ASSESSING THE IMPACT OF SELECTED METHODS OF REMOVAL OF INVASIVE ALIEN *ACACIA MEARNSII* AND *EUCALYPTUS CAMALDULENSIS* ON FYNBOS RIPARIAN ECOSYSTEM FUNCTION

REPORT TO THE

WATER RESEARCH COMMISSION

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EXECUTIVE SUMMARY

Management of alien invasive plants is a major challenge in South Africa, especially in the fynbos biome. Riparian environments are especially vulnerable as the linear nature of these ecosystems makes seed dispersal more efficient, while the relatively more abundant available resources ensure better seedling establishment than in terrestrial ecosystems. The dynamic nature of flowing waters also make management more challenging, with flowing water and flooding leading to dispersal of seeds and redistribution of sediment and resources within the landscape. The Working for Water (WfW) programme has had significant success in clearing stands of invasive species, though restoration of riparian zones following clearing has been uneven. Various approaches to clearing and managing biomass accumulation are practiced, entailing a variety of interventions. The nature of these interventions, however, may in itself impact negatively on riparian ecosystems. An example is herbicide application, which, in various contexts, has led to altered soil microbial population. Another example is removal of dead biomass through combustion of stacked slash (slash piles). In terrestrial environments burning of slash piles has been shown to lead to scars that remain in the landscape for long periods of time, as well as altered soil physicochemical properties. Loss of soil seedbanks, which are destroyed during high-intensity fires, could be one reason for the lack of recovery of burn scars, while the altered physicochemical properties of the soil may be another factor that may hinder the re-establishment of plant communities. Furthermore, removal of invasive alien biomass off-site may also impact riparian ecology in terms of nutrients lost in woody and foliar biomass (the latter may be suspended and removed by floods following hacking of invasive trees). The result is that restoration of affected riparian zones could be uneven, or delays may occur in the recovery of native vegetation following removal of invasive alien biomass. Considering the role of functioning riparian zones in delivery of ecosystem services, the lack of restoration following clearing may impact communities dependant on services from rivers directly and indirectly. For instance, lack of restoration could cause loss of riparian bank integrity, and incur costs associated with sedimentation of water resources.

Jacobs et al. (2013) showed that invasive *Acacia mearnsii* can induce significant changes in soil and microbial dynamics in fynbos riparian environments. Among others, nitrogen levels in soils are elevated, while CO_2 emissions are greatly enhanced by stands of invasive trees. Soil microbial structure is also altered, and removal of invasive trees does not totally restore soils, though a trajectory towards restoration was found in ecosystem function following clearing of *A. mearnsii* trees. However, the impact of management interventions related to clearing invasive alien trees on riparian function has not received attention. Therefore, the overarching aims of this project are to:

1. Evaluate the impact of recommended levels of herbicides, used to control alien invasive growth and regeneration, on soil microbial diversity and on selected beneficial groups of microbes *in situ* and *ex situ* and in riparian soils from two different longitudinal zones.
2. Determine the impact of slash and burn of *Eucalyptus* spp. and *Acacia mearnsii* biomass on soil microbial diversity and on selected beneficial groups of microbes *in situ* and measure regeneration of various native plant species grown in soil from slash and burn scars *ex situ.*
3. Determine the impact of slash and burn of *Eucalyptus* spp. and *Acacia mearnsii* biomass on soil physical and chemical properties *in situ*.
4. Determine the biomass and nutrient content of *Eucalyptus* spp. and *Acacia mearnsii* trees of different sizes growing at different stem densities in riparian sites from two different longitudinal zones.

Alien invasive tree species have a significant impact on their immediate soil environment and removal of these species allows a gradual return to an uninvaded state. However, the manner of removal may have a long-lasting effect on the outcome of rehabilitation. WfW utilises a number of management approaches to eradicate *Acacia mearnsii* and *Eucalyptus camaldulensis* from riparian fynbos habitats, including mechanical, chemical and biological methods. Similar approaches are in use in terrestrial environments. Chemical alien control methods entail the use of specific herbicides. Among others, *A. mearnsii* is treated with Springbok (a Glyphosate-based herbicide) and Garlon (a Triclopyr-based herbicide), while *Eucalyptus camaldulensis* is treated with Plenum (a Fluroxypyr- and Picloram-based herbicide). Literature suggests that these herbicides could have effects on ecosystem components beyond the targeted invasive species, including the soil environment. Field- and greenhouse-based trials were carried out to investigate the impacts of herbicides specific to combating *A. mearnsii* and *E. camaldulensis* in riparian environments. Soil chemical characteristics, namely pH, electrical conductivity, total available nitrogen, soil available phosphorus, and acid phosphatase activity were studied. Additionally, soil microbial characteristics were studied, which included bacterial diversity and function, and evaluation of community structure.

Soil pH and electrical conductivity (EC) were measured using calibrated pH and EC meters, whilst the total available nitrogen, soil available phosphorus, and acid phosphatase activity were all measured through spectrophotometric analysis. The microbial function study included the Biolog plate technique which uses a micro-titre eco-plate where the average well colour development (AWCD) was determined. Furthermore, automated ribosomal intergenic spacer analysis (ARISA) was done in order to determine levels of similarity within the microbial community and to determine the diversity of the microbial community. Community similarity was determined through the Whittaker similarity index, whilst community diversity was determined using Shannon's diversity index. The study included four separate experiments, including two *in situ* experiments where the potential effects of herbicide were measured in riparian environments, and two *ex situ* experiment where the effects were measured in a more controlled environment, using soils sampled from riparian field sites.

All three herbicides showed a significant effect on soil pH (water) levels. When treated with any of the three herbicides, the soil pH consistently decreased in both the *ex situ* and *in situ* experiments, with up to one pH unit difference. In the *in situ* experiment, the pH decreased from 6.0 (before treatment) to 5.5 (after treatment) and in the *ex situ* experiment, the pH decreased from 5.9 to 5.2. This was also found for the other herbicide treatments (Triclopyr and the Fluroxypyr and Picloram treatment) and one can conclude that these herbicides acidify the soil within a six-week period following their application. Furthermore, total available nitrogen increased significantly with Glyphosate and Fluroxypyr and Picloram treatment *in situ*. This was however not consistent throughout all the experiments. The other soil chemical characteristics showed little or no change irrespective of the type and concentration of herbicide used. Similarly, the soil microbial characteristics studied showed little change after herbicide treatment. The bacterial diversity scores remained relatively stable throughout the course of the study, ranging between 1.7 and 2.7. The similarity scores also revealed low levels of separation and dissimilarity between microbial communities before and after a given herbicide treatment.

These results imply that herbicides have little to no effect on soil microbial communities or soil nutrient levels in the short term. It is likely that soil microbes are influenced by the plant community more than applied herbicides, and the effects of herbicides may only manifest with induced changes in the plant community, or over a much longer period than the duration of these experiments. The consistent decrease in soil pH could, however, have implications for restoration in the medium and longer term. A significant body of evidence exists suggesting soil microbes are affected by soil pH,

to such an extent that soil microbial communities are structured by soil pH and can be affected by changes in soil pH. This is also the case for fynbos riparian soils, where soil microbes are strongly affected by soil pH. The use of chemical management should only be utilised when necessary as the effects are rather variable and many aspects of the soil environment require more in-depth research. It is recommended that other herbicides and different active ingredients are tested in future studies with the focus of testing effects at different concentrations and testing the effects over extended periods of time. Additionally, studying the microbial communities in more depth with the aid of microbial species identification can help to determine if and which fynbos microbial genera are being affected.

The burning of slash piles as part of biomass management post removal of *Acacia* and *Eucalyptus* biomass is practiced in terrestrial as well as some riparian environments. Four riparian sites, two previously invaded by each of the species that are the subject of the project, viz. *A. mearnsii* and *E. camaldulensis*, as well as one terrestrial site, namely Blaauwberg, cleared of *A. saligna,* were included in the study. The aim of the study was to evaluate the seasonal and spatial effects of burning of slash piles of *Acacia* spp. and *Eucalyptus* spp. biomass on soil physicochemical properties. Burning was conducted in spring 2014 at the Hermon and Blaauwberg sites, and in winter 2015 for all other study areas. *A. mearnsii* and *A. saligna* piles had a volume of between 21.01 and 88.17 m^3 prior to burning, which took place on cool, windless days, while pre-packed *E. camaldulensis* stacks had a volume of between 93.93 and 116.68 m^3 which were burned by local contractors. Samples were collected from the topsoil layer, 0-10 cm depth, prior to burning, and subsequently, from within the burn scars (from the centre, from an intermediate position, i.e. between the centre and the edge, and from the edge), from the soil matrix (about 2 m away from the edge of the burn scar), from a recovering reference site and from an invaded reference site. The collected samples were subjected to laboratory analyses for pH, EC, total carbon (C) and nitrogen (N), available N, available phosphorus (P; Bray No 2), exchangeable cations and soil hydrophobicity.

At the riparian study areas, within areas affected by fire (burn scars from burning of slash piles), soil pH (water) increased significantly after fire and was still high at the end of the sampling period (one year after fire). This was with the exception of the Wit River area, an *A. mearnsii*-cleared area, where soil pH increased significantly after fire and returned to pre-fire levels after 3-4 months. Electrical conductivity increased significantly after fire and returned to pre-fire levels by the end of the sampling period, except at Wit River, where EC did not change after fire. Available P increased in all study areas (including Wit River) and eventually returned to pre-fire levels within less than a year after burning. Exchangeable cations increased significantly and declined within the medium term. Total C and N responded differently across study areas; they remained unchanged at Hermon and decreased significantly at Rawsonville. Available N was not initially affected by fire at any of the study areas, but later showed higher levels within fire scars in *Acacia*-invaded areas. Hydrophobicity increased only at Rawsonville (*Acacia mearnsii*) as a result of fire and was not affected by fire at other areas. An emerging theme was that the Wit River riparian area, cleared of *Acacia mearnsii*, responded with lower amplitude (soil pH, EC, cations) and also returned to pre-fire soil physicochemical conditions quicker than any of the other areas. This may be due to a flood event immediately following the fire treatment, which suggests moisture may have mobilised nutrient-enriched ash and/or leaching of ions from the soil profile, which lead to a rapid recovery of some properties.

The results from the terrestrial site indicate a delay in recovery of some of the physicochemical properties, e.g. EC and cations did change immediately after fire, but only increased significantly in the subsequent season. Soil pH and available P increased significantly after burning but reached

their peak in summer, when the soil was relatively dry. This contrasts with the relatively moister soils encountered in riparian environments, where mineral soil physicochemistry was immediately affected, and suggests that the moisture regime of locations where burning of slash pile takes place may be an important factor that might influence the fate of burn scars. Most of the riparian areas were also burned during a relatively moister part of the year (winter and spring).

Burn scars are often observed in managed landscapes as semi-circular patches that are not covered by vegetation. In fynbos environments, soil properties within these scars are different from the surrounding landscape, and this may contribute to a lack of recovery that has been observed on some fire scars, even several years after fire. Indeed, in some cases, the chemistry of the burn patches has not returned to pre-fire conditions after one year, and there is no trajectory towards pre-fire soil chemical conditions. This may result in persistence of bare patches, especially if these are hydrologically disconnected from streams, as in the case of riparian sites elevated above the active channel. These bare patches may require active management efforts to aid in re-establishing indigenous vegetation and reduce the potential of erosion by wind or eventual occupation by invasive seedlings. Invasive seedlings may be more plastic and more adaptable in terms of soil chemistry for establishment and growth or able to overcome some limitations to compete for elevated nitrogen or phosphorus.

Burning of slash piles may also affect soil microbial communities, therefore we also investigated the impact of slash and burn of *Eucalyptus camaldulensis* and *Acacia mearnsii* biomass on riparian soil microbial diversity and on selected beneficial groups of microbes *in situ*; only riparian areas formed part of the study. Soil chemical properties which are sensitive to fire, e.g. soil pH, EC, ammonium, nitrate, and phosphate were recorded along with soil bacterial and fungal diversity and structure. The soil chemical properties were compared to the microbial diversity and spatial distribution patterns to determine their association with the soil microbial communities.

The four riparian sites in this part of the study were either invaded by *A. mearnsii* or *E. camaldulensis*. Automated intergenic spacer analysis (ARISA) fingerprinting was employed to evaluate the bacterial and fungal community composition before the fire, within two weeks after the fire and after a year. The microbial diversity profiles of ARISA were determined by means of the Shannon's (*H'*) and Simpson complement (1-*D*) indices, whereas the microbial community structure was evaluated by means of analysis of similarity (ANOSIM), cluster analysis and non-metric multidimensional scaling. The association between soil chemical properties and microbial diversity was determined by employing Pearson correlation coefficient analysis, and the association with the microbial community structure was determined by employing principle component analysis.

This study showed that burning slash piles of *Eucalyptus* biomass has a greater impact on microbial diversity and community structure than burning slash piles of *Acacia mearnsii* biomass. The areas exposed to the burning of slash piles of *Eucalyptus* biomass resulted in microbial mortality whereas the areas exposed to the burning of slash piles of *Acacia* biomass resulted in a variation of microbial abundance. All the sites showed a microbial community shift after the effect of fire. The Bainskloof site, an *A. mearnsii*-invaded site, experienced significant winter flooding after the fire event, which altered the response of fire on soil microbial communities, relative to the other sites. At the *Eucalyptus*-invaded sites bacterial growth showed a steep decrease after the effect of fire and remained relatively low even after a year. Further, the bacterial community structure after a year formed a unique community which was graphically distinct from the community structure before and immediately after the burning of the piles. Fungal diversity showed minimal variation after fire and fungal community structure after a year remained similar to the community structure immediately after the burning of *Eucalyptus* slash piles. In all the sites, soil pH was strongly related to bacterial

diversity and community structure. No s chemical property was closely related to soil fungal diversity or community structure.

This suggests that changes in pH could have a major impact on soil microbial diversity and, as suggested earlier, this could have long term consequences as pH did not return to pre-fire levels over the course of the year that monitoring was conducted on soil chemical properties at these sites. This could affect the ability of the soil to supply propagules that will adequately infect the roots of native plant species with mycorrhiza, an association shown to be crucial in competitive interactions. Some fungal-plant associations are also highly specific, and if some propagules of some groups of soil microbes e.g. ericoid mycorrhiza are not present, this may influence the establishment of native species in burned areas post fire.

Regeneration of various native plant species in burn scars may significantly affect restoration outcomes after clearing of invasive alien biomass. An *in situ* survey (in both *A. mearnsii*- and *E. camaldulensis*-invaded areas) and an *ex situ* experiment were carried out using a study area where *Eucalyptus camaldulensis* slash piles had been burned some months earlier.

Major differences were evident in diversity, cover and abundance of scars of slash piles up to one year after experimental burning (*in situ* survey). Further analyses revealed that invasive plant abundance was higher in scars left by experimental burning, which is consistent with some other studies carried out in North American ecosystems. When considered against results from the *ex situ* experiment, where germination by the invasive *E. camaldulensis* was faster that the native *A. karroo*, invasive species may have a competitive advantage in these highly disturbed environments. *E. camaldulensis* germinated faster and in higher numbers compared to native species, possibly as a result of smoke induced chemical components still present in soil from underneath burned piles. However, over the longer term (8 weeks), these individuals fell behind in terms of growth compared to the individuals growing in unburned soils. This may be due to elevated nutrient levels leading to toxicity, or a lack of nutrients in these soils. This suggests that burned areas represent a harsh environment for seedlings to establish themselves in, as some nutrients may be more available, or a nutrient limitation may result, which is detrimental to seedling establishment and survival in the longer term. The results are also consistent with the findings of significantly altered soil chemical properties and microbial diversity and structure, which may also play a role in how different plant species germinate and establish in burn scars.

Removal of biomass and, concomitantly, nutrients may also affect riparian functioning. The nutrient content of *E. camaldulensis* and *Acacia mearnsii* trees of different sizes growing at different stem densities in riparian areas was measured and models developed predicting biomass and nutrient stocks in biomass. The study focussed on determining the influence of ontogeny and stem density extremes on biomass distribution and nutrient dynamics within these invasive trees. Destructive sampling was carried out of both species growing at two riparian areas each; *E. camaldulensis* was sampled at sites in Wolseley and Alfalfa, while *A. mearnsii* was sampled at a site in Bainskloof and a site at Alfalfa. Samples were wet-weighed in the field, after which dry mass was determined in the lab by oven-drying samples at 40°C until a stable dry mass was achieved. All tree components were treated separately (leaves, stem, bark and branches and twigs). Dried samples were ground through a 50 µm screen and subjected to combustion to determine nutrient stocks locked up in biomass. Samples were separated in terms of their age group (juvenile, sapling, and adult) and also based on their occurrence in a dense stand or as a freestanding individual tree. Samples were treated separately throughout. This data was then used to develop allometric models which could be used to determine biomass and nutrient stocks by measuring stem the BD of samples in the various categories.

The biomass models of various components over BD show that there are distinct differences in growth form between plants from dense stands and freestanding ones. The steeper slopes in most components (except stem biomass) of freestanding trees show an accelerated increase of biomass with a similar increase in stem diameter. There were no discernible differences between stem biomass increase per unit increase of BD between plants from dense stands and those not, while leaves from *E. camaldulensis* showed site-specific allometry. This, by extension, translates into separate, site-specific models for the species' leaf biomass. All other components could be modelled across sites for both species with fairly high coefficients of variation (R^2 values in regressions), and thus high predictability.

Nutrient modelling was treated the same as biomass sampling, i.e. biomass partitioning, differentiation between plants from different life stages, and separation based on stem densities. Nutrient modelling in most cases was done by determining the total nutrient content of various components, and drawing regressions of nutrient mass over stem BD. An exception was made for leaf nutrient stocks of *E. camaldulensis* due to high levels of uncertainty, where nutrient stocks were modelled over leaf biomass. This, however, requires the user to also determine leaf biomass (by using the leaf biomass models provided here) before being able to determine total nutrient stocks.

Overall, these biomass and nutrient models could be useful in various aspects of landscape management, including cost analyses of biomass removal, decision-making regarding use of excess biomass, gathering information on carbon cycling, and planning for nutrient exports from cleared areas. All models should, however, be used with care and with regard for site differences that could distort their accuracy.

Some clear outcomes and recommendations from the study can be shown. Soil pH changed both with herbicide application and with burning of slash piles, but in opposite directions. Herbicides impacted soil through declines in pH, and can also add nutrients to the soil; however, it is unclear whether these impacts are long term or whether the impacts are ephemeral. In the case of fire, soil pH changed by up to two units, and remained high even after a year of monitoring. Other soil properties, notably phosphorus and cations, are also altered, and plants grown in soils from burn scars may be affected by these altered soil chemical conditions, resulting in modified plant competitive interactions. Some alterations to soil microbial diversity and structure in response to fire were found; however, the impact of persistent high pH levels on soil microbial communities and soil-plant interactions (nutrient uptake and symbioses) remains to be determined. The role of floods that can 'reset' the template and mitigate the impacts of fire has been documented, but a more comprehensive study needs to be undertaken to determine the relationship between fire and floods in riparian ecosystems. The alternative to burning biomass is removal of biomass, and as was shown here, large amounts of nutrients can also be removed in biomass (especially wood in the form of wood chips or whole wood). Some of the negative consequences of the approaches to clearing may need to be traded off against the longer-term benefits of removal of woody invasive species from ecosystems.

Several aspects of the research are novel, for instance, the development of biomass and nutrient models specifically for stands of invasive alien plants, as opposed to plantations. Another aspect of the research that can be considered novel is the tracking succession of microbial communities in fire scars, coupled with physicochemical changes post fire. However, the research was constrained by several factors, of which the temporal and spatial alignment of student projects was one. Due to students starting their projects at different times, mainly due to the time it took to recruit students, the initial plan to use the same sites for all student projects had to be abandoned. Thus only some sites were used by multiple projects while other sites were used by only one or two students. This

made aligning the data and trends obtained complex, though clear trends were nonetheless obtained. Another aspect that deserves mention is the loss of some sites halfway through the study due to clearing efforts by landowners. This impacted some aspects of the study, though not irreparably so.

The results present several mechanisms whereby riparian restoration can be thwarted by interventions meant to achieve positive outcomes for ecosystem services affected by invasive alien trees. Considering the major investment in restoration of ecosystem services through the WfW programme, some strategic research is needed on how the processes underlying provision of ecosystem services are affected by invasive alien trees and their management in riparian environments. Some gaps in our understanding of the responses to interventions emerging out of this research include the following, which may serve as recommendations for future research:

- Longer-term responses of soil microbial communities and soil physicochemical properties to the application of herbicides to soils may well show different responses to those reported here, and deserve to be investigated further, and over a longer period.

- Changes in pH, found when soil is exposed to fire and herbicides, have the potential to alter soil microbial communities. The close relationship between bacterial community structure and soil pH in fynbos soils was shown here as well as in a previous study in riparian zones (Slabbert, 2012). Should alterations in soil pH be long term or even irreversible, this may have consequences for soil microbial composition and structure. However, this needs to be investigated further.

- It is recommended that future research into the impacts of herbicides explores evaluating different herbicides with different active ingredients as well as the adjuvants involved.

- It is also recommended that impacts of herbicides on microbial biomass and microbial community compositional changes should be explored, for instance through pyrosequencing, which would allow identification of soil microbial populations.

- The medium- to longer-term fate of soils within burn scars should be investigated given the potential impact on soil microbial and soil functioning.

- The ability of native riparian plant species to regenerate in soils physicochemically altered by herbicides or fire has not been investigated, but may be a good topic for a future study. Similarly, altered soil microbial diversity following fire, and its relationship to native plant species, especially regarding symbioses, also need to be investigated.

- Improving our understanding of competitive interactions between native and invasive seedlings in cleared landscapes and how these are affected by altered soil chemical and microbial properties could also be a good topic for future research.

- Investigating the role of flooding on soil chemical and microbial properties, and how flooding and riparian geomorphology may be used strategically to influence soil conditions and subsequent regeneration of native riparian communities will add to the body of knowledge on riparian restoration.

- To expand the current study, generating biomass and nutrient models for catchments in other provinces and compare to current data, in order to expand the database is an important step to understanding the available biomass for exploitation in commercial and community-based ventures, and deserves some attention.

In interactions between the project team and officials from relevant government agencies it was clear that the outcomes of the project were received well, had an impact on decisions at operational level and also found some traction with officials working on policy. This aligns well with the Water Research Commission's (WRC) goals to inform policy and decision making, which is part of the knowledge tree. The research presented here also aligns with several of the key strategic areas of the WRC, notably within the scope of investigating ecosystem functioning (in this there is a continuity with a previous WRC project, reported in Jacobs et al., 2013) as well as ecosystem restoration, both listed as objectives of KSA2, Water-Linked Ecosystems. While the focus is on how riparian processes are affected by management interventions, there are obvious links to both the terrestrial and aquatic environment through biotic and hydrological connectivity. Many processes that take place within riparian environments have a cascading impact on aquatic environments. For example, the lack of regeneration of vegetation in burn scars after burning of slash piles of *Eucalyptus* and *Acacia* biomass may increase sediment transfer to aquatic environments, impacting both native plant and animal aquatic communities as well as human communities dependant on clean water in adequate amounts. This again mandates due consideration of the consequences of management interventions against the benefits that will accrue when alien invasive species are removed. On the other hand, standing stocks of biomass, C, N and P in invasive alien stands in riparian environments may be exploited for bioenergy, local energy requirements and other uses by human communities, and the models developed here will aid in quantifying available stocks. This directly relates to several of the Lighthouses developed as part of the WRC research scope, for example the Water-Energy-Food Security and the Green Village Lighthouses.

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DECLARATION

Parts of this report consist of work produced during one completed BSc Conservation Ecology 4th year project, one completed MSc, two ongoing MSc and one ongoing PhD study. There will thus be some overlap between the theses produced and this report.

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AMF	Arbuscular Mycorrhizal Fungi
AMR	Average Metabolic Response
ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
ARISA	Automated Ribosomal Intergenic Spacer Analysis
AWCD	Average Well Colour Development
BD	Basal Diameter
CFU	Colony Forming Units
CMD	Community Metabolic Diversity
DNA	Deoxyribonucleic Acid
EC	Emulsifiable Concentrate
EC	Electrical Conductivity
EtBr	Ethidium Bromide
FR	Field Rate
H’	Diversity
HSD	Honest Significant difference
IAP	Invasive Alien Plant
LSD	Least Significant Difference
ME	Micro Emulsion
MUB	Modified Universal Buffer
NMDS	Non-metric Multidimensional Scaling
NMR	Net Mineralisation Rate
OTU	Operational Taxonomic Unit
PCA	Principle Component Analysis
PCC	Pearson Correlation Coefficient
PCR	Polymerase Chain Reaction
SL	Soluble Liquid

SOM	Soil Organic Matter
WfW	Working for Water
WRC	Water Research Commission

CHAPTER 1: Introduction

1.1 BACKGROUND

Management of invasive alien plants is a major challenge in South Africa, especially in the fynbos biome, where many invasive trees have invaded terrestrial and riparian environments. Riparian environments are especially vulnerable as the linear nature of these ecosystems enhances invasion, and also makes management more challenging due to the dynamic nature of flowing waters. Sediment dynamics ensure that propagules are transported within river systems, while disturbance in terms of floods, drought and occasional fires all influence establishment of seedlings and influences competitive outcomes in favour of invasive species (Richardson et al., 2007). Furthermore, riparian environments are the recipients of material, including invasive alien propagules, from elsewhere in the landscape (Jacobs et al., 2007), thus enhancing the invasibility of these environments. Riparian ecotones are hotspots for processes that underlie ecosystems services such as production of clean water and immobilisation of toxins, and act as habitat for riparian plant and animal species. A decline in the ability of riparian environments to produce ecosystem services could severely impact downstream, dependant human communities. Alien invasive plants have been shown to impact water resources, and a major clearing effort, coordinated by the Working for Water (WfW) programme, is making inroads into the large stands of alien trees in riparian and terrestrial environments (le Maitre et al., 2016).

Various approaches to clearing and managing subsequent biomass accumulation are practiced. However, the nature of these interventions may in itself impact negatively on ecosystems. An example is herbicide application, a widely applied practice, which, according to literature on various ecosystems, leads to alteration of soil microbial structure, composition and diversity. Another example is removal of dead biomass through combustion of slash piles consisting of trunks and branches of invasive alien trees after clearing. Burning of slash piles has been shown to lead to scars that remain in the landscape for years to decades, altered regeneration of vegetation and altered soil physicochemical properties. Loss of soil seedbanks, which are destroyed during high-intensity fires, may be one reason for the lack of recovery of burn scars, while the altered physicochemical properties of the soil may be another factor that may hinder the re-establishment of native vegetation. Furthermore, removal of biomass in the form of whole wood or wood chips off site may also impact riparian ecology in terms of nutrients lost, while foliar biomass may be suspended and removed by floods following clearing of invasive trees. The result is that restoration may be uneven, or delays may occur in the recovery of native vegetation following removal.

1.2 RATIONALE

The invasive alien tree *Acacia mearnsii* can induce significant changes to soil processes and soil microbial dynamics in fynbos riparian environments (Jacobs et al., 2013). Among others, nitrogen levels in the soils are elevated, soil phosphatase (an enzyme involved in converting organic phosphorus (P) to plant available P) is enhanced in soils associated with invasive stands, while CO_2 emissions are greatly enhanced, apparently by roots within stands of invasive trees. Soil bacterial and fungal structure is also altered, and removal of invasive trees does not totally restore soils, even after more than seven years of clearing and follow-ups, though the restoration trajectory is towards pre-invasion conditions. As an example, nitrogen levels returned to pre-invasion levels several years after removal of invasive stands, while nitrate levels remained high. Major changes to bacterial structure took place following invasion, with some legacy effects observed in both soil chemical and microbial

properties. However, the impact of management interventions in the riparian zone has not received attention. This includes the impact of herbicides, often found to impact soil microbial communities negatively (Busse et al., 2000; Zabaloy et al., 2012). Fire is used as a tool to reduce biomass which remains following clearing by government agencies as well as private landowners, though some evidence, locally as well as in literature, suggests that impacts of stacking and burning of biomass may be long lasting (Korb et al., 2004). The impacts may include both soil physicochemical and microbial alterations to the soil environment, influencing the establishment of native species post clearing and burning. Lastly, Blanchard and Holmes (2008) found removal of biomass to be the most effective in promoting restoration of riparian environments post clearing, although it can introduce unintentional consequences such as in relation to export of nutrients in the form of wood biomass, which could be detrimental to nutrient cycling and riparian processes in the long term (Naiman and Decamps, 1997; Hefting et al., 2005).

1.2.1 Aims

Given the background outline above, the overarching aims of the project are to:

1. Evaluate the impact of recommended levels of herbicides, used to control alien invasive growth and regeneration, on soil microbial diversity and on selected beneficial groups of microbes *in situ* and *ex situ,* and in riparian soils from two different longitudinal zones.
2. Determine the impact of slash and burn of *Eucalyptus* spp. and *Acacia mearnsii* biomass on soil microbial diversity and on selected beneficial groups of microbes *in situ,* and measure regeneration of various native plant species grown in soil from slash and burn scars *ex situ.*
3. Determine the impact of slash and burn of *Eucalyptus* spp. and *Acacia mearnsii* biomass on soil physical and chemical properties *in situ*.
4. Determine the biomass and nutrient content of *Eucalyptus* spp. and *Acacia mearnsii* trees of different sizes growing at different stem densities in riparian sites from two different longitudinal zones.

1.3 PROJECT SCOPE AND EXTENT

The project included field surveys and experiments on three fynbos rivers, namely the Berg, Breede and Wit (also known as the Witte), as well as greenhouse experiments carried out at Stellenbosch University. A terrestrial site, Blaauwberg, was also added to compare the effect of burning of slash piles on soil properties.

1.4 DIVISION OF LABOUR

Four students worked on the major aspects of the project:

- Roderick Juba, PhD, worked on the biomass and nutrient content of *Eucalyptus camaldulensis* and *Acacia mearnsii* trees of different sizes growing at different stem densities in riparian sites from two different longitudinal zones.

- Tshepo Maubane, MSc, worked on the responses of fynbos soils' physicochemical properties to burning of slash piles of *Acacia* spp and *Eucalyptus camaldulensis* biomass.

- Liam Cogill, MSc, worked on the impact of recommended levels of herbicides, used to control alien invasive growth and regeneration, on soil microbial diversity in riparian soils from two different longitudinal zones.
- Ricardo Smart, MSc, worked on the impact of slash and burn of *Eucalyptus camaldulensis* and *Acacia mearnsii* biomass on soil microbial diversity and on selected beneficial groups of microbes *in situ*.

These students are Black or Coloured males, which is in line with some of the goals in terms of transformation and redress of the WRC Knowledge Tree.

A fifth project, part of a 4th year BSc Conservation Ecology project by Ludwig van der Merwe, formed part of the *in situ* vegetation survey, while a greenhouse trial was conducted on regeneration of various native plant species grown in soil from slash and burn scars *ex situ*.

1.5 STRUCTURE OF THE REPORT

Chapter 1 provides a short introduction to the report, and outlines the structure of the report, while Chapter 2 provides a brief overview of the literature. Chapter 3 summarises a study on the impact of recommended levels of herbicides used to control alien invasive growth and regeneration on soil microbial diversity in riparian soils, and Chapter 4 deals with the impact of burning of slash piles as part of biomass management post removal of *Acacia* and *Eucalyptus* biomass. Chapter 5 expands on the impact of fire as a management tool and documents the impact of slash and burn of *Eucalyptus camaldulensis* and *Acacia mearnsii* biomass on soil microbial diversity and on selected beneficial groups of microbes *in situ*. Chapter 6 deals with regeneration of various native plant species *in situ* and when grown in soil from slash and burn scars *ex situ*, while Chapter 7 summarises a study on the biomass and nutrient content of *Eucalyptus camaldulensis* and *Acacia mearnsii* trees of different sizes growing at different stem densities in riparian sites from two different longitudinal zones. Finally, Chapter 8 summarises the study and makes some recommendations.

CHAPTER 2: Literature review

2.1 INTRODUCTION

Research on riparian zones, their ecology and functioning, is of increasing importance in South Africa, especially against a background of water management inefficiencies and drought, resulting in severe water shortages in several provinces of South Africa (as of 2015). Riparian environments play a critical role in ecosystems services relating to the generation of clean water such as removal of sediment, nutrients and toxic substances from streams and rivers, and also play a critical role as habitat for a host of animals, plants and microbes that underlies hydrological ecosystems services (Bardgett et al., 2001; Naiman et al., 2005; Jacobs et al., 2007). However, riparian zones are some of the most degraded ecosystems, with invasive alien plants as one of the main drivers of loss of riparian biota and riparian function (Richardson et al., 2007). In South Africa, riparian zones are particularly vulnerable as woody invasive alien plants, notably *Acacia mearnsii* and *Eucalyptus* spp., including *E. camaldulensis,* have invaded long stretches of many rivers. In the fynbos biome, the WfW programme has, however, managed to clear a significant part of riparian zones, especially of rivers that contribute significantly to the water supply of urban areas. Several approaches are used for clearing alien invasive species, many of which have been subjected to research in terms of effectiveness when considering post-removal recovery of riparian zones, focussing on re-establishment of native plant species (Holmes et al., 2008). However, the ecosystem impact of various clearing methods as well as management of remaining biomass on site has not been considered in great detail.

2.2 HERBICIDE IMPACT IN RIPARIAN ZONES

WfW utilises various herbicides to suppress regeneration of invasive alien plants (IAPs) in riparian fynbos ecosystems in the Western Cape (Blanchard and Holmes, 2008). They apply specific concentrations and dosages of herbicides to woody IAPs in relation to the target plant's life stage and species. Some of the most ubiquitous invasive alien trees present in the riparian fynbos include *Acacia mearnsii* and *Eucalyptus camaldulensis*. Common herbicides used for controlling the *A. mearnsii* populations are Springbok (active ingredient: Glyphosate) and Garlon (active ingredient: Triclopyr), whilst the herbicide Plenum (active ingredients: Picloram and Fluroxypyr) is used for *E. camaldulensis* (Bold, 2007). These herbicides have been reported to be effective in the short term for suppressing the growth of the afore-mentioned invasive alien trees, but regeneration of IAPs may occur in the long term (Wolmarans and Swart, 2014). Additionally, several studies concluded that the active ingredients of these herbicides have potential detrimental effects on soil functioning and soil processes (George et al., 2009).

The impact of the active ingredients of commonly used herbicides on soil processes, soil microbial populations and ultimately nutrient stocks and fertility may impact native plant communities in several ways, e.g. symbioses between native plants and soil microorganisms may be altered (Wilson and Hartnett, 1997). The effect may manifest through the toxic nature of the herbicide, which may alter microbial community structure through hindering population persistence of *Rhizobium* species (Wolmarans and Swart, 2014). These microbes are important for fixing nitrogen and regulating nitrogenous compounds in the rhizosphere and have symbiotic relationships with *Acacia* species. The regulatory ability of microorganisms such as *Rhizobium*, which partake in these important metabolic activities, is vital for the survival of many native plant species (van der Heijden et al., 2008; Barton and Northup, 2011). Another potential negative impact of the usage of herbicides containing these active ingredients includes its ability to alter the acidity of the soil (Wolmarans and Swart, 2014). The

toxic nature of the herbicide could be strong and long lasting enough to increase the acidity of the soil, decrease soil fertility or weaken native plant species' metabolism, making them more susceptible to attack by pathogens (Wolmarans and Swart, 2014). This would directly impact soil functioning, and might be unfavourable for the persistence of local microorganisms and native plant populations alike, in addition to modifying nutrient availability to plants. In addition, it has been found that some microbes such as *Pseudomonas, Arthrobacter, Bacillus*, and *Xanthomonas* species are adept at breaking down herbicides (Busse et al., 2000). These microbes can, in fact, use nutrients such as N, released from compounds in herbicides, as energy sources, while also decomposing and detoxifying herbicides in the soil. The impacts of herbicides have typically been studied in monoculture agricultural settings and not often *in situ* or in controlled microcosm environments *ex situ* where the field environment is simulated. It is therefore important to study these potential herbicide impacts on a microbial and soil chemical level in both *in situ* and *ex situ* environmental settings.

Soil microorganisms are drivers of turnover of nutrients in the ecosystem, by breaking down complex organic molecules and releasing organic nutrients which are then utilised by the plant community as well as other microbes (Barton and Northup, 2011). Microorganisms have a low homeostasis and high surface to volume ratio and this enables them to react quickly to changes in the environment and act as an early warning signal (Stefanowicz, 2006). As such, microorganisms are usually the first organisms to react to chemical and physical changes in the environment (Barton and Northup, 2011). Their observed changes are precursors to alterations in the health and viability of the ecosystem as a whole. A disturbance to the metabolic activities of microorganisms (e.g. carbon metabolism) could yield altered amounts of nutrients in the soil (Stefanowicz, 2006). Herbicides could be such a disturbance where the toxic nature of the active ingredients alters the soil chemical characteristics as well as the soil microbial structure and function (Wolmerans and Swart, 2014), or may be utilised as a carbon, nitrogen or phosphorus source by microbes (Gaertner et al., 2011; Magadlela et al., 2015). However, different microbes may respond to disturbances in different ways, which could be tested using an index of soil microbial functional diversity. The Biolog® Eco-plates and similar approaches have been used successfully, and have been used successfully to show the ability of active (colony-forming) microbes to utilise carbon sources added through addition of herbicides (Zabaloy et al., 2012).

In fynbos ecosystems, nitrogen and phosphorus are limited and the native plant community is therefore dependent on symbioses with microorganisms such as mycorrhizal fungi for survival. Amino acids in the soil matrix are microbially mineralised to produce plant available NH_4^+ as an intermediate product (Kirchman, 2012). Various soil microbial groups such as *Alpha-proteobacteria* and the *Actinomycetes* play important roles as nitrogen fixers, processors of carbon, as well as regulators of nutrients in soils (Liu et al., 2011; Kirchman, 2012). Additionally, among other vital microbial associations, *Planctomycetes* are especially important where they partake in the Anommox reaction to cycle nitrogen and produce essential end products (Fuerst, 1995). Furthermore, protozoans are also vital for maintaining nitrogen stocks in soils as well as other nutrients and depend on certain bacterial genera as a food source (Kirchman, 2012). Bacteria are therefore not only an important regulator of nutrients in the soil, but also act as a food source in the soil matrix.

2.3 IMPACT OF FIRE ON RIPARIAN ZONES

Approaches for clearing IAPs include cutting and leaving biomass on site, removing biomass for alternative uses such as wood chips and other applications (Holmes et al., 2008), as well as ring-barking or another form of killing large standing trees in place. Removing or destroying biomass on site was found to be the most effective way to ensure recovery of native species, though other factors

may also influence the trajectory of restoration. Thus, cutting and leaving biomass on site may prevent restoration in places, and may be followed by stacking and burning (Holmes et al., 2008), which is a widely practised procedure used to reduce wildfire risk and manage excess biomass (Cilliers et al., 2004; Rhoades et al., 2015). The process involves stacking plant biomass in piles of different sizes which are allowed to dry and then burned on site.

Depending on the load characteristics and microclimatic conditions, slash pile burning may be very severe and significantly different from natural wildfires (Certini, 2005; Rhoades et al., 2015). Intense fires that last longer (such as burning of slash piles) may produce temperatures ranging between 500 and 700°C or more (Korb et al., 2004), which are capable of penetrating deep into soil profiles, altering soil physical and chemical properties, soil microbial communities and soil eco-hydrological properties. Esquilín et al. (2007) recorded temperatures of about 300°C beneath the centre and 175°C beneath the boundary zone of the slash pile. Hubbert et al. (2015) reported soil surface temperatures of approximately 200°C for a prolonged period of more than 30 hours under large fuel stacks. Depending on the length of the period of exposure to high temperatures, soils may change for a short period but recover, or change for a long period but recover, or may change permanently and not recover (Certini, 2005). However, burning of slash piles on site may be the only option where removal proves difficult, if fire risk is to be mitigated and recovery of vegetation to take place (Behenna et al., 2008). The extent of alterations to soil chemical properties and processes will primarily vary depending on fire severity and soil texture (Oswald et al., 1998; Giardina et al., 2000b). Loss of soil nutrients through volatilisation often occurs at high fire temperatures, which impacts on the nutrient stock and cycling (Neary et al., 1999). Immediately after combustion of plant biomass, nutrients concentrate in the ash and ultimately get incorporated and enrich the soil surface (Mohamed et al., 2007; Schafer and Mack, 2010). As a result of ash incorporation, nutrient concentrations in the soil surface layer generally increase immediately after the fire, and eventually decrease seasonally as a result of run-off, leaching and wind which may displace nutrient-rich ash (Giardina et al., 2000b; Certini, 2005; Mohamed et al., 2007; Schafer and Mack, 2010).

Soil organic matter (SOM) supplies crucial soil macro-nutrients such as such as nitrogen and phosphorus, and micronutrients such as boron (Sparks, 2003). Soil organic matter becomes consumed as a result of fire (Certini, 2005); the quantity of SOM consumed or lost during burning of slash piles is largely influenced by the fire severity, soil moisture content, and the depth, texture and presence of stable organic compounds such as humus compounds (Forgeard and Frenot, 1996; Certini, 2005; Neill et al., 2007). Forgeard and Frenot (1996) exposed soil to 150°C and 300°C temperature regimes and found that considerable SOM consumption occurred at 300°C within the 0–2.5 cm soil layer. Granged et al. (2011) also observed that high fire temperatures would consume considerable amounts of soil organic matter. Significant SOM consumption may affect subsequent decomposition processes and thus impact soil nutrient stock and cycling. Organic carbon is utilised by microbes during the decomposition process, which leads to the release of micro- and macro-nutrients into the soil, producing CO_2 in the process (Sparks, 2003). Thus, SOM destruction by fire will have major implications for soil carbon composition. Research has shown that fire-induced temperatures may have non-significant effects on total carbon if the fire is less severe (Hinojosa et al., 2012; Fultz et al., 2016) but may significantly reduce total carbon when fires are prolonged, with high temperatures (Johnson et al., 2011; Switzer et al., 2012). In addition to changing soil carbon composition, fire may also alter forms of SOM, and thus affect decomposition processes (Nave at al., 2011). Changing decomposition processes affect both carbon and nitrogen cycling, and as a result indirectly influence the carbon: nitrogen ratio (Nave et al., 2011, Naudé, 2012).

Soil nutrients in the mineral soil are also consumed at different temperatures (Certini, 2005). For instance, soil nitrogen, including plant available nitrogen (i.e. ammonium and nitrate), becomes

volatilised or transformed to gaseous form by less intense fires which produce temperatures of approximately 200°C (Schafer and Mack, 2010). This suggests that nitrogen is highly susceptible to consumption by elevated temperatures during burning of slash piles. However, given variable conditions, post-fire soil nitrogen concentrations may remain unchanged (Hinojosa et al., 2012; Fultz et al., 2016) or might significantly decline (Switzer et al., 2012; Badía et al., 2014). Plant available nitrogen also show varying results after fire; for instance, ammonium (NH_4^+-N) may increase significantly (Hernández et al., 1997; Kulmala et al., 2014; Fultz et al., 2016), decrease significantly (Kutiel and Naveh, 1987) or remain unchanged (Switzer et al., 2012). Nitrate (NO_3^--N) has been also reported to significantly increase (Hernández et al., 1997; Kulmala et al., 2014). Declines in soil available N could have major consequences for native fynbos species as plant growth is typically constrained by nitrogen (Power et al., 2010).

Phosphorus (P) is a soil macro-nutrient that is required for plant growth (Power et al., 2010; Naudé, 2012) and is considered to be stable, immobile and not easily leached through most soils (Sparks, 2003; Hinsinger, 2001). However, at high fire temperatures, soil P may change into unstable and water-soluble forms, which make it more likely to be leached into the soil profile or into flowing streams (Galang et al., 2010). Further, soil P volatilisation often occurs at a temperature of approximately 774°C. Post fire, soil available P has often significantly increased (Romanya et al.,1994; Giardina et al., 2000a; Badía et al., 2014), which may be due to an elevated pyromineralisation rate of organic P from combustion of organic compounds (Galang et al., 2010). On the other hand, Castelli and Lazzari (2002) and Wang et al. (2013) reported non-significant effects on available P as a result of burning. Higher availability of P could benefit native species germinating in post-fire environments as plant growth is limited by P to a greater extent even than N (Power et al., 2010), though alterations to soil pH may ensure lower availability of P through formation of complexes with iron or aluminium.

Soil exchangeable cations, i.e. calcium (Ca), sodium (Na), potassium (K) and magnesium (Mg) are important nutrients for plants and are less vulnerable to heat as they may persist longer than N, P, or C and volatilise only at considerably higher temperatures (Verma and Jayakumar, 2012). As outlined in Neary et al. (1999), Ca, Mg, Na and K volatilise at temperatures of approximately 1240°C, 1107°C, 880°C and 760°C respectively which may not be reached by most fires. However, after fire, cations become abundant on the soil surface as they are released from surface accumulated ash (Certini, 2005). The elevated concentrations of these plant important cations on the soil after fire may lead to high pH and electrical conductivity (Certini, 2005; Kim et al., 1999; Menzies and Gillman, 2003; Switzer et al., 2012).

Apart from the direct impacts of fire on soil microbial populations, microbial activity and soil chemical reactions are also greatly, though indirectly, affected by modified pH levels after fire (Sparks, 2003; Certini, 2005). Soil heating and abundant base cations on the soil surface after fire result in high pH values (Certini, 2005). Higher fire-induced pH values may modify microbial activity and the release and availability of certain soil-bound nutrients (Iglesias et al., 1997; Kulmala et al., 2014). These surface abundant base cations affect not only soil pH, but also soil electrical conductivity (EC), which often become elevated (Hernández et al., 1997) or, on rare occasions, decrease after burning (Iglesias, 2010). In addition, high base cations on the surface after fire will also affect soil parameters such as sodium adsorption ratio and the capacity of soil to exchange for cations (Sparks, 2003).

Coarse textured soils that are high in organic matter may naturally develop hydrophobicity or water repellency, which inhibits water infiltration and promotes erosion (Doerr et al., 2000; Dekker et al., 2001; Fox et al., 2007). This is as a result of SOM decomposition; certain soils may temporarily repel water especially when the soil is dry as opposed to when it is moist (Doerr and Thomas, 2000).

However, hydrophobicity may develop in soils as a result of burning of slash piles of plant biomass which may release waxes that coat soil particles, making it hydrophobic (Mirbabaei et al., 2013). Post fire, Fox et al. (2007) and Jeyakumar et al. (2014) have observed a significant increase in hydrophobicity; on the other hand, Inbar et al. (2014) indicated a considerable drop in hydrophobicity as a result of burning. On steep landscapes such as narrow valleys, hydrophobicity may lead to movement of large soil sediments by water, and on flat regions hydrophobicity might contribute to the soil surface and subsurface drying because infiltration or wetting is hindered (Neary et al., 1999). The differential responses of soil hydrophobicity to fire may be the result of an interaction between fire and soil properties such as texture; sandy soils are more likely to show hydrophobicity compared to clay soils (Doerr et al., 2000).

Slash pile scars develop on soil surfaces as a result of heating, which alters soil properties to such a degree that vegetation re-establishment is hindered and soil remains bare (Korb et al., 2004). Severe fire, with heavy fuel distributed in dense patches, may form large scars which are affected more in the centre and less towards the edge (Korb et al., 2004; Rhoades et al., 2015). These usually semi-circular surface scars often occupy a significant part within a larger ecosystem, where their presence may cause soil sediments to be unstable and highly vulnerable to erosion by water, particularly in steeply sloped areas (Neary et al., 1999). Soil sediments are naturally stabilised by surface vegetation cover; however, this might not be the case in soils exposed to high fire intensities where fire has altered soil properties and it can no longer support vegetation establishment (Neary et al., 1999; Korb et al., 2004). Intense burning, such as of slash piles, often destroys seeds within the upper part of the soil surface, providing an opportunity for surrounding vegetation to establish within the scar (Halpern et al., 2014). This could pose a problem to land managers, especially if the surrounding vegetation is invasive, as it has been reported that the surrounding invasive vegetation may to be more likely to establish on bare fire scars (Korb et al., 2004). Native vegetation establishment may be slow or fast depending on the properties of the burn scar and environmental conditions (Halpern et al., 2014), or in some cases, might require treatment such as seeding in combination with mulching (Fornwalt and Rhoades, 2011).

Fire is a major factor that can induce shifts in microbial community structure (Neary et al., 1995; Dooley and Treseder, 2012; Ferrenberg et al., 2013; Reazin et al., 2016). The shift is associated with a major increase in heat in the top two centimetres of the soil (Sun et al., 2008; Dooley and Treseder, 2012) which has been shown to have more abundant microbial communities (Hart et al., 2005). The heat impact of fire is promoted by (i) the burning of above-ground plant material (van Wilgen and Richardson, 1985); (ii) the heat transfer to the surface layer of soil (Dooley and Treseder, 2012) and (iii) the long duration of fire (Hart et al., 2015). Ultimately, fire results in microbial mortality (González-Pérez et al., 2004; Hart et al., 2005) which alters the structure of soil microbial communities after fire, resulting in communities that are different from unburned sites (Hamman et al., 2007). Different microbial species have different sensitivities to heat; selective mortality of certain microbial communities and resilience or resistance to fire in others may result in altered communities post fire (Hart et al., 2005). Among microbial groups, fungal communities appears to be more sensitive to fire, perhaps due to the production of fire-resistant spores in some bacteria.

As mentioned, fire can have a major impact on soil physicochemical properties, which in turn affects soil microbial communities (Smith et al., 2008; Shen et al., 2016; Sun et al., 2016). For instance, Bååth and Arnebrant (1994) and Bárcenas-Moreno and Bååth (2009) as well as Dooley and Treseder (2012) noted that changes in pH following fire may affect bacterial diversity directly. Carbon availability to microbes following fire may also affect microbial communities; the release of organic material from heat-sensitive microbial communities after the fire serve as an energy source for heat-tolerant bacteria (Daiz-Ravina et al., 1996). In contrast, Barreiro et al. (2016) showed that fungal

biomass remained relatively low for some time after fire on soils. Therefore, fire may impact microbial communities directly or indirectly, and a decrease in microbial abundance may lead to a decline in microbial activity (Jha et al., 1992).

Fire intensity may also play a role. Reazin et al. (2016) showed that low-intensity fires resulted in less alteration to microbial communities compared to high-intensity fires. However, this picture is somewhat complicated by the findings of Xiang et al. (2014), who observed that the relative abundance of alpha-*Proteobacteria*, *Acidobacteria*and delta-*Proteobacteria* decrease in both low and high fire intensity whereas relative abundance of *Bacteriodetes* and beta-*Proteobacteria* seemed to increase in low and high fire intensity exposure. Furthermore, recent studies have found that microbial diversity after the effects of fire remained relatively low in comparison to a control for up to two years (Xiang et al., 2014; Sun et al., 2016).

2.4 IMPACT OF BIOMASS REMOVAL OF RIPARIAN ZONES

Riparian ecotones are hotspots for processes that underlie ecosystems services such as production of clean water, immobilisation of toxins and sediment deposition and production, depending on the geomorphological context. With increasing understanding of riparian and aquatic processes, the roles of various nutrients in supporting riparian ecosystem services are also becoming clear. Invasive trees in these riparian zones can grow in very dense stands and can reach large sizes (van Wilgen et al., 2012), which implies that significant amounts of nutrients are locked up in the biomass. Under natural conditions, nutrients eventually find their way to riparian soils and the aquatic environment where they are considered allochthonous subsidies, important to support food webs and ecological function (Naiman et al., 2005). Invasive trees may also add nutrients to riparian stocks such as nitrogen (Jacobs et al., 2013; Chamier et al., 2012), fixed by invasive legumes from atmospheric sources, and modify other nutrient cycles by sequestering soil phosphorus and microelements (Yelenik et al., 2004; 2007).

Acacia mearnsii is one of the most invasive woody plant species in the Western Cape, and is often referred to as a transformer species, as it is known to change soil chemical and biological properties, while also influencing growth of neighbouring plant species (Naudé, 2012; Boudiaf et al., 2013). Tye and Drake (2011) suggested that the species also affects nutrient cycles more than native *Acacia* species such as *A. karroo*. Tye and Drake (2011) also pointed to a possible positive feedback loop between N fixation in *A. mearnsii* and its uptake of N_2. This leads to eventual enrichment of soil nutrients such as N, and has far-reaching ecological impacts, including establishment of other nuisance plant species, and eutrophication in river systems, while reducing ecosystem services. This is also true for other invasive *Acacia* species, including *A. saligna*, *A. cyclops*, *A. longifolia,* and *A. dealbata* (Stock et al., 1995; Gaertner et al., 2011; Le Maitre et al., 2011). The notorious invasiveness of the species along riparian zones has been ascribed, in part, to its large investment in propagules, which are easily transported downstream by rivers, or stored in the soil to germinate at a later stage (Richardson et al., 2007).

Eucalyptus camaldulensis is another widely distributed species, which has become naturalised and invasive along riparian zones in South Africa. Nutrient dynamics within the various biomass components of *E. camaldulensis* trees have been studied in Brazil (Bernardo et al., 1998; Harrison et al., 2000), and in New Zealand and Australia (Francis and Sheldon, 2002) and generalisations have been made for a wide range of eucalypt species in forests and plantations (Judd et al., 1996). Findings from these studies suggest that, even though *E. camaldulensis* partitions its resources to various tree components based on physical environmental conditions, a strong relationship could exist between nutrient partitioning, the age of the stand, and the spacing between individual trees.

Greater spacing, for instance, could result in an increase in concentrations of N and P in bolewood and taproots. Harrison et al. (2000) suggested this might be due to a decrease in competition as spacing increases.

Data is available on the indirect effects of stands of *A. mearnsii* on nutrient cycling in fynbos soils (i.e. associations with root bacteria and increases in soil enzymes: Naudé, 2012; Slabbert, 2012; Kambaj Kambol, 2013; Slabbert et al., 2014), but nutrients released directly from plant material have not yet been quantified and this exposes a gap in knowledge of the total inputs of nutrients to the affected ecosystem. There is also biomass and nutrient data available for the species when it has been grown in plantations (Dovey and du Toit, 2004). This data provides a solid baseline for the measurement of tree biomass in managed plantations, and the partitioning of nutrients and biomass to various tree components. Dovey and du Toit (2004) also provide allometric models to predict these variables in plantations. There are, however, major differences between plantation and forest dynamics, including growth form of trees (allometry), environmental stressors, tree life stages, and internal factors, such as phenology. There are also management activities in plantations directed at increasing the volume and quality of merchantable wood per unit area used. For instance, there has been a growing awareness of the value of growing forestry trees in deliberate mixtures with predetermined species to enhance their productivity (Forrester et al., 2005; Kelty, 2006; Nicholls et al., 2006). Some trees have been found to increase stand level productivity through positive interactions with forestry species such as complementary resource use, and increase in nitrogen availability (in the case of nitrogen-fixing species) (Forrester et al., 2006a; Kelty, 2006). Forrester et al. (2006b) even reported twice the amount of above-ground biomass production in *Eucalyptus globulus* when grown with *A. mearnsii* than when it is grown in *E. globulus* monocultures, as measured after 11 years. Other physical management practices such as pruning (Pinkard, 2002; Montagu et al., 2003), residue management, spacing, weed control, and fertilizer additions during early life stages (de Moraes Goncalves et al., 2004; Muñoz et al., 2008) also affect wood quality and overall tree allometry in plantations. Management of plantations thus typically results in significant differences in productivity, and data generated from such systems cannot easily be compared to wild forest systems.

Another difference between managed plants and those growing in wild forests (or invasives growing in riparian and other environments) is naturally occurring periods of change that aren't buffered by management practices to maximise yield. Fife et al. (2008), for instance, suggested that evergreen trees may move nutrients between different tree components during different stages of the plants' lifetime to accommodate its changing needs as it enters various life stages. This has also been found by Lödige et al. (2014), who showed in a greenhouse experiment that differences in tree size of *Fagus sylvatica* (European beech) and *Picea abies* (Norway spruce) correlated with differences in partitioning of above-ground biomass. They also noted that environmental conditions such as drought and lack of sufficient light could (to a lesser extent than tree size) skew above-ground biomass partitioning. Poorter and Nagel (2000) term this change in allocation as a result of tree growth "ontogenic drift", pointing out that larger plants typically have to invest relatively more resources in support structures and leaf area. Importantly, they also note that, even though ontogeny plays a big role in allocation of biomass, specific environmental conditions can also be instrumental in determining the form and functioning of individual plants in their specific environments. Wu et al. (2008) agree with this, showing that drought conditions around seedlings of *Sophora davidii* (David's mountain laurel) resulted in greater root growth, while minimising height, leaf mass and leaf area, and total biomass. Canham et al. (1996) tested four woody species: *Acer rubrum*, *Pinus strobus*, and the light- and water-tolerant species *Acer saccharum* and *Quercus rubra*. Contrary to the later findings by Wu et al. (2008), they found that the latter two species did not change their root allocation patterns in response to a decrease in available soil resources, while the former two species showed a significant reduction in relative belowground growth as a response to increased resources.

It is also likely that nutrient transport within trees is greatly influenced by season. Seasonal variations in nutrient concentrations have also been documented for litter of the leguminous *A. saligna and A. cyclops* (Witkowski, 1991). This is the result of annual events such as flowering, and differences in soil available nutrients (e.g. nutrient losses due to flooding of the riparian zone). It is thus important to quantify seasonal differences in allocation of nutrients to various tree components of *A. mearnsii* and *E. camaldulensis* to determine whether allometric models to predict standing stocks of these nutrients can be applied throughout the year. Trees can store large amounts of nutrients and water and only mobilise these during periods of stress or change (Waring, 1987), implying temporal shifts in nutrient stocks. Seasonal changes in nutrient partitioning may be easily seen in deciduous trees, where leaves are lost, and resources have to be allocated again to stimulate and sustain growth of new leaves. Waring (1987) presented a model to predict carbon allocation in trees, suggesting that tissue involved with photosynthesis (foliage), and the development of new roots usually enjoy great inputs of carbon, while the rest of the available carbon is allocated to storage, stem growth, and production of defensive compounds. Ecological theory also suggests that the parts of the plant most limited in terms of available resources will enjoy relatively greater growth to increase acquisition of that resource. Drought-induced stress would, for instance, reduce shoot growth and cause a change in nutrient allocation to belowground components, increasing root growth (Ericsson et al., 1996).

To get a comprehensive view of the mechanics of plant form and function, Diaz et al. (2016) investigated global datasets containing information on adult plant height, stem specific density, leaf area, leaf mass, leaf nitrogen percentage, and diaspore mass. Through a six-variable principal component analysis, they showed that globally there are distinct differences in allocation patterns between components of trees from different functional backgrounds (e.g. woody vs non-woody). They also showed that plant size and leaf strategy (leaf area and leaf mass per unit area) are the two most consistently differing traits between plants of different functional types. It is thus important to consider these traits and discuss them within the sphere of general resource allocation patterns of invasive trees along Western Cape riparian zones, and to test whether these can be predicted at a stand level.

There is extensive research done on stoichiometry and allometry in ecology, including how these are affected by, for instance, nutrient availability and other environmental variables (*see* Weiner and Thomas, 1992; Müller et al., 2000; Sterner and Elser, 2002; Kollman et al., 2004; Weiner, 2004), as well as self-regulating mechanisms as a response to stem densities (Mohler et al., 1978; Pretzsch, 2002). Literature discussing temporal movements of nutrients within standing trees is, however, not readily available for *A. mearnsii* and *E. camaldulensis*, making assumptions about the accuracy of allometric models predicting nutrient stocks (and their relative positions) in trees difficult. To enhance the accuracy of such models, it is necessary to document seasonal differences in nutrient allocation (and perhaps wood density for biomass measurements) within these trees. Also, it may be useful to investigate the effect of sunlight intensity on nutrient dynamics in leaves and whether there are significant differences between sunny side and shaded leaves. Givnish and Vermeij (1976) discussed leaf size and shape as an evolutionary trait to optimise harvesting of light energy, and to optimise leaf temperature for photosynthesis when the leaf is active, while preventing mortality when the leaf is not active. Rozendaal et al. (2006) also noted physical differences in leaves from sunny and shaded sides of trees. They attributed this to temperature regulation, and reported higher nutrient contents in leaves more exposed to sunlight. Differences can also sometimes be seen in the colour of leaves harvested from sunny and shaded sides of a tree, leaves on the sunny side being slightly darker (Bergen, 1904). An experiment on caterpillars' inclination for leaves from the sunny side of northern hemisphere trees showed that caterpillars fed from leaves from the sunny side had significantly more body mass than those from the shaded side (Moore et al., 1998). This study concluded that leaves on the sunny side typically had higher nitrogen content than others, and also had lower moisture content. Plasticity in leaf physical and chemical characteristics within species as a response to sunlight intensity could thus

be an important variable when discussing stand level nutrient dynamics, especially in forests where shading plays a major role in tree growth (Popma et al., 1992; Rozendaal et al., 2006).

