg kg^{-1})	105.88	46.54	480.20	46.40	3.05	NEWX = LOG10(X)

For soil Na ($cmol_c$ kg^{-1}), soil Zn (mg kg^{-1}), soil Na (%) and soil N (%) it was decided to remove outliers after transformation of the data because these properties still delivered skewness levels outside the ±2 limits as seen in Addendum A. However, after outliers were removed data for Zn (mg kg^{-1}) and soil N (%) were still transformed afterwards. The normal distribution histogram figures, first and final normal distribution, for variables with skew data, is attached in Appendix A. The descriptive statistics for data where outliers were removed and transformation was performed can be seen in Table 4-20.

Table 4-20: Descriptive statistics after outliers was removed and data transformed. (*Transformed data. ** Outliers removed and transformed).

Property	Mean	Min	Max	Std.Dev.	Skewness
Soil P (mg kg^{-1})*	1.05	0.30	2.59	0.41	1.28
Soil Na ($cmol_c$ kg^{-1})	0.08	0.03	0.22	0.04	1.30
Soil Zn (mg kg^{-1})**	0.08	0.04	0.26	0.04	1.60
Soil Fe (mg kg^{-1})*	1.41	0.43	2.18	0.19	-0.69
Soil C (%)*	0.19	0.06	0.61	0.06	0.90
Soil N (%)	0.03	0.00	0.09	0.01	1.08
Soil Na (%)**	0.69	0.29	1.20	0.17	0.28
Plant Fe (mg kg^{-1})*	1.99	1.67	2.68	0.16	0.62

After all the necessary outliers were removed and data transformations performed, multiple regression analysis was performed again. All of the models resulted in being highly significant ($p<0.001$) (Table 4-21). It was noted that the models for all the treatments had higher R^2 values compared to the models for all the data and both depths. The R^2 of the regression models where more or less the same compared to that of the raw data (Table 4-11). Only for treatment D the regression model with the transformed data were a little higher and for treatment D a lot higher. For treatment C the regression model with the raw data had a higher R^2.

Table 4-21: Statistics summary for each model with transformed data

	R^2	Adjusted R^2	Std. Error of estimate	P
All data	0.46	0.42	8.93	<0.001
Depth 0-5 cm	0.51	0.42	8.93	<0.001
Depth 5-20 cm	0.58	0.51	8.24	<0.001
Treatment A	0.75	0.64	6.70	<0.001
Treatment B	0.78	0.68	7.00	<0.001
Treatment C	0.67	0.53	7.48	<0.001
Treatment D	0.72	0.60	7.90	<0.001

4.4.1. All data

Compared to the regression model for the raw data (Table 4-12) the new model with the adjusted (outliers removed and data transformed) data (Table 4-22) have much more chemical properties making a significant (<0.001) contribution to regrowth. When discussing the effect of each chemical property it is being discussed with its effect on growth in combination with the other significant chemical properties in model. It was noted that all the exchangeable cations had a significant effect on the model for average length of regrowth and the influence (b) of each was large. Soil exchangeable ($cmol_c$ kg^{-1}) Na and Ca both had a big negative influence on the regrowth model, while K and Mg both had positive influences. Soil P (mg kg^{-1}) had a negative influence on the model

contradicting the moderate positive influence of plant P in the same model. The negative influence of soil P can be because of the excess soil P levels of soils under treatment D (reached maximum of 391.25 mg kg^{-1}). Exchangeable acidity had a positive influence on the regrowth model. Soil C (%) also made a positive contribution to the model. Soil N (%) however had a negative influence on the regrowth model. In a study by Maistry *et al* (2015) that looked at the effect of different N:P ratio applications to rooibos it was found that at low P concentrations (10 µM) increased N application leads to little increase or decrease in rooibos total dry matter (N:P ratios ranged between 9 and 63). At higher P concentrations (100 µM) the increased application of N (100 to 700 µM) led to a ± eightfold increase in total dry matter (N:P rations ranged between 1 and 6) (Maistry *et al.*, 2015). The average N (%) concentration for all the data thus could have been to high compared to the P (mg kg^{-1}) content of the soil and thus had a high N:P ratio which also according to Maistry et al (2015) result in no or decrease in plant total dry matter.

Table 4-22: Multiple regression results for average length of regrowth when using all the data (*transformed)

Properties	b	Std.Err. of b*	t	p Value
Soil H+ (cmol kg^{-1})	48.595	17.120	2.839	0.005
*Soil P (mg kg^{-1})	-8.073	2.066	-3.907	0.000
Soil Na ($cmol_c$ kg^{-1})	-133.393	56.175	-2.375	0.018
Soil K ($cmol_c$ kg^{-1})	253.240	89.821	2.819	0.005
Soil Ca ($cmol_c$ kg^{-1})	-90.047	23.014	-3.913	0.000
Soil Mg ($cmol_c$ kg^{-1})	128.269	46.084	2.783	0.006
*Soil Zn (mg kg^{-1})	-51.296	19.252	-2.664	0.008
Soil Mn (mg kg^{-1})	-1.316	0.393	-3.351	0.001
Soil B (mg kg^{-1})	-27.842	11.278	-2.469	0.014
*Soil C (%)	45.202	16.742	2.700	0.007
Soil N (%)	-143.228	56.334	-2.542	0.011
*Soil Na (%)	35.241	10.799	3.263	0.001
Soil Ca (%)	1.909	0.463	4.126	0.000
Plant C (%)	0.650	0.282	2.309	0.021
Plant P (%)	93.198	28.106	3.316	0.001
Plant K (%)	14.736	5.301	2.780	0.006
Plant Ca (%)	-24.956	13.709	-1.820	0.069
Plant Mg (%)	-23.767	13.127	-1.811	0.071
*Plant Fe (mg kg^{-1})	-14.144	4.968	-2.847	0.005
Plant Zn (mg kg^{-1})	-0.688	0.308	-2.233	0.026
Plant Al (mg kg^{-1})	0.098	0.033	2.959	0.003

4.4.2. Soil depth

The 0-5 cm depth regrowth model resulted in less significant soil chemical properties than the 5-20 cm model. For plant chemical properties it was the opposite.

Table 4-23: Multiple regression results for average length of regrowth when using data (*transformed) from the two different sampling depths (0-5 cm and 5-20 cm).

Model	Properties	b	Std.Err. of b*	t	p Value
Depth 0-5 cm	*Soil P (mg kg^{-1})	-5.532	2.756	-2.008	0.046
	Soil K ($cmol_c$ kg^{-1})	324.433	156.870	2.068	0.040
	Soil Ca ($cmol_c$ kg^{-1})	-96.188	33.682	-2.856	0.005
	*Soil Zn (mg kg^{-1})	-99.049	33.766	-2.933	0.004
	Soil Mn (mg kg^{-1})	-1.855	0.562	-3.303	0.001
	Soil Ca (%)	1.549	0.796	1.947	0.053
	Plant C (%)	0.764	0.412	1.853	0.066
	Plant P (%)	110.334	41.668	2.648	0.009
	Plant K (%)	16.229	7.997	2.029	0.044
	Plant Mg (%)	-40.283	18.717	-2.152	0.033
	Plant Al (mg kg^{-1})	0.120	0.048	2.502	0.013
Depth 2-20 cm	Soil H+ (cmol kg^{-1})	138.993	28.593	4.861	0.000
	*Soil P (mg kg^{-1})	-8.084	3.472	-2.329	0.021
	Soil Na ($cmol_c$ kg^{-1})	-278.685	88.672	-3.143	0.002
	Soil Ca ($cmol_c$ kg^{-1})	-153.057	40.619	-3.768	0.000
	Soil Mg ($cmol_c$ kg^{-1})	238.010	75.815	3.139	0.002
	Soil B (mg kg^{-1})	-80.090	17.262	-4.640	0.000
	*Soil C (%)	79.541	23.952	3.321	0.001
	*Soil Na (%)	80.750	16.380	4.930	0.000
	Soil Ca (%)	3.445	0.719	4.792	0.000
	Plant K (%)	16.578	7.052	2.351	0.020
	*Plant Fe (mg kg^{-1})	-17.255	6.932	-2.489	0.014

4.4.3. Treatments

Soil P (mg kg^{-1}) had no significant (<0.05) effect in the regrowth model for treatment A, C and D.

Table 4-24: Multiple regression results for average length of regrowth when using data (*transformed) from treatment A and B (A = bare soil, B = added mulch)

Model	Properties	b	Std.Err. of b*	t	p Value
Treatment A	Soil Mn (mg kg^{-1})	-2.793	1.01	-2.766	0.007
	*Soil C (%)	123.977	37.671	3.291	0.002
	*Soil Na (%)	82.357	35.731	2.305	0.024
	Soil Soluble S	-2.716	1.024	-2.653	0.010
	Plant N (%)	-6.37	3.249	-1.961	0.054
	Plant K (%)	31.375	11.544	2.718	0.008
	Plant Cu (mg kg^{-1})	-2.396	1.066	-2.248	0.028
	Plant Mn (mg kg^{-1})	0.066	0.029	2.295	0.025
Treatment B	*Soil P (mg kg^{-1})	52.777	16.091	3.28	0.002
	Soil Na ($cmol_c$ kg^{-1})	-268.579	133.619	-2.01	0.048
	Soil Mg ($cmol_c$ kg^{-1})	521.141	204.903	2.543	0.013
	Soil Mn (mg kg^{-1})	-6.306	1.062	-5.935	0.000
	Soil B (mg kg^{-1})	-51.469	25.447	-2.023	0.047
	*Soil Na (%)	125.4	27.908	4.493	0.000
	Plant N (%)	-10.407	4.25	-2.448	0.017
	Plant Ca (%)	-68.156	36.059	-1.89	0.063
	Plant Cu (mg kg^{-1})	2.661	1.437	1.852	0.068
	Plant Mn (mg kg^{-1})	0.09	0.041	2.216	0.030

Table 4-25: Multiple regression results for average length of regrowth when using data (*transformed) from treatment C and D (C = natural mulch and D = added enriched mulch)

Model	Properties	b	Std.Err. of b*	t	p Value
Treatment C	Soil pH (KCl)	-18.089	6.326	-2.859	0.005
	Soil K ($cmol_c$ kg^{-1})	1118.39	241.045	4.64	0.000
	Soil Ca ($cmol_c$ kg^{-1})	-405.976	102.675	-3.954	0.000
	Soil Mg ($cmol_c$ kg^{-1})	295.926	137.943	2.145	0.035
	*Soil Zn (mg kg^{-1})	-183.81	64.895	-2.832	0.006
	*Soil C (%)	132.433	36.683	3.61	0.001
	Soil K (%)	-11.789	3.842	-3.068	0.003
	Soil Ca (%)	6.08	1.521	3.999	0.000
	Soil Soluble S	-2.316	0.826	-2.803	0.006
	Plant N (%)	-8.119	3.997	-2.031	0.046
	Plant P (%)	174.353	66.092	2.638	0.010
	*Plant Fe (mg kg^{-1})	-32.564	12.602	-2.584	0.012
	Plant Zn (mg kg^{-1})	-1.089	0.575	-1.895	0.062
	Plant Al (mg kg^{-1})	0.132	0.06	2.212	0.030
Treatment D	Soil pH (KCl)	-35.203	7.723	-4.558	0.000
	Log® soil resistance	-30.844	13.535	-2.279	0.026
	Soil H+ (cmol kg^{-1})	86.695	39.941	2.171	0.033
	*Soil P (mg kg^{-1})	-19.846	5.385	-3.685	0.000
	Soil Cu (mg kg^{-1})	-53.379	20.454	-2.61	0.011
	*Soil Zn (mg kg^{-1})	123.766	39.262	3.152	0.002
	Soil Mn (mg kg^{-1})	-3.136	1.032	-3.039	0.003
	*Soil Fe (mg kg^{-1})	-20.606	8.668	-2.377	0.020
	Soil N (%)	-462.004	111.345	-4.149	0.000
	Soil Ca (%)	4.298	1.311	3.279	0.002
	Plant N (%)	8.203	3.244	2.529	0.014

When looking at all the models there were certain soil and plant chemical properties that repeated its significant ($p<0.05$) effect three or more times out of the seven models for average length of regrowth. It was soil chemical properties P (mg kg^{-1}), Na ($cmol_c$ kg^{-1}), K ($cmol_c$ kg^{-1}), Ca ($cmol_c$ kg^{-1}), Mg ($cmol_c$ kg^{-1}), Zn (mg kg^{-1}), Mn (mg kg^{-1}), C (%) and Na (%). In the regrowth models for all the data, depth 0-5 cm, 5-20 cm and treatment D soil P had a negative influence on the regrowth, but for all three of these models data of treatment D were included. Treatment D received a phosphate fertilizer (MAP) which led to excess phosphate in the soil especially in the 0-5 cm depth. For treatment B (added mulch) however P had a big positive influence on the regrowth model. Joubert *et al* (1987) found optimum growth of rooibos seedlings at plant available P levels of 15 to 20 mg kg^{-1}. The average amount of plant available P for treatment B for the whole year (July 2015 – June

2016) ranged between 3 and 26.92 mg kg^{-1} with an average of 10.56 mg kg^{-1} (data not shown). These P levels however are for 2 year and older plants. The positive influence of P in the treatment B model thus shows that these levels (covering the 15 to 20 mg kg^{-1} concentration levels), in combination with the other nutrients, had a positive influence on regrowth. For treatment D the plant available P (mg kg^{-1}) levels ranged between 4 and 391.25 mg kg^{-1} (data not shown). This big increase in P, especially in the 0-5 cm layer, was very high and led to the negative effect on regrowth.

In three models soil Na ($cmol_c$ kg^{-1}) had a negative significant (<0.05) influence, which included the models with all the data, depth 5-20 cm and treatment B model soil Na had a big negative influence. Soil Na saturation (%) however had a positive influence on the models for all the data, depth 5-20 cm, treatment A and treatment B. From this it is possible to see that soil Na played a definite role in some regrowth models.

Soil K ($cmol_c$ kg^{-1}) had a positive significant influence on the regrowth models for all the data, depth 0-5 cm and treatment C. Soil K levels ranged between 0.03 and 0.22 $cmol_c$ kg^{-1} with an average of 0.08 $cmol_c$ kg^{-1} for all the data. Joubert *et al* (1987) found optimum growth of rooibos seedlings at 60 mg kg^{-1} K (Bray II). When converting the $cmol_c$ kg^{-1} to mg kg^{-1} the soil K ranged between 15.64 and 119.06 mg kg^{-1} with an average of 38.41 mg kg^{-1} (Table 4-3). The model for all the data (R^2=0.46) was not as strong as it was for the treatment C model. The soil K (mg kg^{-1}) levels for treatment C ranged from 15.64 to 102.07 mg kg^{-1} with an average of 36.01. These K levels covered the concentration levels of 60 (mg kg^{-1}) for optimal growth of rooibos seedlings.

Soil Ca ($cmol_c$ kg^{-1}) had negative influence in all the regrowth models where it had a significant (<0.05) effect. These models include the models for all the data, 0-5 cm and 5-20 cm depth data; and treatment C data. Smith (2014) found that Ca has significant positive effect on aboveground biomass. His study included fields of different ages (0 to 60 yrs) and the soil Ca ranged between 0.15 and 0.43 $cmol_c$ kg^{-1}. In this study the soil Ca levels ranged between 0.28 and 1.4 $cmol_c$ kg^{-1} with an average of 0.56 $cmol_c$ kg^{-1} (Table 4-3). This average Ca $cmol_c$ kg^{-1} is higher than the maximum concentration levels in the study of Smith (2014) and can be the reason for the negative influence on the regrowth model.

Soil Mg ($cmol_c$ kg^{-1}) had a significant positive influence on the regrowth models for all the data, 5-20 cm depth, treatment B and treatment C data. In the regrowth model for treatment B soil Mg had the biggest influence compared to the other models. Joubert *et al* (1987) found that added Mg

suppressed rooibos growth, probably because of its antagonistic effect on the uptake of Ca, N, P and K. The study of Joubert *et al* (1987) was done on rooibos seedlings harvested at 5 months old. It was recommended that a higher Mg concentration of 0.2 me/100 g with a saturation of 10% is too high for rooibos seedling growth. Smith (2014) found no significant correlation between aboveground biomass and soil Mg. The Mg content of soils in this study ranged between 0.15 and 0.85 $cmol_c\ kg^{-1}$ with an average of 0.34 $cmol_c\ kg^{-1}$. This is higher than the recommendation of Joubert *et al* (1987), but it had a positive influence on the regrowth of older rooibos plants.

Soil Zn (mg kg^{-1}) had significantly negative influences in the regrowth models for all the data, depth 0-5 cm and treatment C except for treatment D the effect was positive. Mn (mg kg^{-1}) had a significantly negative influence on the models for all the data, depth 0-5 cm, treatment A, B and D. Smith (2014) found that soil Mn showed a significant negative correlation with aboveground biomass. No other research has been done that contained information on the effect of different levels of trace elements on the growth of rooibos plants. In the study of Smith (2014) the soil Zn ranged between 0.20 to 0.55 mg kg^{-1}. In this study the soil Zn ranged between 0.1 and 0.8 mg kg^{-1} (all data).

Soil C (%) had a significantly (<0.05) positive influence on the model for all the data, treatment A and C. Smith (2014) also found a significantly positive correlation between soil C (%) and aboveground biomass.

The plant nutrients that were significant in three or more of the models included N (%), P (%), K (%), Al (mg kg^{-1}) and Fe (mg kg^{-1}). Plant N (%) content had a significant negative influence in the regrowth models of treatment B and C, but for treatment D the influence of plant N was positive. This means that the uptake of N at treatment A, B and C had a negative influence on growth while at treatment D it had a positive influence. For treatment A, B and C the soil N:P ratios were 39, 41 and 47 (data not shown) respectively which can be the reason for the negative effect and it correlates with results of Maistry *et al* (2015). For treatment D the N:P ratio was 7 (data not shown) and explains the positive effect of plant N which correlates with the results of Maistry *et al* (2015).The influence (b) of plant N (%) however is very small compared to the other chemical properties in the model.

Plant Fe (mg kg^{-1}) had a significantly negative influence in the models for all the data, depth 5-20 cm and treatment C. This negative effect on regrowth is the opposite of the significantly positive effect that Smith (2014) found on above-ground biomass. For this study the plant Fe content ranged between 46.54 and 480.20 mg kg^{-1} with an average of 105.88 mg kg^{-1}. This is lower than the

averages that ranged between 196.40 and 377.47 in the study of Smith (2014) in Clanwilliam. This indicates that the Fe uptake in this study in the Nieuwoudtville Bokkeveld region might be too low. The positive influence (b) of plant Al (mg kg^{-1}) was very small compared to the other chemical properties. Smith (2014) however found a negative correlation between foliar Al and aboveground biomass. For this study the plant Al ranged between 33 and 190 mg kg^{-1} with an average of 81.28 mg kg^{-1}. In the study of Smith (2014) the average plant Al levels ranged between 150 and 350 mg kg^{-1} which are much higher than the levels for this study. This indicates that the plant Al levels in this study might have been low enough to have had a positive influence on growth.

Plant P (%) had a significant positive influence in all the regrowth models except for the models of depth 5-20 cm, treatment A and B its influence was not significant. This result however contradicts the negative correlation that Smith (2014) found between plant P (%) and aboveground biomass for rooibos. The influence (b) of plant P in the models was also big except for the model of the 0-5 cm depth data and treatment D. This positive influence of plant P (%) on the regrowth models thus means that the uptake of P, in combination with the effects of the other nutrients, leads to better growth of the rooibos plant. Plant K (%) had a significant positive influence in the regrowth models for all the data, 0-5 cm depth data and treatment B data. This means that the uptake of K is also important for the growth of the rooibos plant. This result supports the finding of Smith (2014) that found plant (foliar) K has a significant positive correlation with aboveground biomass.

4.5. Conclusion

With the help of statistical analysis, it was possible to see the variation in data and extract the most important information out of the complex and big dataset. With the PCA score plots it was already possible to see that there was a difference in plant and soil chemical data between the different sampling times throughout the season between July 2015, September 2015, January 2016 and June 2016. The PCA loadings plot made it easy to see which soil and plant chemical properties, and growth properties were positively or negatively correlated, and even not correlated at all. After completion of the multiple regression models for the raw data it was decided to look at the normal distribution and skewness of the data for all the chemical properties. Skew data were transformed and the multiple regression models were repeated, but this time only for the growth parameter average length of regrowth. This led to more soil and plant chemical properties having a significant influence in the model. Soil P, Mg and K had the biggest positive influences on the regrowth models. Only in the treatment D model soil P had a big negative influence because of the excess P that was added through fertilizer. Plant P also had a large positive influence. Soil Ca and N however had the biggest negative influence in some of the models. The soil Ca levels might have been too high and

the soil N levels too low. One important thing that was also noted was that the N:P ratio is of great importance for the uptake of N by the plant which can lead to better growth. Only the plant N (%) of treatment D had a small positive influence on the regrowth model which according to Maistry *et al* (2015) can be because of the N:P ratio in the soil.

After multiple regression analysis was performed again there were certain chemical properties that repeated its significant effect in three or more models. The soil chemical properties included P (mg kg^{-1}), Na ($cmol_c$ kg^{-1}), K ($cmol_c$ kg^{-1}), Ca ($cmol_c$ kg^{-1}), Mg ($cmol_c$ kg^{-1}), Zn (mg kg^{-1}), Mn (mg kg^{-1}), C (%) and Na (%). The plant chemical properties included Na (%) en plant N (%), P (%), K (%), Al (mg kg^{-1}) and Fe (mg kg^{-1}). In **Chapter 5** the cycling and pools of nutrients including C, N, P, K, Na, K, Ca, Mg, Zn, Mn, Fe and Al were the focus when changes over time and differences between mulch treatments were discussed.

5. EFFECT OF DIFFERENT RESIDUE TREATMENTS ON THE ROOIBOS NUTRIENT CYCLE AND POOLS

5.1. Introduction

No research has been done on the role of the natural litter layer in the rooibos nutrient cycle and what effect it have on the soil and plant nutrient status and also quantified plant uptake throughout the year. Smith (2014) studied the effect of rooibos plant litter compost on the soil quality and rooibos yield. A noticeable effect was found on the pH, EC, total microbial biomass and exchangeable cations when rooibos litter compost was applied at a rate of 20 t ha^{-1}, but no noticeable effect was found on soil N, soil C, P (Bray II) and micronutrients (Fe, Cu, Zn, Mn and B). The removal of the litter layer (mulch) during seed harvesting may play a role in the rooibos plants that die in the field for no obvious reason, while other plants in the same field thrive.

Mulches are used to conserve soil moisture, suppress weeds and plant diseases, and moderate soil temperatures. The potential of mulches to increase organic matter, improve soil structure and to establish nutrient cycling patterns similar to natural ecosystems has been recognized (Tiquia *et al.*, 2002). The main hypothesis for this chapter was that when harvesting rooibos seed, there is a negative interference with the nutrient cycle and pools of the individual rooibos plant leading to less nutrient uptake. The harvesting of rooibos seed includes the removal of the natural litter layer (mulch) that the plant produced and the thin topsoil layer.

The study was implemented to assess the changes in soil and plant nutrient status for plants under different residue management treatments over time. The objective of this study was to investigate the effect of rooibos seed harvesting (the clearing of natural mulch), added mulch (non-enriched and enriched) and natural mulch (undisturbed) on the soil and plant nutrient status and also uptake over a one-year period. The hypothesis is that the mulch and natural mulch treatments would show a higher increase in soil and plant nutrients over the one-year period then the no mulch treatment. Thus confirming that by removing the mulch layer under the plant will lead to faster depletion of soil nutrients and less uptake by plant.

5.2. Materials and methods

Trials were done in already established (four fields) rooibos fields across the Nieuwoudtville Bokkeveld region and analysis was done as described in **Chapter 3**.

5.2.1. Plant dry biomass prediction and nutrient uptake calculation

One of the aims for the study was to determine the plant nutrient content (mg per plant) and uptake during four times throughout the one year. Only the rooibos plant parts of the trials on the Cloudskraal farm were harvested in January (time of harvest for this field). It was decided to formulate a calculation using the height, width and leaf to wood mass ratio to predict the dry biomass (kg), harvested part, of the plant (allometric approach). Change in plant size is also accompanied by change in plant biomass (Northup *et al*, 2004). To harvest plants four times in one year would have been too destructive. This is a non-destructive estimation of plant biomass. Harvest, drying and weighing is thus not needed (Northup *et al*, 2004).

The height and the width of the plant were used to calculate the volume of the plant. Roughly, the rooibos plant has the form of two cones together connecting at their bases. The volume formula of a cone was used to calculate the plant volume. In order to incorporate the leave to wood mass ratio in the formula it was multiplied with the plant volume as seen in Equation 5-1.

Equation 5-1: Calculation of x value that was correlated with plant dry matter yield (kg)

$$X = \left(\left(\pi \left(\frac{width}{2} \right)^2 \right) \times \left(\frac{\frac{height}{2}}{3} \right) \right) \times 2 \times dry\ leaf{:}\ wood\ mass\ ratio$$

The X value that contained growth information of the plant (Equation 5-1) was then correlated with the yield dry matter (kg) from Cloudskraal. A polynomial (2nd order) gave the best regression line with a R^2 of 0.77 (Figure 5-1). The equation for the regression line was used to predict the dry matter yield for each plant. Dry matter yield (kg) and the nutrient concentration were then used to calculate the plant nutrient content (mg kg^{-1}). The difference in nutrient content (mg/plant) between different times thus show if uptake or loss occurred.

