The effect of fire scars on microbial diversity of Fynbos soil

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Declaration

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Researcher: Ricardo Virgill Smart Date: December 2017

Dedication

This thesis is dedicated to Jerobiam M. Julies. Thank you for always being there for me, even through the most difficult time of my life. I appreciate your constant motivation and support.

I would also like to dedicate this thesis to my best friend Tyrone Jafta. Though I have lost you through a terrible accident during the course of this degree, our memories kept me going. I miss you, Tyrone. Rest in Peace.

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Summary

Microbial communities (bacteria, archaea, fungi, protista and viruses) are essential for the maintenance of a healthy balance in soil ecosystems. There are many factors that influence and disrupt this balance, including invasive species and fire events which disturb the properties and microhabitats of soil. Riparian zones are not typically exposed to fire. However, when the riparian zones are exposed to fire, it may have significant consequences for the natural patterns and processes of a soil ecosystem and the soil microbial communities. Invasive alien woody species such as *Acacia* and *Eucalyptus* spp. have become ubiquitous across riparian environments, affecting water and nutrient cycling and reducing plant diversity. However, the approaches to clear invasive species may also have negative consequences for ecosystem functioning. The 'slash and burn' technique is a biomass management tool that uses the felling of invasive stands, which are then stacked to build a pile (from dead plant biomass) and burnt. This study determined the effect of burning (the 'slash and burn' technique) of invasive biomass (*Acacia* and *Eucalyptus* spp.) on soil bacterial and fungal diversity and community structure in fynbos riparian zones (Western Cape, South Africa).

The sites chosen for this study were within fynbos regions invaded by *Acacia mearnsii* (also known as black wattle) or *Eucalyptus camaldulensis* (river red gum). Four study sites were chosen, each at different statuses of invasion. These sites were Bainskloof, Rawsonville, Robertson and Wellington. Before the mechanical removal of invasive species, the Bainskloof and Rawsonville sites consisted predominantly of *A. mearnsii*, with a small percentage cover of *Eucalyptus* spp. at the Rawsonville site. The Robertson and Wellington sites consisted predominantly of *E. camaldulensis*, with a small percentage cover of *Acacia spp.* at the Wellington site. Changes in the microbial diversity and community structure were assessed using automated ribosomal intergenic spacer analysis (ARISA) fingerprinting. Microbial diversity profiles of ARISA were determined by means of the Shannon (*H'*) and Simpson's complement (1-*D*) indices. Microbial community structure profile of ARISA was evaluated by means of Analysis of Similarity (ANOSIM), cluster analysis and non-metrical multidimensional scaling (NMDS). The Pearson correlation coefficient (PCC) analysis was used for the correlation between the chemical properties and microbial diversity (*H'*). Whereas, the principle component

analysis (PCA) was used to determine which chemical properties may explain the variation of microbial community structure post-fire.

This study showed that the 'slash and burn' of *Eucalyptus* biomass had a greater impact on the soil microbial communities compared to the 'slash and burn' of *Acacia* biomass. The data indicated that the 'slash and burn' of *Acacia* biomass (Bainskloof) did not affect the bacterial diversity (*H'*) post-fire. In contrast, the 'slash and burn' of *Eucalyptus* biomass (Robertson and Wellington; also Rawsonville, where some *Eucalyptus* biomass was present in the piles) led to a steep decrease in bacterial diversity (*H'*) immediately post-fire which remained relatively low a year after the burn event. Furthermore, the 'slash and burn' of *Acacia* and *Eucalyptus* biomass had no effect on the fungal diversity (*H'*). This, in turn, resulted in no variation of fungal diversity (*H'*) within and between invasion sites throughout the study.

Post-fire, all sites demonstrated a shift in microbial community structure. In addition, all the sites showed three distinct bacterial community structures separated by different sample times. The unique microbial community structure in the Bainskloof site, a year after the burn event, could be due to the disturbance of a flood. The unique bacterial community structures in the *Eucalyptus* (Robertson and Wellington) and Rawsonville sites, a year after the burn event, are likely due to the successional changes of the bacterial communities after the 'slash and burn'. Furthermore, the fungal community structures post-fire and a year after the burn event in the *Eucalyptus* sites could not be delineated as separate clusters. This was in contrast to the results in the Rawsonville site where the post-fire fungal community structure was different from the community structure a year after the burn event. Moreover, the fungal community structures in the *Eucalyptus* and Rawsonville sites a year after the burn event were similar. This similarity could possibly be due to the post-fire dominant fungal species that are beneath the soil surface layer where fire occurred or from adjacent areas around the burnt piles. These post-fire dominant fungal species have the capacity to disperse into the burnt areas by means of mycelial expansion from deeper to surface soil profiles or from the margins of the burnt piles into the burnt areas.

The sites exposed to the 'slash and burn' of *Eucalyptus* biomass showed that soil pH served as the strongest soil abiotic indicator for bacterial diversity (*H'*). This finding was not evident in the Bainskloof site, which was exposed to the 'slash and burn' of *Acacia* biomass. In this study, all the sites showed that the 'slash and burn' of *Acacia* and *Eucalyptus* biomass leads to an increase in soil pH. However, the bacterial diversity (*H'*) showed different trends between invasion sites post-fire. The 'slash and burn' of *Eucalyptus* biomass resulted in a decrease in bacterial diversity (*H'*). Whereas, the 'slash and burn' of *Acacia* biomass did not affect the bacterial diversity (*H'*) post-fire. As for the fungal communities, no soil abiotic properties served as a useful indicator for the fungal diversity (*H'*).

The soil pH, EC and PO_4 concentration explained the most variation of microbial communities in the sites exposed to the 'slash and burn' of *Eucalyptus* biomass. These trends were not observed after the 'slash and burn' of *Acacia* biomass at the Bainskloof site. At this site, no variation in EC and PO_4 concentration was recorded immediately post-fire. However, EC and PO_4 concentration a year after the burn event was relatively higher compared to the conditions pre-fire. As for the sites exposed to the 'slash and burn' of *Eucalyptus* biomass, the soil pH, EC and PO_4 concentration showed a steep increase immediately post-fire which remained relatively high a year after the burn event.

'Slash and burn' of *Eucalyptus* biomass left a patch where the fynbos vegetation did not recover. It is possible that the 'slash and burn' of *Eucalyptus* biomass may have damaged the roots and mycorrhizal fungi in the soil that consequently decreased the rate and capacity of recolonization in burnt areas. For future research, it will be useful to investigate the effect of 'slash and burn' of invasive biomass on specific functional groups (i.e. mycorrhizal fungi, ammonifiers and N-fixers) in the riparian zones of fynbos. It will also be of value to evaluate the recovery of these functional groups (if possible) post-fire and to determine what it means for the restoration of fynbos vegetation.

'Slash and burn' of *Acacia* biomass, however, is unclear due to the interference of a flood that occurred at the Bainskloof site during the trial period. The flood disturbed the burnt areas and led to the re-establishment of *A. mearnsii*. Therefore, for future research, an observational study

may be considered to assess whether fynbos vegetation will recover after 'slash and burn' of *Acacia* biomass. Taken together, the results demonstrated a shift in microbial communities post-fire. However, the microbial diversity (*H'*) remained the similar after the 'slash and burn' of *Acacia* biomass.