Besides its use in forestry, species like *A. mearnsii* is used within rural areas as building material, for firewood, but also for the production of wood chips for exportation. Pulp made from *A. mearnsii* chips, in particular, is in great demand in Asian markets, which was estimated in 2011 to support a $4.3 billion industry, based on the export of chips from countries like South Africa, Brazil, and Indonesia (see Griffin et al., 2011). This, however, inevitably leads to nutrient export as well and potentially to net losses of nutrients and organic matter in the environment. This activity could effectively deplete nutrient reserves in plantations, or at least significantly disrupt nutrient cycles (Homyak et al., 2008). By studying nutrient concentrations in these wood chips, it is possible to quantify these losses, and possibly suggest alternative management practices.

CHAPTER 3: The impact of recommended levels of herbicides, used to control alien invasive growth and regeneration, on soil microbial diversity in riparian soils from two different longitudinal zones

3.1 INTRODUCTION

WfW, a widely recognized restoration initiative, utilises herbicide treatment to control invasive alien plants (IAPs) in riparian fynbos ecosystems in the Western Cape (Blanchard and Holmes, 2008; Holmes et al., 2008). WfW apply specified concentrations and dosages of herbicides to woody IAPs in relation to the target plant's life stage and species. Some common IAPs present in riparian fynbos include *Acacia mearnsii* and *Eucalyptus camaldulensis*. The herbicides recommended for controlling the *A. mearnsii* populations are Springbok (active ingredient: Glyphosate) and Garlon (active ingredient: Triclopyr), whilst the herbicide used for *E. camaldulensis* is Plenum (active ingredients: Picloram and Fluroxypyr) (Bold, 2007). The active ingredients in the herbicide target certain enzymatic or photosynthetic processes which induces abnormal growth, inhibition of cell division or the disruption of certain important enzymatic pathways (Kearney and Kaufman, 1975; Peterson et al., 1994; Powles and Yu, 2010). These herbicides have been reported to be effective in the short term for suppressing the growth of the afore-mentioned invasive alien trees, but regeneration may occur in the long term (Wolmarans and Swart, 2014). Additionally, several studies have concluded that the active ingredients of these herbicides have potential detrimental effects on soil functioning and soil processes (George et al., 2009). The impact of herbicides on soil microbial populations and microbially mediated soil processes have not been tested in local conditions.

The use of certain herbicides could lead to alterations in the chemical and biological characteristics of soils exposed to the herbicides. There have been several recorded instances of how herbicides and the active ingredients they contain influence the chemical conditions of the soil (Likens et al., 1970). For instance, herbicides have been shown to acidify soils where they've been used (Wolmarans and Swart, 2014). Herbicides containing phenylurea have also been shown to decrease microbial diversity as well as CFUs (colony forming units) in soil (Fantroussi et al., 1999). Fungal growth inhibition with the treatment of herbicides has also been reported where the active ingredients of the herbicide suppress spore germination and rate of hyphal extension of the mycelia (Wilkinson and Lucas, 1969). The xenobiotic compounds found within the chemical structure of herbicides can also alter mineralisation rates and other solubilisation processes (Perucci et al., 2000). There are, however, reports of certain herbicides (such as Glyphosate) stimulating the growth of microbial genera in the soil. These microbes are essentially able to use the active ingredient as a food source and hence increase their growth and biomass in the given soil. The findings of the effects of herbicides on microbes are, however, rather inconsistent, making it important to further study their potential impact on soil processes (Mijangos et al., 2009). Further evaluation of the impacts of different herbicides at different concentrations on microbial activity and diversity will promote our understanding of how herbicides affect the simplest and most important forms of an ecosystem.

In this chapter, we report on the impact of various widely used herbicides on soil chemical and microbiological properties, specifically in riparian environments. The aim is to assess the implications this treatment could have for future chemical management of riparian fynbos ecosystems in the Western Cape of South Africa. The three herbicides chosen included different active ingredients (Glyphosate, Triclopyr, and Fluroxypyr and Picloram) and were applied at different dosages and concentrations as to using a log dose response approach (10%, 100%, 1000%). Various soil chemical characteristics were evaluated before and after a given herbicide treatment was applied. This was

done to compare natural soil in its normal condition as opposed to when it has been exposed to herbicide (Mijangos et al., 2008). The soil chemical characteristics covered in this chapter include soil available NH_4^+, NO_3^-, PO_4^{3-}, N mineralisation rate, acid phosphatase activity, pH and electrical conductivity.

The specific aims of the chapter are:

1. To evaluate the impact of recommended levels of herbicides, used to control alien invasive growth, on soil microbial community structure (including microbial diversity and richness), *in situ*. The impacts of herbicide on microbial community structure will be expressed through OTUs (operational taxonomic units) produced by ARISA.

2. To evaluate the impact of different types and dosages of herbicides on soil microbial activity and function. The impacts will be measured through the study of changes in the metabolic potential of microbial communities, *ex situ*.

3. To evaluate the impact of recommended levels of herbicides on soil chemical characteristics, *in situ* and *ex situ*. Soil chemical parameters will include analysis of NO_3^-, NH_4^+ availability, phosphatase activity, and nitrogen mineralisation rate.

3.2 MATERIALS AND METHODS

3.2.1 Sampling and experimental design *in situ*

3.2.1.1 Experiment 1

Four *in situ* sites were selected, two of which were dominated by *Acacia mearnsii* and the other two by *Eucalyptus camaldulensis* (see Table 3.1) along the catchments of the Breede River and Wit River in the Western Cape. The areas chosen for experimentation consisted of dense stands of *A. mearnsii* and *E. camaldulensis.* This meant that the soil samples taken were representative of soils invaded by *A. mearnsii* or *E. camaldulensis* and not of an uninvaded soil where only native plant species were present. Additionally, the sites had to be within the vicinity of a river, but at the same time not too close to the river so that herbicide spillage into the river could be prevented.

Selected individual trees were treated with either a full recommended dosage of herbicide (100% of the recommended concentration/field rate) or 0% the recommended concentration/field rate, which serves as control. The treatments included three different types of herbicide, each with its own active ingredient. *A. mearnsii* trees were treated with Triclopyr and Glyphosate, whilst *E. camaldulensis* trees were treated with a mixture of Picloram and Fluroxypyr.

Herbicides were applied with a knapsack sprayer (16 ℓ). The spray was applied evenly in a mist, using a conical nozzle, and application took place in warm, cloudless and windless weather conditions to prevent spray drift and to enhance the setting of the herbicide in the targeted plant and surrounding soil. *A. mearnsii* seedlings were treated with herbicide in a foliar spray form and, for mature *E. camaldulensis,* trees were selected, trees were cut down and then the herbicide was sprayed on the cut stump. All treatments were replicated five times. The field rate for the herbicides applied is displayed in Table 3.1.

Soil sampling took place using a steel cylinder (5 cm diameter) in the immediate vicinity of the individual tree to collect soils that would be exposed to the mist spray during herbicide application. The soil samples were collected to a depth of four centimetres and placed into appropriately labelled Ziploc bags and stored in a refrigerator at 4°C within two hours of collection. All soil chemical analysis was done within 48 hours of collection as certain properties of the soil (such as nitrogen mineralisation rate and acid phosphatase activity) are sensitive and could change over time. The first sampling took place just before treating the respective samples with the given herbicide treatment. The second sampling included repeating the soil collection a month after the given herbicide application took place.

3.2.1.2 Experiment 2

This experiment was conducted in autumn on site in fynbos riparian areas along stretches of the Breede River in Alfalfa, in the Western Cape of South Africa. Two primary sites were selected for experimentation. One was a set area of riparian fynbos invaded and dominated by *A. mearnsii* trees and the other invaded and dominated by *E. camaldulensis* trees. The areas chosen for experimentation consisted of dense stands of *A. mearnsii* and *E. camaldulensis,* respectively. As with experiment 1, the sites had to be within the vicinity of a river, but at the same time not too close to the river, so that herbicide spillage into the river could be prevented.

The herbicide treatment included three different types of herbicide. *A. mearnsii* tree seedlings were treated with Triclopyr and Glyphosate, whilst *E. camaldulensis* were treated with a mixture of Picloram and Fluroxypyr. Each of these herbicide treatments were applied at three different rates, including 0 × field rate (control), 1 × field rate and 10 × field rate (Crouzet et al., 2010). Herbicides were applied with a knapsack sprayer (16 ℓ) as described in Section 3.2.1.1. All treatments were replicated five times. The field rate for the herbicides applied is displayed in Table 3.2 below. Soil sampling and analysis followed the protocol described in experiment 1.

Soil sampling took place in three stages throughout the course of this study: 1) Pre-treatment (right before herbicide application); 2) Post-treatment 1 (one week after herbicide application); and post-treatment 2 (six weeks after herbicide application). This allows measurement of the effects of the given concentration and dose of herbicide on the soil chemical and microbial characteristics over time.

Table 3.1. *In situ*, Experiment 1. The different treatments are displayed with the respective active ingredient included. Each of the treatments' field sites are given along with the GPS co-ordinates. Each of the herbicides is applied at four different rates: 0 × FR (field rate), and 1 × FR.

EC = Emulsifiable concentrate; SL = Soluble liquid; ME = Micro emulsion; FR = field rate

Experiment 1				
Locality	**GPS co-ordinates**	**Target IAP species**	**Treatments at FR**	**Active ingredient:**
Alfalfa A (Breede River)	33°46'5.12"S; 19°32'6.107"E	*Acacia mearnsii* seedlings	Garlon 0.75 ℓ/ha: • 0 × FR • 1 × FR	Triclopyr (as butoxy ethyl ester) 480 g/ℓ EC
			Springbok 4.5 ℓ/ha: • 0 × FR • 1 × FR	Glyphosate (as isopropylamine salt) 360 g/ℓ SL
Alfalfa B (Breede River)	33°46'1.528"S; 19°32'23.243"E	*Eucalyptus camaldulensis* adults	Plenum 9 ℓ/ha: • 0 × FR • 1 × FR	Fluroxypyr + Picloram 80 g/ℓ ME
Bainskloof (Wit River)	33°32'37.93"S, 19°10'28.19"E	*Acacia mearnsii* seedlings	Garlon 0.75 ℓ/ha: • 0 × FR • 1 × FR	Triclopyr (as butoxy ethyl ester) 480 g/ℓ EC
			Springbok 4.5 ℓ/ha: • 0 × FR • 1 × FR	Glyphosate (as isopropylamine salt) 360 g/ℓ SL
Rainbow (Breede River)	33°39'57.838"S, 19°24'2.815"E	*Eucalyptus camaldulensis* adults	Plenum 9 ℓ/ha: • 0 × FR • 1 × FR	Fluroxypyr + Picloram 80 g/ℓ ME

Table 3.2. *In situ*, Experiment 2. The different treatments are displayed with the respective active ingredient included. Each of the treatments' field sites are given along with the GPS co-ordinates. Each of the herbicides is applied at three different rates: 0 × FR (field rate), 10 × FR and 1 × FR.

EC = Emulsifiable concentrate; SL = Soluble liquid; ME = Micro emulsion, FR = field rate

Experiment 2				
Locality	**GPS co-ordinates**	**Target IAP species**	**Treatments at FR**	**Active ingredient**
Alfalfa A (Breede River)	33°46'5.12"S; 19°32'6.107"E	*Acacia mearnsii* seedlings	Garlon 0.75 ℓ/ha: • 0 × FR • 0.1 × FR • 1 × FR	Triclopyr (as butoxy ethyl ester) 480 g/ℓ EC
			Springbok 4.5 ℓ/ha: • 0 × FR • 0.1 × FR • 1 × FR	Glyphosate (as isopropylamine salt) 360 g/ℓ SL
Alfalfa B (Breede River)	33°46'1.528"S; 19°32'23.243"E	*Eucalyptus camaldulensis* adults	Plenum 9 ℓ/ha: • 0 × FR • 0.1 × FR • 1 × FR	Fluroxypyr + Picloram 80 g/ℓ ME

3.2.2 Sampling and experimental design *ex situ*

3.2.2.1 Experiment 1

This experiment took place in autumn and was set up in the Forestry Department nursery of the University of Stellenbosch. The experiment used a microcosm approach to evaluate the effects of herbicides on soils. Plant trays (32 cm x 28 cm x 8 cm in dimension) were set up with different combinations of soil types and seeds that are representative of a common riparian fynbos habitat, and which could be found along the Breede River and Wit River. The aim of this microcosm was to design an experimental environment that simulated to an extent the conditions of riparian fynbos ecosystems in the Western Cape.

The soil media used to fill the plant trays were collected from the Alfalfa A and B field sites (see Table 3.2). A set number of trays were filled with soils collected in the immediate vicinity of *Acacia mearnsii* trees (found at the Alfalfa A site) whilst the other trays were filled with soils collected from around *Eucalyptus camaldulensis* trees (found at the Alfalfa B site). Once the trays were filled with soil, seeds of various plant species commonly found within riparian fynbos zones were planted and left to germinate and grow alongside the plant species already found within the seedbank. Special care and attention went into ensuring the germination of *Acacia mearnsii* and *Eucalyptus camaldulensis* seeds as well as growing them in each of their respective trays. Two native species were planted. namely *Brabejum stellatifolium* and *Acacia karroo.* With the above-mentioned species being planted and grown together with that of the species already in the seed bank, a "micro-community" could be present in each tray.

Herbicide treatment took place with the use of a knapsack sprayer as described in Section 3.2.1.1. The herbicides used are shown in Table 3.3. All trays with *E. camaldulensis* seedlings were exposed to Plenum (Fluroxypyr and Picloram) treatment, whilst trays containing *A. mearnsii* seedlings were treated with Springbok (Glyphosate) or Garlon (Triclopyr). Each herbicide was applied at four different concentrations in order to compare the impacts of the dosage of a given herbicide on soil chemical and microbial properties. The four concentrations followed a dilution series to simulate a log dose response. These concentrations include a 0% herbicide concentration, 10% concentration, 100% concentration, and 1000% concentration.

The soil samples were taken with the use of a sterile soil scoop or spoon, transferred into a Ziploc bag, labelled, sealed and refrigerated for preservation. All analyses were done within two days from the initial soil sampling, as soil chemical and microbial properties of the soil might change after two days.

3.2.2.2 Experiment 2

This experiment includes monitoring the given herbicides' effects on the soil chemical and microbial characteristics in a controlled microcosm environment. Soil cores were collected in PVC cylinders (15 cm height and 10 cm width) from the *in situ* sites (*A. mearnsii* and *E. camaldulensis* field sites described above) and brought back to a temperature controlled room for incubation (Sierra, 1997). The soil cores were kept in darkness to prevent influence from varying light intensity on the soil samples as well as to prevent any of the seeds remaining in the seed bank to germinate. Each of the soil cores received a particular concentration and type of herbicide application as described in Table 3.3. Soil cores were

sealed with PVC caps underneath to make a soil-core container. Parafilm was cast over each soil-core top to regulate the soil moisture content in each container over time (Roslycky, 1982).

Each soil core was sampled from three times during this study. One sample was taken before the respective herbicides were applied (pre-treatment). Another was taken a week after the herbicides were applied (post-treatment 1), and then again six weeks after the herbicides were applied (post-treatment 2). The soil samples collected from the soil cores were analysed for the given soil chemical and soil microbial characteristics at all three sampling stages, namely, pre-treatment; post-treatment 1 and post-treatment 2.

The herbicide treatment included three different types of herbicide, each with its own active ingredient. *A. mearnsii*-invaded soils were treated with Triclopyr and Glyphosate, whilst *E. camaldulensis*-invaded soils were treated with a mixture of Picloram and Fluroxypyr. Each of these herbicide treatments was applied at four different rates including 0 × field rate (control), 0.1 × field rate, 1 × field rate and 10 × field rate (Crouzet et al, 2010). All treatments were replicated five times. Herbicides were applied through a conical nozzle to ensure an even spread and application of the herbicide in a mist form. The respective concentration of herbicide applied to the soil cores could hence, to an extent, be regulated in the soil cores.

3.2.3 Soil chemical analysis

3.2.3.1 Soil pH

The pH levels of the soil samples were measured using a HI-8424 pH meter. Each soil sample was sieved (2 mm) and thereafter weighed into 10 g samples. The 10 g sample was then mixed into solution with distilled water in a 1:5 ratio in a 50 ml plastic vial. The soil mixture was then thoroughly stirred for 30 minutes to mix, after which the suspension was left to settle for a minimum of 30 minutes. Once the soil was completely settled at the bottom of the vial, the recordings were done. The HI-8424 pH meter was calibrated to a range of pH 4–7. After calibration, the pH of each sample was recorded to one decimal place.

3.2.3.2 Electrical conductivity

Electrical conductivity was recorded with a HI-8733 conductivity meter. Each sample was sieved (2 mm) and weighed to 10 g samples. Distilled water was added to each soil sample in a plastic vial to a ratio of 1:5. The solution was then shaken to allow for thorough mixing of the soil with the distilled water, after which the solution was left for at least 30 minutes to give the soil a chance to settle. The calibration was set to 12.88 mS at a temperature of 2˚C before taking measurements. The recordings were taken to one decimal place.

Table 3.3. *Ex situ*, Experiments 1 and 2. The different treatments are displayed with the respective herbicide as well as the active ingredient included. Each of the herbicides is applied at four different rates: 0 × FR (field rate), 0.1 × FR, 1 × FR, 10 × FR. The types of soil, the herbicide and the associated concentrations are the same for experiment 1 and 2.

EC = Emulsifiable concentrate; SL = Soluble liquid; ME = Micro emulsion, FR = field rate

Target soil of IAP species	**Treatments at FR**	**Active ingredient**
Acacia mearnsii-invaded soil	Garlon 0.75 ℓ/ha: • 0 × FR • 0.1 × FR • 1 × FR • 10 × FR.	Triclopyr (as butoxy ethyl ester) 480 g/ℓ EC
Acacia mearnsii-invaded soil	Springbok 4.5 ℓ/ha: • 0 × FR • 0.1 × FR • 1 × FR • 10 × FR.	Glyphosate (as isopropylamine salt) 360 g/ℓ SL
Eucalyptus camaldulensis-invaded soil	Plenum 9 ℓ/ha: • 0 × FR • 0.1 × FR • 1 × FR • 10 × FR.	Fluroxypyr + Picloram 80 g/ℓ ME

3.2.3.3 Soil available nitrate

Soil available nitrate was determined spectrophotometrically. Sieved soil samples (2 mm sieve) of 10 g were weighed and thoroughly mixed with 25 ml of 0.5 M K_2SO_4 in sterile 50 ml plastic vials. This solution was then shaken for an hour and thereafter filtered through Whatman no.40 filter paper. The filtrate was mixed with two reagents before final measurements were taken. The first of the reagents included a 5% salicylic acid solution, whilst the second of the reagents was a solution of 4 M sodium hydroxide. An amount of 0.5 ml of each samples' filtrate was mixed with 1 ml of the salicylic acid and left for a total of 30 minutes. Thereafter, 10 ml of the sodium hydroxide reagent was added to the mixture and left for an hour before reading the absorbance at 410 nm with the use of a spectrophotometer.

3.2.3.4 Soil available ammonium

The determination of available ammonium in the soil samples were done through colorimetric methods. Soil samples were sieved (2 mm) and weighed to 10 g each. Thereafter, the soils were thoroughly mixed with 25 ml of 0.5 M K_2SO_4 solution in sterile 50 ml plastic vials. This solution would then be shaken for an hour and filtered through Whatman no.40 filter paper. The filtrate extracted was mixed with two reagents to induce colour development. The reagents included reagent N1 which consisted of sodium salicylate, sodium citrate, sodium tartate, sodium nitroprusside and distilled water; reagent N2 consisted of sodium hydroxide, sodium hypochlorite and distilled water. A volume of 0.1 ml of each samples' filtrate was mixed with 5 ml of reagent N1 and left for 15 minutes. After 15 minutes, 5 ml of reagent N2 was mixed into solution and thereafter left for an hour for the mixture to stabilise and to allow for colour development to take place. The samples were then measured with a spectrophotometer at an absorbance of 655 nm.

3.2.3.5 Nitrogen mineralisation rate

Assessing the nitrogen mineralisation rate involved measuring the rate at which ammonium was being mineralised in the soil on a daily time step. Soil samples were sieved (2 mm) and weighed out to 10 g. These samples were then mixed with 25 ml of distilled water in a sterile 50 ml plastic vial. The samples were thereafter placed into incubation at a constant temperature of 27°C for seven days. After the seven days of incubation, the sample solutions were extracted with 0.5 M K_2SO_4 and filtered through Whatman no.40 filter paper. The filtrate extracted was mixed with reagents N1 and N2. Having measured the absorbance on the first and seventh day, one can then calculate the change in available ammonium over time and hence the rate at which ammonium was being mineralised per day.

3.2.3.6 Soil available phosphorus

To evaluate the amount of available inorganic PO_4^{3-} the Bray no.2 method was used (Tabatabai, 1982). Soil samples were sieved (2 mm) and weighed to 6.7 g each. Each soil sample was then mixed with 50 ml Bray no.2 solution (HCl and NH_4F) in sterile plastic vials. The mixing process included mixing each sample for 40–50 seconds with a high-speed shaker. The solution was then

filtered through Whatman no.40 filter paper. Thereafter, 20 ml of the filtrate was mixed with 10 ml of Boric acid which is a mixture consisting of boric acid and ammonium molybdate. The ANSA (amino-naphol sulphonic acid) reducing agent was then added at a volume of 10 ml. The ANSA reducing agent consists of sodium sulphite (anhydrous) and potassium metabisulphite. The mixture thus far was then adjusted to a total volume of 50 ml with distilled water. The complete mixture was left for 20 minutes for colour development to take place. After the 20 minutes had elapsed, the absorbance was determined at 660 nm with a spectrophotometer.

3.2.3.7 Evaluating acid phosphatase activity

Phosphatase activity was measured spectrophotometrically. The soil samples are from soils that are fairly acidic, which is best measured using the acid phosphatase method (Booi, 2011). The extraction procedure included weighing 1 g of 2 mm sieved soil and mixing it with 0.2 ml toluene in a sterile 50 ml plastic vial. After mixing, 4 ml of MUB (modified universal buffer) was added secondarily. The MUB reagent is a solution made up of 1 N NaOH, citric acid, boric acid, maleic acid, tris (hydroxymethyl) aminomethane and distilled water. The MUB was modified to a pH of 6.5 to continue with the acid phosphatase method. After this reagent was added, 1 ml of 0.025 M p-nitrophenyl phosphatase solution was added and the content shaken for a few seconds to allow mixing. The 0.025 M p-nitrophenyl reagent was produced by dissolving disodium p-nitrophenyl phosphate tetrahydrate in MUB at the pH level of 6.5. After mixing of all the above, the vials were sealed and incubated for an hour at 37°C. Thereafter, the vials were uncapped and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH was added. This was then shaken again to allow for mixing, after which the soil suspension would be filtered through no.12 filter paper. A spectrophotometer was then used to read the absorbance values of the samples at 402 nm.

3.2.4 Microbial analysis

3.2.4.1 DNA extraction

The Zymo DNA extraction protocol includes the extraction and isolation of DNA from a specified soil sample. For each sample. 0.25 g of fresh soil was used and the extraction carried out according to the manufacturer's instructions.

3.2.4.2 Gel electrophoresis

After DNA extraction, gel electrophoresis was performed to check for the presence of DNA. A 15 agarose gel was used, stained with EtBr (ethidium bromide). The EtBr binds to the DNA and allows for the DNA to be visualised under UV light.

3.2.4.3 ARISA

The ARISA (Automated Ribosomal Intergenic Spacer Analysis) method is based on the Polymerase Chain Reaction. The method is aimed at lysing eluted DNA samples in an effort to further analyse the

bacterial diversity richness. Extracted DNA was amplified using a fluorescently labelled (6-carboxy-fluorescein) forward primer (ITSF-FAM-(5'-GTCGTAACAAGGTAGCCGTA-3')) and the reverse primer (ITSReub-(5'-GCCAAGGCATCCACC-3')). A 10 µl reaction mixture was prepared as follow: 4.1 µl ddH2O, 5 µl KapaTaq ready mix (Kapa Biosystems, South Africa), 0.2 µl of each of the forward and reverse primers and 0.5 µl genomic DNA. The PCR conditions were as follows: 95°C for 5 minutes (1 cycle), followed by 38 cycles of 95°C for 45 seconds, 56°C for 50 seconds and 72°C for 70 seconds and a final cycle at 72°C for 7 minutes. The presence of DNA was confirmed on an 1% agarose gel stained with Ethidium Bromide (EtBr) under UV light.

An automated Genetic Analyser ABI 3010XI (Central Analytical Facility at Stellenbosch University) was used for capillary analysis using the Liz 1200 size standard. The raw data was expressed as electropherograms using different fluorescent intensities and fragment lengths and analysed using Gene Mapper® Version 5 Software (Applied Biosystems, United States). After performing size calling according to the size marker, the genotypes table was exported to Microsoft Excel (2016) for further analysis. Peak height was preferred over peak size.

3.2.4.4 Biolog plate technique

The Biolog plate technique includes the usage of microtiter eco-plates which contain various carbon substrates. The different carbon substrates are found within wells containing a colourless tetrazolium dye. These wells allow one to test for the extent to which the microbial community can metabolize carbon substrates in a given soil sample. The assay was set up by making a slurry with the soil sample and diluting it to contain 10^5 colony forming units (CFUs) for every 100 µl.

Once the relevant dilution was calculated, the inoculum 100 µl was pipetted into each of the 96 wells on the eco-plate. After inoculating each well, the plate was covered and labelled appropriately. The microtiter plate was then placed into a plastic container with moist paper towels and incubated at 25°C. Over time, the microorganisms in the inoculum will induce colour formation in the wells through formazan production (which is a result of the carbon substrates being oxidised). The plate was placed into a microplate reader set at an absorbance of 590 nm every 12–24 hours for a week. Colour development was measured spectrophotometrically and calculations such as AMR (average metabolic response), CMD (community metabolic diversity) and AWCD (average well colour development) were done to determine the extent to which microbes in a given soil sample can metabolise carbon substrates. The results were then collated and used to draw up a community level physiological profile.

3.2.5 Statistical analyses

For *in situ* experiment 1, the soil properties evaluated include total soil available N and soil available P. For *in situ* experiment 2, the soil properties evaluated include pH, electrical conductivity, total soil available N, N mineralisation, soil available P and phosphatase activity. For the *ex situ* experiment, the soil properties evaluated include pH, electrical conductivity, soil available N, N mineralisation, soil available P and phosphatase activity. The statistical analysis included a Shapiro-Wilks test to test for the normality of a given data set's distribution. Additionally, a One-way ANOVA was done to test for significant differences between samples and between different herbicide concentration treatments. Non-parametric analyses were also carried out as some of the data were shown to be non-normally

distributed. The Kruskal-Wallis ANOVA was done to further test for significant differences between the soils' response to different herbicide concentrations.

3.3 RESULTS

3.3.1 *In situ*, Experiment 1

3.3.1.1 Soil chemical results

Soil available nitrogen is important in riparian fynbos as it is especially limited in the soil matrix. Indigenous fynbos plant species have adopted specialised mechanisms for acquiring this limited nutrient in a form that is soluble and ready for uptake.

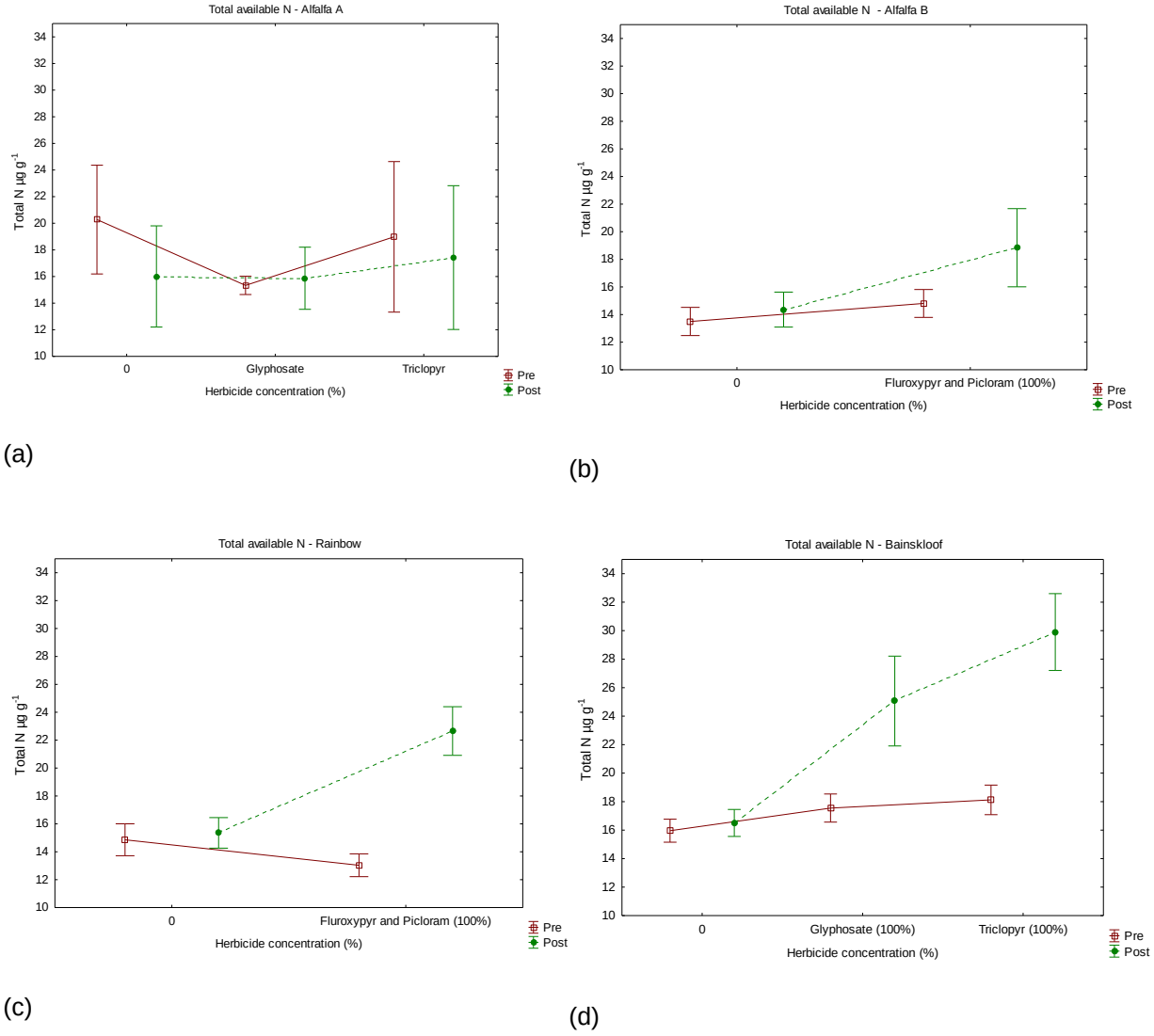


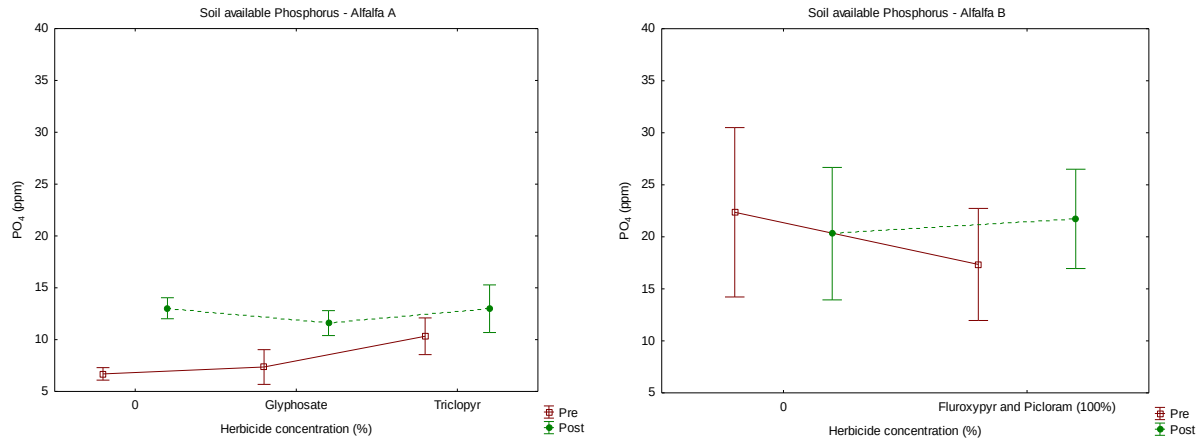
Figure 3.1: Total available N for soils before and after herbicide treatment took place at the following sites: (a) Alfalfa A, (b) Alfalfa B, (c) Rainbow, (d) Bainskloof. The soil available nitrogen is shown across the three different herbicide concentrations (0% and 100% of the recommended WfW concentration). The point represents the mean and the whisker the standard error.

These forms include nitrate (NO_3^-) and ammonium (NH_4^+). Certain fynbos plant species are known to form symbiosis with belowground microbes where the microbes fix nitrogen. It is hence important to evaluate the potential changes in this limiting resource with herbicide treatment. The total available N over time with herbicide application is given in Figure 3.1; it is the sum of the soil available NO_3^- and NH_4^+ measured in N-µg g^{-1}.

The soils subjected to treatment of Glyphosate and Triclopyr at the Alfalfa A site displayed no significant effect of the herbicide on the total soil available N (One-way ANOVA: F=0.40 and p=0.68 and F=0.05 and p=0.96; Figure 3.1). The total N in the soil remained fairly stable from pre-treatment to post-treatment, and ranged between 12 and 24 N-µg g^{-1}. There a slight increase in the total N when applying the Fluroxypyr and Picloram herbicide at a 100% concentration. A one-way ANOVA revealed no significant difference between the pre-treatment to post-treatment samples. Furthermore, the Rainbow site also showed an increase in the total available soil N when applying the Fluroxypyr and Picloram herbicide at a 100% concentration. A one-way ANOVA showed a significantly higher amount of total available N in the soils than before treatment (F=10.60 and p=0.01).

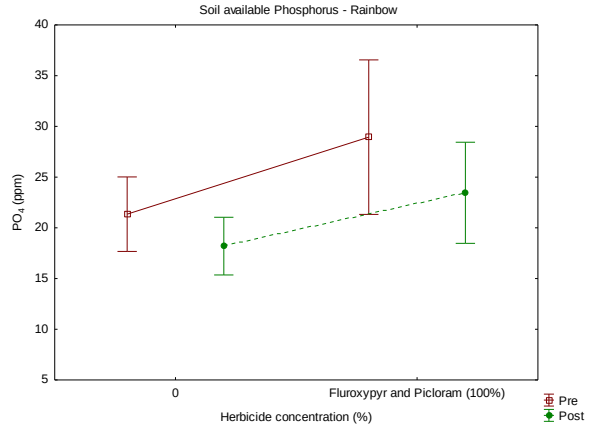
The soil available P at the Alfalfa A site remained fairly constant before herbicide treatment up until four weeks after treatment (Figure 3.2). A one-way ANOVA revealed no significant differences when comparing the soils before and after the herbicide treatment ($p > 0.05$). The available phosphorus generally kept within a range of between 5 and 15 ppm PO_4^{3-} . Alfalfa B and Rainbow showed no trend with regard to the Fluroxypyr and Picloram herbicide having an effect on the soil available P.

A one-way ANOVA showed no significant difference between the pre-treatment to post-treatment samples for either of these two sites. The Glyphosate and Triclopyr herbicide treatment at the Bainskloof site also showed little effect of the herbicide on the soil available P. There was, however, a slight increase in the PO_4^{3-} from pre-treatment to post-treatment samples when applying either of the two herbicides. No significant difference was found between the pre- and post-treatment samples, which suggests no significant effects of the Glyphosate and Triclopyr herbicide on the available P in the soil over the treatment period.



(a) (b)

Figure 3.2: Soil available phosphorus for soils before and after herbicide treatment took place at the following sites: (a) Alfalfa A, (b) Alfalfa B, (c) Rainbow, (d) Bainskloof. The soil available phosphorus is shown across the three different herbicide concentrations (0% and 100% of the recommended WfW concentration). The point represents the mean and the whisker the standard error.



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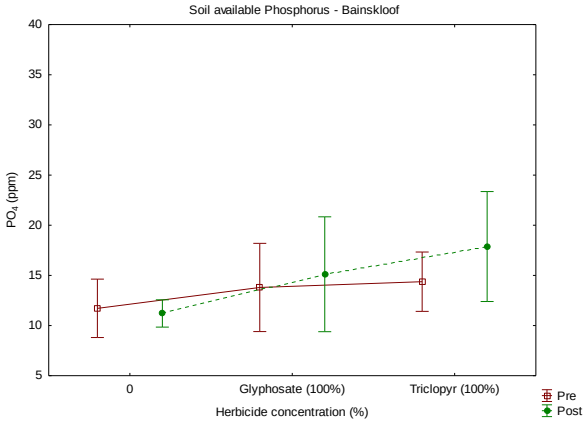


(d)

Figure 3.2 (Continued): Soil available phosphorus for soils before and after herbicide treatment took place at the following sites: (a) Alfalfa A, (b) Alfalfa B, (c) Rainbow, (d) Bainskloof. The soil available phosphorus is shown across the three different herbicide concentrations (0% and 100% of the recommended WfW concentration). The point represents the mean and the whisker the standard error.

3.3.2 *In situ*, Experiment 2

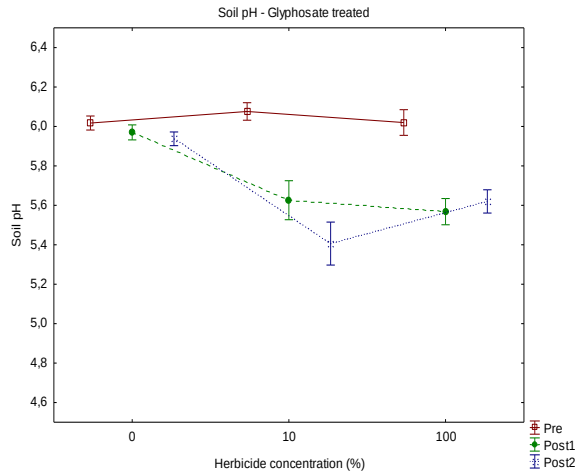
3.3.2.1 Soil chemical results

The results below were obtained *in situ* during the winter season of 2016 (*in situ* experiment 2). Various soil chemical properties were evaluated before and after certain herbicides had been applied and the herbicides were applied at different concentrations.

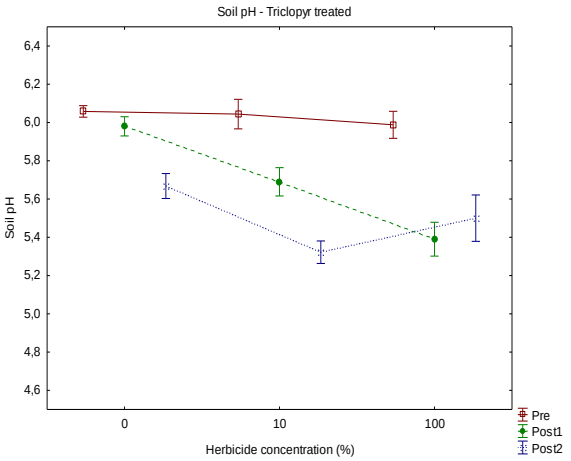
Soil pH is a good indicator of the fertility and general health of soil. Fynbos riparian soils are fairly acidic, but if the pH is too low or too high, problems for the survival and persistence of local microorganisms could result, as well as for the availability of plant nutrients (e.g. phosphorus). Shifts in the above-ground plant community could also take place with changes in the soil's acidity, where the soil quality is degraded or left in an unfavourable condition for plant growth.

The soil pH data for the soils treated with the Glyphosate-based herbicide (Springbok herbicide) had a normal distribution (Shapiro-Wilks test: $p < 0.05$). Glyphosate displayed an acidifying effect on the soil when applied (Figure 3.3a) at either of the concentrations. The pH of the soils treated with the 10% and 100% concentration was significantly lower than the pH of the 0% concentration soils (control samples) one week after and six weeks after treatment (One-way ANOVA: $F=7.41$ and $p=0.01$; $F=10.74$ and $p=0.00$ for the 10% and100 % soils, respectively). The soils treated with Triclopyr also showed an acidifying effect where the soils treated with the 10% and 100% concentrations seemed to decrease in pH one week after treatment and six weeks after treatment (Figure 3.3b). A one-way ANOVA revealed that one week after treatment, soil pH was significantly lower than the pH of the pre-treatment soils ($F=16.48$ and $p=0.00$). It is clear that the soil pH level drops over the six-week period after treatment with Triclopyr in the form of Garlon. The Fluroxypyr and Picloram-based herbicide (Plenum) had a similar effect on the soil pH level. The samples taken one week after treatment with the 10% and 100% concentration of herbicide, had a significantly lower pH than the control (pre-

treatment) samples (One-way ANOVA: F=43.88 and p=0.00). Based on the available data, there appears to be a consistent decrease in the pH of the soil within a six-week period after applying this herbicide.



(a)



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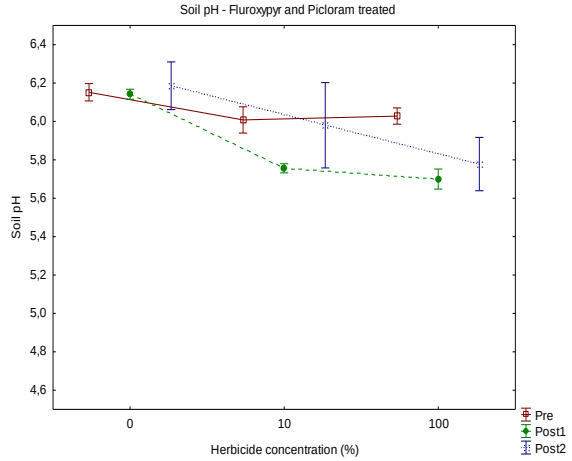
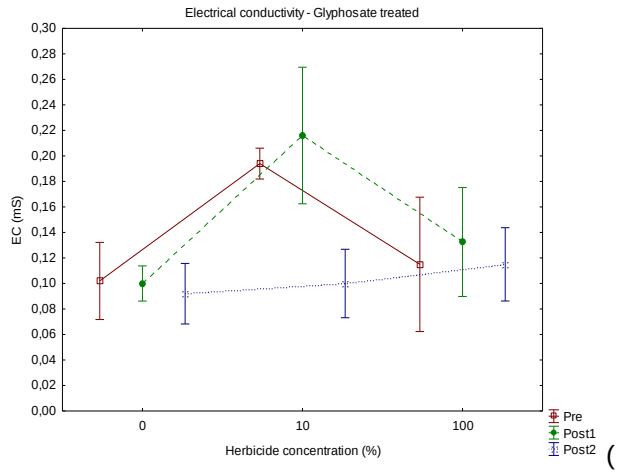


(c)

Figure 3.3: The pH for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The pH is shown across the three different herbicide concentrations (0%, 10%, and 100% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

Electrical conductivity (EC) is a measurement that correlates with key soil properties such as soil texture, drainage conditions, level of organic matter, and particularly salinity. It is important to test for potential effects of herbicides on EC, as it can indicate changes in the soil which may indirectly alter respective soil microbial communities or plant communities.



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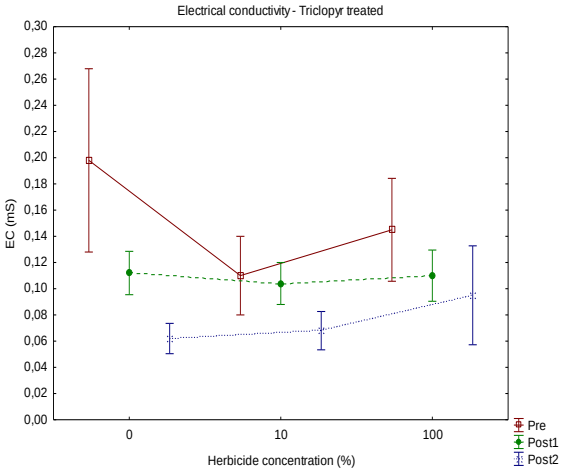

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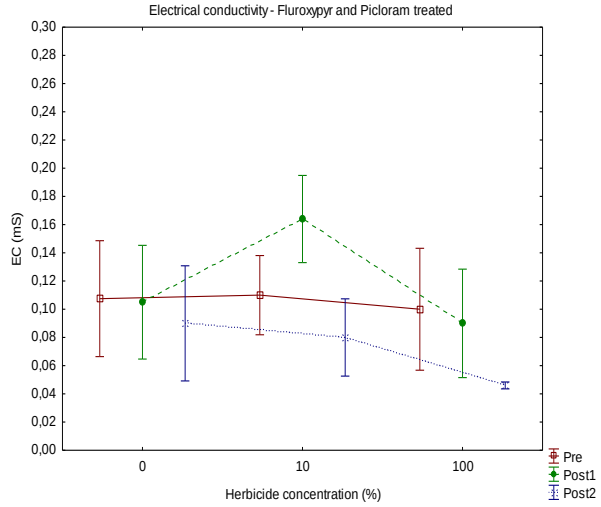


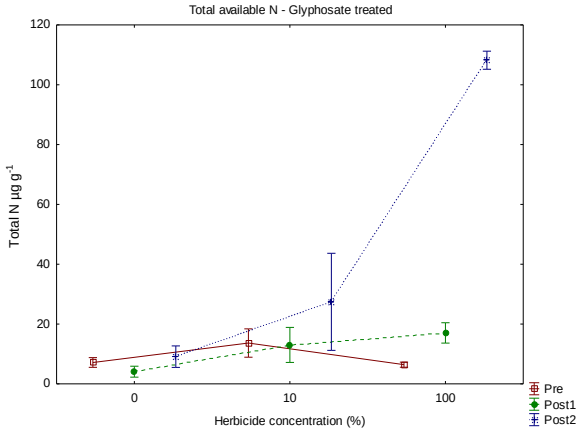
(c)

Figure 3.4: The electrical conductivity for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The EC is shown across the three different herbicide concentrations (0%, 10%, and 100% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

The EC of the soils treated with Glyphosate showed a high degree of variability ranging between 0.04 and 0.3 m/S, and hence displayed no effects of the herbicide treatment (Figure 3.4). A one-way ANOVA expressed no significant differences between the different concentration groups at any of the sampling occasions (pre-, post 1, and post 2) ($p > 0.05$). Similarly, the soil samples treated with the Triclopyr herbicide showed no significant differences (one-way ANOVA: $p > 0.05$). A Kruskal-Wallis ANOVA was done to justify the non-normal distribution and no significant differences were found ($p > 0.05$). The Fluroxypyr- and Picloram-based herbicide (Plenum) similarly showed no impact on the EC of the soil where the EC remained rather stable throughout the six weeks following the application

of the herbicide. Furthermore, a one-way ANOVA and Kruskal-Wallis showed no significant differences between the different concentration treatments before and after the treatment.

The total N in Figure 3.5 is the sum of the soil available NO_3^- and NH_4^+ measured in N-µg g^{-1} of the *in situ* experiment.



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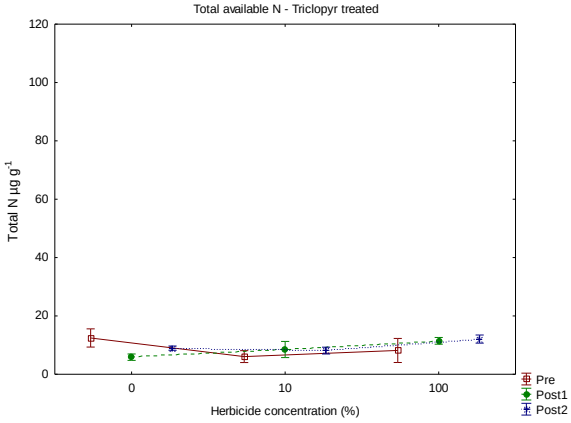

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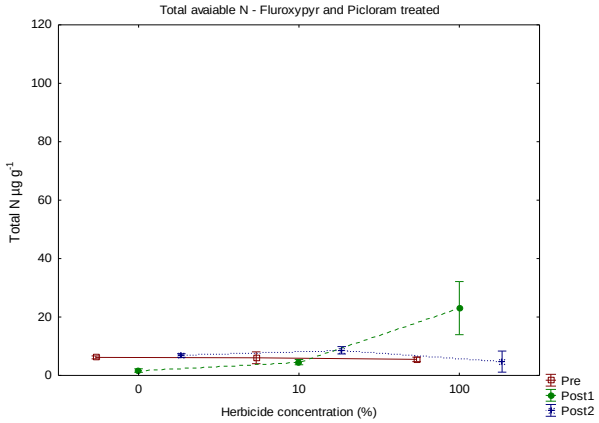


(c)

Figure 3.5: Total soil available N before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The total available N is shown across the three different herbicide concentrations (0%, 10%, and 100% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

In soils treated with Glyphosate, the total available N greatly increases after the six-week period (Figure 3.5a). The 10% concentration treatment showed an increase in total N by approximately 10 units of N-µg g^{-1}, while the 100% treatment showed an almost fivefold increase in N-µg $g^{-1,}$ as opposed to the control soils (0% concentration soils). In addition, a one-way ANOVA showed that after one week and after six weeks, soil total N was significantly higher than that of the pre-treatment

soils (F=13.90 and p=0.01; F=354.24 and p=0.00 for post 1 and post 2 respectively). The total soil available N was stable for the soils treated with the Triclopyr-based herbicide (Garlon) where the soil total N remained within an interval range of 5 to 20 N-µg g^{-1} despite the treatment of 10% or 100% of the Triclopyr concentration (Figure 3.5.b). A one-way ANOVA did, however, reveal a significant difference within the samples after one week (F=12.41 and p=0.01). No effect of the Triclopyr treatment on the total soil available N could be observed. The total soil available N within the soils treated with Fluroxypyr and Picloram similarly remained stable throughout the six weeks, except when looking at the soils treated with 100 % of the herbicide concentration a week after application (post 1) (Figure 3.5 c). It appeared that the soil available N (N-µg g^{-1}) increased slightly with the 100% treatment. A Kruskal-Wallis ANOVA revealed that the total soil N was significantly higher at one week after treatment than at pre-treatment and the six-week sampling stages (H=5.33 and p=0.02).

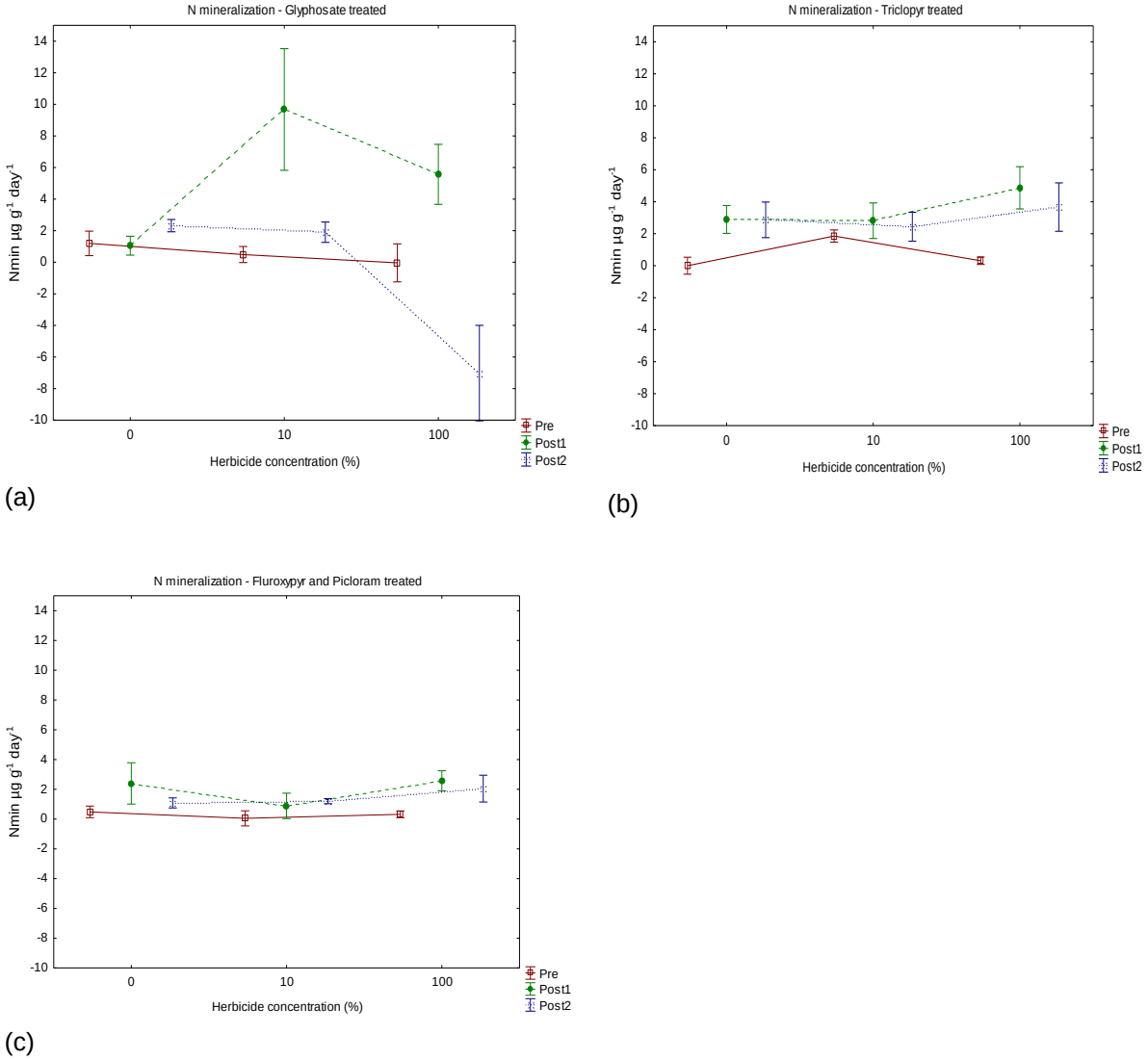
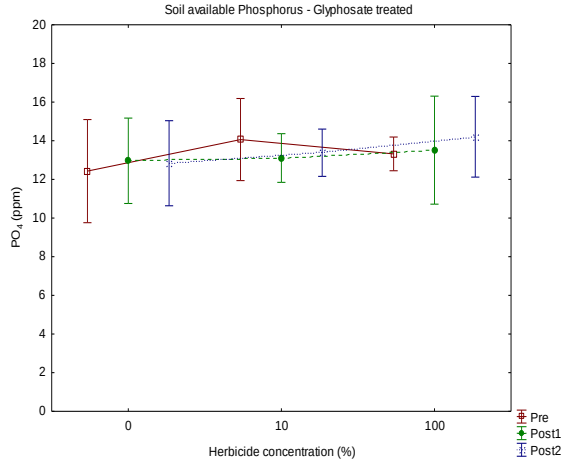


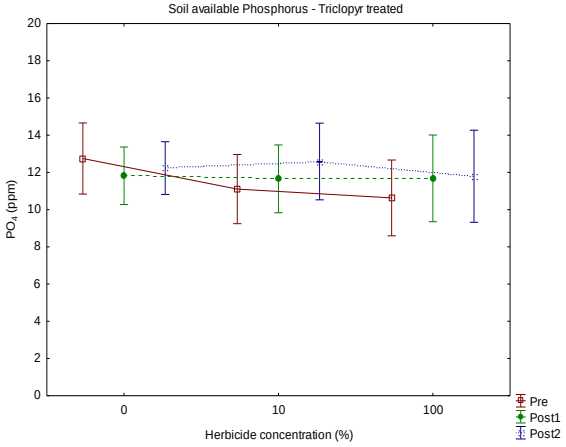
Figure 3.6: Nitrogen mineralisation rate for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The NMR is shown across the three different herbicide concentrations (0%, 10%, and 100% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

Nitrogen mineralisation rate is a metric expressing the rate at which organic nitrogen is being converted to inorganic forms of nitrogen. The process includes measuring the NH_4^+ available before and after incubation under anaerobic conditions. Incubation takes place over a duration of 7 days so that one can calculate a rate of mineralisation per day. The units used to express nitrogen mineralisation include $\mu g\ g^{-1}\ day^{-2}$.

Plant available forms of phosphorus in the soil includes PO_4^{3-}. It is an important determinant of soil fertility and is rather limited in the soils of fynbos habitats. Indigenous plants of the fynbos depend on this form of phosphorus for growth.



(a)



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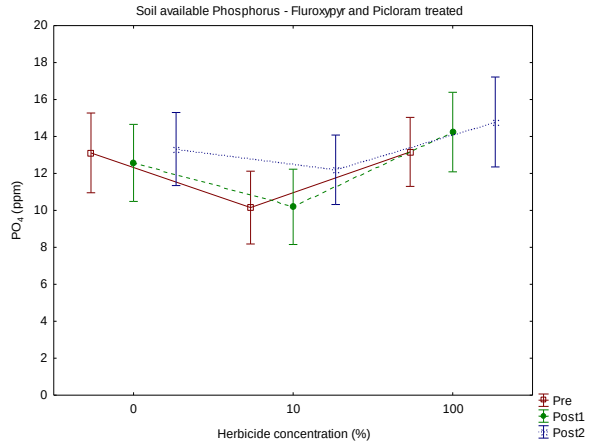


(c)

Figure 3.7: Soil available phosphorus for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The available phosphorus is shown across the three different herbicide concentrations (0%, 10%, and 100% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

The soil available phosphorus remained constant throughout the course of the study. The sample sets for all three herbicide treatments expressed a normal distribution (Shapiro-Wilks test: $p > 0.05$) and a one-way ANOVA revealed no significant differences between the herbicide concentration treatments or between the different sampling stages. This indicated no effect of herbicide type, concentration, or sampling stage on the phosphorus levels in the soil. The phosphorus remained stable even under the 100% treatment of the different herbicides and ranged between 8 and 18 ppm PO_4^{3-} (Figure 3.7). Soil available phosphorus is not affected by these three herbicides (Glyphosate, Triclopyr, and Fluroxypyr and Picloram) at a 10% or 100% concentration.

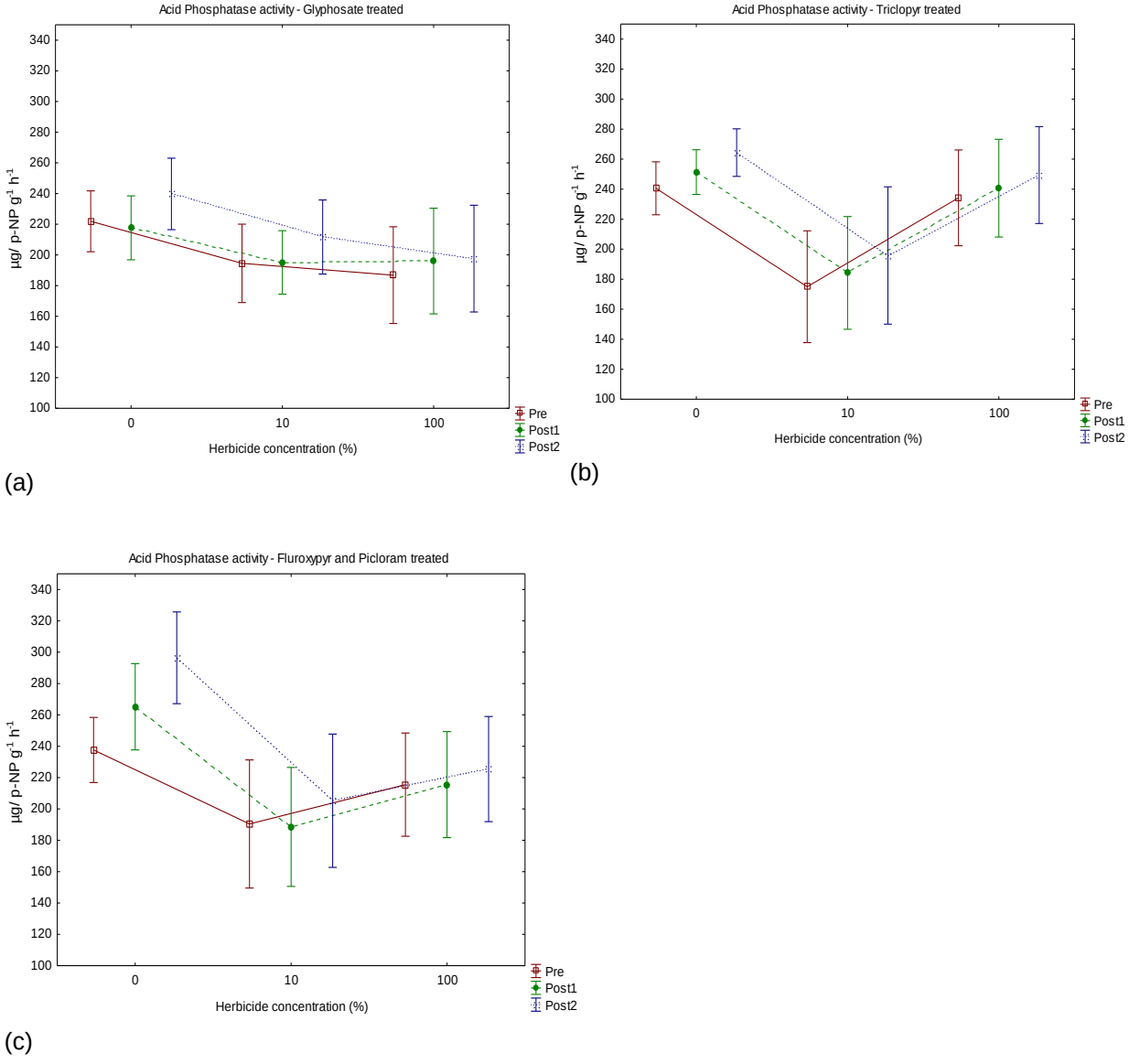


Figure 3.8: Acid phosphatase activity for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The phosphatase activity is shown across the three different herbicide concentrations (0%, 10%, and 100% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

Acid phosphatase monoesterase activity is an indication of the rate at which ester-phosphate bonds are hydrolysed in neutral to acidic soils. This hydrolysis leads to the production of phosphate in its plant available form. Evaluating any potential changes in acid phosphatase activity as a result of herbicide treatment would give insight into the effects exerted on the phosphate end product which is vital for both plant and microbial growth.