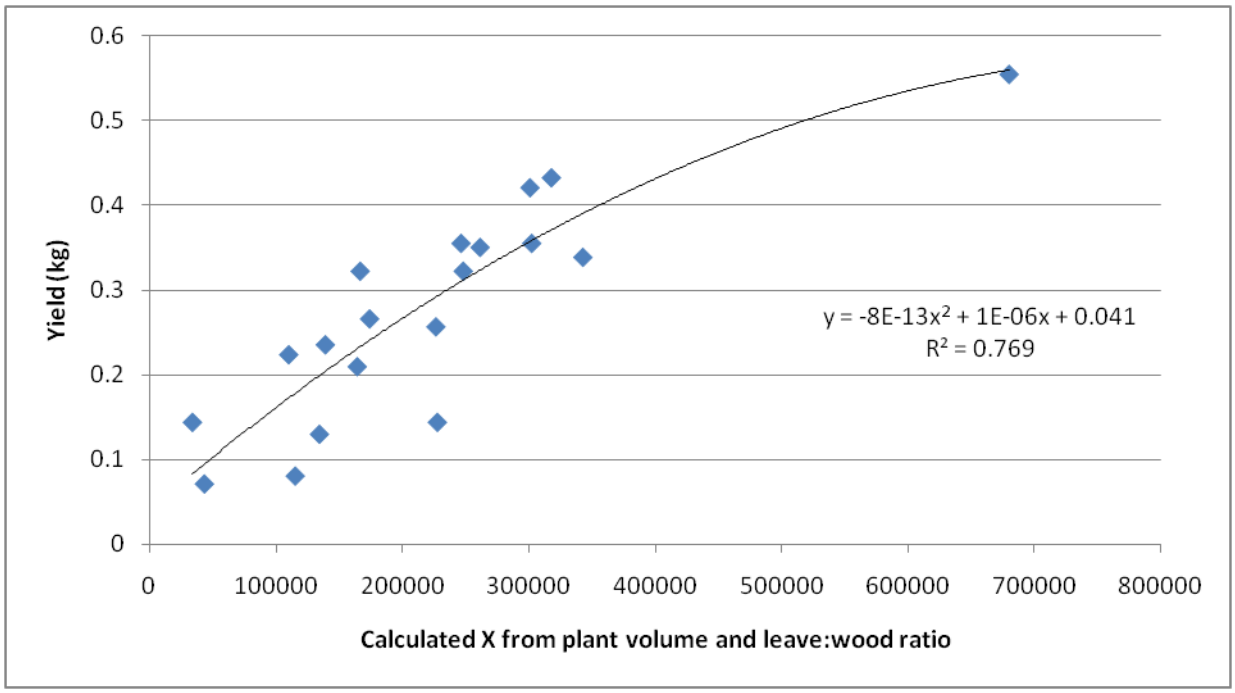

Figure 5-1: Yield (kg) and calculated X value with polynomial (2 order) regression line. Regression line equation and R^2 included.

5.3. Results and discussion

In **Chapter 4** the nutrients C, N, P, K, Na, K, Ca, Mg, Zn, Mn, Fe and Al were identified as nutrients that had a significant effect on the growth parameter average length of regrowth. For Al, only plant data was available, and thus only the change in plants over time and between treatments, were discussed. For C only the effect of each treatment over time on the total soil carbon was discussed. With the cycling of nutrients between soil, plant and mulch the following nutrients were discussed: N, P, K, Na, K, Ca, Mg, Zn, Mn and Fe. The results include plant, mulch and soil analysis which forms part of the nutrient cycle seen in Figure 5-2. The soil nutrients discussed here are of the 20 cm topsoil.

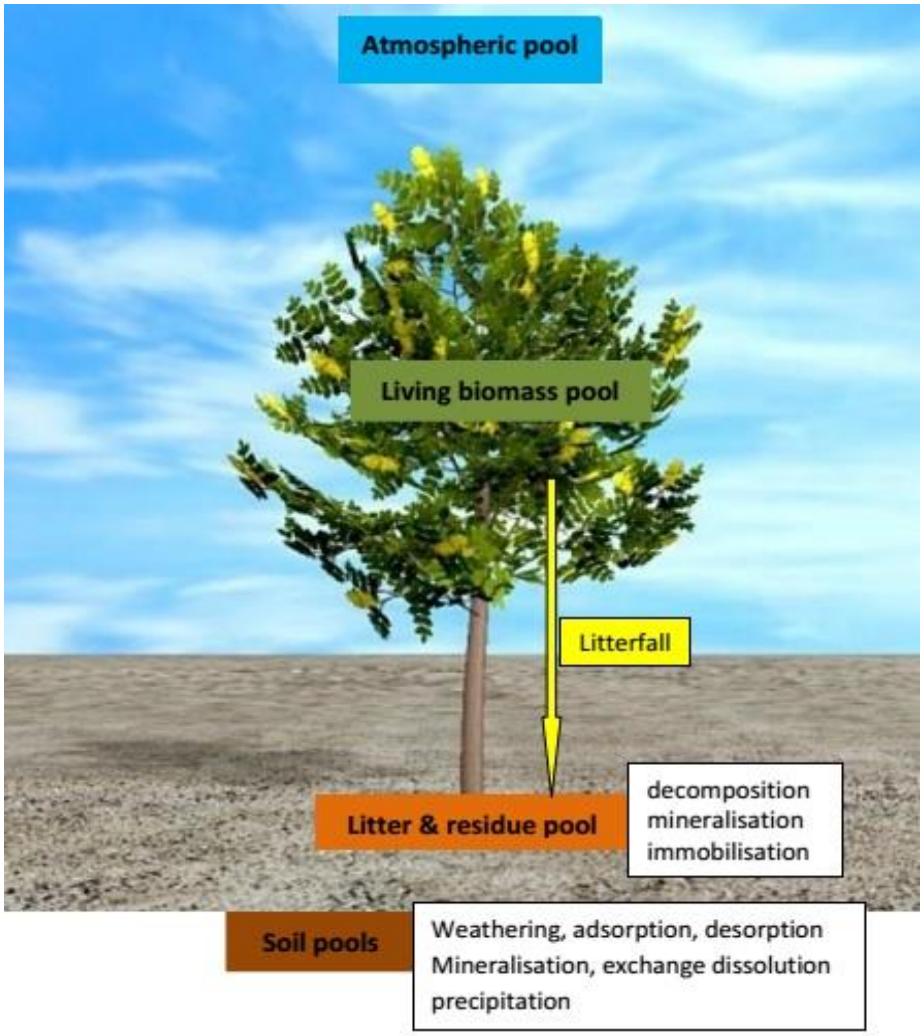

Figure 5-2: Representation of some major nutrient pools, losses, additions and cycling within in a plant ecosystem.

5.3.1. Soil pH

For all four treatments the soil pH (KCl) increased from July 2015 to January 2016. The increase from July to September however was bigger. The no mulch treatment (A) showed a very small increase from July 2015 to September 2015 compared to that of the mulch treatments. Mulching material and soil composition determine the effect the mulch would have on the soil pH (Ni *et al*, 2016) and this study the rooibos mulch led to an increase. From January 2016 to June 2016 there were however a decrease in soil pH. The decrease for treatment A was very small and for treatment D very big. Over the one year period there was an increase in soil pH for all treatments except treatment D. Treatment D received the MAP fertilizer. Ammonium based fertilizers can have an acidifying effect on the soil (McGowen et al, 2001). Nitrification (ammonium to nitrate) acidify the soil by generating two H^+ ions for ammonium molecule that's nitrified to nitrate. The acidifying effect of the P ion ($H_2PO_4^{-1}$) in monoammonium phosphate is very little in soils with pH lower than

7.2. Nitrogen application was thus the main contributor to the lowering of the soil pH under treatment D.

Table 5-1: Average soil pH for each treatment for all four treatments during sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment).

Treatment	Sampling time			
	July	September	January	June
A	4.54	4.57	4.65	4.64
B	4.39	4.50	4.62	4.54
C	4.38	4.57	4.60	4.53
D	4.46	4.60	4.64	4.38

5.3.2. Nitrogen

It is visible from the four sampling times during one year (July 2015 to June 2016) that the plant N concentration for all the treatments increased from July to September, but showed a decrease from September 2016 to June 2016 (Table 5-2). Stassen (1987) also found that the N (%) concentration in rooibos plants was highest in September (1.5 %) and low during January (1.1 %). This pattern was also seen by Lotter et al (2014) with plant N (%) the high in September and low in February. The big increase in plant N (%) concentration from July 2015 to September 2015 with increase in plant N content (mg/plant) thus means that during this period the N uptake by the plant were high. This increase in plant N was also accompanied by a decrease in the soil N for all treatments (correlation however between soil and plant N were very weak). The big decrease in plant N (%) concentration from September to January with decrease in plant N content (mg/plant) for treatment A means that little or no N uptake took place. This big decrease in plant N content with treatment A is mainly because of weaker growth compared to the other treatments and less N uptake. The plant N (%) concentration in this study (Table 5-2) was at the same levels than the N (%) that Smith (2014) found in the older rooibos fields that ranged between 0.8 and 1.3 % for June.

From September 2015 to January 2016 for treatment B, C and D there was an increase in the plant N content, compared to treatment A (decrease), and a decrease in plant N concentration, which means that there was still a highly active uptake of N especially for treatment B. In **Chapter 6** the study concluded that treatment B and D conserved the most soil water in dry periods just after rain, compared to the other treatments, which could be the reason for the higher N uptake during this time

Table 5-2: Growth and nitrogen concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (%)	1.33	1.86	1.24	1.17
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	1904.15	3305.79	2970.89	1231.93
	Soil (%)	0.04	0.02	0.03	0.03
B	Plant (%)	1.43	1.75	1.38	1.23
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	1985.33	3295.72	4061.37	1381.96
	Mulch (%)		1.75	1.51	1.20
	Mulch (mg)		3500.00	3010.00	2397.07
	Soil (%)	0.04	0.03	0.05	0.03
C	Plant (%)	1.35	1.79	1.32	1.32
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	1953.02	3256.47	3511.77	1313.65
	Mulch (%)			1.13	1.59
	Soil (%)	0.04	0.03	0.03	0.03
D	Plant (%)	1.43	1.93	1.42	1.41
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	2075.68	3614.71	3908.14	1393.98
	Mulch (%)		1.75	1.59	1.46
	Mulch (mg)		3500.00	3170.00	2920.93
	Soil (%)	0.04	0.03	0.04	0.03

. Treatment A showed the smallest increase in growth from September 2015 to January 2016. The weighed rooibos mulch (200 g) added in September 2015 showed a decrease in N (%) to January, which can be the reason for the big increase in soil N. However, N leaches from fresh litter at a much slower rate compared to Mg and K (Aerts and Chapin, 1999). The MAP fertilizer added at treatment D seemed to have had no effect on the soil N (%). Nitrogen is mostly present in the organic form and the addition of MAP fertilizer thus may have affected the inorganic pool in the soil, but has little change to affect the total N %. The dry combustion technique is also not sensitive enough to pick up very small changes in soil N content.

After January the plants were harvested which contributed to the big decrease in N content (mg/plant) from January to June, and thus just show ± how much N were removed with harvest. The total soil N in July 2015 and June 2016 for this study were the same as what Smith (2014) found in the rooibos soils of Clanwilliam (2 year and older plants) which ranged between 0.01 and 0.04. The decrease in soil N from July 2015 to September 2015 was the biggest for treatment A.

5.3.3. Phosphorus

For all treatments from July 2015 to September 2015 the plant P (%) concentration increased, but from September 2015 to June 2016 there was a decrease. The increase from July 2015 to September 2015 meaning P uptake by plant was very high during this period. Stassen (1987) found that plant P (%) concentration was highest in September (0.10 %) and lowest from January to June (0.05 %). The plants for all treatments had a decrease in plant P content (mg/plant) from September 2015 to January 2016, which means that P uptake during this period was very low. The decrease in plant P (%) concentration in the plant can be attributed to the dilution effect with increased growth during this period.

Table 5-3: Growth and phosphorus concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (%)	0.05	0.12	0.07	0.05
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	66.24	215.05	120.83	50.19
	Soil (mg kg^{-1})	9.94	9.06	10.32	6.77
B	Plant (%)	0.05	0.12	0.07	0.06
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	60.73	203.46	191.38	69.05
	Mulch (%)		0.09	0.05	0.03
	Mulch (mg)		180.00	95.00	66.67
	Soil (mg kg^{-1})	9.50	8.94	11.06	8.80
C	Plant (%)	0.04	0.11	0.06	0.05
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	62.46	197.12	156.50	51.65
	Mulch (%)			0.03	0.03
	Soil (mg kg^{-1})	9.25	8.94	11.40	6.25
D	Plant (%)	0.05	0.13	0.08	0.08
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	68.98	220.58	195.02	77.79
	Mulch (%)		0.09	0.10	0.08
	Mulch (mg)		180.00	190.00	166.67
	Soil (mg kg^{-1})	11.06	11.44	88.63	60.44

Treatment A had the biggest decrease in plant P (%), thus compared with the very little uptake of P for all the treatments treatment A had the smallest uptake. Treatment A also showed the smallest increase in growth (kg/plant) for this time. Treatment D was the only treatment that had no plant P

(%) decrease from January 2016 to June 2016, thus there was higher uptake of P because of the added MAP fertilizer. The plant (foliar) P Smith (2014) found ranged between 0.03 and 0.09 % in June.

All treatments, except D, showed a very small decrease in soil P (mg kg^{-1}) from July 2015 to September 2015. Treatment showed a very small increase. There was thus very little change in soil P (mg kg^{-1}) during this period. All treatments showed an increase in soil P (mg kg^{-1}) from September 2015 to January 2016 and a decrease from January 2016 to June 2016. The added mulch for treatment B showed a decrease in P content (mg) from the day it was applied in September 2015 till June 2016. Phosphorus, the same as N, leaches from mulches at a much slower rate (Aerts and Chapin, 1999) and thus the small decrease seen in Table 5-3 for treatment B. The P content for the mulch at treatment D in September 2015 was the analysis on the mulch only before it was enriched with MAP. The very small increase in mulch P (mg) content means that after the 10 g of MAP were applied most of it moved from the mulch to the soil and thus the very big increase in soil P (mg kg^{-1}). The mulch thus did not slowly release the fertilizer as initially thought it would. From January 2016 to June 2016 the mulch P concentration then decreased, the same as for treatment B and C.

The soil P (mg kg^{-1}) of these soils was between the minimum and maximum soil P (mg kg^{-1}) found at Clanwilliam which were between 1 and 17 (mg kg^{-1}) (Smith, 2014).

5.3.4. Sodium

There was an increase in plant Na (mg kg^{-1}) concentration and content (mg/plant) from July 2015 to January 2016. However, the increase from September 2015 to January 2016 for treatment A, were smaller compared to treatment B, C and D. The big increase in the plant Na (mg kg^{-1}) till January 2016 could possibly resulted from the mist from the west, which is also a source airborne salts. The plant Na (mg kg^{-1}) for rooibos plants in a study at Clanwilliam had a maximum of 3000 (mg kg^{-1}) in June (Smith, 2014), compared to the maximum of 5742.55 (mg kg^{-1}) (Table 5-4) in the Bokkeveld plateau for this study. Because of this external source of Na it was not possible to use the plant Na content (mg/plant) in Table 5-4 to discuss plant Na uptake. The mulch Na concentration and content for treatment B and D showed a small decrease from September 2015 to January 2016, but the decrease to June 2016 were much larger. This same trend was observed with plant Na (mg kg^{-1}).

Table 5-4: Growth and sodium concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (mg kg^{-1})	3486.50	4977.00	6150.56	2670.50
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	534.20	1000.54	1478.66	298.57
	Soil ($cmol_c$ kg^{-1})	0.07	0.05	0.08	0.08
B	Plant (mg kg^{-1})	3598.40	5157.20	8285.50	3120.60
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	523.14	995.14	2442.57	339.89
	Mulch (mg kg^{-1})		5556.67	3992.25	1368.33
	Mulch (mg)		1111.33	798.45	273.67
	Soil ($cmol_c$ kg^{-1})	0.07	0.07	0.10	0.11
C	Plant (mg kg^{-1})	3426.75	5742.55	6232.00	3316.64
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	504.19	981.90	1673.31	307.32
	Mulch (mg kg^{-1})			4720.50	736.67
	Soil ($cmol_c$ kg^{-1})	0.06	0.06	0.10	0.08
D	Plant (mg kg^{-1})	3899.15	5325.10	6979.60	3766.83
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	581.64	1003.23	1926.80	397.16
	Mulch (mg kg^{-1})		5556.67	4909.75	1357.67
	Mulch (mg)		111.33	981.95	271.53
	Soil ($cmol_c$ kg^{-1})	0.06	0.06	0.15	0.09

For all the mulch treatments (B, C and D) there was no change in soil Na ($cmol_c$ kg^{-1}) from July 2015 to September 2015. Treatment A showed a decrease in soil Na ($cmol_c$ kg^{-1}) during this period. From September 2015 to January 2016 there was an increase in soil Na ($cmol_c$ kg^{-1}) for all the treatments. From January 2016 to June 2016 a treatment A showed no change, treatment B showed an increase, treatment C a decrease, and treatment D also a decrease. The soil Na ($cmol_c$ kg^{-1}) seemed to be not affected by the treatments that were applied. The average soil exchangeable Na ($cmol_c$ kg^{-1}) in June at Clanwilliam in the study of Smith (2014) ranged between 0.04 and 0.09 $cmol_c$ kg^{-1} which is almost at the same level for this study, which ranged between 0.05 and 0.15 $cmol_c$ kg^{-1} (Table 5-4).

5.3.5. Potassium

The K (%) concentration increases in the plant from July 2015 to September 2016 was very high, but showed a small decrease from September 2015 to January 2016. The small decrease in concentration was almost the same for all the treatments. This decrease is a combination of less K

uptake and the dilution effect of growth. Stassen (1987) found a higher rooibos plant K (%) during September (0.3 %), with the plants reaching their minimum K (%) in January (0.18 %). In this study however the difference in the plant K (%) between September 2015 and January 2016 was much smaller. The plant K (%) in a study at Clanwilliam ranged between 0.3 and 0.43 % (Smith, 2014). The plant K (%) concentrations in this study correlated well with that of Smith (2014). From July 2015 to September 2016 there was an increase in plant K content, thus the uptake of K during this period were very high, but from September 2015 to January 2016 the uptake were not as great (smaller difference in K content). Only treatment A showed a decrease in plant K content from September 2015 to January 2016 meaning there were little or no uptake compared to that of the mulch treatments.

Table 5-5: Growth and potassium concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (%)	0.30	0.59	0.43	0.35
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	413.07	1042.43	1027.42	374.86
	Soil ($cmol_c\ kg^{-1}$)	0.08	0.07	0.10	0.08
B	Plant (%)	0.32	0.58	0.51	0.42
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	454.88	1085.00	1460.54	468.79
	Mulch (mg kg^{-1})		0.57	0.31	0.14
	Mulch (mg)		1140.00	615.00	286.67
	Soil ($cmol_c\ kg^{-1}$)	0.07	0.07	0.11	0.11
C	Plant (%)	0.31	0.60	0.52	0.37
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	440.47	1092.71	1312.85	368.48
	Mulch (mg kg^{-1})			0.22	0.08
	Soil ($cmol_c\ kg^{-1}$)	0.08	0.07	0.10	0.08
D	Plant (%)	0.31	0.62	0.52	0.41
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	440.37	1145.90	1393.26	410.62
	Mulch (mg kg^{-1})		0.57	0.34	0.16
	Mulch (mg)		1140.00	675.00	313.33
	Soil ($cmol_c\ kg^{-1}$)	0.07	0.09	0.13	0.11

The biggest change in exchangeable soil K ($cmol_c\ kg^{-1}$) occurred between September 2015 and January 2016 which resulted in an increase. From January 2016 to June 2016 it was only treatment B that showed no decrease in soil K ($cmol_c\ kg^{-1}$) compared to the other treatments. Smith (2014) found exchangeable soil K levels that ranged between 0.05 and 0.12 $cmol_c\ kg^{-1}$ in June in rooibos fields with 2 year and older plants. The soil K levels, 0.08 to 0.11, at the Bokkeveld Plateau falls within these range (Table 5-5). For treatment B and D there was a big decrease in mulch K content (mg) from September 2015 to January 2016 and for all three mulch treatments there were a smaller decrease from January 2016 to June 2016. K is very mobile and easily leaches from fresh litter, thus leaches at a much higher rate than N, P and Ca (Aerts and Chapin, 1999). The loss of K content from the mulch however did not lead to an increase in the soil K ($cmol_c\ kg^{-1}$).

5.3.6. Calcium

The plant Ca (%) concentration increased from July 2015 to September 2015 for all four treatments. From September 2015 to January 2016 there was a big decrease in plant Ca (%) and then from January 2016 to June 2016 there were an increase in plant Ca (%) for all the treatments. Treatment B and C showed the smallest decrease in plant Ca (%) from September 2015 to January 2016 and the biggest increase from January 2016 to June 2016. Stassen (1987) also found a high Ca (%) concentration in September (0.45 %) with a big decrease to January (0.28 %) and then an increase to June (0.37 %). The plant Ca content (mg/plant) increased from July 2015 to September 2015 and decreased to January 2016. There was thus little or no uptake from September 2015 to January 2016. Treatment B showed the smallest decrease in plant Ca content (mg/plant), thus although treatment B showed the biggest growth meaning greater dilution of Ca (%) there were still a higher uptake compared to the other treatments. The increase in plant Ca (%) from January to June 2016 means that there was also Ca uptake during this period. The plant Ca (%) values found in June 2015 (Table 5-6) were lower than the 0.15 to 0.29 % reported by Smith (2014). Exchangeable soil Ca increased from July 2015 to January 2016 for all treatments with a smaller increase from September 2015 to January 2016. From January 2016 to June 2016 there was a decrease in soil Ca.

Table 5-6: Growth and calcium concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (%)	0.14	0.22	0.09	0.14
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	207.55	384.64	180.91	136.89
	Soil (%)	0.55	0.60	0.63	0.50
B	Plant (%)	0.14	0.21	0.10	0.16
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	201.42	393.43	293.34	180.13
	Mulch (%)		0.37	0.31	0.29
	Mulch (mg)		733.33	620.00	580.00
	Soil (%)	0.46	0.52	0.59	0.50
C	Plant (%)	0.15	0.20	0.09	0.15
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	215.74	360.80	231.55	152.61
	Mulch (%)			0.29	0.32
	Soil (%)	0.43	0.52	0.55	0.41
D	Plant (%)	0.15	0.24	0.11	0.15
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	216.71	433.39	292.11	148.24
	Mulch (%)		0.37	0.33	0.32
	Mulch (mg)		733.33	650.00	640.00
	Soil (%)	0.51	0.63	0.67	0.52

In the study of Smith (2014) the exchangeable soil Ca ($cmol_c\ kg^{-1}$) for fields with 2 year and older rooibos plants ranged between 0.21 and 0.43 $cmol_c\ kg^{-1}$ in June, lower than the levels in the Bokkeveld Plateau rooibos soils (Table 5-6). The increase from July 2015 to January 2016 can be because of the increase in soil pH during this time (Table 5-1) during this time. A weak positive correlation (R^2=0.49) was found between soil exchangeable K ($cmol_c\ kg^{-1}$) and soil pH (Table 4-4). From the mulches for treatment B and D showed a small decrease in Ca content (mg) while for treatment C there was a small increase from January 2016 to June 2016. Ca do not leach as easily from litter like Mg and K and thus the small decrease and even increase (Aerts and Chapin, 1999).

5.3.7. Magnesium

For all the treatments there was an increase in plant Mg (%) concentration from July 2015 to September 2015. From September 2015 to January 2016 there was a small decrease in plant Mg (%), all treatments, followed by an increase till June 2016 only for treatment A, C and D. Treatment B

showed no change. This small decrease in plant Mg (%) during time period when growth is high explaining the increase in plant Mg content (mg/plant) from September 2015 to January 2016. Treatment A and C showed a slightly bigger decrease in plant Mg (%) during this period, and thus the smaller increase in plant Mg content (mg/plant). Stassen (1987) found almost the same pattern in plant Mg (%) concentration change with Mg (%) concentration being the highest in September (0.22 %) and the lowest in January (0.19 %). For this study the lowest plant Mg (%) concentration was in July 2015. The increase in plant Mg content (mg/plant) from July 2015 to January 2016 means that there was a high uptake of Mg for both periods. Treatment A showed the smallest increase in plant Mg content (mg kg^{-1}). The plant Mg (%) concentration in June 2016 correlated well with what Smith (2014) found in Clanwilliam for rooibos plants.

Table 5-7: Growth and magnesium (Mg) concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (%)	0.16	0.21	0.18	0.20
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	233.39	375.37	386.72	209.03
	Soil (%)	0.29	0.31	0.35	0.31
B	Plant (%)	0.16	0.22	0.21	0.21
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	229.47	408.96	611.85	245.04
	Mulch (%)		0.28	0.22	0.21
	Mulch (mg)		553.33	445.00	426.67
	Soil (%)	0.28	0.29	0.35	0.34
C	Plant (%)	0.17	0.21	0.18	0.21
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	215.74	378.24	483.54	213.22
	Mulch (%)			0.21	0.20
	Soil (%)	0.28	0.30	0.34	0.28
D	Plant (%)	0.17	0.23	0.21	0.23
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	242.70	421.53	573.26	229.26
	Mulch (%)		0.28	0.23	0.22
	Mulch (mg)		553.33	465.00	433.33
	Soil (%)	0.30	0.35	0.40	0.33

The exchangeable soil Mg ($cmol_c\ kg^{-1}$) increased continuously from July 2015 to January 2016 and from there on it decreased. The increase from June 2015 to January 2016 can be because of the increase in soil pH during this time (Table 5-1) during this time. A positive correlation (R^2=0.61) was found between soil exchangeable Mg ($cmol_c\ kg^{-1}$) and soil pH (Table 4-4). The increase in soil Mg ($cmol_c\ kg^{-1}$) from September 2015 to January 2016 for treatment A and C was smaller. In the study of Smith (2014) the exchangeable soil Mg ($cmol_c\ kg^{-1}$) for fields with 2 year and older rooibos plants ranged between 0.03 and 0.18 $cmol_c\ kg^{-1}$ in June which is much lower than the average found in the Bokkeveld Plateau rooibos fields (Table 5-7). According to Aerts and Chapin (1999) Mg is very mobile and easily leaches from litter, but looking at the change in mulch Mg content (mg) in Table 5-7 for treatment B, C and D there were only a small decrease in the Mg content (mg). This means that Mg leaching from the mulch was not that great especially from January 2016 to June 2016.