Opsomming

Mikrobiese gemeenskappe (bakterieë, archaea, fungus, protista en virusse) is noodsaaklik vir hul funksie om ’n gesonde balans te behou in grond. Daar is baie faktore wat hierdie balans beïnvloed en ontwrig, insluitende uitheemse bome en brande wat die eienskappe en mikrohabitats van grond versteur. Rivieroewers ervaar gewoonlik nie ’n brand nie, maar indien wel kan dit moontlik ’n beduidende invloed hê op die natuurlike patrone en prosesse van die grond ekosisteem en die grondmikrobiese gemeenskappe. Uitheemse indringer bosagtige spesies soos *Acacia* en *Eucalyptus* spp. het alomteenwoordig geword in oeweromgewings, en het sodoende die water- en voedingstofsiklusse beïnvloed en het plantdiversiteit verminder. Pogings om die indringerspesies te verwyder het egter negatiewe gevolge vir die funksionering van die ekosisteem. Die ‘sny-en-brand’ tegniek word gebruik om die biomassa te beheer en bestaan uit die afkap van uitheemse bome, waarvandaar ’n ophoping van dooie plant biomassa gemaak word en daarna gebrand word. Hierdie studie fokus op die effek van vuur (die ‘sny-en-brand’ tegniek) van uitheemse bome (*Acacia* en *Eucalyptus* spp.) op die grondbakteriese en fungus diversiteit en gemeenskapstruktuur in riveroewers van fynbos (Wes-Kaap, Suid-Afrika).

Die fynbos rivieroewers wat bestudeer word in hierdie studie is bedreig deur *Acacia mearnsii* (black wattle) of *Eucalyptus camaldulensis* (river red gum). Vier studie areas was gekies, waarvan twee van die studie areas bedreig was deur *A. mearnsii* en *E. camaldulensis*, onderskeidelik. Voor die verwydering van uitheemse bome, was die Bainskloof en Rawsonville areas gedomineer deur *A. mearnsii*, met ’n lae persentasie van *Eucalyptus* spp. by die Rawsonville area. Die Robertson en Wellington areas was gedomineer deur *E. camaldulensis*, met ’n lae persentasie van *Acacia spp.* by die Wellington area. Veranderinge in die mikrobiese diversiteit en gemeenskapstruktuur was bepaal deur die geoutomatiseerde ribosomale intergeniese spasie analise (ARISA) vingerafdruk metode. Hierdie metode is gebruik om die grondmikrobiese diversiteit en gemeenskapstruktuur te analiseer. Die mikrobiese diversiteitsprofiel van ARISA was bepaal met die gebruik van die Shannon (*H’*) indeks en Simpson komplement (1-*D*) indeks. Die mikrobiese gemeenskapstruktuur profiel van ARISA is geëvalueer met behulp van die Analise van Soortgelykheid (ANOSIM), kluster analise, en die nie-metriese multidimensionele skaling (NMDS). Die Pearson-korrelasiekoëffisiënt (PCC) analise is gebruik om die korrelasie tussen die chemiese komponente en die mikrobiese diversiteit te

bepaal. Die beginselkomponent-analise (PCA) is gebruik om te bepaal watter chemiese eienskappe die variasie in mikrobiese gemeenskapstruktuur na die brand veroorsaak.

Die studie bewys dat die 'sny-en-brand' van *Eucalyptus* biomassa 'n groter impak het op die mikrobiese gemeenskappe, in vergelyking met die 'sny-en-brand' van *Acacia* biomassa. Die 'sny-en-brand' van *Acacia* biomassa (Bainskloof) het geen effek op die bakteriese diversiteit (*H'* en 1-*D*) gehad nie. In teenstelling, het die 'sny-en-brand' van *Eucalyptus* biomassa (Robertson en Wellington; sowel as Rawsonville, wat 'n aantal *Eucalyptus* biomassa bevat het binne die ophopings), tot 'n afname in bakteriese diversiteit (*H'* en 1-*D*) gelei, wat gevolglik konstant gebly het tot 'n jaar na die brand. Met betrekking tot die fungus diversiteit (*H'* en 1-*D*), het die 'sny-en-brand' van *Acacia* en *Eucalyptus* biomassa geen effek op die fungus diversiteit (*H'* en 1-*D*) gehad nie. Die fungus diversiteit was soortgelyk tussen studie areas, voor en na die brand.

Na die 'sny-en-brand' van *Acacia* en *Eucalyptus* biomassa, het al die studie areas 'n verskuiwing in grondmikrobiese gemeenskapstruktuur getoon. Die bakteriese gemeenskapstruktuur, in al die studie areas, was verskillend by elke monsternemingsessie. Die oorstroming in die Bainskloof area het gelei tot 'n unieke mikrobiese gemeenskapstruktuur, 'n jaar na die brand. Die unieke bakteriese gemeenskapstrukture in die *Eucalyptus* en Rawsonville areas, 'n jaar na die brand, is as gevolg van die opeenvolgende veranderinge van bakteriese gemeenskappe na die brand. Die fungus gemeenskapstruktuur na die brand, in die *Eucalyptus* areas, was soortgelyk aan die gemeenskapstruktuur 'n jaar na die brand. In teenstelling, die fungus gemeenskapstrukture in die Rawsonville area, na die brand en 'n jaar na die brand, was verskillend. Verder, die fungus gemeenskapstrukture in die *Eucalyptus* en Rawsonville areas, 'n jaar na die brand was soortgelyk. Hierdie ooreenkoms kan moontlik toegeskryf word aan die dominante fungus spesies na die brand, wat voorkom onder of aan die rante van die verbrande grondoppervlakte. Hierdie dominante fungus spesies het die vermoë om oor die verbrande grondoppervlakte te versprei deur middel van miselium uitbreiding van dieper na grondoppervlak, of van die rante na binne die verbrande grondoppervlakte.

Die areas wat gebrand was met die *Eucalyptus* biomassa het getoon dat die grond pH as 'n sterk abiotiese indikator dien vir die grondbakteriese diversiteit (*H'*). Dit was nie die geval in die

Bainskloof area wat deur die *Acacia* biomassa gebrand was nie. Al die studie areas het getoon dat die 'sny-en-brand' van *Acacia* en *Eucalyptus* biomassa die grond pH verhoog het, maar die neiging in bakteriese diversiteit (*H'*) na die brand was verskillend. Na die 'sny-en-brand' van *Eucalyptus* biomassa was daar 'n afname in bakteriese diversiteit (*H'*). In teenstelling, was die bakteriese diversiteit (*H'*) nie geaffekteer na die 'sny-en-brand' van *Acacia* biomassa nie. In hierdie studie, was daar geen korrelasie getoon tussen die abiotiese eienskappe en fungus diversiteit (*H'*) nie.

Die grond pH, elektriese geleidingsvermoë (EC) en fosfaat (PO_4) konsentrasie verduidelik die meeste variasie tussen die mikrobiese gemeenskapstrukture na die brand, in die areas wat gebrand was met die 'sny-en-brand' van *Eucalyptus* biomassa. Hierdie tendense was nie gevind na die 'sny-en-brand' van *Acacia* biomassa (Bainskloof) nie. Na die 'sny-en-brand' van *Acacia* biomassa was daar geen variasie in EC en PO_4 konsentrasie getoon nie. In teenstelling, die areas wat gebrand was met die 'sny-en-brand' van *Eucalyptus* biomassa het getoon dat daar 'n toename is in grond pH, EC en PO_4 konsentrasie na die brand, wat gevolglik konstant gebly het tot 'n jaar na die brand.

Die 'sny-en-brand' van *Eucalyptus* biomassa het gelei tot brandletsels wat die plantegroei van fynbos spesies verhoed het. Dit is moontlik dat die 'sny-en-brand' van *Eucalyptus* biomassa die wortels en mikorrisa in die grond vernietig het wat gevolglik die herstel van plantegroei in gebrande areas beïnvloed het. Vir verdere navorsing sal dit nuttig wees om die effek van vuur (die 'sny-en-brand' tegniek) van uitheemse bome op spesifieke funksionele groepe (soos byvoorbeeld mikorrisa en N-fikseerders) in die oewersones van fynbos te bestudeer. Dit sal ook van waarde wees om die herstel van hierdie funksionele groepe (indien moontlik) na die brand te evalueer en te bepaal wat hierdie effek op die herstel van fynbosplantegroei mag hê.