The acid phosphatase activity showed a normal distribution across all herbicide treatments (Shapiro-Wilks test: $p > 0.05$; Figure 3.8). In addition, no significant differences were detected in the phosphatase activity between samples taken at different sampling stages and samples treated with different concentrations of herbicide. This was the case for all three herbicide treatments (One-way ANOVA: $p > 0.05$). No changes in phosphatase activity when treated with 10% or 100% concentration of the herbicide (Figure 3.8) were detected over the course of the experiment. The acid phosphatase activity of the soils ranged between 140 and 340 µg *p*-NP g^{-1} dry soil.

3.3.2.2 Soil microbial diversity

Shannon's diversity index is based upon the bacterial community structure before herbicide treatment (pre-treatment), a week after treatment (post 1), and six weeks after treatment (post 2). The diversity index is a quantitative measure used to show the diversity (different species) in the samples, whilst showing the distribution of these species. The number of OTUs (operational taxonomic units, analogous to species) is shown along with Shannon's diversity values.

Treatment with Glyphosate (Springbok herbicide) had little effect on the diversity of the microbial communities in the samples. There was, however, a slight decrease in the number of OTUs a week after treatment at a 100% concentration (Table 3.4). The number of OTUs did however increase and return to pre-treatment levels six weeks after the herbicide application. Additionally, the data expressed a normal distribution for the microbial community across all sampling stages (pre-treatment, post 1 and post 2) (Shapiro-Wilks test: $p > 0.05$). A one-way ANOVA revealed that the number of OTUs present under the 100% Glyphosate treatment a week after treatment, was significantly lower than the microbial communities present in the soils of the 0% and 10% concentration treatments. This suggests that Glyphosate treatment at 100% could have an effect on the number of OTUs present in the soil, but only after a week and the effect doesn't seem to persist over time.

The bacterial communities present in the soils treated with Triclopyr showed no change with the treatment at 10% or 100% concentration (Table 3.4). Shannon's diversity scores remained consistent from before (pre-treatment) to a week after (post 1), to six weeks after (post 2) irrespective of the concentration of the Triclopyr herbicide applied. A one-way ANOVA did however indicate a significant difference in the 10% concentration ($F=5.58$ and $p=0.04$). This suggests that there may have been an effect of the Triclopyr-based herbicide on the OTUs in the soil where the number of OTUs increased six weeks after treatment at the 10% concentration.

Treatment with Fluroxypyr and Picloram seemed to have very little impact on soil bacterial diversity as minor variation was observed and Shannon's diversity scores remained constant regardless of the concentration of herbicide applied (Table 3.4). Treatment with Fluroxypyr and Picloram additionally showed no significant impact on the number of OTUs as there was considerable variation within samples. A one-way ANOVA showed no significant differences between the different sampling stages (pre-treatment, post 1 and post 2).

Table 3.4: Means and standard deviation of Shannon's diversity values and number of OTUs for bacterial communities in soils treated with Glyphosate (Springbok herbicide), Triclopyr (Garlon herbicide), and Fluroxypyr and Picloram (Plenum herbicide). The herbicides were applied at four different concentrations: 0%, 10%, and100% of the recommended concentration.

Glyphosate			**Triclopyr**			**Fluroxypyr and Picloram**		
Pre-treatment	**Shannon's-diversity index**	**Number of OTUs**	**Pre-treatment**	**Shannon's-diversity index**	**Number of OTUs**	**Pre-treatment**	**Shannon's-diversity index**	**Number of OTUs**
0%	0.030 ± 0.001	57.00 ± 2.94	0%	0.028 ± 0.002	42.33 ± 1.70	0%	0.020 ± 0.001	47.00 ± 7.26
10%	0.031 ± 0.002	49.00 ± 4.24	10%	0.025 ± 0.001	39.67 ± 3.30	10%	0.022 ± 0.001	53.67 ± 0.94
100%	0.028 ± 0.000	49.33 ± 8.01	100%	0.024 ± 0.003	50.67 ± 8.99	100%	0.020 ± 0.002	35.67 ± 15.58
Post 1	**Shannon's-diversity index**	**Number of OTUs**	**Post 1**	**Shannon's-diversity index**	**Number of OTUs**	**Post 1**	**Shannon's-diversity index**	**Number of OTUs**
0%	0.026 ± 0.003	55.33 ± 4.50	0%	0.027 ± 0.000	40.00 ± 6.53	0%	0.023 ± 0.001	46.67 ± 2.36
10%	0.028 ± 0.000	51.33 ± 14.43	10%	0.028 ± 0.001	36.33 ± 3.68	10%	0.019 ± 0.004	33.33 ± 2.36
100%	0.028 ± 0.000	22.67 ± 5.19	100%	0.028 ± 0.001	51.33 ± 3.09	100%	0.022 ± 0.001	28.33 ± 12.04
Post 2	**Shannon's-diversity index**	**Number of OTUs**	**Post 2**	**Shannon's-diversity index**	**Number of OTUs**	**Post 2**	**Shannon's-diversity index**	**Number of OTUs**
0%	0.028 ± 0.000	42.67 ± 5.73	0%	0.026 ± 0.004	56.33 ± 3.86	0%	0.019 ± 0.000	49.67 ± 3.68
10%	0.029 ± 0.001	42.67 ± 10.21	10%	0.025 ± 0.002	49.67 ± 4.92	10%	0.021 ± 0.002	36.33 ± 7.54
100%	0.028 ± 0.004	48.33 ± 9.39	100%	0.027 ± 0.001	49.33 ± 1.70	100%	0.022 ± 0.001	53.33 ± 5.31

Similar results were obtained with the Kruskal-Wallis ANOVA ($p > 0.05$). This suggests that the Fluroxypyr- and Picloram-based herbicide has no effect on the diversity of microbial communities, at least over the short term as was the case in this experiment.

Non-metric dimensional scaling plots (NMDS plots) is a gradient analysis which plots samples according to a dissimilarity matrix. The NMDS plots were generated through a Whittaker-dissimilarity index, which produced a dissimilarity matrix. The samples were compared to one another in an effort to display any grouping in the microbial community before and after a given herbicide treatment.

The bacterial communities found within soils treated with Glyphosate (Springbok herbicide) seem to express significant cluster patterns when the community structures at the pre-treatment, post 1, and post 2 sampling stages are compared.

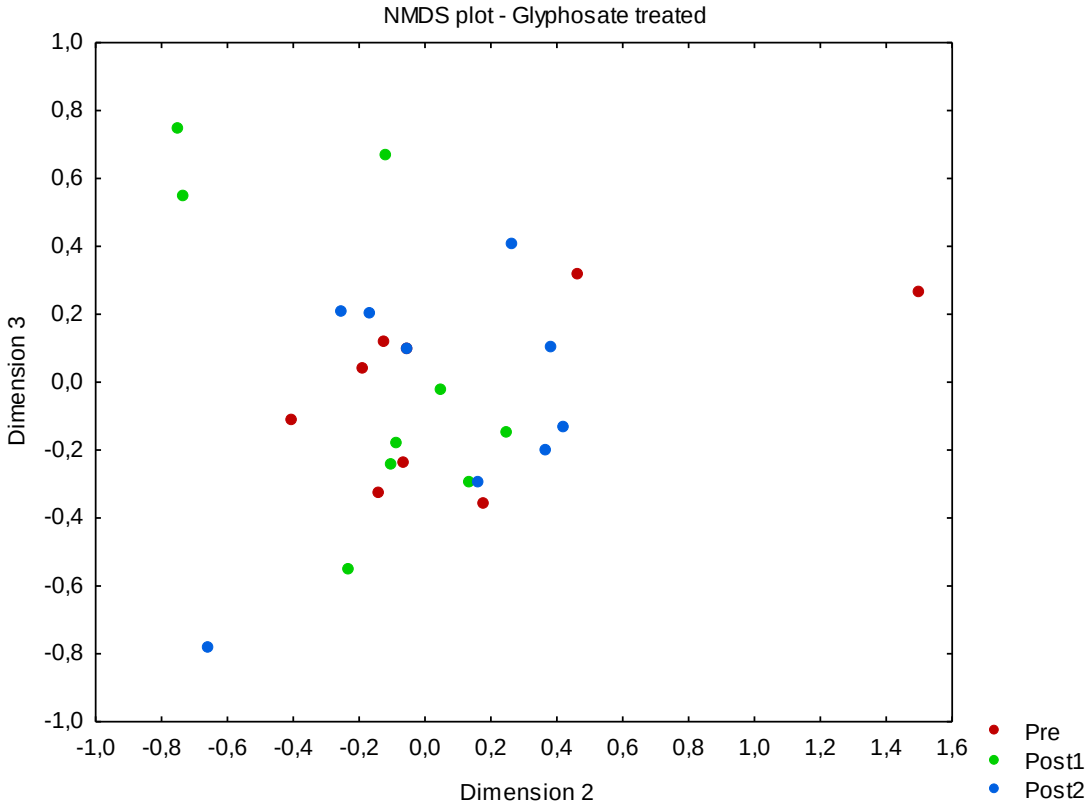


Figure 3.9: Non-metric multidimensional scaling plot for the distribution of bacterial communities in soils treated with Glyphosate. The NMDS analysis was generated through the Whittaker similarity index, which shows the bacterial distribution before herbicide treatment (pre-treatment), a week after treatment (post 1), and six weeks after treatment (post 2).

An ANOSIM revealed that the microbial community structures were similar before herbicide treatment (pre-treatment) and after (post 1 and post 2) ($R=0.105$). The treatment of Triclopyr (Garlon) produced similar results with no noticeable effect on the bacterial community structure within the six weeks

following its application (ANOSIM: R=0.187 and p=0.004; Figure 3.10).

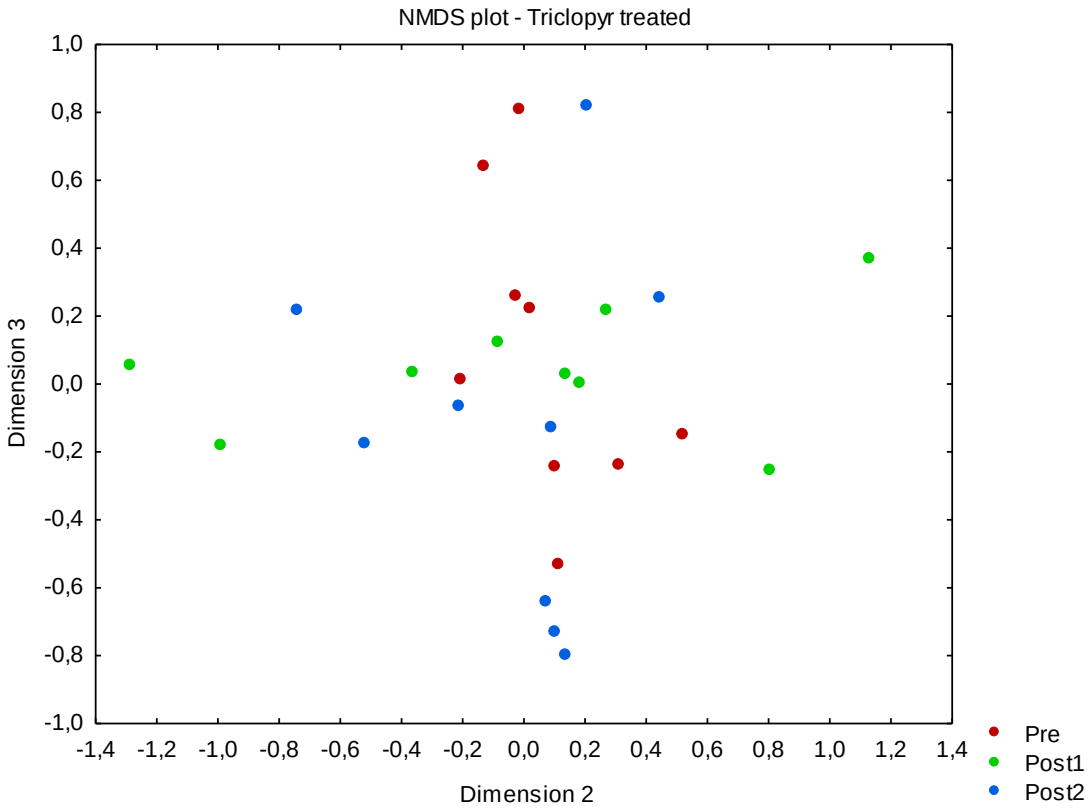


Figure 3.10: Non-metric multidimensional scaling plot for the distribution of bacterial communities in soils treated with Triclopyr. The NMDS analysis was generated through the Whittaker similarity index, which shows the bacterial distribution before herbicide treatment (pre-treatment), a week after treatment (post 1), and six weeks after treatment (post 2).

The soils treated with Fluroxypyr and Picloram (Plenum) displayed the same trend as the Glyphosate- and Triclopyr-treated soils (Figure 3.9 and Figure 3.10 respectively), where the microbial community structure remained unchanged with herbicide treatment (Figure 3.11). Additionally, no significant difference between these groups was shown (ANOSIM: R=0.052 and p=0.087).

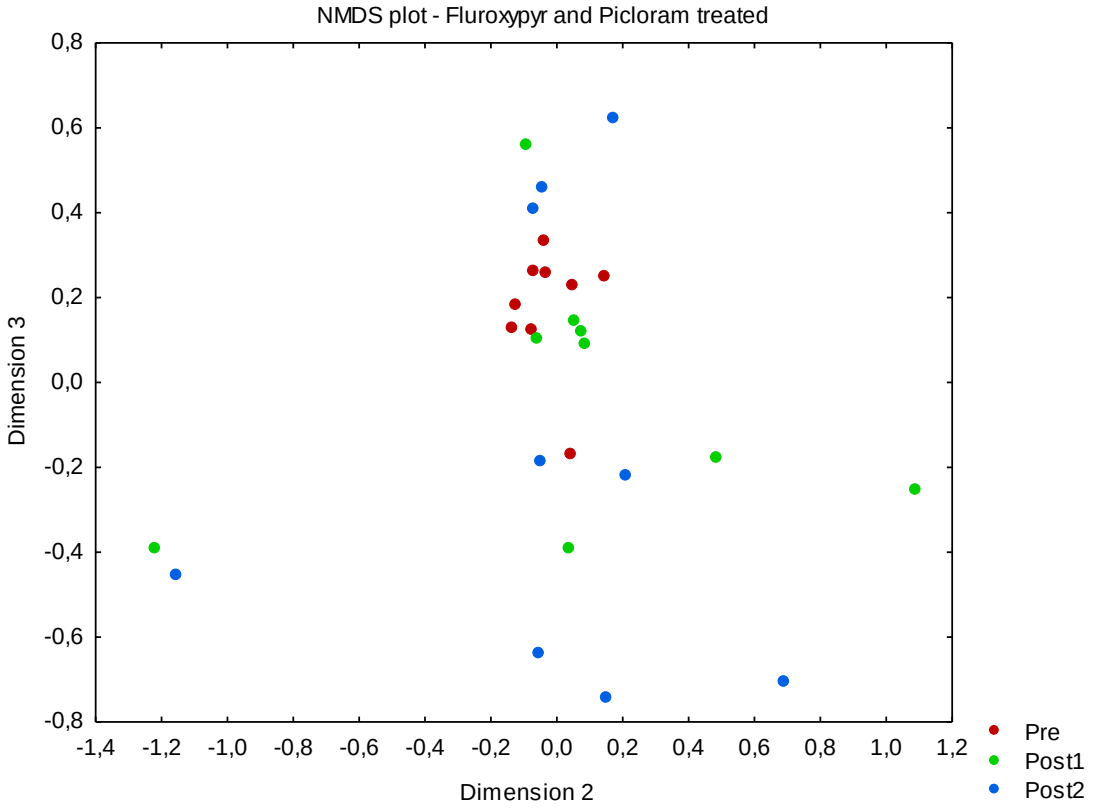


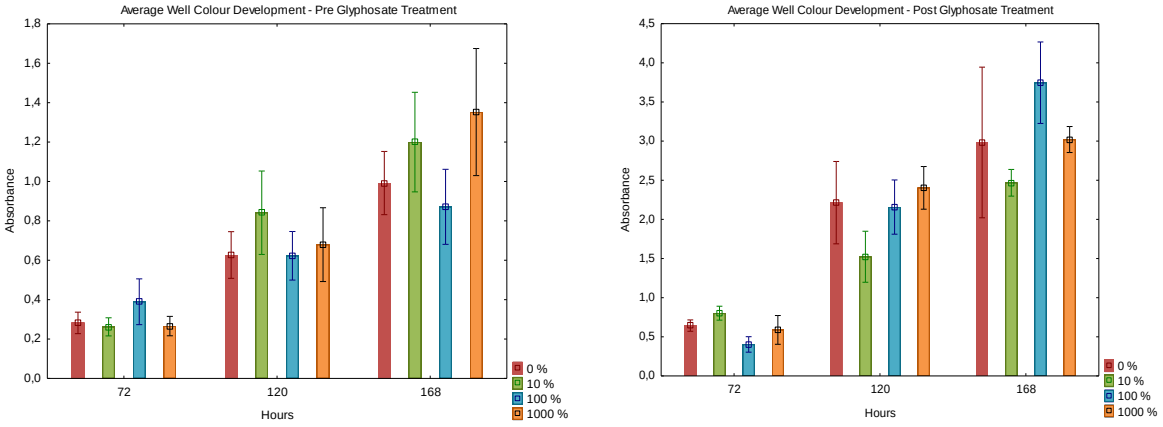
Figure 3.11: Non-metric multidimensional scaling plot for the distribution of bacterial communities in soils treated with Fluroxypyr and Picloram. The NMDS analysis was generated through the Whittaker similarity index, which shows the bacterial distribution before herbicide treatment (pre-treatment), a week after treatment (post 1) and six weeks after treatment (post 2).

3.3.3 *Ex situ*, Experiment 1

The results below have been obtained *ex situ* during the autumn season of 2015.

3.3.3.1 Soil microbial physiological profiles

Carbon substrate utilisation patterns were evaluated in an effort to describe the functional diversity (metabolic potential) of the microbial community. The absorbance of the micro-titre wells was converted into average well colour development (AWCD). The absorbance is displayed for 72, 120 and 168 hours after incubation. The standard error is presented in the bar graph along with the respective absorbance values.

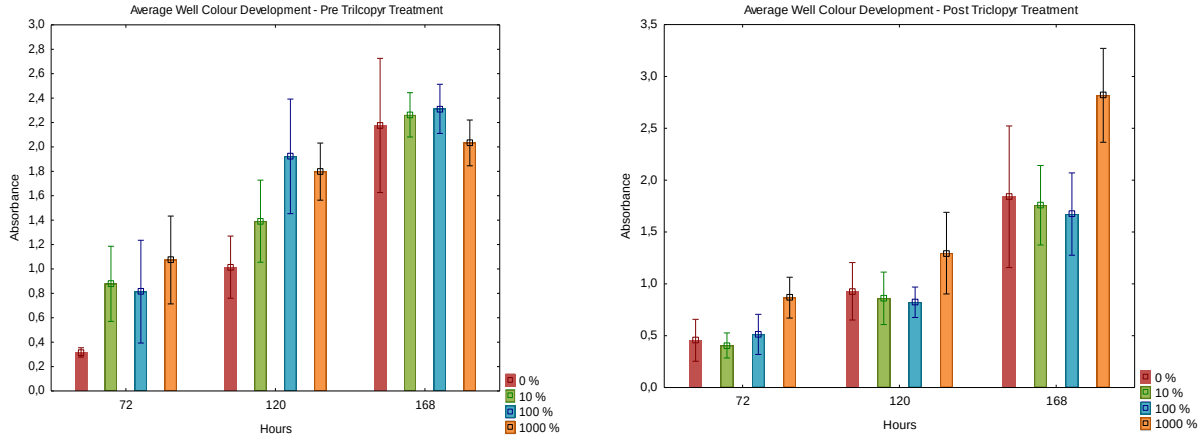


(a) (b)

Figure 3.12: AWCD for soils treated with Glyphosate in the form of Springbok. The AWCD is displayed over time (hours). The 0%, 10%, 100% and 1000% concentration treatment results are displayed for pre-treatment (a) and post treatment (b). The whiskers represent standard deviation.

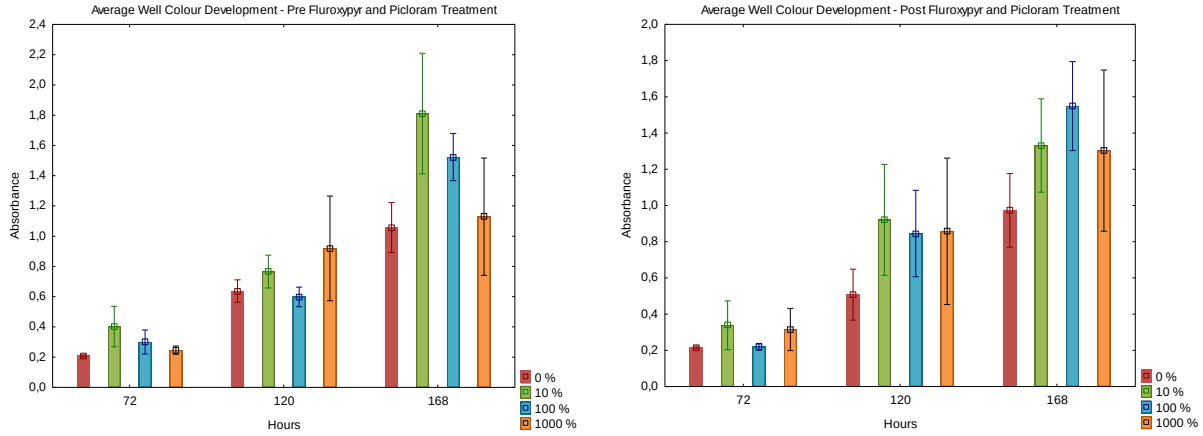
The carbon utilisation of the microbial community appeared unaffected by the Glyphosate herbicide. The absorbance increased uniformly with time across all concentration levels (Figures 3.12a and b). This can be further described with the community metabolic diversity (CMD) which showed that the number of positive wells responding to the inoculum generally increased from 26, after 72 hours of incubation, to 75 after 168 hours of incubation. A one-way ANOVA showed no significant difference among the different concentration levels from pre- to post ($p > 0.05$). It was however revealed that the CDM, after 168 hours' incubation time, were significantly higher than after 72 hours of incubation (Tukey HSD test: $p=0.01$). This meant that the AWCD increased significantly with time regardless of the concentration of the Glyphosate herbicide used. No inhibitory effect of either concentration treatment on the metabolic potential (carbon substrate utilisation) could therefore be detected.

The soil treated with Triclopyr showed no effect of the herbicide on the carbon utilisation capabilities of the microbial community. The absorbance shows a stable increase in carbon substrate utilisation across time where the average well colour development after 72 hours ranged between 0.3 and 1.0, increasing to between 1.5 and 3.0 after 168 hours of incubation (Figures 3.13a and b). The number of positive well responses (CMD) similarly showed a general increase, ranging from 12 to 47 after 72 hours of incubation, and 52 to 83 after 168 hours of incubation. A Tukey HSD test revealed the AWCD after 72 hours was significantly lower than after 168 hours ($p=0.02$). Therefore, AWCD increased significantly with time, but no significant differences between the concentration treatments could be observed when comparing pre- to post treatment. The Triclopyr herbicide seems to have no adverse or inhibitory effect on the microbial communities' potential to metabolise carbon substrates.



(a) (b)

Figure 3.13: AWCD for soils treated with Triclopyr in the form of Garlon. The AWCD is displayed over time (hours). The 0%, 10%, 100% and 1000% concentration treatment results are displayed for pre-treatment (a) and post treatment (b). The whiskers represent standard deviation.



(a) (b)

Figure 3.14: AWCD for soils treated with Fluroxypyr and Picloram in the form of Plenum. The AWCD is displayed over time (hours). The 0%, 10%, 100% and 1000% concentration treatment results are displayed for pre-treatment (a) and post treatment (b). The whiskers represent standard deviation.

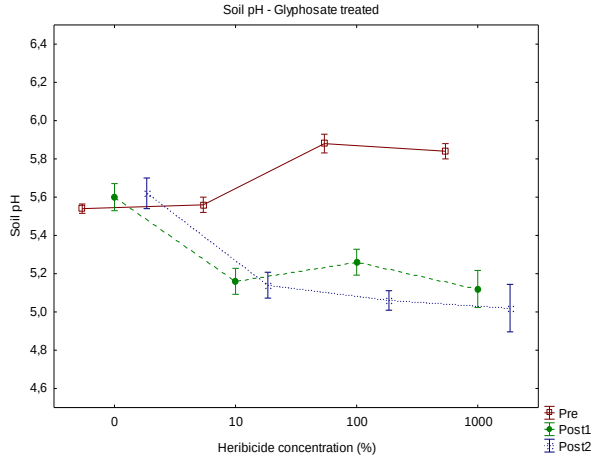
The microbial communities of the samples treated with Fluroxypyr and Picloram (Figure 3.14) showed a similar trend to those of the samples treated with Glyphosate and Triclopyr (Figures 3.12 and 3.13 respectively). There were no pronounced impacts of any of the concentrations of herbicide on the metabolic capabilities of the microbial communities. The number of positive well responses averaged 19.5 after 72 hours of incubation, increasing to 70.0 after 168 hours of incubation. There was, however, a high of variability in the AWCD, especially after 120 and 168 hours of incubation. A one-way ANOVA revealed that the AWCD of the samples exposed to 100% herbicide concentration were

significantly higher than the controls (0% concentration) post treatment (F=11.26 and p=0.01). A Tukey HSD test additionally revealed that absorbance after 168 hours was significantly higher than after 72 hours (p=0.02), showing that the AWCD increased significantly with time irrespective of the concentration of herbicide applied to the soil. No effects of the herbicide on the carbon substrate utilisation of the microbial community could therefore be detected.

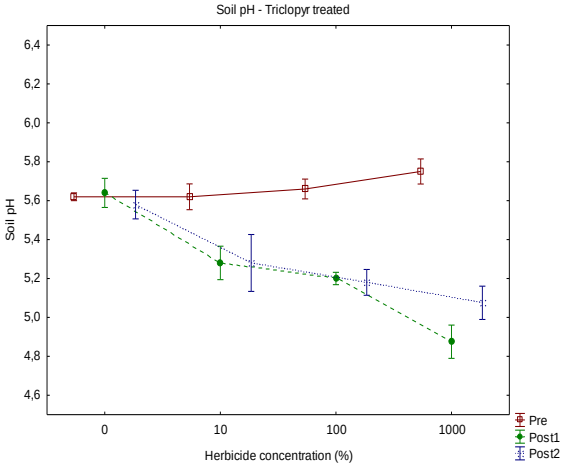
3.3.4 *Ex situ*, Experiment 2

3.3.4.1 Soil chemical results

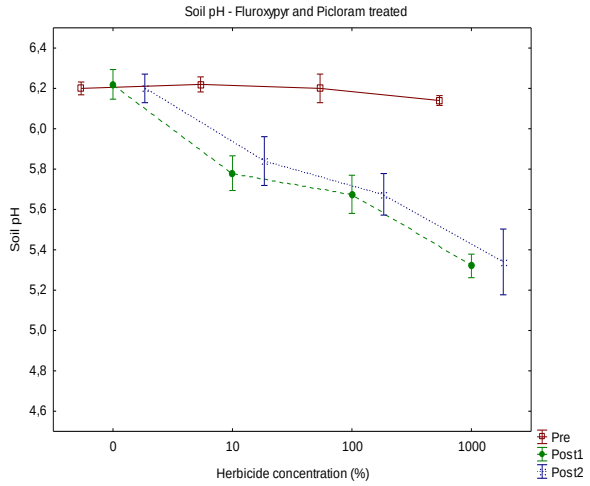
The soils treated with Glyphosate showed a consistent decrease in pH when herbicide treatment took place (Figure 3.15a), similar to what was found in previous experiments.



(a)



(b)



(c)

Figure 3.15: The pH for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The pH is shown across the three different herbicide concentrations (0%, 10%, 100% and 1000% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

A one-way ANOVA: showed that the soil pH at the post 1 and post 2 sampling stages was significantly lower than the pH of the pre-treatment samples (F=18.14 and p=0.00; F=4.68 and p=0.02 for post 1 and post 2 respectively). A Kruskal-Wallis ANOVA also showed a significant difference at the post 1 and post 2 sampling stage when comparing the different treatments (H=14.29 and p=0.00; H=8.27 and p=0.04 for post 1 and post 2 respectively). There was also a clear decrease in the pH levels before and after Glyphosate treatment.

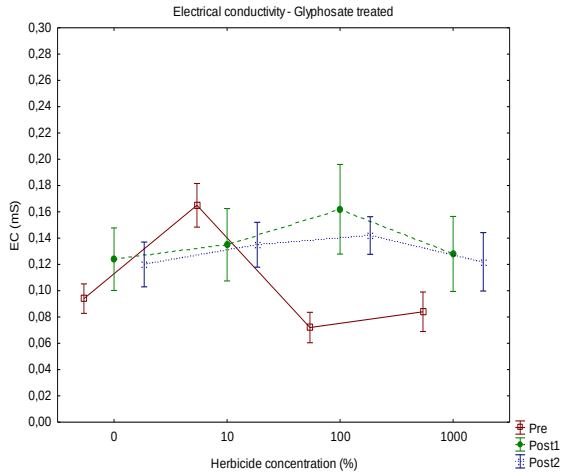
The pH in the soils treated with the Triclopyr herbicide showed a similar trend as seen in the soils treated with Glyphosate, where the pH decreased with the application of herbicide at the 10%, 100% and 1000% concentration levels (Figure 3.15b). A one-way ANOVA showed a significant difference at all the sampling stages ($p < 0.05$).

The Fluroxypyr- and Picloram-treated soils displayed the same decrease in the soil pH when the given herbicide treatment took place. A one-way ANOVA revealed a significantly lower pH in the soils when the herbicide was applied at the 10%, 100%, or1000 % concentration level (F=23.82 and p=0.00; F=9.08 and p=0.00 for post 1 and post 2 respectively). A Kruskal-Wallis ANOVA expressed the same significant differences (H=14.80 and p=0.00; H=11.98 and p=0.01 for post 1 and post 2 respectively). It can, therefore, be concluded that the soil pH consistently decreases with the treatment of any of the three given herbicides.

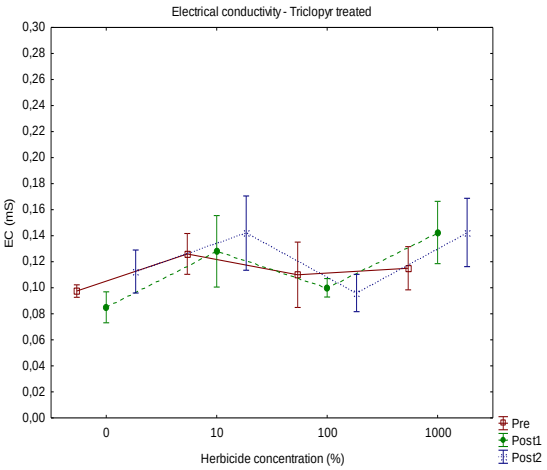
The EC in the soils treated with Glyphosate displayed large fluctuations and degrees of variability over time (Figure 3.16a). There seemed to be no effect of the Glyphosate-based herbicide on EC when applied at any of the concentration levels. A one-way ANOVA showed a significant difference within the pre-treatment samples at the different concentration levels (F=8.55 and p=0.00), which suggests that the EC was variable before the herbicide treatment even took place.

The EC in the Triclopyr-treated soils showed no change and remained generally stable, ranging between 0.06 and 0.18 m/S. No significant differences were found between the concentration treatments when comparing pre-treatment to post 1 and post 2 samples (One-way ANOVA: $p>0.05$).

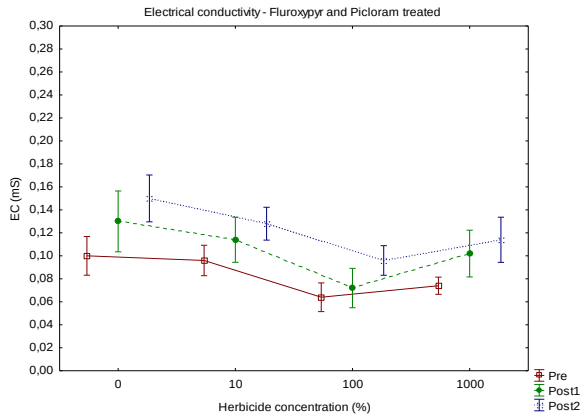
The soils treated with Fluroxypyr and Picloram displayed stability throughout the sampling stages (pre-treatment, post 1, and post 2), as seen in the soils treated with Triclopyr. No significant difference was shown, yet again, when comparing the different concentration treatments within the pre, post 1 and post 2 sampling stages (One-way ANOVA: $p > 0.05$).



(a)



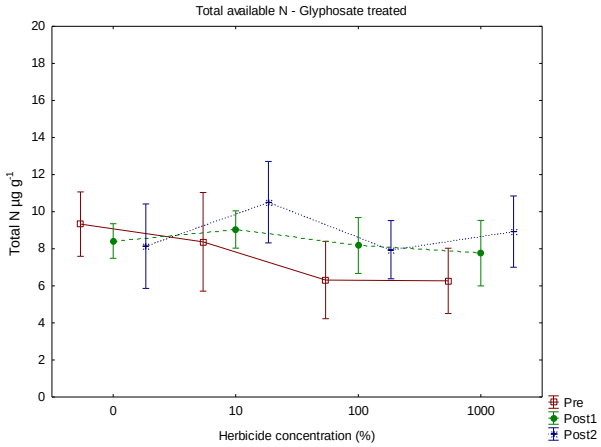
(b)



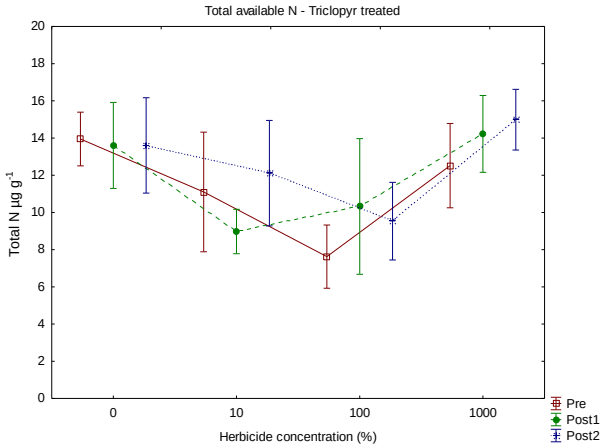
(c)

Figure 3.16: The EC for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The EC is shown across the three different herbicide concentrations (0%, 10%, and 100% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

Application of any of the herbicides had no significant effect on the total soil available N (Figure 3.17). A One-way ANOVA showed no significant difference between the samples before and after treatment ($p > 0.05$). The total soil available N varied between 4 and 17 N-µg g^{-1}.



(a)



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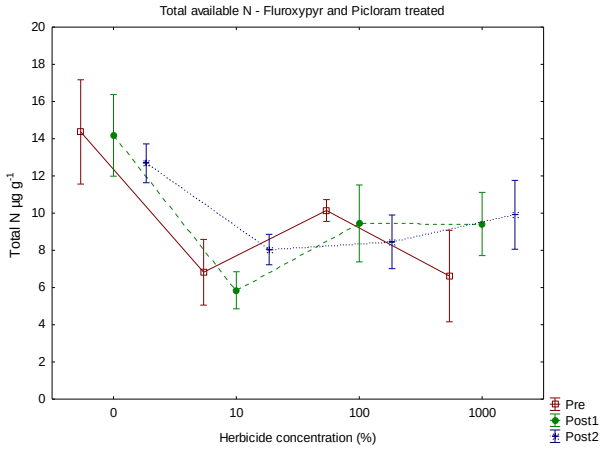
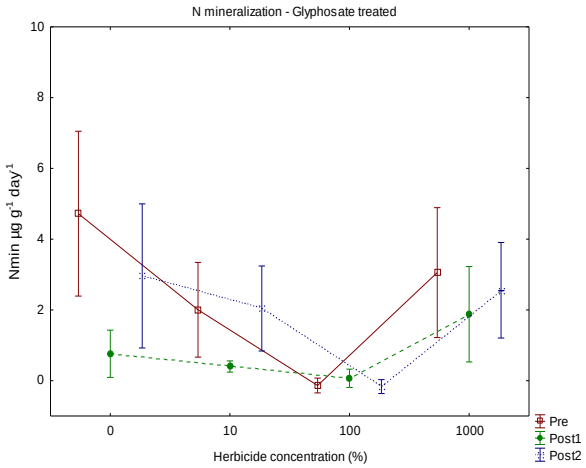


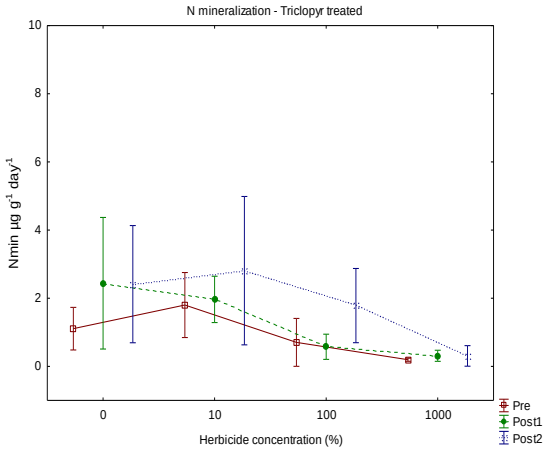
(c)

Figure 3.17: Total soil available N before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The total available N is shown across the three different herbicide concentrations (0%, 10%, 100% and 1000% of the recommended WfW concentration). The point represents the mean and the whisker the standard error.

The samples treated with herbicides showed no effect on the nitrogen mineralisation rates (Figure 3.18a). A One-way ANOVA confirmed that no significant differences among the nitrogen mineralisation rates before herbicide treatment, a week after treatment (post 1), and six weeks after treatment (post 2) ($p > 0.05$) were observed. In general, the samples showed considerable variation and thus the application of the herbicides had no significant effect on the soil mineralisation rates.



(a)



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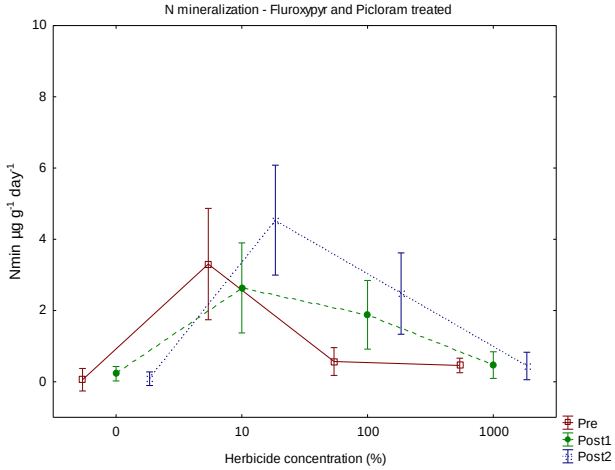
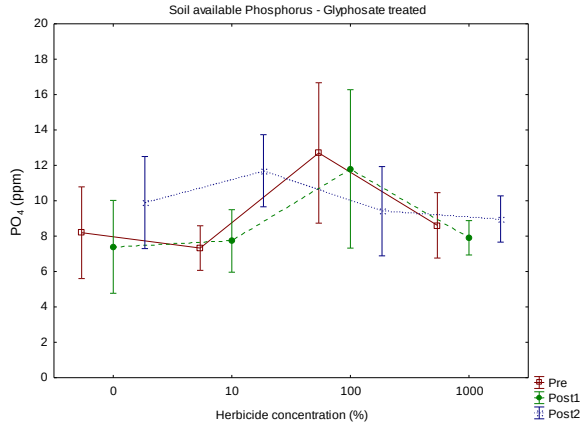


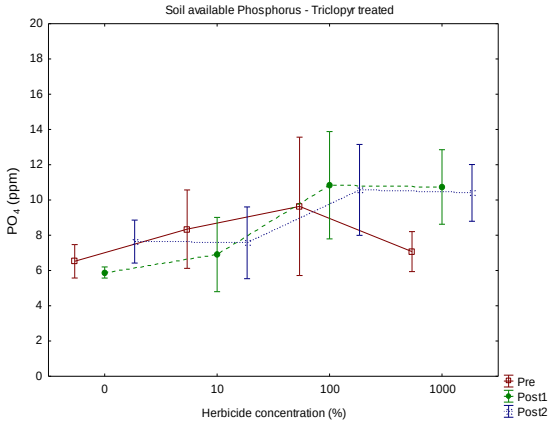
(c)

Figure 3.18: Nitrogen mineralisation rate for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The NMR is shown across the three different herbicide concentrations (0%, 10%, 100% and 1000% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

The phosphorus levels in the soil were very variable throughout the course of the experiment regardless of the herbicide. A One-way ANOVA showed no significant difference between the soils treated with the different concentrations from before to after herbicide treatment ($p > 0.05$). Thus none of the herbicides induced changes to phosphorus levels in the soil (Figure 3.19). The available phosphorus in the soil generally ranged between 2 and 18 PO_4^{3-} (ppm).



(a)



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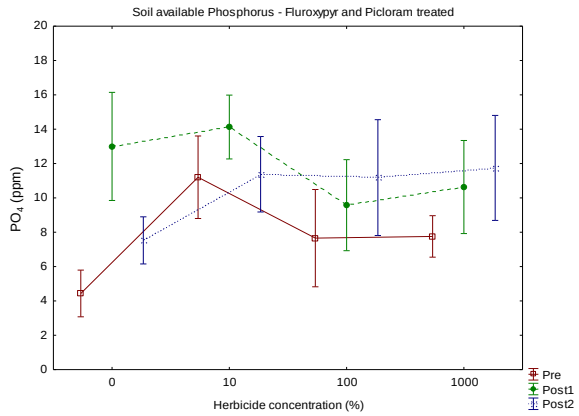
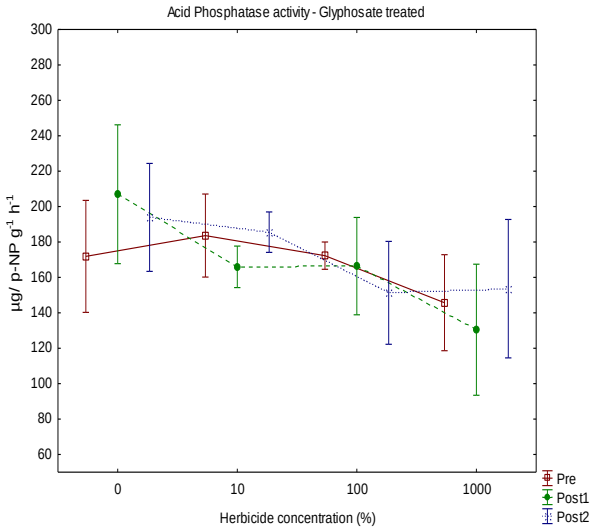


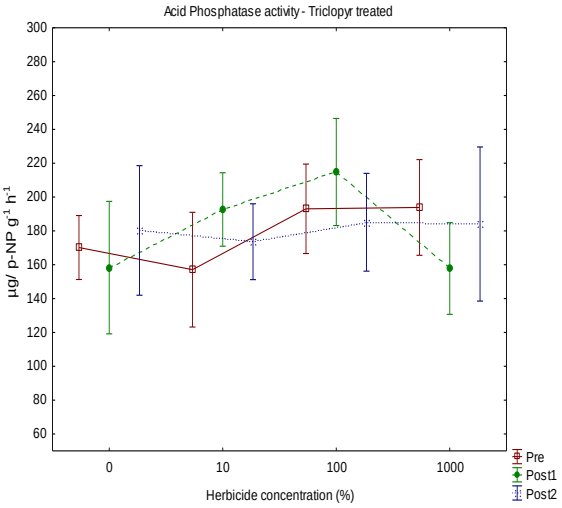
(c)

Figure 3.19: Soil available phosphorus for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The available phosphorus is shown across the four different herbicide concentrations (0%, 10%, 100% and 1000% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

The soils treated with herbicides displayed no change in acid phosphatase after application. The phosphatase activity rate remained stable from pre-treatment to post 1 to post 2 irrespective of the concentration of herbicide applied (Figure 3.20).



(a)



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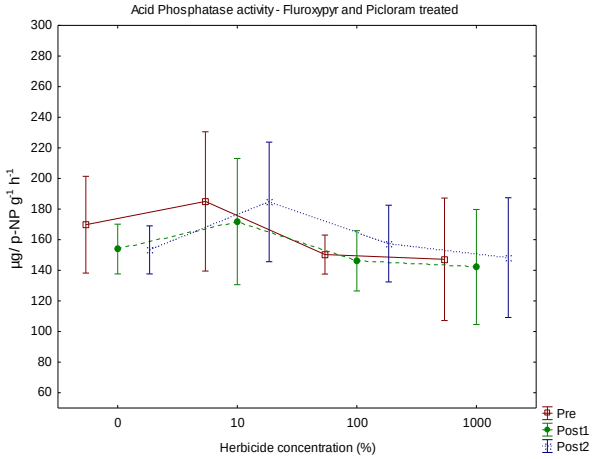


(c)

Figure 3.20: Acid phosphatase activity for soils before and after Glyphosate (a), Triclopyr (b), and (c) Picloram and Fluroxypyr treatment is displayed. The phosphatase activity is shown across the four different herbicide concentrations (0%, 10%, 100% and 1000% of recommended WfW concentration). The point represents the mean and the whisker the standard error.

Acid phosphatase activity in the soils remained between 80 and 250 µg/p-NP.g^{-1}.h^{-1} across all three different herbicide treatments. This was confirmed using one-way ANOVA and Kruskal-Wallis ANOVA ($p > 0.05$). Acid phosphatase activity rates in the soil were not impacted by any of the herbicides at a concentration of 10%, 100%, and 1000% over a six period.

3.3.4.2 Soil microbial diversity

The soil microbial diversity mirrors the environmental data, and appear to be relatively unaffected. The number of OTUs in soils treated with 100% and 1000% Glyphosate concentration showed a slight decrease from the before sample to a week after treatment. There was, however, an increase in

the OTUs over the six weeks after treatment (for the soils treated with the 100% and 1000% concentration), A One-way ANOVA showed a significant difference between the concentration groups before and a week after herbicide treatment ($F=4.79$; $p=0.03$ and $F=5.11$; $p=0.03$ respectively).

The soils treated with Triclopyr (Garlon) showed no convincing effect on the diversity of the bacterial communities as Shannon's diversity scores remained stable throughout the study (Table. 3.5). The deviation from the mean additionally remained low being less than 0.01 units. Furthermore, the number of OTUs showed no consistent trend across the different concentration groups from pre-treatment to post 1 to post 2 as there seems to be high variability around the mean. The bacterial communities six weeks after the treatment (post 2) displayed a significant difference between the concentration groups where the 10% and 100% groups had a significantly lower diversity than the 0% and 1000% groups (One-way ANOVA: $F=6.92$; $p=0.01$). Despite the significant difference found between the bacterial communities six weeks after treatment, it seems as though Triclopyr had no convincing effect on the bacterial communities in the six-week period following treatment.

The bacterial communities in the soils treated with Fluroxypyr and Picloram (Plenum) expressed no deviation according to Shannon's diversity analysis (Table.3.5). Shannon's diversity scores generally kept within a range of 1.9 to 2.2. These scores are similar to the scores expressed in the soils treated with Glyphosate and Triclopyr. The OTUs remained rather variable and seemed to be unaffected by the herbicide. However, a Kruskal-Wallis ANOVA expressed a significant difference between samples before herbicide treatment ($p=0.036$) and a week after treatment ($p=0.047$). However, the standard deviation of the OTUs was high and one could therefore make no clear deductions about the impact of the herbicide on microbial diversity.

Table 3.5: Means and standard deviation of Shannon's diversity values and number of OTUs for bacterial communities in soils treated with Glyphosate (Springbok herbicide), Triclopyr (Garlon), and Fluroxypyr and Picloram (Plenum). The herbicides were applied at four different concentrations: 0%, 10%, 100% and 1000% of the recommended concentration.

Glyphosate			**Triclopyr**			**Fluroxypyr and Picloram**		
Pre-treatment	**Shannons-diversity index**	**Number of OTUs**	**Pre-treatment**	**Shannons-diversity index**	**Number of OTUs**	**Pre-treatment**	**Shannons-diversity index**	**Number of OTUs**
0%	0.040 ± 0.000	59.33 ± 4.11	0%	0.039 ± 0.000	47.00 ± 2.83	0%	0.039 ± 0.000	54.00 ± 2.16
10%	0.039 ± 0.000	50.00 ± 2.16	10%	0.040 ± 0.002	41.33 ± 13.07	10%	0.039 ± 0.000	53.67 ± 0.47
100%	0.036 ± 0.001	26.33 ± 1.70	100%	0.037 ± 0.001	21.67 ± 8.18	100%	0.035 ± 0.004	33.33 ± 14.27
1000%	0.037 ± 0.003	37.00 ± 18.06	1000%	0.039 ± 0.002	40.00 ± 16.31	1000%	0.033 ± 0.000	21.33 ± 3.09
Post 1	**Shannons-diversity index**	**Number of OTUs**	**Post 1**	**Shannons-diversity index**	**Number of OTUs**	**Post 1**	**Shannons-diversity index**	**Number of OTUs**
0%	0.040 ± 0.001	57.00 ± 4.32	0%	0.040 ± 0.001	54.33 ± 4.64	0%	0.038 ± 0.000	51.00 ± 4.32
10%	0.038 ± 0.004	47.00 ± 22.63	10%	0.037 ± 0.002	30.33 ± 13.89	10%	0.037 ± 0.001	48.00 ± 9.09
100%	0.033 ± 0.000	18.67 ± 0.47	100%	0.039 ± 0.001	41.00 ± 13.59	100%	0.034 ± 0.003	28.33 ± 11.26

Glyphosate			**Triclopyr**			**Fluroxypyr and Picloram**		
1000%	0.034 ± 0.001	22.67 ± 3.40	1000%	0.036 ± 0.003	31.00 ± 14.99	1000%	0.034 ± 0.003	18.00 ± 5.66
Post 2	**Shannons-diversity index:**	**Number of OTUS:**	**Post 2**	**Shannons-diversity index:**	**Number of OTUs:**	**Post 2**	**Shannons-diversity index**	**Number of OTUs**
0%	0.039 ± 0.001	41.33 ± 6.55	0%	0.041 ± 0.000	55.33 ± 4.03	0%	0.038 ± 0.000	49.67 ± 4.11
10%	0.037 ± 0.001	32.33 ± 10.62	10%	0.037 ± 0.001	38.33 ± 3.30	10%	0.036 ± 0.001	37.00 ± 7.26
100%	0.038 ± 0.002	48.67 ± 9.81	100%	0.038 ± 0.002	37.33 ± 10.21	100%	0.038 ± 0.001	52.67 ± 4.92
1000%	0.038 ± 0.002	52.00 ± 7.79	1000%	0.040 ± 0.000	57.67 ± 2.05	1000%	0.034 ± 0.002	30.00 ± 7.12

There was no change in the microbial community structure when treating the soil with Glyphosate (Springbok) as seen in the non-metric dimensional plot (Figure 3.21). There are no clusters or form of separation of the pre-treatment from post 1, and post 2 microbial communities. A high degree of similarity was revealed when doing an analysis of similarity (ANOSIM: R=0.146 and p=0.006).

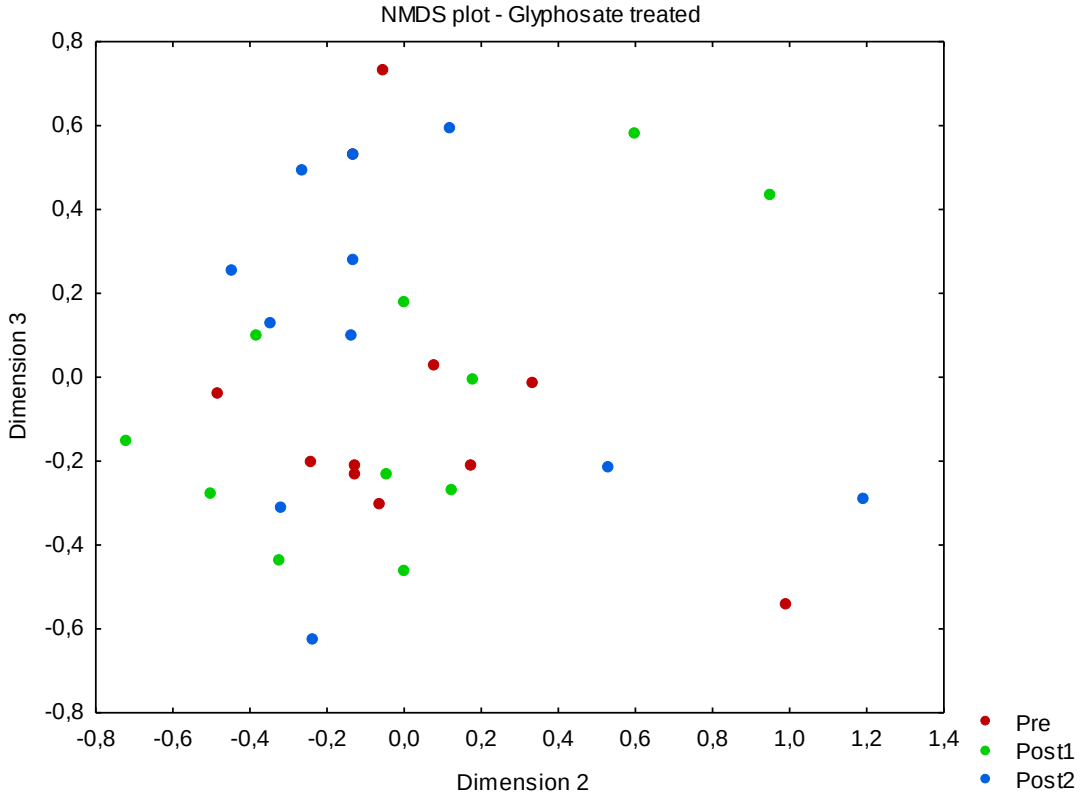


Figure 3.21: Non-metric multidimensional scaling plot for the distribution of bacterial communities in soils treated with Glyphosate. The NMDS analysis was generated through the Whittaker similarity index, which shows the bacterial distribution before herbicide treatment (pre-treatment), a week after treatment (post 1), and six weeks after treatment (post 2).

The soils treated with Triclopyr in the form of Garlon seem to express no strong dissimilarities when comparing the structure of the microbial community from before to after treatment. Thus Triclopyr had no impact on the bacterial community structure over the six-week period following its application.

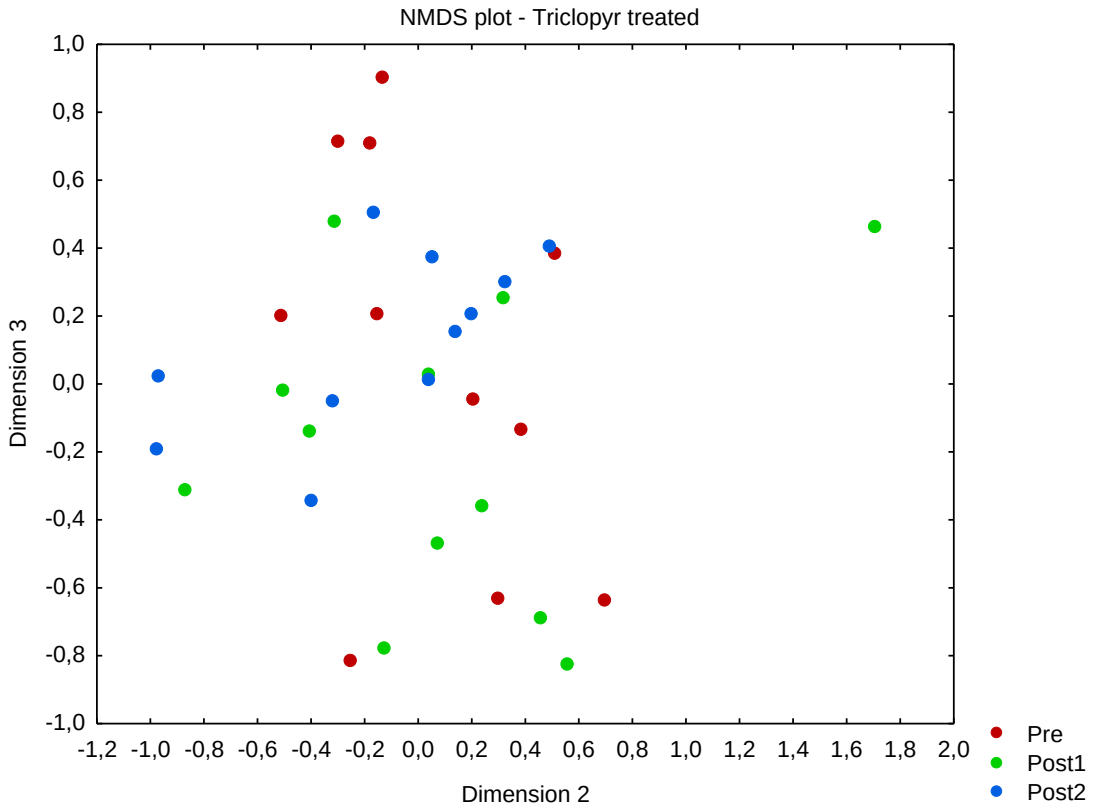


Figure 3.22: Non-metric multidimensional scaling plot for the distribution of bacterial communities in soils treated with Triclopyr. The NMDS analysis was generated through the Whittaker similarity index, which shows the bacterial distribution before herbicide treatment (pre-treatment), a week after treatment (post 1), and six weeks after treatment (post 2).

An ANOSIM expressed a minor degree of dissimilarity when comparing the microbial community before and after herbicide treatment (R=0.072 and p=0.037). Thus the Triclopyr herbicide had no clear impact on the bacterial community structure over the six-week period following its application.

Fluroxypyr and Picloram treatment in the form of Plenum showed no convincing effects on the bacterial community structure (Figure 3.23). No clusters or strong separation within the microbial community were found from before (pre-treatment) to a week (post 1) or six weeks (post 2) after the given herbicide treatment.

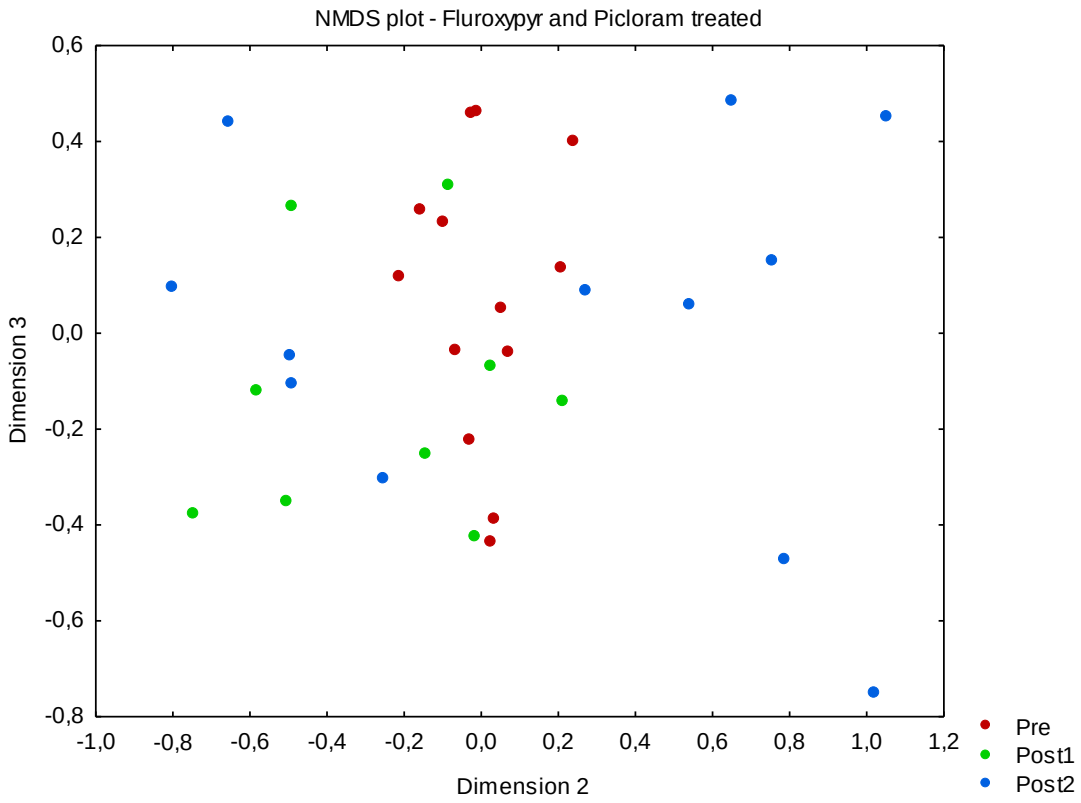


Figure 3.23: Non-metric multidimensional scaling plot for the distribution of bacterial communities in soils treated with Fluroxypyr and Picloram. The NMDS analysis was generated through the Whittaker similarity index, which shows the bacterial distribution before herbicide treatment (pre-treatment), a week after treatment (post 1) and six weeks after treatment (post 2).