5.3.8. Iron

The change in plant Fe concentration (mg kg^{-1}) from July 2015 to September 2015 between the mulch treatments were different and seemed to be not affected by the treatments. Treatment A and B showed an increase in plant Fe (mg kg^{-1}), while treatment C and D showed a decrease. However, the change from September 2015 to January 2016 (decrease) and from January 2016 to June 2016 (increase) was the same for all the treatments.

Stassen (1987) found a rooibos plant norm of 225 mg kg^{-1} for plants in field conditions and 67 mg kg^{-1} for 3-year-old rooibos which were harvested in March. Smith (2014) found plant Fe (mg kg^{-1}) concentrations in June that ranged between 196.4 and 377.47 mg kg^{-1} for 2 year and older plants. For this study the plant Fe (mg kg^{-1}) averages ranged between 93.61 and 119.84 mg kg^{-1} (Table 5-8) which is much lower than that of Smith (2014). The plant Fe (mg kg^{-1}) concentration falls in the sufficient or normal category of 100 to 500 mg kg^{-1} for plants (Pais and Jones Jr, 1997). All the treatments, except treatment D, showed an increase in plant Fe content (mg kg^{-1}) from July 2015 to September 2016 (At this point fertilizer at treatment D was not mixed with the mulch and thus was the same as treatment B). There was thus big variation in Fe uptake during this period when looking at the plant Fe contents (mg/plant) for treatment B and D in this time. From September to January treatment D showed the smallest decrease in Fe content (mg/plant) and treatment A the biggest decrease. The decrease from January 2016 to June 2016 is the result of the amount removed (negative) with harvest in January 2016 plus the Fe uptake (positive) from January 2016 and June.

Table 5-8: Growth and iron (Fe) concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (mg kg^{-1})	101.72	112.73	76.37	101.49
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	15.43	20.86	17.98	10.22
	Soil (mg kg^{-1})	26.14	29.19	30.25	27.65
B	Plant (%)	107.35	119.64	68.99	119.84
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	15.60	23.04	21.03	13.99
	Mulch (mg kg^{-1})		614.53	472.43	11123.98
	Mulch (mg)		122.91	94.49	2224.80
	Soil (mg kg^{-1})	26.73	32.32	25.33	39.69
C	Plant (%)	115.59	110.24	70.80	106.04
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	17.63	20.60	18.87	10.66
	Mulch (mg kg^{-1})			2086.13	11182.50
	Soil (mg kg^{-1})	24.33	25.42	29.46	33.16
D	Plant (%)	163.88	109.80	75.29	93.61
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	23.71	20.69	20.64	9.32
	Mulch (mg kg^{-1})		614.53	665.15	2031.70
	Mulch (mg)		122.91	133.03	406.34
	Soil (mg kg^{-1})	28.51	28.5	26.65	36.72

When looking at soil Fe (mg kg^{-1}) the changes from July 2015 to January 2016 for all the treatments no trends were observed and looked completely random and unaffected by the treatments. During this time the soil Fe (mg kg^{-1}) concentration ranged between 24 and 33 mg kg^{-1}. However, when just looking at the change between July 2015 and June 2016 (1-year period) the soil Fe (mg kg^{-1}) concentration for the mulch treatments (B, C and D) showed a very big increase compared to the very small increase for treatment A. Organic matter increase plant-available Fe, because the organic acids chelates with Fe, preventing precipitation keeping it soluble. At Clanwilliam in the study of Smith (2014) the soil Fe (mg kg^{-1}) concentration ranged between ± 25 and 108 mg kg^{-1}, but without the farm that had excessively high soil Fe (mg kg^{-1}) it ranged between 25 and 65 mg kg^{-1}. These concentration levels were a little higher than the average found in the Bokkeveld Plateau rooibos fields (Table 5-8). The mulches added to treatment B and D had a much lower Fe concentration (mg kg^{-1}) in January 2016 compared to that of treatment C. The increase in the mulch Fe concentration

from January 2016 to June 2016 was much bigger for treatment B and C compared to that of treatment D.

5.3.9. Zinc

For all the treatments the plant Zn concentration (mg kg^{-1}) increased from July 2015 to September 2015, followed by a decrease till January 2016 and then again with a very small decrease for treatment A, C and D and increase for treatment B to June 2016. Stassen (1987) found a Zn concentration norm of 11 mg kg^{-1} which only correlates with the Zn (mg kg^{-1}) concentrations in September 2015 for this study (Table 5-10).

Table 5-9: Growth and zinc (Zn) concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (mg kg^{-1})	5.80	12.43	7.92	7.62
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	0.80	2.18	1.61	0.80
	Soil (mg kg^{-1})	0.21	0.13	0.16	0.17
B	Plant (%)	5.11	12.33	6.59	8.15
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	0.69	2.19	1.93	0.89
	Mulch (mg kg^{-1})		23.29	9.76	10.03
	Mulch (mg)		4.66	1.95	2.01
	Soil (mg kg^{-1})	0.18	0.13	0.19	0.24
C	Plant (%)	5.00	11.39	6.77	6.69
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	0.74	2.03	1.72	0.67
	Mulch (mg kg^{-1})			3.73	5.66
	Soil (mg kg^{-1})	0.15	0.14	0.15	0.21
D	Plant (%)	5.02	11.97	7.43	7.11
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	0.69	2.14	1.96	0.73
	Mulch (mg kg^{-1})		23.29	11.37	24.17
	Mulch (mg)		39.55	34.53	32.77
	Soil (mg kg^{-1})	0.22	0.18	0.20	0.26

Smith (2014) found plant Zn concentrations (June) that ranged between 14.2 and 18.16 mg kg^{-1} which is higher compared to this study in June. The plant Zn concentrations found in this study for July 2015, January 2016 and June 2016 was also lower than the normal values of 10 to 100 mg kg^{-1} for plants (Pais and Jones Jr, 1997). Treatment A and C also had the biggest decrease in plant Zn

content (mg/plant) from September 2015 to January 2016 which means less Zn uptake during this period compared to treatment B and D.

All treatments showed a decrease in plant available soil Zn (mg kg^{-1}) from July 2015 to September 2015, and a continuous increase from September 2015 to June 2016. From January 2016 to June 2016 only treatment B showed an increase in plant Zn. Smith (2014) found soil Zn (mg kg^{-1}) concentration levels that ranged between 0.23 and 0.58 % in June at Clanwilliam. These concentration values were higher than what was found in this study during June (Table 5-9). The mulches (treatment B and D) showed a decrease in Zn content from September 2015 to January 2016 and then an increase (treatment B, C and D) from January 2016 to July 2016. This increase in Zn can possibly be because of a Zn based pesticide that could have been applied after harvest.

5.3.10. Manganese

For all treatments the plant Mn concentration (mg kg^{-1}) increased from July 2015 to September 2015, followed by a big decrease till January 2016 and then again with an increase to June 2016. In the study of Stassen (1987) a plant Mn concentration norm of 47 mg kg^{-1} was found which only correlates with the January 2016 Mn (mg kg^{-1}) concentrations in this study (Table 5-10). Smith (2014) found Mn concentration levels that ranged between 134 and 142 mg kg^{-1} which is much higher than the Mn concentrations for this study in June 2016. Treatment A and C had the smallest increase in plant Mn content (mg/plant) from July 2015 to September 2015 and treatment A, B and C had the smallest decrease from September 2015 to January 2016, which means that there was less uptake of Mn for these treatments during these periods.

Treatment D showed much higher plant Mn concentrations from September 2015 to June 2016. This can be attributed to the high soil P levels. Excess P in soil leads to P toxicity and also Mn accumulation in the leaves (Hawkins *et al*, 2008). The plant available soil Mn (mg kg^{-1}) concentration for treatment A and C showed a decrease and for treatment B and D an increase from July 2015 to September 2015. The biggest decrease was showed by treatment A. From July 2015 to September 2015 treatment B and D was the same mulch treatment, but D showed the biggest increase in soil Mn (mg kg^{-1}).

Table 5-10: Growth and manganese (Mn) concentrations in the plant, mulch and soil for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (mg kg^{-1})	56.28	106.89	43.54	61.19
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.10
	Content (mg/plant)	7.80	17.16	8.54	6.46
	Soil (mg kg^{-1})	4.54	4.12	3.98	4.00
B	Plant (%)	63.25	110.58	36.50	67.03
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	8.23	19.44	10.73	7.63
	Mulch (mg kg^{-1})		197.73	157.75	144.60
	Mulch (mg)		39.55	31.55	28.92
	Soil (mg kg^{-1})	3.65	3.78	3.74	4.31
C	Plant (%)	64.89	98.29	38.19	69.93
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	9.93	17.97	9.72	6.83
	Mulch (mg kg^{-1})			138.15	136.77
	Soil (mg kg^{-1})	3.71	3.62	3.31	3.24
D	Plant (%)	78.75	130.48	56.66	96.53
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	10.83	22.37	14.57	9.98
	Mulch (mg kg^{-1})		197.73	172.65	163.87
	Mulch (mg)		39.55	34.53	32.77
	Soil (mg kg^{-1})	3.44	3.97	4.02	4.61

From September 2015 to January 2016 it was only treatment D that showed a small increase. Treatment A and C had the biggest decrease in soil Mn (mg kg^{-1}) during this period. From January 2016 to June 2016 treatment B and D showed the same big increase while treatment A showed almost no change with a slight increase for treatment C. Smith (2014) found soil Mn (mg kg^{-1}) concentration levels that ranges between 2 and 10.2 mg kg^{-1} in June which correlates with the Mn concentration levels found in June for this study (Table 5-10). For treatment B, C and D the mulch Mn content (mg) decreased continuously from September 2015 to June 2016.

5.3.11. Aluminium

All treatments showed an increase in plant Al concentration (mg kg^{-1}) from July 2015 to September 2015. The biggest Al uptake took place between July 2015 and September 2015 with increase in plant Al (mg kg^{-1}) concentration and plant Al content (mg/plant). Smith found plant Al (mg/kg) concentrations that ranged between 150 and 350 mg kg^{-1} in June which is much higher than the concentration levels found in June for this study (Table 5-11).

Table 5-11: Growth and aluminium (Al) concentrations in the plant and mulch for all four treatments during different sampling times (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Property	Sampling time			
		July	September	January	June
A	Plant (mg kg^{-1})	69.20	79.90	76.78	98.39
	Dry matter yield (kg/plant)	0.14	0.18	0.22	0.11
	Content (mg/plant)	10.56	14.87	17.22	9.87
B	Plant (%)	69.95	85.00	63.80	101.10
	Dry matter yield (kg/plant)	0.14	0.19	0.30	0.11
	Content (mg/plant)	10.08	16.12	19.19	9.87
	Mulch (mg kg^{-1})		190.00	317.50	1675.87
	Mulch (mg)		38.00	63.50	335.17
C	Plant (%)	73.75	84.45	59.30	92.55
	Dry matter yield (kg/plant)	0.15	0.18	0.26	0.10
	Content (mg/plant)	11.38	15.72	15.40	4.53
	Mulch (mg kg^{-1})			667.50	1896.67
D	Plant (%)	74.60	81.90	66.20	86.00
	Dry matter yield (kg/plant)	0.15	0.19	0.27	0.10
	Content (mg/plant)	11.25	15.30	17.84	8.56
	Mulch (mg kg^{-1})		190.00	370	1103.33
	Mulch (mg)		38.00	74.00	220.67

5.3.12. Summary

Treatment A and C had a significant decrease in total soil C (%), but for treatment B and D there were an increase which was not significant (Table 5-11). For all the treatments there was a decrease in total soil N (%) and plant N (%) over the year. With plant P (%) it was only for treatment A that there was no change. For treatment B and D there were a significant increase in plant P (%). Only for treatment D there was a significant increase in soil P (mg kg^{-1}) because of the added fertilizer. Treatment B and D was the only mulch treatment that showed a significant increase in soil and plant K concentration. For treatment A and C the soil K ($cmol_c$ kg^{-1}) was the same in July 2015 and June 2016, thus no change but for treatment B and D there was an increase (not significant). There was a

decrease in plant Na (%) over the one-year period for all treatments. The added mulch treatments (B and D) showed a significant increase in soil Na ($cmol_c\ kg^{-1}$). The plant and soil Ca increased over the one-year period for treatment B and for treatment D there were only an increase in soil Ca ($cmol_c\ kg^{-1}$). However, no significant changes were found in plant and soil Ca concentration over the one-year period for all the treatments. All the treatments led to the significant increase in plant Mg over the one-year period, but only for treatment A it was not significant.

Table 5-12: The effect each treatment had on the concentration of the nutrients over a one year period (July 2015 to June 2016). "–" for decrease, "+" for increase and "*" for significance. (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

Treatment	Nutrient pool	Nutrient concentration increase or decrease										
		C	N	P	K	Na	Ca	Mg	Fe	Zn	Mn	Al
A	Plant		-	NC	+	-	NC	+	+	+*	+	+
	Soil	-*	-*	-	NC	+	-	+*	NC	-*	-	
B	Plant		-	+*	+*	-	+	+*	+	+*	+	+
	Soil	+	-*	-	+*	+*	+	+*	+*	+	+	
C	Plant		-	+	+	-	NC	+*	-	+*	+	+
	Soil	-*	-	-	NC	+	-	NC	+*	+	-	
D	Plant		-	+*	+*	-	NC	+*	-*	+*	+	+
	Soil	+	-	+*	+*	+*	+	+*	+*	+	+*	

For all treatments, except treatment A, soil Fe ($mg\ kg^{-1}$) increased (significant for B, C and D) over the one-year period. Treatment D showed a significant decrease in plant Fe, which can be attributed to the antagonistic affect P has on Fe uptake. Treatment A and B showed an increase in plant Zn ($mg\ kg^{-1}$) and treatment C and D a decrease (all not significant). For all treatments there was a significant increase in plant Zn ($mg\ kg^{-1}$). Over the one year period the plant Zn ($mg\ kg^{-1}$) showed an increase of ± the same size (Table 5-9) for all the treatments and thus was not affected by the treatments. Only for treatment A was there a significant change in soil Zn ($mg\ kg^{-1}$) which was a decrease. The plant Mn ($mg\ kg^{-1}$) concentration for all treatments increased all was not significant, thus was unaffected by the treatments. Treatment A and C showed a decrease in soil Mn ($mg\ kg^{-1}$), not significant, while there was an increase for treatment B and D (significant). For all the treatments there was a not significant plant Al ($mg\ kg^{-1}$) increase and was this increase was unaffected by the treatments.

Only between treatment A and B there was a significant ($P<0.05$) difference in the increase of estimated dry matter. Although treatment A was not significantly different from treatment C and D treatment A showed the lowest estimated dry matter increase (Figure 5-3).

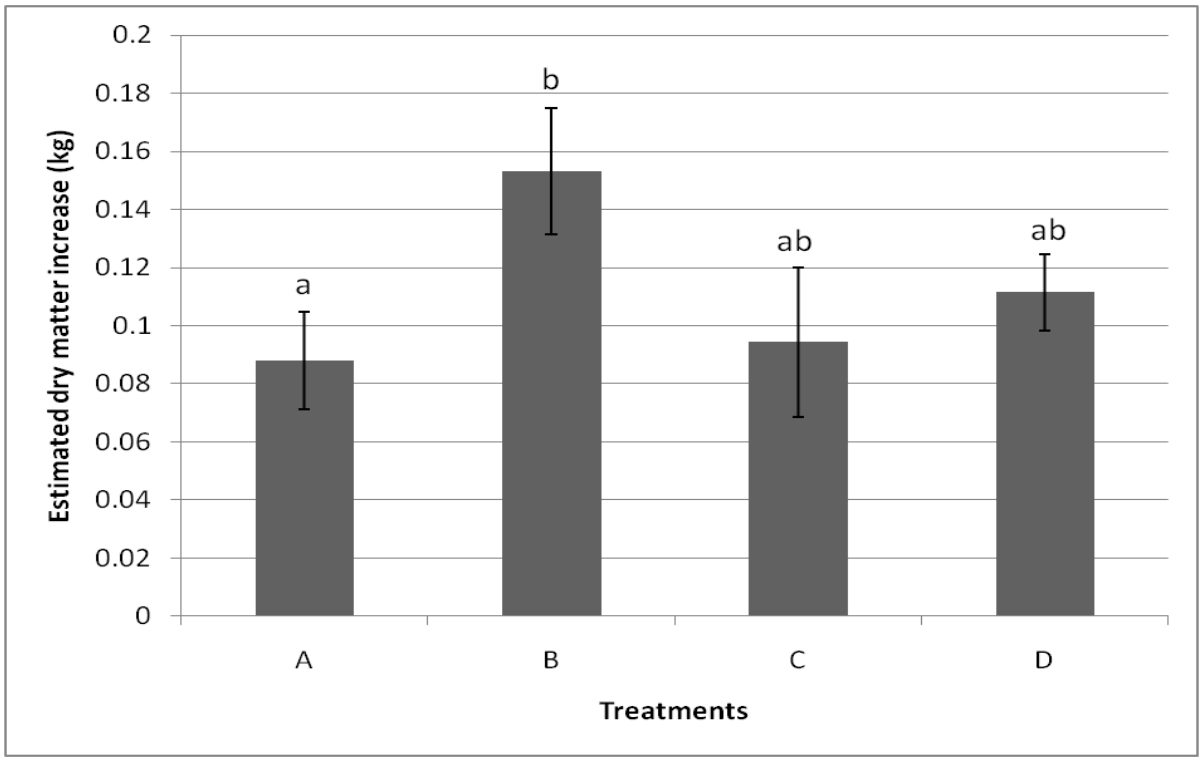

Figure 5-3: The estimated dry matter increase (kg) for the four different treatments from June 2015 to January 2016. Means ± s.e. The letters of significance differ between the treatments. (A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment)

5.4. Conclusions

The macronutrients N, P and K in the plant showed a comparable increase from July 2015 to September 2015, but from September 2015 to June 2016 there was a decrease. The plants with mulch treatment all showed an increase in plant N content from September 2015 to January 2015 while the plants with treatment D showed a decrease. Although there was very little variation in soil N, from July 2015 to September 2015 there was a decrease for all the treatments. However, the total change in plant N from July 2015 to June 2016 showed no big difference between A and the mulched treatments and thus unaffected by the treatments. All treatments showed a decrease in soil N from July 2015 to September 2015 followed by lots of variation in changes from September 2015 to June 2016 which seemed to be unaffected by the treatments. Over the one-year period there was a total decrease of soil N for all treatments (significant for A and B). From September 2015 to January 2016 treatment D had the biggest increase in soil P because of the added fertilizer. Over the one-year period the plants with treatment B, C and D (B and D was significant) showed an increase in plant P. The mulch treatments thus led to a better uptake of P. Soil K increased from September 2015 to January 2016 for all treatments, followed by a decrease till June 2016 except for treatment D. For all the mulch treatments the increase in plant K content from July 2015 to January 2016 were much bigger compared to that of treatment A. Over the one-year period all the treatments showed an

increase in plant K (only significant for treatment B and D). The added mulch treatment thus led to a better uptake of K.

For both soil Ca and Mg there was an increase from June 2015 to January 2016 followed by a decrease till June 2016. The plant Ca and Mg for both increased from July 2015 to September 2015 and decreased from September 2015 to January 2016. The decrease till September for Ca was very big and for Mg small. From January 2016 to June 2016 the plant Ca increased a lot while for Mg the increase was very small to almost no change. Over the one-year period not one treatment had a significant effect on the total change in soil and plant Ca. Treatment B and D however showed a average increase in soil Ca and plant Ca just for treatment D. All the treatments led to the significant increase in plant Mg over the one-year period, but only for treatment A it was not significant.

Plant Fe (mg kg^{-1}) showed a big decrease from September to January 2016 followed by an increase from January 2016 to June 2016. From July 2015 to September 2015 there was a small increase in soil Fe (mg kg^{-1}). The change from September 2015 to January 2016 showed big variation between treatments. From January 2016 to June 2016 the soil Fe (mg kg^{-1}) increased for all treatments. Over the one-year period there was a significant total increase in soil Fe for all the mulch treatments.. The uptake of Fe by plant was not affected by growth because of no significant changes in plant Fe (mg kg^{-1}). Plant Zn showed a big increase from July 2015 to September 2015 followed by a big decrease till January 2016 and almost no change till June 2016. Soil Zn decreased from July 2015 to September 2015 and then increased continuously till June 2016. Over the one-year period there was a total significant increase for all the treatments in plant Zn. Only treatment A showed a significant change in soil Zn over the year with a decrease compared to the other treatments that all showed an increase (not significant). The treatments did not affect the uptake of Zn uptake, which was very small compared to the macro nutrients. Both plant Mn and Al increased from July 2015 to September 2015 followed by a decrease, big decrease for Mn, till January 2016 and an increase till June 2016 for all treatments. The changes in soil Mn over the year period were very small. Over the one-year period there was only a significant change in soil Mn for treatment D which was an increase. The uptake of Mn and Al was not influenced by the different treatments. Treatment A and C both led to the total significant decrease in soil carbon. The added mulch treatments (B and D) thus led to no significant decrease in total soil C over the one-year period.

Ultimately treatment A led to the lowest estimated dry matter increase till January 2016, being only significant different from treatment B, which had the highest estimated dry matter increase. These treatments were only done over a one-year period and there is the possibility that when the treatments continued for longer the impact might have been greater.

6. EFFECT OF THE DIFFERENT RESIDUE TREATMENTS ON THE SOIL WATER CONTENT, TEMPERATURE AND EC.

6.1. Introduction

The Nieuwoudtville area has a semi-arid Mediterranean climate which gets an average yearly rainfall of 500 to 650 mm on the escarpment and drops to around 350 mm in Nieuwoudtville (Manning and Goldblatt, 1997). Most of the rainfall is in the winter months from May to September according to the 2009-2014 average climate data. According to Mooney and Rundel (1979) and Lötter *et al* (2014) plants in the Mediterranean region takes up nutrients during the winter when it is most wet and when the plants have higher photosynthetic rates. It is thus of great importance to conserve soil moisture in the topsoil (0-30 cm), where most of the feeder roots of the rooibos plant exists, to promote nutrient uptake for longer periods.

The biggest reason why mulch/litter layers increase water availability is because of reduced evaporation (Facelli and Picket, 1991). Soil surface water evaporation significantly affects crop water use efficiency. Out of the total evapotranspiration, surface evaporation accounts for 25-50% (Liu *et al.*, 2002). Mulch layers also inhibit light penetration to the soil surface that prevents weed seedlings from growing (Ossom *et al.*, 2001). When weed is absent it can improve water use efficiency significantly.

The aim of this study was to compare the volumetric water content (VWC), temperature and electrical conductivity (EC) at the 5 cm and 20 cm depth for the four residue treatments at different times if the season; before flowering in September, after flowering before and after harvest, and during active nutrient uptake in April/May. Soil specific calibration for the probes was done. The hypothesis is that the soil of the treatment where the natural mulch is removed (A) would have a lower VWC and EC over the long-term compared to the other treatments (B, C and D).

6.2. Results and discussion

The 5TE probe data (VWC, Temp and EC) for different times of the season are showed with different weather conditions at Cloudskraal. The probe data was inspected and given according to the different phenological stadia, the same time when samples were taken. All the volumetric water content (VWC) data displayed for each day, except for the cumulative data, where readings taken at 18:00.

6.2.1. September and October 2015 (before flowering)

The September to October period is just before flowering. The weather data from 23 September to 23 October 2016 are presented in Table 6-1. The reason why earlier data is not shown is because of the subsoil probes that were not yet installed at that time. The weather data presented in Table 6-1 was not from the weather station on the farm, but the weather station of the ARC 8.8 kilometres away (-31.38386, 19.07949) (weather station at farm did not work in that time). The maximum temperature for this time period was 33.58 °C and the minimum 1.51 °C, the precipitation totalled at 4.83 mm.

Table 6-1: Weather data for the 23 September to 23 October 2015 time period (ARC, 2016)

Date	Tmax (°C)	Tmin (°C)	Rain (mm)	Date	Tmax (°C)	Tmin (°C)	Rain (mm)
2015/09/23	25.56	5.70	0	2015/10/09	32.20	6.57	1.27
2015/09/24	24.08	4.75	0	2015/10/10	18.41	4.88	2.29
2015/09/25	24.99	4.21	0	2015/10/11	26.49	2.83	0
2015/09/26	22.19	7.95	0	2015/10/12	21.52	5.09	0
2015/09/27	16.01	4.48	0	2015/10/13	17.50	7.24	0
2015/09/28	21.65	1.51	0	2015/10/14	20.47	6.77	0
2015/09/29	14.87	2.36	1.27	2015/10/15	18.34	3.61	0
2015/09/30	16.35	5.96	0	2015/10/16	27.23	0.26	0
2015/10/01	21.81	3.94	0	2015/10/17	33.58	7.48	0
2015/10/02	23.43	3.77	0	2015/10/18	31.46	6.00	0
2015/10/03	24.52	2.79	0	2015/10/19	27.94	10.02	0
2015/10/04	27.32	4.58	0	2015/10/20	29.46	10.89	0
2015/10/05	24.72	5.38	0	2015/10/21	24.02	11.19	0
2015/10/06	28.74	3.13	0	2015/10/22	32.33	11.80	0
2015/10/07	28.41	1.58	0	2015/10/23	31.39	14.40	0
2015/10/08	25.98	8.29	0				

The soil volumetric water content (VWC) of treatment A, B and C at both depths are shown in Figure 6-1. Figure 6-1 shows data of a dry time period with small increase in wetness on the 26th September. The temperature readings of these probes are shown in Table 6-1.