Die 'sny-en-brand' van *Acacia* biomassa is onbekend as gevolg van die oorspoeling in die Bainskloof area gedurende die proefperiode. Die vloed het die verbrande oppervlakte versteur en het gelei tot die herstel van *A. mearnsii*. Op grond hiervan, mag dit van waarde wees om te bepaal of die fynbos plantegroei sal herstel na die 'sny-en-brand' van *Acacia* biomassa. Neem kennis dat daar 'n verskuiwing in mikrobiese gemeenskappe getoon was en dat die mikrobiese diversiteit (*H'*) konstant gebly het na die 'sny-en-brand' van *Acacia* biomassa.

CHAPTER ONE

Literature Review

1.1. Invasive species in fynbos

In South Africa, approximately 72,000 km^2 of the native vegetation of the Cape Floristic Region (CFR) has been lost. The remaining native or near-pristine fynbos covers 18,000 km^2 (Burgoyne *et al.* 2005; Moran & Hoffman 2012; Myers *et al.* 2000). Of the remaining fynbos vegetation, ~78% is protected, with 60% and 18% are found in mountainous and lowland regions, respectively (Moran & Hoffman 2012; Myers *et al.* 2000). The loss of fynbos vegetation is mainly due to land use practices such as agriculture, forestry and urban areas, and the displacement of native plants through alien plant invasion (Moran & Hoffman 2012; Myers *et al.* 2000). The CFR, which is a biodiversity hotspot of global significance, hosts around 8400 indigenous species, of which ~80% are endemic species (Moran & Hoffman 2012; Myers *et al.* 2000; Van Wilgen 2009).

Alien plant invasion poses a major threat to fynbos vegetation. A large proportion of the fynbos region is characterised as critically endangered (Figure 1.1). In 1997, the Working for Water (WfW) program introduced the combined application of mechanical removal and chemical control to remove the invasive species and promote the recovery of fynbos vegetation (Marais & Wannenburgh 2008; Moran & Hoffman 2012; Prins *et al.* 2004; Van Wilgen *et al.* 2012). The National Environmental Management: Biodiversity Act (Act 10 of 2004) promulgated actions to control alien plant invasion in fynbos, aimed at reducing the area of ecosystems under threat, reduce the extinction of indigenous species, prevent the loss and degradation of soil structure, prevent loss of function of threatened ecosystems and protect regions of high conservation importance (South Africa 2011). Over R133 million has been spent to control various invasive species listed in Table 1.1 (Marais & Wannenburgh 2008; Moran & Hoffman 2012). In the CFR mechanical removal and subsequent chemical control are being used widely to reduce alien plant invasion.

Although the fynbos vegetation is threatened by a large number of invasive species, this study only focuses on *Acacia* and *Eucalyptus spp.* These species are already widely distributed over the fynbos vegetation region (Figure 1.2) (Constantinides & Fownes 1994; Forsyth *et al.* 2004; Holmes & Cowling 1997a; Ruwanza *et al.* 2013; Tererai *et al.* 2013; Tucker & Richardson 1995;

Wilson *et al.* 2011; Witkowski 1991; Yelenik *et al.* 2004). Invasive *Acacia* spp. (family Fabaceae) made its appearance in fynbos more than 150 years ago, originating from Australia (Allsopp & Cherry 2004; Tucker & Richardson 1995; Vosse *et al.* 2008). The persistence of invasive *Acacia* spp. in fynbos is partially attributed to their capacity to release seeds immediately after maturation, the rapid accumulation of seed banks in the soil and their rapid growth rate (Holmes & Cowling 1997b; Tucker & Richardson 1995; Werner *et al.* 2008). Another reason for the persistence of invasive *Acacia* spp. in fynbos is their high germination success after a fire in the dry summer and autumn seasons (Holmes & Cowling 1997b). Invasive species of the genus *Eucalyptus* (family Myrtaceae) also originate from Australia, and their introduction to fynbos occurred during the late 19th century (Teraria *et al.* 2013; Vosse *et al.* 2008). Allelochemicals, which are produced by *Eucalyptus* spp., results in regeneration failure of natural vegetation, causing a reduction in biodiversity in areas invaded by these species (Pellissier & Souto 1999; Tererai *et al.* 2013; Zhang *et al.* 2010). The roots of *Eucalyptus* spp. and rhizosphere soils in *Eucalyptus* invaded areas contain high concentrations of allelochemicals, which in turn, increases the abundance of *Eucalyptus* spp. (Zhang *et al.* 2010; Zhang *et al.* 2012).

Acacia and *Eucalyptus* spp. are both major threats to the fynbos vegetation. Both genera have specific adaptations that allow them to outcompete native species (Morris *et al.* 2011; Tererai *et al.* 2013). *Acacia* spp. are more widely distributed in fynbos compared to *Eucalyptus* spp. (Figure 1.2), and their litter production is greater (Constantinides & Fownes 1994; Zhang *et al.* 2012). A recent study suggested that the growth rate of *Eucalyptus* spp. is faster than *Acacia* spp., which increases their ability to produce more biomass compared to *Acacia spp.* over the same time period (Zhang *et al.* 2012). Both *Acacia* and *Eucalyptus* spp. produce litter that influence soil and biogeochemical processes, which ultimately alters the ecosystem functioning (Morris *et al.* 2011; Slabbert *et al.* 2012). Constantinides and Fownes (1994) reported that the phosphorus (P) concentration in the leaves and litter of *Acacia* and *Eucalyptus* spp. are similar. However, the nitrogen (N) concentration of *Acacia* spp. litter is relatively higher due to the plant's ability to fix atmospheric nitrogen (Constantinides & Fownes 1994). Enriched litter increase the availability of N, which increases the abundance and growth rate of *Acacia* spp. (Werner *et al.* 2008).