Furthermore, an ANOSIM expressed very little dissimilarity between the pre, post 1 and post 2 microbial communities (R=0.095), yet showed a significant difference between them (p=0.038).

3.4 DISCUSSION

The three herbicides tested showed little effect on most of the soil chemical aspects studied, with some notable exceptions. Aspects such as electrical conductivity, soil available phosphorus and acid phosphatase activity remained stable regardless of the type of herbicide applied, even at 1000% of the recommended field. Few studies have looked at the effect of herbicides on soil chemical characteristics, however, a previous study showed little to no effect of herbicides on soil nutrients or on the microbial activity in the soil (Wiedenhamer and Callaway, 2010), which is somewhat similar to what was found in this study.

Soil pH seemed to present pronounced changes with herbicide application. It was observed that the soil pH decreased consistently with the concentration of the treatment of any of the three herbicides to the soil. The pH was significantly lower a week after and six weeks after the given herbicide treatment took place. The stronger concentration treatments (100% and 1000%) seemed to decrease the pH more than the weaker concentrations (10%). It can be concluded that Glyphosate, Triclopyr, and Fluroxypyr and

Picloram all have an acidifying effect on the soil. However, as the experiment was only monitored for six weeks, the change may not be permanent. It may well be possible that the pH could return to a more basic pH at a later stage. Furthermore, the effect of the herbicide on soil pH could be as a result of the adjuvants included in the mixture of the given herbicide and not necessarily the active ingredient itself. It is possible that the active ingredients in Glyphosate, Triclopyr, and Fluroxypyr and Picloram are not responsible for the acidifying effect observed. It was shown in Howe et al. (2004) and Peixoto (2005) that the adjuvants in the herbicide tested were more toxic than the active ingredient included.

Some changes in nitrogen availability and mineralisation was observed after herbicide treatment. Although not all differences were significant, total N seemed to increase with the Glyphosate herbicide applied at 100% concentration *in situ* (experiment 2). Similarly, total N increased considerably a month after Glyphosate and Triclopyr was applied at 100% concentrations *in situ* (experiment 1). Nitrogen mineralisation rate increased after Glyphosate treatment *in situ* (experiment 2), but decreased to very low rates six weeks after treatments. It seems as though the Glyphosate-based herbicide could elevate nitrogen mineralisation levels within a week, but this effect diminished thereafter. Rhoades et al. (2002) reported similar trends where the herbicide treatment significantly increased the nitrogen levels in the soil. Additionally, Reddy et al. (2003) also reported an increase in the soil nitrogen levels when chemical clearing of plants took place, yet linked this effect to the decomposing dead root and shoot biomass in the soil. This could be the effect responsible for not only the change in nitrogen levels, but for the change in the soils' pH in this study. During the plants stress induced by herbicide exposure, the plant could respond and release certain exudates and release nutrients to the soil matrix when it dies. This elevation in nitrogen levels could be related to the plants' response to the herbicide treatment, as the nitrogen levels in the soils of the *ex situ* experiment remained stable and rather unaffected where there was only bare soil and no plants present. This is in line with Mijangos et al. (2006), who found that there was no significant impact of herbicide and fertilizer on N levels in a microcosm experiment (*ex situ*).

Furthermore, the microbial community structure and metabolic activity in the soil showed no changes when treated with a given herbicide. The results of the *ex situ* experiment 1 displayed a steady metabolic potential of the bacterial communities throughout the four weeks after e herbicide type treatment. This has also been observed by Crouzet et al. (2010) and Wardle and Parkinson (1990), where the herbicides tested induced no significant or adverse effects on the microbial biomass and microbial activity. Zabaloy et al. (2012) reported that even herbicides applied at ten times the field rate showed no substantial effect on microbial richness, function and activity. Widenfalk et al. (2008) also showed no effect on bacterial activity when the herbicides Glyphosate and Captan were applied. Furthermore, the results showed that little to no effect on the microbial community structure was observed after different herbicide treatments, even at 100% and 1000% of the recommended levels. The diversity scores, as well as the similarity scores, presented very little variation or change over six weeks. Thus the herbicides tested (Glyphosate, Triclopyr, and Fluroxypyr and Picloram) had no adverse effect on the diversity or structure of the bacterial communities in the soil after application, though longer-term changes to soil microbial communities cannot be excluded. Weaver et al. (2007), report minimal effects of herbicide on the community structure of microorganisms. It appears that no effects of the given herbicides exist when regarding the microbial community structure and microbial communities' metabolic potential, at least in the short term, as was the case in these experiments. Alternatively, if there are any impacts on these aspects of the soil microbial community, they could only potentially occur within the first few days of treatment and then dissipate by the first week after treatment. The microbial community seems to retain its original state (community structure and metabolism) within the first week after herbicide treatment.

In summary, the herbicides tested only showed a consistent significant effect on soil pH levels (*in situ* and *ex situ*) and partly some effect on the soils' nitrogen and nitrogen mineralisation levels (*in situ*). No effects were exerted on the other soil aspects covered, including electrical conductivity, soil phosphorus,

and phosphatase activity. Moreover, the effects of the herbicides on the soil microbial community structure and community metabolic potential were minimal. It is necessary that we continue testing herbicides and the potential impact they could have on soil microbial communities and soil chemical characteristics, especially using experiments with a duration of months to years. The interactive effects of allelochemicals and herbicides remain largely unknown (Weidenhamer and Callaway, 2010). It is recommended that future research studying the impacts of herbicides goes deeper into evaluating different herbicides with different active ingredients and the adjuvants involved. We should include testing the given herbicides at higher concentration levels and for longer periods of time and even more frequently in order to gain a better insight and assess herbicide risk. It is also recommended that we focus on covering other aspects of the microbial community present in the soil and how they respond to herbicide treatment. Such an aspect could include identification of microbial populations in the soil as well as evaluating effects on microbial biomass. Having identified microbial species in the soil before and after herbicide treatment can to an extent give us an indication of how important genera such as *Desulfovibrio* and *Rhizobia* are affected. These microbial genera process nutrients in the soil which are important for uptake by native plant species (*Desulfovibrio* regulates sulphur in the soil, whilst *Rhizobia* regulates nitrogen in the soil; Stafford et al., 2005). With this information, we would know which microbial genera are affected by certain herbicides and thus know the implications of applying a certain herbicide or chemical management scheme for the soils' inhabitants.

CHAPTER 4: The response of fynbos soils' physicochemical properties to burning of slash piles of Acacia spp. and Eucalyptus camaldulensis biomass

4.1 INTRODUCTION

The removal and management of invasive alien plants (IAPs) are among the great challenges currently faced by land management organisations in South Africa (Richardson and van Wilgen, 2004). Successful management or control of IAPs such as the Australian tree species *Eucalyptus camaldulensis* and *Acacia mearnsii*, widespread riparian invaders, would contribute towards restoring and sustaining natural ecosystems which are currently threatened by such tree species (van Wilgen et al., 2012). Presently, in South Africa, WfW and other organisations utilise different physical, chemical and biological methods to clear IAPs from ecosystems (Holmes et al., 2008). However, clearing of these areas leads to accumulation of excess IAP biomass within ecosystems, which requires further management in order to reduce risk of wildfire (Holmes et al., 2008; Chamier et al., 2012; Rhoades et al., 2015). Preferably, the excess biomass should be sold or used on secondary markets such as bioenergy and timber (Holmes et al., 2008; Mugido et al., 2014). Alternatively, when there is no secondary market or the site is inaccessible, the excess biomass may be burned on site, often through the process of burning of slash piles. This process might be effective in destroying IAP biomass, however, it also exposes the soil and surrounding environment to higher temperatures than those of natural wildfires (Rhodes et al., 2015), leading to short-, medium- or long-term changes in soil chemical, physical and biological properties and thus the inability of the surface soil to establish and support vegetation (Korb et al., 2004; Certini, 2005).

The level of damage to the soil depends on fire severity, which is influenced by several factors, including microclimatic conditions and the amount of wet or dry fuel available (Certini, 2005). Fire would have its greatest impact on the soil surface and less impact as distance into the soil profile increases (Certini, 2014). Soil moisture levels may also reduce the level of severity and extent of heat penetration into the soil profile. This suggests that dry soil profiles are more likely to experience higher fire temperatures than those that are moist (Busse et al., 2005), because part of the heat produced by the fire will be consumed by evaporation of soil moisture, thus delaying heat transfer through the soil profile (Beadle, 1940). Busse et al. (2005) conducted an experiment on dry and moist soils in which masticated shrub mulch was burned over the two soil surfaces. They recorded higher temperatures (600°C) on dry soils than on moist soils (400–500°C), the latter more closely resembling riparian soils, and the former terrestrial soils. Affected soils often develop burn scars which may persist for several years and may be challenging to manage or re-vegetate (Korb et al., 2004).

The development and persistence of these scars is often attributed to altered soil properties and the lack of a soil-stored seed bank, with viable seeds killed off by the elevated soil temperatures (Cilliers et al., 2004; Korb et al., 2004). During burning surface soil nutrients are released from the soil through volatilisation and/or mobilisation into different horizons of the soil profile (Neary et al., 1999; Galang et al., 2010). Immediately after burning, nutrient concentrations on the surface depend on the composition of the soil deposited ash which is variable depending on the combusted biomass properties (Certini, 2005; Bodi et al., 2014). Post fire, available P often significantly increases in soil (Romanya et al.,1994; Giardina et al., 2000a; Badía et al., 2014), although in some instances it may remain unchanged (Castelli and Lazzari, 2002; Wang et al., 2013). Similarly, the effects on available N have been shown to be variable, where ammonium may increase significantly (Hernández et al., 1997; Kulmala et al., 2014; Fultz et al., 2016), significantly decrease (Kutiel and Naveh, 1987) or remain unchanged (Switzer et al., 2012). Nitrate has been also reported to significantly increase after fire (Hernández et al., 1997; Kulmala et al., 2014). Exchangeable cations have in most cases been reported to increase after burning (Kim et al., 1999; Menzies and Gillman, 2003; Switzer et al., 2012). These increased cation concentrations have been linked to or contributed to increases in soil EC and pH (Certini, 2005). The fire-affected parameters

usually decrease gradually within time to pre-fire and non-burned area levels. This is with the exception of soil pH, which have been reported to increase immediately after fire due to both heat and ash on the soil surface and persist longer than the other affected parameters (Neary et al., 1999; Rhoades et al., 2004), though soil temperature may play a role in the magnitude of change in pH (DeSandoli, 2014).

Fynbos fires, which are fuelled by finer restiods and ericoids, are often less intense than burning of slash piles and thus have a lesser impact on soils (Kraaij and van Wilgen, 2014). Although there has not been much reported on the response of soils to fynbos fires (Kraaij and van Wilgen, 2014), there are some authors that have observed a loss in cations and phosphorus (De Ronde, 1990). Stock and Lewis (1986) reported an increase total and available N which could be the result of enriched ash on the soil surface. Within the Western Cape of South Africa, how the existence of IAPs and approaches to their clearing affects the Western Cape fynbos soils remains an unanswered question, particularly regarding soil chemistry and soil processes and if and how they recover after burning (Chamier et al., 2012; van Wilgen et al., 2012; Jacobs et al., 2013). This study aimed to evaluate the effects of burning of slash piles of *Acacia* spp. and *Eucalyptus camaldulensis* biomass on soil physicochemical properties. The study will answer three key questions (i) how will burning of slash piles of *Acacia* spp. and *Eucalyptus camaldulensis* biomass affect soil physicochemical properties over the course of one year? (ii) what are the spatial impacts of burning of slash piles of *Acacia* spp. and *Eucalyptus camaldulensis* biomass on physicochemical properties (iii) Thirdly, what is the trajectory followed by physicochemical properties subsequent to burning of biomass of *Acacia* spp. and *Eucalyptus camaldulensis*?

4.2 MATERIAL AND METHODS

4.2.1 Site description

The study was conducted in 2014 and 2015 at four riparian areas and one terrestrial area within the Western Cape, South Africa, where the climate is Mediterranean with cool wet winters and warm dry summers (Rebelo et al., 2006). The riparian study areas were located along the Wit River (termed Wit River; $33^{0}32'36.06''$S, $19^{0}10'17.90''$E; dominant invasive species *A. mearnsii*), along the Breede River near Rawsonville (Rawsonville; $33^{0}43'43.43''$S, $19^{0}28'25.63''$E; *A. mearnsii*), along the Breede River near Robertson (Robertson; $33^{0}50'17.08''$S, $19^{0}52'28.33''$E; *E. camaldulensis*) and along the Berg River near Hermon (Hermon; $33^{0}28'42.83"$S, $18^{0}56'13.83''$E; *E. camaldulensis*. The terrestrial site was located at Blaauwberg Nature Reserve (Blaauwberg; $33^{0}45'14.61''$S, $18^{0}29'35.30''$E; *A. saligna*). Information on the sizes of piles, soil information and timing of the experimental burning is given in Table 4.1.

Blaauwberg Nature Reserve is located in the north of the City of Cape Town municipal area in the Western Cape, South Africa. The study area is characterised by deep acidic sandy soils and it experiences a Mediterranean type climate, which is cool and wet in winter, and warm and dry in summer. Most of the rainfall is received between the months of May and August and the mean annual rainfall is 575 mm (Rebelo et al., 2006; Krupek et al., 2016). The native vegetation is dominated by fynbos, with Proteaceae, Ericaceae, Restionaceae and Asteraceae prominent; however, native plant species have almost entirely been displaced by the Australian invasive species *Acacia saligna* which has invaded the area and occurs in dense stands (Krupek et al., 2016).

Table 4.1: Description of slash piles in terms of the primary biomass, pile size, texture and burn season volume

Study area	Study species	Average pile size (m^3)	Soil texture (tested)	Burn season
Hermon	*Eucalyptus camaldulensis*	116.68	Loamy	Spring 2014
Blaauwberg	*Acacia saligna*	28.30	Sandy	Spring 2014
Wit River	*Acacia mearnsii*	21.01	Loamy sand	Winter 2015
Rawsonville	*Acacia mearnsii*	88.17	Sandy loam	Winter 2015
Robertson	*Eucalyptus camaldulensis*	93.93	Sandy clay loam	Winter 2015

Volume = (length x width x height x π) ÷ 6 = volume m^3 (Busse et al., 2013).

4.2.2 Experimental design

The study areas were divided into the burn site which comprised the centre, intermediate and edge of the burn scar (Figure 4.1), and non-burn site which consisted of the soil matrix approximately 2 m from the edge of the burn scar (Figure 4.1), the invaded reference site and the recovering reference site. At all sites the invaded reference area consisted of predominantly trees of the same invasive plant species cleared from the sites. At Hermon, Wit River and Blaauwberg, the recovering site consisted of an area which had been cleared and is currently recovering. On the other hand, at Robertson and Rawsonville, the recovering site was an area that represents local native vegetation. This was due to the lack of areas that had been cleared and were recovering. Different sized slash piles were selected and burning was conducted in spring 2014 at Blaauwberg and Hermon, and in winter 2015 at Robertson, Rawsonville, and Wit River (Table 4.1).

4.2.3 Sampling and laboratory methods

Soil sampling was conducted over a period of approximately one year post fire. Samples were collected using a metal tube, from a depth of 0–10cm, on five occasions, namely a few days after the experimental burn took place, within two weeks post fire and once in three subsequent seasons. At all riparian study areas, a total of 52 soil samples were collected on each sampling occasion, three samples from each of eight piles (viz. the centre, intermediate position, i.e. between the edge and centre, and the edge of the burn scar (Figure 4.1), one sample from the soil matrix outside each of eight piles (about 2 m from the edge of the burn scar), ten soil samples from the recovering site, and ten samples from the invaded site. This sampling procedure was followed at the terrestrial study area, however, only five slash piles were selected and as a result collected samples totalled 40.

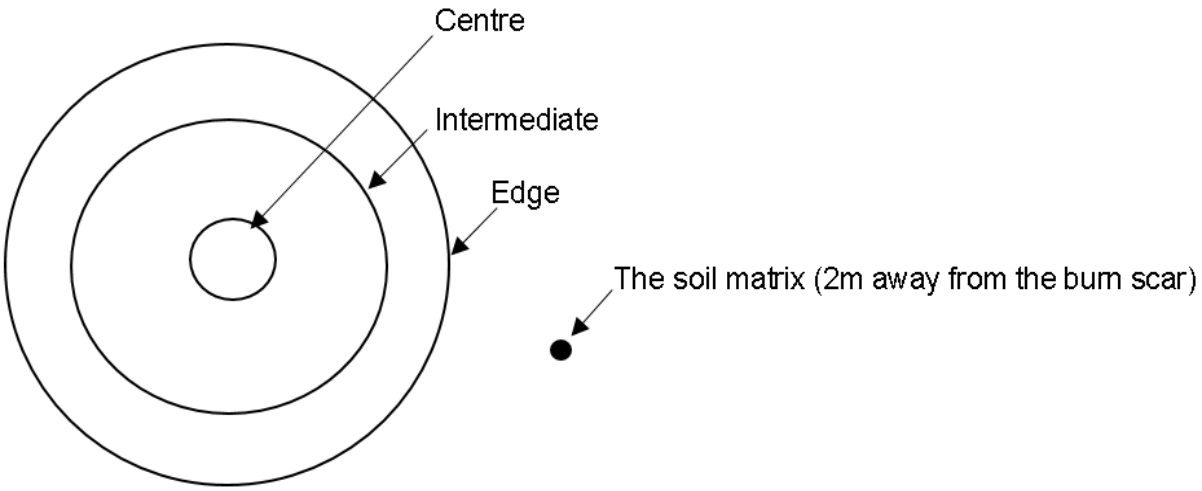


Figure 4.1: Depiction of the centre, intermediate and edge sampling positions within the burn scar, and the soil matrix

Field-collected soil samples were sealed in plastic bags and transported to the laboratory. On arrival, the soil samples were sieved through a 2 mm sieve to remove rocks, pebbles and gravel (Mataix-Solera et al., 2013). Thereafter, the gravimetric soil water content was determined by oven drying samples at 105°C for 48 h (Schafer and Mack, 2010). Soil subsamples were treated with hydrogen peroxide to remove excess organic matter, then soil texture was determined using the hydrometer method, by dispersing 40–50 g soil with 100 ml sodium hexametaphosphate (HMP) solution in an electric mixer for five minutes and measuring buoyancy using an ASTM 152H hydrometer (Gee et al., 1986). Soil pH was tested by mixing 10 g of soil and 20 ml distilled water for 30 minutes (1:2) and analysed with a calibrated Hanna pH meter HI 8424 (Robertson et al., 1999). Electrical conductivity of the soil was measured using a 1:2 mixture of soil and distilled water and a calibrated Hanna HI 8733 conductivity meter (Hanna HI 8733) (Miller and Curtin, 2007).

Available nitrogen (NH_4^+-N and NO_3^--N) was extracted using a 0.5 M K_2SO_4 solution, then later analysed using a Genesys 20 spectrophotometer. The salicylic acid method was used to measure NO_3^--N concentrations and the indophenol blue method for NH_4^+-N concentrations; procedures are as described in Anderson and Ingram (1993). Exchangeable cations were extracted with 1 M NH_4OAc (Simard, 1993) and analysed with a Varian 240 FS atomic absorption spectrometer. Only the Blaauwberg, Hermon and Rawsonville study areas' soils were analysed for exchangeable cations. Available P was extracted using the P-Bray 2 solution according to Bray and Kurtz (1945), and the concentration was determined with a spectrophotometer (Genesys 20) based on the molybdenum blue method (Olsen et al., 1982).

4.2.4 Data analyses

The data was analysed using the Statistica version 13 software package (Dell Inc., 2015). All parameters were checked for normality using the Kolmogorov-Smirnov test. Two-way analyses of variance (ANOVA) were used to determine the burn treatment effects seasonally, viz. pre-fire, post fire, summer, autumn and winter. One-way ANOVAs were used to determine the burn treatment effects by location, viz. the centre, intermediate, edge, matrix, and reference sites. Where the computed ANOVAs showed significance ($p < 0.05$), the Bonferroni post-hoc tests were performed.

4.3 RESULTS

The results presented here for all parameters are based on seasonal data from Blaauwberg, Hermon, Robertson, Rawsonville and Wit River, with the exception of exchangeable cations which is based only on data from the Hermon, Rawsonville and Blaauwberg study areas.

4.3.1 Chemical properties

4.3.1.1 Soil pH

In riparian soils, pH within the burn scars increased significantly after burning at all the study areas, i.e. Hermon ($p < 0.01$; Figure 4.2a), Robertson ($p < 0.01$; Figure 4.2b), Rawsonville ($p < 0.01$; Figure 4.2c) and Wit River ($p < 0.01$; Figure 4.2d); In contrast, the soil pH of the non-burned sites remained unchanged at all study areas across all seasons (Figure 4.2). At the end of the sampling period (approximately one year after burning), burn scar soil pH values were still significantly higher than both pre-fire and non-burn sites at all study areas, with the exception of Wit River which had returned to pre-fire levels in summer (about four months after burning).

Within the terrestrial burn scars (Blaauwberg, Figure 4.2), soil pH (water) increased significantly ($p < 0.01$) after burning of slash piles, and continued to increase ($p < 0.01$) to reach a maximum in summer and it had not returned to pre-fire levels at the end of the sampling period, i.e. winter. There was a difference of 2.90 pH units between the summer values on and off the burn scar. In contrast, the soil pH within the non-burn sites (viz. the matrix and two reference sites) remained unchanged throughout the sampling period (Figure 4.2e).

4.3.1.2 Electrical conductivity

At three of the four study areas, soil EC within the burn scars increased significantly after fire compared to the non-burn sites which had remained seasonally unchanged (Figure 4.3). At Hermon, soil EC increased significantly after fire ($p < 0.01$) and returned to pre-fire levels by the end of sampling (about a year after burning) (Figure 4.3a). At Robertson, burn scar soil EC increased significantly ($p < 0.01$) after burning and was still significantly higher ($p=0.02$) than pre-fire in about a year after burning (Figure 4.3b). Burn scar soil EC at Rawsonville also increased significantly ($p < 0.01$) as a result of fire, but had returned to pre-fire levels in spring (approximately 3 months after burning) (Figure 4.3c). In contrast, the fourth study area (i.e. Wit River) did not experience changes in soil EC within the burn scar after burning and remained unchanged in spring and summer. However, a spike in EC occurred in both the burn and non-burn sample areas in autumn (almost a year after burning) when soil EC values became significantly higher than pre-fire at both the burn ($p=0.01$) and the non-burn sites ($p <0.01$; Figure 4.3d).

At Blaauwberg, soil EC within the burn scar did not increase immediately after burning; the effects of fire were delayed and were only noticeable in summer (i.e. the season after post fire), when EC increased significantly ($p=0.01$) and continued to increase ($p <0.01$) to reach its maximum in autumn before returning to pre-fire levels (Figure 4.3e). There was a difference of 790.72 µS between the autumn values on and off the burn scar. On the other hand, the non-burn sites, viz. the matrix and the two reference sites, did not show any major changes through all the seasons.

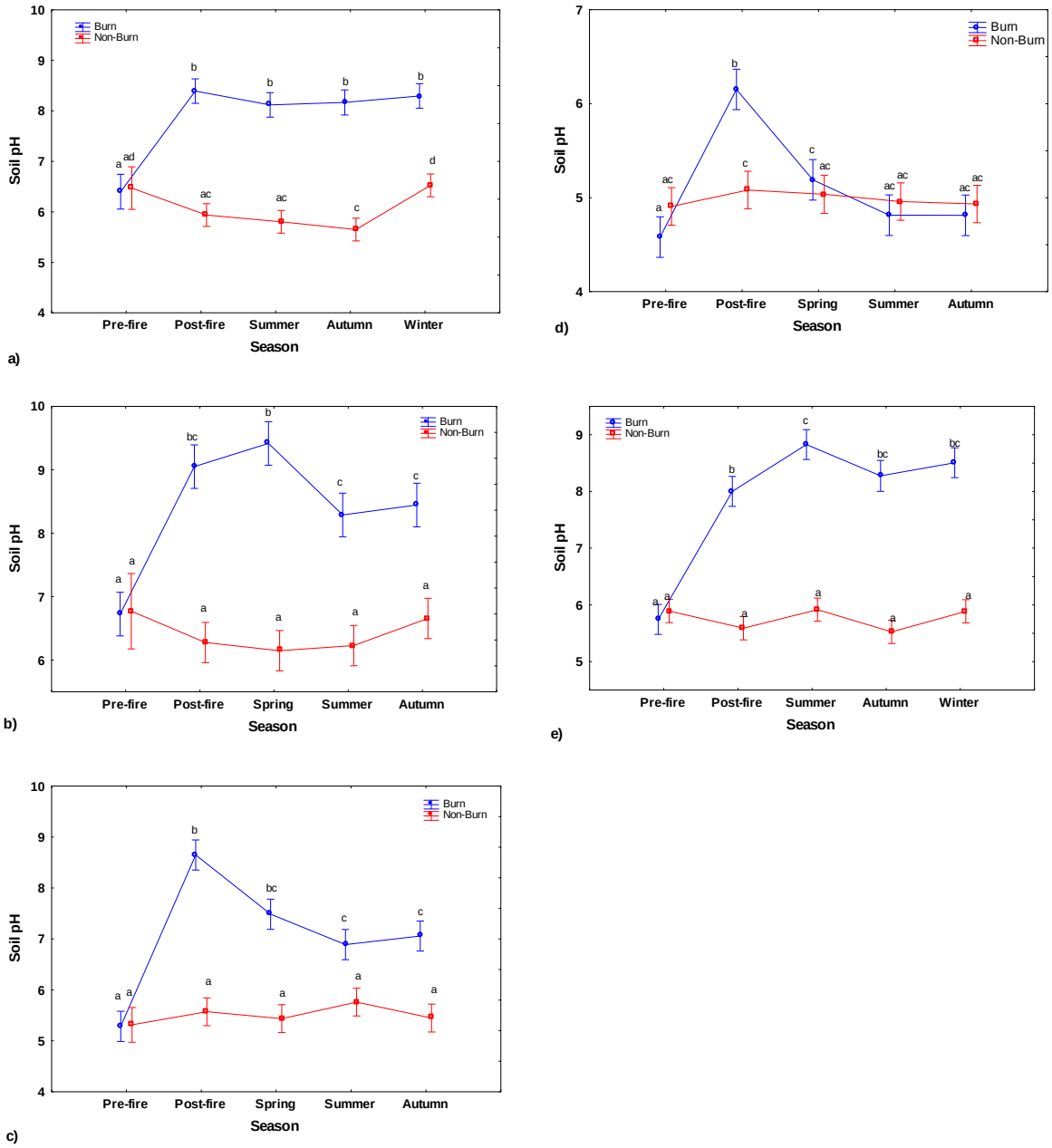


Figure 4.2: Seasonal soil pH values within the burn and the non-burn treatment sites at the (a) Hermon (b) Robertson (c) Rawsonville (d) Wit River, and (e) Blaauwberg study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p < 0.05$).

4.3.1.3 Exchangeable cations

Exchangeable cations (i.e. Ca, Mg, Na, and K) within the burn scar increased after fire at the riparian sites, Hermon and Rawsonville, while remaining unchanged at the non-burn sites. At Hermon cations within the burn scar significantly increased ($p < 0.01$) after burning and had returned to pre-fire levels approximately a year after burning, while remaining unchanged in the non-burn sites (Figure 4.4a). Similarly, at Rawsonville, burn scar cation concentrations also increased significantly ($p < 0.01$) after fire and eventually returned to pre-fire levels after approximately a year (Figure 4.4b).

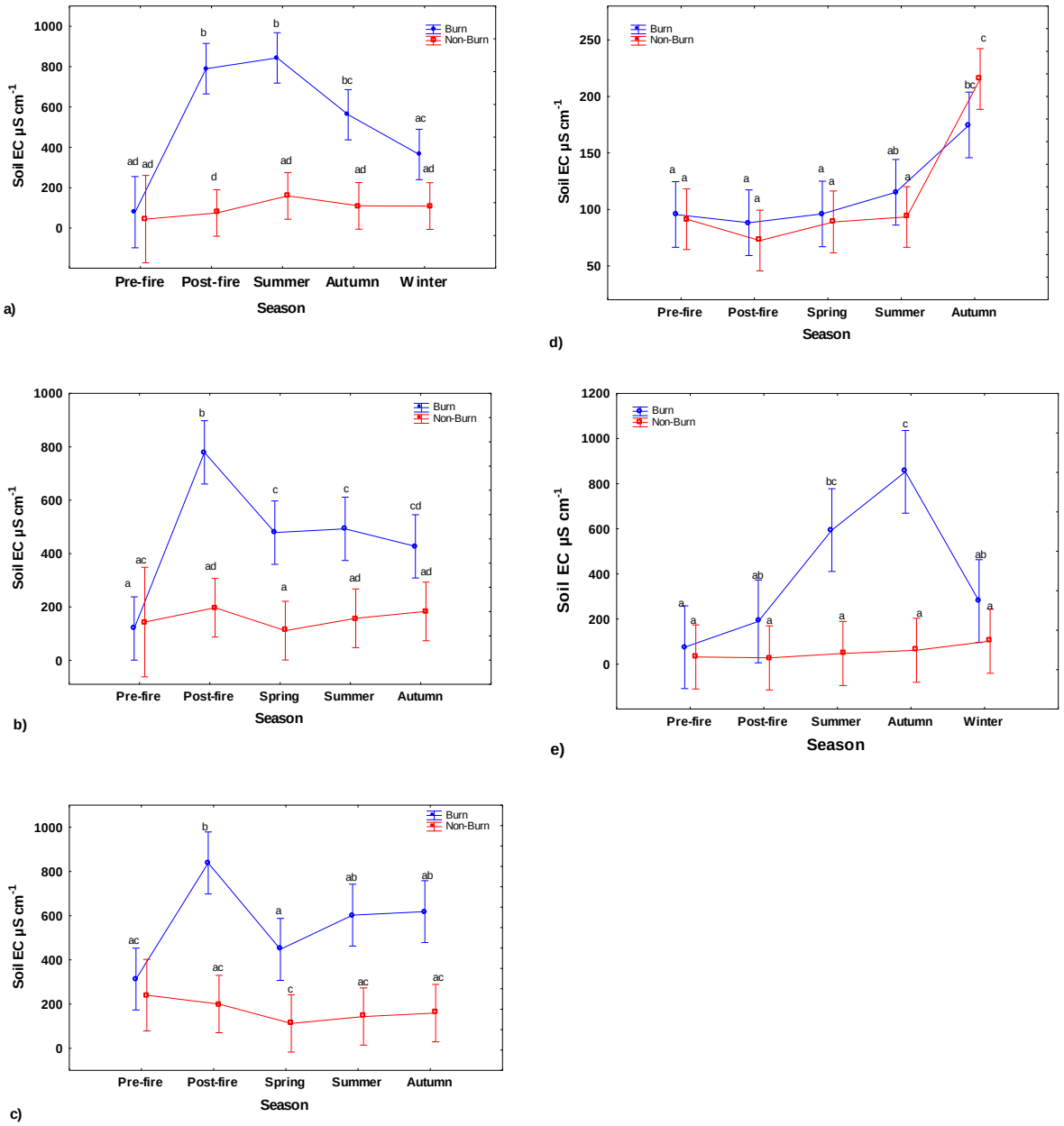


Figure 4.3: Seasonal soil EC values within the burn and the non-burn treatment sites at (a) Hermon, (b) Robertson, (c) Rawsonville and (d) Wit River and (e) Blaauwberg study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p < 0.05$).

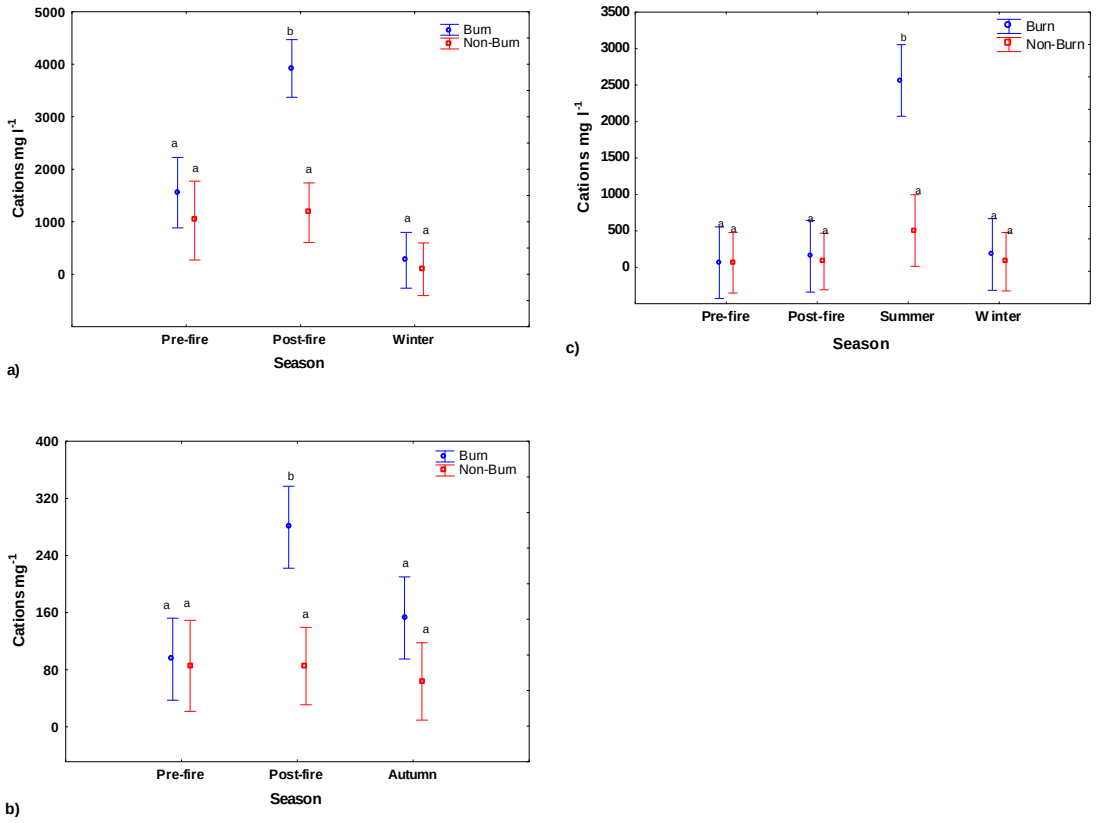


Figure 4.4: Seasonal cation concentrations within the burn and the non-burn treatment sites at (a) Hermon, (b) Rawsonville and (c) Blaauwberg study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p < 0.05$).

Within the burn scars at the Blaauwberg site, exchangeable cations (i.e. Ca, Mg, Na and K) did not increase immediately after fire; the increase appeared to be delayed and only became significantly higher than non-burn areas in summer (one season post fire); and by the end of the sampling period, exchangeable cations had returned to pre-fire levels. There were no significant changes in exchangeable cation concentrations within the non-burn treatment sites (Figure 4.4c).

4.3.1.4 Available P

At all riparian study areas, available P concentrations consistently increased as a result of burning. At Hermon, available P concentrations within the burn scar increased significantly ($p < 0.01$) after fire, and continued to increase to reach a peak in summer, and had returned to pre-fire levels at the end of the sampling period, approximately a year after burning (Figure 4.5a). At Robertson, available P concentrations did not change significantly in post-fire season; a significant increase only came about in spring ($p < 0.01$) (three months post fire), and P concentrations had returned to pre-fire levels in summer. On the other hand, the non-burn site available P levels did not show any significant changes seasonally, there was, however, a trend showing a decrease after the pre-fire season (Figure 4.5b). At Rawsonville, available P concentrations within the burn scar significantly increased ($p < 0.01$) after fire and then returned to levels similar to those of pre-fire in spring, three months after burning. On the contrary, the non-burn sites followed a trend opposite to that of the burn scar, where available P decreased significantly ($p < 0.01$) after fire, and was still lower at the end of sampling (Figure 4.5c). At Wit River, available P concentrations within the burn scar increased significantly ($p < 0.01$) after fire and

had not returned to pre-fire levels at the end of the sampling period (about a year after burning). Available P concentrations within the non-burn sites also increased significantly (p=0.02) in the post-fire season, remained at this level in spring and returned to pre-fire levels in summer (Figure 4.5d).

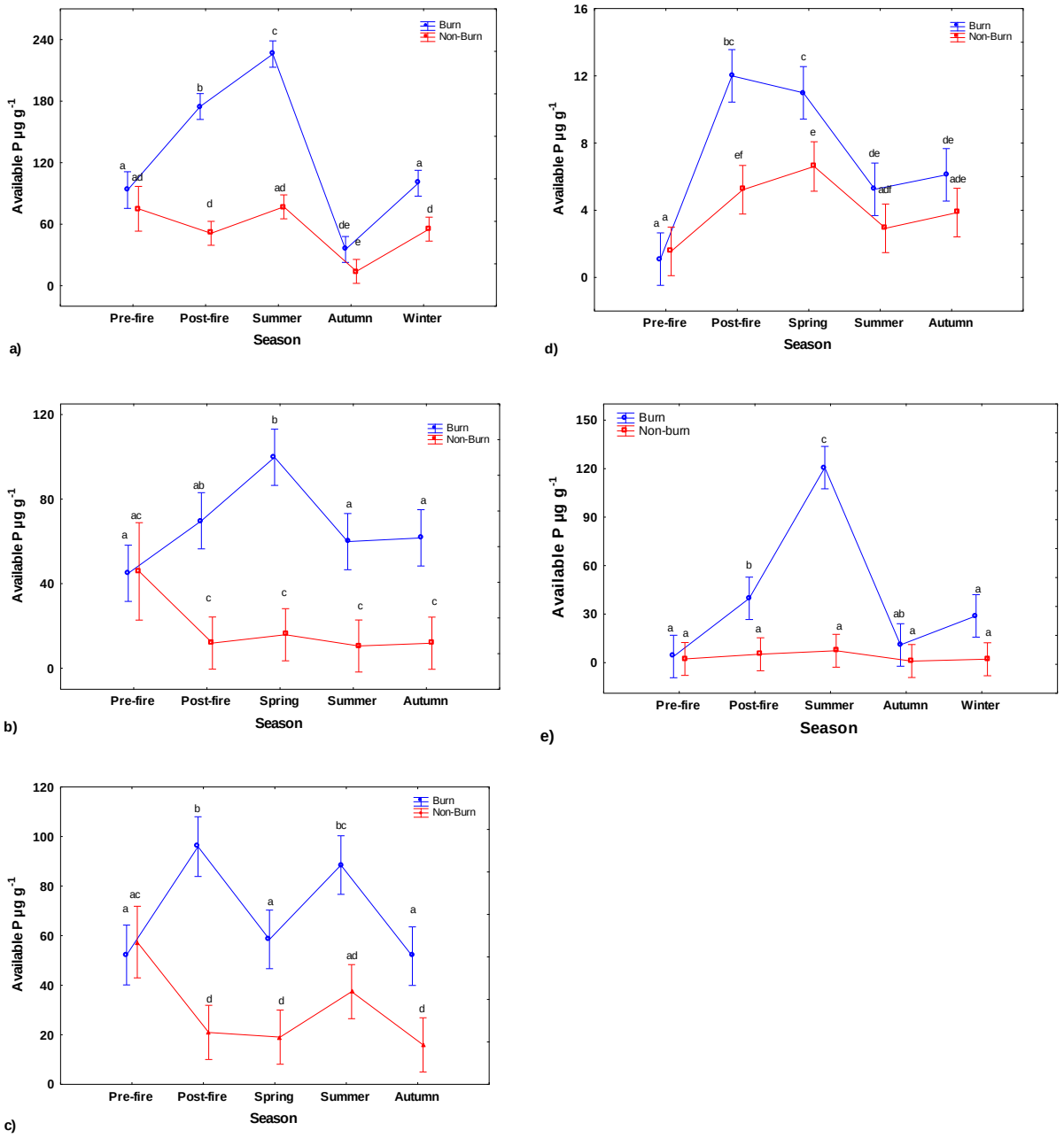


Figure 4.5: Seasonal available P levels within the burn and the non-burn treatment sites at (a) Hermon, (b) Robertson, (c) Rawsonville (d) Wit River and (e) Blaauwberg study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p < 0.05$).

At Blaauwberg, available P increased significantly (p=0.01) after fire and continued to increase ($p < 0.01$) to reach its highest concentrations in summer, and eventually returned to pre-fire levels in autumn, approximately six months after burning. The actual values in summer were 7.35 µg g^{-1} in non-

burn soils and 120.63 µg g^{-1} in burn scar soils, a difference of two orders of magnitude. The non-burn treatment sites were not affected by the fire and no differences were found through all the seasons (Figure 4.5e).

4.3.1.5 Available N

Total available N did not change immediately after fire either in the terrestrial or the riparian sites, within both the burn and non-burn treatment sites. However, in the subsequent seasons, concentrations of available N followed varying trends at the various study areas. At Hermon, the available N levels remained relatively unchanged throughout the sampling period (Figure 4.6a). At Robertson, in spring (about three months after burning), there were significant drops in available N levels within both the burn scar ($p < 0.01$) and the non-treatment sites ($p=0.01$) (Figure 4.6b). At Rawsonville, six months after burning (summer), available N levels within the burn scar became significantly higher ($p < 0.01$) than the non-burn sites, and continued in this pattern towards the end of sampling (Figure 4.6c). Similarly, at Wit River, available N concentrations in the last season of sampling were significantly higher ($p < 0.01$) in the burn scar than in the non-burn sites (Figure 4.6d). At Blaauwberg, soil available N did not change immediately after fire within the burn scar and remained unaltered throughout the sampling period. The non-burn sites also did not change immediately after fire, but levels were significantly lower than the burn scar ($p < 0.01$) in autumn (Figure 4.6e).

4.3.2 Physical properties

The effects of burning of slash piles on seasonal hydrophobicity (time taken for a water drop to infiltrate soil) varied among the study areas. At Hermon, in burn scars, the soil was non-hydrophobic with an average residence time of 5.00 s and it remained non-hydrophobic post fire (Table A1), which was also the case for the non-burn areas. At Robertson, neither spatial nor temporal data showed any major trends, and soil was on average strongly hydrophobic throughout the study period, including pre-fire (Table A2). At Wit River, soils tended to be strongly, or even severely, hydrophobic, with no particular trends spatially or temporally (including pre-fire) (Table A3). In contrast, Rawsonville showed higher hydrophobicity in the burn area, with the centre, intermediate and edge positions of the burn scar showing strongly hydrophobic soils, and the soil matrix, the recovering and invaded reference sites slightly hydrophobic (Table 4.2). Pre-fire, both burn and non-burn areas showed slight hydrophobicity, with only the burn areas showing increasing hydrophobicity over time as the drier summer period approached.

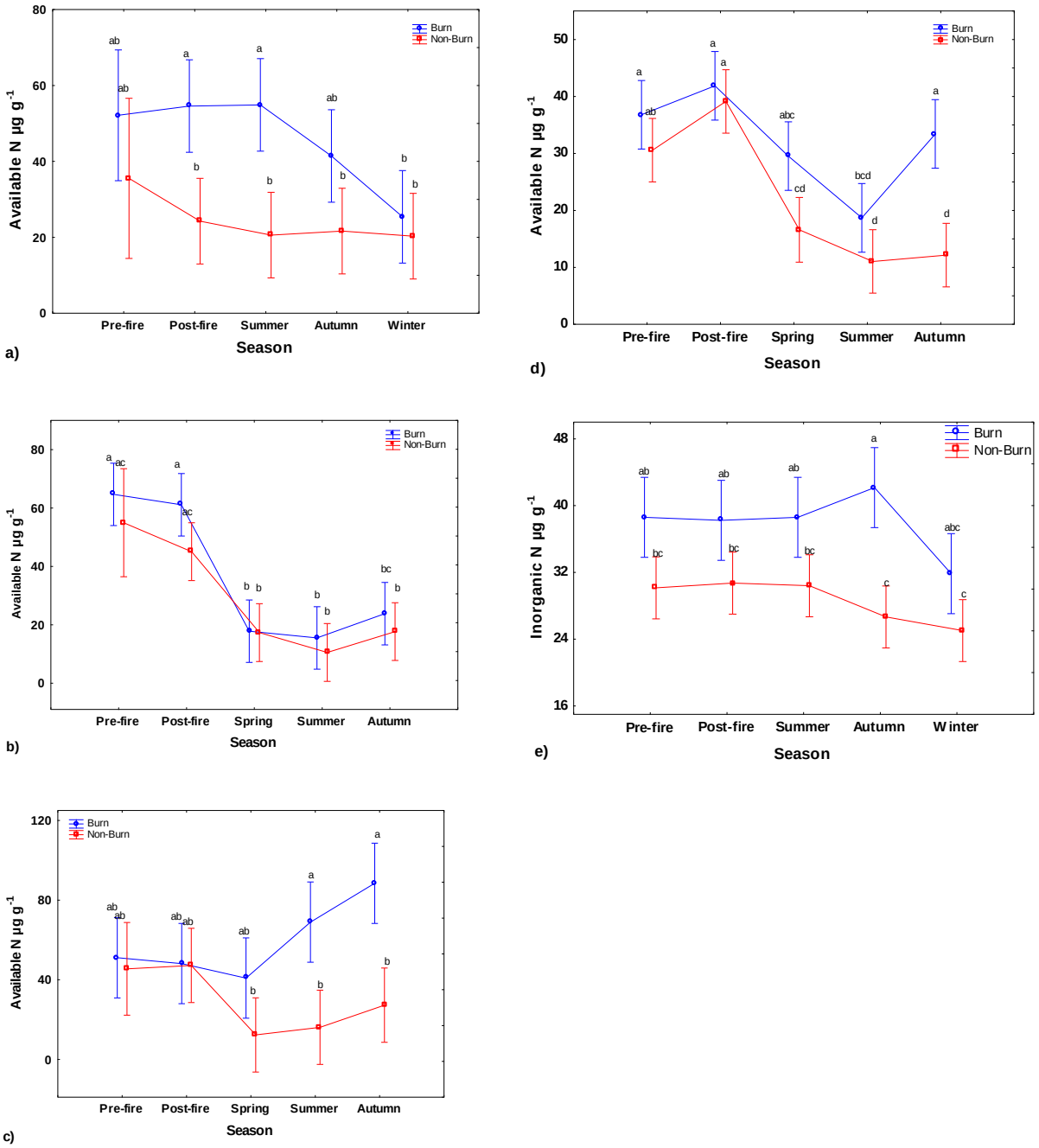


Figure 4.6: Seasonal soil available N levels within the burn and the non-burn treatment sites at (a) Hermon, (b) Robertson, (c) Rawsonville, (d) Wit River and (e) Blaauwberg study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p < 0.05$).

Table 4.2: (a) Seasonal hydrophobicity values between the burn and non-burn treatment sites at Rawsonville study area. (b) Spatial hydrophobicity values between the burn and non-burn sampling positions at Rawsonville study area. Means represent time in seconds for the Water Drop Penetration Test at each sampling location, with standard errors in brackets.

a) Seasonal hydrophobicity values

	Burn treatment		**Non-Burn treatment**	
Season	**Time (s)**	**Category**	**Time (s)**	**Category**
Pre-fire	5.00 (±0.00)	Non-hydrophobic	5.94 (±0.74)	Slightly hydrophobic
Post fire	14.92 (±9.79)	Slightly hydrophobic	5.00 (±0.00)	Non-hydrophobic
Spring	115.42 (±75.23)	Strongly hydrophobic	5.89 (±0.62)	Slightly hydrophobic
Summer	494.58 (±153.64)	Strongly hydrophobic	8.07 (±1.91)	Slightly hydrophobic
Autumn	234.67 (±123.40)	Strongly hydrophobic	6.68 (±0.94)	Slightly hydrophobic

b) Spatial hydrophobicity values

Location	**Treatment**	**Time (s)**	**Category**
Centre	**Burn**	311.53 (±117.83)	Strongly hydrophobic
Intermediate	**Burn**	190.25 (±90.50)	Strongly hydrophobic
Edge	**Burn**	142.91 (±73.20)	Strongly hydrophobic
Matrix	**Non-burn**	8.88 (±1.80)	Slightly hydrophobic
Recovering	**Non-burn**	5.50 (±0.35)	Slightly hydrophobic
Invaded	**Non-burn**	5.35 (±0.35)	Slightly hydrophobic

At Blaauwberg, during pre-fire, the soil was already strongly hydrophobic within the fire scar and it remained this way post fire and was still strongly hydrophobic at the end of the sampling period. On the non-burned sites, the soil was strongly hydrophobic pre-fire and at the end of sampling it was severely hydrophobic (Table 4.3).

Table 4.3: (a) Seasonal hydrophobicity values between the burn and non-burn treatment sites at Blaauwberg study area; and (b) Spatial hydrophobicity values between the burn and non-burn sampling positions at Blaauwberg study area. Means represent time in seconds for the water drop penetration test at each sampling location, with standard errors in brackets.

a) Seasonal hydrophobicity values

	Burn treatment		**Non-burn treatment**	
Season	**Time (s)**	**Category**	**Time (s)**	**Category**
Pre-fire	233.87 (±79.17)	Strongly hydrophobic	127.12 (±40.73)	Strongly hydrophobic
Post fire	504.40 (±134.43)	Strongly hydrophobic	108.25 (±33.81)	Strongly hydrophobic
Summer	458.13 (±137.02)	Strongly hydrophobic	1155.48 (±164.71)	Severely hydrophobic
Autumn	919.60 (±206.14)	Severely hydrophobic	1385.32 (±116.53)	Severely hydrophobic
Winter	363.20 (±119.62)	Strongly hydrophobic	1158.08 (±133.57)	Severely hydrophobic

b) Spatial hydrophobicity values

Location	**Treatment**	**Time (s)**	**Category**
Centre	**Burn**	503.75 (±141.19)	Strongly hydrophobic
Intermediate	**Burn**	421.45 (±113.66)	Strongly hydrophobic
Edge	**Burn**	758.80 (±151.02)	Severely hydrophobic
Matrix	**Non-burn**	814.75 (±148.58)	Severely hydrophobic
Recovering	**Non-burn**	1030.13 (±141.96)	Severely hydrophobic
Invaded	**Non-burn**	965.00 (±115.35)	Severely hydrophobic

Streamflow for 2015 showed a steep increase some days after the piles were burned (26 May 2015) at Bainskloof (Wit River site). This data is shown in Figure 4.7.

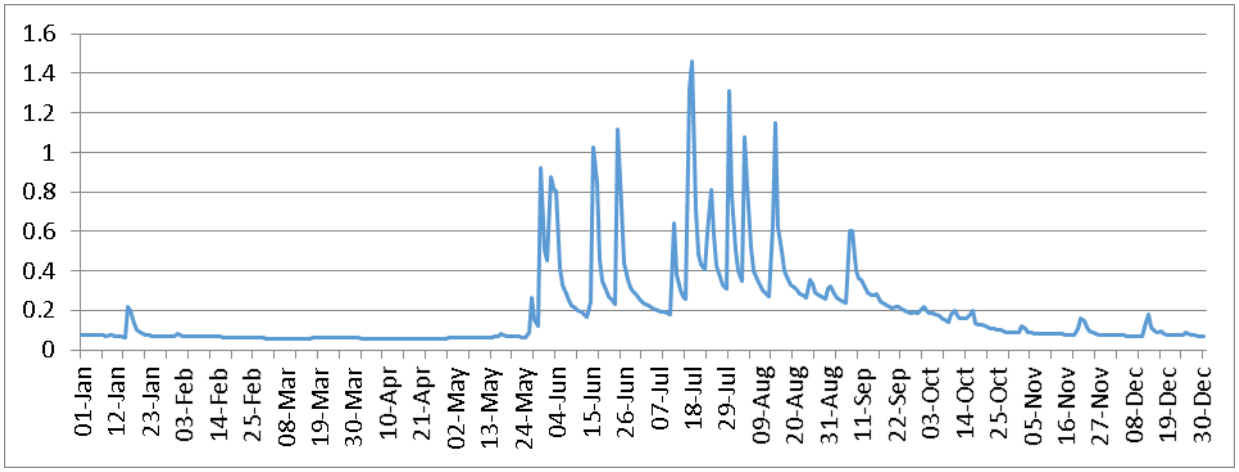


Figure 4.7: Streamflow for 2015 at the Wit River site (Bainskloof).

4.4 DISCUSSION

The burning of *Acacia* spp. and *Eucalyptus camaldulensis* biomass affected soil physicochemical properties variably. The results show that soil pH, EC, cations and available P respond immediately to the effects of burning of slash piles at all study areas; in contrast, available N did not show a direct response to fire. At all study areas (terrestrial and riparian), soil pH increased significantly after burning, with an increase by up to 4.4 pH units at Hermon and Robertson study area and by up to 3.4 pH units in Rawsonville study area (however, only 1.8 units at Wit River). An increase in soil pH is consistent with results obtained by Arocena and Opio (2003) and Granged et al. (2011).

Soil pH remained significantly higher than non-burn areas for more than one year after fire at three of the study areas. Granged et al. (2011) observed an increase in soil pH in nutrient-poor acidic soils in South-Western Spain which has a Mediterranean climate, and the pH remained high for more than one year. Rhoades et al. (2004) also detected soil pH increases which persisted for more than one year, similar to the results presented here, where after one year, no trajectory towards pre-fire levels was observed. These results were observed in terrestrial sites, similar to Blaauwberg, which showed the same trend as three of the four riparian sites, naming a significant increase in pH, which remained significantly higher than unburned areas, which suggest that persistently high soil pH after slash pile burning may be a feature in Mediterranean soils. The persistence in high soil pH may either be due to slow dissolution rates, i.e. slow release of base cations from the ash, which results in pH increase or to the denaturing of organic acids during burning (Bodi et al., 2014; Thomaz et al., 2014; Xue et al., 2014). The Wit River study area was the exception, where soil pH increased after burning and returned to pre-fire levels within four months after burning. The Wit River study area was exposed to flooding which could have washed away the surface deposited ash or leached the concentrated base cations through the soil profile, resulting in the speedy recovery of the soils (Tomkins et al., 1991).

The effects of flooding can also be seen with soil EC, where at Wit River soil EC remained unchanged immediately after burning. This supports the notion that base cations could have been washed away or leached through the soil profile. In contrast, at Robertson, Rawsonville and Hermon, soil EC increased immediately after fire, with an increase of more than 700 µS cm^{-1} at Hermon (*Eucalyptus*) to become significantly higher than both the pre-fire levels and the non-burn sites. Norouzi and Ramezanpour (2013) and Inbar et al. (2014) also observed an increase in soil EC as a result of burning. However, EC had returned to pre-fire and non-burn levels within a year after burning at all sites. Similar to the lack of a consistent response experienced at Bainskloof for pH, soil EC showed a trend entirely dissimilar to that of the other sites, suggesting that flooding overrided the effect of the fire.

The effects on EC almost mirror those on exchangeable cations. At both Hermon and Rawsonville cations increased immediately after burning and had returned to pre-fire and non-burn levels within a year. At Hermon, cation concentrations more than doubled as a result of burning of slash piles. Kennard and Gholz (2001) and Arocena and Opio (2003) reported a significant increase in exchangeable cations within soils that were exposed to burning of slash piles, which is similar to this study. Ando et al. (2014) observed an increase in K and Ca after burning of slash pile on sandy clay loam soils, mirroring an increase in EC. These cation-enriched soils eventually returned to their pre-fire levels as the surface deposited ash is leached or mobilised, and the topsoil leached (Bodi et al., 2014). The link between cations and soil EC can also be seen at Blaauwberg (terrestrial), where both these parameters (EC and cations) did not increase immediately after burning; instead, the effects were delayed and only appeared three months after burning. Burning here took place towards the end of the rainy season, and also, terrestrial soils may be less moist than riparian soils, as they are not closely linked to flowing streams (Naiman and Decamps, 1997; Naiman et al., 2005). Due to the lower soil moisture, base cations could have been released at slow rates from the enriched ash (Tomkins et al., 1991; Bodi et al., 2014).

Implications of cation-enriched soils include increased pH and EC (as observed), which may have an influence on soil microorganisms, leading to altered decomposition processes (Bodi et al., 2014).

Available P concentrations were affected by fire at all of the study areas. At Hermon, Rawsonville, Wit River and Blaauwberg the effects were observed immediately after burning, whilst at Roberson the effects were delayed. At Wit River the increase was within both the burn scar and the non-burn treatment areas. At Blaauwberg available P levels reached their peak in summer at 120.63 µg g^{-1} which was 15 times more than the non-burn sites. Within a year of sampling, the concentrations had returned to pre-fire levels or/and were similar to the non-burn treatment sites. Pourreza et al. (2014) also reported an increase in available P after severe burning on sandy clay loam soils. Ando et al. (2014) conducted a burning experiment involving slash piles in eastern Zambia on sandy loam soils and found that topsoil available P increased. Alcañiz et al. (2016) also reported an increase in available P after prescribed fire which returned to pre-fire level within a year. Within the fynbos, an increase in available P may be beneficial to native vegetation; however, such a large increase may be detrimental to some fynbos species which may experience P toxicity (Power et al., 2010). Yelenik et al. (2004) also suggested that some indigenous fynbos plants may be negatively affected by elevated levels of both N and P, which might instead be beneficial to invasive legumes such as *Acacia* species, which require P as part of the N-fixing mechanism (Vitousek et al., 2004). The rapid decline in P concentrations from summer to autumn may be due to uptake from microbes, since fire has the ability to transform soil P to microbial available P (i.e. orthophosphate, Certini, 2005), thus conversion to unavailable P, though leaching of finer soil particles from the topsoil cannot be excluded.

At all study areas available N was not immediately affected by burning of slash piles. Contrary to the findings of this study, available N has been reported to increase after fire (Oswald et al., 1998; Fernandez et al., 2009; Schafer and Mack, 2010), which has been attributed to increased N mineralisation resulting from fire-induced changes in soil temperature, soil pH and microbial activity (Wan et al., 2001). On occasion, available N (NH_4^+-N) has been shown not to significantly change on burned soils (e.g. Switzer et al., 2012). However, at Rawsonville (*Acacia*-invaded), within six months after burning, available N levels within the burn scar became higher than the non-burn treatment sites and continued to increase towards the end of sampling. This trend was also seen at Wit River (also *Acacia*-invaded), however the increase here started to occur with a year of sampling. This medium-term increase in available N might be due to increased N mineralisation resulting from increased pH and elevated soil temperatures during and immediately following burning of leguminous biomass (Wan et al., 2001), or may be related to the likely lower burn temperatures of *Acacia*-dominated slash piles. Naudé (2012) found higher available N in *Acacia*-invaded riparian areas, but no difference in N mineralisation rate, compared to natural riparian zones, suggesting modified N cycling in *Acacia*-invaded areas. *Eucalyptus*-invaded areas (Hermon and Robertson) did not show any clear trends in terms of plant available N, suggesting that burning of biomass may have different consequences for riparian soil biogeochemistry depending on which invader constitutes the bulk of the biomass. Even if N may be more available in patches affected by slash burning (e.g. *Acacia* affected riparian reaches), invasive seedlings may be able to outcompete native species for this resource, as suggested by Morris et al. (2011).

Fire did not change hydrophobicity in a major way, which contrasts with many other similar studies (e.g. Mirbabaei et al., 2013 and Jeyakumar et al., 2014). Fox et al. (2007)) observed a significant increase in hydrophobicity following fire; however, Inbar et al. (2014) found a lowering of hydrophobicity following fire. The general lack of a response to fire found in this study is likely due to the already hydrophobic or severely hydrophobic soils present at the sites before fire. The impact of fire on this aspect appeared negligible, except where hydrophobicity was low before the fire, as in Rawsonville.

The study aimed to answer two key questions (i) how will burning of slash piles of *Acacia* spp. and *Eucalyptus camaldulensis* biomass affect soil physicochemical properties? (ii) Secondly, what is the

trajectory followed by physicochemical properties subsequent to burning of biomass of *Acacia* spp. and *Eucalyptus camaldulensis*? The results show that there may be consistencies in how slash pile burning affects some soil physicochemical properties within fynbos. Parameters such as soil pH, EC, cations and available P showed almost similar trends across the study areas (riparian and terrestrial), where they increase after burning and return to pre-fire and/or to non-burn treatment site levels. This is with the exception of soil pH which remained persistently high for more than a year of sampling. The results also suggest that other parameters such as available N may not respond immediately to the effects of fire; instead addition factors (e.g. nitrification), may play a role in their responses. The results suggest that precipitation and soil moisture are essential for the recovery of soil after burning; this was seen at Wit River, where flooding might have led to a speedy recovery of soil pH and no effects on soil EC. The effects of soil moisture were also seen at Blaauwberg where both soil EC and cations had a delayed response to the effects of burning, indicating that in low moisture soils, the release of base cations from enriched ash may be slow.