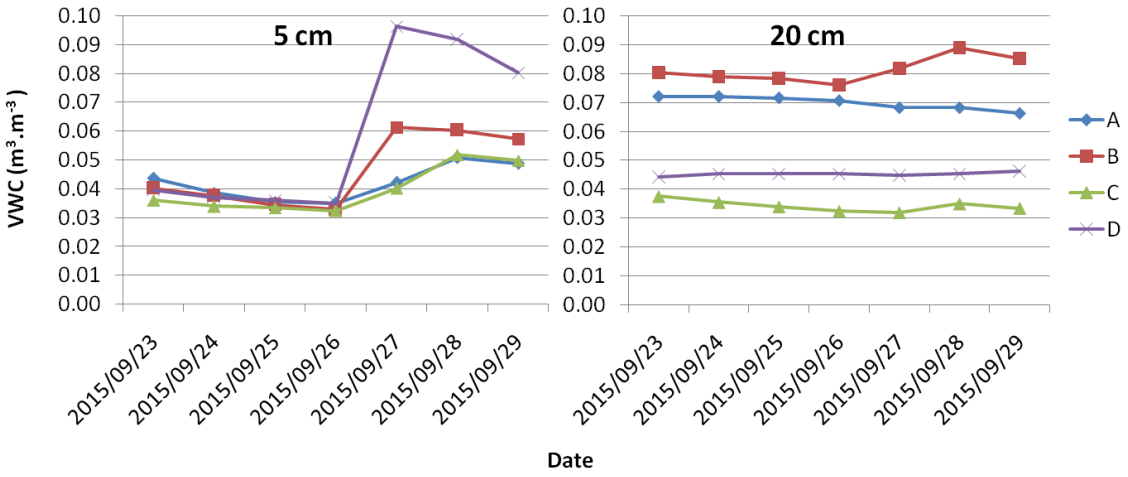

Figure 6-1: VWC for treatment A, B, C and D during a dry period (23 – 29 September 2015), A = bare soil treatment, B = added mulch treatment, C = natural leaf litter and D = added enriched mulch.

In Figure 6-1 can be seen that the VWC for all the treatments in the 5 cm depth were very the same before the increase in VWC on the 26th. At the 20 cm the difference in VWC between the different treatments was bigger.

The weather data in Table 6-1 show no rain on the 26th or 27th but it is possible that there were a small amount of rain on the farm that explain the VWC increase in the 5 cm depth. Treatment D showed a very fast increase in VWC compared to the other treatments, and this could have resulted from the hygroscopic nature of the fertilizer, which helps the soil to retain the moisture. The VWC at both depths for treatment C and D was also the same, compared to the other treatments where there were big differences.

During the dry period, the maximum temperature for the 5 cm deep bare soil treatment was 31.2°C and for the added rooibos mulch treatment 28.5°C. For the subsoil it was 20°C and 21.8°C respectively (Table 6-2). The litter layer of treatment B, C and D managed to maintain a lower maximum soil temperature at the 5 cm layer but had a slightly higher minimum temperature compared to treatment A. At the 20 cm depth it was the opposite, with treatment A having the lower maximum and higher minimum soil temperature.

Table 6-2: Soil temperatures for the different treatments at two depths (23 – 25 September 2015)

Date		Temperature (°C)							
		A (5 cm)	A (20 cm)	B (5 cm)	B (20 cm)	C (5 cm)	C (20 cm)	D (5 cm)	D (20 cm)
2015/09/23	Max	29.4	19.6	28.0	21.4	27.9	21.1	25.8	22.0
	Min	11.2	15.1	11.8	14.8	11.7	14.9	13.0	15.9
2015/09/24	Max	30.2	19.8	27.4	21.4	28.2	20.8	26.6	19.3
	Min	12.3	15.7	12.7	15.3	12.8	15.5	13.4	16.2
2015/09/25	Max	31.2	20.0	28.5	21.8	28.2	21.1	26.6	22.0
	Min	12.3	15.8	13.0	15.5	11.7	14.9	13.0	15.9

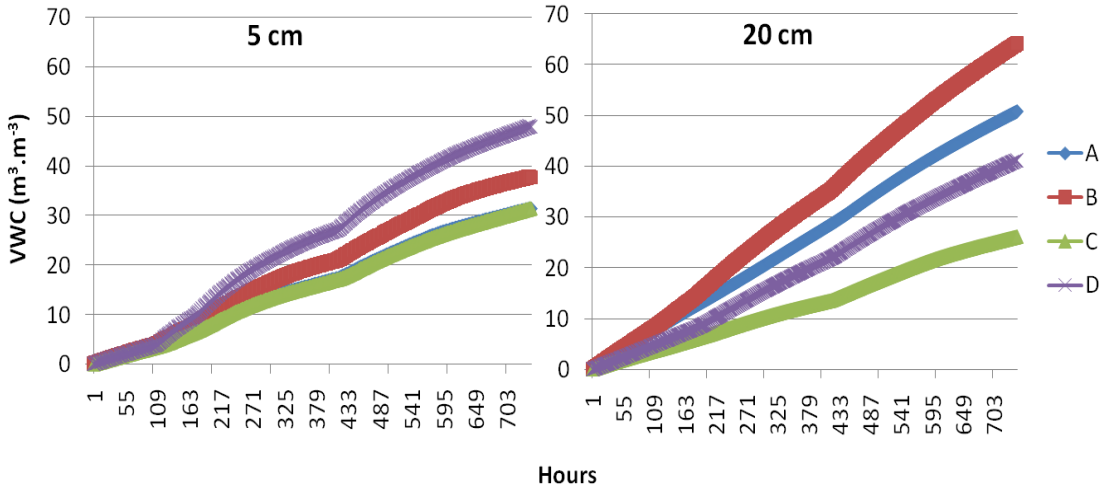

Figure 6-2: Cumulative (hourly) VWC for treatment A, B, C and D (23 September to 23 October 2015), A = bare soil treatment, B = added mulch treatment.

In Figure 6-2 the cumulative (hourly) VWC from 23 September to 23 October 2015 for each treatment and depth is shown. The total precipitation for this time period was only 4.83 mm. This graph helps to show, which treatment had the higher soil VWC for the longest during the whole month period. Figure 6-2 (left) shows that the two added mulch treatments (B and D) had the highest cumulative VWC at the 5 cm depth compared to the treatment A and C. At the 20 cm depth treatment B had the highest cumulative VWC and treatment A the second highest. The lower VWC values for C at the 20 cm depth can be because of gravel in the way which affects the VWC readings taken by the probe.

During August and September of 2015 there was a lot of dense mist on the plateau in the mornings as seen in Figure 6-3. This mist is then intercepted by the rooibos plant where it condensates. Either the droplets fell to the ground (through fall) or they moved via stemflow to the soil. In Figure 6-4 the

effect the mist has on the topsoil VWC is displayed. The photo was taken around 9 a.m. when the mist was still dense.

Figure 6-3: Pictures taken on Cloudskraal on the morning of 24 August 2015.

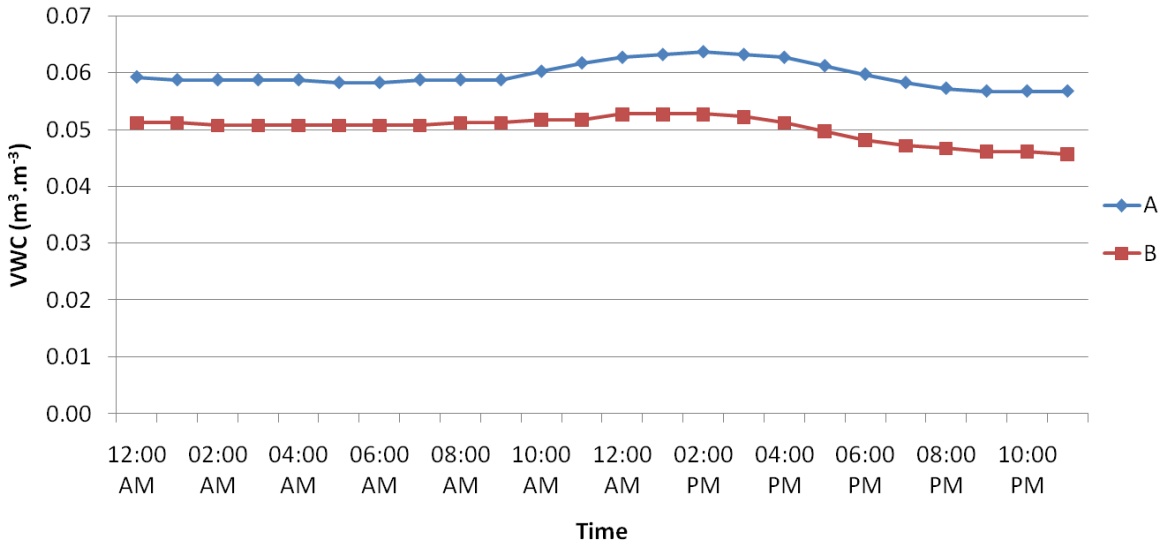

Figure 6-4: The hourly VWC topsoil (5 cm) readings for treatment A and B on 24 August 2015, A = bare soil treatment, B = added mulch treatment.

In Figure 6-4 can be seen that after 08:00 AM there is a small increase in the VWC of the bare soil treatment (A) and the added mulch treatment (B). The added mulch treatment (B) had a lower increase in VWC that can be attributed water retention by the litter layer. It is possible for the litter to limit or reduce plant water availability through retention by litter (Facelli and Picket, 1991). Figure 6-4 shows that the mist on and near the escarpment do add to the soil VWC, and these rooibos plants do benefit from, compared to rooibos plants that are further away from the escarpment.

6.2.2. January and February 2016 (after flowering)

The data presented here is from the time period of 10 January 2016 to 10 February 2016, after flowering, and the weather data for this time period is presented in Table 6-3. During this time the total precipitation for the study site was 57.6 mm and the temperature reached a maximum of 43°C and a minimum of 12.88°C. Compared to older weather data January 2015 was an exceptionally wet month.

Table 6-3: Weather data for the 10 January to 10 February 2016 time period

Date	Tmax (°C)	Tmin (°C)	Rain (mm)	Date	Tmax (°C)	Tmin (°C)	Rain (mm)
2016/01/10	39.50	15.57	0	2016/01/26	26.88	13.56	0
2016/01/11	36.40	16.81	0	2016/01/27	27.17	13.17	0
2016/01/12	33.40	19.00	0	2016/01/28	30.46	14.13	0
2016/01/13	33.85	18.81	0	2016/01/29	37.38	15.38	0
2016/01/14	33.22	16.14	0	2016/01/30	38.83	17.38	0
2016/01/15	30.46	14.80	0	2016/01/31	40.76	19.00	0
2016/01/16	39.50	16.81	8	2016/02/01	43.00	19.57	0
2016/01/17	41.81	20.14	20	2016/02/02	34.37	15.00	0
2016/01/18	35.44	19.95	0	2016/02/03	26.98	13.94	0
2016/01/19	33.64	20.33	0	2016/02/04	32.70	12.88	0
2016/01/20	38.64	19.85	0	2016/02/05	38.83	17.57	0
2016/01/21	28.85	18.05	18	2016/02/06	28.56	15.09	0
2016/01/22	30.05	19.09	11.6	2016/02/07	32.29	15.09	0
2016/01/23	31.88	18.62	0	2016/02/08	36.08	14.42	0
2016/01/24	25.32	14.61	2	2016/02/09	39.05	16.81	0
2016/01/25	25.81	14.13	0	2016/02/10	32.29	18.62	0

The soil volumetric water content for each treatment and two depths are shown in Figure 6-5 and Figure 6-6. The data are presented at two different small times which are short before harvest and short after harvest of each day at 18:00. The temperatures for each probe over the same study period are also shown in Table 6-3 and Table 6-4.

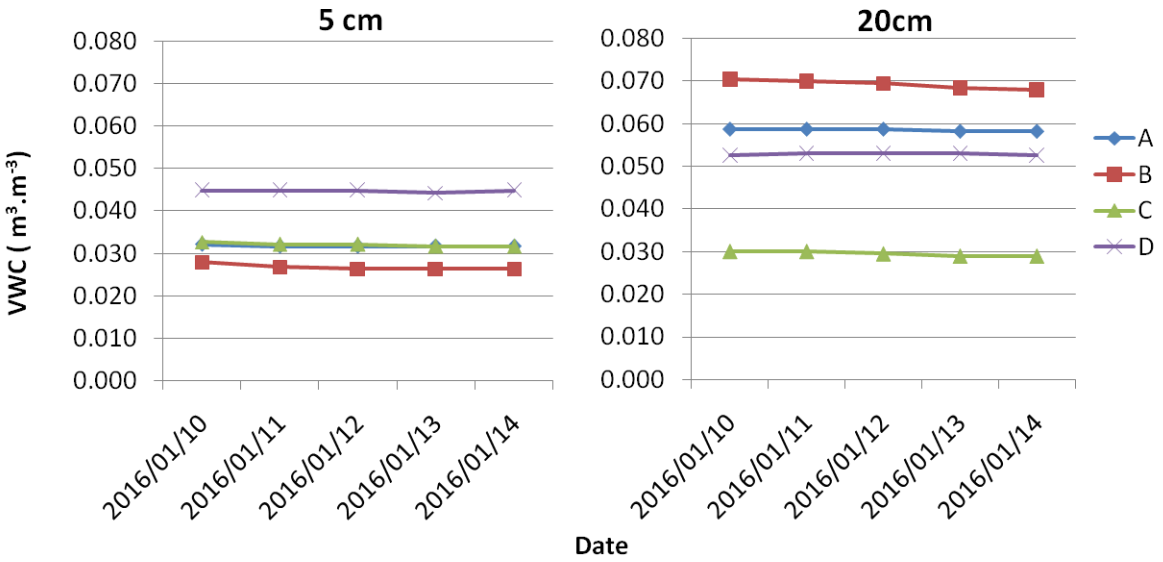

Figure 6-5: VWC for all four treatments during a dry period (10 – 14 January 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

The VWC data in Figure 6-5 represent a dry time period in January 2016. It was noted that for all the treatments the VWC in the subsoil (20 cm) was higher and in the topsoil (5 cm) lower, except for treatment C and D where the VWC at both depths was almost the same. The added mulch treatment (B) had the lowest VWC at 5cm depth but the highest at the 20cm depth. Treatment D which is also a mulch treatment, but enriched with fertilizer, showed the opposite at the 5cm depth by having the highest VWC at the 5 cm depth. The VWC of treatment A and C was the same at the 5 cm depth, but treatment C was the lowest at the 20 cm depth.

At treatment B the soil temperature (5 cm) reached a maximum of 45.9°C and at treatment D 33.9°C (Table 6-3) while the atmospheric temperature was 39.5°C on the 10th of January. This was still before harvest which means the size of the shade of plants can influence the top soil temperature. The plant of treatment D was 94 cm in height and 95 cm in canopy width, while the plant of treatment B was 83 cm in height and 80 cm in canopy width. Not only does the size of the plants result in intercepting more or less direct sunlight but also the plants directly next to it. The smaller plant of treatment B had no plants directly next to it, thus received a lot of direct sunlight on the dark coloured mulch which could've led to the very high soil temperature at the 5 cm depth. The high temperature at the 5 cm depth can lead to the lowering of the relative humidity (RH) and thus lower VWC readings. In general the colour of the mulch does play a role in the performance of plant growth. In a study of Decoteau *et al* (1989) that focused on the effect of different coloured polyethylene mulches on tomato yields it was found that darker (black) coloured mulches led to higher yields compared the lighter coloured (silver and white) ones. The colour of the surface mulch

can thus change the plant microclimate (quantity of light and root zone temperature) (Decoteau *et al.*, 1989).

When the rooibos topsoil root system of the treatment C plant was exposed it was discovered that this plant was in fact 3 plants, not one, that could have contributed to the lower VWC levels in the 5 cm and 20 cm depth.

Table 6-4: Soil temperatures for the different treatments at two depths (10 – 14 January 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

		Temperature (°C)							
Date		A (5 cm)	A (20 cm)	B (5 cm)	B (20 cm)	C (5 cm)	C (20 cm)	D (5 cm)	D (20 cm)
2016/01/10	Max	36.5	30.4	45.9	33.8	40.4	32.0	33.6	30.5
	Min	23.4	27.0	23.5	27.0	24.4	27.0	24.8	27.3
2016/01/11	Max	35.6	30.4	43.7	33.6	39.8	32.2	33.9	30.7
	Min	25.0	28.0	25.1	28.1	25.9	28.1	26.3	28.2
2016/01/12	Max	34.4	30.2	42.5	33.2	38.6	31.7	33.2	30.5
	Min	24.8	27.9	24.9	28.0	25.7	28.0	26.0	28.1
2016/01/13	Max	33.7	30.4	41.7	32.8	38.1	31.5	32.6	30.1
	Min	25.0	27.0	25.1	27.9	25.8	27.8	26.1	28.0
2016/01/14	Max	32.4	29.6	40.7	32.2	37.0	30.8	31.1	29.9
	Min	23.6	27.4	23.9	27.4	24.7	19.0	25.2	27.6

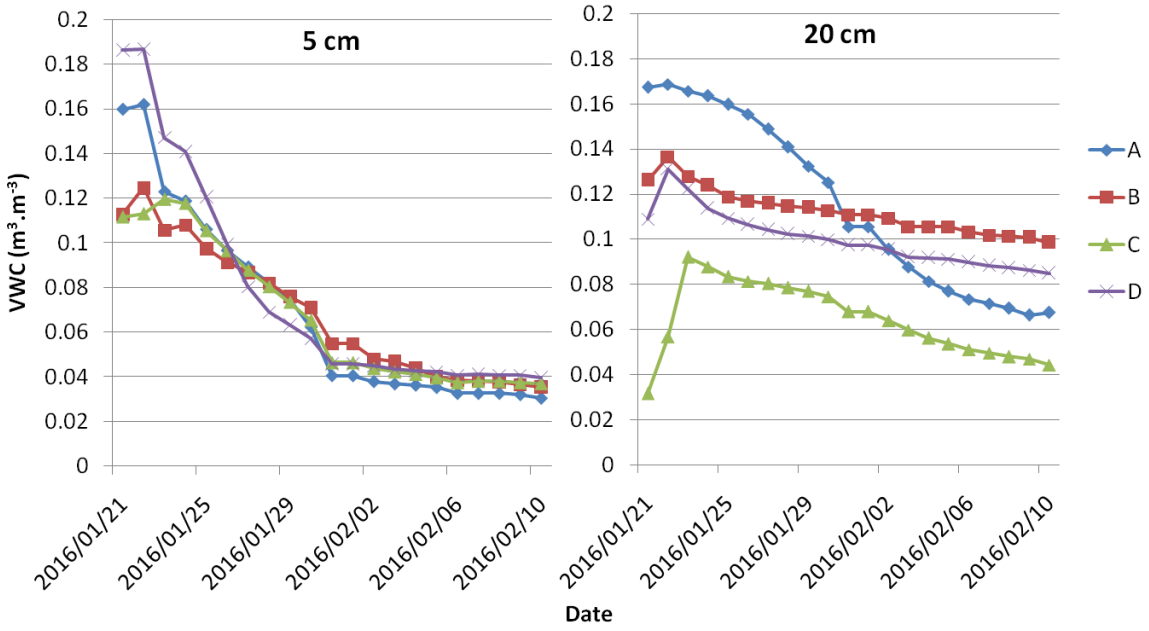

Figure 6-6: VWC for all four treatments during a wet time period, after rain (21 January – 10 February 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

Figure 6-6 show that after rain on the 17th, 21st and 22nd of January 2016 (Table 6-4) there was a much higher VWC in the topsoil (5 cm) of the added enriched rooibos mulch treatment (D) and the bare soil treatment (A). The added mulch (B) and natural leaf litter treatment (C) had a lower VWC at the topsoil (5 cm). After the last rain on the 22nd there was a drastic decrease in VWC for treatment A and D at the 5 cm depth compared to the steady decrease for treatment B and C. When the soils reached field capacity after more than 10 days the VWC for all the treatments were almost the same. Although differences was very small at the 5 cm depth treatment A had the lowest VWC and D the highest.

At the subsoil (20 cm) after the rain the bare soil treatment (A) had the highest VWC, the added mulch (B) and added enriched mulch treatment (D) had a lower VWC, and treatment C the lowest. It was noted that there were a drastic decrease in VWC for treatment A after the last rain, while treatment B, C and D showed the same steady decrease. After a few days when the soils reached their field capacity at the 20 cm depth, treatment B and D had the highest VWC, and treatment C the lowest. The low readings at treatment C can be the result of gravel in the way of the probe.

When looking at the dynamics and change of VWC, Table 6-5, in the soil over time it shows that treatment A and D had a drastic decrease in VWC at the 5 cm depth. Treatment B and C showed the same decrease at the 5 cm depth. Because treatment A, the bare soil treatment had no mulch this change can be attributed to mainly higher evaporation. With treatment D the higher nutrient content could have led to better growth thus higher transpiration. This then also means that much of nutrient uptake can instead occur at the 5 cm depth. At the 20 cm depth only treatment A showed a drastic decrease and big change in VWC over time, while the decrease for treatment B, C and D showed the same decrease. The bigger decrease for treatment A at the 20 cm depth can be attributed to higher rate of transpiration. In a previous study it was demonstrated that in the 5 cm layer of the soil surface mulching reduced soil surface evaporation significantly. The 5 cm mulch layer reduced surface evaporation up to 40% compared to the bare soil water losses (McMillen, 2013). Ramakrishna et al., (2006) also found that the amount of moisture stored up to a depth of 90 cm in the soil profile there was a significant greater under the mulch over the bare soil. Thus as expected the evaporation from the surface of the bare soil was much greater (Ramakrishna *et al.*, 2006).

Table 6-5: Summary of the difference in soil VWC between wet peak of the soil after rain and soil field capacity, A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

Depth	Treatment			
	A	B	C	D
5 cm	0.13	0.09	0.08	0.15
20 cm	0.10	0.03	0.05	0.04

Unfortunately the electrical conductivity (EC) the probes measured were very low and always ranged between 0 and 0.02 dS m^{-1} during this time and it was thus hard to compare EC values, which the probes only registered when soil was moist enough (data not shown). For this reason it was decided to look at the exchangeable Na ($cmol_c$ kg^{-1}) of the soil samples, from the treatments where the probes were installed, taken during this time. In Table 6-6 it can be seen that treatment A and D had the highest Na ($cmol_c$ kg^{-1}). For treatment B, C and D the difference between the 5 cm and 20 cm depth were only 0.01 $cmol_c$ kg^{-1}, but for treatment A it was 0.02 $cmol_c$ kg^{-1}. This bigger difference can be the result of a high evaporation rate at the 5 cm depth and thus the higher exchangeable Na ($cmol_c$ kg^{-1}) compared to the other treatments. The high exchangeable Na for treatment D can be the result of the bigger plant (leaf area) capturing more mist/fog from the air containing salts.

Table 6-6: Summary of the exchangeable Na ($cmol_c$ kg^{-1}) in January 2016 for the soils where probes were installed, A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment. (Values with stars show great VWC difference over time)

Depth	Treatment			
	A	B	C	D
5 cm	0.14	0.08	0.06	0.17
20 cm	0.12	0.07	0.07	0.18

In Table 6-7 the soil temperatures are given for the end of the wet time period when most of the soil water drained and the soils reached its field capacity. This time period was after the rooibos plants of the treatments have been harvested. In this time treatment A, B and C had very high maximum soil temperatures of 46.7°C, 46.4°C and 44.4° respectively while treatment D had a lower topsoil temperature of 37.5°C in the 5 cm depth. The maximum soil temperatures in the 20 cm depth was highest with treatment B at 35.3°C and lowest with treatment D at 31.9°C. The 5 cm depth soil temperature for treatment A and B was very the same after harvest confirming that the size of the plant influenced the soil temperature before harvest before January (Table 6-4). The very small difference for the 5 cm depth soil temperature can be attributed to the dark colour of the rooibos

mulch which can absorb more light compared to other crop mulches, while the soils with very light colour helps it to reflect more light and thus do not have the same build up of heat compared to other darker soils. Other research also found that mulches moderated the soil temperature (8 cm depth) by increasing the daily minimum temperature and decreasing the daily maximum temperature (Maggard *et al.*, 2012). Mulch moderated soil temperature, decreasing daily maximum and increasing daily minimum temperatures (Table 6-7).