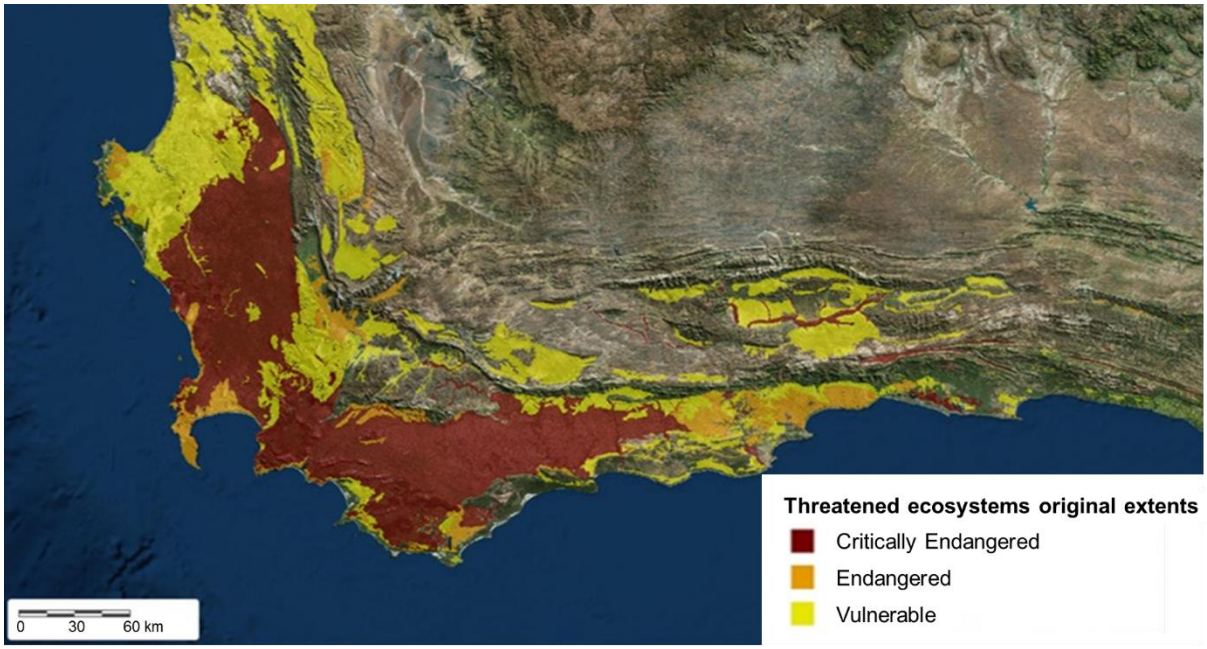


Figure 1.1: Fynbos vegetation was assigned to three categories, namely critically endangered, endangered, vulnerable or protected (clear areas) (South Africa 2011, South African National Biodiversity Institute 2006).

Table 1.1: Invasive species in fynbos (adapted from Marais & Wannenburgh 2008).

Genus		
Acacia spp.	*Hakea spp.*	*Psidium guajava*
Arundo donax	*Jacaranda mimosifolia*	*Ricinus communis*
Caesalpinia spp.	*Lantana camara*	*Rubus spp.*
Cereus spp.	*Melia azederach*	*Salix spp.*
Cestrum spp.	*Opuntia spp.*	*Sesbania punicea*
Chromolaena odorata	*Pinus spp.*	*Solanum spp.*
Eucalyptus spp.	*Populus spp.*	
Eichhornia crassipes	*Prosopis* spp	

(a) *Acacia* spp.

(b) *Eucalyptus* spp.

Figure 1.2: Fynbos vegetation is threatened by *Acacia* and *Eucalyptus* spp. Kotze *et al.* (2010) determined the range, abundance and total density (% invasive) of *Acacia* and *Eucalyptus* spp. These species are already widely distributed over the fynbos vegetation region.

1.2. Invasive species and competition for resources

Acacia and *Eucalyptus* spp. are highly competitive and can often outcompete indigenous fynbos species for resources such as light, nutrients, space and water. Consequently, canopy cover, seed production, richness and diversity of indigenous fynbos species in alien invaded areas decrease with time (Holmes & Cowling 1997a; Holmes & Cowling 1997b; Morris *et al.* 2011; Yelenik *et al.* 2004; Zhang *et al.* 2012). This competitive advantage, in turn, increases canopy cover, seed production, richness and diversity of invasive species in invaded areas (D'Antonio & Vitousek 1992; Gordon 1998; Rajcan & Swanton 2001).

1.2.1. Light

Indigenous fynbos plant species are generally smaller than *Acacia* and *Eucalyptus spp.* (Figure 1.3) (Morris *et al.* 2011; Van Wilgen & Richardson 1985; Van Wilgen *et al.* 1990; Witkoski 1991). This gives rise to an assemblage of indigenous fynbos species beneath the *Acacia* and *Eucalyptus* canopy, known as fynbos understorey (Holmes & Cowling 1997b; Zhang *et al.* 2012). Understorey species face the difficulty of obtaining sufficient light as the height, density and canopy cover of *Acacia* and *Eucalyptus spp.* limit light penetration (Tererai *et al.* 2013). Furthermore, the expansion of invasive species to cover available horizontal and vertical space leads to increased shading of surface areas (Werner *et al.* 2008). The enlargement of invasive species cover in newly invaded habitat patches is promoted by the absence of native pests and pathogens in invaded areas (Morris *et al.* 2011). Consequently, the abundance of invasive species increases, which results in an increase in canopy cover (Zhang *et al.* 2012). All these factors, along with the wide distribution of invasive species, significantly decrease the light resources for indigenous fynbos species.

1.2.2. Nutrients

Indigenous fynbos species in *Acacia* and *Eucalyptus* invaded areas are exposed to soils that are considerably enriched. Gaertner *et al.* (2011) found that the soil chemical properties in *Acacia* and *Eucalyptus* invaded areas in fynbos are significantly different compared to the fynbos vegetation reference sites. These include carbon (C), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), N and soil pH (Gaertner *et al.* 2011). Fynbos vegetation is known to grow

in soils with low nutrient concentrations (Stock & Lewis 1986; Yelenik *et al.* 2004). However, the high N concentration on the soil surface layer, enhanced by *Acacia* litter production and leaf input, promotes a competitive advantage for *Acacia* spp. over the indigenous fynbos species (Werner *et al.* 2008). The enriched soils of *Eucalyptus* invaded areas in fynbos could be due to the high photosynthetic and growth rates of *Eucalyptus* spp., as well as the soil microbial communities in the rhizospheres of invaded areas that help with the degradation of plant biomass and the high litter nutrient concentrations of the *Eucalyptus* spp. (Gaertner *et al.* 2011). The nutrient enrichment, after the clearing of *Acacia* and *Eucalyptus* spp., could potentially promote secondary invasion over the recovery of indigenous fynbos species (Gaertner *et al.* 2011; Yelenik *et al.* 2004).

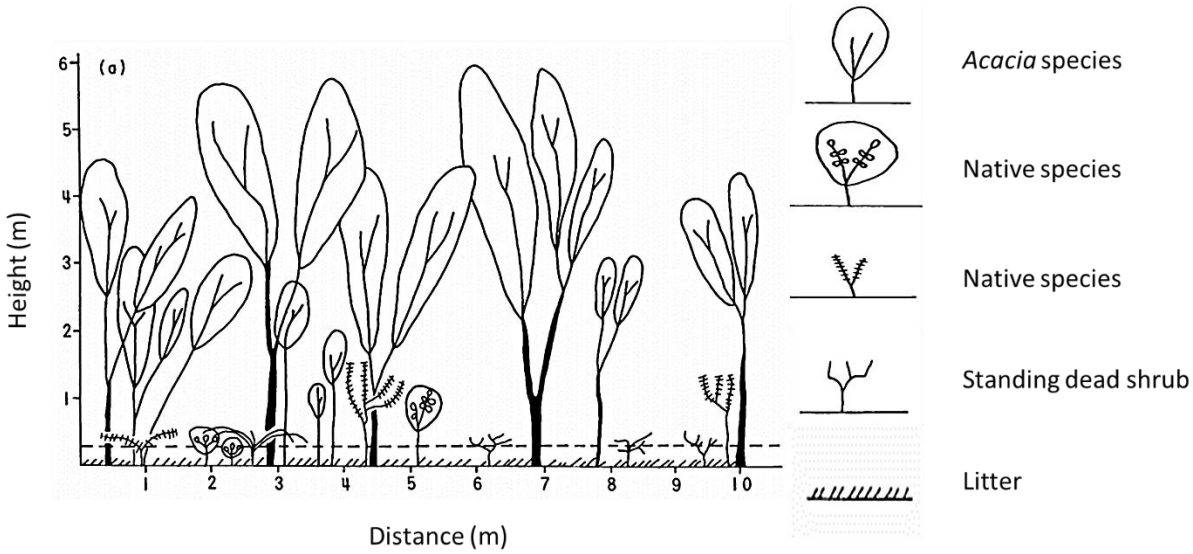


Figure 1.3: Fynbos vegetation profile of invaded areas (from Van Wilgen & Richardson 1985).