Our findings suggest that burning of slash piles has the effect of changing acidic, nutrient-poor fynbos soils (Rebelo et al., 2006) into high pH and nutrient-enriched soils, though the effect is only temporary for nutrients. This may influence the ability of the burned soils to establish and support fynbos vegetation, and thus affect ecosystem rehabilitation processes. Considering the large amount of funding spent on restoring ecosystem services to invaded riparian environments, these results suggest that appropriate outcomes may be hampered by altered soil conditions that may favour secondary invasion, a lag in regeneration, or discourage regeneration of native species. Indeed, anecdotal evidence exists that some scars remain in riparian landscapes long after slash piles have been burned, while evidence from North American forests suggests that terrestrial sites exposed to burning of slash piles may remain low in vegetation cover even decades after the burn event (Korb et al., 2004). This suggests that some trade-off may exist where careful consideration should be given to the effect of fire on soil ecology against clearing of IAPs and subsequent burning of slash piles in riparian and terrestrial areas, though more evidence from terrestrial sites is needed.

CHAPTER 5: The impact of slash and burn of *Eucalyptus camaldulensis* and *Acacia mearnsii* biomass on soil microbial diversity and on selected beneficial groups of microbes *in situ*

5.1 INTRODUCTION

Infrequent fires are essential for the germination of indigenous fynbos species (Keeley and Bond, 1997; van Wilgen and Richardson, 1985). High temperatures and dry heat enhance the desiccation of seed coats and like other Mediterranean environments, smoke as well as heat promotes the growth of embryos (Brown, 1993; Keeley and Fotheringham, 2000). It has been shown that the presence of large tracts of invasive species such as *Acacia* and *Eucalyptus* spp. leads to fires at higher frequency and with higher intensity, which may affect subsequent succession of fynbos (Holmes and Cowling, 1997). While some indigenous fynbos species may re-sprout or geminate after fire, these are quickly outcompeted by invasive seedlings in the post-fire environment. Holmes and Cowling (1997) found that two fire cycles in an *Acacia*-invaded terrestrial area led to a drastic decrease (~70%) in indigenous fynbos species.

Soil microbial communities are an important factor influencing regeneration of native and invasive species after fire in fynbos ecosystems. The soil ecosystem is a complex and dynamic biological system and offers a variety of microhabitats for prokaryotic and eukaryotic microorganisms, including bacterial and fungal communities (Bååth and Arnebrant, 1994; Fierer et al., 2006;. Nannipieri et al., 2003;. Prober et al., 2015). There is a close link between available nutrients in soil and microbial communities. Microbes play an important role in all known biological reactions (Craine et al., 2010; Jha et al., 1992). At the base of soil ecology are microbial communities that assist in the decomposition of organic matter in the soil, leading to CO_2 emissions, which are greatly modified with changes in land-use that affect soil microbial organisms (Dooley and Treseder, 2012). On the other hand, the stability of microbial communities is dependent on availability of nutrients in the soil. The nutrient availability, in turn, is influenced by the transformations mediated by microbial biomass (Fierer et al., 2006; Hu et al*.,* 1999). The cycling of nutrients, which are retained by soil organic matter (SOM), functions to maintain soil structure that contains water for plant use and helps microbial community structures to recover more rapidly after a disturbance (Alexrood et al., 2002; Willey et al., 2008). Therefore, it is necessary to investigate the effect of environmental characteristics, plant invasion and fire on soil microbial communities.

Soil microbial communities are affected by various environmental characteristics (Buckley and Schmidt, 2001). Nonetheless, they have the capacity to adapt and survive across a wide range of physicochemical properties in soil (Robertson et al., 1997; Willey et al., 2008). It is crucial to note that soil microbial communities follow different trends in different environmental conditions. For example, the bacteria beta-*Proteobacteria* and *Bacteriodetes* spp. are dominant in carbon-rich environments (e.g. soils with high organic matter content) and their growth rate is relatively high in non-limiting soil environments, whereas *Acidobacteria* spp. are dominant in nutrient-poor environments and their abundance and growth rate decrease with increased concentration of C (Fierer et al., 2007; Hu et al., 1999). In addition, there are also some species such as alpha-*Proteobacteria*, *Firmicutes* and *Actinobacteria* spp. that remain steady in response to varied C availability in soil (Fierer et al., 2007). Moreover, it is important to note that environmental changes may lead to a variation in microbial diversity. For example, neutral to basic soils may be associated with higher bacterial diversity, whereas more acidic soils may be associated with lower bacterial diversity.

Information is lacking regarding the impact of invasive alien trees on terrestrial and riparian soil processes and microbial dynamics in South Africa. Slabbert et al. (2014) found that *Acacia mearnsii*

alters microbial community composition and diversity whereas soil fungi appear to be more resistant to changes following alien invasion in fynbos. These changes are influenced by IAP-mediated alteration to soil nutrient and other chemical properties (e.g. pH and available P). Soil pH and available P are also altered by fire. Soil microbial community structure is altered significantly by fire (Dooley and Treseder 2012; Ferrenberg et al., 2013; Neary et al., 1995; Reazin et al., 2016). The greatest alteration usually takes place in the top two centimetres of the soil (Dooley and Treseder, 2012; Sun et al., 2008) where the microbial abundance is highest (Hart et al., 2005). Burning of leaf litter enhances the shift in microbial structure and dynamics (Dooley and Treseder, 2012; van Wilgen and Richardson, 1985) usually resulting in mortality of microbes (González-Pérez et al., 2004; Hart et al., 2005), which alters the structure of soil communities post fire and results in an altered community structure compared to unburned areas (Hamman et al., 2007).

Tolerance to fire differs among fungi and bacteria; in general, fungi have been found to be more sensitive to fire than bacteria (Bárcenas-Moreno and Bååth, 2009; González-Pérez et al*.,* 2004; Smith et al*.,* 2008). The ability and capacity of bacteria to recover from the effect of heat may be the result of their ability to form spores which can withstand high temperatures (Murrell and Scott, 1966), in contrast to fungi where only a few species form heat-tolerant spores (Bárcenas-Moreno and Bååth, 2009). Other factors such as soil pH, phosphorus and water content also influence survival of fungi and bacteria post fire (Shen et al., 2016; Smith et al., 2008; Sun et al., 2016). Studies by Bååth and Arnebrant (1994), Bárcenas-Moreno and Bååth (2009) and Dooley and Treseder (2012) suggest that soil bacterial diversity is more likely to increase following an increase in soil pH post fire, whereas the diversity of soil fungi showed minimal variation.

Fire intensity also plays an appreciable role in the shift in soil microbial community structure (Reazin et al., 2016; Xiang et al., 2014). A high-intensity fire, which typically has a longer duration than a low-intensity fire, has a greater effect on soil fungal communities in comparison to a low-intensity fire (Reazin et al., 2016). In contrast, Xiang et al. (2014) observed that the relative abundance of alpha-*Proteobacteria*, *Acidobacteria*, *Plantobacteria* and delta-*Proteobacteria* spp. decreases following both low and high fire intensity, whereas relative abundance of *Bacteriodetes* and beta-*Proteobacteria* spp. seemed to increase following low and high intensity fires. The impact of fire on microbial communities seems to persist after fire. Two recent studies found that microbial diversity post fire remained relatively low for up to two years in comparison to a control (Sun et al., 2016; Xiang et al., 2014).

Invasive species in many parts of the Cape Floristic Region are removed by means of mechanical management strategies for commercial purposes such as timber. However, the remaining bark, branches, leaves, stems and twigs of these invasive species (dead biomass), after the mechanical removal of invasive species, may become a major problem. This is due to the dead plant material that might be able to ignite under the right conditions and suitable fuel content, while inhibiting the natural recovery of native plant communities in cleared areas. In some cases, removal of any biomass may be challenging due to inaccessibility. Up to 710 km^2 of *Acacia*-invaded areas have been treated and are current standing on 90 km^2 condensed areas, whereas ~67 km^2 *Eucalyptus*-invaded areas have been treated and 20 km^2 condensed areas still remain (Marais and Wannenburgh, 2008). One efficient management strategy to remove the remaining plant material of *Acacia* and *Eucalyptus* spp. is the ‘slash and burn’ method, where material is stacked and fire is used to reduce the dead biomass.

The effect of fire on soil microbial communities differs among ecosystems and fire types (Dooley and Treseder, 2012). Riparian zones are not typically exposed to fire, thus removal of IAP biomass using fire may have significant and negative impacts on soil microbial dynamics. In some instances, the intensity of the fire will leave a scar, where the vegetation does not recover. In this study, emphasis was placed on the effect of burning of slash piles of invasive biomass on microbial diversity and community structure in riparian zones of fynbos soil. No previous studies that have been identified have focussed on the

effect of burning of slash piles of invasive biomass on soil microbial composition in fynbos or in riparian zones. The objectives of the study were as follows:

1. Determine the relative microbial richness of each site where burning of slash piles of *Acacia* and *Eucalyptus* biomass took place, by means of ARISA, at three stages, namely pre-fire, post fire and a year after the burn event.

2. Compare the microbial diversity of all three sample collections to one another to evaluate the impact of burning of slash piles of *Acacia* and *Eucalyptus* biomass. This study also includes their association towards the soil chemical properties.

3. Determine the dissimilarity of microbial community structure within and between invasion sites, respectively.

4. Observe the spatial distribution patterns of microbial communities in riparian zones of different fynbos regions affected by burning of slash piles of *Acacia* and *Eucalyptus* biomass.

5.2 MATERIALS AND METHODS

5.2.1 Site description

The sites chosen for this study were fynbos vegetation (Western Cape, South Africa) invaded by *Acacia mearnsii* or *Eucalyptus camaldulensis*, situated next to rivers (riparian zones). Four sites were chosen, which had different invasion statuses (Table 5.1). The Bainskloof site was in the riparian zone of the Wit River. It has been invaded predominantly by *A. mearnsii.* The Rawsonville site was on the Bree River and has been predominantly invaded by *A. mearnsii* with a small percentage cover of *Eucalyptus camaldulensis*. The Robertson site was also on the Bree River. It consisted predominantly of *E. camaldulensis.* The Wellington site was on the Berg River and has been predominantly invaded by *E. camaldulensis* with a small percentage of *Acacia* spp. cover

5.2.2 Sample collection

Sites were selected in areas where cleared invasive plant material was stacked (piled). Three piles were collected from the Wellington site, of which two piles were located next to the invasive biomass and the other pile was surrounded by farmland. Five piles were collected from the Rawsonville and Robertson sites, respectively. The piles were divided into three sections from which soil samples were taken, i.e. the centre, intermediate and off-pile sampling points. The centre samples were collected from soil in the middle of the piles, while the intermediate samples were collected from the area between the centre and edge of the pile. These two sample points were representative of the burned piles, whereas the off-pile soil samples that were collected from outside the piles (~2 m away) served as controls. Each section had three replicates of which the microbial richness, diversity and community structure within a specific pile were determined.

Fresh soil samples from each pile were collected at three different time periods. These time periods were pre-fire, post fire and a year after the burn event. Pre-fire samples were taken in June-July 2015 from undisturbed soil samples before the piles were assembled. Thereafter, each pile was built from *Eucalyptus* or *Acacia* woody biomass, depending on the particular site. After the piles were assembled and burned, post-fire samples were collected in August-September 2015. The last sampling session took place one year after the first sampling.

Table 5.1: Study sites along with dominant invasive species and soil description.

Site	River	Co-ordinates	Dominant invasive species
Wellington	Berg River	-33˚ 64' S, 18˚ 96' E	*E. camaldulensis*
Rawsonville	Bree River	-33˚ 54' S, 19˚ 16' E	*A. mearnsii*
Robertson	Bree River	-33˚ 72' S, 19˚ 47' E	*E. camaldulensis*
Bainskloof	Wit River	-33˚ 84' S, 19˚ 87' E	*A. mearnsii*

5.2.3 Soil analysis

Up to 500 g of the surface soil (0–10 m) was collected with a handheld spade. Soil samples were air dried on paper bags by spreading out each sample in a thin layer. Thereafter, the samples were sieved through a 2 mm sieve filter to remove larger particles and sent to the Soil Science department at the University of Stellenbosch for analyses which included soil pH, electrical conductivity (EC), ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}).

5.2.4 Molecular characterisation

Soil microbial DNA extractions were performed within 24 hours of sampling. For DNA extraction a soil microbe DNA kit was used (Zymo Research USA). Two hundred and fifty milligrams of soil were used for DNA extraction according to the manufacturer's instructions. One microliter of the purified DNA was loaded onto 1% (w/v) agarose gel stained with ethidium bromide (0.5 µg/ml), for separation in 1 X TAE buffer. Gel electrophoresis was performed at 80 V for 40 minutes. The gel was stained with ethidium bromide and visualised under ultraviolet light.

5.2.4.1 PCR amplification and primers

Purified DNA samples were prepared in triplicate for polymerase chain reaction (PCR). The PCR mixture (10 µl) contained 2 µl of DNA, 0.2 µl of each 500 nM primer, 2.6 µl of Milli-Q water and 5 µl of 2 X Kapa Taq ready mix (KapaBiosystems, South Africa). The thermal cycling reaction conditions for bacterial and fungal automated ribosomal spacer intergenic spacer (ARISA) are summarised in Table 5.2 and Table 5.3. The primers used for bacterial and fungal ARISA are listed in Table 5.4. The GeneAmp PCR system 9700 was used to perform PCR reactions. Two microlitres of the purified DNA were loaded onto 1% (w/v) agarose gel stained with ethidium bromide (0.5 µg/ml), for separation in 1 X TAE buffer. Gel electrophoresis was performed at 80 V for 40 minutes. The gel was stained with ethidium bromide and visualised under ultraviolet light. Triplicate PCR samples were pooled and sent to the DNA Sequencing Facility at the Central Analytical Facility at Stellenbosch University for capillary electrophoresis.

Table 5.2: The thermal cycling reaction conditions of PCR for bacterial ARISA.

Steps	**Temperature**	**Time**	**Cycles**
Initial denaturation	95°C	5 min	1
Denaturation	95°C	45 s	
Annealing	56°C	50 s	34
Extension	72°C	70 s	
Final extension	72°C	7 min	1
	4°C	Hold	

5.2.4.2 Automated ribosomal intergenic spacer analysis (ARISA)

ARISA was used to characterise the microbial community structure. The fluorescent data was examined by the use of ABI 3010xl Genetic analyser. The amplicons of ARISA were separated by means of capillary electrophoresis according to different fragment size and fluorescent intensity. GeneMapper 5.0 software was used for the conversion of electropherogram data of the separated components. LIZZ 1200 size standard was used for bacterial and fungal ARISA with base pair sizes between 100-1000bp in length. Peak heights were analysed to minimize inaccuracy in contrast to peak sizes that are influenced by the variation in an area. The bin size for ARISA profiles was 3 bp, as it shared the highest number of peaks in correlation to other bin sizes ranging from 1 to 7 bp (Slabbert et al., 2010). For further analysis, the data retrieved from GeneMapper 5.0 software was transferred to Microsoft Excel (Microsoft Corporation).

Table 5.3: The thermal cycling reaction conditions of PCR for fungal ARISA.

Steps	**Temperature**	**Time**	**Cycles**
Initial denaturation	94°C	5 min	1
Denaturation	94°C	30 s	
Annealing	54°C	45 s	36
Extension	72°C	50 s	

Final extension	72°C	7 min	1
	4°C	Hold	

Table 5.4: The primers used in this study.

ARISA	**Primer (F/R)**	**Oligonucleotide sequence**
Bacteria	ITSF*[a] (forward)	5'-GCCAAGGCATCCACC-3'
	ITSReub[a] (reverse)	5'-GTCGTAACAAGGTAGCCGTA-3'
Fungi	ITS5*[b] (forward)	5'-GGAAGTAAAAGTCGTAACAAGG-3'
	ITS4[b] (reverse)	5'-TCCTCCGCTTATTGATATGC-3'

* - 5' end-labelled FAM fluorescent dye (carboxyl-fluorescein)

a - from Cardinale et al. (2004)

b - from Slemmons et al. (2013) and White et al. (1990)

5.2.5 Data analysis

To investigate the effect of burning of slash piles of invasive biomass (*Acacia and Eucalyptus camaldulensis*) on soil microbial (bacterial and fungal) communities in riparian zones of fynbos, the data was collated according to the study sites allocated in Table 5.1. The different fragment sizes (also interpreted as peaks) generated for ARISA profiles were normalised by applying a threshold to exclude background fluorescent data (Navarrete et al., 2010). The threshold excluded peak heights that were lower than 0.5% of the total fluorescent data of each sample (Slabbert et al., 2010). The peaks obtained in the analysis are represented as theoretical operational taxonomic units (OTUs). Accordingly, the average microbial OTUs of each pile for all three sample collections were determined to calculate the Shannon diversity (*H'*) and Simpson complement (1-*D*) indices.

Shannon's diversity (*H'*) and Simpson complement (1-*D*) indices were calculated to compare the microbial diversity pre-fire, post fire and a year after the burn event. The *H'* index (Equation 5.1) uses the proportional abundance (p_i) to describe the evenness and species abundance of each sample. Whereas the 1-*D* index (Equation 5.2) uses p_i to quantify the abundance of dominant species, by taking the number of species present per sample into account. As a result, a higher *H'* score reflects a greater species diversity, while a higher 1-*D* score increases the chance that two species from different samples represent the same species. In the equation, the p_i is defined as the fraction of each different fragment size (peak) of the total relative proportion peaks per sample.

Shannon's diversity is defined by:

$$H' = -\sum_{i=1}^{n} p_i \log(p_i)$$

Equation 5.1

The Simpson's complement index is determined by:

$$1 - D = 1 - \sum_{i=1}^{n} (p_i)^2$$

Equation 5.2

The data matrices, constructed using the Whittaker similarity (S_w) index, were used for bacterial and fungal ARISA profiles to estimate the dissimilarity coefficients of bacterial and fungal communities within the respective sites for all three sampling sessions. The S_w index units ranged from 0 to 1, with 0 indicating the level of dissimilarity and 1 indicating a completely identical association. The b_{i1} and b_{i2} variables compare the fraction of different fragment sizes of two distinct samples.

The Whittaker similarity index was calculated by:

$$S_w = 1 - \sum_{i=1}^{n} \frac{|b_{i1} - b_{i2}|}{2}$$

Equation 5.3.

The microbial diversity (*H'*) of all the samples within a specific site was compared to evaluate whether the microbial diversity was similar in all the piles, for all three sample collections. A one-way ANOVA was used to conduct these comparisons. The 1-*D* index was used to confirm the results.

In order to assess the effect of fire on the microbial diversity, the control was compared to the burned samples. A factorial ANOVA test was used to analyse this comparison and the post-hoc Tukey Honest Significant difference (HSD) test was used to confirm the ANOVA results (Statistica version 13 – Statsoft). The variation within and between invasion sites was analysed in the same manner.

The Pearson correlation coefficient (PCC) analysis and principle component analysis (PCA) were used to compare and measure the level of association between the microbial diversity (*H'*) and community structure, respectively, and the soil chemical properties. The correlation coefficient (*r*) units from PCC analysis indicate the strength of linear regression which ranged from +1 to -1, with +1 implying a strong positive correlation, 0 implying no correlation and -1 implying a strong negative correlation between two variables. Furthermore, the PCA was used to illustrate any graphical separations of microbial community by means of Bray-Curtis distance, to determine which soil chemical properties contributed to the variation of microbial community structure after the burning of slash piles.

To illustrate the composition of the microbial community pre-fire, post fire and a year after the burn event in the invaded sites, cluster analysis was performed to analyse the distance relationship of similarity (as measured by *Sw* index) for all three sample collections (Slabbert et al*.,* 2010). A complete linkage clustering was conducted with Statistica ver. 13 (Stat soft) by means of 1-Pearson r distance correlation.

The Bray-Curtis similarity index was applied to assess the strength of dissimilarity (R-value) of the microbial community structure by using Analysis of Similarity (ANOSIM) (Slabbert et al., 2014). The Bray-Curtis index registered the presence of peaks as 1 and the absence of peaks as 0. The R-value of the ANOSIM comparison was calculated using R 3.2.2 software. The results of the R-value and the Bray-Curtis distance ranged from 0 to 1 which are opposite in meaning compared to the *Sw* index. This means that 0 indicates the level of similarity and 1 indicates a complete dissimilarity association.

To observe the spatial distribution patterns of microbial communities, NMDS was performed to analyse the microbial community distance matrices of all three sample collections within and between invaded areas. A Scree test for every NMDS analysis was performed to calculate the number of dimensional scaling. The stress value of each NMDS plot was accepted, if the stress value remained < 0.10.

5.3 RESULTS

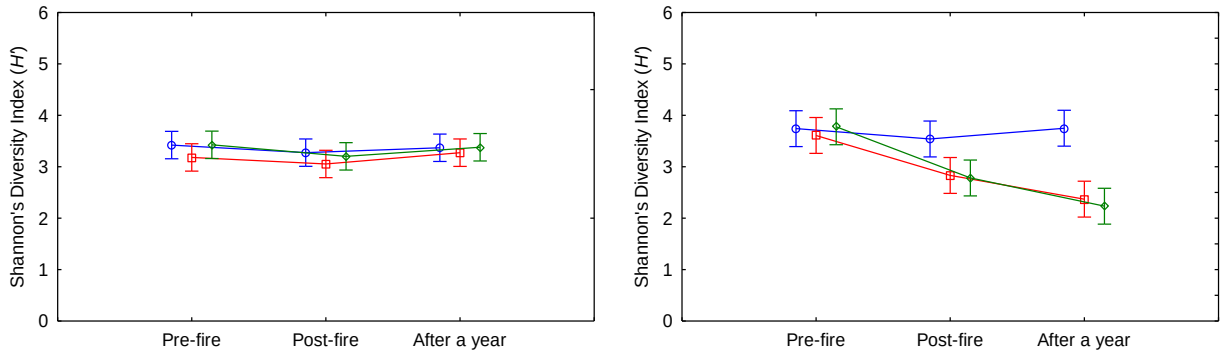
5.3.1 Bacterial diversity pre-fire

The bacterial richness and diversity of each study site is presented in Table 5.5. All the sites indicated that the bacterial diversity (*H'*) pre-fire was similar (not significantly different) in all the soil samples, apart from the Wellington site. The bacterial diversity (*H'*) in the soil samples of pile 3 from the Wellington site is significantly lower in comparison to piles 1 and 2 with $p = 0.003$ and $p = 0.011$, respectively. Based on this finding, pile 3 was excluded from further analysis.

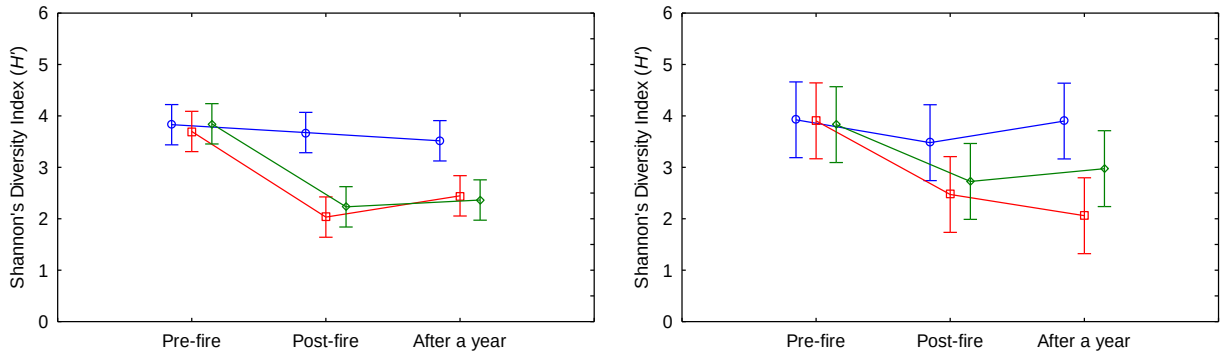
The bacterial diversity (*H'*) in the soil samples underneath the piles pre-fire ranged from 3.30 to 3.77. Robertson obtained the highest diversity score (*H*' = 3.77), followed by Wellington (*H*' = 3.73), Rawsonville (*H*' = 3.69) and then Bainskloof (*H*' = 3.30) (Table 5.5). The bacterial diversity at different sampling points (control, centre and intermediate samples), in respective sites, showed no significant difference pre-fire.

5.3.2 Effect of burning of slash piles of invasive biomass on bacterial diversity

The bacterial diversity (*H'*) in the soil samples underneath the piles showed two different trends post fire (Table 5.5). In the Bainskloof site, the bacterial diversity remained similar post fire (from *H'* = 3.30 to 3.13) and a year after the burn event (*H'* = 3.33). The Rawsonville, Robertson and Wellington sites showed a decrease in bacterial diversity (*H'*) post fire which remained similar a year after the burn event. It was apparent that the bacterial diversity (*H'*) in the centre and intermediate samples was similar for all the sites throughout the study (Figure 5.1). This observation was also found by the Simpson's complement (1-*D*) index (data not shown). Based on these findings, the centre and intermediate samples were pooled for further analysis and represent the burned samples



(a) (b)



(c) (d)

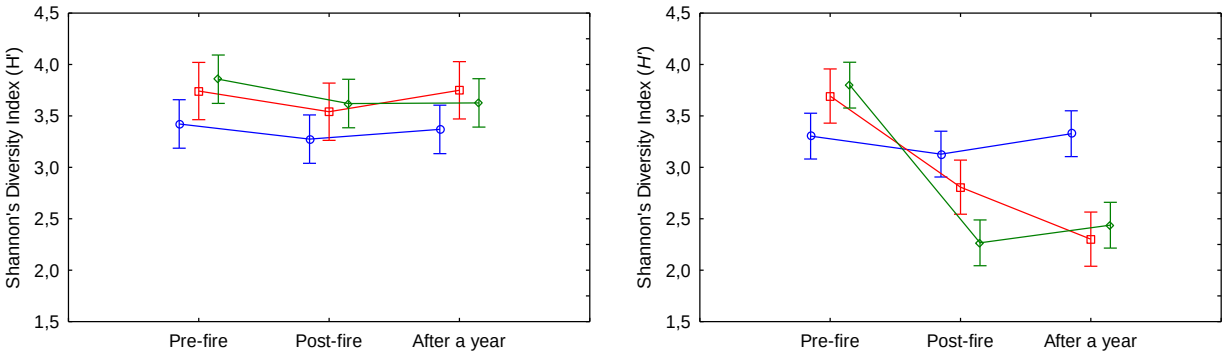
Figure 5.1: The effect of burning of slash piles of invasive biomass on bacterial diversity (H') in the four study sites. The blue represents the bacterial diversity of the control, whereas the red (centre) and green (intermediate) represent the bacterial diversity in the soil samples underneath the piles. Significant results are indicated with a (*): Bainskloof (p=0.972), Rawsonville*, (p=0.001), Robertson* (p=0.002), Wellington (p=0.178)

Table 5.5: The bacterial richness and diversity of the four sites at all three sample times. The diversity was determined by means of Shannon's (*H'*) and Simpson's complement (1-*D*) indices. The standard deviation is indicated in brackets.

	Pre-fire		**Post fire**		**A year after burn event**	
	Off-Pile	**Burned Area**	**Off-Pile**	**Burned Area**	**Off-Pile**	**Burned Area**
Bainskloof						
Richness	46.4 (6.63)	41.7 (7.35)	41.4 (12.3)	36.5 (9.84)	42.0 (3.76)	38.6 (7.76)
H'	3.42 (0.28)	3.30 (0.29)	3.28 (0.56)	3.13 (0.44)	3.37 (0.19)	3.33 (0.25)
1-*D*	0.94 (0.03)	0.94 (0.03)	0.93 (0.05)	0.92 (0.06)	0.94 (0.02)	0.95 (0.02)
Rawsonville						
Richness	50.8 (3.66)	51.2 (6.78)	46.4 (5.46)	29.2 (8.00)	53.0 (3.46)	19.2 (11.9)
H'	3.74 (0.14)	3.69 (0.26)	3.54 (0.27)	2.81 (0.33)	3.75 (0.09)	2.30 (0.62)
1-*D*	0.97 (0.01)	0.97 (0.02)	0.96 (0.02)	0.90 (0.03)	0.97 (0.01)	0.84 (0.09)
Robertson						
Richness	58.4 (3.45)	53.1 (5.80)	49.4 (7.30)	16.3 (7.68)	49.0 (10.6)	23.3 (13.2)
H'	3.83 (0.19)	3.77 (0.18)	3.68 (0.26)	2.13 (0.43)	3.52 (0.42)	2.41 (0.65)
1-*D*	0.97 (0.01)	0.97 (0.01)	0.97 (0.01)	0.83 (0.06)	0.95 (0.04)	0.84 (0.07)
Wellington						
Richness	55.7 (8.39)	50.8 (9.91)	45.0 (10.6)	30.7 (12.5)	57.3 (5.86)	22.7 (16.0)
H'	3.79 (0.23)	3.73 (0.25)	3.27 (0.52)	2.91 (0.55)	3.84 (0.15)	2.33 (0.72)
1-*D*	0.97 (0.01)	0.97 (0.01)	0.91 (0.06)	0.91 (0.06)	0.97 (0.01)	0.84 (0.07)

5.3.3 Variation in bacterial diversity between invasion sites

The *Eucalyptus* sites were compared to the *Acacia* sites to evaluate whether a significant difference exists. The bacterial diversity (*H'*) in *Eucalyptus* sites pre-fire was higher than in the *Acacia* sites; however, no significant difference was obtained (Figure 5.2). After the burning of slash piles of invasive biomass, the bacterial diversity (*H'*) in the soil samples underneath the piles (burned areas) in the *Eucalyptus* and Rawsonville sites followed a similar trend, and no significant difference was recorded between these two sites (Figure 5.4). This finding was in contrast to the comparison between the *Eucalyptus* and Bainskloof sites. The Tukey HSD test of this comparison recorded a significant difference post fire and a year after the burn event (p <0.001), respectively.



(a) Off-Pile (p= 0.881) (b) Burned Area * (p < 0.001)

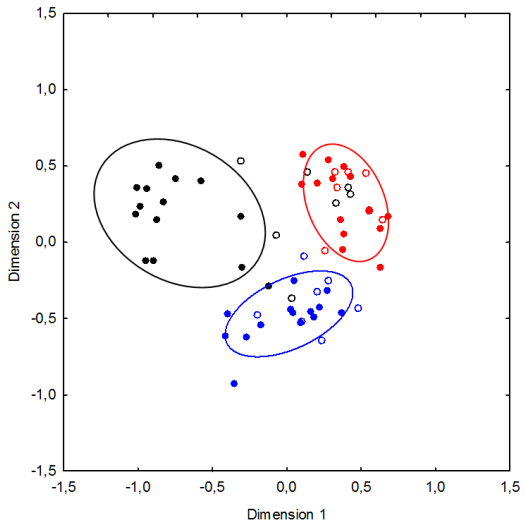
Figure 5.2: The effect of burning of slash piles of *Acacia* biomass on bacterial diversity (H') compared to the effect of burning of slash piles of *Eucalyptus* biomass on bacterial diversity (H'). Blue indicates the bacterial diversity in Bainskloof, red in Rawsonville and green in *Eucalyptus* sites. Significant results are indicated with a (*).

5.3.4 Bacterial community structure

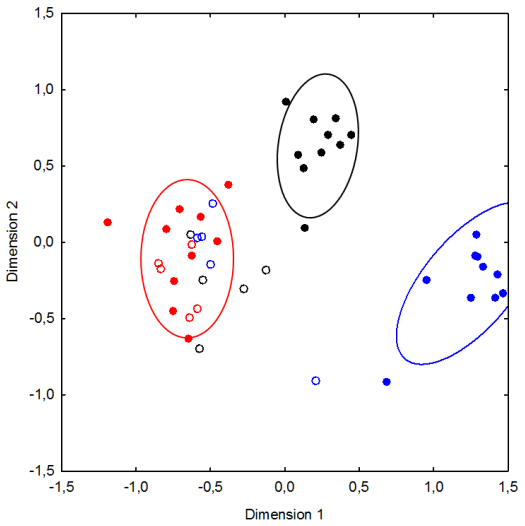
Three distinct bacterial communities (separated by different sample times) are expected in the soil samples underneath the piles for each respective site based on the ANOSIM results in Table 5.6. The NMDS plots are illustrated in Figure 5.3.

Table 5.6: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) was used to compare the bacterial communities in the soil samples underneath the piles of all three sample times in respective sites. The p-values are indicated in brackets.

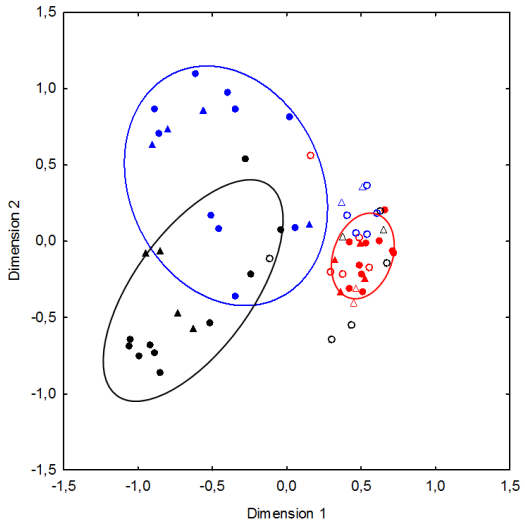
Sites	**Pre-fire vs. post fire**	**Pre-fire vs. after a year**	**Post fire vs. after a year**
Bainskloof	0.799 (0.001)	0.640 (0.001)	0.799 (0.002)
Rawsonville	0.988 (0.006)	1.000 (0.007)	0.954 (0.002)
Robertson	0.717 (0.001)	0.615 (0.001)	0.427 (0.002)
Wellington	1.000 (0.034)	0.750 (0.028)	0.688 (0.025)



(a) (nine dimensions, stress = 0.069)



(b) (four dimensions, stress = 0.093)

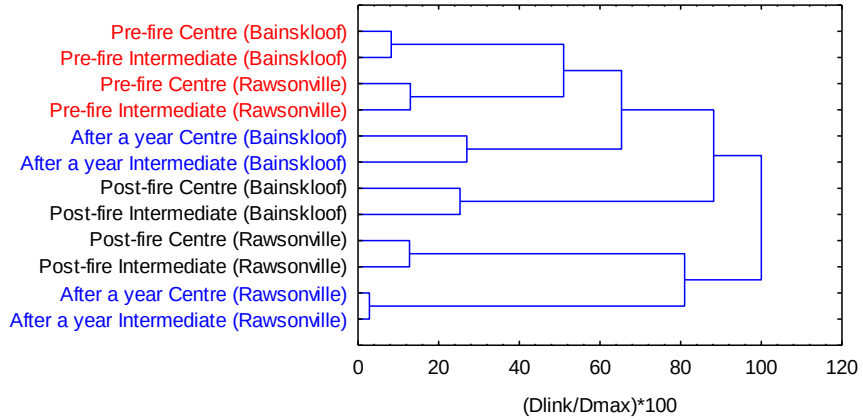


(c) (six dimensions, stress = 0.094)

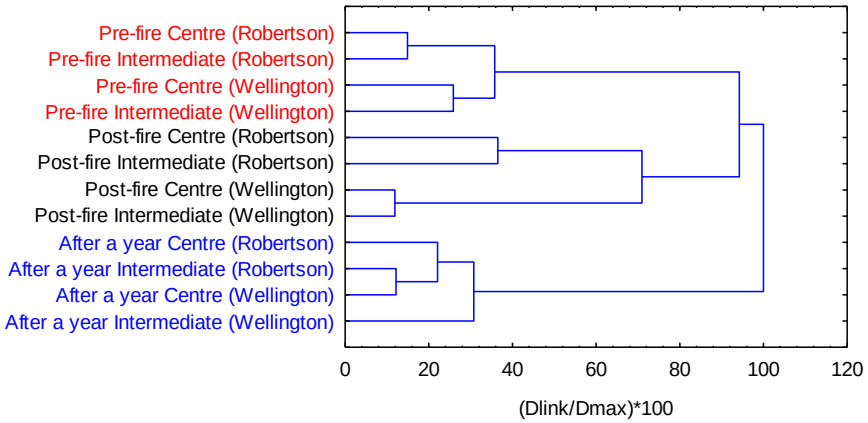
Figure 5.3: Non-metric multidimensional scaling (NMDS) of bacterial community structure of the control (empty circles) and the soil samples underneath the piles (filled circles) for three sample times in respective sites. Red represents the bacterial community structure pre-fire, black represents post fire and blue represents a year after the burn event. For the *Eucalyptus* sites, the bacterial community structure of soil samples from Robertson is indicated as circles and from the Wellington site is indicated as triangles. Ellipses denote 75% confidence levels. (a) Bainskloof, (b) Rawsonville, (c) *Eucalyptus* sites

5.3.5 Variation of bacterial community structure within invasion sites

The cluster analysis in Figure 5.4 illustrates that the bacterial communities within invasion sites pre-fire are site specific. The bacterial communities in Bainskloof and Rawsonville followed two different trends after the burning of slash piles of *Acacia* biomass. The bacterial communities in Bainskloof and Rawsonville were different throughout the study (Table 5.7). Based on these findings, Bainskloof and Rawsonville were separated for further analysis. As for the bacterial communities in Robertson and Wellington, a similar trend was observed after the burning of slash piles of *Eucalyptus* biomass (Figure 5.4b). Table 5.7 showed that Robertson and Wellington shared similar bacterial communities throughout the study. Based on these findings, Robertson and Wellington were grouped for further analysis.



(a)



(b)

Figure 5.4: The Whittaker similarity index along with the cluster analysis was used to compare the bacterial communities of all three sample times in the respective invasive sites. (a) *Acacia*, (b) *Eucalyptus camaldulensis*

Table 5.7: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) was used to compare the bacterial communities in the soil samples underneath the piles at all three sample times within invasion sites. The p-values are indicated in brackets.

Invasive sites	**Pre-fire**	**Post fire**	**After a year**
Bainskloof compared to Rawsonville	0.240 (0.001)	0.786 (0.001)	0.898 (0.001)
Robertson compared to Wellington	0.071 (0.323)	0.212 (0.132)	0.120 (0.738)

5.3.6 Variation in bacterial community structure between invasion sites

When the bacterial community structure of each site was compared to one another, three distinct groupings were displayed pre-fire (Figure 5.5a). The *Eucalyptus* and Rawsonville sites showed an R = 0.565 and R = 0.240, respectively compared to the Bainskloof site pre-fire, while an R = 0.240 existed between these sites (Table 5.8). Post fire and a year after the burn event, the soil samples underneath the piles (burned areas) presented two distinct bacterial community structures (Figure 5.5b). One grouping presented the bacterial community structure of the Bainskloof site and the other grouping was a combination of the *Eucalyptus* and Rawsonville sites. The ANOSIM confirmed a similarity of bacterial community structure between the *Eucalyptus* and Rawsonville sites post fire and a year after the burn event (Table 5.8).

Table 5.8: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) was used to compare the bacterial communities in the soil samples underneath the piles at all three sample times between invasion sites. The p-values are indicated in brackets.

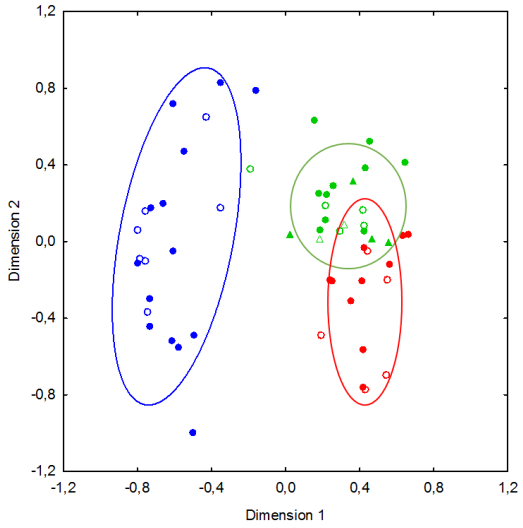
Invasive sites	**Pre-fire**	**Post fire**	**After a year**
Bainskloof compared to *Eucalyptus* sites	0.565 (0.001)	0.734 (0.001)	0.626 (0.001)
Rawsonville compared to *Eucalyptus* sites	0.240 (0.002)	0.046 (0.227)	0.051 (0.188)

5.3.7 Fungal diversity pre-fire

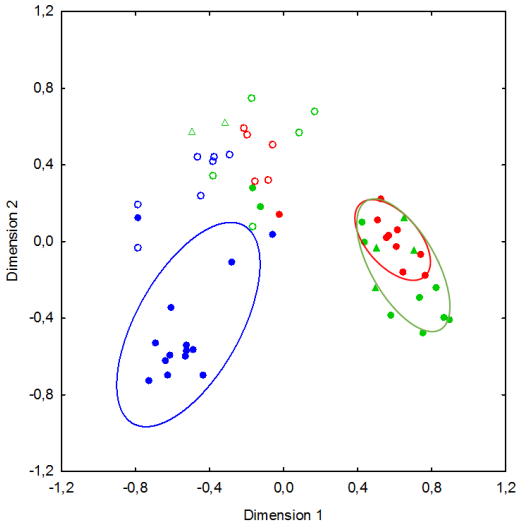
Table 5.9 summarises the fungal richness and diversity of all the sites. The fungal diversity (*H'*) in the soil samples from Rawsonville and Robertson recorded no significant difference when all the soil samples in the respective sites, pre-fire, were compared to one another. As for the Bainskloof and Wellington sites, the post-hoc (Tukey HSD) test indicated that the fungal diversity (*H'*) in the soil samples of one pile was significantly lower in comparison to the other piles for both sites. Based on this observation, these piles were excluded from further analysis.

The fungal diversity (*H'*) in the soil samples underneath the piles pre-fire ranged from 2.52 to 2.71 (Table 5.9). Wellington obtained the highest diversity score (H' = 2.71), followed by Bainskloof (H' = 2.67), Rawsonville (H' = 2.65) and then Robertson (H' = 2.52). No significant difference was observed from

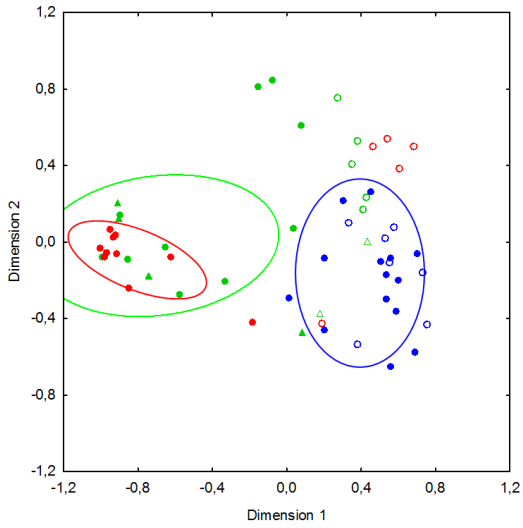
one-way ANOVA when the fungal diversity at different sampling points (control, centre and intermediate), in respective study sites, was compared.



(a) (nine dimensional, stress = 0.064)



(b) (nine dimensional, stress = 0.059)



(c) (nine dimensional, stress = 0.059)

Figure 5.5: Non-metric multidimensional scaling (NMDS) of bacterial community structure of the control (empty circles) and the soil samples underneath the piles (filled circles) of (a) pre-fire, (b) post fire and (c) a year after the burn event sample times. The blue indicates the community structure in Bainskloof, red in Rawsonville and green in *Eucalyptus* sites. For the *Eucalyptus* sites, Robertson was indicated as circles and Wellington was indicated as triangles. Ellipses denote 75% confidence levels.

Table 5.9: The fungal richness and diversity of four sites at all three sample times. The diversity was determined by means of Shannon's (*H'*) and Simpson's complement indices (1-*D*). The standard deviation is indicated in brackets.

	Pre-fire		**Post fire**		**A year after burn event**	
	Control	Underneath the piles	Control	Underneath the piles	Control	Underneath the piles
Bainskloof						
Richness	25.7 (4.96)	25.8 (3.26)	28.0 (7.12)	28.2 (7.70)	21.6 (4.47)	25.2 (4.85)
H'	2.68 (0.27)	2.67 (0.23)	2.86 (0.43)	2.77 (0.69)	2.44 (0.32)	2.78 (0.30)
1-*D*	0.89 (0.04)	0.89 (0.04)	0.91 (0.07)	0.87 (0.14)	0.85 (0.07)	0.90 (0.04)
Rawsonville						
Richness	29.0 (1.58)	26.2 (6.65)	27.0 (4.85)	15.2 (11.8)	29.6 (6.22)	28.1 (5.36)
H'	2.96 (0.18)	2.65 (0.44)	2.57 (0.79)	2.28 (0.99)	2.79 (0.35)	2.68 (0.34)
1-*D*	0.93 (0.02)	0.88 (0.07)	0.81 (0.23)	0.83 (0.18)	0.89 (0.05)	0.87 (0.06)
Robertson						
Richness	23.0 (3.08)	26.8 (8.08)	27.8 (1.79)	25.6 (7.62)	32.8 (6.46)	25.8 (10.4)
H'	2.49 (0.39)	2.52 (0.66)	2.73 (0.18)	2.59 (0.55)	3.01 (0.32)	2.60 (0.48)
1-*D*	0.86 (0.07)	0.83 (0.14)	0.89 (0.04)	0.87 (0.10)	0.92 (0.04)	0.87 (0.05)
Wellington						
Richness	25.3 (3.05)	26.3 (4.41)	22.0 (7.93)	31.0 (6.03)	35.3 (4.16)	23.8 (10.8)
H'	2.71 (0.16)	2.71 (0.30)	2.50 (0.50)	3.05 (0.30)	3.15 (0.17)	2.41 (0.74)
1-*D*	0.90 (0.02)	0.89 (0.04)	0.87 (0.06)	0.93 (0.03)	0.93 (0.02)	0.83 (0.13)

5.3.8 Effect of burning of slash piles of invasive biomass on fungal diversity

The fungal diversity (*H'*) in the soil samples underneath the piles (burned areas) at *Acacia*- and *Eucalyptus*-invaded sites showed two different trends post fire. The *Acacia* sites (Bainskloof and Rawsonville) showed that the fungal diversity (*H'*) in the centre samples of piles, post fire, is significantly different compared to the fungal diversity (*H'*) in the intermediate position samples of piles (determined by the Tukey HSD test ($p < 0.050$)) (Figure 5.6). However, a year after the burn event, the fungal diversity (*H'*) of the centre and intermediate samples was similar. As for the *Eucalyptus* sites, no significant difference was recorded when the fungal diversity (*H'*) of the centre and intermediate samples of Robertson and Wellington were compared (Figure 5.6). In addition, the Simpson's complement (1-*D*) index confirms the fungal diversity trend of centre and intermediate samples for all three sample times in the respective sites.

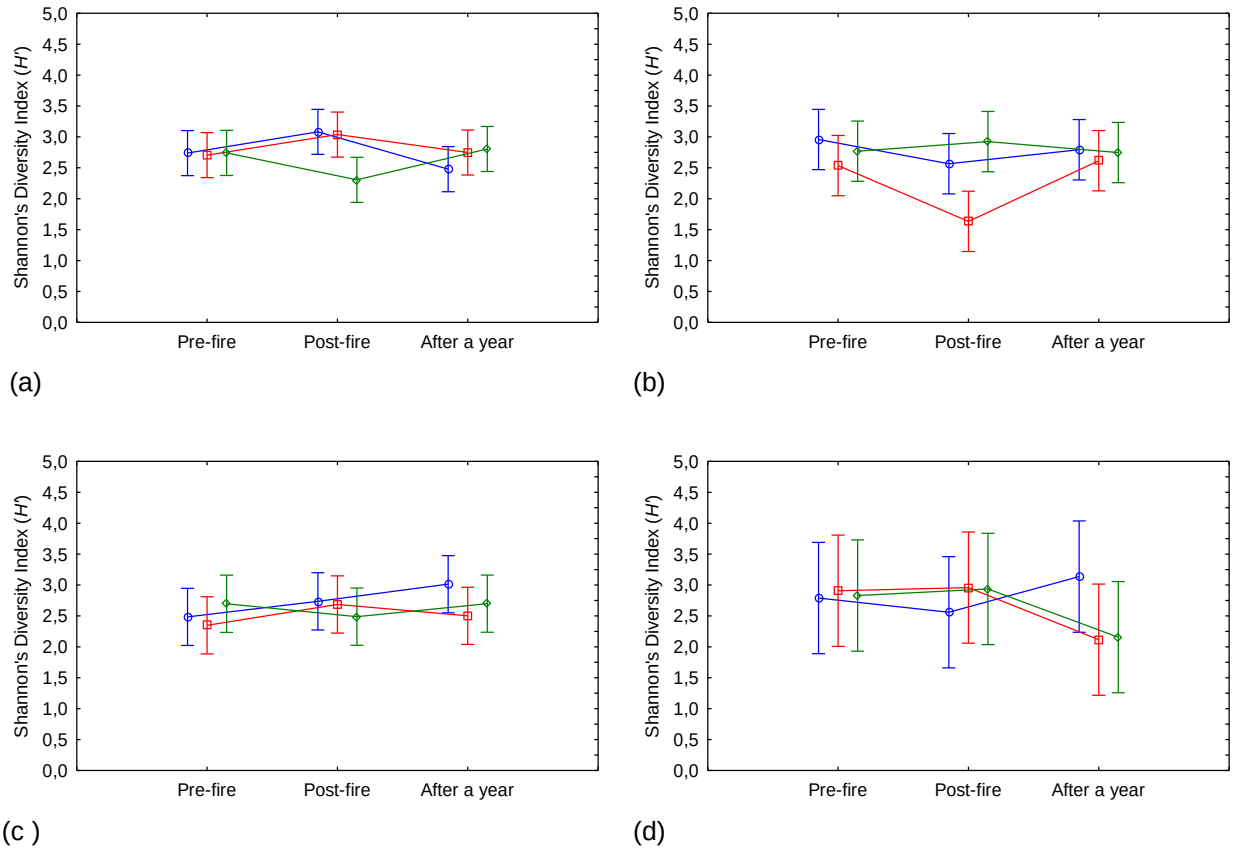
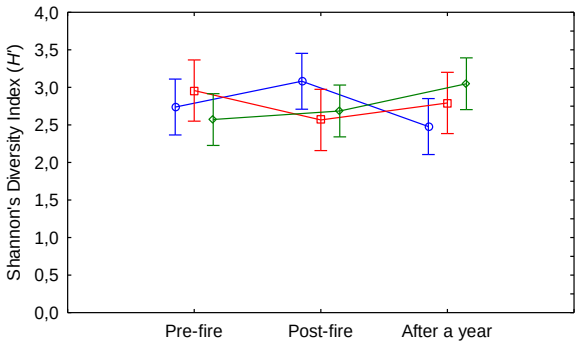


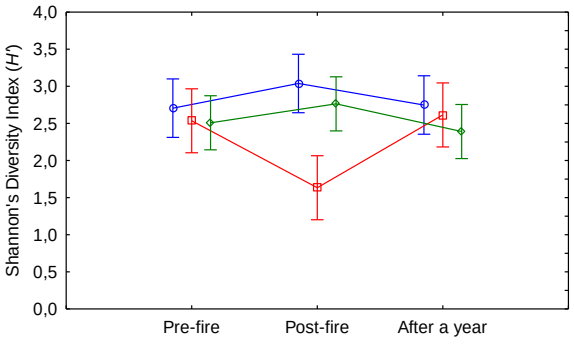
Figure 5.6: The effect of burning of slash piles of invasive biomass on fungal diversity (*H'*) in the four study sites. The blue represents the fungal diversity of the control, whereas the red (centre) and green (intermediate) represent fungal diversity in the soil samples underneath the piles. Significant results are indicated with a (*): (a) Bainskloof* (p= 0.037), (b) Rawsonville (p= 0.142), (c) Robertson (p= 0.590), (d) Wellington (p= 0.403).

5.3.9 Variation in fungal diversity between invasion sites

The fungal diversity (*H'*) in the *Acacia* and *Eucalyptus* sites was similar pre-fire. The control samples obtained a significant difference of *p*= 0.046, however, the post-hoc test reveals no significant difference between the sites (Figure 5.7a). The fungal diversity (*H'*) of the centre samples in Rawsonville (*Acacia* site) was significantly lower than the *Eucalyptus* sites post fire. Nonetheless, no significant difference was recorded a year after the burn event (Figure 5.7b). The fungal diversity of the intermediate samples for all the sites was similar throughout the study (Figure 5.7c).



(a)



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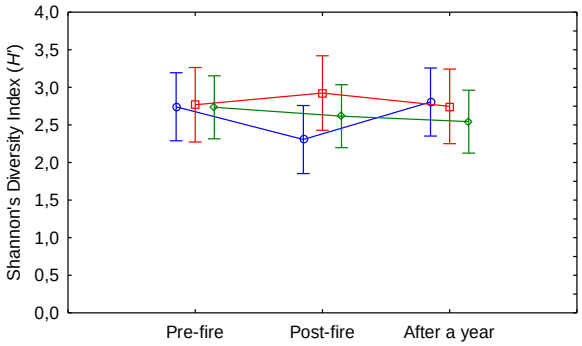


(c)

Figure 5.7: The effect of burning of slash piles of *Acacia* biomass on fungal diversity (*H'*) compared to the effect of burning of slash piles of *Eucalyptus* biomass on fungal diversity (*H'*). The blue indicates the fungal diversity in Bainskloof, red in Rawsonville and green in *Eucalyptus* sites. Significant results are indicated with a (*).(a) Control* (*p*=0.046); (b) Centre samples* (*p*= 0.016); (c) Intermediate samples (*p*= 0.338)

5.3.10 Fungal community structure

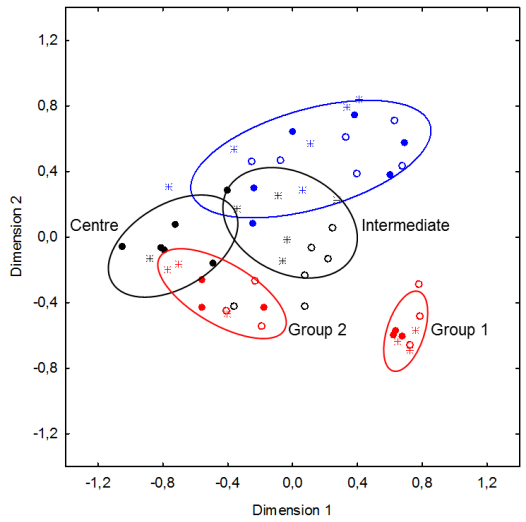
The fungal community structures of all the sites were analysed for all three sample times to evaluate the strength of dissimilarity after the effect of burning of slash piles of invasive biomass. Three distinct fungal communities (separated by different sample times) are expected in the soil samples underneath the piles for each respective site based on the ANOSIM results in Table 5.10, apart from the Robertson site. The fungal communities post fire and a year after the burn event in the Robertson site were similar.

Table 5.10: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) was used to compare the bacterial communities in the soil samples underneath the piles at all three sample times in respective sites. The *p*-values are indicated in brackets.

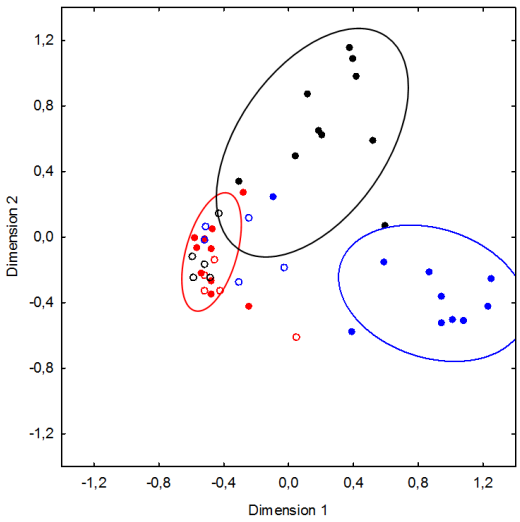
Sites	**Pre-fire vs. Post fire**	**Pre-fire vs. After a year**	**Post fire vs. After a year**
Bainskloof	0.334 (0.001)	0.552 (0.001)	0.464 (0.001)
Rawsonville	0.551 (0.001)	0.862 (0.001)	0.658 (0.001)
Robertson	0.645 (0.001)	0.651 (0.001)	0.063 (0.080)
Wellington	1.000 (0.025)	0.958 (0.033)	0.406 (0.019)

The NMDS plots in Figure 5.8 indicated two groupings of fungal community structures in the Bainskloof site pre-fire and post fire. The meaningful groupings of fungal community structure pre-fire in Bainskloof are due to the dissimilarities in the soil samples underneath respective piles (Figure 5.8). It appears that the fungal communities pre-fire underneath piles 1, 2 and 4 are different from the fungal communities underneath piles 5, 6 and 7.

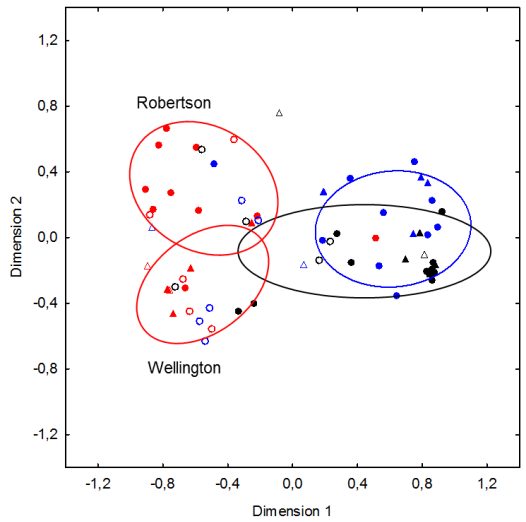
Furthermore, the meaningful groups post fire are due to the dissimilarities between the centre and intermediate samples (R = 0.246, p= 0.041). A year after the burn event, no distinct fungal community structures in the Bainskloof site were formed (R = 0.048, p = 0.573). As for Rawsonville, Robertson and Wellington sites, no meaningful groupings of fungal community structure pre-fire were found in respective sites. Secondly, no dissimilarity between the fungal community structure of the centre and intermediate samples throughout the study was recorded.



(a)



(b)



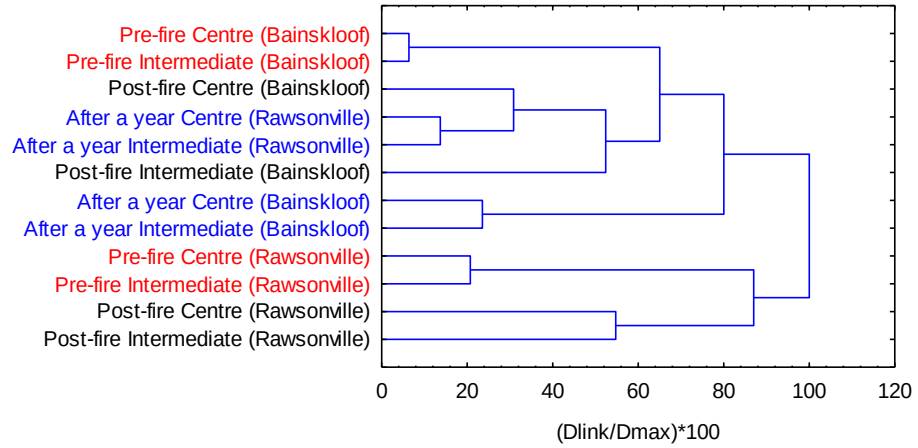
(c)

Figure 5.8: Non-metric multidimensional scaling (NMDS) of fungal community structure of the control (empty circles) and burned areas (filled circles) for all three sample times in respective sites. Red presents the fungal community structure pre-fire, black presents post fire and blue presents a year after the burn event. For the *Eucalyptus* sites, the fungal community structure of Robertson is indicated as circles and of the Wellington site is indicated as triangles. Ellipses denote 75% confidence levels. (a) Bainskloof (nine dimensions, stress = 0.054), (b) Rawsonville (eight dimensions, stress = 0.053), (c) *Eucalyptus* sites (nine dimensions, stress = 0.049)

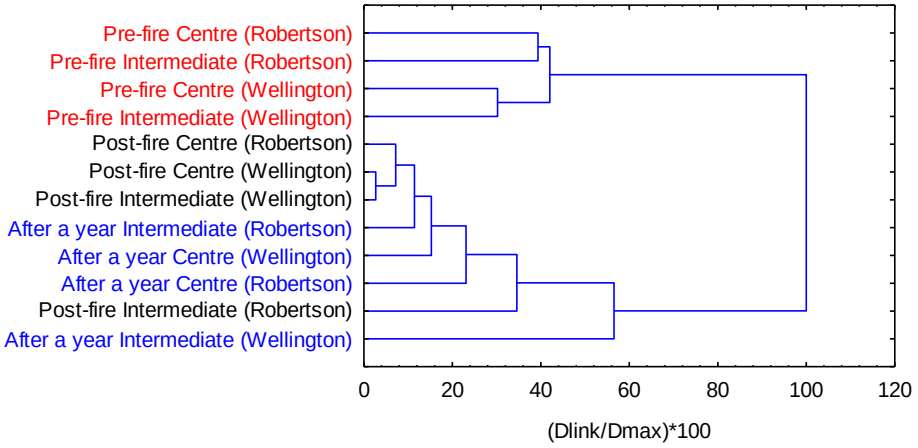
5.3.11 Variation of fungal community structure within invasion sites

In Figure 5.9, the fungal communities within invasion sites pre-fire are site specific. The cluster analysis in Figure 5.9a illustrated that the fungal communities in Bainskloof and Rawsonville (*Acacia* sites) followed two different trends after the burning of slash piles of *Acacia* biomass. As for the *Eucalyptus* sites, the

fungal communities in Robertson and Wellington followed a similar trend after the burning of slash piles of *Eucalyptus* biomass (Figure 5.9b).