Table 6-7: Soil temperatures for the different treatments at two depths, A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment

		Temperature (°C)							
Date		A (5 cm)	A (20 cm)	B (5 cm)	B (20 cm)	C (5 cm)	C (20 cm)	D (5 cm)	D (20 cm)
2016/02/07	Max	43.1	31.3	42.8	33.9	41	32.2	35.8	30.8
	Min	23.7	27.1	24.4	26.8	24.5	24.7	25.2	27.3
2016/02/08	Max	44.4	31.6	44.2	34.3	42.8	32.8	36.6	31.2
	Min	23.4	27.2	23.8	26.6	24.2	26.7	25	27.3
2016/02/09	Max	46.7	32.4	46.4	35.3	44.4	33.7	37.5	31.9
	Min	24.3	27.7	24.6	27.3	25.1	27.4	25.8	27.9
2016/02/10	Max	44.5	32.1	44	34.6	42.9	33.1	36.9	31.6
	Min	24.6	28.4	24.9	27.9	25.4	28	26.2	28.5

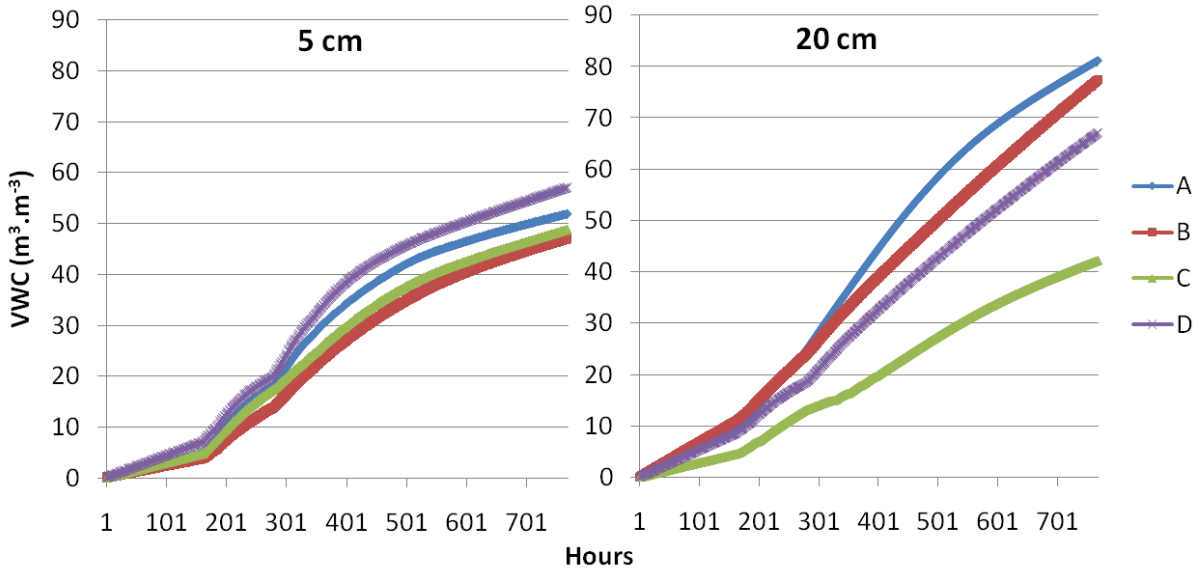

Figure 6-7: Cumulative VWC (hourly) for all four treatments at both depths from (10 January to 10 February 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

After a one month period from 10 January to 10 February the cumulative VWC in the 5 cm depth for the added enriched mulch treatment (D) was highest with the added mulch treatment (B) having the lowest cumulative VWC (Figure 6-7). At the 20 cm depth the cumulative VWC for the bare soil treatment (A) was the highest, with the added mulch (B) and added enriched mulch treatment (D) having the 2nd and 3rd highest cumulative VWC. The probe at the 5 and 20 cm depth for treatment C gave error readings when the soil moisture was very high. During this time period the total precipitation was 57.6 mm. It was noted that the increase in VWC at the 5 cm depth with treatment A and D was almost the same, but as time went on treatment D conserved more water. For treatment A at both depths there is a more notable decrease in VWC compared to the other treatments when conditions begun to get dry. All the rain for this month period was received in the first half up until 301 hours in Figure 6-7 where the difference in soil VWC at the 5 cm depth between the treatments was small. After this a distinct decrease was noted for treatment A when rain was absent for a long time, thus dry conditions.

In Figure 6-8 the rainfall for this month period and the soil profile (30 cm) VWC for each treatment are shown in the same graph. The VWC readings for the 5 cm probe represented the top 10 cm of the topsoil profile while the VWC readings of the probe at the 20 cm depth represented the next 20 cm depth (10 – 30 cm). The gravelly E horizon started at the 30 cm depth. The VWC of the topsoil profile were thus calculated as followed:

$$Soil\ profile\ (30\ cm)VWC = \ VWC\ (5\ cm)\left(\frac{10}{30}\right) + \ VWC\ (20\ cm)\left(\frac{20}{30}\right)$$

From Figure 6-8 can be seen that in the beginning before harvest the total VWC for treatment B was the highest while for treatment C the lowest. Again the low total VWC for treatment C may be attributed to gravel in the way of the probe. It is also visible that when it rain and the soil became really wet the readings for treatment C were problematic. After last rain on the 24th and harvest at the end of January it was noted that all the mulch treatments, except for treatment C, conserved soil moisture compared to treatment A (bare soil).

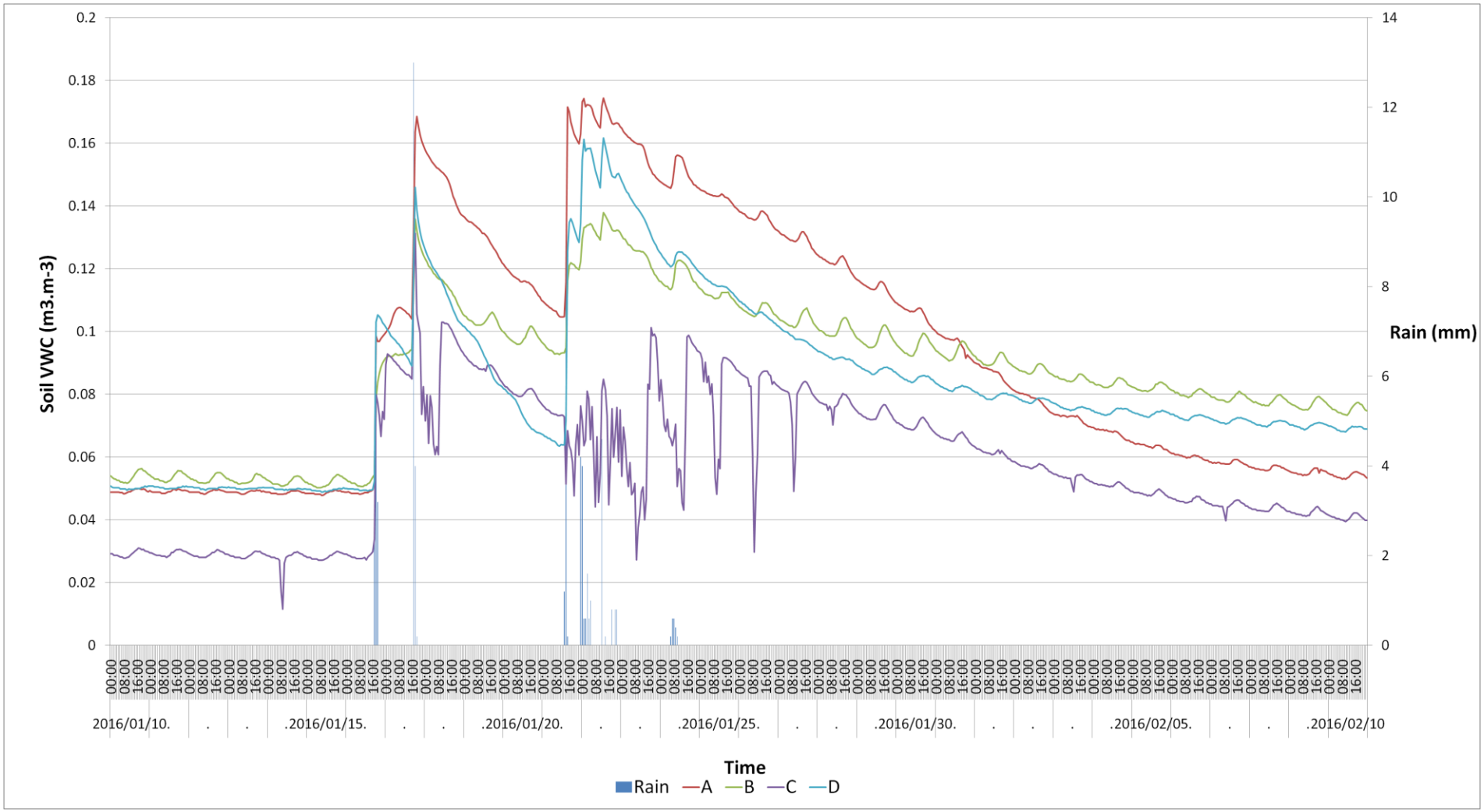

Figure 6-8: Soil profile (30 cm) VWC for all four treatments and rainfall from 10 January 2016 – 10 February 2016. A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

6.2.3. April and May 2016 (beginning of active nutrient uptake)

Total precipitation of 63.4 mm was recorded for the 6 April 2016 to 6 May 2016 period, near the average of previous years. The temperature reached a maximum of 35.76 °C and a minimum of 3.88 °C. Compared to older weather data the precipitation for this period was a little higher.

Table 6-8: Weather data for 6 April to 6 May 2016

Date	Tmax (°C)	Tmin (°C)	Rain (mm)	Date	Tmax (°C)	Tmin (°C)	Rain (mm)
2016/04/06	28.66	11.33	0	2016/04/22	23.77	11.72	16
2016/04/07	28.26	12.69	0	2016/04/23	21.28	9.77	1
2016/04/08	28.95	14.61	0	2016/04/24	25.42	8.88	0
2016/04/09	31.06	15.95	0	2016/04/25	17.95	7.38	0
2016/04/10	33.12	17.76	0	2016/04/26	23.87	5.14	0
2016/04/11	35.76	13.17	0	2016/04/27	24.74	3.88	0
2016/04/12	28.56	11.24	0	2016/04/28	23.58	7.88	0
2016/04/13	28.36	12.69	0	2016/04/29	14.33	7.18	43.2
2016/04/14	31.17	12.59	0	2016/04/30	18.33	4.62	0
2016/04/15	33.37	16.71	0	2016/05/01	21.66	5.45	0
2016/04/16	32.6	15.76	0	2016/05/02	23.39	7.58	0
2016/04/17	27.76	16.43	0	2016/05/03	24.16	8.88	0
2016/04/18	34.06	13.94	0	2016/05/04	27.27	9.47	0
2016/04/19	30.96	17.38	0	2016/05/05	29.95	12.21	0
2016/04/20	26.78	12.4	0	2016/05/06	27.08	12.98	0
2016/04/21	19.38	11.82	4.2				

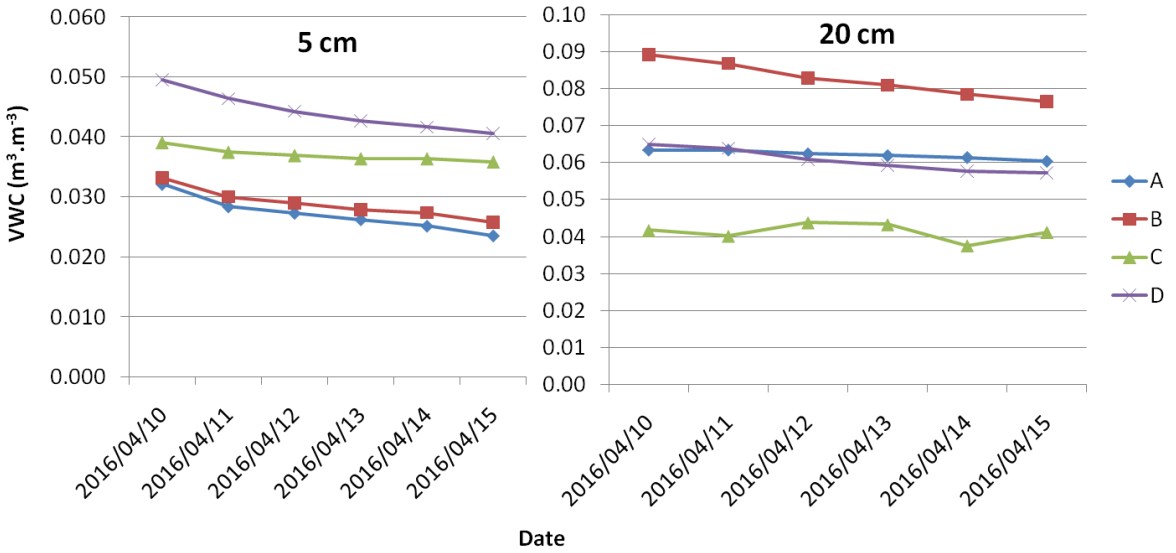

Figure 6-9: VWC for all the treatments during a dry time period (10 – 15 April 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

During the dry period, illustrated in Figure 6-9, when rain was absent the added enriched mulch treatment (D) and natural mulch treatment (C) had the highest VWC at the 5 cm depth while the VWC of the added mulch treatment (B) were lower and the bare soil treatment (A) the lowest. At the 20 cm depth the added mulch treatment (B) had the highest VWC, the added enriched mulch (D) and bare soil treatment (A) had a lower VWC. The natural mulch treatment (C) had the lowest VWC throughout this time period which can be the result of gravel in the probe's way. The VWC for treatment D at the 5 and 20 cm depth were very similar. It was noted that the VWC trend in Figure 6-9 for both depths was the same as for the VWC at the end of the time period for Figure 6-6 when soils were at field capacity. At the 5 cm depth treatment A had the lowest VWC that meaning evaporation was higher.

Table 6-9 show the soil temperatures for each treatment and it was noted that treatment A, B and D had high soil temperatures at both depths. Treatment B and C had the highest maximum soil temperatures of 35.1°C and 34.6°C respectively at the 5 cm depth, and a maximum temperature of 26.4°C and 26.1°C respectively at the 20 cm depth.

Table 6-9: Soil temperatures for the different treatments at two depths (12 – 15 April 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

Date		Temperature							
		A (5 cm)	A (20 cm)	B (5 cm)	B (20 cm)	C (5 cm)	C (20 cm)	D (5 cm)	D (20 cm)
2016/04/12	Max	28.4	23.9	31.2	25.1	30.0	24.8	27.3	24.6
	Min	17.9	21.0	17.3	20.6	18.3	17.2	19.0	21.3
2016/04/13	Max	30.3	24.3	33.4	25.7	33.0	25.4	28.7	24.3
	Min	17.7	20.7	17.2	20.3	18.0	20.6	18.7	20.9
2016/04/14	Max	30.9	24.4	34.6	26.0	33.8	25.5	29.2	24.4
	Min	17.4	20.5	16.9	20.1	17.7	20.4	18.4	20.8
2016/04/15	Max	31.6	24.9	35.1	26.4	34.6	26.1	30.2	25.0
	Min	19.2	21.2	18.8	21.0	19.5	21.2	20.1	21.6

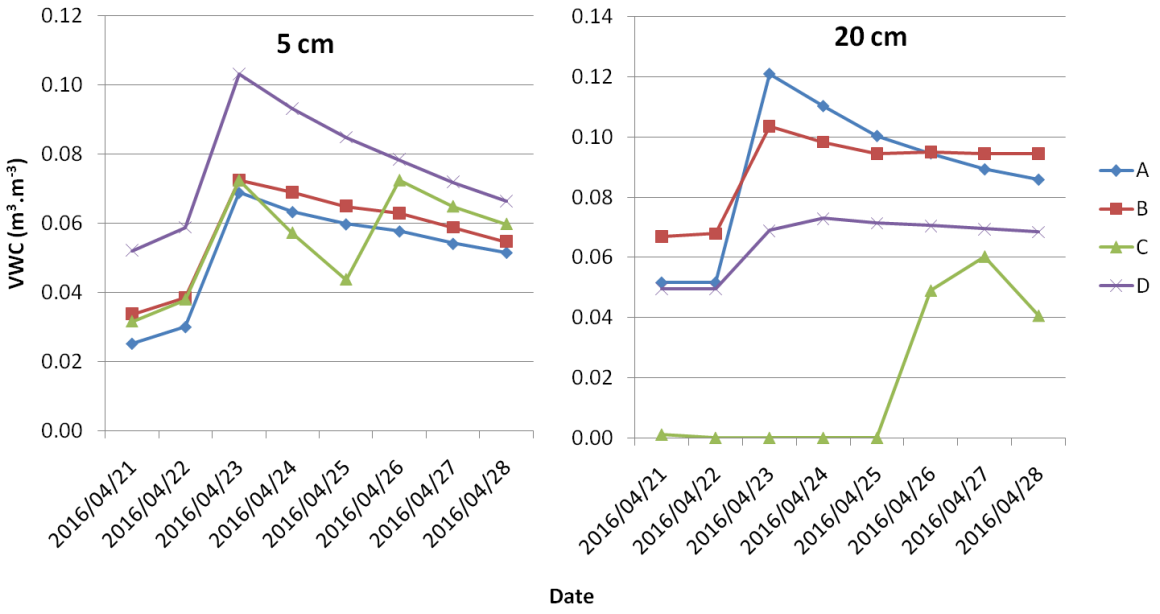

Figure 6-10: VWC for all the treatments during a wet time period (21 – 28 April 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

Again the probes for treatment C gave problematic readings when the soil was too wet and was thus ignored till the 27th. After rain on the 21st and 22nd treatment D had the highest VWC at the topsoil while treatment A had the lowest VWC throughout this time period (Figure 6-10), but all the treatments showed identical increase over this time. At the 20 cm depth a very quick increase in VWC is visible for treatment A but also a fast decline after one day. This means that the water infiltrated from the 5 cm depth to the 20 cm depth faster than the rest. After the smaller VWC increase for treatment B and D in the 20 cm depth, the soil moisture was conserved for longer compared with treatment A. At the end of this time period treatment B had the highest VWC and the bare soil treatment (A) the second highest VWC.

In Table 6-10 the soil temperatures are shown of a few days after the rain. All the treatments, except for the added enriched mulch treatment (D), reached a maximum topsoil temperature of ± 26°C in this time. Treatment C and D had the highest 20 cm soil temperature of 10.9°C. It was noted that treatment A at the 5 cm depth had the same decrease rate compared to B and C. This is different when compared to Figure 6-6. This difference can be attributed the difference in temperature between these times. In January the air temperatures were much higher which could have led to higher evaporation.

Table 6-10: Soil temperature for the different treatments at two depths (25 – 28 April 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

Date		Temperature							
		A (5 cm)	A (20 cm)	B (5 cm)	B (20 cm)	C (5 cm)	C (20 cm)	D (5 cm)	D (20 cm)
2016/04/25	Max	21.5	18.6	20.3	18.7	21.5	-5.5	20.2	19.1
	Min	12.9	16.0	13.3	15.8	1.1	-31.0	13.9	16.4
2016/04/26	Max	25.4	19.3	26.0	20.1	25.2	20.0	22.0	18.7
	Min	9.4	13.9	9.8	13.3	-4.3	-27.5	10.7	14.4
2016/04/27	Max	25.3	19.3	25.3	19.9	25.3	20.0	22.2	18.7
	Min	10.0	14.1	10.2	13.5	10.9	6.1	10.9	14.4
2016/04/28	Max	26.0	19.9	26.2	20.6	26.1	20.6	23.2	19.4
	Min	11.8	14.9	11.9	14.4	1.6	-19.1	12.6	15.3

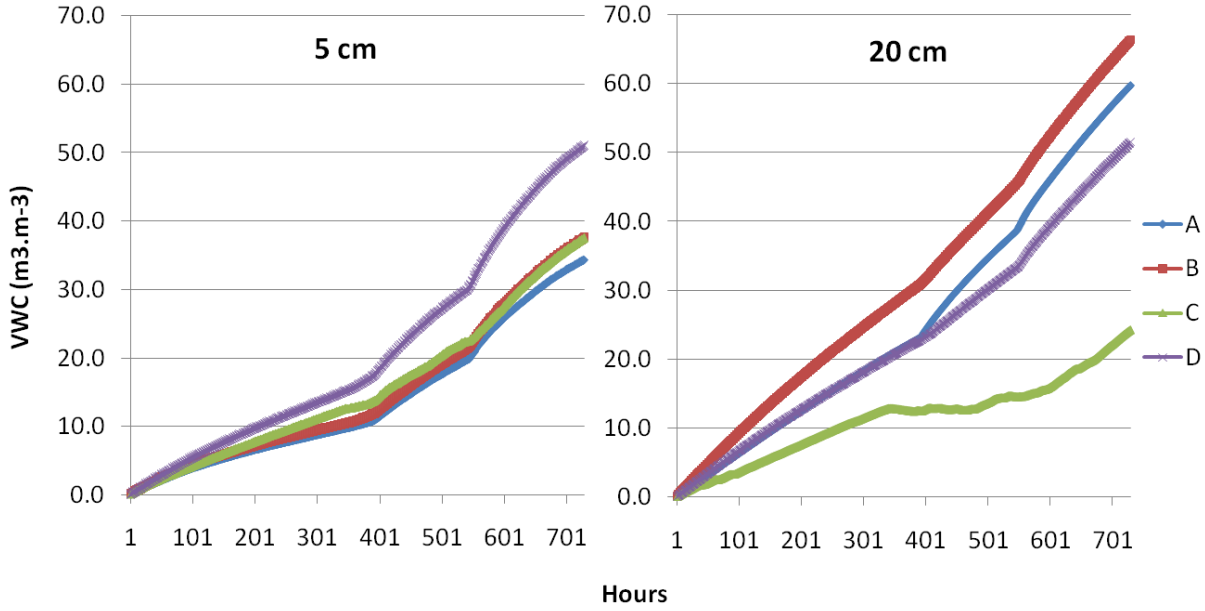

Figure 6-11: Cumulative VWC (hourly) for all four treatments at both depths (6 April to 6 May 2016), A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

The readings of treatment C was ignored (Figure 6-11). After a one month period from 6 April to 6 May 2016 the added enriched mulch treatment (D) had a much higher cumulative VWC in the 5 cm depth and the bare soil treatment (A) had the lowest cumulative VWC. In the 20 cm depth the added mulch treatment (B) had the highest cumulative VWC with the bare soil treatment (A) a bit lower and the added enriched mulch treatment the lowest out of the 3 treatments. In this month time period the total precipitation was 63.4 mm, the highest compared to the other time periods discussed in this chapter. It was noted that in the end at the 5 cm depth of treatment A, the VWC decreased more drastically compared to the other treatments which can be attributed to

evaporation. With the other treatments the mulch acted as soil water evaporation barrier. During this time period rainfall was high and frequent (Table 6-8). This led to the small difference between treatment A and B at the 5 cm depth.

Figure 6-12 shows that in the beginning of the time period (when rain was absent) the mulch treatments (B and D) had a higher VWC in its soil profile compared to treatment A (bare soil).

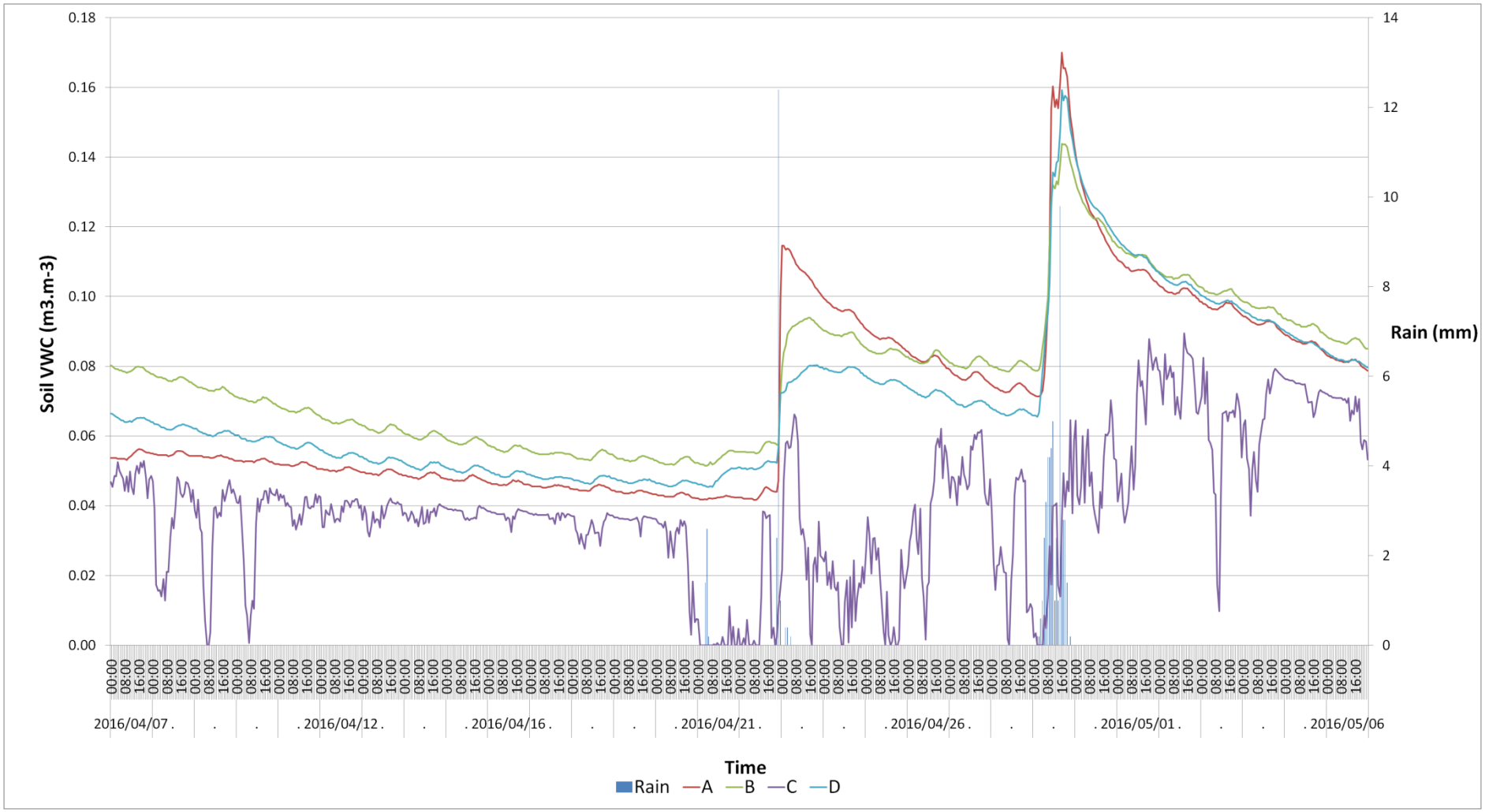

Figure 6-12: Soil profile (30 cm) VWC for all four treatments and rainfall from 7 April 2016 – 6 May 2016. A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment

6.2.4. Root excavation after study

After the trials were done in June 2016 the 30 cm deep soil profile was dig on the side of the plant where the probes were installed. An 8×8 cm block grid was then put next to the root system of the plant and a picture taken as seen in Figure 6-13. This was done to help see the size of the topsoil root system and how this might have impacted the VWC of the topsoil.