1.2.3. Space

A fundamental component that enhances the distribution of invasive species is wind (Holmes & Cowling 1997a). The wide wind dispersal of invasive propagules may invade spaces where fynbos vegetation could otherwise flourish. *Acacia* and *Eucalyptus* spp. are larger in comparison to indigenous fynbos species (Figure 1.3), which means that the former species are more exposed to wind than the latter. This promotes the dispersal seed of invasive species and limits the seed distribution of indigenous fynbos species (Van Wilgen & Richardson 1985). Furthermore,

fynbos experiences average winds of 150-250 km.day^{-1} during warm, dry summers and 160-200 km.day^{-1} during cold, wet winters (Kraaij *et al.* 2013; Witkoski 1991). This wind exposure of fynbos (and invaded) landscapes distributes the seeds of invasive species over long distances, which could potentially promote the establishment of invasive species into new areas. Seeds may also be distributed by ants, birds and through vertebrate dispersal (Holmes & Cowling 1997a; Knight & MacDondald 1991; Tucker & Richardson 1995).

1.2.4. Water

Acacia and *Eucalyptus* spp. are both major threats to the water resources in fynbos vegetation. Key factors which influence the water availability, in areas with high water holding capacity, are the root system of invasive species and their rapid production of root mass (Bouilett *et al.* 2002; Morris *et al.* 2011). This root system promotes hydraulic redistribution in *Eucalyptus* spp., which transports water from wet to dry areas via their roots (Ruwanza *et al.* 2013). The dry areas are generally the soil surface layers, which are exposed to high levels of evaporation. In addition, Bouilett *et al.* (2002) observed that *Eucalyptus* spp. in a recently invaded site (~1-year old) extended their roots up to 3 m deep. *Acacia* spp., on the other hand, have a relatively higher drought tolerance and may persist and thrive during the dry fynbos summer conditions (Crous *et al.* 2012; Werner *et al.* 2008). This capacity, along with wind exposure during summer, could also be important factors for the successful expansion of *Acacia* spp. The establishment of *Acacia* spp. into new habitats could potentially limit the water availability in invaded areas, which consequently, could result in a decrease in the richness of indigenous fynbos species (Gaertner *et al.* 2011). Thus, *Acacia* and *Eucalyptus* spp. are both excessive water users, however, with *Acacia* spp. being the prime water users in South Africa (Le Maitre *et al.* 2000).

1.3. Riparian zones in fynbos

Riparian zones, which are essential for conveying crucial resources such as energy and materials between freshwater and terrestrial ecosystems, are critical zones for soil microbial processes underlying many ecosystem services and harbour a unique biodiversity (Naiman & Décamps 1997; Slabbert *et al.* 2014). The WfW program aims to remove the majority of the invasive species in fynbos, particularly in the riparian zones and water catchment areas. Over R55 million has been spent (1997-2006) to clear *Acacia* (~R45 million) and *Eucalyptus* spp. (~R10 million)

from the South African riparian zones (Marais & Wannenburg 2008, Prins *et al.* 2004; Van Wilgen *et al.* 2012). Along with other factors mentioned, the successful expansion of invasive species along the fynbos riparian zones, may be attributed to the frequent flooding that results in the water-aided dispersal of seeds (Tererai *et al.* 2013; Vosse *et al.* 2008). This becomes a competitive advantage for invasive species, which outcompete the indigenous fynbos species in the riparian zones (Crous *et al.* 2012; Tererai *et al.* 2013). The removal of invasive species in fynbos riparian zones is still a major hurdle.

1.4. Fire in fynbos

Fire disturbs the properties and microhabitats of soil, which in turn, affects the soil microbial communities that are essential for the maintenance of a healthy balance in soil ecosystems. There are many factors that influence and disrupt this balance, including invasive species and fire events (Dooley & Treseder 2012; Ferrenberg *et al.* 2013; Neary *et al.* 1995; Reazin *et al.* 2016; Slabbert *et al.* 2014; Vosse *et al.* 2008). The use of fire ('slash and burn' technique) in fynbos to reduce invasive biomass and to enhance recovery appears to be a cost-effective and efficient management strategy (Blanchard & Holmes 2008; Holmes 2001). The 'slash and burn' technique comprises of the felling of invasive stands, which is then stacked to build a pile from dead plant biomass, and burnt.

Riparian zones are not typically exposed to fire. However, when the riparian zones are exposed to fire, it may have significant consequences for the natural patterns and processes of a soil ecosystem and the soil microbial communities (Dooley & Treseder 2012; Ferrenberg *et al.* 2013; Neary *et al.* 1995; Reazin *et al.* 2016). Stacking of biomass significantly enhances fire risk in riparian zones, when left to dry. In some instances, the fire, either managed or accidental, will leave a scar, where the native vegetation does not recover. This results in uneven restoration, or may even give a competitive advantage to species involved in secondary invasion (Maubane & Jacobs 2016).

1.4.1. Effect of fire

Fire has a major effect on soils (Raison 1979). Fire changes the water relations, microhabitats, structure and porosity in soil, which in turn, has an influence on natural patterns and processes

of a soil ecosystem (Bowman *et al.* 2009; Brooks *et al.* 2004; Fayos 1997; Neary *et al.* 1999; Raison 1979). Fire also alters the soil temperature and moisture, which could potentially generate changes in the microbial communities (Batten *et al.* 2006). An increase in soil temperature decreases the soil matric potential, which could inhibit soil respiration and promote the senescence of plant species (Reynolds *et al.* 2015). Moreover, the behaviour of fire is determined by the intensity and the rate of spreading, and weather conditions which could enhance dry soil conditions through increasing in hydrophobicity after a fire event (Alexander *et al.* 2014; Brooks *et al.* 2004; Miller & Urban 1999; Neary *et al.* 1999; Rothermel 1983). Factors and weather conditions that promote the fire intensity and spread rate are listed in Table 1.2.

Table 1.2: Factors that control the behaviour of fire in ecosystems. The behaviour of fire is determined by the intensity and spread rate of the fire, and weather conditions. However, the intensity and spread rate of the fire, and weather conditions are determined by the factors listed below (Alexander *et al.* 2014; Brooks *et al.* 2004; Miller & Urban 1999; Neary *et al.* 1999; Rothermel 1983).

Fire intensity	Fire spread	Weather conditions
Canopy-bulk density	Wind direction	High temperature
Ground fuels	Fire size	Low humidity
Surface fuels	Fire probability	Strong wind
Fuel moisture content	Susceptibility of species	Slightly clouded
Fuel surface to volume ratio	Fire tolerance class of species	Drought
Slope steepness	Spatial configuration	
Fire height		

1.4.2. Promotion of fire conditions in fynbos

The South African National Biodiversity Institute (SANBI) has recorded various fire events in fynbos from 1927 until 2015 (CapeNature Fires, 2016). It is apparent that the fire events in fynbos have increased substantially over the past three decades (Figure 1.4). Natural fires in fynbos (natural vegetation) could ignite and spread under high air temperatures (>20°C) and low relative humidity (<40%) in the presence of wind and low fuel moisture content (dead and live biomass of indigenous fynbos species) (Van Wilgen & Richardson 1985). These conditions are usually found during summer to early autumn (December-March) (Kraaij *et al.* 2013; Witkowski

1991). Natural fire in fynbos is difficult to ignite under low air temperatures (<15°C) and high relative humidity (>50%) in extreme cloudy weather conditions (Van Wilgen & Richardson 1985). These conditions are prevalent during winter to early spring (June-September) (Kraaij *et al.* 2013; Witkoski 1991).