(a)



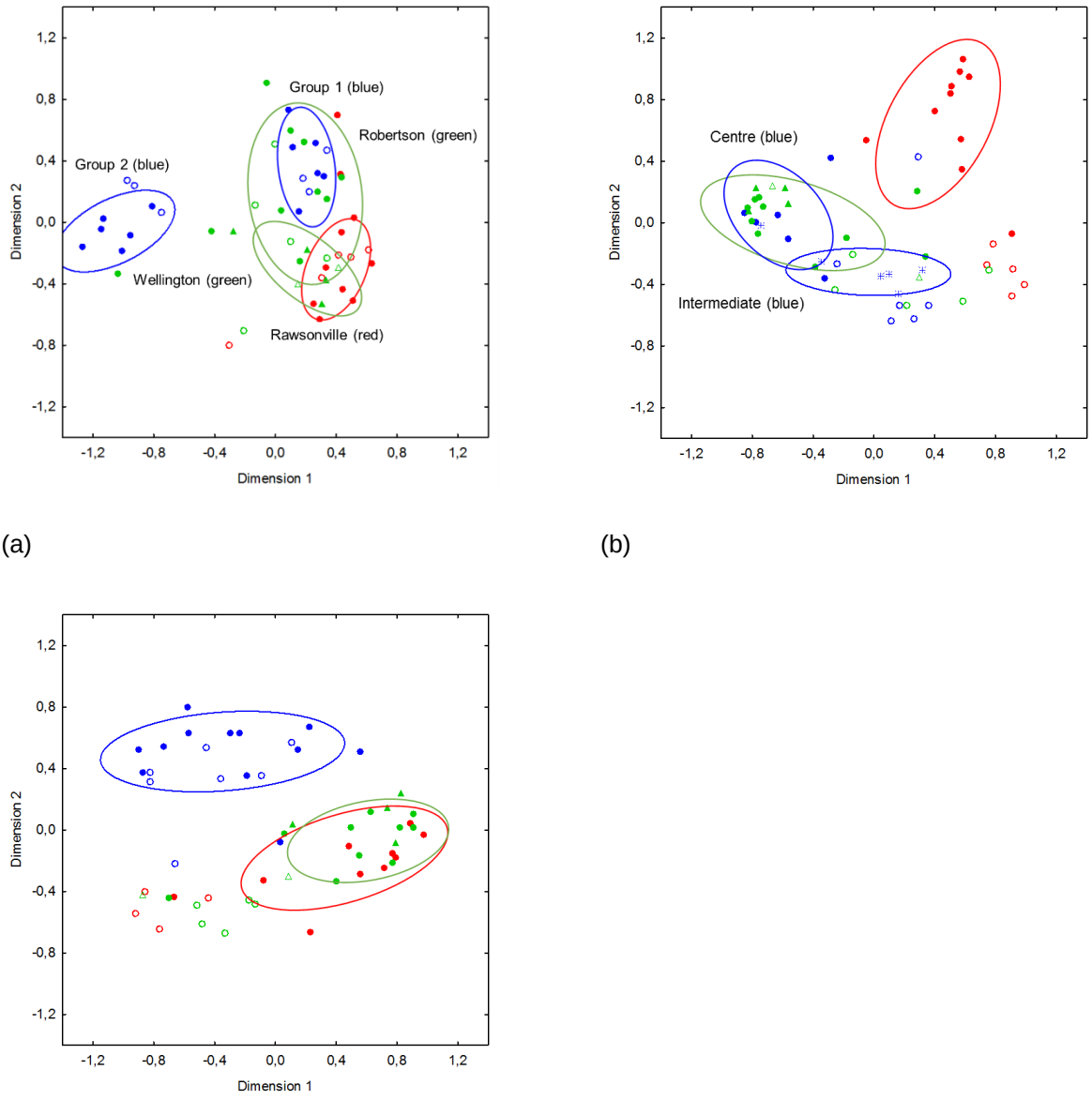
(b)

Figure 5.9: The Whittaker similarity index along with the cluster analysis was used to compare the fungal communities of all three sample times in respective invasive sites. (a) *Acacia* sites, (b) *Eucalyptus* sites

5.3.12 Variation of bacterial community structure between invasion sites

When the fungal community structure of all the sites was compared to one another for all three sample times: four groupings were displayed pre-fire (Figure 5.10a); three groupings were displayed post fire (Figure 5.10b) and two groupings were displayed a year after the burn event (Figure 5.10c). Pre-fire, the fungal community structure of the *Eucalyptus* (Robertson) site was similar to group 2 of the Bainskloof site (R = 0.206, *p*= 0.058), whereas Rawsonville, Wellington and group 1 of the Bainskloof sites formed three separate groupings. Post fire, the fungal community structure of the centre samples in the Bainskloof site was similar to the *Eucalyptus* sites (Robertson and Wellington) (R = 0.072, *p*= 0.267). The fungal community structure of the Rawsonville site and the intermediate samples of the Bainskloof site formed two distinct groupings post fire (Figure 5.10b). A year after the burn event, the fungal community structure

of the *Eucalyptus* and Rawsonville sites was similar (R = 0.038, *p*= 0.213), whereas the Bainskloof site formed a distinct grouping (Figure 5.10c).



(a) (b)

(c)

Figure 5.10: Non-metric multidimensional scaling (NMDS) of fungal community structure of the control (empty circles) and the soil samples underneath the piles (filled circles) of (a) pre-fire, (b) post fire and (c) a year after the burn event sample times. Ellipses denote 75% confidence levels.

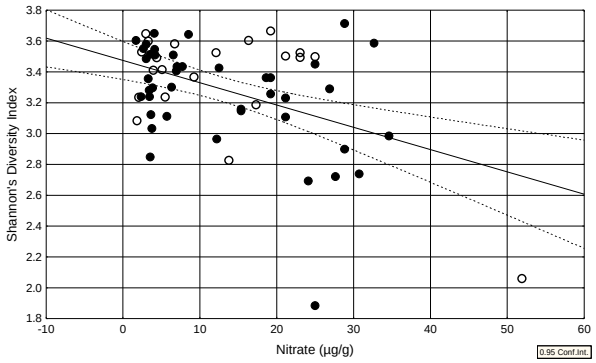
5.3.13 Relationship between soil chemical properties and microbial composition

When the soil chemical properties and bacterial diversity (*H'*) of the Bainskloof site were correlated to one another, the NO_3 concentration indicated a significant correlation with bacterial diversity (*H'*) with an r = -0.451 (Table 5.11). As for the Rawsonville and *Eucalyptus* sites, the soil pH, EC and PO_4 concentration indicated a negative correlation with bacterial diversity (*H'*) (Table 5.11). The soil pH showed the strongest correlation coefficient in the Rawsonville and *Eucalyptus* sites with r = -0.721 and r = -0.746, respectively. Figure 5.11 illustrates an overview of the significant correlations between soil chemical properties and

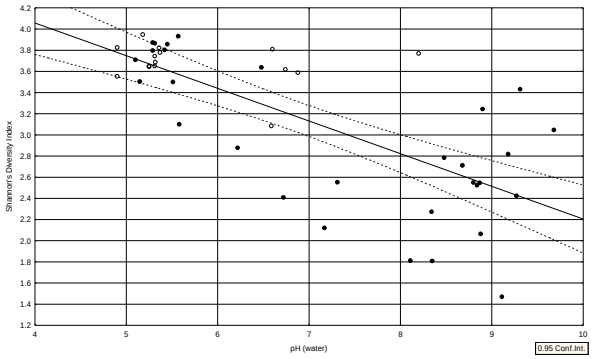
bacterial diversity (*H'*). No significant correlation was recorded between soil chemical properties and fungal diversity (*H'*), apart from the NH_4 concentration in the *Eucalyptus* site which obtained an r = -0.339 (Table 5.12).

Table 5.11: Pearson correlation coefficient (PCC) analysis between soil chemical properties and bacterial diversity (*H'*). The *p*-values are indicated in brackets.

	Bainskloof	**Rawsonville**	***Eucalyptus* sites**
pH (water)	-0.115 (0.370)	-0.721 (<0.001)	-0.746 (<0.001)
EC (µS/cm)	0.018 (0.888)	-0.579 (<0.001)	-0.604 (<0.001)
NH_4 (µg/g)	-0.229 (0.071)	-0.113 (0.459)	0.0818 (0.524)
NO_3 (µg/g)	-0.451 (<0.001)	0.003 (0.984)	0.237 (0.061)
PO_4 (µg/g)	0.194 (0.128)	-0.610 (<0.001)	-0.446 (<0.001)

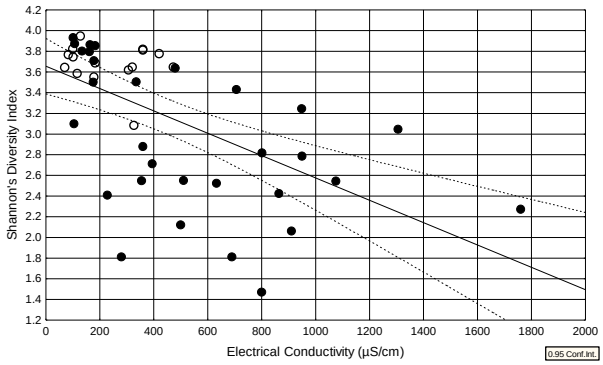


(a)



(b)

Figure 5.11: Pearson correlation coefficient (PCC) analysis between soil chemical properties and bacterial diversity (H') of all the sites pre-fire (red), post fire (black) and a year after the burn event (blue). The controls are indicated as empty circles and the soil samples underneath the piles as filled circles. For the *Eucalyptus* sites, Robertson is indicated using circles whereas Wellington is indicated using triangles. (a) Bainskloof: Nitrate (r = -0.451), (b) Rawsonville: pH (r = -0.721), (c) Rawsonville: EC (r = -0.579), (d) Rawsonville: Phosphate (r = -0.610), (e) *Eucalyptus* sites: pH (r = -0.746), (f) *Eucalyptus* sites: EC (r = -0.604), (g) *Eucalyptus* sites: Phosphate (r = -0.446)



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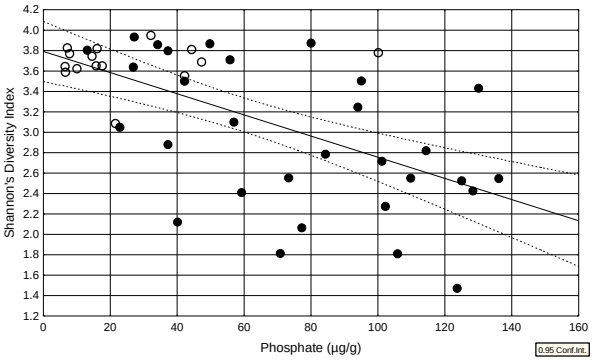

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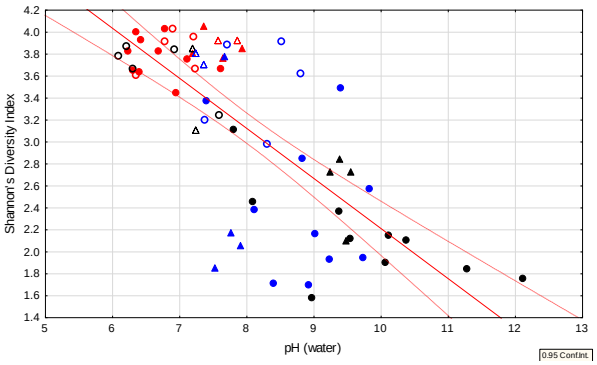

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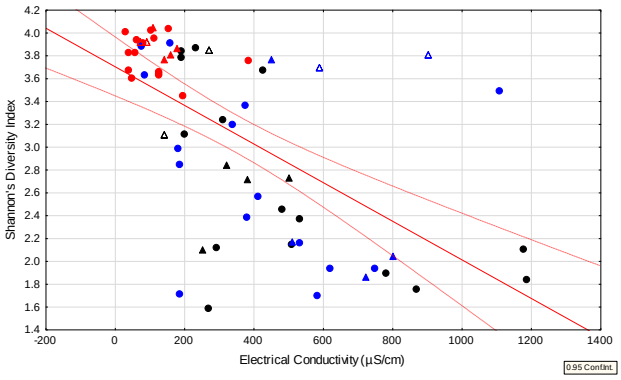

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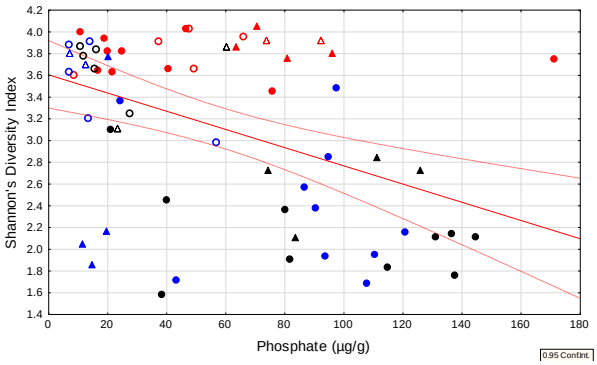


(g)

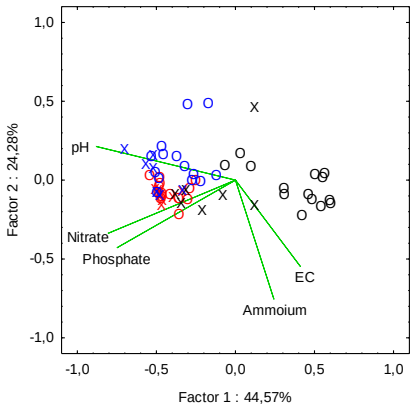
Figure 5.11 (Continued): Pearson correlation coefficient (PCC) analysis between soil chemical properties and bacterial diversity (H') of all the sites pre-fire (red), post fire (black) and a year after the burn event (blue). The controls are indicated as empty circles and the soil samples underneath the piles as filled circles. For the *Eucalyptus* sites, Robertson is indicated using circles whereas Wellington is indicated using triangles. (a) Bainskloof: Nitrate (r = -0.451), (b) Rawsonville: pH (r = -0.721), (c) Rawsonville: EC (r = -0.579), (d) Rawsonville: Phosphate (r = -0.610), (e) *Eucalyptus* sites: pH (r = -0.746), (f) *Eucalyptus* sites: EC (r = -0.604), (g) *Eucalyptus* sites: Phosphate (r = -0.446)

Table 5.12: Pearson correlation coefficient (PCC) analysis between soil chemical properties and fungal diversity (H'). The p-values are indicated in brackets.

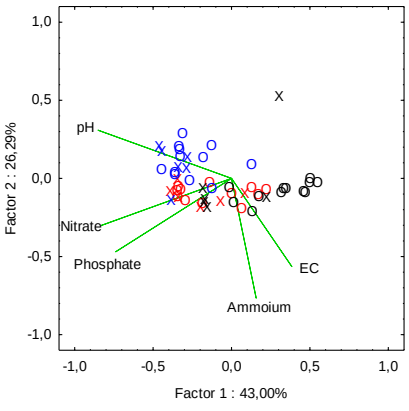
	Bainskloof	**Rawsonville**	***Eucalyptus* sites**
pH (water)	0.189 (0.172)	-0.290 (0.054)	0.127 (0.321)
EC (µS/cm)	0.066 (0.636)	-0.136 (0.372)	0.080 (0.532)
NH_4 (µg/g)	0.123 (0.358)	-0.251 (0.097)	-0.339 (0.007)
NO_3 (µg/g)	0.142 (0.307)	-0.210 (0.167)	0.112 (0.381)
PO_4 (µg/g)	0.235 (0.087)	0.179 (0.239)	0.203 (0.111)

5.3.14 Principle component analysis

The first principle component of the Bainskloof site correlated strongly with soil pH, NO_3 and PO_4 concentration (Figure 5.12a-b), whereas the second principle component correlated with the EC and NH_4 concentration. According to Figure 5.12a-b, the bacterial and fungal community structure post fire could be differentiated from the community structure pre-fire and a year after the burn event along the first principle component. The first principle component of the *Eucalyptus* and Rawsonville sites was determined by pH, EC and PO_4 concentration, whereas the second principle component was determined by NH_4 and NO_3 concentration (Figure 5.12c-f). The soil samples underneath the piles in the *Eucalyptus* and Rawsonville sites illustrated a clear separation between the bacterial and fungal community structures pre-fire, and the community structures post fire and a year after the burn event (Figure 5.12c-f).

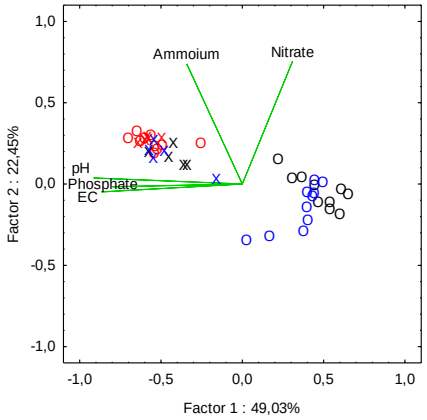


(a)



(b)

Figure 5.12: Principle component analysis (PCA) of soil chemical properties and microbial community structures of all the sites pre-fire (red), post fire (black) and a year after the burn event (blue). The controls are indicated as x and the soil samples underneath the piles as o. For the *Eucalyptus* sites, Robertson is indicated in the same way, using x and o, whereas the controls at Wellington are indicated with a *, and the soil samples underneath the piles with a +. (a) Bainskloof - Bacterial community structure, (b) Bainskloof - Fungal community structure, (c) Rawsonville - Bacterial community structure, (d) Rawsonville - Fungal community structure, (e) *Eucalyptus* sites - Bacterial community structure, (f) *Eucalyptus* sites - Fungal community structure.



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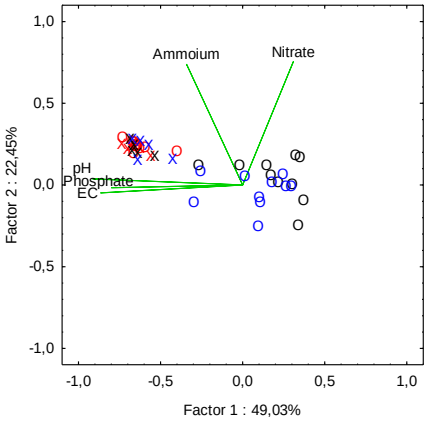

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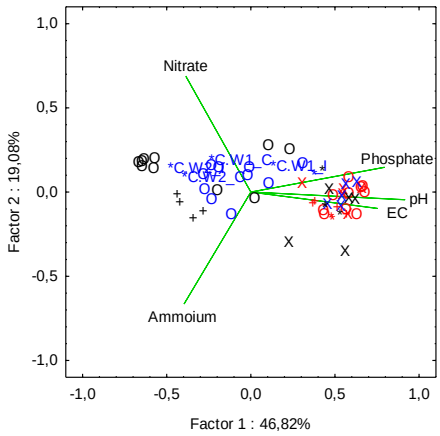

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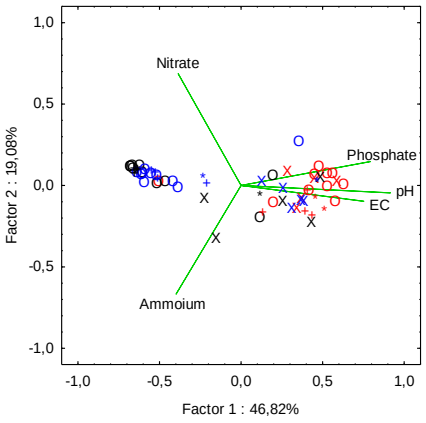


(f)

Figure 5.12 (Continued): Principle component analysis (PCA) of soil chemical properties and microbial community structures of all the sites pre-fire (red), post fire (black) and a year after the burn event (blue). The controls are indicated as x and the soil samples underneath the piles as o. For the *Eucalyptus* sites, Robertson is indicated in the same way, using x and o, whereas the controls at Wellington are indicated as * and the soil samples underneath the piles as +. (a) Bainskloof - Bacterial community structure, (b) Bainskloof - Fungal community structure , (c) Rawsonville - Bacterial community structure, (d) Rawsonville - Fungal community structure, (e) *Eucalyptus* sites - Bacterial community structure, (f) *Eucalyptus* sites - Fungal community structure.

5.4 DISCUSSION

Alien plant invasion poses a major threat to fynbos (Moran and Hoffman, 2012; Myers et al., 2000). The establishment of *Acacia* and *Eucalyptus* spp. in fynbos vegetation has led to a decrease in the abundance of microbial diversity and fynbos plant species (Slabbert et al., 2014; Vosse et al., 2008). The establishment of *Acacia* and *Eucalyptus* spp. in fynbos vegetation, in turn, promotes a phase transition (from native to invaded fynbos ecosystem) which has a significant impact on the natural patterns and processes of an ecosystem, physical-chemical properties and soil microbial communities (Morris et al., 2011; Stock et al., 1995; Witkowski, 1991). Invasive species in many parts of the Cape Floristic Region have been removed by means of mechanical management strategies for commercial purposes such as

timber. However, the remaining bark, branches, leaves, stems and twigs of these invasive species (dead biomass), as well as biomass left in inaccessible places, pose a major threat to restoration and also a wildfire risk. This is due to the dead plant material that might ignite under the right conditions and suitable fuel content, while inhibiting the natural recovery of native plant communities in cleared areas. One efficient management strategy to remove the remaining plant material of *Acacia* and *Eucalyptus* spp. (after mechanical removal) is the 'slash and burn' (burning of slash piles) method, where material is stacked and fire is used to reduce the dead biomass. In this study, the emphasis was on the effect of burning of slash piles of *Acacia* and *Eucalyptus* biomass on microbial diversity and community structure in riparian zone fynbos soil.

5.4.1 Effect of burning of slash piles of invasive biomass on bacterial diversity

After the mechanical removal of invasive biomass (pre-fire) in riparian zones of fynbos, the data recorded no significant difference in bacterial diversity within and between invasion sites. After the removal of *Acacia* and *Eucalyptus* biomass through experimental stacking and burning, two different trends with regards to the bacterial diversity in the samples underneath the piles were observed. Bacterial diversity was not affected by the burning of slash piles of *Acacia* biomass in the Bainskloof site. The bacterial diversity remained similar post fire and a year after the burn event. In contrast, all the other sites that were exposed to the burning of slash piles of *Eucalyptus* biomass indicated a significant decrease in bacterial diversity which remained similar a year after the burn event. Furthermore, the effect of burning of slash piles of *Eucalyptus* biomass resulted in a decrease in bacterial richness. These results suggest that burning of slash piles of *Eucalyptus* biomass might have resulted in greater heat-induced changes compared to the burning of slash piles of *Acacia* biomass; the latter tends to burn less intensely. It is also possible that burning of slash piles of *Eucalyptus* biomass could have led to bacterial mortality (González-Pérez et al., 2004; Hart et al., 2005), which may have resulted in a significant decrease in bacterial richness and diversity.

5.4.2 Effect of burning of slash piles of invasive biomass on bacterial community structure

Results from the NMDS plots showed that each site presented three distinct bacterial communities separated by different sampling times. This suggests that the burning of slash piles of *Acacia* and *Eucalyptus* biomass resulted in a shift in bacterial community structure (Dooley and Treseder, 2012; Ferrenberg et al., 2013; Neary et al., 1995; Reazin et al., 2016). Previous studies suggested that the shift may be explained by the heat impact of fire which is greater in the top 2 cm of the soil (Dooley and Treseder, 2012; Sun et al., 2008) where the microbial communities are high in abundance (Hart et al., 2005).

It was interesting to observe that the bacterial community structure of the Rawsonville site was significantly similar to the *Eucalyptus* sites post fire, seeing as the piles at Rawsonville were predominantly built from *Acacia* biomass with a small percentage of *Eucalyptus* biomass. This finding supports the previous suggestion that burning of slash piles of *Eucalyptus* biomass might have led to higher soil temperatures compared to the burning of slash piles of *Acacia* biomass. In addition, the bacterial community structure of the Rawsonville and *Eucalyptus* sites remained similar a year after the burn event. However, the bacterial community structure a year after the burn event is different from the community structure post fire. It is possible that the influence of seasonal changes could have promoted a unique bacterial community structure a year after the burn event which was different from those presented pre-fire and post fire (Ferrenberg et al., 2013).

A year after the burn event, Bainskloof (*Acacia* site) experienced a flood which disturbed the piles. No differentiation between the bacterial community structures of the control and the soil samples underneath the piles (burned areas) was evident. This contention was supported by the lack of significant changes in EC and the rapid decline in pH to pre-fire levels after the flood, as found in Chapter 4.

5.4.3 Effect of burning of slash piles of invasive biomass on fungal diversity

Little is known about fungal composition in riparian zones of fynbos. Nonetheless, Slabbert et al. (2010) have stated that the microbial communities in fynbos are profoundly diverse and are closely associated with the above-ground flora. The present study found that the fungal diversity in fynbos areas cleared of *Acacia* and *Eucalyptus* biomass was similar.

The sites cleared of *Acacia* biomass indicated two fungal diversity trends post fire which were in contrast to one another. According to the data from this study, it appears that exposure to burning of slash piles in Bainskloof had a greater impact on the fungal diversity in the intermediate areas of the burned piles in comparison to the centre areas. This observation was in contrast to the Rawsonville site. The variation of fungal diversity in the soil samples underneath the piles (burned areas) between Bainskloof and Rawsonville might be influenced by the stacking and rearrangement of the *Eucalyptus* biomass in the Rawsonville site. As for the sites cleared of *Eucalyptus* biomass, the fungal diversity in Robertson and Wellington followed a similar trend post fire. The fungal diversity a year after the burn event, in the respective sites, was similar to the fungal diversity pre-fire. When the fungal diversity of all the sites was compared to another, no significant difference was recorded between the control, centre and intermediate samples pre-fire and a year after the burn event, respectively.

5.4.4. Effect of burning of slash piles of invasive biomass on fungal community structure

Results from the NMDS plots showed that each study site presented a unique fungal community structure. These observations are similar to what was reported by Slabbert et al. (2010) who found that the fungal communities in fynbos are site specific. A possible explanation is that some fungal communities are more host-specific towards plant roots than bacterial communities (Barreiro et al., 2016). Another explanation could be that the removal of invasive biomass has a significant impact on fungal community composition (Bååth and Arnebrant, 1994).

The presence of recently recovered fynbos shrublands or grass (after the removal of invasive biomass by means of mechanical management) in the invaded areas is a key factor which could have also promoted the distinct fungal community structures (Morris et al., 2011; van Wilgen and Richardson, 1985; van Wilgen et al., 1990; Witkowski, 1991). All these sites appear to fall into different natural fynbos vegetation types with different species composition (Ruwanza et al., 2012), thus the unique sets of plant species may significantly affect the fungal community composition belowground.

The Bainskloof site presented two distinct fungal community structures pre-fire, which suggests that fungal communities in the soil samples underneath the piles were not homogeneously distributed. The spatial distribution of fungal communities (Morris, 1999) could have been influenced by soil moisture patterns and texture (Morris and Boerner, 1999), the presence and absence of plant species at particular areas on the same site (Ettema and Wardle, 2002; Urbanová et al., 2015) and the availability of invasive plant material on the surface layer of the soil (removed biomass). The fungal community structure of the Robertson site pre-fire was closely related to the group 2 fungal community structures of the Bainkloof site. This result

was rather surprising, seeing that Robertson was predominantly invaded by *Eucalyptus camaldulensis* whereas Bainskloof was predominantly invaded by *Acacia* spp.

All the sites indicated a shift in the fungal community structure after the effect of burning of slash piles of invasive biomass. Bainskloof showed two distinct fungal community structures post fire which were separated by the centre and intermediate samples. An interesting finding in this study was that the fungal community structure in the centre samples of Bainskloof was similar to the *Eucalyptus* sites. This result was rather surprising, as these sites were exposed to the burning of slash piles of different invasive biomass and the duration of the respective fires was also different. In this study, the burning of slash piles of *Acacia* biomass (dead material) resulted in smouldering for ~2 to ~5 hours, whereas the burning of slash piles of *Eucalyptus* biomass resulted in smouldering lasting for between 3 hours and up to 9 days. Based on the classification reported by Reazin et al. (2016) that a low-intensity fire generally burns for a shorter period of time than a high-intensity fire, it can be assumed that the fire intensity of *Eucalyptus* biomass is greater than the fire intensity of *Acacia* biomass.

After the burning event, Bainskloof experienced a flood which disturbed the piles. Flooding in riparian zones is a common disturbance that results in water-aided dispersal of *Acacia* seeds (Tererai et al., 2013; Vosse et al., 2008). The effect of flooding may have caused the fungal community variation between the centre and intermediate samples post fire to be similar a year after the burn event. Additionally, some *Acacia* growth was visible in the burned areas and no separation of fungal community structures was evident between the control, centre and intermediate samples. Furthermore, the fungal community structure in Bainskloof, pre-fire and a year after the burn event, was significantly different. The fungal community variation pre-fire and a year after the burn event in Bainskloof could be due to the absence or removal of *Acacia* biomass and the presence of recovered fynbos species pre-fire compared to the Bainskloof site a year after the burn event which was dominated by *Acacia* species with a small percentage of fynbos species.

The fungal community structure in the Rawsonville sites, a year after the burn event, was closely related to the fungal community structure of the *Eucalyptus* sites. A possible reason for this finding was explained by Reazin et al. (2016). They stated that post-fire dominants—beneath the soil surface where fire occurred or from adjacent areas around the burned piles—have the capacity to distribute over the burned areas by means of mycelial expansion from deeper to surface soil profiles or from the margins of the burned piles into the burned areas (Reazin et al., 2016). The adjacent areas around the burned piles indicated plant growth in the sites not exposed to burning of slash piles of *Eucalyptus* biomass, whereas the burned areas did not indicate any plant growth. This might be due to the effect of burning of slash piles of *Eucalyptus* biomass which could have destroyed the roots and mycorrhizal fungi in the soil that consequently decreased the recolonization capacity and rate in burned areas (Bååth et al., 1995).

It was only evident a year after the burn event that the Robertson site experienced secondary invasion by *Acacia* spp. A year after the burn event, the control areas in the Robertson site were predominantly covered by *Acacia* spp. After the removal of *Eucalyptus* biomass (primary invasion), *Acacia* spp. had the competitive advantage over the fynbos species to compete for essential resources such as light, nutrients, space and water. Based on the availability of these resources and the distribution of seeds by ants, birds, vertebrates and wind dispersal, the biomass of *Acacia* spp. became more abundant in the riparian zones (D'Antonio and Vitousek, 1992; Gordon, 1998; Holmes and Cowling, 1997; Knight and MacDonald, 1991; Rajcan and Swanton, 2001; Tucker and Richardson, 1995).

5.4.5 Soil chemical properties associated with microbial diversity and community structure

Previous studies reported that bacterial diversity is more likely to increase along with soil pH post fire (Bååth and Arnebrant, 1994; Bárcenas-Moreno and Bååth, 2009; Dooley and Treseder, 2012). Soil pH plays a significant role in fynbos vegetation (Prins et al., 2004), and showed the strongest association towards the soil bacterial composition (Fierer and Jackson 2006; Kim et al., 2014; Lauber et al., 2009; Osborne et al., 2011). The present study, along with previous studies, found that fire increases soil pH (Barreiro et al., 2016; Jensen et al., 2001). The increase in soil pH post fire could be attributed to the alkaline nature of ashes (Bååth and Arnebrant, 1994; Hernández et al., 1997). The sites exposed to burning of slash piles of *Eucalyptus* biomass (Rawsonville, Robertson and Wellington) indicated a significant correlation between soil pH and bacterial diversity.

Other soil chemical properties, such as EC and PO_4 concentration, also showed a significant correlation with bacterial diversity. EC, which measures the soluble cations in soil, increased post fire (Kutiel and Naveh 1987). The increase is due to the combustion of organic matter which promotes the concentration of soluble cations (Hernández et al., 1997). EC decreases gradually post fire due to the fixation of the salt, precipitation and leaching (Hernández et al., 1997; Kutiel and Naveh 1987). Furthermore, the production of polyphosphate (soluble forms) which is deposited in ash (DeBano and Conrad, 1978; Khanna and Raison, 1986) has increased post fire. Previous studies indicated that total and inorganic P are likely to increase post fire (Kutiel and Naveh, 1987; Romanya et al., 1994) and the fire intensity is directly proportional to the soil P concentration (Reinhart et al., 2016; Schaller et al., 2015). Most literature focussed on the total and inorganic P post fire and limited studies have taken the PO_4 concentration in soil into consideration (DeBano and Conrad, 1978; Romanya et al., 1994; Schaller et al., 2015). This finding along with the EC correlation is interesting. Only one previous study was identified that reported on any association between EC and PO_4 concentration with bacterial diversity (Slabbert, 2012). Furthermore, the PCA further indicated that soil pH, EC and PO_4 concentration as first principle components and NH_4 and NO_3 concentration as second principle components could separate the bacterial and fungal community structures in the sites exposed to burning of slash piles of *Eucalyptus* biomass, for the three sample times.

Although the bacterial diversity in the Bainskloof site remained similar throughout the study, it appears that it was significantly correlated with the NO_3 concentration in the soil. This result was unexpected. Wan et al. (2001) documented that NO_3 concentration remains relatively low post fire and increases gradually as time progresses to a concentration which is threefold greater than the pre-fire condition and could remain high for half a year to one year. In this study, however, the NO_3 concentration returned to pre-fire conditions a year after the burn event.

The only correlation found between soil chemical properties and fungal diversity was the NH_4 concentration presented in *Eucalyptus*-dominated sites. This result was also unexpected. Previous studies recorded that the total N and NH_4 concentration in the top two centimetres of soil increases immediately post fire (Hernández et al., 1997; Stock and Lewis 1986; Thanos and Rundel 1995). These increased concentrations may be promoted by the conversion of organic forms into inorganic NH_4-N and NO_3-N (Neary et al., 1999), and the N input of ash and insoluble soil organic N over the surface layer (Grogan et al., 2000). In this study, the NH_4 concentration in *Eucalyptus*-invaded sites post fire was similar to pre-fire conditions, and the control indicated a decrease in NH_4 concentration post fire. The decrease in NH_4 concentration is not due to the effect of burning of slash piles of *Eucalyptus* biomass and these observations was not evident in the other sites. It is possible that the variation of NH_4 concentration in the *Eucalyptus* sites could have promoted the association with fungal diversity.

In this study, the ARISA fingerprinting technique was effectively used to assess microbial diversity and community structure in invaded fynbos areas. Using ARISA, trends in microbial diversity and community structure after exposure to different burn treatments were observed. The investigations have shown

conflicting results. It was apparent that burning of slash piles of *Eucalyptus* biomass had a greater impact on soil microbial communities than the burning of slash piles of *Acacia* biomass. Burning of slash piles of *Eucalyptus* biomass left a scar where the fynbos vegetation did not recover. The effect of burning of slash piles of *Acacia* biomass is still unclear due to the interference of the flood.

Data showed that the microbial diversity (both bacteria and fungi) was similar after the mechanical removal of *Acacia* and *Eucalyptus* biomass (pre-fire). However, the microbial community structure in each site was different. The dominant bacterial species in *Acacia*-invaded areas have already been identified (Slabbert et al., 2014), but no previous studies have attempted to identify the bacterial species in *Eucalyptus*-invaded areas. For future research, it might be of value to investigate the bacterial and fungal species that are present in *Acacia*- and *Eucalyptus*-invaded areas. These findings may provide information regarding the possible contribution of these bacterial and fungal species towards: (i) the difference in microbial community structure between *Acacia*- and *Eucalyptus*-invaded areas, (ii) the dominant species present post fire, (iii) the succession of microbial communities after exposure to seasonal changes and (iv) the effect of flooding on microbial community structure.

Soil microbes underlie many riparian ecosystem services, which are essential for producing ecosystem goods that society depends on. Fungal and bacterial communities underneath *Eucalyptus camaldulensis* piles were affected more by burning compared to *Acacia mearnsii* piles, and, after one year, community structure different from the pre-fire community structure was evident. This suggests that alterations to microbial communities may be long lasting, with unknown impacts on soil processes, soil plant interactions and restoration of plant communities in affected patches. Lack of critical microbial partners in symbioses with native plants may lead to a lag or failure of native plant recovery following clearing and burning of slash piles. This, in turn, can cascade into higher level effects on ecosystem services, such as bank stabilisation, which needs plant cover to be re-established, and eventually to production of clean water, which may be affected greatly by mobilisation of sediment from un-vegetated patches, such as those affected by burning of slash piles.

CHAPTER 6: Regeneration of various native plant species grown in soil from slash and burn scars ex situ

6.1 INTRODUCTION

Fire is ubiquitous in the fynbos biome, and is a fundamental driver of fynbos ecology. There is significant evidence that fire played a major role on the development of the Cape flora (Keeley et al., 2011), and that current fire regimes may have been relatively constant back to the Middle Miocene climate optimum (Bytebier et al., 2011). Regeneration of fynbos vegetation following fire can follow a variety of strategies, and most fynbos plant species are adapted to fire using one of these survival or persistence strategies (e.g. epicormic shoots, heat stimulated germination, re-sprouting from the base) (Kraaij and van Wilgen, 2014).

Riparian environments are less likely to experience frequent fires compared to adjoining tracts of terrestrial fynbos (Pettit and Naiman, 2007), though with changing environmental conditions, incidences of fire in riparian environments are also increasing. Major environmental changes in fynbos riparian zones are associated with land-use change and invasion by alien invasive plants, especially woody species from Australia (*Acacia* spp. and *Eucalyptus* spp.) and Central America (*Pinus* spp.) (van Wilgen et al., 2012). The accumulation of biomass by these fast-growing species represents a major fire risk when sufficient fuel has accumulated, and management of biomass, both as stands of mature invasives and upon clearing of stands of *Acacia* and *Eucalyptus* spp., remains a major challenge for conservation and land management agencies and private landowners.

The WfW programme employs different methods, or a combination thereof, for invasive tree clearing (Holmes et al., 2008). Blanchard and Holmes (2008) carried out a study where three initial clearing techniques were identified, as used by WfW, namely:

- fell only (where trees are felled and slash left on site);
- fell and remove (where slash is removed from the riparian zone);
- slash pile burning (the slash is left on site for a period of time before it is burned).

The study concluded that the clearing treatment which is used can have a significant impact on the recovery of indigenous vegetation with regard to species composition, species richness, growth form structure and vegetation structure. The study also found that although the slash pile burning method is the best method for reducing woody alien species, this method may compromise or prolong the recovery time of indigenous vegetation. Since secondary invasion by alien herbaceous species occurred where natural riparian vegetation did not recover, the fell and remove method was recommended as the best of the three in promoting the re-establishment of indigenous vegetation.

Many of the areas where clearing of IAPs occurs are inaccessible, making removal of slash from the site a cumbersome task. Thus, the slash pile burning method is still utilised to dispose of excess biomass from clearing sites, despite recommendations that fell and remove is the best method (Behenna et al., 2008). Since slash pile burning is unavoidable in some cases, Behenna et al. (2008) suggested it only be used if the underlying soils are moist. This method has been used recently across sites in the southern part of the Western Cape. However, the effectiveness of this method in restoration of degraded, alien-invaded habitats is largely unknown since research on this subject is still ongoing (Blanchard, 2008).

Slash burning mainly involves burning of plant debris (organic matter). The fire will affect the underlying soil or rock differently depending on the properties of the fire. Soil nutrients will also be affected by the fire-induced temperatures. After the fire, the concentration of nutrients in the soil will be influenced by the composition. In most cases, soil nutrient concentrations improve after fire, especially in the top layer of the soil. However, the improvements may be short term as they are affected by external factors. Factors such

as leaching, run-off and wind could result in removal of post-fire nutrient concentrations (Giardina et al., 2000b; Certini, 2005; Schafer and Mark, 2010).

Exposure of soils to extremely high temperatures could result in loss of viable seeds, hamper re-sprouting or kill belowground plant parts and lead to a lack of, or delay in, regeneration on affected patches (Korb et al., 2004; Rhoades et al., 2015). Korb et al. (2004) suggested that high temperatures create a seed limitation by killing viable seeds and may influence establishment of remaining seeds or seeds dispersed from surrounding unaffected areas through a lack of mycorrhizal fungi in fire-affected areas. Rhoades et al. (2015) suggested that, over the medium term, soil alterations may affect regeneration, though recovery of soil takes place over the longer term. However, fire-affected areas remained poorly established by woody species up to 50 years post fire, with grasses and forbs dominating the affected areas.

Invasive plant species have been observed at higher frequencies in fire-affected patches compared to non-burned areas, especially invasive grasses and forbs. Shive et al. (2013) found that although cover of exotic species decreased over time, frequency of invasive greases and forbs increased over time on fire-affected patches. Barclay et al. (2004) also found invasive grasses to dominate woody ecosystems following fire in North America.

The aim of this study is to determine vegetation recovery of fire-burned scars following IAP removal and burning of slash. The type (native, non-native or invasive), growth and life history patterns of plants was monitored in order to determine if the burning of slash successfully removed woody invasive alien plant propagules and promoted re-establishment of indigenous plant species. This study was carried out in order to give recommendations for managers, decision makers and conservation efforts tasked with the recovery of indigenous vegetation.

The following research questions guided the study:

- How do different native and invasive species germinate and grow in burned and non-burned riparian soils?
- How do riparian environments recover following biomass removal using stacking and burning of slash, compared to nearby unburned areas?

6.2 MATERIALS AND METHODS

6.2.1 *Ex situ*

6.2.1.1 Study area

The study was conducted at four different sites across the southern part of the Western Cape (Figure 1). The sites are along fynbos riparian zones and were all cleared by WfW, except the Bainskloof site, which was cleared and burned by workers employed by the owner. Each site was burned at a different time, thus are expected to have different states of succession. The sites have a long history of primarily *Eucalyptus spp* and *Acacia mearnsii* invasion. The sites used were the same as used in the study on slash burning effects on soil physicochemical properties, reported earlier (Chapter 4).

- Rawsonville Site (33°43’44.12” S 19°28’26.30” E)
- Robertson Site (33°49’43.33” S 19°52’33.61”)
- Hermon Site (33°28’41.27” S 18°56’14.14” E)
- Bainskloof Site (Wit River; 33°32’42.99” S 19°10’11.32” E)

6.2.1.2 Sampling method

The experiment was conducted in a greenhouse at the Department of Forestry of Stellenbosch University. Some seeds were bought from Silverhills Seeds (*Acacia karroo*, *Brabejum stellatifolium*, *Metrosideros angustifolia*) while others were collected in the field (the Berg River valley, *A. karroo*) or obtained from the Forestry Department, Stellenbosch University (*Eucalyptus camaldulensis*). *Acacia karroo* and *A. mearnsii* seeds were scarified in concentrated sulphuric acid (H_2SO_4) and saturated overnight to increase chances of germination. The seeds were then removed from the acid and rinsed three times with water before they were sowed in the soil. The depth the seeds were sowed was relative to their seed size, i.e. 1 mm-sized seeds were sowed 1 mm deep in the ground.

Unburned soil was collected on Riverside farm which is situated in Rawsonville and ash was collected in Hermon. All the soils were sifted by passing through a 2 mm sieve in order to remove most of the seeds from the seedbank. Some of the soils were thoroughly mixed to ensure soil consistency and placed in 185 x 140 x 50 mm plant trays. Seeds of each of the above-mentioned five plant species were sowed in all four different soil types, namely, burned soil mixed ash (BA), burned soil (BN), non-burned soil mixed with ash (NA) and non-burned soil (NN). For example, seed of *A. karroo* was sowed in BA, BN, NA and NN soil types. Each plant species had five replicas per soil type. The ash trays included a 1 cm thick ash layer that was derived from *E. camaldulensis, i.e.* this specific plant species. The automated irrigation system of the greenhouse was used to water the seeds twice a day.

The seeds were monitored and the number of seeds germinated, plant height and leaf circumference were recorded. Seed germination count, daily growth and leaf circumference were recorded for the first time one week after the seeds were sown. Thereafter, seedlings were monitored consecutively every two to three days for a period of eight weeks.

6.2.2 *In situ*

Each study site was composed of areas subjected to slash piles that burned, where woody alien invasive vegetation was piled and burned. Areas that were cleared of woody alien invasive vegetation were left with no immediate intervention after clearing. The piles varied in diameter (see Table 4.1). The burning of the piles took place after the rainy season or after the start of the rainy season, when soils were relatively moist. This was done to keep fire intensity low, and to ensure safety and mitigate the risk of run-away fires. The time of the fire treatment varied, and is given in Table 4.1. The no-burn treatment received no burning. The study sites were then left to recover for a period of time (time varied between sites). A transect-belt method (Eberhardt, 1978) that consists of five contiguous 1 x 1 m quadrats (Figure 2) was used. This method was selected in order to include the various positions within the fire scars (centre, intermediate position, edge). Within each quadrat, the Whittaker method was used to measure the species richness and abundance (Stohlgren et al., 1995). Percentage vegetation cover was also estimated by calculating projected canopy cover downwards on the ground relative to the size of the quadrat. The diversity was then calculated by using the Simpson index (McIntosh, 1967). Each plant species was documented.

The diversity of the “burned’ and no-burn treatments at each study site was calculated using the Simpson index, in order to determine which treatment had a higher biodiversity rating.

6.2.3 Statistical analysis

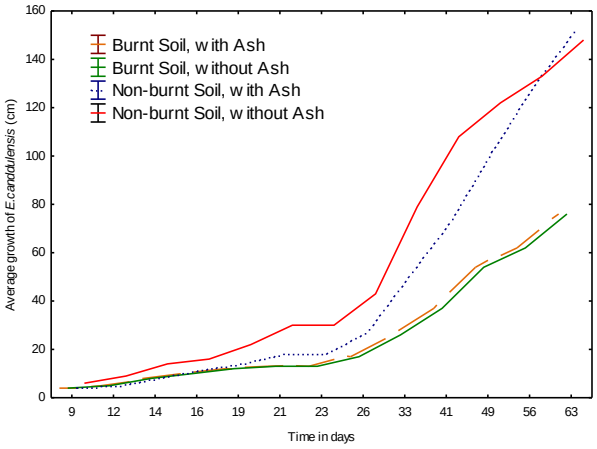
A One-way ANOVA was performed, in order to determine if there was a significant difference in abundance between the burn and no-burn treatments at each of the study sites. Either a Fisher LSD or Tukey HSD Post-hoc test was performed, depending on whether the data was normally or non-normally distributed. A Fisher LSD post-hoc test was done if the data was non-normally distributed (non-parametric) and a Tukey HSD post-hoc test was done if the data was normally distributed (parametric).

6.3 RESULTS

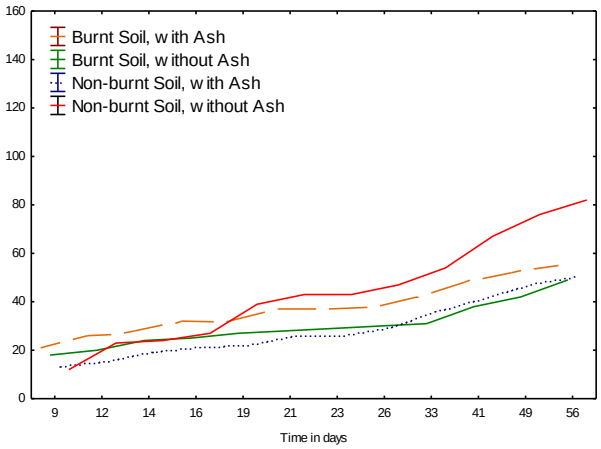
6.3.1 *Ex situ*

Of the five species planted, only two germinated in sufficient number to draw any conclusions. The native species *A. karroo* germinated only in low numbers, and also showed relatively slow growth compared to the invasive species *E. camaldulensis* (Figure 6.1). The non-burned soil without additional ash showed the best germination, with 8 out of 30 seeds that germinated and established. The rest of the treatments did not show differences, but germination was lower than in non-burned soil. The treatment where soils were sampled from non-burned areas, with no additional ash added, showed the best height growth over time, followed by the soil from burned areas with added ash. However, the treatments burned soil with ash, burned soil without ash and non-burned soil with ash showed similar growth over time.

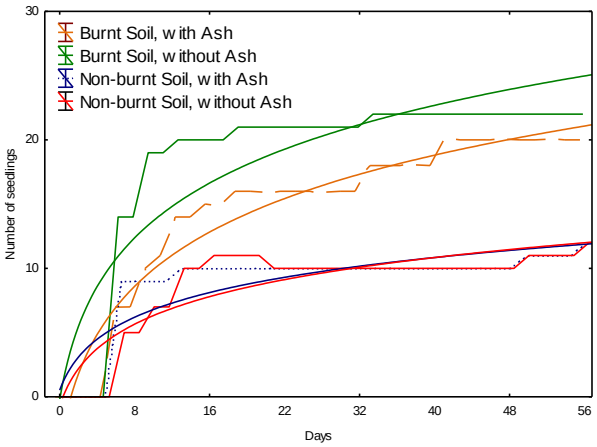
Eucalyptus camaldulensis showed strong germination and fast growth compared to the native species *A. karoo* (Figure 6.1). The burned soil with and without ash showed the strongest germination, while non-burned soil showed lower numbers of seedlings that germinated, though this was still stronger than the native *A. karroo* seedlings. However, despite strong germination of *E. camaldulensis* seeds in soils from burned areas, growth over time showed a declining rate compared to seedlings growing in unburned soils (Figure 6.1). At the end of the experiment after 8 weeks, the *E. camaldulensis* seedlings growing in soils from burned areas reached heights of about 78 cm, while those on non-burned soils reached heights of 145 cm (Figure 6.1a). Visual inspection of *E. camaldulensis* seedlings also showed that seedlings growing in soils from burned areas, with and without ash, showed a red colouration (typical of young seedlings) at the end of the experiment compared to those growing in soils unaffected by slash pile burning (Figure 6.2). In either the burned or the non-burned treatments, addition of ash did not change growth substantially (Figure 6.1).



(a)



b)



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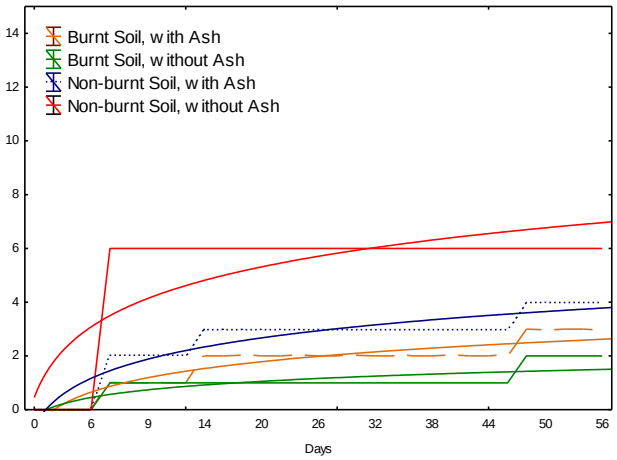
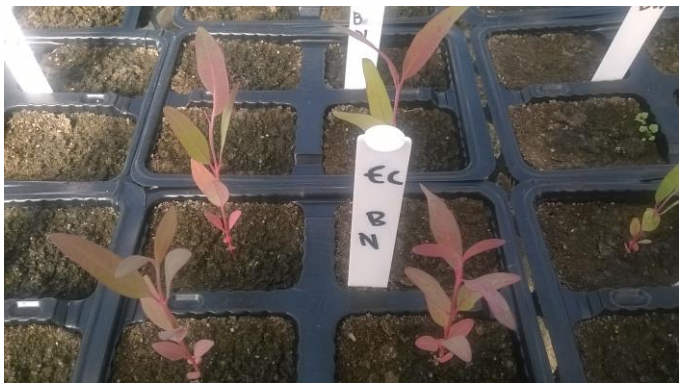


(d)

Figure 6.1: Growth of *E. camaldulensis* seedlings (a), and (b) *A. karroo* seedlings over the course of 9 weeks, with seedlings growing in pots in a greenhouse; (c) shows the number of *E. camaldulensis* seedlings that germinated, while (d) shows the number of *A. karroo* seedlings that germinated.

(a)



(b)

Figure 6.2: Differences in colouration of seedlings after 9 weeks of growth. (a) shows the seedlings growing in non-burned soil and (b) in burned soil.

6.3.2 *In situ*

6.3.2.1 Plant and functional type composition

In order for areas affected by IAPs to recover to their pre-invasion state, a certain level of indigenous propagules must still be present in the ecosystem. The likelihood of an area naturally recovering to its previous state can be determined by looking at the proportion of indigenous to non-native or invasive species. If an area has a low presence of indigenous vegetation the probability of the area recovering to its previous state is unlikely. Most study areas had a high proportion of plant species that are non-native or invasive (Figure 6.3), with Bainskloof showing the highest ratio of non-native to native species. At Robertson and Rawsonville, no native species were present within the burned areas, while the unburned areas did show the presence of some native species. Hermon, the site that was burned first (Figure 6.3d), showed the highest proportion of invasive species for both burned and unburned, with hardly any difference between the two treatments.

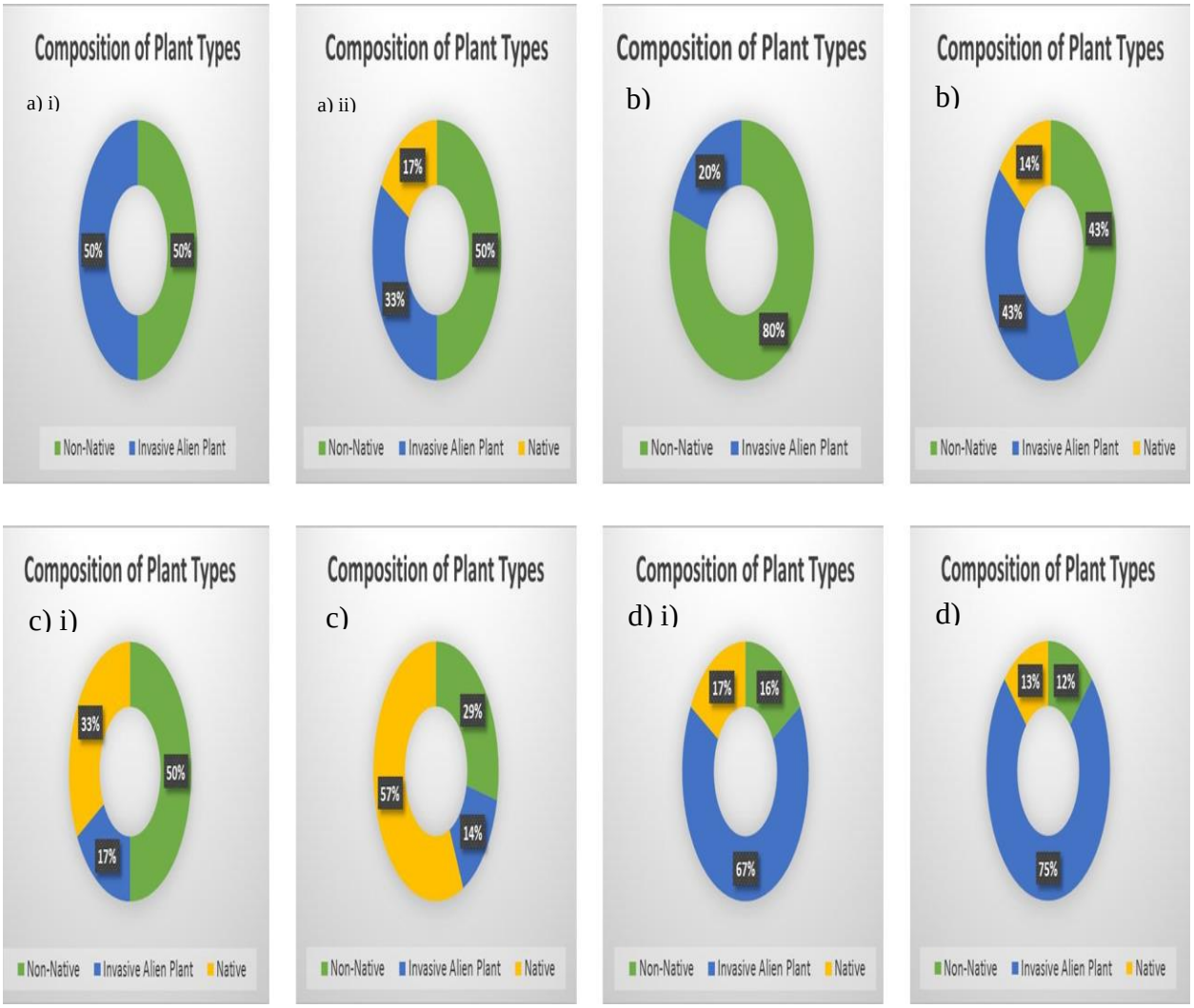


Figure 6.3: Pie charts that illustrating the number of species of each of the categories, native, non-native and invasive between the burned and unburned areas at the different study sites. A) Robertson; B) Rawsonville; C) Bainskloof i) and D) Hermon. Unburned i) Burned ii)

6.3.2.2 Total vegetation cover

There was a significant difference in vegetation cover between the burn and non-burn treatments at the Robertson, Rawsonville and Hermon study sites ($p<0.05$), with the non-burn treatment having a higher vegetation cover than the burn treatment (Figure 6.4). There was, however, not a significant difference in vegetation cover between the burn and non-burn treatments at the Bainskloof study site ($p>0.05$), with the non-burn treatment having a higher, but not significantly higher, vegetation cover than the burn treatment (Figure 6.4c). There was a significant difference in vegetation cover between the burn and burn treatments across all the study sites ($p<0.05$), with the non-burn treatment having a significantly higher vegetation cover than the burn treatment.

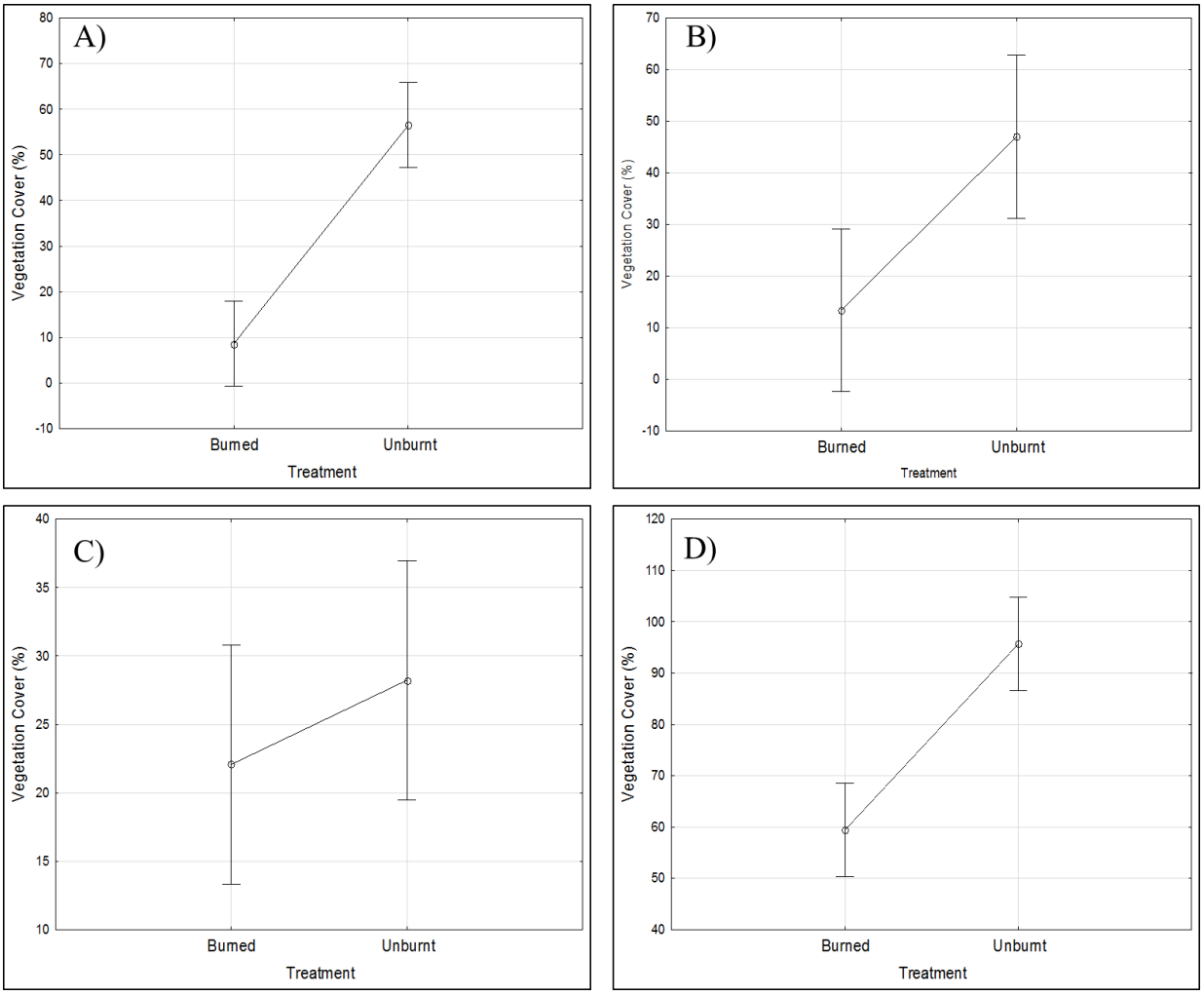


Figure 6.4: Graphs that illustrate the difference in vegetation cover between the burn and non-burn treatments at the different study sites. A) Robertson, B) Rawsonville, C) Bainskloof, D) Hermon.