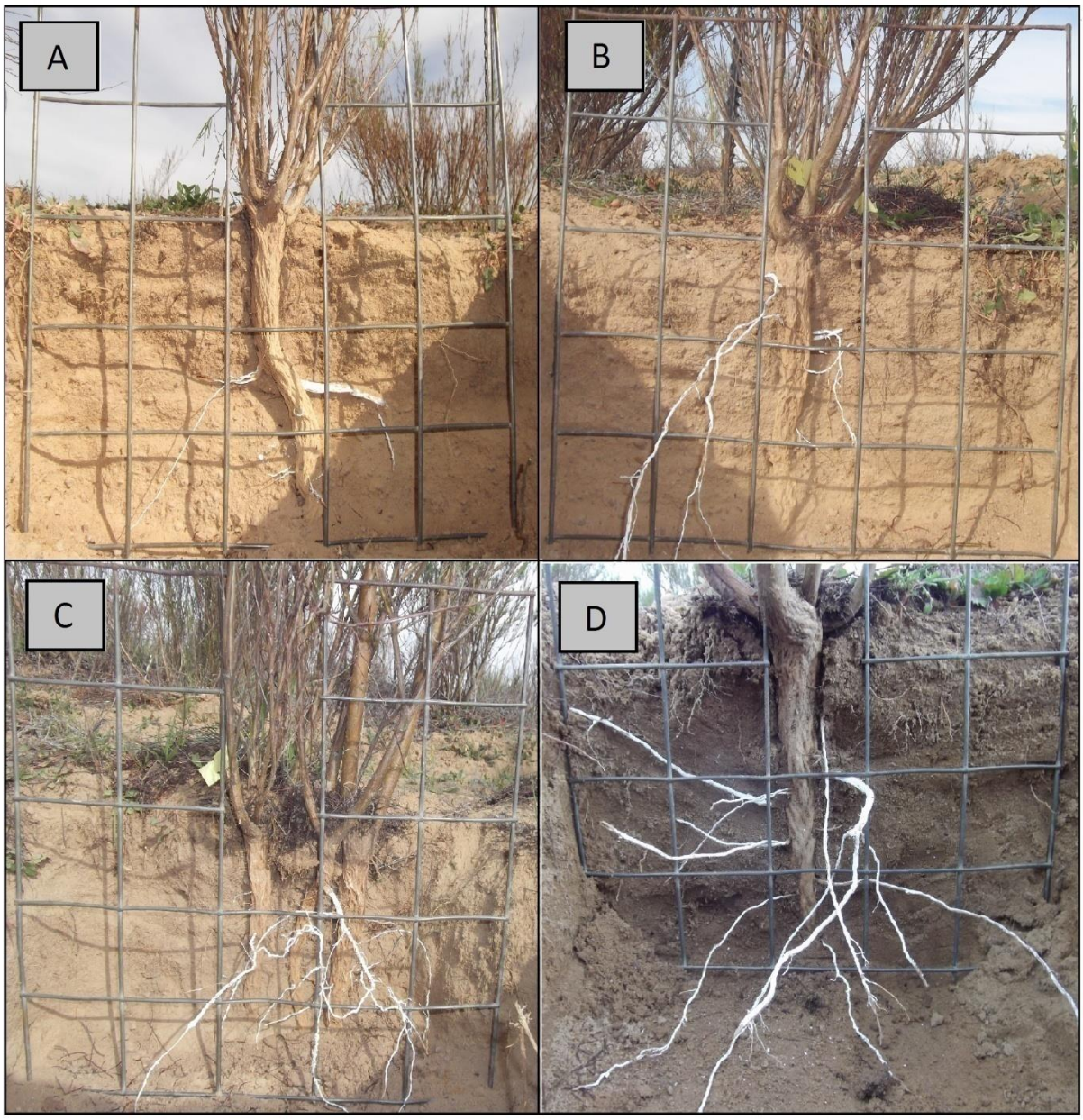

Figure 6-13: Pictures taken of the root system for each plant where the probes where inserted, A = bare soil treatment, B = added mulch treatment, C = natural mulch treatment and D = added enriched mulch treatment.

After topsoil (30 cm) root excavation it was possible to look at the root growth and spread for the rooibos plant of each treatment. Most of the visible (white) roots occurred between the 8 cm and

30 cm depth of the profile. In the top 8 cm depth there was also very fine roots visible, but was too difficult to colour and prone to breaking trying to remove the soil around it.

6.3. Conclusion

It was visible that the size of the plants where the probes was installed played a big role in the volumetric water content and temperature of the soils before the rooibos plant was harvested at the end of January. The leaf interception of both light and rain played a large part in the status of the VWC and the temperature of the soils. The bigger plants intercepted more light during the day than the smaller/thinner plants. This led to a higher soil temperature at the 5 cm depth for the smaller plants compared to the bigger plants that cast a shadow over the top soil. During precipitation some of the rain gets intercepted by the plant, which evaporates later, and some reaches the soil under the plant via throughfall and stemflow. Bigger plants intercept more rain compared to smaller plants and this leads to less water reaching the soil. The topsoil of the bigger plants (during this time the plant of treatment A was bigger) thus shows a slower increase in VWC and lower VWC than the smaller plant (treatment B) (Figure 6-1). This was all before harvest.

After harvest at end of January during times when there were frequent and high rainfall the VWC in the topsoil profiles were more or less the same (April and May), but when looking at the dynamics it was noted that with treatment A (bare soil) when dry weather prevailed (after 25 January 2016) the decrease was much faster compared to the mulched treatments. It thus shows that during times of sufficient and frequent rain the mulch treatment does not offer much of an advantage over the bare soil treatment. However, when its dry the mulch treatments conserved more water for longer. The mulched treatments moderated the soil temperature by increasing the daily minimum temperature and decreasing the daily maximum temperature at the 20 cm depth.

7. NEAR-INFRARED SPECTROSCOPY FOR SOIL AND PLANT CHEMICAL CHARACTERIZATION

7.1. Introduction

Smaller farmers also need to increase productivity to survive financially and the one way to do that is with precision farming. With precision farming soil nutrient management is very important and is required to prescribe correct fertilizer practises. Making precision farming for smaller farmers possible a less expensive method of analyzing soil and plant material is necessary. To find a cheaper analytical method that offers rapid and accurate analysis near-infrared reflectance spectroscopy (NIRS) was tested. This method with the help of chemometrics was used to see if it can successfully predict the soil and plant chemical parameters. The aim of this study was to evaluate the potential of NIRS to predict various chemical components in soil and rooibos plant material from the Nieuwoudtville rooibos region in the Northern Cape.

Over the last two decades there was a increase in interest for NIR spectroscopy in soil science (Stenberg *et al.*, 2010). Near infrared spectroscopy is a low cost method that can either be used for complementing or substituting of traditional characterization methods. A large number of samples, collected and analyzed, are required to capture the spatial variability. When calibration is done the prediction of multiple soil properties simultaneously is possible (Bilgili *et al.*, 2010).

7.2. Data analyses

7.2.1. Chemical analysis (reference data)

In **Chapter 3** the chemical analysis methods for the soil and plant chemical properties is discussed.

7.2.2. Principle component analysis (PCA)

A chemometric software package namely SIMCA^{TM} (Version 14, 2015, MKS Umetrics, Sweden) was used to evaluate the spectral and reference data. The use of this software is great for illustrating the statistics graphically. In chemometrics is PCA one of the most powerful and important analysing methods as well in a lot of other areas (Bro and Smilde, 2014). PCA is a simple, non-parametric method where relevant or the most important information can be extracted from complex data sets, by reducing a complex data set to a lower dimension (Shlens, 2014). PCA was done with the goal to compress the data, reduce dimensionality, decrease redundancy, and to create dynamics of the system by identifying how different variables work together. Within the dataset PCA also contains the maximum amount of variance. The two principal components (PC) in a PCA are a set of linearly uncorrelated variables that show the most variation. The first PC, a linear combination of x-variables,

accounts for the maximum variance possible in a dataset, where the second PC is accounting for the maximum of the remaining variation possible. With PCA complex information can be presented visually and makes it possible to see patterns or trends and outliers in the structure of the PCA.

PCA of soil spectra and reference data

For spectra PCA model 4 PC's were selected that accounted for 99.8% of the variance. The PCA t-scores for component 1 and 2, illustrated in Figure 7-1, covered the maximum variation in the x-space with R2X values of 0.918 and 0.0414. In Figure 7-1 can be seen that the data points (scores) are coloured according to the region in the Bokkeveld Plateau and in Figure 7-2 the data points are coloured according depth. The PCA score scatter plot show no clear groupings between the regions, but a few possible spectral outliers is visible.

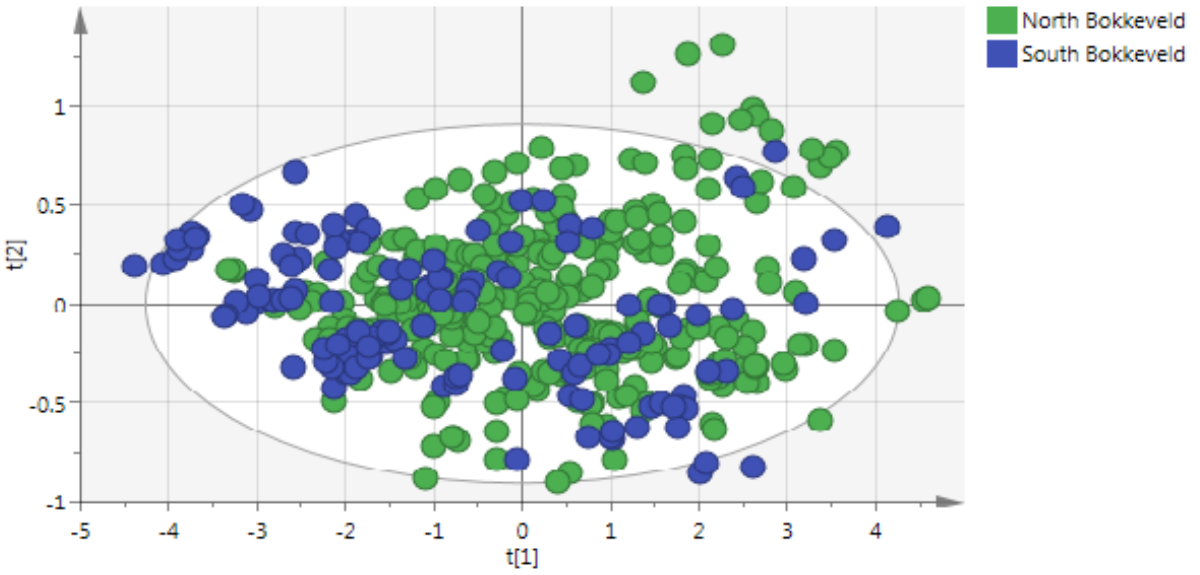

Figure 7-1: PCA score scatter plot for the soil spectral data and coloured according region.

No groupings between the topsoil and subsoil in the Figure 7-2 is visible similar to Figure 7-1.

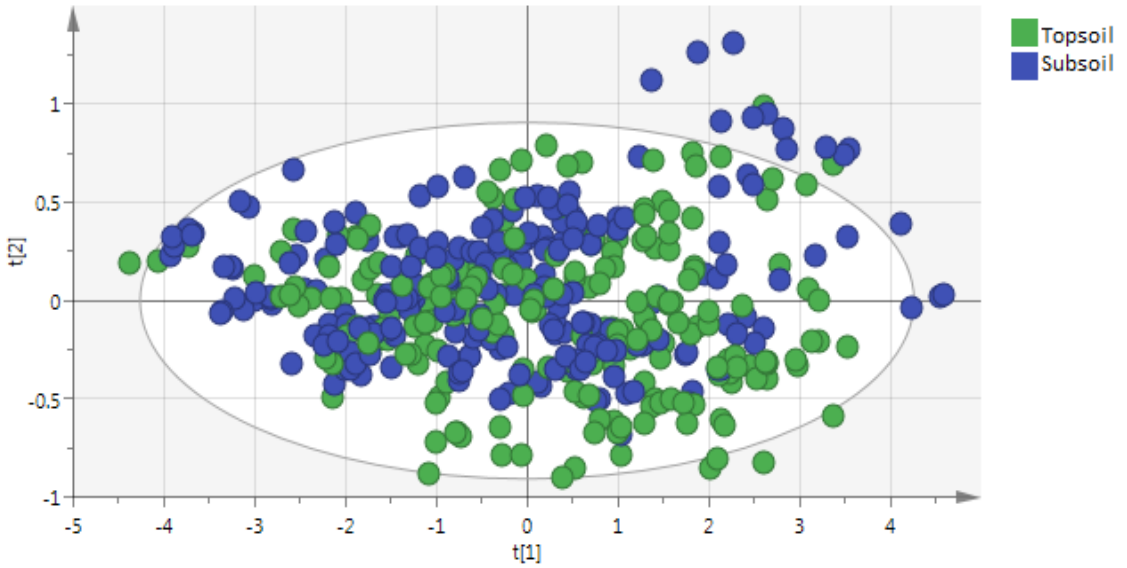

Figure 7-2: PCA score scatter plot for the soil spectral data and coloured according depth.

The PCA model for the reference data, illustrated in Figure 7-3 and Figure 7-4, have 2 PC's selected that accounted for 49.2% of the variance. The PCA t-scores for the two components covered the maximum variation in the x-space with R2X values of 0.346 and 0.146. In Figure 7-3 and Figure 7-4 the data points were coloured according the region and depth.

In Figure 7-3 groupings, not definite, of the two regions is visible. Most of the blue South Bokkeveld data points are located in the left bottom corner of the PCA score scatter plot.

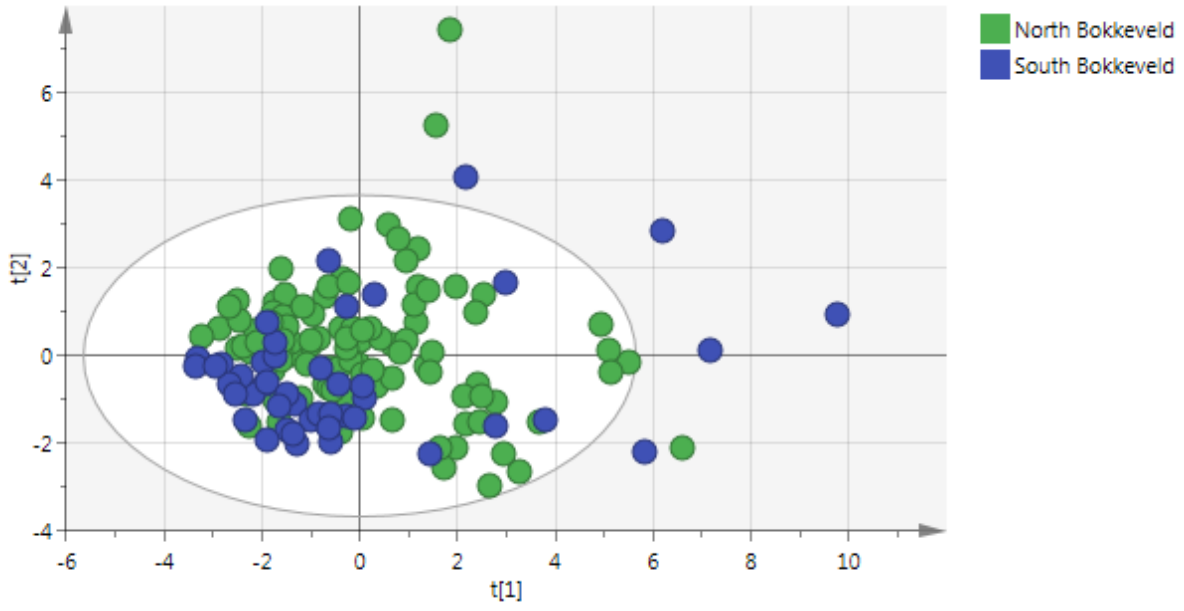

Figure 7-3: PCA score scatter plot for the soil reference data and coloured according region.

In Figure 7-4 there are also two groupings visible between the topsoil (5 cm) and subsoil (20 cm) data points. Most of the topsoil data points are located in the bottom half of the PCA score scatter plot and the subsoil data points in the top half.

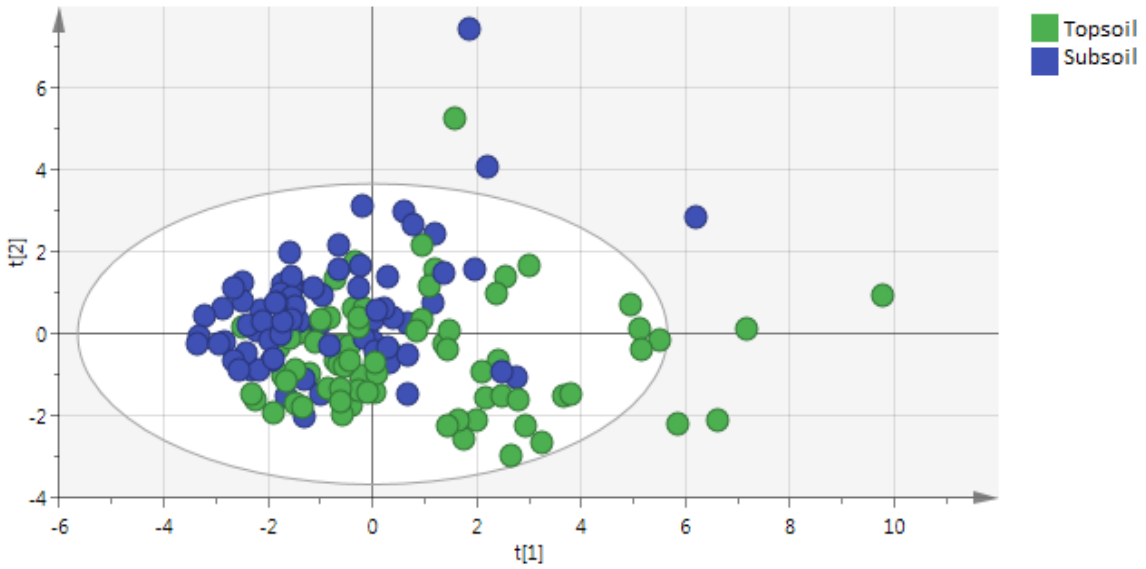

Figure 7-4: PCA score scatter plot for the soil reference data and coloured according depth.

PCA of plant spectra and reference data

6 PC's was selected for this PCA model (Figure 7-5) and they accounted for 99,7% of the variance. The PCA t-scores of PC 1 and 2 covered the maximum variation in the X-space with R2X values of 0.628 and 0.344. Plant samples were taken from 4 different farms and coloured accordingly in the PCA model. In Figure 7-5 groupings between the farms is visible. The Cloudskraal and Oorlogskloof samples are grouped together on the top part of the PCA, while the De Dammetjies and Grootlaagt samples are grouped in the lower part of the PCA. This PCA shows that the plant spectra of the most southern sites, De Dammetjies and Grootlaagt, are different from the northern sites.

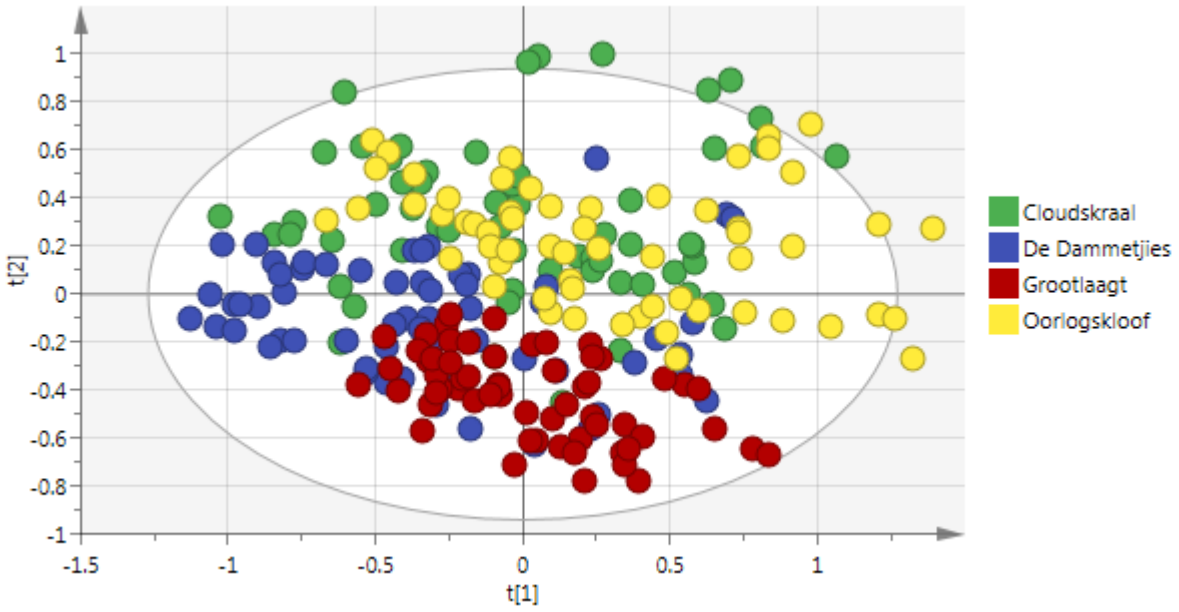

Figure 7-5: PCA score scatter plot of the leaf spectral data and coloured according to farm.

For the PCA of the leaf reference data only one PC was identified which accounted for 25.6% of the variation in X with a R2X value of 0.256 (Figure 7-6).

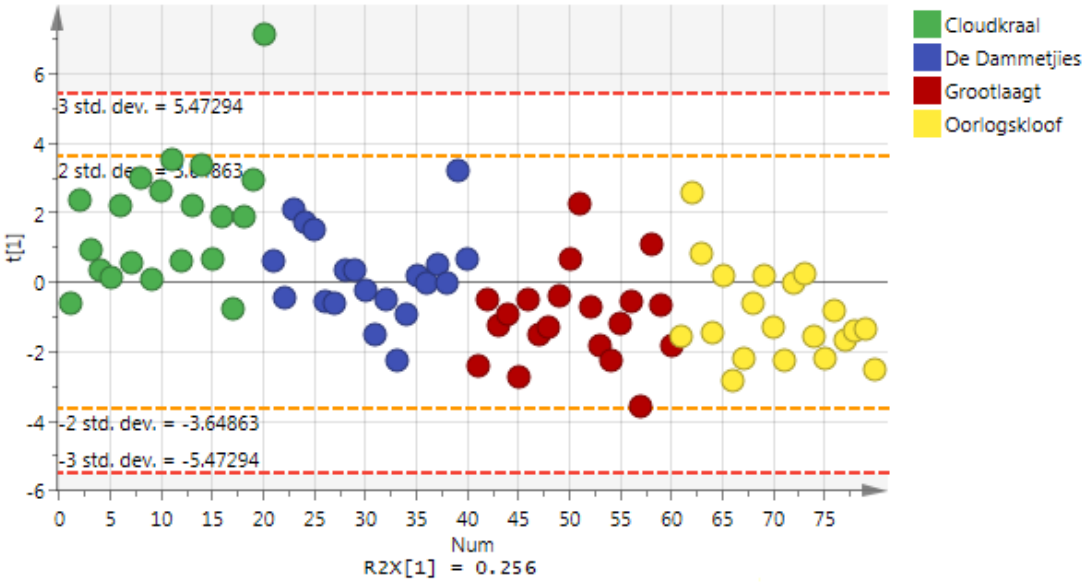

Figure 7-6: PCA score scatter plot for the leaf reference data and coloured according to farm

From the spectral and reference data PCA score scatter plots of the soil and plant samples possible outliers were detected. The data points that were far placed from the centre of the PCA were considered as possible outliers.

7.2.3. PLSR modelling

A chemometric analysis namely partial least-squares regression (PLSR), a multivariate calibration technique, was used to calibrate the spectral data with the laboratory (reference) soil and leaf data. The calibration models were then validated using test set validation. With quantitative spectral analysis is PLSR as a modelling technique in chemometrics very popular (Rossel *et al.*, 2006). PLS models are based on the principal components (PCA) (Zornoza *et al.*, 2008) of the independent (X) and dependent data (Y). PLS build a regression model between the calculated PC scores, not the original data.

PLSR was used to build a predictive model using both the NIR spectra data (X) and the reference data (Y), which is the soil/plant chemical analysis done in the lab. PLSR helps to find a covariance between Y and X with the goal to predict Y from X. The prediction equation is given below:

$$Y = b, X$$

Y is the target parameter and it relates to the various soil properties analysed in the laboratory. The same goes for the plant properties analysed in the laboratory. With the help of the calibration function (b) and the NIR spectral data (X) the specific soil or plant property can be predicted. Building this prediction model both the NIR spectra matrix and the matrix of the soil and plant analysed chemical properties (reference data) is used. The soil NIR spectra matrix consists of 456 scans, 3 scans per sample, which are used for calibration, and all the absorbance values from 800 to 2777.7nm. The plant NIR spectra matrix consists of 240 scans, 3 for each sample, and all the absorbance values between 1063 and 2355 nm. These matrixes were the source for the X-variables in the prediction model. The chemical results of the soil and plant analysed in the laboratory was the matrix for the y-variables. The soil chemical results matrix consists of all the analyzed and calculated soil properties (15) for each of the 152 soil samples. The leaf chemical results matrix consists of all the analyzed leaf properties (13) for each of the 80 plant samples. These matrixes were the source of the Y-variables in the prediction equation (Zornoza *et al.*, 2008). OPUS 7.2 software was used to build the predictive models for each chemical property of the soil and plant samples.

Data pre-processing of the spectral data eliminate, fully or partly, the systematic errors caused by various factors and therefore it is very important (Galvez-Sola *et al.*, 2015). It also ensure that there is a good correlation between the concentration values and spectral data (Bruker-Optics, 2011). Pre-processing methods of OPUS 7.2 software includes the following: no spectral data pre-processing, constant offset elimination, straight line subtraction, vector normalization (SNV), min-max normalization, multiplicative scattering correction (MSC), first derivative, second derivative, first

derivative + straight line subtraction, first derivative + vector normalization (SNV) and first derivative + MSC. All pre-processing methods were selected for which the OPUS software generated a prediction model and the model with the smallest RMSECV was selected.