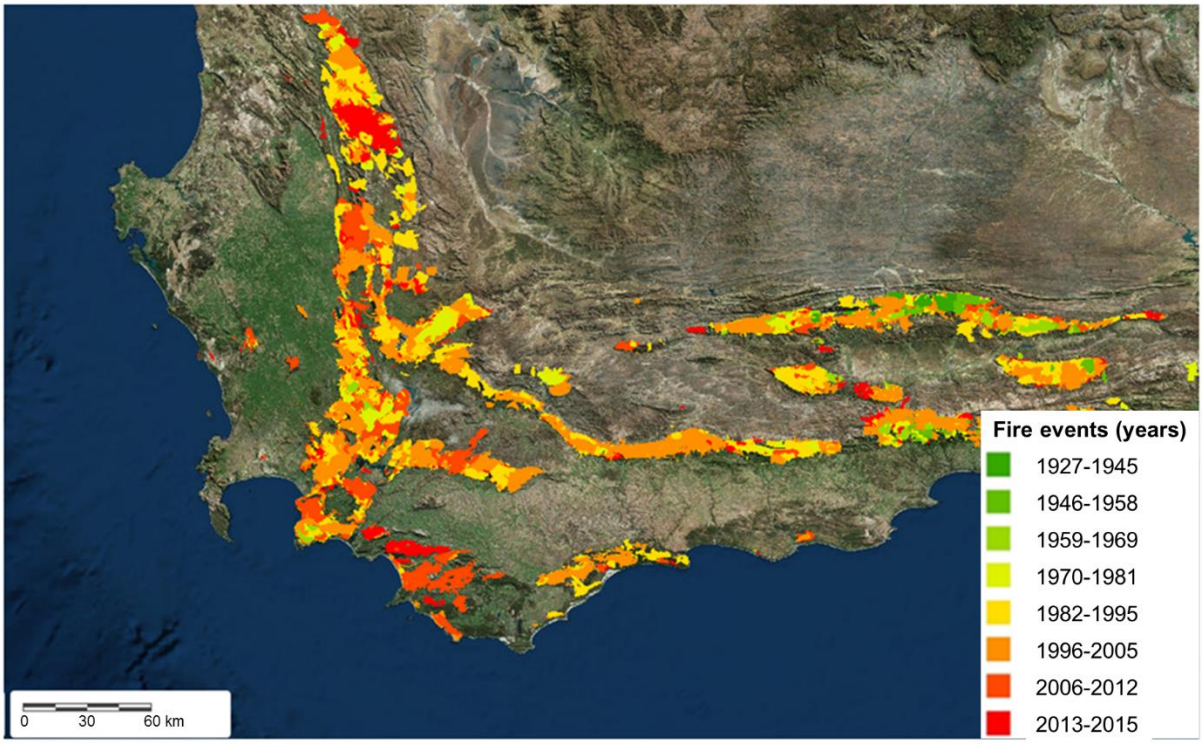


Figure 1.4: Fire events in fynbos since 1927 until 2015. SANBI's fire management recorded the fire event to promote future analysis to include the frequency of fire occurrence, the return intervals of fire, and the veld age (CapeNature Fires 2016).

1.4.3. Fire in invaded fynbos areas

Fire in invaded fynbos areas, which have not been cleared from invasive species, would mostly be sustained by the fuel moisture content of the fynbos understorey that is beneath the invasive canopy (Van Wilgen & Richardson 1985). This is due to the height differences between invasive and indigenous fynbos species (Figure 1.3), and the inability of fynbos species to ignite the canopy of invasive species (Van Wilgen & Richardson 1985). In areas which have been cleared of invasive species, the fire behaviour is different. Holmes (2001) found that fire was sustained by the dead plant biomass of invasive species on the soil surface layers, which resulted in a

greater fire intensity in comparison to a fire which was sustained by fynbos species (plant biomass only). In addition, the clearance of invasive stands contributes to additional dead fuel moisture content along with the fynbos species on the soil surface layers. This, in turn, generates a fire with a greater heat release and soil heating compared to a fire which is predominantly sustained by fynbos species (Holmes 2001). This is especially the case when invasive biomass is stacked (Maubane & Jacobs 2016). Moreover, the large amount of fuel available for fire in invaded areas enhances both the risk and intensity of fires and is a major challenge for conservation agencies.

1.4.4. Effect of fire on soil chemical properties

Immediately post-fire, the total N and NH_4-N concentration in the top two centimetres of soil increases (Hernández *et al.* 1997; Stock & Lewis 1986; Thanos & Rundel 1995). This is due to the conversion of organic forms of soil N into the production of inorganic NH_4-N and NO_3-N, and the N input of ash and the insoluble soil organic N over the soil surface layer (Grogan *et al.* 2000; Neary *et al.* 1999). Post-fire, the NH_4-N concentration remains relatively high for a short duration of time, which later decreases by means of NH_4 volatilisation or consumption (Neary *et al.* 1999; Stock & Lewis 1986; Wan *et al.* 2001). In contrast, the NO_3-N concentration remains relatively low post-fire due to inadequate heat-induction to initiate NO_3 volatilisation (Choromanska & DeLuca 2002; Wan *et al.* 2001). Factors that could increase the NO_3-N concentration post-fire are rain and fires that reach soil temperatures up to 400 °C (Bosatta & Agren 1995; Choromanska & DeLuca 2002; Thanos & Rundel 1995). Furthermore, Choromanska and DeLuca (2002) reported that the soil water potential is a crucial factor which could influence the NH_4-N and NO_3-N post-fire. Soils with low water potentials that are exposed to fire for the first time have lower NH_4-N and NO_3-N concentrations compare to soils that were previously exposed to fire (Choromanska & DeLuca 2002). In general, soil available N (NH_4-N and NO_3-N) is known to have some relationship with soil microbial dynamics, and perhaps especially so in low-fertility fynbos soils (Choromanska & DeLuca 2002; Slabbert *et al.* 2014).

Electrical conductivity (EC) measures soluble cations (i.e. calcium (Ca), magnesium (Mg) and potassium (K)) in soil (Kutiel & Naveh 1987; Pereira *et al.* 2017). The EC increases immediately post-fire due to the combustion of organic matter which promotes the concentration of soluble

cations (Hernández *et al.* 1997; Kutiel & Naveh 1987). Nonetheless, the EC subsequently decreases gradually post-fire due to the fixation of the salt, precipitation and leaching (Hernández *et al.* 1997; Kutiel & Naveh 1987). The change in cations are closely related to the change in pH post-fire, and this has some implications for soil microbial structure in fynbos soils (Slabbert *et al.* 2014).

P is a vital element for plant growth and could lead to P-starvation when the concentration is too low. The low soil P concentration could, in turn, inhibit the growth of plants. The total and inorganic P concentration increase immediately post-fire, as it has been reported that the soil P concentration is directly proportional to the fire intensity (Kuiel & Naveh 1987; Reinhart *et al.* 2016; Romanya *et al.* 1994; Schaller *et al.* 2015). Other studies suggested that the pre-fire P stocks in plants and litter are found in ash or the charred plant remains post-fire (DeBano & Conrad 1978; Muñoz-Rojas *et al.* 2016). Furthermore, the phosphate (PO_4) concentration also increases immediately post-fire (DeBano & Conrad 1978; Khanna & Raison 1986). This is due to the soil heating and the mineralisation of P that promotes the production of polyphosphate (soluble forms) which is deposited in ash (DeBano & Conrad 1978; Khanna & Raison 1986). Slabbert *et al.* (2014) found that more than any other nutrient, soil PO_4 is a nutrient closely related to soil microbial structure in fynbos soils.