6.3.2.3 Invasive alien plant abundance

There was no significant difference in invasive alien plant abundance between the burn and no-burn treatments at the Robertson study site ($p>0.05$), with the no-burn treatment having a higher abundance than the burn treatment (Figure 6.5a). There was a significant difference in invasive alien plant abundance between the burn and no-burn treatments at the Rawsonville study site ($p<0.05$), with the no-burn treatment having a higher invasive alien plant abundance than the burn treatment (Figure 6.5B).

There was no significant difference in invasive alien plant abundance between the burn and no-burn treatments at the Bainskloof study site ($p>0.05$), with the unburned treatment having a higher invasive alien plant abundance than the burned treatment (Figure 6.5C). There was a significant difference in invasive alien plant abundance between the burn and no-burn treatments at the Hermon study site ($p<0.05$), with the no-burn treatment having a higher invasive alien plant abundance than the burn treatment (Figure 6.5D). There was no significant difference in invasive alien plant abundance between the burn and no-burn treatments across all sites ($p<0.05$), with the no-burn treatments having significantly higher invasive alien plant abundance than the burn treatments.

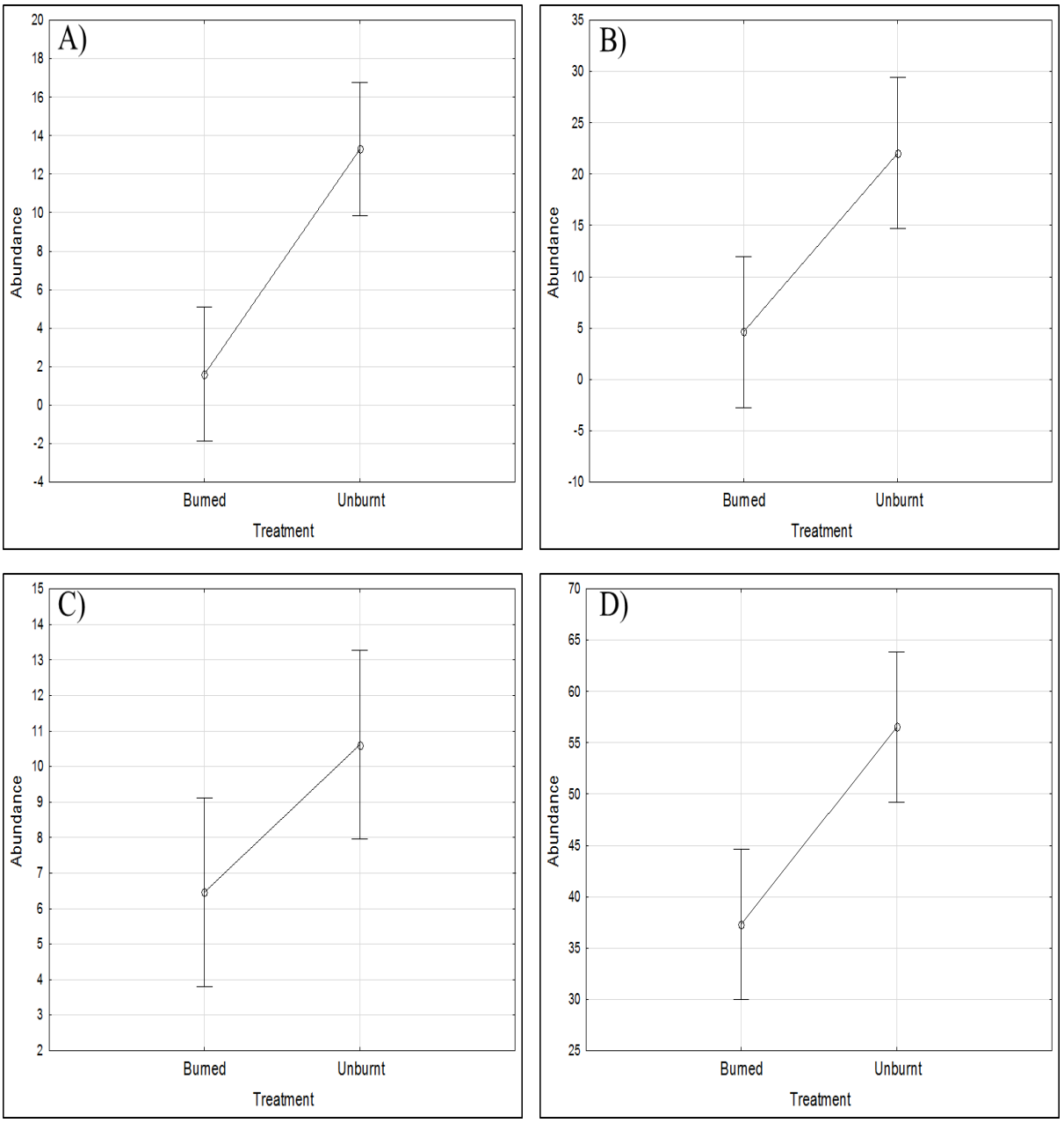


Figure 6.5: Graphs that illustrates the difference in IAP abundance between the burn and non-burn treatments at the different study sites. A) Robertson, B) Rawsonville, C) Bainskloof, D) Hermon.

6.3.2.4 Species richness

There was a significant difference in overall species richness between the burn and non-burn treatments at the Robertson study site ($p<0.05$), with the no-burn treatment having a higher species richness than the burn treatment (Figure 6.6). There was also a significant difference in species richness between the burn and no-burn treatments at the Rawsonville study site ($p<0.05$), as well as at the Bainskloof study site ($p<0.05$), with the unburned treatment having a higher species richness than the burned treatment in both cases (Figure 6.6C). Also at the Hermon study site the unburned treatment had significantly higher species richness compared to the burned treatment (Figure 6.6). A One- Way ANOVA was performed to determine possible differences in species richness between burn and no-burn treatments across all study sites. There was a significant difference in species richness between the burn and no-burn treatments across all sites ($p<0.05$), with the no-burn treatments having significantly higher abundance than the burn treatments.

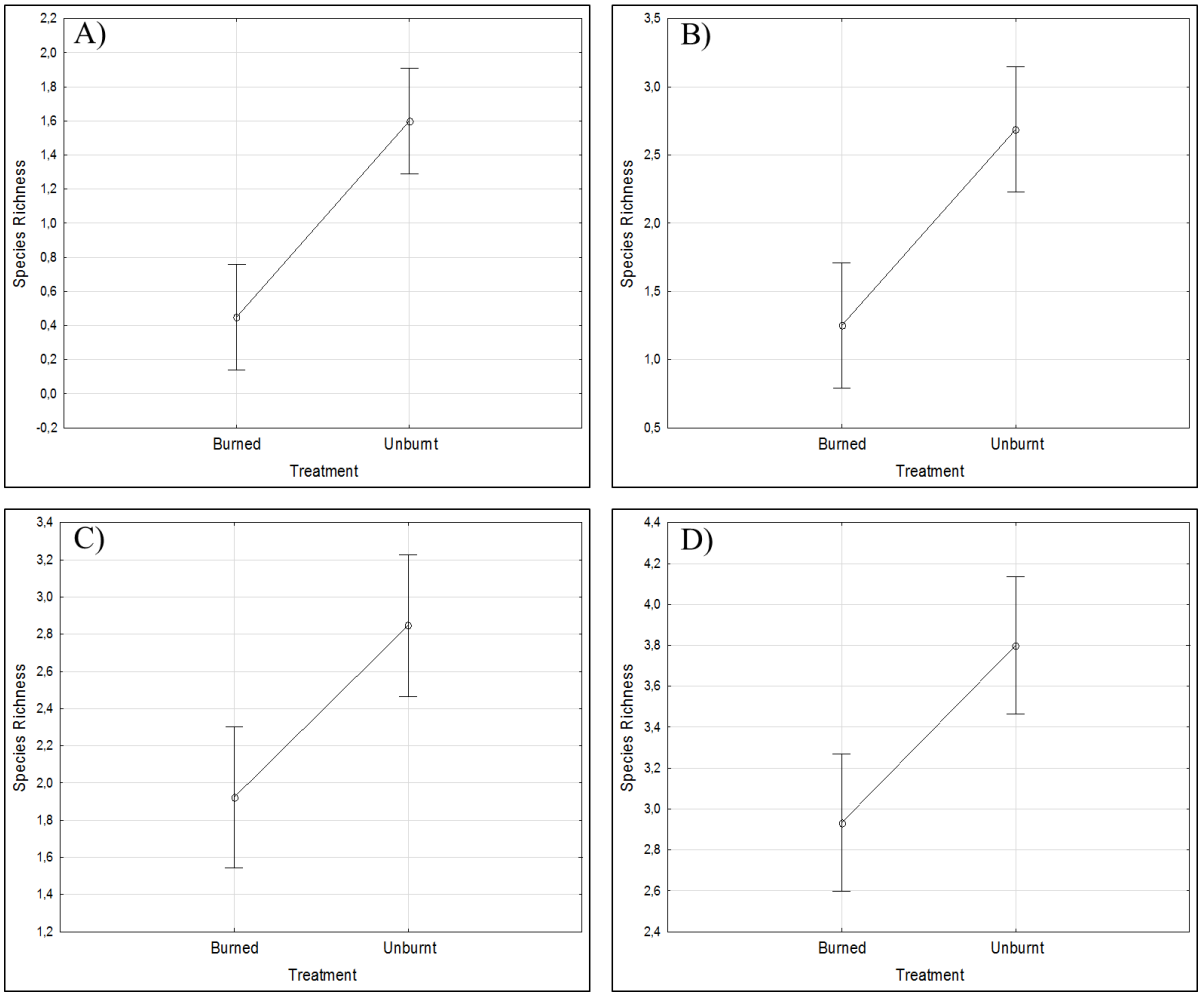


Figure 6.6: Graphs that illustrates the difference in species richness between the burned and 'unburned' treatments at the different study sites. A) Robertson, B) Rawsonville, C) Bainskloof, D) Hermon.

6.3.2.5 Diversity

Interestingly, the Robertson and Rawsonville study sites, which were at an early successional state (cleared last), had a higher biodiversity rating at the burn treatments than the no-burn treatments according to the index (Table 6.1). The study sites at a later successional state (Bainskloof and Hermon), had a higher biodiversity rating at the no-burn treatments than the burn treatments according to the index (Table 6.1). This can be explained by the fact that areas that are dominated by one or two species are considered to be less diverse than areas which have several different species with similar abundances. The Simpson index takes into account the number of species present, as well as the relative abundance of each species. We know that as species richness and evenness increase, so too does diversity. Therefore, although the study sites at early successional states (Robertson and Rawsonville) have a higher species richness and abundance, their evenness is lower therefore their diversity index is lower. The sites at a later successional state (Bainskloof and Hermon) had a more equally shared distribution of species between the two treatments, with diversity favouring the no-burn treatments.

Table 6.1: Table that shows the calculated Simpson Index between different plot types at the different study sites. Highest Simpson Index value at each study is indicated in bold.

SITE	**Robertson**		**Rawsonville**		**Bainskloof**		**Hermon**	
Plot type	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned
S (Number of species)	4	6	6	7	6	7	6	8
N (Total number of individuals)	32	257	74	353	78	127	535	788
Simpsons Index (D)	2.19	1.55	2.91	2.42	3.43	4.24	2.43	2.95

6.3.2.6 Community similarity

To further understand to what extent the burn and no-burn treatments' vegetation differed from each other, the study proceeded by determining the similarity between the treatments using the Sorenson's Coefficient. The coefficient uses a value falling between 0 and 1; the closer the value is to 1 the higher the similarity is. The outcome of the similarity test helps support previous assumptions to some extent, since it was found that at all the study sites there was a high similarity between the two treatments (Table 6.2). This also help support the assumption made earlier, that the stack and pile burning method does not suffice in promoting the re-establishment of native/indigenous plants. This is evident from the high similarity between the plots, probably due to the inability of fire scars to prevent the re- establishment of IAPs and other non-native plants in the area.

Table 6.2: Table that shows the calculated Sorenson's Coefficient between the two plots (burn and non-burn) at the different study sites

Study Sites	Robertson	Rawsonville	Bainskloof	Hermon
Sorenson's Coefficient (CC)	0.60	0.62	0.77	0.71

6.4 DISCUSSION

6.4.1 *Ex situ*

Only two species germinated in sufficient number to warrant further analyses, namely *Acacia karroo* and *Eucalyptus camaldulensis*. The native species *A. karroo* germinated only in small numbers compared to the invasive species *E. camaldulensis*, and also grew slower. The invasive species germinated in larger numbers in the burned soil, possibly the effect of smoke-derived compounds on a species that grows in its native range in fire-driven ecosystems (Bradstock et al., 2012). Similar responses can be seen in local fynbos species which germinate upon fire queues, with the absence of smoke typically resulting in low germination (Boucher et al., 2004; Brown, 1993). However, this response was not evident in *A. karroo*, which is confined to ephemeral river valleys in Karoo environments, occasionally occurring in riparian environments alongside perennial rivers in the fynbos and succulent Karoo biomes (Mucina and Rutherford, 2006) (and also in the forest and savanna biome; Archibald and Bond, 2003). Instead, a mixed response was seen, with seeds germinating in both unburned and burned treatments, contrasting with the consistently fire-induced initial response by *E. camaldulensis*. These responses may indicate a higher response in burned patches in the invasive species compared to native species; however, much more information is needed, warranting further studies to compare native and invasive plant species' responses.

Growth in *E. camaldulensis* showed the opposite response compared to germination. Over the course of nine weeks, growth of seedlings in soils from burned areas fell behind those of non-burned areas, regardless of whether additional ash was added. The seedlings from burned areas were half the height of the seedlings from the non-burned areas, and also appeared to have a different foliage colouration compared to the other treatments. *Eucalyptus* seedlings are known to go through a phase where foliage appears red, which later disappears. However, seedlings in the burned soils appeared to be still in this phase when compared to non-burned seedlings. The lack of growth and the discolouration may be an indication that burning may have induced either a nutrient limitation or toxicity, which suggests that these seedlings may be outcompeted by seedlings that may be better adapted to the soil chemical conditions. *A. karroo* also does not show fast growth, and would be unlikely to be able to compete with *E. camaldulensis* growing in fire scars. It is unclear from these results which of these two species are able to establish successfully and grow in fire scars, where cover also appears to be low for the first year post fire. It seems more likely that herbaceous, specifically alien invasive grass, species might be better adapted to grow in fire scars, as was found in studies from the North American continent (Korb et al., 2004; Rhoades et al., 2015). More studies are needed to identify which species might be able to grow fast enough to be competitive in the post fire environment.

6.4.2 *In situ*

The study found significant differences in abundance between the two initial clearing methods at all the study sites and across all the study sites, therefore indicating that the biomass management

method has a significant influence on abundance of recovering vegetation (clearing and removal versus slash burning). This result is also supported by a study done by Blanchard (2008), which found similar results. From the results, we also see that the burn (slash pile burning) treatment greatly inhibits abundance of regenerating plant species compared to the unburned treatment. The significantly higher abundance found at the Hermon study site compared to the other study sites, could be explained by the timing of the burning. The slash pile at the Hermon study site was burned eight months prior to the other sites, meaning that the site had eight months longer to recover, thus more plants had regenerated in this period. This suggests that a longer time period may be necessary for plants to establish in burn scars due to seed limitation, soil conditions that are not conducive to germination and establishment of plants, as shown by Chapter 4. Fire is also known to significantly reduce mycorrhizal fungal propagules in the top few centimeters of soil (Deka and Mishra, 1983). Arbuscular mycorrhizal fungi (AMF) are known to have mutualistic relationships with plants and generally promote plant growth (Haskins and Gehring, 2004). AMF can therefore be critical to the survival of many species, and a reduction in mycorrhizal fungal propagules may reduce the re-establishment of the plant community by reducing the abundance of mycorrhiza-dependent plant species (Haskins and Gehring, 2004). The reduction in AMF, seed limitation and soil conditions therefore could be some of the reasons that can explain the lack of abundance at the burn treatments. However further research would be required to confirm this assumption.

The study found significant differences in vegetation cover between the burned and non-burned treatments at all the study sites (except the Bainskloof study site) and across all the study sites. This indicates that the method of clearing of biomass has a significant influence on vegetation cover of plant species in cleared riparian landscapes. This result is also supported by a study done by Blanchard and Holmes (2008), which found similar results in fynbos riparian sites. The “slash and burn” treatment (slash pile burning), appears to not promote recovery of vegetation after alien clearing, at least not in the first few months. This can be seen by the significantly lower vegetation cover of the burn treatment compared to the non-burn treatment. The Bainskloof study site experienced a flood, which could explain the non-significant difference in vegetation cover between the burn and non-burned treatment. Flowing water could have dispersed seeds from adjacent seed banks where they were deposited on the previously burned areas. This could serve as an explanation to why the non-burn treatments had a significantly higher abundance across all the sites and within the study sites excluding Bainskloof.

The study found significant differences in species richness between the two initial clearing methods within each study site and across all the study sites. This indicates that the initial clearing method has a significant influence on the species richness of recovering vegetation. Blanchard (2008) also found that after the initial alien clearing, in areas where slash piles were burned, a significantly lower species richness was present compared to areas that were not burned. Fire is known to affect soil structure, nutrient cycling and soil pH (see Chapter 4). It is therefore possible that the alteration of these soil properties due to the fire at the burn treatment, made conditions for germination of some plant species unfavourable. Seeds and seedlings in the seedbank may remain dormant due to unfavourable conditions. The IAPs in contrast are more resilient against these changes and therefore their seeds may germinate, even in conditions not favoured by native species. This could help explain why the ‘unburned’ treatment had a significantly higher species richness than the burn treatment.

The high community similarity that was found between the two treatments over all of the study sites is an indication of similar plant species regenerating after the alien clearing effort. This could be due to the seedbank of the two treatments, containing seeds or seedlings of similar plant species. However the fire at the burn treatment likely led to the mortality of several plant seeds in its seed bank. An

alternative suggestion is that the high presence of IAPs at both treatments accounted for the high similarity.

This study therefore discourages the use of the slash pile burning method, and is in line with a previous study (Blanchard and Holmes, 2008). Where it is logistically and financially possible, the fell and remove method should be used, since previous studies have found it best in terms of vegetation recovery (Blanchard and Holmes, 2008). Alternatively, wood can be removed for use within local communities for firewood, structural wood or other uses, while finer material may still remain on site.

CHAPTER 7: The biomass and nutrient content of *Eucalyptus camaldulensis* and *Acacia mearnsii* trees of different sizes growing at different stem densities in riparian sites from two different longitudinal zones

7.1 INTRODUCTION

The South African government reported that revenue generated from the export of forestry products has doubled in the period from 1998 to 2008 from less than R7 billion to almost R15 billion, effectively making South Africa a net exporter of forest products, as reported in the State of the Forests Report (2009). Apart from its importance in increasing the Country's GDP (approx. 1.2% of total GDP), plantations are often located in or nearby poverty-stricken areas, assisting with job creation and education in these areas. However, invasion of these forestry species into natural South African ecosystems has caused various ecological problems such as increases in biomass and litterfall (influencing fire dynamics), decreases in stream flow (*Acacia* spp*., Pinus* spp*., Eucalyptus* spp.), changes in soil water repellency (*Eucalyptus* spp.) and changes in soil nutrient dynamics (Yelenik et al., 2004; Jacobs et al., 2013). Riparian ecotones are hotspots for processes that underlie ecosystems services such as production of clean water and immobilisation of toxins, and act as habitat for riparian plant and animal species. With increasing understanding of riparian and aquatic processes, the roles of various nutrients in supporting riparian ecosystem services are also becoming clear. Invasive trees in these riparian zones can grow in very dense stands and can reach large sizes, which implies that significant amounts of nutrients are locked up in biomass. Under natural conditions, nutrients eventually find their way to riparian soils and the aquatic environment where they are considered allochthonous subsidies, important to support food webs and ecological function. Invasive trees may add elements to riparian nutrient stocks such as nitrogen (fixed by invasive legumes from atmospheric sources), but may sequester others such as phosphorus and microelements.

Invasion by woody alien species in South Africa is thus an important economic and ecological problem. This is evident in the Western Cape region, where large-scale invasions in riparian zones by species such as *Acacia mearnsii (Black wattle)* and *Eucalyptus camaldulensis* (River red gum) have caused significant losses in natural plant diversity and reduced low flows, subsequently necessitating the input of government funds aimed at their control and eventual eradication. WfW, aimed at eradicating alien plants mainly along river systems, has spent just under R700 million on alien clearing and education programmes in the year 2013-2014.

A cost analysis by de Wit et al. (2001) suggested that the losses in value of natural fynbos in South Africa as a result of invasion could, in total, amount to US $11.75 billion (R149.67 billion), total losses in water would amount to the value of US $3.2 billion (R40.76 billion), the net cost of managing just *A. mearnsii* was estimated at US $1.4 billion (R17.83 billion), and it would cost about US $60 million (R764.25 million) annually to clear invasive alien plants over a period of 20 years. These costs significantly affect estimations of the net economic benefits (or costs) of the forestry sector. Conversely, the quick-growing nature of the plants has long provided rural communities with a reliable source of goods such as firewood, timber, and bark (sold for tannins). In addition to this, WfW, coupled with the regenerative ability of invasive species, provides relatively stable income streams to disadvantaged communities where the plants occur. It is thus important to explore more ways in which woody invasive trees can be utilised to the economic and ecological benefit of people and ecosystems affected by them, in order to minimise the amount of water resources lost to woody tree invasion, while also minimising the net financial cost of clearing.

Acacia mearnsii is one of the most invasive woody plant species in the Western Cape. It is often referred to as a transformer species, as it is known to change soil chemical and biological properties, while also influencing growth of neighbouring plant species (Naudé, 2012; Boudiaf et al., 2013). Tye and Drake (2011) suggested that the species also affects nutrient cycles more than native *Acacia* species such as *A. karroo*, leading to eventual enrichment of soil nutrients such as N, and has far-reaching ecological impacts, including establishment of other nuisance plant species and eutrophication in river systems, while also reducing ecosystem services. This is also true for other invasive *Acacia* species, including *A. saligna*, *A. cyclops*, *A. longifolia,* and *A. dealbata* (Stock et al., 1995; Gaertner et al., 2011; Le Maitre et al., 2011). The notorious invasiveness of the species along riparian zones has been ascribed, in part, to its large investment in propagules, which are easily transported downstream by rivers, or stored in the soil to germinate at a later stage (Richardson et al., 2008).

Eucalyptus camaldulensis, another widely distributed species, has become naturalised and invasive along riparian zones in South Africa. Nutrient dynamics within the various biomass components of *E. camaldulensis* trees have been studied in Brazil (Bernardo et al., 1998; Harrison et al., 2000), and in New Zealand and Australia (Judd et al., 1996; Francis and Sheldon 2002). Findings from these studies suggest that, even though *E. camaldulensis* partitions its resources to various tree components based on physical environmental conditions, a strong relationship could exist between nutrient partitioning, the age of the stand, and the spacing between individual trees. Greater spacing, for instance, could result in an increase in concentrations of N and P in bolewood and taproots. Harrison et al. (2007) suggested this might be due to a decrease in competition as spacing increases.

Data is available on the indirect effects of stands of *A. mearnsii* on nutrient cycling in fynbos soils (i.e. associations with root bacteria and increases in soil enzymes; Naudé, 2012; Slabbert, 2012; Kambaj Kambol, 2013; Slabbert et al., 2014), but nutrient concentrations released directly from plant material have not yet been quantified. This exposes a gap in our knowledge of the total inputs of nutrients to the affected ecosystem. There is also biomass and nutrient data available for the species when it has been grown in plantations (Dovey and du Toit, 2004) which provides a solid baseline for the measurement of tree biomass in managed plantations, and the partitioning of nutrients and biomass to various tree components. Dovey and du Toit (2004) also provide allometric models to predict these variables in plantations. There are, however major differences between plantation and forest dynamics, including growth form of trees (allometry), environmental stressors, tree life stages, and phenology. There are also management activities in plantations directed at increasing the volume and quality of merchantable wood per unit area used (Pinkard, 2002; Montagu et al., 2003; de Moraes Goncalves et al., 2004; Forrester et al., 2005; Kelty, 2006; Nichols et al., 2006; Muñoz et al., 2008). Management of plantations thus typically results in significant differences in productivity, and data generated from such systems cannot easily be compared to wild forest systems.

Another difference between managed plants and those growing in wild forests is naturally occurring periods of change that aren't buffered by management practices to maximise yield. This has implications for the resulting growth form of trees and also greatly impacts the subsequent nutrient distribution within trees. Fife et al. (2008) suggested that an evergreen tree may move nutrients between different tree components during different stages of the plant's lifetime to accommodate its changing needs as it enters various life stages. This has also been found by Lödige et al. (2014), who showed in a greenhouse experiment that differences in tree size of *Fagus sylvatica* (European beech) and *Picea abies* (Norway spruce) correlated with differences in partitioning of above-ground biomass. Environmental conditions such as drought and lack of sufficient light could also (to a lesser extent than tree size) skew above-ground biomass partitioning but will not be discussed here.

Assessment of available literature suggests that quantitative research on nutrient dynamics and temporal and spatial shifts in nutrient stocks of either *A. mearnsii* or *E. camaldulensis* is currently lacking. In the Western Cape, several studies have focussed on soil nutrient dynamics as affected by invasive alien plants and the legacy after they have been cleared. Although studies of soils associated with these plants can provide a fairly good idea of the state of nutrients in an area, such studies do not provide a holistic view of nutrient dynamics and transfer between ecological components. Studies of soil in isolation also do not provide much insight into temporal changes in nutrient dynamics within plants, or response to differences in trees' spatial arrangement (dense vs freestanding). Such studies also do not provide information on potential nutrient losses following harvesting of the trees, chipping and use of those chips elsewhere, including export to overseas markets. To advance towards a holistic view of nutrient dynamics in invaded riparian zones, it is important to include studies of this element.

This study aimed to quantify the biomass and standing stocks of nutrients inside stands of *A. mearnsii* and *E. camaldulensis* along riparian zones in the Western Cape. It was also designed to provide data on seasonal retranslocation of nutrients to different tree components. The study uses data on standing biomass and stocks of nutrients in the species tested to generate allometric models which can be used to predict these variables through simple (linear or non-linear) regressions and to address possible nutrient losses from previously invaded and cleared riparian systems.

7.2 MATERIALS AND METHODS

7.2.1 Site description and methodology

Four sites were identified, all located in riverine ecosystems in the Boland region of the Western Cape. Two are invaded by *A. mearnsii* and two by *E. camaldulensis*. These sites are: Bainskloof (*A. mearnsii*), Wolseley (*E. camaldulensis*), and Alfalfa (one *A. mearnsii site* and one *E. camaldulensis site*).

For biomass measurements and determinations of nutrient content for allometric scaling, six individuals of each species were sampled destructively from three life stages (juvenile, sapling, and adult) at the four sites. Sampling within sites was done separately in areas of dense stands and areas with freestanding trees. These were handled separately throughout the study to accommodate differences in growth dynamics and hence biomass and nutrient partitioning. Trees were harvested destructively and wet mass measurements were taken of all above-ground tree parts. All subsamples were weighed, including a subsample which was dried to constant mass, and weighed again for biomass determinations. Biomass estimations included inventory of the trees used, and explicit compartmentalisation taking into consideration the different dimensions of the trees. The trees were thus divided into leaves, twigs and branches, and the trunk, which was divided into stem and bark and sawed into logs for ease of weighing. Samples were ground to a powder after which a subsample was subjected to combustion for nutrient determinations.

Measurements were taken of the trees' basal diameter (BD). Subsamples of all tree parts (after being weighed in the field) were dried at 40°C in the laboratory, until a stable dry mass was achieved. Data gathered from subsamples was used to extrapolate to values for total biomass of various components per tree, which were secondarily extrapolated to stand level.

Nutrients tested for included total N, total P, total C, base cations (Ca^{+}, K, Mg, Na), and a range of micronutrients (Z, Fe, Cu, Mn). Tests for nutrients were done through dry combustion of powdered plant material. The results generated were used to produce allometric equations to predict standing

stocks of these nutrients in the different components of trees of the different species. This data, combined with biomass data, can ultimately be used to extrapolate findings to a landscape level, and to produce composite allometric models to predict nutrient standing stocks in entire stands of *A. mearnsii* and *E. camaldulensis*.

7.3 RESULTS

7.3.1 Biomass

Trees were broken up into their various components, and also treated according to their stand density (i.e. dense vs freestanding). A factorial ANOVA was carried out to determine how the biomass components change as a result of these two variables for both species.

7.3.1.1 Eucalyptus camaldulensis

Figure 7.1 shows that, as *Eucalyptus camaldulensis* trees grow older, there is a general downwards trend in the percentage of the total biomass that is occupied by the leaf component.. There are significant differences in the leaf component between the different life stages, with the adult eucalypt trees growing in dense stands consistently having lower percentage of leaves than those at lower densities.

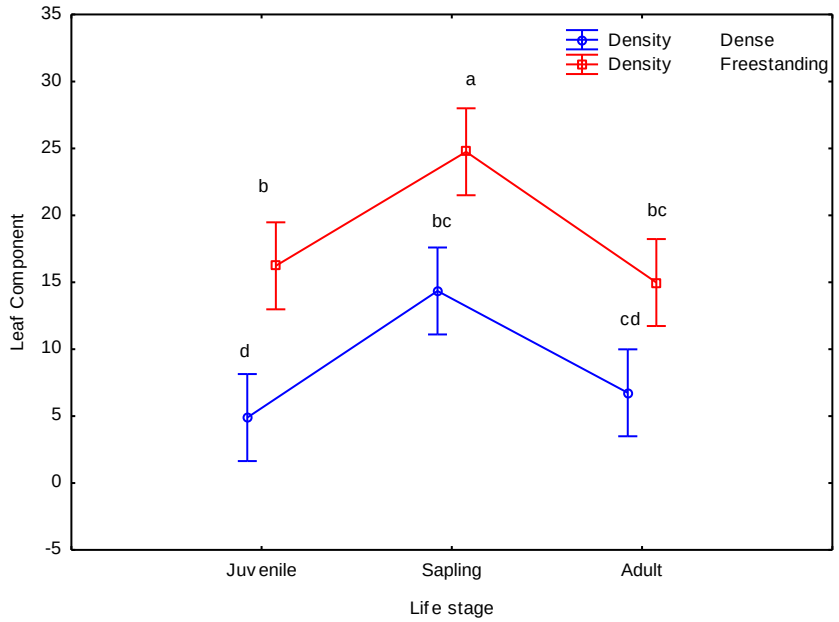


Figure 7.1: Change in leaf component of *Eucalyptus camaldulensis* between three life stages: juvenile, sapling, and adult. Letters above error bars show where significant differences were found (Fisher's LSD test; $p<0.05$).

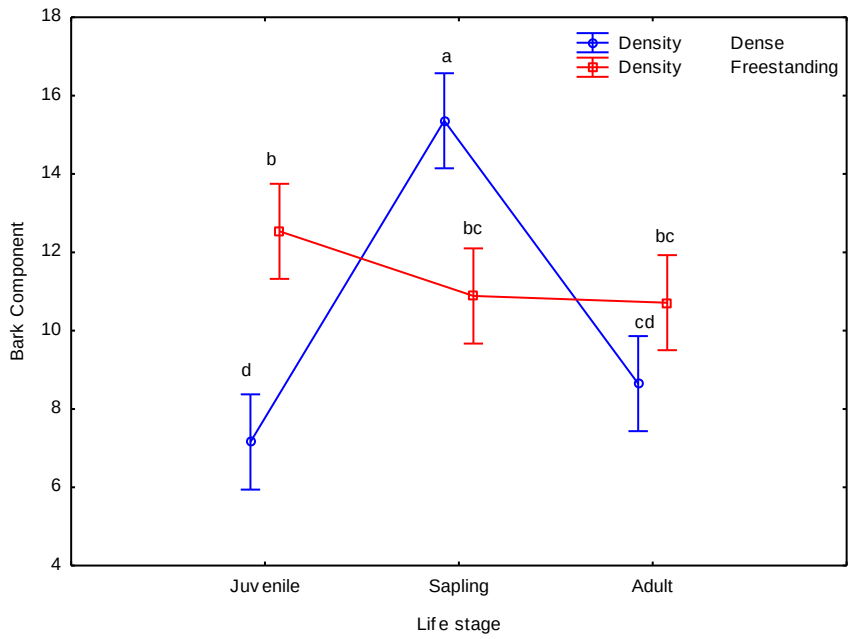


Figure 7.2: Change in bark component *of Eucalyptus camaldulensis* between three life stages: juvenile, sapling, and adult. Letters above error bars show where significant differences were found (Fisher's LSD test; $p<0.05$)

Figure 7.2 shows a slight decrease in bark component from juvenile to adult in freestanding trees. It also shows that saplings growing in dense stands had a significantly greater bark proportion than any of the other categories. Adults and juveniles that grow in dense stands also have a significantly lower bark component than other categories (LSD; $p<0.05$).

Figure 7.3 shows the percentage change of the twig and branch component in *E. camaldulensis*, between sites of two different densities and between different life stages. There was a significant difference between trees from different life stages in dense stands (LSD; $p<0.05$). The twig and branch component peaks for dense stands at the sapling stage and then decreases towards the adult stage. For freestanding trees, there is a constant but non-significant decrease in twigs and branches component from juvenile to adult.

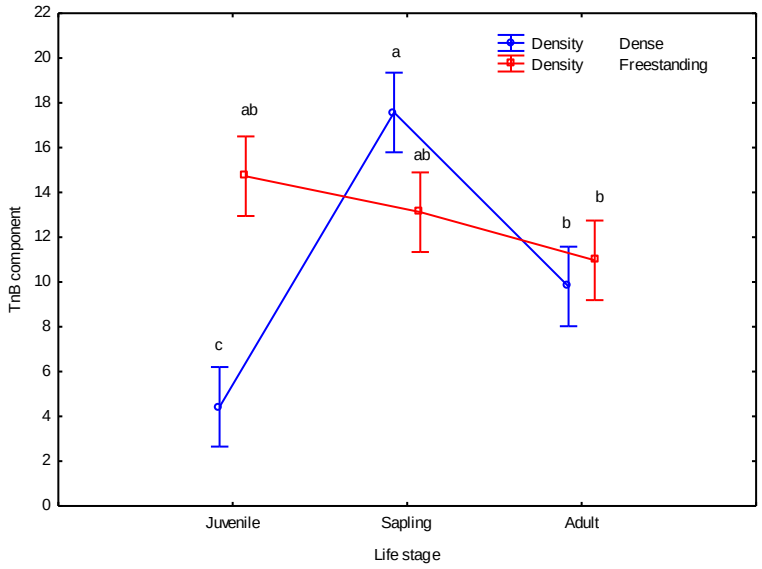


Figure 7.3: Change in twig and branch component of *Eucalyptus camaldulensis* between three life stages, juvenile, sapling, and adult. Letters above error bars show where significant differences were found (Fisher's LSD test; $p<0.05$)

The stem component of freestanding trees is greatest at the adult stage, but differed significantly only from the sapling stage (LSD; $p=0.0238$). The lowest percentage of stem component is found in saplings for both density extremes. In both cases, stem component is significantly less than at the adult stage, and in dense stands also significantly less than the juvenile stage.

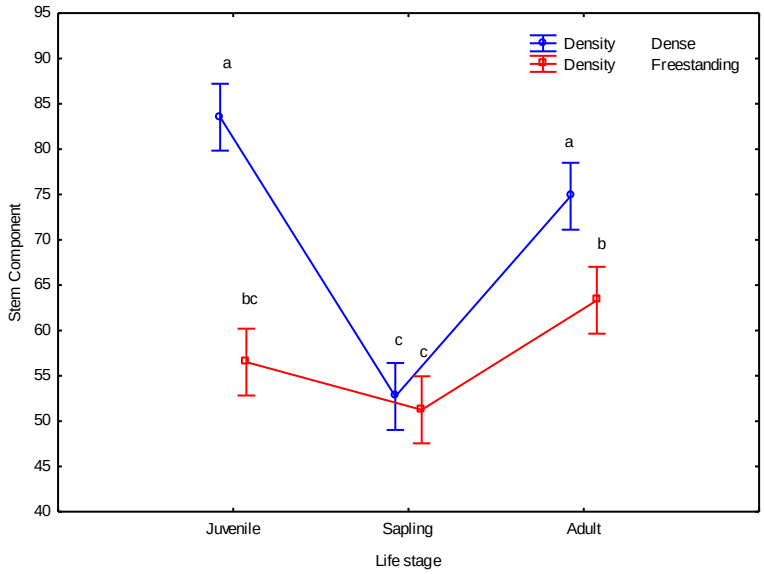


Figure 7.4: Change in stem component of *Eucalyptus camaldulensis* between three life stages**:** juvenile, sapling, and adult. Letters above error bars show where significant differences were found (Fisher's LSD test; $p<0.05$)

7.3.1.2 Acacia mearnsii

The leaf component *of Acacia mearnsii* declines significantly with progression of growth stage (Figure 7. 5). The only significant difference, however, was between adults and saplings from dense stands and juvenile trees from freestanding stands, the former two having a significantly lower leaf component (LSD; $p<0.05$). The leaf component of the within life stages between the different densities differed significantly for juveniles and adults (LSD; $p<0.05$), but not for saplings (LSD; $p=0.742$).

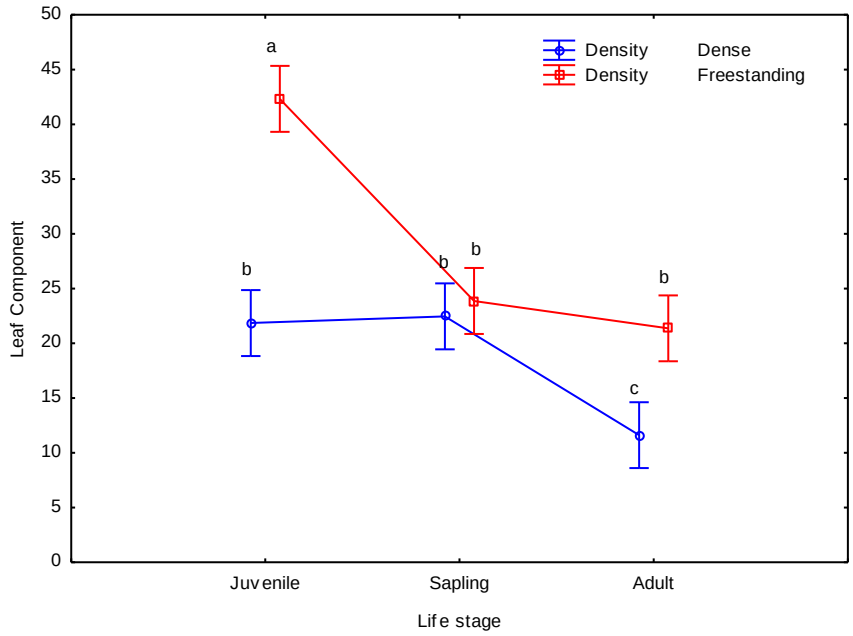


Figure 7.5: Change in leaf component of *Acacia mearnsii* between three life stages: juvenile, sapling, and adult. Letters above error bars show where significant differences were found (Fisher's LSD test; $p<0.05$)

The bark component of *A. mearnsii* also decreases significantly as trees grow bigger, with adult plants having significantly less biomass attributed to the bark component than juvenile trees (Figure 7.6). The bark component of freestanding trees remained unchanged throughout the various life stages (LSD; $p\geq0.05$).

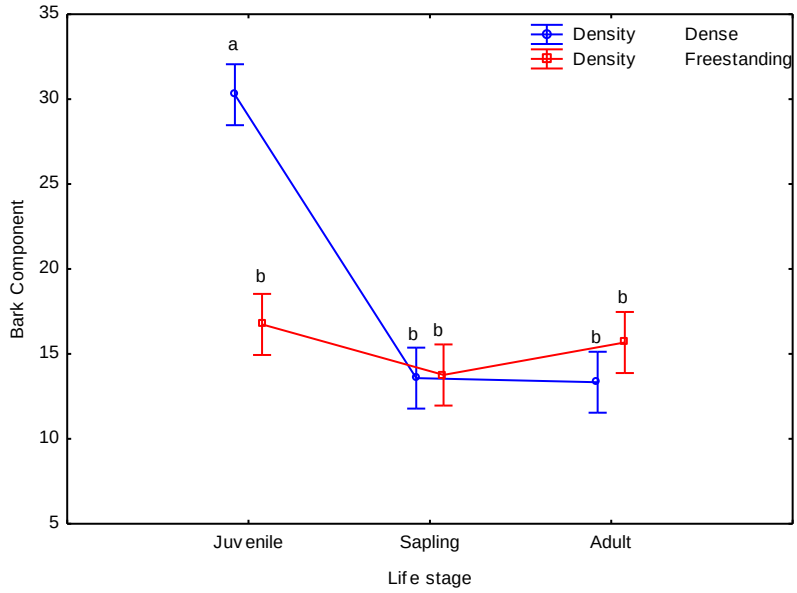


Figure 7.6: Change in bark component of *Acacia mearnsii* between three life stages: juvenile, sapling, and adult. Letters above error bars show where significant differences were found (Fisher's LSD test; $p<0.05$)

The stem component of the total biomass of *A. mearnsii* increases significantly over the different life stages for both density categories (factorial ANOVA; $p<0.05$). Adult trees from dense stands had a significantly greater stem biomass component than that of juveniles and saplings from both densities (LSD; $p<0.05$), while freestanding adults do not increase their stem biomass component after the sapling stage.

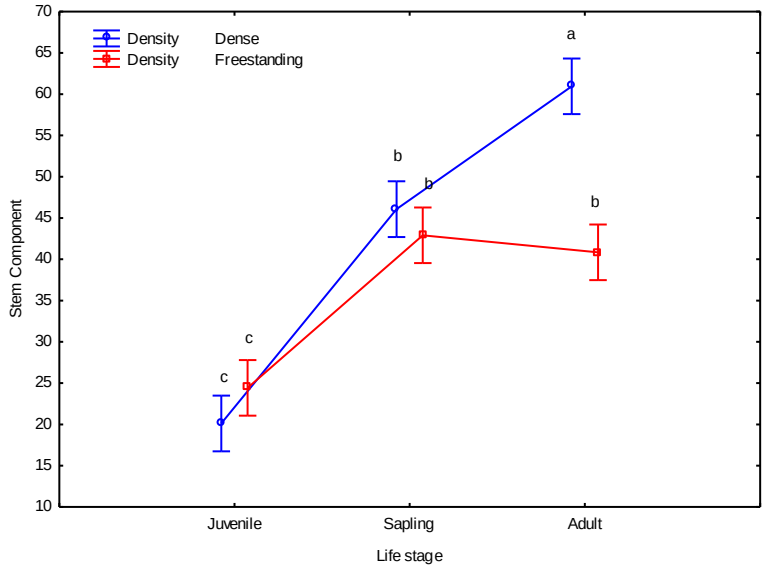


Figure 7.7: Change in stem component of *Acacia mearnsii* between three life stages: juvenile, sapling, and adult. Letters above error bars show where significant differences were found (LSD; $p<0.05$)

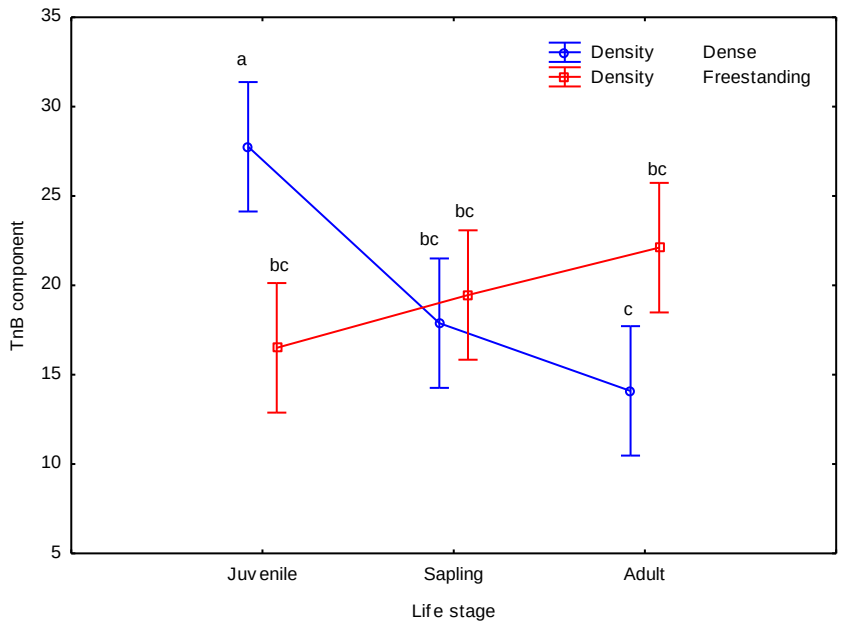


Figure 7.8: Change in twig and branch components of *Acacia mearnsii* between three life stages: juvenile, sapling, and adult. Letters above error bars show where significant differences were found (Fisher's LSD test; $p<0.05$)

The twig and branch components of freestanding individuals increase constantly but not significantly (LSD; $p\geq0.05$) from juvenile to the adult stage, while the twig and branch components decrease significantly in the dense stands (LSD; $p<0.05$).

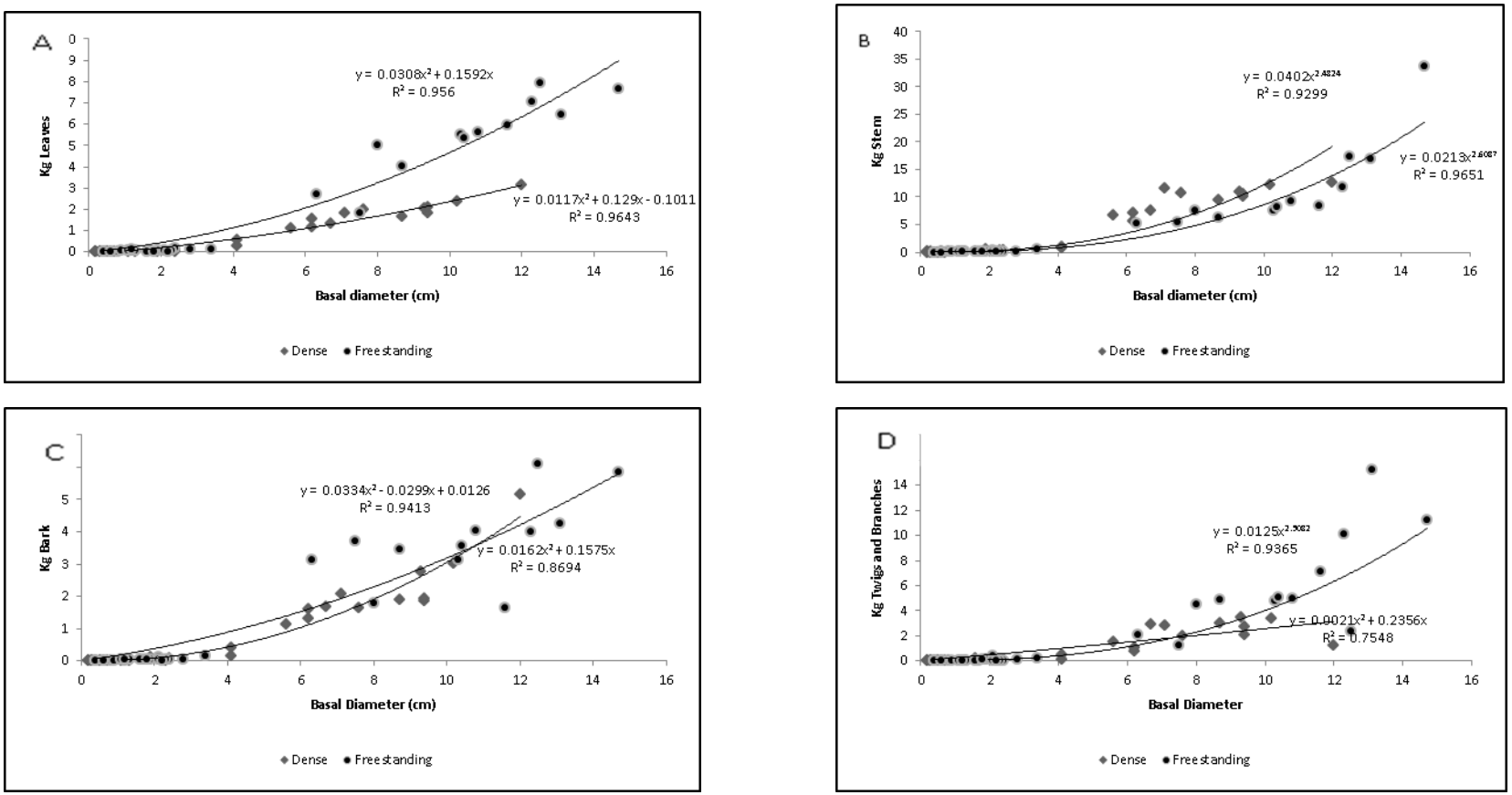


Figure 7.9: Best-fit regression models of biomass data over all size ranges of *Acacia mearnsii*, distinguishing between dense stands and freestanding trees. Biomass data is fitted over stem basal diameter (BD); A=leaf biomass, B=stem biomass, C=bark biomass, D=twigs and branches biomass.

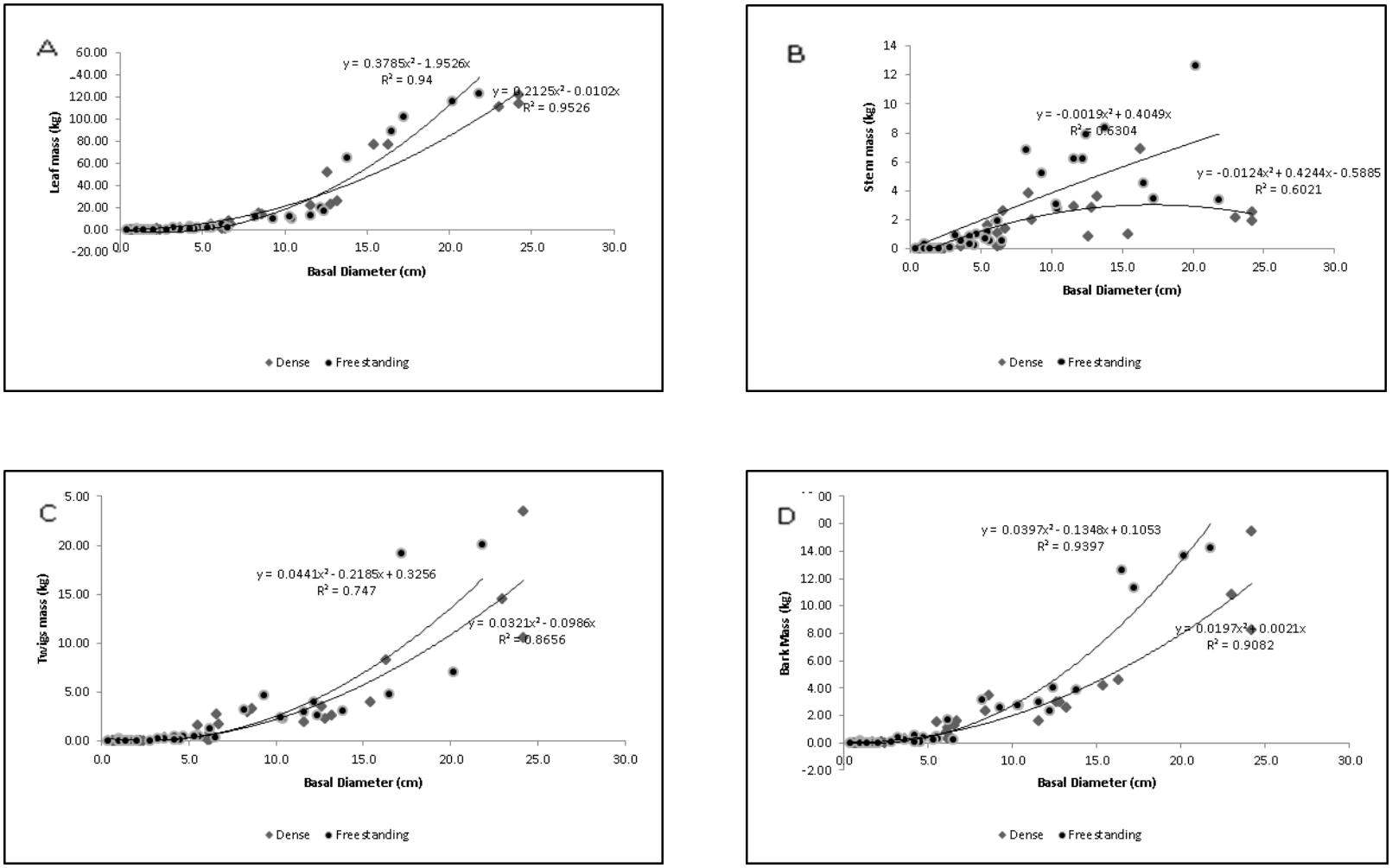


Figure 7.10: Best-fit regression models of biomass data over all size ranges of *Eucalyptus camaldulensis*, distinguishing between dense stands and freestanding trees. Biomass data is fitted over stem basal diameter (BD); A=leaf biomass, B=stem biomass, C=twigs and branches biomass , D=bark biomass.

Because there was a significant site difference in leaf growth between Alfalfa and Wolseley, site-specific models are presented below to accommodate this discrepancy (Figure 7.12).

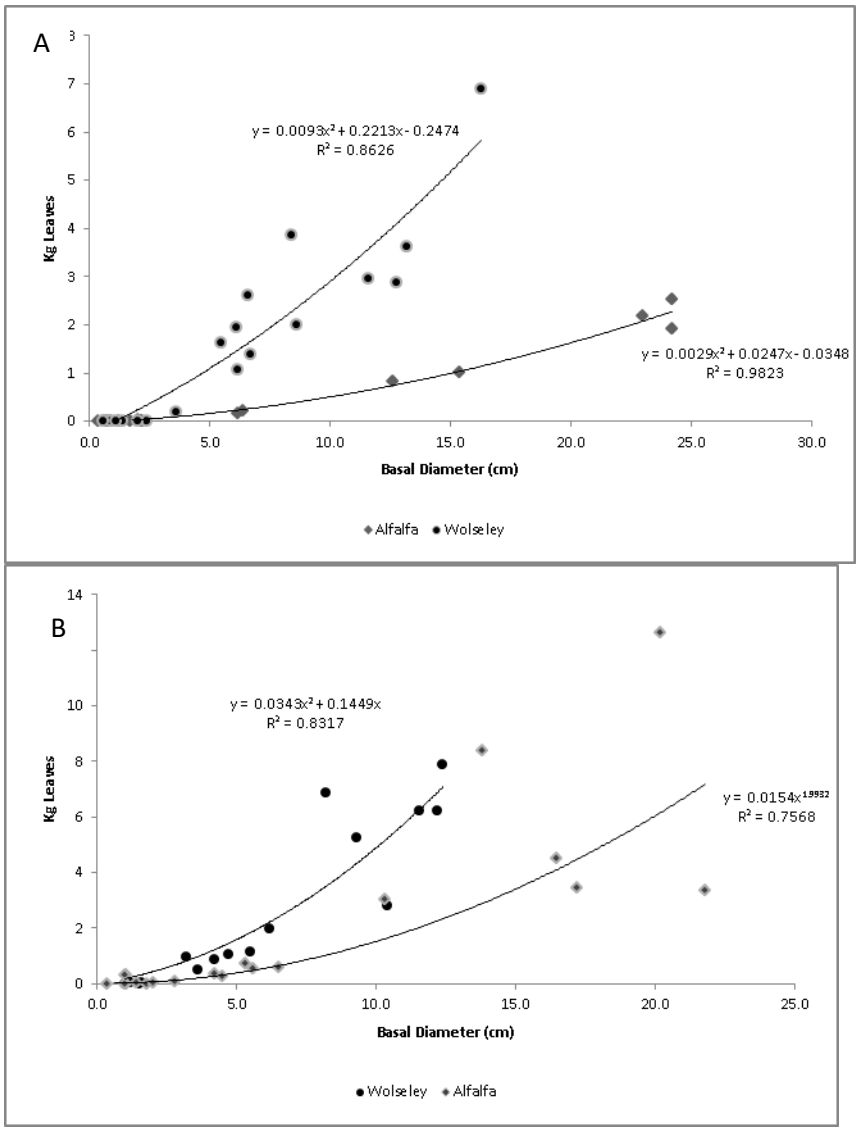


Figure 7.11: Best-fit regression models of leaf data separately for the two sites used for *Eucalyptus camaldulensis*, distinguishing between dense stands and freestanding trees. Biomass data is fitted over stem basal diameter (BD); A=dense stands, B=freestanding trees

Tables 7.1 and 7.2 give best-fit regression models of biomass data over all size ranges of *A. mearnsii* and *E. camaldulensis.*

Table 7.1: Allometric equations generated from best-fit regression models of biomass data over all size ranges of *A. mearnsii*, distinguishing between dense stands and freestanding trees

	Dense stands		**Freestanding trees**	
Component	**Regression**	**R^2**	**Regression**	**R^2**
Stem	$y = 0.040x^{2.482}$	0.929	$y = 0.021x^{2.608}$	0.965
Leaves	$y = 0.011x^2 + 0.129x - 0.101$	0.964	$y = 0.159x$	0.956
Bark	$y = 0.235x$	0.754	$y = 0.012x^{2.508}$	0.936
Twigs and branches	$y = 0.033x^2 - 0.029x + 0.012$	0.941	$y = 0.157x$	0.869

Table 7.2: Allometric equations generated from best-fit regression models of biomass data over all size ranges of *E. camaldulensis*, distinguishing between dense stands and freestanding trees

Component	**Dense stands**		**Freestanding trees**	
	Regression	**R^2**	**Regression**	**R^2**
Stem	$y = 0.197x^2 + 0.409x - 2.063$	0.953	$y = - 1.952x$	0.94
Leaves	$y = 0.304x$	0.557	$y = 0.404x$	0.630
Bark	$y = 0.002x$	0.908	$y = 0.039x^2 - 0.134x + 0.105$	0.939
Twigs and branches	$y = 0.034x^2 - 0.098x$	0.865	$y = 0.044x^2 - 0.218x + 0.325$	0.747

Correlation matrices were created for both species to show the relationship of various components to one another (Tables 7.3 and 7.4).

Table 7.3: Correlation matrix of various above-ground *A. mearnsii* components to one another and to stem basal diameter (BD)

Variable	**BD**	**Leaf biomass**	**Stem biomass**	**Bark biomass**	**Twigs biomass**
BD	1.000000	0.907437	0.905723	0.929120	0.818757
Leaf biomass	0.907437	1.000000	0.846183	0.904310	0.868653
Stem biomass	0.905723	0.846183	1.000000	0.892943	0.807023
Bark biomass	0.929120	0.904310	0.892943	1.000000	0.754378
Twigs biomass	0.818757	0.868653	0.807023	0.754378	1.000000

Table 7.4: Correlation matrix of various above-ground *E. camaldulensis* components to one another and to stem basal diameter (BD)

Variable	Leaf biomass	Stem biomass	Bark biomass	Twigs biomass	BD
Leaf biomass	1.000000	0.540151	0.596127	0.419310	0.671772
Stem biomass	0.540151	1.000000	0.938120	0.876872	0.908317
Bark biomass	0.596127	0.938120	1.000000	0.903313	0.889669
Twigs biomass	0.419310	0.876872	0.903313	1.000000	0.832363
BD	0.671772	0.908317	0.889669	0.832363	1.000000

7.3.2 Nutrients

Tables 7.5 through 7.12 present results from modelling of various nutrients over stem basal diameter, with the exception of Table 7.9, which presents models as leaf nutrients over leaf biomass.