Detecting outliers

Each component was handled separately, thus was there a different calibration and validation set for each component. In the PCA's (Figure 7-1 to Figure 7-6) possible spectral outliers were identified as the data points found far from the centre of the PCA model. When one of these outliers was responsible for a bad calibration model, confirmation that it was also a calibration outlier; they were excluded from dataset to prevent it from skewing the prediction model. If all three spectra of one sample were excluded the reference data (Y) of the sample was also excluded. The basis for calibration outliers of the chemical variables in the calibration set was because of big true sample differences and a result of poor chemical analysis in the laboratory (Todorova *et al.*, 2014). For example, sample RB 3-2; 48A-2 and 49A-2 have Zn (mg kg^{-1}) values of 108.3; 7.3 and 7.4 respectively compared to the average of 1.27 mg kg^{-1} for other samples. With RB 3-2 there was definitely an analysis error in the lab while with 48A-2 and 49A-2 the Zn levels could have just been very high in the soil.

7.2.4. Model selection

The performance of the prediction models were evaluated with the help of the following calibration statistics: coefficient of determination (R^2), root mean square error of cross validation (RMSEP) and the ratio of performance deviation (RPD). Root mean square error of estimation (RMSEE) was also used for evaluation of the calibration model. R^2 values suggest how strong the prediction model for the specific property is. It is given as the percentage of variance present in the true component values, which is reproduced in the regression. RMSEP is based on an error of a number (n) of calibration samples and is computed as follows:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(y_i - y_{pred})^2}{n}}$$

where y_i indicates the measured reference values (observed) and y_{pred} the predicted values while *n* the number of samples. RMSEP estimate the variation of the predicted and reference values of a validation set (Zude, 2009). The smaller the RMSEP of the model, the stronger the prediction will be.

Ratio of performance deviation (RPD) was calculated as the ratio of the standard deviation (SD) of the reference data to the RMSEP:

$$RPD = \frac{SD}{RMSEP}$$

The prediction model assessment guidelines of (Chang and Laird, 2002) was used to categorize the models as follow:

1) 0.8≤R^2≤1.0; RPD>2 is good;
2) 0.5≤R^2≤0.8; 1.4≤RPD≤2.0 is satisfactory and;
3) R^2<0.5; RPD<1.4 is unreliable.

These thresholds has been widely used by the soil community since the paper of (Chang and Laird, 2002) had been published. Larger RPD values indicate better fitting models (Bellon-Maurel and McBratney, 2011).

7.3. Results (Soil)

7.3.1. Spectral features

The spectral features of all the soil samples can be seen in Figure 7-7. Absorbance was recorded as log 1/R, and diffuse reflectance is R. In Figure 7-7 show that spectral data from 12500-3600 cm^{-1} was saved. When calibration started an interactive region of 9403.3-4247.1 cm^{-1} was selected because there is not much spectral information (spectral noise) above 9403.3 cm^{-1} and the very strong absorption peak needs to be excluded (Bruker-Optics, 2011). Prominent absorption spikes can be seen at 7069 cm^{-1}, 5195 cm^{-1} and 4530 cm^{-1} (1415nm, 1925nm and 2208nm) in the soil spectrum. The first two spikes are associated with overtones of O-H bonds (Oinuma and Hayashi, 1965; Ben-Dor *et al.*, 1997) while the last spike is associated with methyl (Clark, 1999).

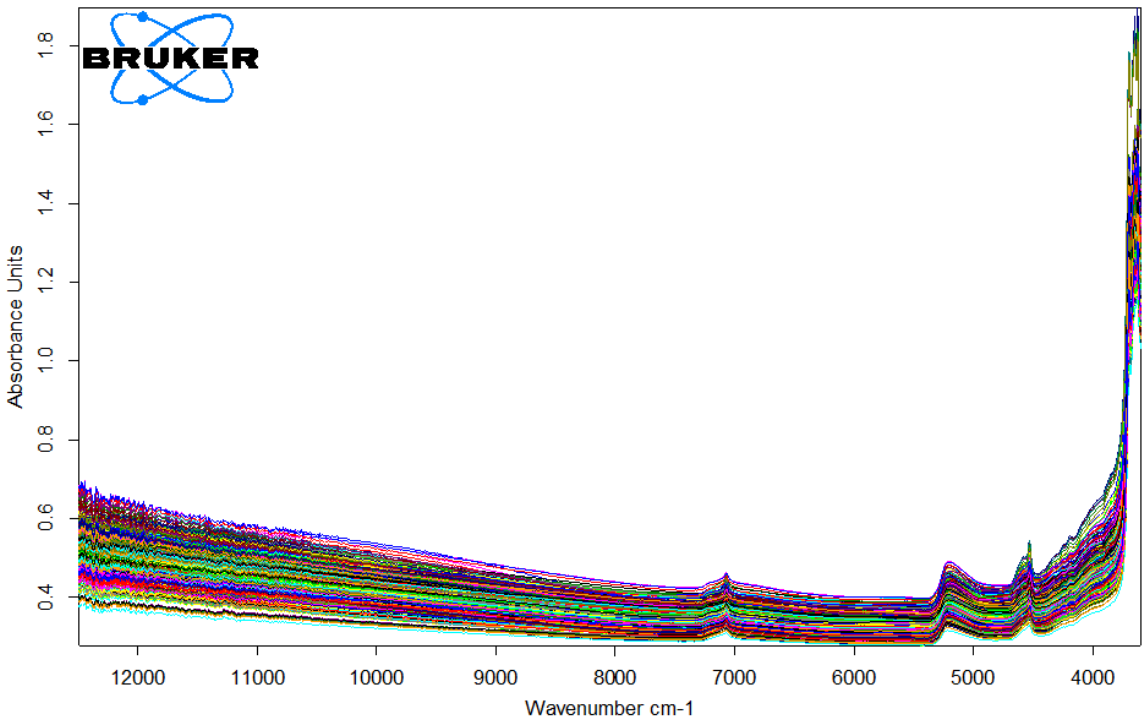

Figure 7-7: Spectral absorbance of all 152 soil samples

7.3.2. Soil chemical properties

In Table 7-1 the main statistics of the soil chemical results (outliers removed) of the selected samples for each property is shown under the reference column. The predicted statistics using test set validation are shown under the predicted column. The standard deviation (SD) of pH from the reference data is very small although soil samples is collected from a very big area, but it is the typical soil pH for which rooibos plants are adjusted to. The big difference between the maximum (max) and minimum (min) of P (mg kg^{-1}) and K (mg kg^{-1}) is due to some farms fertilize while other, for example organic farmers, do not.

Table 7-1: Summary of soil chemical properties for the reference and predicted datasets

Soil chemical parameter	n	Observed				Predicted			
		Mean	Min	Max	SD	Mean	Min	Max	SD
pH (KCl)	145	4.79	4.30	5.80	0.41	4.78	4.13	5.62	0.31
Resistance (Ohm)	152	4130.30	1040.00	8660.00	2147.30	4149.50	1045.00	6897.00	1343.70
H+ (cmol kg^{-1})	147	0.47	0.25	1.40	0.17	0.46	0.25	1.13	0.12
P (mg kg^{-1})	149	14.03	0.00	51.00	11.65	14.49	-4.82	35.15	8.83
Na ($cmol_c$ kg^{-1})	138	0.05	0.02	0.10	0.02	0.05	0.02	0.09	0.02
K ($cmol_c$ kg^{-1})	148	0.13	0.03	0.44	0.07	0.13	0.02	0.42	0.07
Ca ($cmol_c$ kg^{-1})	144	0.86	0.34	2.09	0.39	0.87	0.23	2.12	0.38
Mg ($cmol_c$ kg^{-1})	153	0.38	0.18	1.21	0.19	0.38	0.09	0.99	0.14
Cu (mg kg^{-1})	153	0.27	0.10	1.20	0.18	0.27	-0.03	0.58	0.08
Zn (mg kg^{-1})	132	1.03	0.10	3.40	0.64	0.96	0.23	1.67	0.32
Mn (mg kg^{-1})	143	6.00	0.90	20.60	3.94	6.28	-1.18	16.20	2.87
B (mg kg^{-1})	151	0.11	0.02	0.48	0.08	0.11	0.02	0.29	0.05
Fe (mg kg^{-1})	137	23.79	9.00	59.00	10.91	25.17	7.20	52.17	8.95
C (%)	143	0.30	0.12	0.84	0.16	0.27	0.05	0.77	0.14
N (%)	153	0.06	0.01	0.25	0.04	0.05	0.03	0.12	0.01

OPUS Software was used to build several prediction models for each property. The software builds over 300 different models with different pre-processing methods and spectral regions. The model with the lowest RMSEP was then manually chosen as the best prediction model for the specific property. In Table 7-2 the best calibration model with its spectral range and pre-processing method for each property can be seen. The R^2 value for the validation set ranges between 32.32 and 78.73 and the RPD's between 1.22 and 2.18. B (mg kg^{-1}) had the lowest R^2 value and RPD of 32.32 and 1.22 while Mg (mg kg^{-1}) had the highest with 78.73 and 2.18 respectively.

Table 7-2: PLSR statistics with spectral range and pre-processing methods for soil samples

Soil chemical parameter	Calibration model			Validation model						
	R 2	RMSEE	RPD	R 2	RMSEP	RPD	Bias	rank	Region	Pre-processing
pH	75.80	0.20	2.03	68.17	0.23	1.77	0.00	10	5450.1-4246.7	1st Derivative
Resistance (Ohm)	46.09	1540	1.36	38.10	1680	1.27	-19.20	5	4601.6-4246.7	MSC
H^+ ($cmol_c$ kg^{-1})	79.59	0.06	2.21	67.88	0.09	1.79	0.02	9	9685.2-6086.5 & 4655.5-3934.3	1st Derivative
P (mg kg^{-1}) - Bray II	68.69	6.09	1.79	55.38	7.76	1.50	-0.45	10	11147.1-9708.4 & 8994.8-3961.3	MSC
Na ($cmol_c$ kg^{-1})	60.86	0.01	1.60	40.50	0.02	1.30	0.00	10	7502.1-4597.7	1st Derivative + SNV
K ($cmol_c$ kg^{-1})	79.68	0.03	2.22	74.38	0.04	1.98	0.00	8	9403.7-8451 & 6102-4246.7	2nd Derivative
Ca ($cmol_c$ kg^{-1})	78.34	0.21	2.15	71.65	0.21	1.88	-0.01	8	7867-7340.1 & 6827.1-4759.7	No spectral data pre-processing
Mg ($cmol_c$ kg^{-1})	75.98	0.09	2.04	78.73	0.09	2.18	0.01	6	6102-4246.7	2nd Derivative
Cu (mg kg^{-1})	26.01	0.12	1.16	37.18	0.14	1.26	0.00	7	8890.7-7340.1 & 6287.1-6306.4 & 5797.3	Min-Max normalization
Zn (mg kg^{-1})	30.02	0.55	1.20	35.67	0.53	1.26	0.07	5	4521-4597.7	SNV
Mn (mg kg^{-1})	64.07	2.47	1.67	51.36	2.36	1.44	-0.27	9	9403.7-8451 & 4601.6-4246.7	Straight line subtraction
B (mg kg^{-1})	46.62	0.06	1.37	32.32	0.07	1.22	0.00	6	7502.1-6098.1 & 4601.6-4246.7	Straight line subtraction
Fe (mg kg^{-1})	64.17	6.67	1.67	71.95	5.76	1.95	-1.35	8	9403.7-8370 & 7857-7340.1 & 6827.1-5793.4 & 5280.4-4246.7	No spectral data pre-processing
C (%)	79.13	0.07	2.19	66.44	0.09	1.79	0.02	9	5450.1-4597.7	2nd Derivative
N (%)	37.65	0.02	1.27	35.70	0.03	1.29	0.01	7	9403.7-8370 & 7344-6823.3 & 6310.3-5793.4 & 5280.4 4759.7	Constant offset elimination

The R^2 and RPD of the validation set were used to evaluate the predictive power of the model for each component. The only property with a validation RPD above 2 was Mg ($cmol_c$ kg^{-1}) which according to Chang & Laird (2002) is a good predictive model. The models that showed a RPD above 1.6 was the pH, H ($cmol_c$ kg^{-1}), K ($cmol_c$ kg^{-1}), Ca ($cmol_c$ kg^{-1}), Fe (mg kg^{-1}) and C (%) prediction models and is therefore satisfactory according Chang & Laird (2002). The prediction models of resistance (Ohm), P (mg kg^{-1}), Na (cmolc kg^{-1}), Cu (mg kg^{-1}), Zn (mg kg^{-1}), Mn (mg kg^{-1}), B (mg kg^{-1}) and N (%)

were poor because of low RPD values below 1.5 and thus unreliable. When using the R^2 value for model evaluation the pH, H ($cmol_c\ kg^{-1}$), P ($mg\ kg^{-1}$), K (($cmol_c\ kg^{-1}$), Ca($cmol_c\ kg^{-1}$), Mg ($cmol_c\ kg^{-1}$), Mn ($mg\ kg^{-1}$), Fe ($mg\ kg^{-1}$) and Cu ($mg\ kg^{-1}$) prediction models classify as satisfactory and the rest as unreliable.

R^2 and RPD of the pH model were in range compared to other studies where the R^2 ranges from 0.66 to 0.95 and RPD from 1.68-4.45. The R^2 value of other studies for P varied between 0.53 and 0.65 and is also in range with the R^2 value of 0.27 (26.88) in this study. The RPD value of 1.50 for this study is lower than the RPD values of around 1.7 in other studies (Zornoza *et al.*, 2008; Mashimbye, 2013; Pienaar, 2014; Wang *et al.*, 2014).

Carbon (C) and nitrogen (N) prediction models of this study appear to be weaker compared to models of other studies. The studies of Chang & Laird (2002) and (Chang *et al.*, 2001) showed R^2 values from 0.85 and higher, and the RPD's was 2.52 and higher for C and N prediction models. The study of Wang *et al* (2014) had a R^2 value of 0.75 for the N prediction model which is much higher than the 0.36 (35.70) in this study. In this study the R^2 value for C was 0.66 (66.44) and the RPD 1.79 which is lower compared to the studies mentioned above.

The power of the predictive models for the exchangeable cations in this study was similar to other studies. The R^2 value of 0.41 (40.50) and RPD of 1.3 for Na ($cmol_c\ kg^{-1}$) was very low compared to the R^2 of 0.76 and RPD of 2.06 of Pienaar (2014), but is slightly higher than R^2 values for other studies which were 0.13 and 0.09 (Chang *et al.*, 2001; Zornoza *et al.*, 2008). The R^2 value and RPD of Ca ($cmol_c\ kg^{-1}$), Mg ($cmol_c\ kg^{-1}$) and K ($cmol_c\ kg^{-1}$) was also similar to those of other studies (Chang *et al.*, 2001; Zornoza *et al.*, 2008; Pienaar, 2014). Chang et al (2001), Zornoza et al (2008) and Pienaar (2014) got R^2 values that ranged from 0.44 to 0.95 and RPD's that ranged from 1.34 to 2.46 for Ca. For Mg they got R^2 values that ranged from 0.72 to 0.91 and RPD's that ranged from 1.89 to 2.2. With K they got R^2 values that ranged from 0.65 to 0.79 and RPD's that ranged from 1.44 to 2.19. The reason why exchangeable cations such as Ca and Mg had better calibration and predictive models compared to the rest is because of its strong dependence on organic matter and clay. These minerals or nutrients are mainly controlled by organic matter type and clay content, which the variable charges of the its functional groups are responsible for the adsorption of the different cations (Zornoza *et al.*, 2008).

The predictive models for Cu ($mg\ kg^{-1}$) and Zn ($mg\ kg^{-1}$) in study of (Todorova *et al.*, 2014) for heavy metals show a R^2 values of 0.6 and 0.77, and RPD's of 1.82 and 2.01. The R^2 and RPD in this study for these heavy metals were very poor compared to these studies. R^2 ranged from 0.35 (35.67) to 0.37

(37.18). Metals do not absorb in the NIR region. The only way they can be detected is by co-variation with spectrally active constituents in the soil such as organic matter fractions or oxides, sulphides, carbonates, hydroxides and other compounds (Stenberg *et al.*, 2010).

7.4. Results (Plant)

7.4.1. Spectral features

Spectral features of the 80 plant sample can be seen in Figure 2. The same spectral range (12500-3600 cm^{-1}) as for the soil was saved and the same interactive region for calibration was chosen which is from 9403.3-4247.1 cm^{-1}. The very high absorption peak at 4000 cm^{-1} and the spectra above 9500 cm^{-1} is cut out. According to (Ben-Dor *et al.*, 1997) the absorption peaks at 6840 cm^{-1}, 5790 cm^{-1} and 4259 cm^{-1} are associated with OH in water, CH2, cellulose, lignin, starch, pectin, aliphatic C-H, wax, humic acid and glucan. According to (Clark, 1999) the absorption peaks at 5183 cm^{-1} and 4681 cm^{-1} are associated with carboxylic acids and oil. The absorption peak at 4681 cm^{-1}.is associated with polysaccharides (C-O). These are the main absorption peaks for plant NIR spectra and it corresponds with the spectra of Galvez-Sola *et al* (2015).

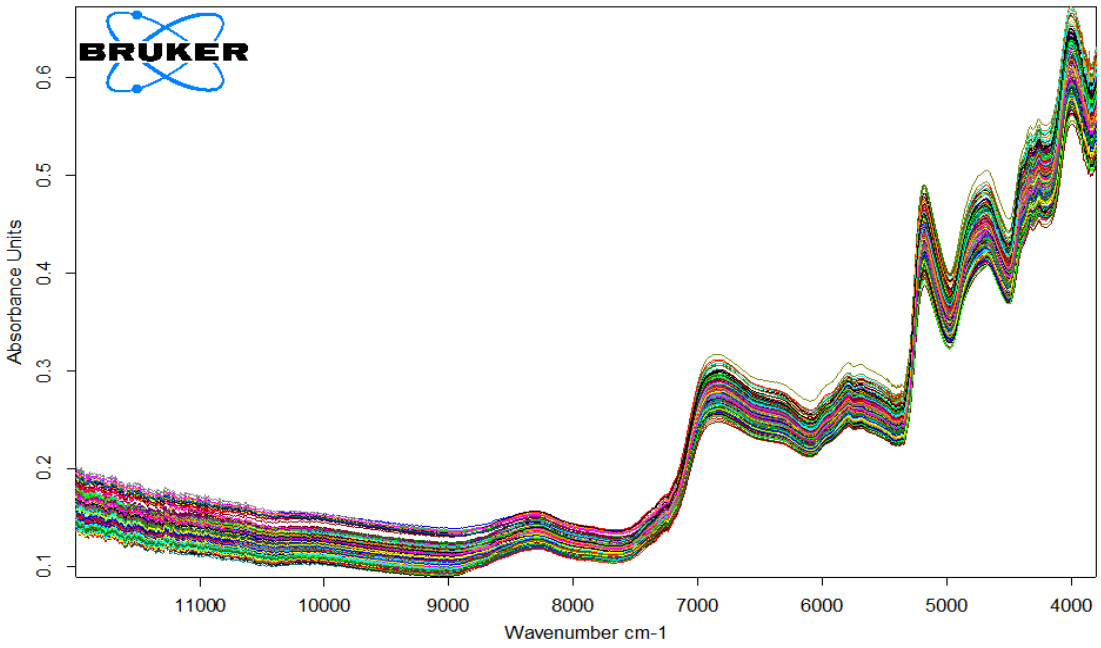

Figure 7-8: Spectral absorbance of all 80 milled plant samples

7.4.2. Plant chemical properties

In Table 7-3 contains the plant chemical statistical data of each component from the reference and predicted dataset. Most of the plant chemical parameters show very small standard deviation (SD) and thus are the results all concentrated and have a small range. A wide range of mineral concentrations in plants is important in building a good and robust predictive model (Galvez-Sola *et al.*, 2015). Na, Fe, Mn, B and Al had high standard deviations.

Table 7-3: Summary of plant chemical properties for the reference and predicted data sets.

Soil chemical parameter	n	Observed				Predicted			
		Mean	Min	Max	SD	Mean	Min	Max	SD
C (%)	81	52.27	47.87	56.73	1.66	51.92	50.80	53.28	0.57
N (%)	81	1.39	1.16	1.88	0.18	1.38	1.10	1.72	0.15
P (%)	78	0.05	0.03	0.09	0.01	0.05	0.03	0.08	0.01
K (%)	71	0.30	0.15	0.49	0.08	0.31	0.17	0.39	0.05
Ca (%)	78	0.15	0.11	0.21	0.03	0.15	0.11	0.20	0.02
Mg (%)	80	0.16	0.09	0.21	0.03	0.17	0.12	0.22	0.02
Na (mg kg^{-1})	79	3416.94	1979.00	5263.00	861.09	3565.03	2082.00	5981.00	730.25
Fe (mg kg^{-1})	77	108.26	55.20	193.20	32.44	114.95	62.63	155.90	18.83
Cu (mg kg^{-1})	81	2.43	1.19	6.58	1.15	2.07	0.38	3.65	0.56
Zn (mg kg^{-1})	78	5.28	3.65	9.40	1.47	5.13	3.17	7.61	0.96
Mn (mg kg^{-1})	81	73.92	23.98	178.90	38.60	61.87	33.48	93.59	12.70
B (mg kg^{-1})	77	18.01	9.52	34.30	6.75	18.87	11.81	27.97	3.90
Al (mg kg^{-1})	80	71.89	45.00	120.00	17.41	73.31	47.42	109.80	14.38

The results of the best PSLR model for each plant chemical parameter, generated by the OPUS software, are shown in Table 7-4. The coefficient of determination (R^2) values ranged from 21.09 to 75.77 and the RPD values from 1.16 to 2.09. P, K, Mg and Na had the highest R^2 and RPD values while C (%), Fe, Cu and Mn had the lowest R^2 and RPD values.

Table 7-4: PLSR statistics with spectral range and pre-processing methods for rooibos plant samples

Plant chemical parameter	Calibration model			Validation model						
	R 2	RMSEE	RPD	R 2	RMSEP	RPD	Bias	rank	Region	Pre-processing
C (%)	24.53	1.22	1.15	21.09	1.46	1.16	0.35	2	8451-7498.3 & 5774.1-5446.3	First derivative + MSC
N (%)	62.45	0.13	1.63	51.51	0.12	1.44	0.01	8	5450.1-4246.7	Constant offset elimination
P (%)	75.37	0.01	2.02	75.77	0.01	2.09	0.01	10	9403.7-8451 & 5450.1-4246.7	First derivative
K (%)	71.54	0.04	1.87	60.78	0.05	1.62	-0.01	9	6102-4597.7	Min-Max normalization
Ca (%)	56.93	0.02	1.52	55.13	0.02	1.51	0.00	10	5450.1-4597.7	First derivative
Mg (%)	85.06	0.01	2.59	74.32	0.01	2.00	0.00	10	5450.1-4246.7	SNV
Na (mg kg^{-1})	69.51	478	1.81	64.42	510	1.75	-148	9	6102-4597.7	First derivative + Straight line subtraction
Fe (mg kg^{-1})	38.23	24.90	1.27	29.37	27.10	1.23	-6.63	6	9403.7-6098.1 & 5450.1-4597.7	First derivative + MSC
Cu (mg kg^{-1})	37.58	0.65	1.27	25.27	0.98	1.24	0.36	7	9403.7-8451 & 4848.4-4246.7	First derivative + MSC
Zn (mg kg^{-1})	45.25	0.97	1.35	38.16	1.15	1.28	0.15	8	7502.1-6098.1 & 5450.1-4246.7	First derivative + SNV
Mn (mg kg^{-1})	25.12	24.60	1.16	24.39	33.40	1.23	12.00	5	6102-5446.3	Straight line subtraction
B (mg kg^{-1})	47.49	4.54	1.38	40.03	5.04	1.31	-0.86	9	9403.7-7498.3 & 4601.6-4246.7	Min-Max normalization
Al (mg kg^{-1})	55.02	14.60	1.49	48.97	12.40	1.41	-1.42	9	8451-7498.3 & 5450.1-4597.7	SNV

According to Chang & Laird (2002) only the prediction models of P (%) and Mg (%) was good because of an RPD above 2. The predictive models with a RPD between 1.4 and 2 where N (%), K (%), Ca (%) and Na (mg kg^{-1}) and thus were satisfactory. The predictive models for the other chemical properties had an RPD lower then 1.4 and were thus unreliable. When the R^2 value is used for evaluation then the prediction models of N, P, K, Ca, Mg (%) and Na (mg kg^{-1}) would classify as satisfactory and the rest unreliable.

There is little research done on plant chemical characterization with the help of NIRS. Galvez-Sola *et al* (2015) did research on the estimation of nutritional elements in citrus leaves using NIR reflectance spectroscopy. Galvez-Sola *et al* (2015) used the same Bruker MPA instrument for scanning, but sample preparation was different. Galvez-Sola *et al* (2015) obtained high R^2 and RPD values for the prediction models of N, K, Ca and Mg (g/100g); and Fe and Zn (mg kg^{-1}). All of them had a R^2 and RPD above 0.75 and 1.6 respectively. Only B, Cu and Mn (mg kg^{-1}) had lower R^2 values ranging between 0.36 to 0.53 and RPD's of 1.22 to 1.46.

In Table 7-4 the prediction models for C, B, Cu, Mn and Fe (mg kg^{-1}) had the lowest R^2 and RPD values, corresponding (except for C and Fe) with the research of Galvez-Sola *et al* (2015). The prediction models of C, Fe, Cu, Zn, Mn, B and Al had was very poor with R^2 values ranging from 0.21 (21.09) to 0.48 (48.97), lower than the values of Galvez-Sola *et al* (2015). In the study of Galvez-Sola *et al* (2015) the R^2 values for N, K, Ca and Mg varied between 0.88 to 0.99 compared to the much lower values of 0.52 (51.51) to 0.74 (74.32) of this study.

The reason for the differences in the performance of the prediction models can be because of the different sample preparation that were used and also the chemical composition of the samples used in this study differ from the samples used in other studies. In the other studies the number of samples used to build the calibration models was also very big. For some chemical properties such as Cu in soil the limited variation/range in concentrations (small SD) can alter the building of a good calibration model as seen in this chapter.