Soil pH is a crucial factor for fynbos vegetation and is known to have a significant effect on soil microbial communities (Fierer & Jackson 2006; Kim *et al.* 2014; Lauber *et al.* 2009; Osborne *et al.* 2011; Prins *et al.* 2004). Soil pH increases post-fire and the increase could be attributed to the alkaline nature of ash (Barreiro *et al.* 2016; Hernández *et al.* 1997; Jensen *et al.* 2001). The increased soil pH post-fire could change the availability of soil nutrients, which in turn, could affect the plant recuperation (Pereira *et al.* 2017).

1.4.5. Advantage and disadvantage of fire in fynbos

Natural fires are essential for the germination of fynbos species, though every fynbos region and vegetation type have its own characteristic post-fire recovery and fire return period (Keeley & Bond 1997; Musil & de Witt 1991; Manders & Richardson 1992; Rebelo & Siegfried 1990; Van Wilgen & Richardson 1985). Fire promotes high temperatures and dry heat conditions for the

desiccation of seed coats and supports the growth of seed embryos (Brown 1993; Keeley & Fotheringham 2000). Additional benefits of periodic fire to fynbos germination are listed in Table 1.3. It is hypothesised that areas invaded by *Acacia* and *Eucalyptus* spp. in fynbos experience more frequent fires compared to indigenous fynbos species in its native habitat. However, the *Acacia* spp. are a greater threat for more frequent fires in fynbos than *Eucalyptus spp.* (Allsopp & Cherry 2004). Furthermore, some indigenous fynbos species persist in *Acacia* invaded areas post-fire due to their resprouting ability and the contribution of vertebrate dispersal. Holmes and Cowling (1997b) found that the effect of two fire cycles in *Acacia* invaded sites has led to a drastic decrease (~70%) in indigenous fynbos species.

Table 1.3: Factors that stimulate the seed germination of fynbos species (adapted from Brown 1993).

Factors	Effect
Ethylene and ammonia in smoke	Stimulate seed germination
Chemical factors in plant-derived smoke	Stimulate seed germination
Heat	Fracturing hard seed coats
	Stimulating seed embryos
High temperature	Desiccation of seed coats

1.5. Soil microbial communities

Soil microbial communities play a significant role in the ecology of fynbos. The soil ecosystem is a complex and dynamic biological system that provides a variety of soil microhabitats for prokaryotic and eukaryotic microorganisms, such as bacteria and fungi (Bååth & Arnebrant 1994; Fierer *et al.* 2007; Nannipieri *et al.* 2003; Prober *et al.* 2015). These microbial communities play an important role in all known biological reactions and serve as a major reservoir for the mobilisation of nutrients (Craine *et al.* 2010; Jha *et al.* 1992). These microbial communities also mediate in the decomposition of organic material in soil and are associated with changes in carbon dioxide (CO_2) emission (Dooley & Treseder 2012). The stability of microbial communities is dependent on nutrient availability in the soil, which is influenced by the transformations mediated by microbial biomass (Fierer *et al.* 2007; 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 communities to recover more rapidly after

a disturbance (Alexrood *et al.* 2002). Therefore, it is necessary to investigate the effect of environmental factors, alien plant invasion and fire on microbial communities.

1.5.1. Effect of environmental factors on soil microbial communities

Microbial communities are affected by various environmental factors and have the capacity to adapt and survive across a wide range of physicochemical properties in soil (Buckley & Schmidt 2001; Robertson *et al.* 1997). It is crucial to note that soil microbial communities follow different trends in various environmental conditions (Fierer *et al.* 2007; Hu *et al.* 1999). For example, beta-*Proteobacteria* and *Bacteriodetes spp.* are dominant in C-rich environments and their growth rate is relatively high in non-limiting resources (Figure 1.5) (Fierer *et al.* 2007). However, *Acidobacteria* spp. are dominant in nutrient-poor environments and their abundance and growth rate decrease with the increased C (Figure 1.5) (Fierer *et al.* 2007; Hu *et al.* 1999). In addition, there are also some bacterial species such as alpha-*Proteobacteria*, *Firmicutes* and *Actinobacteria* spp. that remain steady in the variation of C availability in soil (Fierer *et al.* 2007). Moreover, it is also important to note that environmental changes may lead to a variation in soil microbial diversity. For example, neutral to slightly acidic soils may be associated with higher bacterial diversity, whereas more acidic soils may be associated with lower bacterial diversity (Figure 1.6) (Fierer & Jackson 2006).

Soil fungal communities are tree-specific and the soils and litter under different trees could explain a great proportion of the variation in soil fungal communities (Unbanová *et al.* 2015). According to the study conducted by Schmidt and Bolter (2002), it appears that the soil fungal biomass dominated in the presence of vegetation, whereas bacterial biomass dominated in the absence of vegetation. This finding was similar to the study performed by Bååth (1980) which documented that the clear-cutting (stem removal) of trees results in a drastic decrease of soil fungal biomass. The lack of energy sources after the clear-cutting of trees could also lead to a decrease in fungal mycelium and mycorrhizal fungal hyphae (Bååth 1980). Barreiro *et al.* (2016) observed that the addition of *Eucalyptus* wood chips promoted the growth of fungi and inhibited the growth of bacteria in soils.

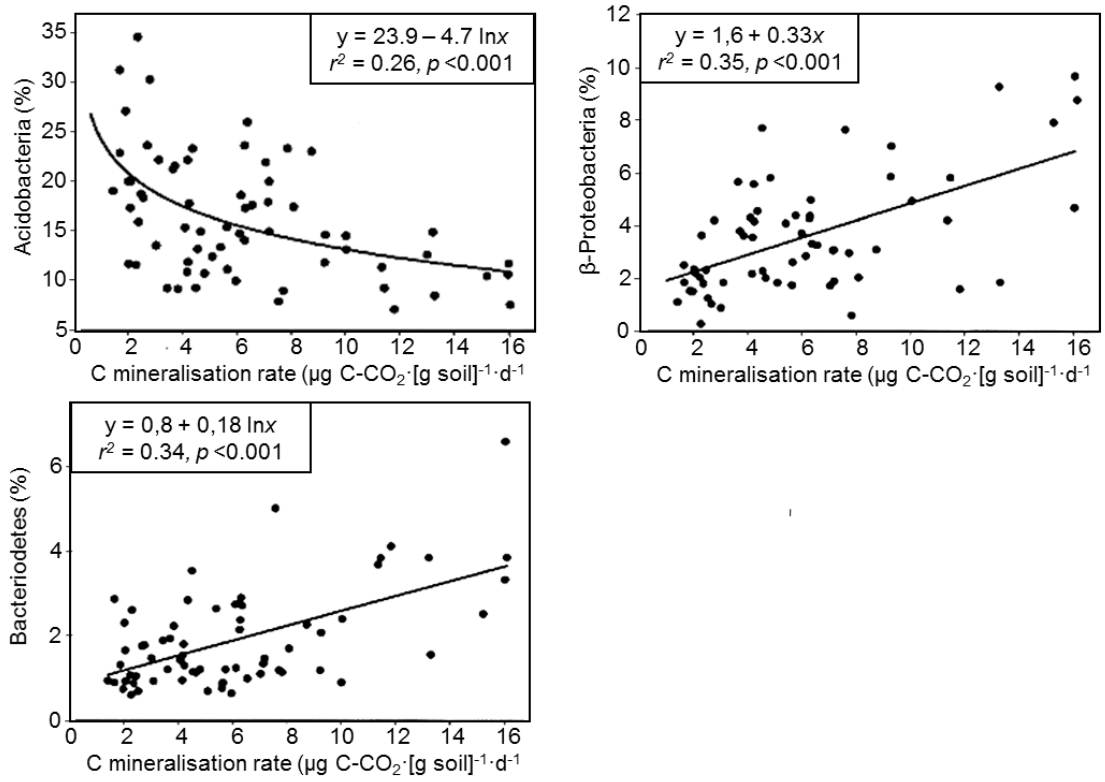


Figure 1.5: The effect of C availability in soil on soil bacterial species (Fierer *et al.* 2007).