Table 7.5: Models of leaf nutrients of *A. mearnsii* over stem basal diameter (x) for dense stands and freestanding individuals

Nutrient	Density			
	Dense	**R^2**	**Freestanding**	**R^2**
N	$y = 0.003x$	0.911	$y = 0.000x^{2.060}$	0.906
P	$y = 1E\text{-}05x^2 + 0.069x$	0.927	$y= 0.018x^{2.309}$	0.930
K	$y = 0.036x^2 + 0.407x - 0.350$	0.936	$y = 0.063x^{2.247}$	0.921
Ca	$y = 0.050x^{2.315}$	0.862	$y = 0.067x^{2.430}$	0.913
Mg	$y = 0.036x^2 + 0.212x - 0.190$	0.937	$y = 0.041x^{2.173}$	0.916
Na	$y = 3.069x^2 + 10.81x$	0.923	$y = 5.06x^2 + 28.99x$	0.906
Fe	$y = 0.555x^{1.973}$	0.898	$y = 2.627x$	0.900
Zn	$y = 0.021x^2 + 0.332x$	0.891	$y = 0.160x$	0.925
B	$y = 0.042x^{2.006}$	0.896	$y = 0.050x^{2.214}$	0.922
C	$y = 10.82x^{2.621}$	0.911	$y = 11.2x^{2.933}$	0.938

Table 7.6: Models of stem nutrients of *A. mearnsii* over stem basal diameter (x) for dense stands and freestanding individuals

Nutrient	Density			
	Dense	**R^2**	**Freestanding**	**R^2**
N	$y = 0.117x^{2.375}$	0.882	$y = 0.068x^{2.595}$	0.883
P	$y = 0.010x^{2.365}$	0.843	$y = 0.005x^{2.646}$	0.934
K	$y = 0.031x^{2.354}$	0.791	$y = 0.025x^{2.414}$	0.931
Ca	$y = 0.031x^{2.443}$	0.905	$y = 0.026x^{2.563}$	0.894
Mg	$y = 0.012x^{2.486}$	0.860	$y = 0.009x^{2.507}$	0.923
Na	$y = 1.741x^{2.475}$	0.895	$y = 1.044x^{2.704}$	0.947
Fe	$y = 0.151x^{1.949}$	0.826	$y = 0.082x^{2.267}$	0.842
Zn	$y = 0.035x^{2.259}$	0.857	$y = 0.022x^{2.388}$	0.943
B	$y = 0.025x^2 + 0.267x - 0.254$	0.913	$y = 0.053x^2 - 0.051x$	0.956
C	$y = 10.82x^{2.621}$	0.911	$y = 11.2x^{2.933}$	0.938

Table 7.7: Models of bark nutrients of *A. mearnsii* over stem basal diameter (x) for dense stands and freestanding individuals

Nutrient	Density			
	Dense	**R^2**	**Freestanding**	**R^2**
N	$y = 1.040x^{1.854}$	0.827	$y = 0.254x^2 + 3.979x$	0.770
P	$y = 0.010x^2 + 0.118x - 0.042$	0.820	$y = 0.009x^{2.421}$	0.833
K	$y = 0.038x^2 + 0.208x$	0.879	$y = 0.083x^2 + 0.316x - 0.126$	0.789
Ca	$y = 0.036x^{1.989}$	0.870	$y = 0.083x^2 + 0.316x - 0.126$	0.789
Mg	$y = 0.307x$	0.752	$y = 0.027x^2 + 0.009x + 0.110$	0.899
Na	$y = 14.66x$	0.820	$y = 15.25x$	0.862
Fe	$y = 1.834x$	0.89	$y = 0.195x^2 + 0.704x - 0.152$	0.853
Zn	$y = 0.089x$	0.933	$y = 0.140x$	0.843
B	$y = 0.027x^{2.182}$	0.880	$y = 0.128x$	0.852
C	$y = 214.9x$	0.929	$y = 24.35x^{2.014}$	0.750

Table 7.8: Models of branch and twig nutrients of *A. mearnsii* over stem basal diameter (x) for dense stands and freestanding individuals

Nutrient	Density			
	Dense	**R^2**	**Freestanding**	**R^2**
N	$y = 0.331x^{1.872}$	0.840	$y = 0.126x^{2.554}$	0.935
P	$y = 0.017x^{1.841}$	0.858	$y = 0.008x^{2.462}$	0.932
K	$y = 0.045x^{1.931}$	0.872	$y = 0.014x^{2.517}$	0.928
Ca	$y = 0.100x^{1.942}$	0.885	$y = 0.049x^{2.612}$	0.944
Mg	$y = 0.043x^{1.788}$	0.84	$y = 0.015x^{2.524}$	0.934
Na	$y = 5.146x^{1.696}$	0.83	$y = 1.641x^{2.563}$	0.924
Fe	$y = 0.493x^{1.613}$	0.858	$y = 0.151x^{2.536}$	0.946
Zn	$y = 0.122x^{1.746}$	0.858	$y = 0.028x^{2.654}$	0.917
B	$y = 0.032x^{1.821}$	0.876	$y = 0.013x^{2.535}$	0.935
C	$y = 0.543x$	0.94	$y = 0.377x$	0.844

Due to the discrepancies in models of leaf biomass as modelled over stem diameter for *Eucalyptus* samples, the nutrient contents for leaves were not modelled over stem BD, but rather over the leaf biomass of their respective trees (Table 7.9). These models are mostly linear.

Table 7.9: Models of leaf nutrients of *E. camaldulensis* over leaf biomass (x) for dense stands and freestanding individuals

Nutrient	Density			
	Dense	**R^2**	**Freestanding**	**R^2**
N	$y = 21.59x$	0.980	$y = 23x$	0.934
P	$y = 1.231x$	0.848	$y = 1.832x$	0.961
K	$y = 6.470x$	0.914	$y = 7.118x$	0.932
Ca	$y = 5.812x$	0.902	$y = 7.018x + 0.640$	0.932
Mg	$y = 2.139x$	0.980	$y = 2.479x$	0.946
Na	$y = 192.8x + 1.935$	0.907	$y= 247.9x$	0.924

Nutrient	Density			
	Dense	**R^2**	**Freestanding**	**R^2**
Fe	y = 7.618x	0.884	y = 11.45x	0.899
Zn	y = 2.278x	0.885	y = 2.197x	0.966
B	y = 2.425x	0.850	y = 3.206x	0.967
C	$y = 0.552x^{1.000}$	1	y = 268.0x	0.795

Table 7.10: Models of stem nutrients of *E. camaldulensis* over stem basal diameter (x) for dense stands and freestanding individuals

Nutrient	Density			
	Dense	**R^2**	**Freestanding**	**R^2**
N	$y = 0.008x^{2.957}$	0.806	$y = 0.433x^2 - 1.955x$	0.951
P	$y = 0.016x^{2.394}$	0.877	$y = 0.018x^{2.358}$	0.885
K	$y = 0.089x^{2.258}$	0.906	$y = 0.053x^{2.596}$	0.901
Ca	y = 0.982x	0.644	$y = 0.033x^{2.486}$	0.865
Mg	$y = 0.016x^{2.261}$	0.865	$y = 0.013x^{2.590}$	0.898
Na	$y = 0.014x^{2.203}$	0.899	$y = 0.009x^{2.527}$	0.884
Fe	$y = 0.003x^{2.386}$	0.828	$y = 0.002x^{2.894}$	0.900
Zn	$y = 0.005x^2 - 0.031x$	829	$y = 0.015x^2 - 0.084x$	0.930
B	$y = 0.006x^2 - 0.047x$	0.935	$y = 0.014x^2 - 0.152x + 0.248$	0.950
C	$y = 106.1x^2 + 345.7x - 1416$	0.948	$y = 163.2x^2 - 388.5x$	0.968

Table 7.11: Models of branch and twig nutrients of *E. camaldulensis* over stem basal diameter (x) for dense stands and freestanding individuals

Nutrient	Density			
	Dense	R^2	Freestanding	R^2
N	$y = 0.014x^{2.334}$	0.627	$y = 0.313x^2 - 1.860x$	0.782
P	$y = 0.003x^{2.387}$	0.877	$y = 0.006x^{2.118}$	0.760
K	$y = 0.013x^{2.455}$	0.874	$y = 0.027x^{2.131}$	0.797
Ca	$y = 0.009x^{2.473}$	0.874	$y = 0.371x^2 - 2.284x$	0.691
Mg	$y = 0.002x^{2.599}$	0.889	$y = 0.083x^2 - 0.460x$	0.730
Na	$y = 0.167x^{2.597}$	0.895	$y = 0.674x^{2.158}$	0.785
Fe	$y = 0.073x^{1.241}$	0.563	$y = 0.640x^2 - 5.053x$	0.866
Zn	$y = 0.015x^{2.856}$	0.863	$y = 0.060x^{1.874}$	0.712
B	$y = 0.001x^{2.754}$	0.904	$y = 0.004x^{2.400}$	0.781
C	$y = 2.883x^{2.491}$	0.895	$y = 1.650x^{2.885}$	0.954

Table 7.12: Table 10: Models of bark nutrients of *E. camaldulensis* over stem basal diameter (x) for dense stands and freestanding individuals

Nutrient	Density			
	Dense	R^2	Freestanding	R^2
N	$y = 78.83x^{2.373}$	0.938	$y = 87.17x^{2.432}$	0.884
P	$y = 7.966x^{2.215}$	0.923	$y = 8.136x^{2.350}$	0.87
K	$y = 7.972x^{2.211}$	0.920	$y = 8.136x^{2.350}$	0.873
Ca	$y = 22.79x^{2.517}$	0.873	$y = 17.06x^{2.584}$	0.889
Mg	$y = 129.1x^{2.099}$	0.790	$y = 82.38x^{2.493}$	0.883
Na	$y = 6.741x^{2.477}$	0.905	$y = 7.906x^{2.258}$	0.840
Fe	$y = 0.495x^{2.239}$	0.836	$y = 0.418x^{2.571}$	0.862
Zn	$y = 0.192x^{2.399}$	0.860	$y = 0.14x^{2.722}$	0.860
B	$y = 0.087x^{2.477}$	0.888	$y = 0.099x^{2.371}$	0.808
C	$y = 3.814x^{2.449}$	0.930	$y = 3.421x^{2.064}$	0.835

7.4 DISCUSSION

The biomass models presented here are generated from best-fit models of the samples. From figures showing variations in allocation of biomass to various tree components as a result of growth, it is clear that these differ enough to warrant sampling from different life stages in order to capture the natural variation in an invasive forest. These models together could possibly be used to accurately predict above-ground biomass of *A. mearnsii* and *E. camaldulensis* growing in invasive stands rather than plantation environments. Models were made for trees from dense stands and freestanding trees. Except for leaf biomass models, all models show similar trends in biomass in relation to an increase in stem diameter, as measured as BD because most juveniles measured did not yet reach sufficient height for measurements at the Diameter of Breast Height (DBH; 1.4 m height).

The percentage of the *E. camaldulensis* biomass occupied by twigs and branches presents a good example of how stem density influences tree allometry. At the juvenile stage, many plants do not develop many branches but rather invest in leaves, which grow directly from the stem. When the plant reaches sapling stage it invests slightly more in branches to increase its capacity for leaf production and, by extension, its photosynthetic efficiency. This allows the plant to grow more quickly through a dense canopy. When reaching a specific height, the lower branches become obsolete as they are shaded by the newly formed canopy; some of the resources stored within these branches are assimilated and redistributed to support growth of new biomass higher up in the plant and for strengthening of supporting structures. Stem density is thus a driver of competition between neighbours. A tree in a dense stand also typically grows taller than a freestanding individual at any specified BD, as it has to compete for light. Results from this study show that this could increase the proportion of biomass allocated to the stem and proportionately reduce that allocated to other components, and this differs significantly from specimens that grow alone. Considering the use of either species for their biomass, these results thus suggest that dense stands typically would provide greater returns for trees with the same stem diameter as freestanding trees.

An important consideration is that environmental variability between growth sites and variability within sampling sites could also influence growth form and biomass allocation in trees. Relatively conspicuous differences such as branch and leaf biomass can easily be described as a response to availability of sunlight. Lödige et al. (2014) found that soil moisture and nutrient content could have a significant influence on above-ground biomass allocation in some trees. This also holdfor temporal changes in requirements within plants, which can result in re-translocation of resources between components. Poorter and Nagel (2000) point out that larger plants typically have to invest relatively more resources in support structures and leaf area. Waring (1987) presented a model to predict carbon allocation in trees, suggesting that tissues involved with photosynthesis (foliage) and the development of new roots usually enjoy great inputs of carbon, while the rest of the available C is allocated to storage, stem growth, and production of defensive compounds. Theoretically, then, the parts of the plant most limited in terms of available resources will enjoy relatively greater growth to increase acquisition of that resource. Drought-induced stress would, for instance, reduce shoot growth and cause a change in nutrient allocation to above-ground components, while increasing root growth (Ericsson et al., 1996).

Differences in soil properties are often complex and in riparian ecotones are dependent on environmental variables such as rainfall, proximity to the flood zone, frequency of fires, and in many cases are a result of invasion, while simultaneously driving growth processes within trees.

Leguminous species such as *A. mearnsii*, for instance have the capacity to enrich soil N and P (which could enhance growth of both the invader and other competing species), while trees in the *Eucalyptus* genus are known to create microsites unfavourable for growth by other plants.

A problematic variable to successfully predict was leaf mass of *E. camaldulensis*. This is likely because of significant site differences between the Alfalfa site and the Wolseley site. Leaf mass at the Alfalfa site remained much lower per unit increase in BD than at Wolseley. These models are thus presented separately for biomass and a different technique was used for nutrient models. This does, however, highlight the possibility of unexpected site differences which could make models such as those presented here, site specific. This is further supported by the correlation matrices (Tables 7.3 and 7.4) which show that leaf biomass had the worst correlation with any of the other tested variables, making it difficult to predict. It is thus highly recommended for application that models are tested first against individuals in sites falling outside this study area through small-scale pilot studies.

Nutrients, after being absorbed from the soil, are allocated to different tree components based on the tree's metabolic or physiological needs. For instance, when available nutrients in the soil become depleted, plants tend to invest into enhancing root growth to maximise nutrient uptake (Hermans et al., 2006). This allocation of nutrients is relatively plastic and largely determines growth form and biomass allocation of trees. With a finite supply of nutrients, translocation within plants implies an internal mechanism which identifies nutritional needs and distributes nutrients accordingly. It also implies trade-offs in terms of growth of certain components, e.g. trees shedding leaves, and saplings losing their lower branches to facilitate growth of leaves in the canopy.

The models presented here could be used separately depending on the needs of the user. Someone studying nutrients would, for instance, not need to calculate biomass first, as nutrient models are based directly on stem diameter (under the assumption of near-perfect biomass measurements).

Modelling biomass and nutrients of *A. mearnsii* and *E. camaldulensis* in Western Cape riparian zones could provide useful information on the state of invasion in the region, and the opportunities presented by it. The models presented here are the first to describe biomass and nutrients in relation to allometry in invasive stands in the region. Although fairly accurate, subtle environmental differences result in differences in growth forms that cannot be modelled through landscape approaches and require site-specific modelling. These results present strong evidence that the growth form of trees as a result of ontogeny do not allow the use of biomass models created from a single-aged stand such as a managed plantation, to predict the biomass of individual trees and stands. The role of stem densities in biomass partitioning of above-ground tree components is also clear. In most cases models were sufficient to, with a sufficiently large sample size and consideration of external factors, be expanded to include other regions.

CHAPTER 8: Conclusions

Programmes such as WfW and other aligned programmes have, with the help of a large ecological research effort, managed to go some way towards addressing the challenge of woody, alien invasive plants in terrestrial and riparian ecosystems in South Africa (van Wilgen and Wannenburg, 2016). Though alien invasive trees are still spreading at an alarming rate, the strategic intervention of the WfW programme in catchments has prevented the loss of water associated with stands of alien invasive trees, and has also allowed rural people to attain a certain dignity that comes with being employed and earning an income, as opposed to the grinding poverty that lack of employment confines South Africa's poor and vulnerable population to (Hough and Prozesky, 2013).

The benefits of WfW and its approaches to removing and managing alien invasive trees are obvious. However, literature suggests that the use of herbicides, fire and removal of biomass may have some consequences for ecosystem functioning. This study was therefore designed to:

1. Evaluate the impact of recommended levels of herbicides, used to control alien invasive growth and regeneration, on soil microbial diversity and on selected beneficial groups of microbes *in situ* and *ex situ* and in riparian soils from two different longitudinal zones.
2. Determine the impact of slash and burn of *Eucalyptus camaldulensis* and *Acacia mearnsii* biomass on soil microbial diversity and on selected beneficial groups of microbes *in situ,* and measure regeneration of various native plant species grown in soil from slash and burn scars *ex situ.*
3. Determine the impact of slash and burn of *Eucalyptus camaldulensis* and *Acacia mearnsii* biomass on soil physical and chemical properties *in situ*.

4. Determine the biomass and nutrient content of *Eucalyptus camaldulensis* and *Acacia mearnsii* trees of different sizes growing at different stem densities in riparian sites from two different longitudinal zones.

The application of herbicides to cut stumps *in situ* and to soils *ex situ* changed soil pH significantly, with major declines seen at levels typically applied in the field, and stronger declines seen with increasing concentrations. In addition, soil nitrogen levels increased significantly with the application of Glyphosate. Soil microbial populations were not affected in a major way; however, as has been shown in several studies, alterations to soil pH may ultimately impact soil microbial populations, and also processes supported by soil microbes. In addition, the study covered soil microbial community responses in the short term, and these results cannot be extrapolated to longer-term microbial responses to herbicide applications.

Soil pH was one of the main soil properties that changed following burning of slash piles in riparian soils. Surprisingly, soil pH did not return to pre-fire levels even up to one year after the fire. Other impacts were also seen, notably in terms of soil pH and cations, which increased following fire, but returned to pre-fire levels one year after the fire. No major differences were evident when comparing the riparian sites to a terrestrial site. Interestingly, soil available N was unaffected by the fire until some months afterwards, when scars where *Acacia mearnsii* slash was burned showed high available N compared to non-burned areas. This implied that P levels are more available initially, but that N levels in burned areas may only become more available over time, and then only in burn scars where *Acacia* biomass was burned. It may be possible the difference between the two categories (*Acacia* and *Eucalyptus* slash plies may be related to the size of the pile and the size of the main members, which may induce hotter fires in the case of *Eucalyptus camaldulensis*; however, this was not determined in the study.

This may also be the reason why the study showed that burning of slash piles of *Eucalyptus* biomass has a more profound impact on microbial diversity and community structure than burning of slash piles of *Acacia mearnsii* biomass. All the sites indicated a microbial community shift after the effect of fire, which may be directly due to mortality of microbial population and a lag phase in recovery, as well as indirectly due to altered soil properties such as pH and soil phosphorus. These alterations in physicochemical and microbial dynamics may also influence regeneration and survival of seedling established on the burn scars. The invasive species *E. camaldulensis* responded very fast in terms of germination when planted in burned soil, but soil nutrient deficiencies or toxicity may affect the survival of species growing in burned soil during the seedlings phase. While some information is available on slash and burn of alien invasive trees in relation to regeneration post fire, it is the first time that a study has specifically investigated soil chemical and microbiological changes subsequent to burning of slash piles, and our study suggests that the negative impact of this practice needs to be carefully traded off against other benefits.

The alternative to biomass management though burning of stacks on site is removal of whole wood or chips that are removed to local or international destinations. Several models were developed that can be used to determine which nutrients are exported and in what quantities, and different models were derived for freestanding trees and trees in dense stands. These models are the first to be developed for biomass and nutrients stocks in invasive stands of *A. mearnsii* and *E. camaldulensis*.

The research was constrained by students starting their projects at different times, mainly due to the different times that students joined the project. Thus our initial plan to use the same sites for all student projects had to be abandoned. Another constraint was the loss of some sites halfway through the study due to clearing efforts by landowners.

This study covered riparian ecosystem responses to several commonly used management interventions. Some aspects not covered, however, deserve some consideration for future studies, including:

- Longer-term responses of soil microbial communities and soil physicochemical properties to application of herbicides to soils may well show different responses to those reported here, and deserve to be investigated further, and over a longer period.

- Changes in pH, found when soil is exposed to fire and herbicides have the potential to alter soil microbial communities, as shown in fynbos soils by the close relationship between bacterial community structure and soil pH shown here as well as a previous study in riparian zones (Slabbert 2012). Should alterations in soil pH be long term or even irreversible, this may have consequences for soil microbial composition and structure. However, this needs to be investigated further.

- It is recommended that future research studying the impacts of herbicides explores evaluating different herbicides with different active ingredients as well as the adjuvants involved.

- It is also recommended that impacts of herbicides on microbial biomass and microbial community compositional changes should be explored, for instance through pyrosequencing, which would allow identification of the soil microbial populations in the soil.

- The medium- to longer-term fate of soils within burn scars should be investigated given the potential impact on soil microbial communities and soil functioning.

- The ability of native riparian plant species to regenerate in soils physicochemically altered by herbicides or fire has not been investigated, but may be a good topic for a future study. Similarly, altered soil microbial diversity following fire and its relationship to native plant species, especially regarding symbioses, also need to be investigated.

- Improving our understanding of competitive interactions between native and invasive seedlings in cleared landscapes and how these are affected by altered soil chemical and microbial properties could also be a good topic for future research.

- Investigating the role of flooding on soil chemical and microbial properties, and how flooding and riparian geomorphology may be used strategically to influence soil conditions and subsequent regeneration of native riparian communities will add to the body of knowledge on riparian restoration.

- To expand the current study, generating biomass and nutrient models for catchments in other provinces and, in order to expand the database is an important step to understanding the available biomass for exploitation in commercial and community-based ventures, and deserves some attention.

The research presented here aligns with several of the key strategic areas of the Water Research Commission (WRC), notably within the scope of investigating ecosystem functioning (in this there is a continuity with a previous WRC project, reported in Jacobs et al., 2013) as well as with ecosystem restoration, both listed under objectives of KSA2, Water-Linked Ecosystems. It also aligns with the Lighthouses developed as part of the WRC research scope, for example the Water-Energy-Food Security and Green Village Lighthouses.

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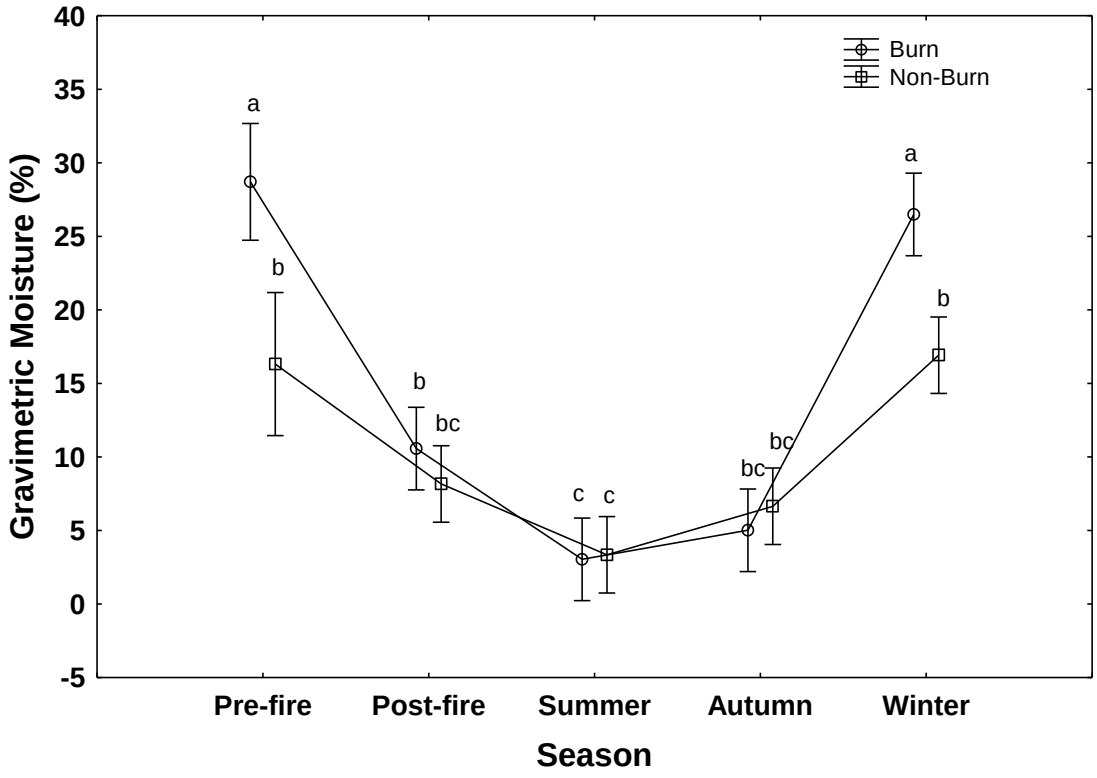
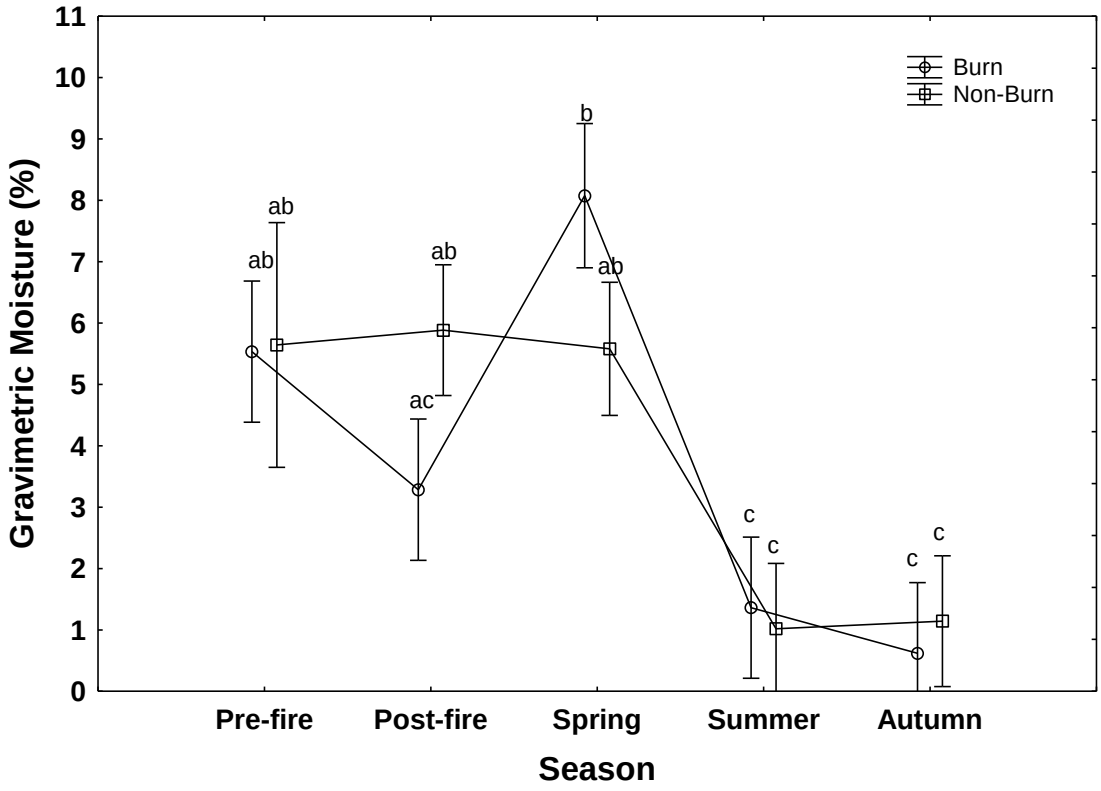
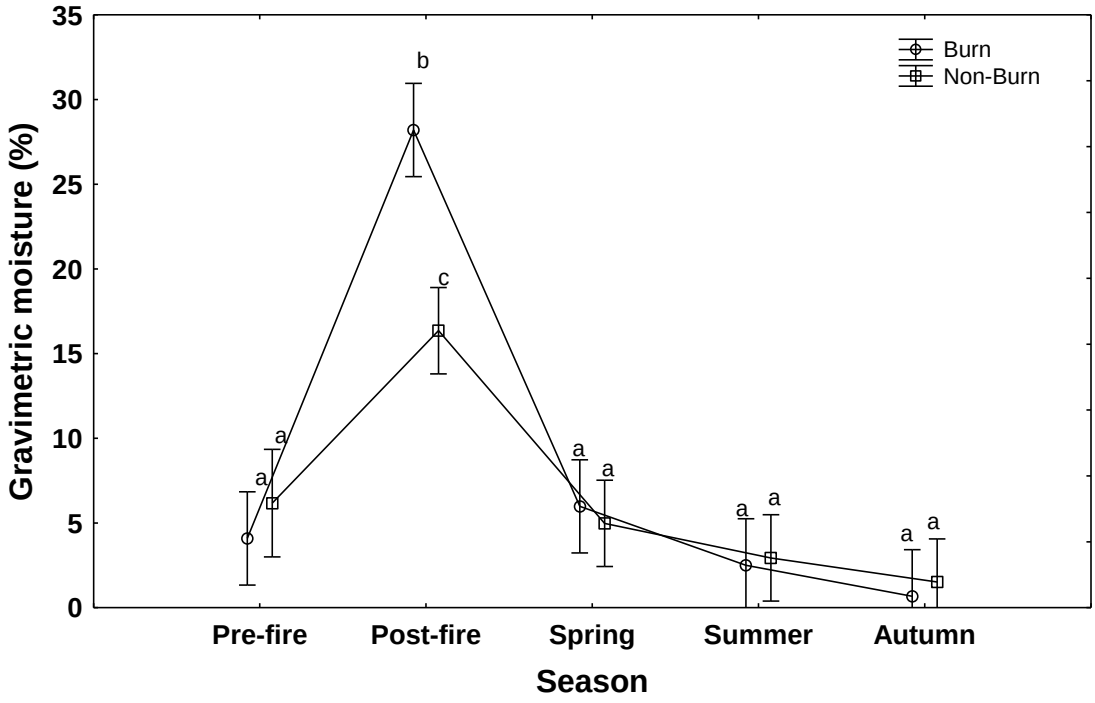
APPENDICES



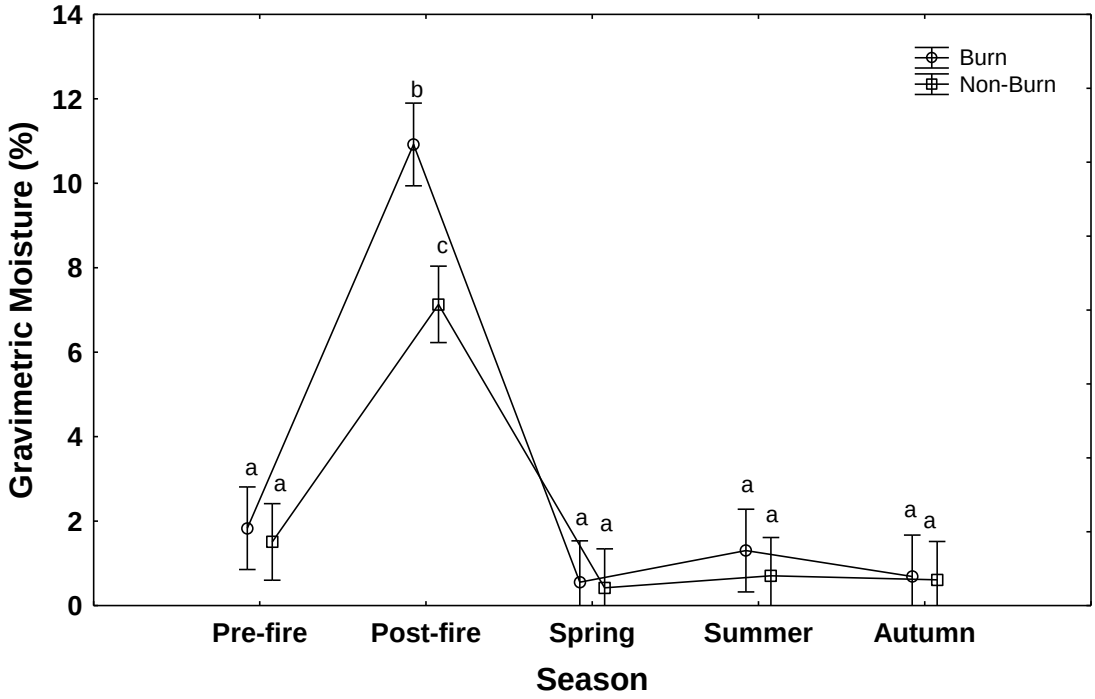
Appendix A1: Seasonal gravimetric soil water content within the burn and the non-burn treatment sites at the Hermon study area. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). The seasonal means are interaction effects based on two-way ANOVA: treatment X season ($F_{[4, 218]}=7.17$, $p<0.01$).



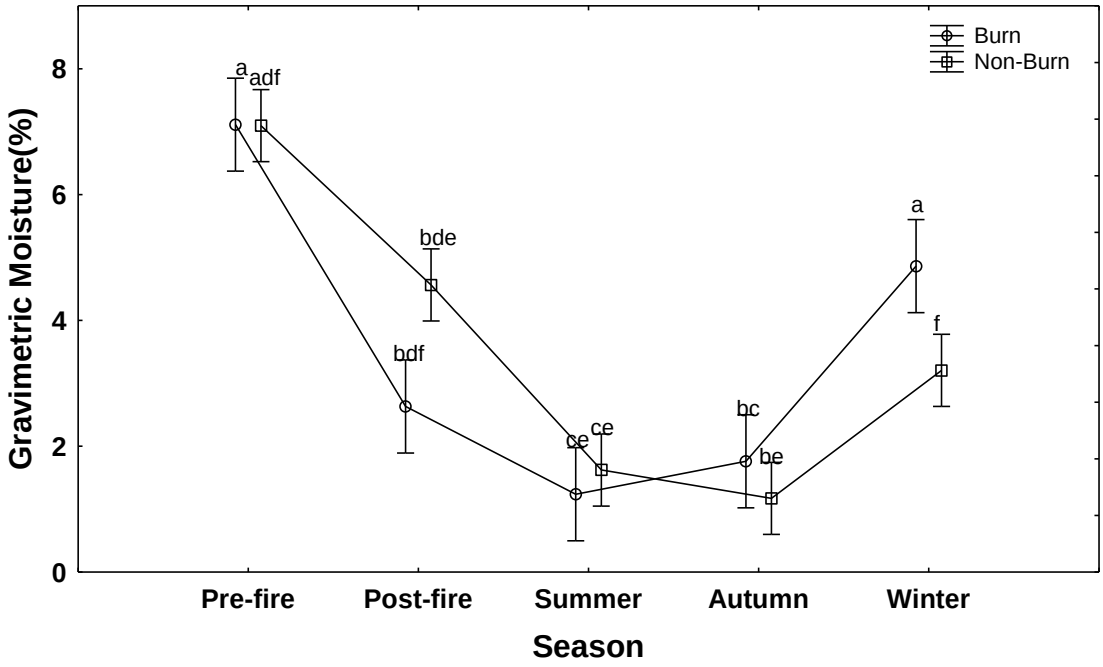
Appendix A2: Seasonal gravimetric soil water content within the burn and the non-burn treatment sites at the Robertson study area. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). The seasonal means are interaction effects based on two-way ANOVA: treatment X season ($F_{[4, 228]}=5.17$, $p<0.01$).



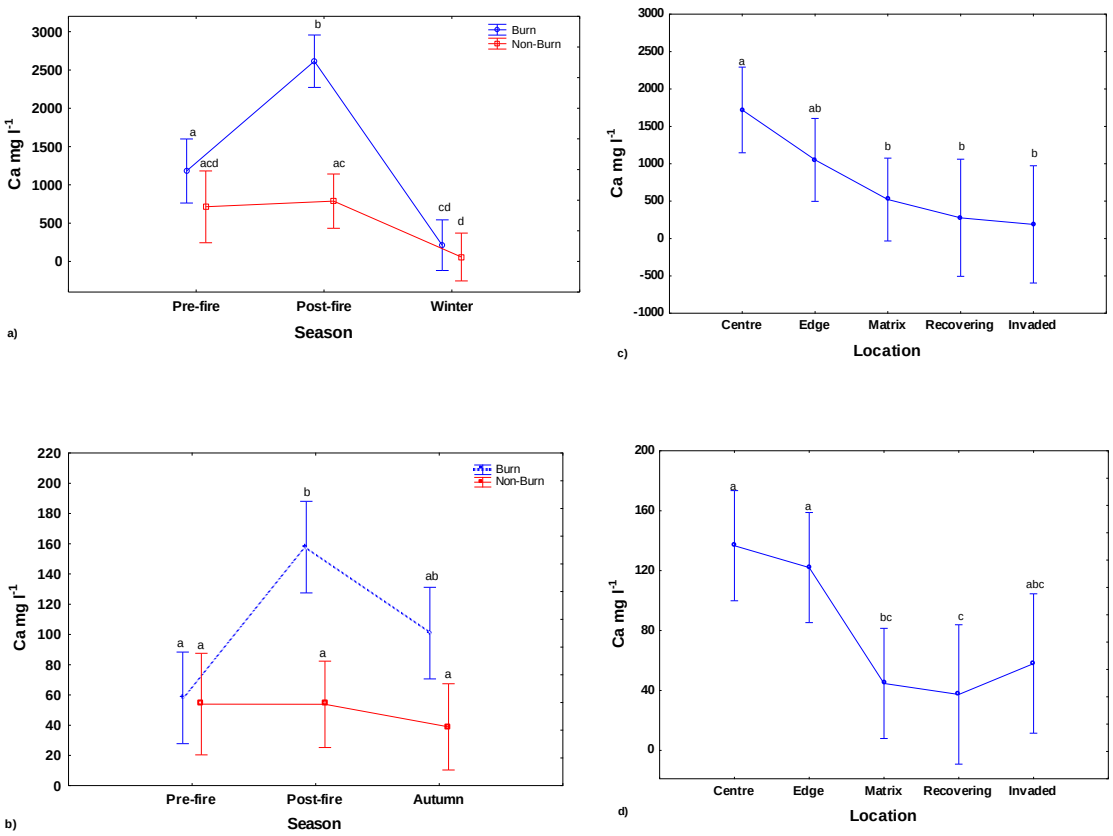
Appendix A3: Seasonal gravimetric soil water content within the burn and the non-burn treatment sites at the Rawsonville study area. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). The seasonal means are interaction effects based on two-way ANOVA: treatment X season ($F_{[4, 240]}=8.65$, $p<0.01$).



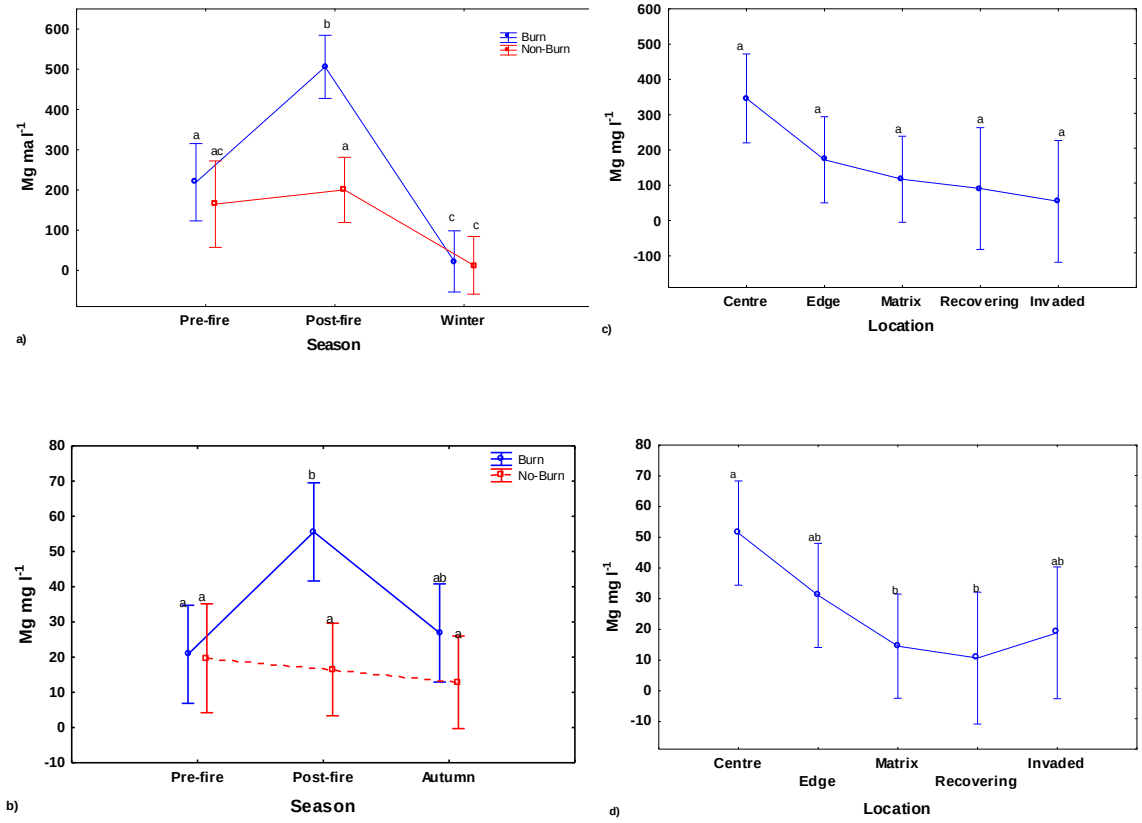
Appendix A4: Seasonal gravimetric soil water content within the burn and the non-burn treatment sites at the Wit River study area. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). The seasonal means are interaction effects based on two-way ANOVA: treatment X season ($F_{[4, 249]}=5.44$, $p<0.01$).



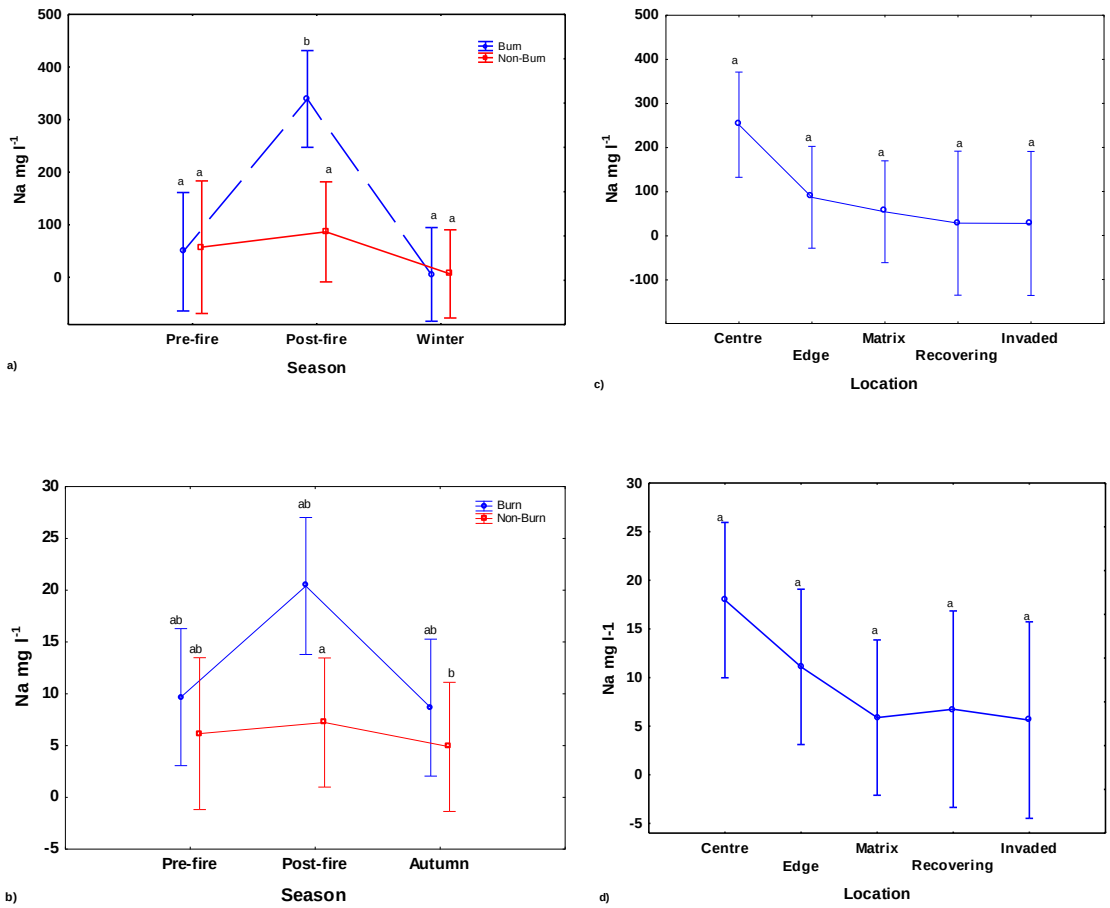
Appendix A5: Seasonal gravimetric soil water content within the burn and the non-burn treatment sites at the Blaauwberg study area. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). The seasonal means are interaction effects based on two-way ANOVA: treatment X season ($F_{[4, 190]}=7.74$, $p<0.01$).



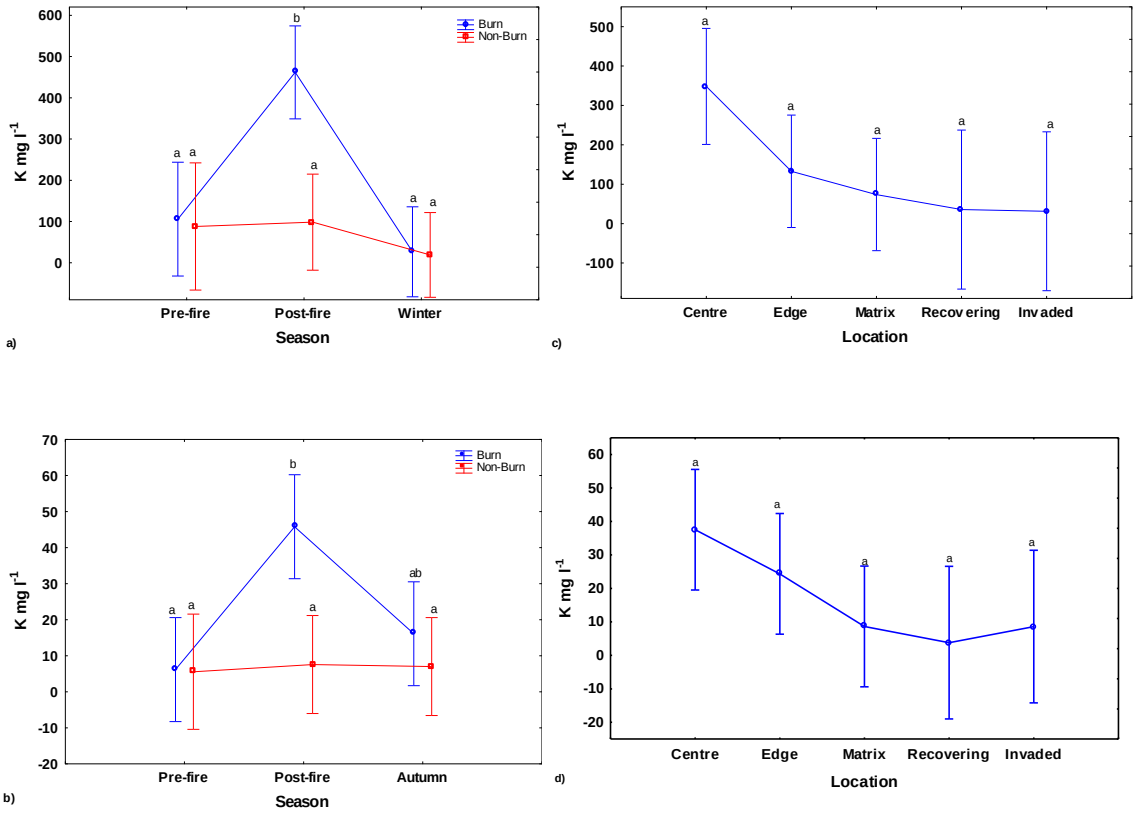
Appendix A6: Seasonal calcium concentrations within the burn and the non-burn treatment sites at (a) Hermon, (b) Rawsonville study areas; and spatial soil magnesium levels within the burn scar and non-burn treatment sampling locations at (c) Hermon, (d) Rawsonville study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). All the seasonal soil calcium concentrations mans are interaction effects based on two-way ANOVA: treatment X season (a) ($F_{[2, 75]} =13.20$, $p<0.01$), (b) ($F_{[1, 91]} =5.21$, $p=0.01$). Spatial calcium concentrations means are based on a one-way ANOVA: location (c) ($F_{[4, 58]} =4.04$, $p=0.01$), Figure(d) ($F_{[4, 63]} =5.56$, $p<0.01$).



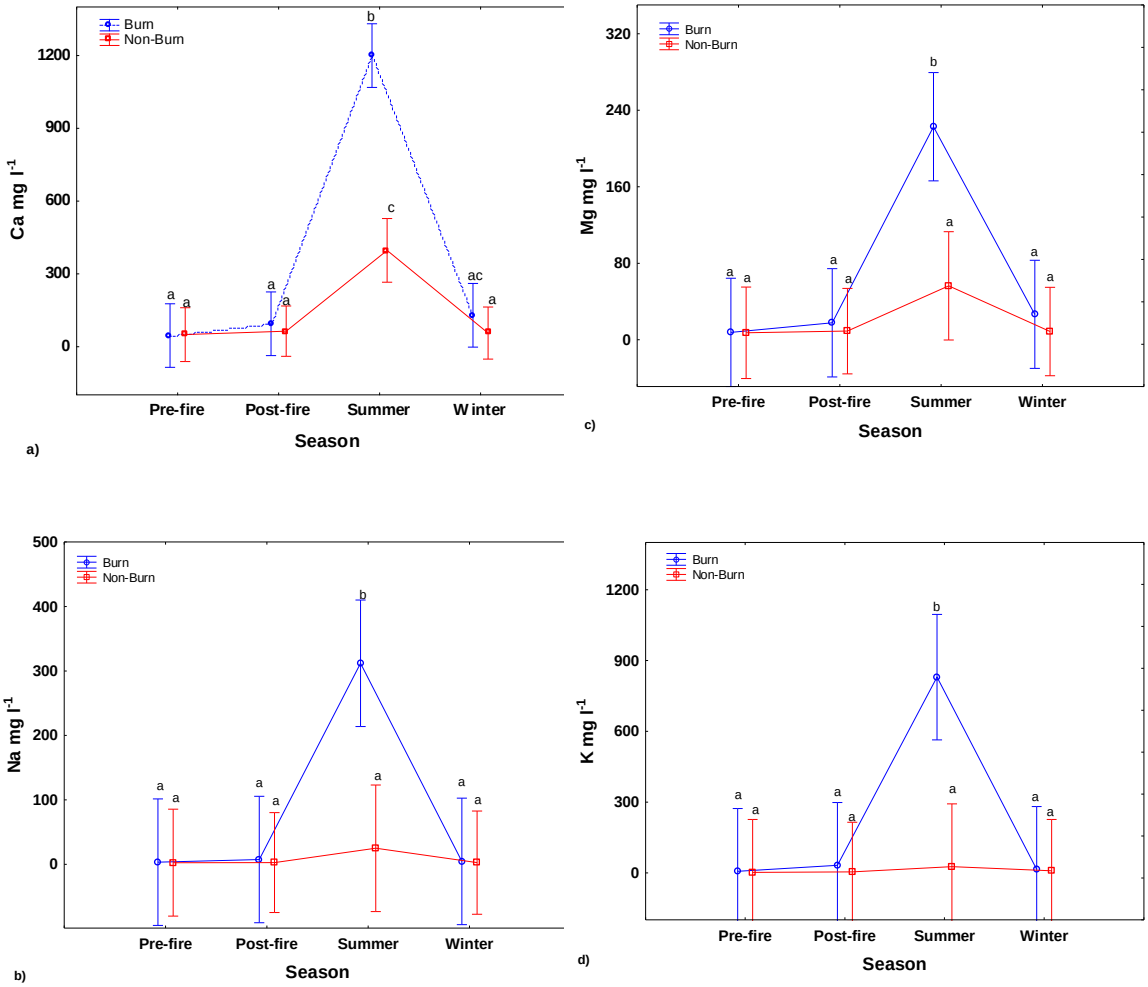
Appendix A7: Seasonal soil magnesium concentrations within the burn and the non-burn treatment sites at (a) Hermon, (b) Rawsonville study areas; and spatial soil magnesium levels within the burn scar and non-burn treatment sampling locations at (c) Hermon, (d) Rawsonville study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). All the seasonal soil magnesium concentrations mans are interaction effects based on two-way ANOVA: treatment X season (a) ($F_{[2, 75]}=7.99$, $p<0.01$), (b) ($F_{[2, 91]}=3.76$, $p=0.02$). Spatial magnesium concentrations means are based on a one-way ANOVA: location (c) ($F_{[4, 58]}=2.81$, $p=0.03$), (d) ($F_{[4, 63]}=3.37$, $p=0.01$).



Appendix A8: Seasonal sodium concentrations within the burn and the non-burn treatment sites at (a) Hermon, (b) Rawsonville study areas; and spatial soil sodium levels within the burn scar and non-burn treatment sampling locations at (c) Hermon, (d) Rawsonville study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). All the seasonal soil sodium concentrations mans are interaction effects based on two-way ANOVA: treatment X season (a) ($F_{[2, 75]}$ =4.77, p=0.01), (b) ($F_{[2, 91]}$ =1.40, p=0.25). Spatial sodium concentrations means are based on a one-way ANOVA: location F(c) ($F_{[4, 58]}$ =2.18, p=0.08), (d) ($F_{[4, 63]}$ =1.57, p=0.19).



Appendix A9: Seasonal potassium concentrations within the burn and the non-burn treatment sites at (a) Hermon, (b) Rawsonville study areas; and spatial potassium levels within the burn scar and non-burn treatment sampling locations at (c) Hermon, (d) Rawsonville study areas. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). All the seasonal potassium concentrations means are interaction effects based on two-way ANOVA: treatment X season (a) ($F_{[2, 75]}=5.60$, $p<0.01$), (b) ($F_{[2, 91]}=3.72$, $p=0.03$). Spatial potassium concentrations means are based on a one-way ANOVA: location (c) ($F_{[4, 58]}=2.78$, $p=0.04$), (d) ($F_{[4, 63]}=2.12$, $p=0.08$).



Appendix A10: Seasonal soil exchangeable cations concentrations within the burned and the non-burned treatment sites. Mean values are shown by different point symbols and vertical bars indicate ± 95% confidence intervals. Letters indicate significant differences between means (Bonferroni tests, $p<0.05$). Graphs are of interaction effects based on two-way ANOVA: treatment X season, Figure5.a ($F_{[3,\ 87]}$ =17.85, $p<0.01$), Figure5.b ($F_{[3,\ 87]}$ =4.33, $p=0.01$), Figure5.c ($F_{[3,\ 87]}$ = 4.01, $p=0.01$) and Figure5.d ($F_{[3,\ 87]}$ =4.55, $p=0.01$).

Table A1: (a) Seasonal hydrophobicity values of the burn and non-burn treatment sites at Hermon, (b) Spatial hydrophobicity values of the burn and non-burn sampling positions at Hermon study area. Means represent time in seconds for the Water Drop Penetration Test at each sampling location, with standard errors in brackets.

a) Seasonal

	Burn treatment		**Non-Burn treatment**	
Season	**Time (s)**	**Category**	**Time (s)**	**Category**
Pre-fire	5.00 (±0.00)	Non-Hydrophobic	5.00 (±0.00)	Non-Hydrophobic
Post-fire	5.00 (±0.00)	Non-Hydrophobic	5.00 (±0.00)	Non-Hydrophobic
Summer	7.38 (±2.29)	Slightly Hydrophobic	5.25 (±0.25)	Slightly Hydrophobic
Autumn	7.25 (±0.87)	Slightly Hydrophobic	6.41 (±0.85)	Slightly Hydrophobic
Winter	11.42 (±4.20)	Slightly Hydrophobic	8.39 (±3.39)	Slightly Hydrophobic

b) Spatial

Location	**Treatment**	**Time (s)**	**Category**
Centre	**Burn**	5.91 (±0.50)	Slightly Hydrophobic
Intermediate	**Burn**	9.81 (±3.06)	Slightly Hydrophobic
Edge	**Burn**	7.56 (±1.94)	Slightly Hydrophobic
Matrix	**Non-burn**	9.19 (±3.03)	Slightly Hydrophobic
Recovering	**Non-burn**	5.00 (±0.00)	Non-hydrophobic
Invaded	**Non-burn**	5.00 (±0.00)	Non-hydrophobic

Table A2: (a) Seasonal hydrophobicity values between the burn and non-burn treatment sites at Robertson study area, (b) Spatial hydrophobicity values between the burn and non-burn sampling positions at Robertson study area. Means represent time in seconds for the Water Drop Penetration Test at each sampling location, with standard errors in brackets.

a) Seasonal

	Burn treatment		**Non-Burn treatment**	
Season	**Time (s)**	**Category**	**Time (s)**	**Category**
Pre-fire	259.33 (±102.53)	Strongly Hydrophobic	480.00 (±289.06)	Strongly Hydrophobic
Post-fire	269.54 (±96.10)	Strongly Hydrophobic	184.46 (±89.59)	Strongly Hydrophobic
Spring	36.79 (±31.02)	Slightly Hydrophobic	77.68 (±63.94)	Strongly Hydrophobic
Summer	111.63 (±75.80)	Strongly Hydrophobic	172.89 (±91.16)	Strongly Hydrophobic
Autumn	280.83 (±110.03)	Strongly Hydrophobic	177.64 (±80.65)	Strongly Hydrophobic

b) Spatial

Location	**Treatment**	**Time (s)**	**Category**
Centre	**Burn**	104.06 (±53.74)	Strongly Hydrophobic
Intermediate	**Burn**	130.84 (±60.32)	Strongly Hydrophobic
Edge	**Burn**	289.19 (±97.38)	Strongly Hydrophobic
Matrix	**Non-Burn**	2489.44 (±91.28)	Strongly Hydrophobic
Recovering	**Non-Burn**	19.38 (±5.61)	Slightly Hydrophobic
Invaded	**Non-Burn**	210.75 (±84.43)	Strongly Hydrophobic

Table A3: (a) Seasonal hydrophobicity values between the burn and non-burn treatment sites at Wit River study area, (b) Spatial hydrophobicity values between the burn and non-burn sampling positions at Wit River study area. Means represent time in seconds for the Water Drop Penetration Test at each sampling location, with standard errors in brackets.

a) Seasonal

	Burn treatment		**Non-Burn treatment**	
Season	Time (s)	Category	Time (s)	Category
Pre-fire	249.42(±85.92)	Strongly Hydrophobic	527.89(±121.47)	Strongly Hydrophobic
Post-fire	206.04(±96.38)	Strongly Hydrophobic	86.29(±56.24)	Strongly Hydrophobic
Spring	668.63(±150.59)	Severely Hydrophobic	480.85(±133.76)	Strongly Hydrophobic
Summer	686.71(±148.11)	Severely Hydrophobic	667.00(±130.91)	Severely Hydrophobic
Autumn	1193.37(±139.84)	Severely Hydrophobic	957.77(±156.63)	Severely Hydrophobic

b) Spatial

Location	**Treatment**	**Time (s)**	**Category**
Centre	**Burn**	596.53 (±124.75)	Strongly Hydrophobic
Intermediate	**Burn**	819.50 (±144.34)	Severely Hydrophobic
Edge	**Burn**	664.97 (±127.16)	Strongly Hydrophobic
Matrix	**Non-burn**	575.22 (±129.06)	Strongly Hydrophobic
Recovering	**Non-burn**	932.15 (±123.28)	Severely Hydrophobic
Invaded	**Non-burn**	134.68 (±55.54)	Strongly Hydrophobic

Table 3.1: (a) Seasonal hydrophobicity values between the burn and non-burn treatment sites at Blaauwberg study area; and (b) Spatial hydrophobicity values between the burn and non-burn sampling positions at Blaauwberg study area. Means represent time in seconds for the Water Drop Penetration Test at each sampling location, with standard errors in brackets.

a) Seasonal

	Burn treatment		**Non-burn treatment**	
Season	**Time (s)**	**Category**	**Time (s)**	**Category**
Pre-fire	233.87 (±79.17)	Strongly Hydrophobic	127.12 (±40.73)	Strongly Hydrophobic
Post-fire	504.40 (±134.43)	Strongly Hydrophobic	108.25 (±33.81)	Strongly Hydrophobic
Summer	458.13 (±137.02)	Strongly Hydrophobic	1155.48 (±164.71)	Severely Hydrophobic
Autumn	919.60 (±206.14)	Severely Hydrophobic	1385.32 (±116.53)	Severely Hydrophobic
Winter	363.20 (±119.62)	Strongly Hydrophobic	1158.08 (±133.57)	Severely Hydrophobic

b) Spatial

Location	**Treatment**	**Time (s)**	**Category**
Centre	**Burn**	503.75 (±141.19)	Strongly Hydrophobic
Intermediate	**Burn**	421.45 (±113.66)	Strongly Hydrophobic
Edge	**Burn**	758.80 (±151.02)	Severely Hydrophobic
Matrix	**Non-burn**	814.75 (±148.58)	Severely Hydrophobic
Recovering	**Non-burn**	1030.13 (±141.96)	Severely Hydrophobic
Invaded	**Non-burn**	965.00 (±115.35)	Severely Hydrophobic