7.5. Conclusion

An NIR spectrometer was used to get spectral absorbance for air dried, sieved soil samples and oven dried and milled plant samples from the Nieuwoudtville rooibos farms in the North and South Bokkeveld region. Individually outliers for each chemical component were removed and the final data set was then used to set up calibration models. Test validation was used to set up the PLSR calibration models. The model with the lowest RMSEE and RMSEP was chosen as the best PLSR calibration and prediction model for each specific chemical component. The study concluded that only Mg ($cmol_c$ kg^{-1}) can be predicted with good accuracy, while pH, H ($cmol_c$ kg^{-1}), K (mg kg^{-1}), Ca ($cmol_c$ kg^{-1}), Fe (mg kg^{-1}) and C (%) models showed satisfactory predictability. The resistance (Ohm), P (mg kg^{-1}), Na ($cmol_c$ kg^{-1}), Cu (mg kg^{-1}), Zn (mg kg^{-1}), Mn (mg kg^{-1}), B (mg kg^{-1}) and N (%) models showed weak predictability. The soils of this study were very low in clay and organic matter, which can be the main reason for the poor calibration models. Clay and organic matter have a primary response in the NIR region (Zornoza *et al.*, 2008).

NIR spectrometer was also used to get the absorbance spectra of rooibos plant samples fine (moderately) milled with a Thomas Wiley Mill. The same procedure as for the soil was used to obtain the PSLR models for each specific chemical component. The study concluded that P (%) and Mg (%) had good predictive models, while the models for N (%), K (%), Ca (%) and Na (mg kg^{-1}) were satisfactory. The predictive models for the rest were unreliable.

In this study and the method used it was not possible to precisely measure the amount and concentration (quantitatively) of elements in the soil samples and milled rooibos plant samples using NIRS. This can be attributed to small variation in the chemical analysis data of the soils and plants and another very important reason can be that the resolution of the analysis results received from the lab was not big enough and this makes it very hard for the software to build a good regression model. There is however the potential to further develop and test new methods for NIRS analysis to build better calibration models that can predict quantitatively more accurate. Other IR instruments can also be used to test for better calibrations.

8. GENERAL CONCLUSION AND FUTURE RESEARCH PROSPECTS

Rooibos farmers face the challenge of increasing the rooibos yields per ha because of greater demand each year while the available land for rooibos cultivation is limited. Research and information on rooibos cultivation practices and its effect on the plant nutrients cycle is very little. This MSc study formed part of a bigger research project aimed at optimising soil fertility and plant nutrient status for increased rooibos tea quality and sustainable rooibos production in the Northern Cape (de Clercq and van der Merwe, 2015). During early excursions to the rooibos farms at Nieuwoudtville it was noted that, although conditions in the rooibos field is the same, rooibos plants differed a lot in growth performance and mortality. It was also noted that the fields where the farmers harvested seed look poorer in growth.

The main objective of the study was thus to investigate the effect of different mulch treatments on the rooibos plant nutrient cycle and growth. One of these treatments included the removal of the litter layer under the plant imitating seed harvesting. The hypothesis was that when interfering with the natural mulching process of the rooibos plant it will lead to weaker growth. This also led to a second objective. This entailed the identification of nutrients that had a significant effect of growth. The important nutrients were then identified with the help of different statistical analysis methods. The third objective was also to investigate the effect of the different mulch treatments on the soil volumetric water content, soil temperature and EC. Lastly NIR spectroscopy was used to investigate if it can be used as an alternative method of soil and plant analysis.

With the help of multiple regression analysis it was possible to identify nutrients that have a significant effect or influence on the average length of regrowth from all the data gathered of the trials over the one-year period. During the process of mining the important data it was noted that for all the treatments except treatment D plant N (%) had a significantly negative effect on the average length of regrowth multiple regression model. Research of Maistry *et al* (2015) showed that at low soil P levels (10 µM) increased soil N (higher N:P ratios) led to weaker growth, but at high P levels (100 µM) (lower N:P ratios) stronger growth and that the N:P ratio in the soil is very important. The uptake of N at treatment D which had the very high P levels and lower soil N:P ratio showed that plant N (%) has a significantly ($p<0.05$) positive ($b=8.203$) effect on average length of regrowth. In this study plant N and P had a positive correlation ($R=0.56$). Treatment A, B an C had high N:P ratios. After multiple regression analysis was performed for all the data together, both depths alone and for all the treatments alone there were certain chemical properties that repeated its significant effect in three or more models. The soil chemical properties included P (mg kg^{-1}), Na ($cmol_c$ kg^{-1}), K ($cmol_c$ kg^{-1}), Ca ($cmol_c$ kg^{-1}), Mg ($cmol_c$ kg^{-1}), Zn (mg kg^{-1}), Mn (mg kg^{-1}), C (%) and % Na

saturation at pH 7. The plant chemical properties included Na (%) en plant N (%), P (%), K (%), Al (mg kg^{-1}) and Fe (mg kg^{-1}). The N:P ratios investigated, possibly provides the best approach in understanding the supposed P toxicity reported on by previous authors.

From July 2015 to September 2015 most of the nutrients including: N, P, K, Ca, Mg, Zn, Mn, Fe and Al there was a very large increase in concentration while growth was taking place. This means that nutrient uptake by the plant was very high during this period. Then from September 2015 to January 2016, during flowering, the concentration of all the nutrients decreased which is the result of increased growth by the plant that leads to the dilution of the nutrient concentration. The plant nutrients K and Mg however showed a small decrease in concentration compared to the other nutrients and also an increase in content (mg/plant). This small decrease in concentration and increase in content thus means that for these nutrients, there was still a high uptake from September 2015 to January 2016. The decrease in soil Mg and Ca from July 2015 to September 2015 was pH related. The correlation between pH and soil Mg (cmolc kg^{-1}) was moderately positive (R^2=0.61) and for Ca it was weakly positive (R^2=0.49). All the treatments showed a decrease in soil and plant N over the one-year period. Only the decrease in soil N for treatment A and B was significant. For all treatments there were an increase in plant and soil Mg, except for treatment C the soil Mg concentration were the same as a year back in 2015. The treatments led to different plant growth performances. Although not statistically significant the increase in estimated dry matter (kg/plant) for treatment C (natural rooibos litter mulch) was greater compared to treatment A (bare soil). Treatment B (added mulch) however had a significantly higher increase in estimated dry matter (kg/plant). For treatment D where the added mulch was enriched with the MAP fertilizer the increase in estimated dry matter (kg/plant) was not significantly higher than treatment A which can be attributed to the too high soil P levels. At treatment D most of the fertilizer just leached from the mulch which can be attributed to the charges of the phosphate and organic matter. Both phosphate and organic matter is negatively charged thus the organic matter would repel the phosphate. The mulch treatments only had a significant effect on the plant P, K and Mg uptake compared to treatment A. All the mulch treatments, although not significant for all, showed an increase in plant P and K. The plant P of treatment A showed no change. The plant and soil Mg for all the treatments showed an increase, but was only significant for all mulch treatments. This higher uptake of P, K and Mg thus can be the reason for the good growth that resulted from treatment B. Although treatment A had a higher soil volumetric water content during wet periods (frequent rain), the mulch treatments conserved more water for longer when weather were dry afterwards. This can also be the reason why treatment B, which conserved the most water, had the highest estimated dry matter increase because the soils were wetter for longer leading to higher nutrient uptake in the topsoil.

Throughout the one year the mulch treatments (B and C) resulted in different soil temperatures to that of the no mulch treatment (A). During summer at the 5 cm depth the mulch treatments had a lower maximum and higher minimum soil temperature, whereas at the 20 cm depth the mulch treatments had a higher maximum and lower minimum soil temperature. During the winter at the 5 cm depth the mulch treatments had a higher maximum and lower minimum soil temperature, whereas at the 20 cm depth the mulch treatments had a lower maximum and higher minimum soil temperature.

NIR spectroscopy was evaluated to help find a cheaper and faster way to analyse soil and plant samples for the rooibos farmers in the Nieuwoudtville Bokkeveld region. It was found that only soil exchangeable Mg ($cmol_c\ kg^{-1}$); plant Mg and plant P can be predicted with good accuracy (RPD>2). NIR spectroscopy was used to help predict soil and plant chemical properties quantitatively accurate but the weak predictive models got for the other nutrients can be because of the very low levels with small variation found in these nutrient poor sandy soils.

Future research projects

The nutrient uptake by the taproot of the plant needs to be quantified and more trials need to be done in cultivated rooibos conditions. Although other research has shown that rooibos doesn't need any extra N for acceptable growth and that it bind enough N by itself the addition of N needs to be investigated. The effect of soil N and P contents with different N:P ratios should be looked at in cultivated field conditions. The effect of Na, K and Mg in the rooibos nutrient cycle also needs more attention and research. Different methods of adding fertilizer to the rooibos plant after plant needs to be investigated, because nutrient needs for younger and older plants are different. Previous research has shown foliar sprays is not effective and that it actually just falls to the ground where the plant will then take it up. In this study it was attempted to add fertilizer with mulch to the plant where it will then be slowly released directly to the rhizosphere, but it gave an unexpected result and possibly too much fertilizer was added.

There is a vast range of infra-red (IR) spectroscopy tools, for example MIR, that can be used to build better predictive calibrations for soil and plant samples. Qualitative instead of quantitative analysis using IR spectroscopy can also be investigated.

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10. APPENDICES

Appendix A: Chapter 4 supplementary data

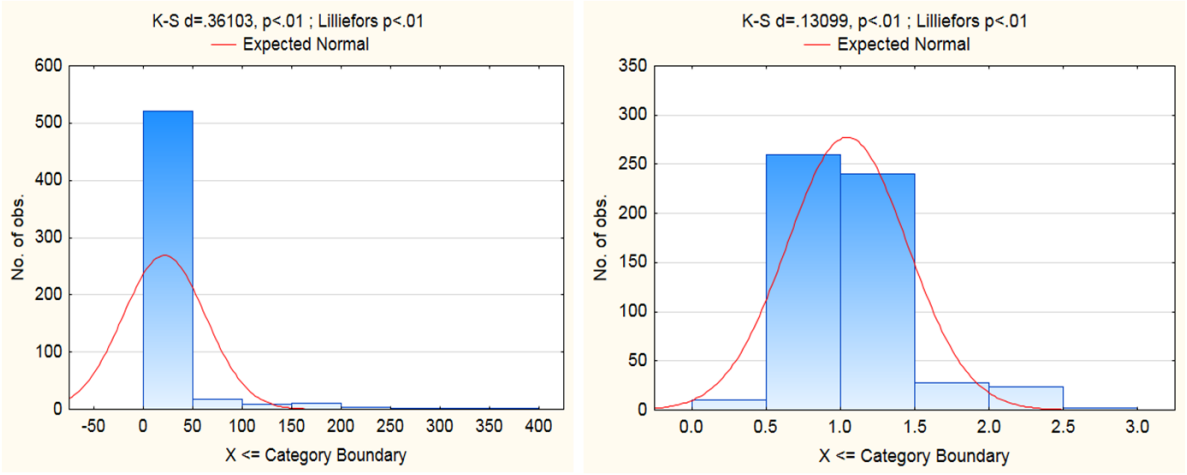

Figure 10-1: Normal distribution for raw data (left) and transformed and/or outliers removed data (right) of soil P (mg kg^{-1})

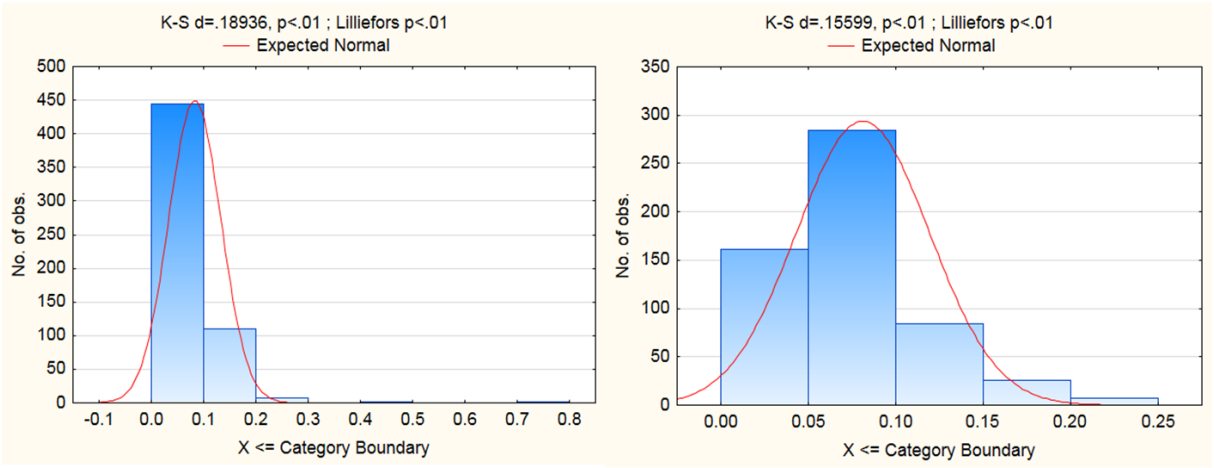

Figure 10-2: Normal distribution for raw data (left) and transformed and/or outliers removed data (right) of soil Na ($cmol_c$ kg^{-1})

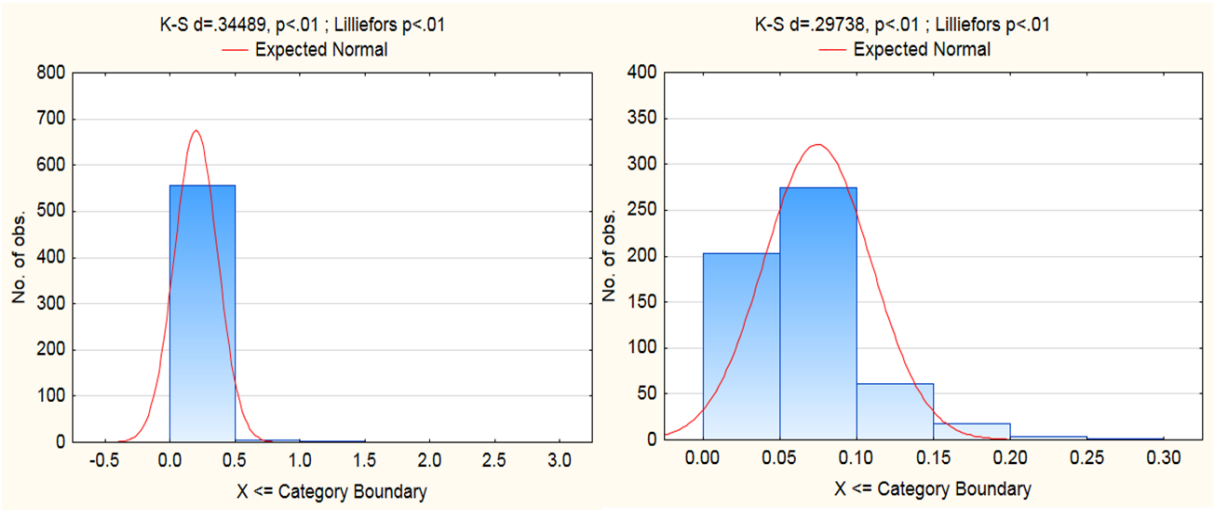

Figure 10-3: Normal distribution for raw data (left) and transformed and/or outliers removed data (right) of soil Zn (mg kg^{-1})

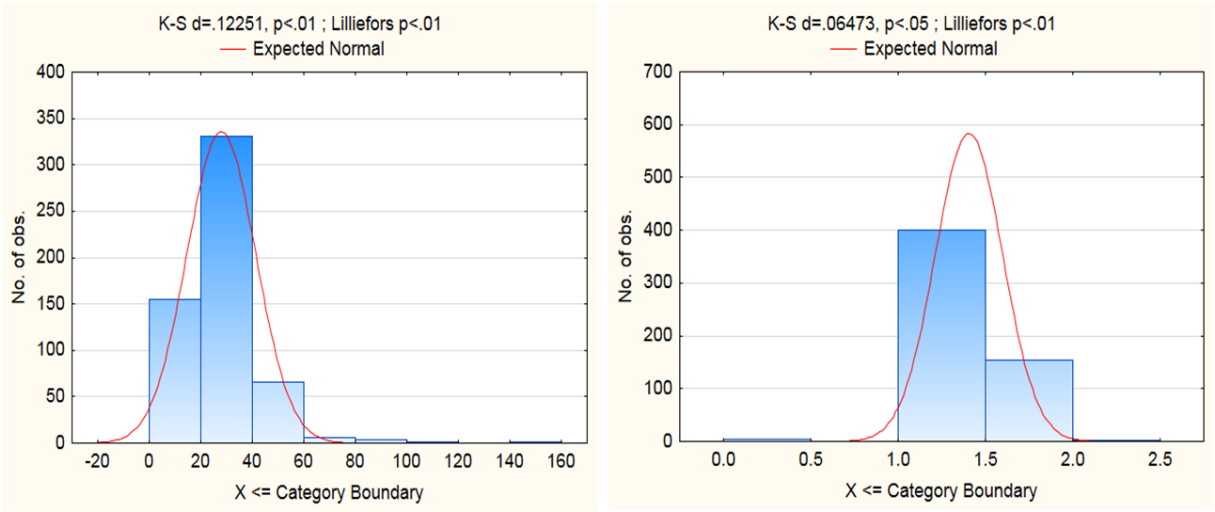

Figure 10-4: Normal distribution for raw data (left) and transformed and/or outliers removed data (right) of soil Fe (mg kg^{-1})

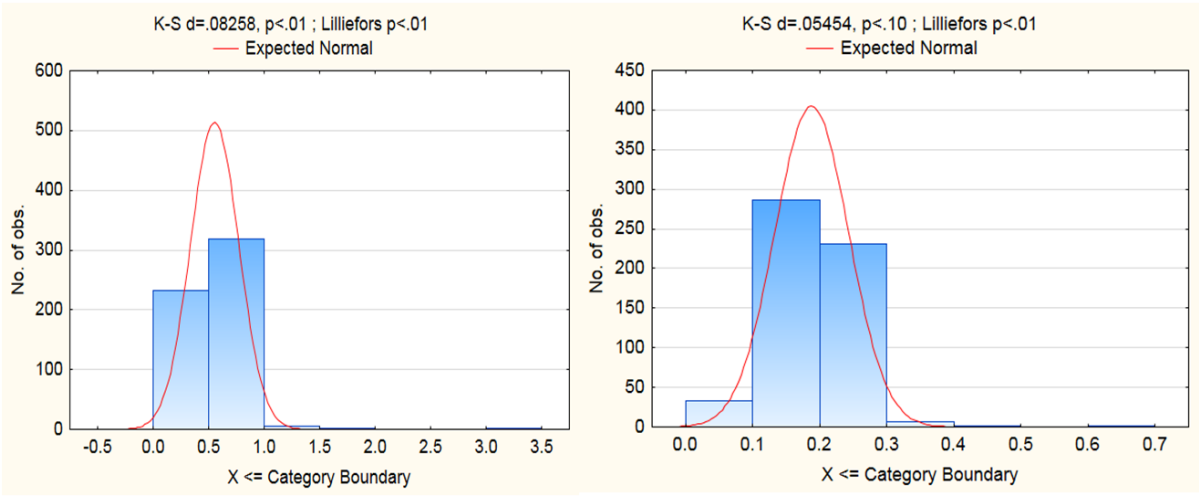

Figure 10-5: Normal distribution for raw data (left) and transformed and/or outliers removed data (right) of soil C (%)

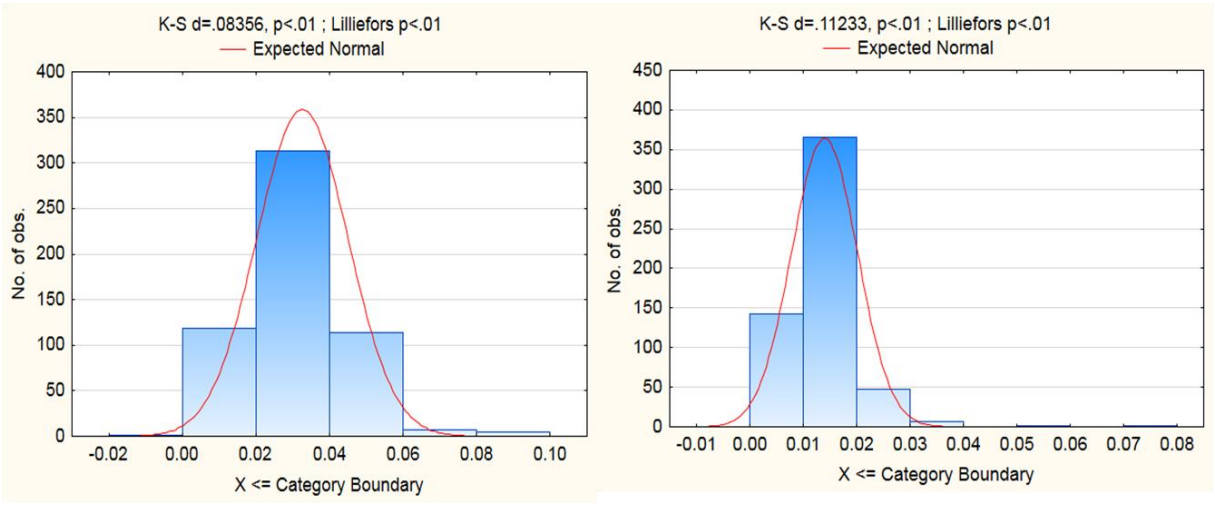

Figure 10-6: Normal distribution for raw data (left) and transformed and/or outliers removed data (right) of soil N (%)

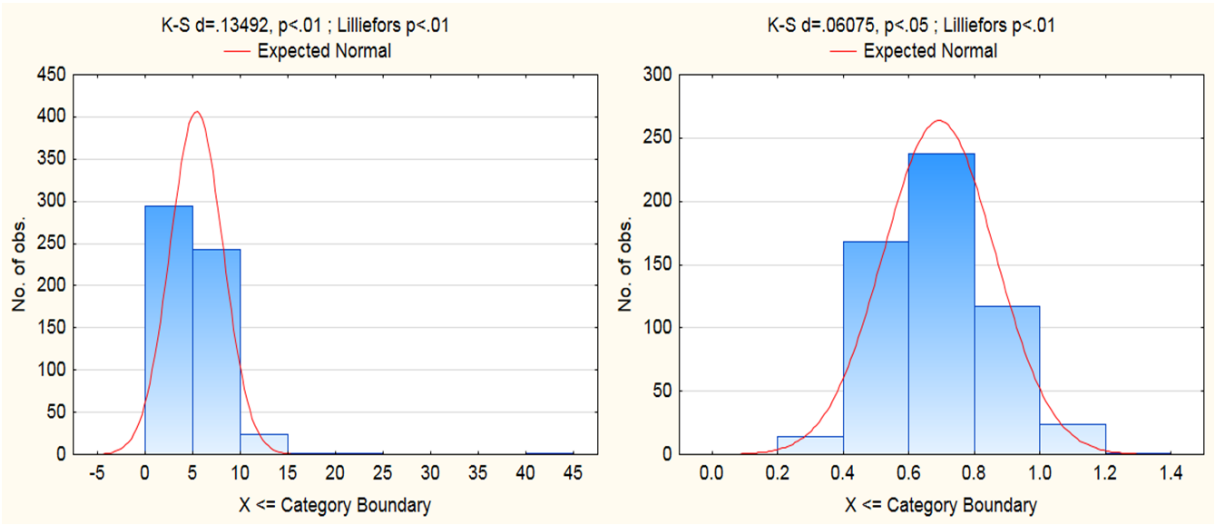

Figure 10-7: Normal distribution for raw data (left) and transformed and/or outliers removed data (right) of soil Na (%)

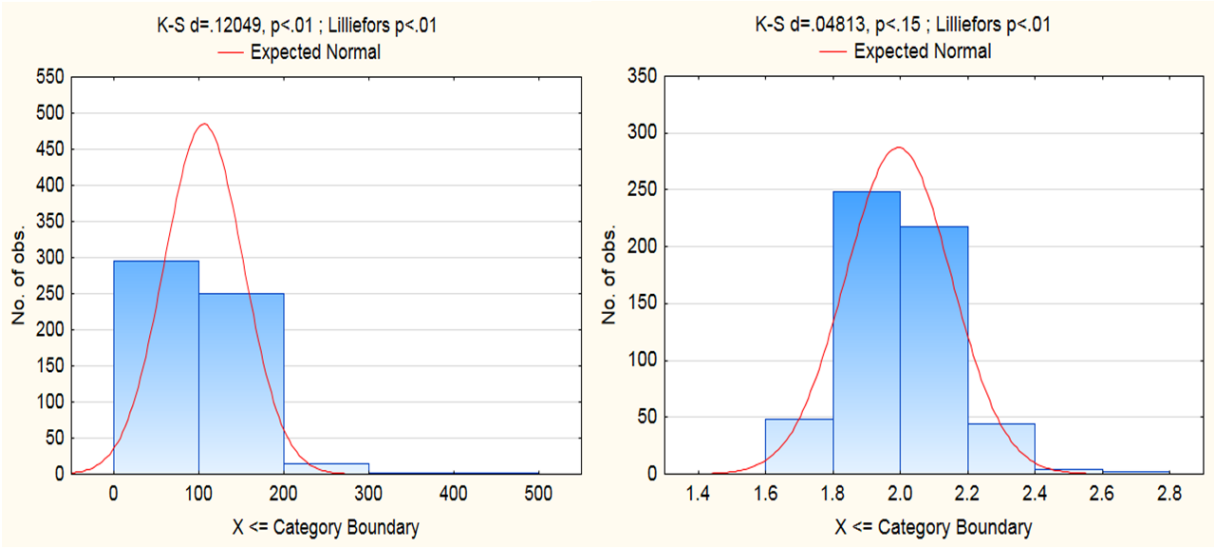

Figure 10-8: Normal distribution for raw data (left) and transformed and/or outliers removed data (right) of plant Fe (%)