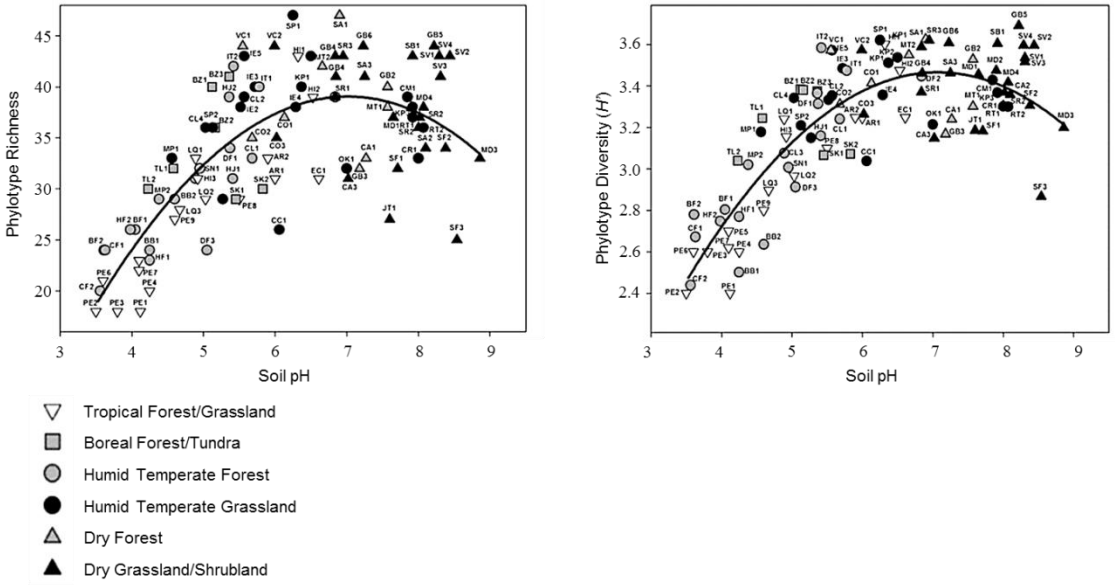


Figure 1.6: The correlation between bacterial richness and diversity, and the increase of soil pH. Neutral to lower acidic soils may be associated with higher bacterial diversity, whereas most acidic soils may be associated with lower bacterial diversity (from Fierer & Jackson 2006).

Plants are crucial for the survival of soil fungal communities and the plant-fungal interaction could be classified as parasitic, saprophytic or symbiotic (Azul *et al.* 2011; Berbee 2001). Saprotrophic fungi are mainly present in soils with high SOM content and play a crucial role in the breakdown of dead plant biomass, which contains essential nutrients for growth and reproduction (Dai *et al.* 2007; Schmidt & Bolter 2002). Ectomycorrhizae are present on the tips of the plant roots and facilitate uptake of various essential nutrients, which are beneficial in the seedling establishment and growth of plant communities (Azul *et al.* 2011). Plant symbionts, however, appear to be extremely sensitive towards plant management practices (Azul *et al.* 2011; Hartmann *et al.* 2012). This might be because soil fungal communities are more host-specific towards plant roots than bacterial communities (Barreiro *et al.* 2016). Moreover, the increase of plant species has a direct impact on the fungal richness, diversity and community structure in soil (Unbanová *et al.* 2015).

1.5.2. Effect of alien plant invasion on soil microbial communities

Alien plant invasion may lead to a decrease in fynbos species, and the invasion of *Acacia* spp. are known to reduce the bacterial diversity in fynbos sites (Slabbert *et al.* 2014; Vosse *et al.* 2008). No previous studies attempted to investigate the soil bacterial diversity in *Eucalyptus* invaded areas in fynbos. The decrease in soil bacterial diversity and fynbos species in invaded sites might be due to the plant-microbe interactions, and the soil nutritional composition in respective areas (Grayston *et al.* 1998; Hart *et al.* 2005; Otsuka *et al.* 2008; Slabbert *et al.* 2014; Yelenik *et al.* 2004). The introduction of alien plant invasion into a native ecosystem could cause soil aggregation and erosion, which in turn, may disturb the soil microhabitats that could alter the soil microbial communities (Batten *et al.* 2006; Wolfe & Klironomos 2005). The variation in the physicochemical properties (soil C, N, moisture, pH and salinity) after alien plant invasion and the presence of different plant species may also alter the microbial communities (Batten *et al.* 2006; Morris *et al.* 2011; Nannipieri *et al.* 2003; Schmidt & Bolter 2002; Stock *et al.* 1995; Witkoski 1991). Additionally, the alteration of microbial communities after alien plant invasion has a striking effect on the fitness of native plant species. The following three examples illustrate this. Firstly, the absence of beneficial soil microbial communities could decrease the re-establishment of indigenous species (Azul *et al.* 2011). Secondly, the change in soil microbial communities affects the rate of biogeochemical cycling and generates environmental conditions

that could be inhospitable to support the growth of indigenous species (Batten *et al.* 2006; Wolfe & Klironomos 2005). And finally, the invasion of some wood-rotting fungal parasites into the sapwood (between the bark and heartwood) could potentially cause the death of indigenous species (Dai *et al.* 2007). In addition, plant species provide vital resources to the soil by means of organic matter input from leaf-litter production and root exudates, including the production of allelopathic compounds which could potentially alter the soil microbial communities (Morris *et al.* 2011; Nannipieri *et al.* 2003; Pellissier & Souto 1999; Stock *et al.* 1995; Tererai *et al.* 2013; Witkoski 1991; Wolfe & Klironomos 2005; Zhang *et al.* 2010).

1.5.3. Effect of fire on soil microbial communities

Fire leads to a shift in soil microbial communities (Figure 1.7) (Dooley & Treseder 2012; Ferrenberg *et al.* 2013; Neary *et al.* 1995; Reazin *et al.* 2016). The shift may be explained by the vertical temperature impact of fire which is greater in the top two centimetres of the soil, where the microbial communities are in abundance (Dooley & Treseder 2012; Hart *et al.* 2005; Sun *et al.* 2008). Through heat-induction, fire results in microbial mortality, which alters the structure of microbial communities post-fire and results in a community structure different than those from unburnt areas (González-Pérez *et al.* 2004; Hamman *et al.* 2007; Hart *et al.* 2005). The structural difference post-fire may be attributed to the microbial-sensitivity towards soil heating (Hart *et al.* 2005).

Researchers have hypothesised that fungi are more sensitive to fire than bacteria (Bárcenas-Moreno & Bååth 2009; González-Pérez *et al.* 2004; Smith *et al.* 2008). This is because bacteria have a higher heat tolerance capacity than fungi (Dunn *et al.* 1985; Hart *et al.* 2015). However, it has been documented that this hypothesis does not apply to all fungi, as some fungi have the capacity to recover from the effect of heat as a result of their heat-tolerant spores, which can withstand high temperatures (Bárcenas-Moreno & Bååth 2009; Murrell & Scott 1966). Soil abiotic properties that have a significant effect on the soil bacterial communities post-fire are soil pH, temperature and water content (Shen *et al.* 2016; Smith *et al.* 2008; Sun *et al.* 2016), all of which may be affected by intense fire. Previous studies reported that bacterial diversity is more likely to increase along with the increase in soil pH post-fire, whereas the diversity of fungi

indicated minimal variation (Bååth & Arnebrant 1994; Bárcenas-Moreno & Bååth 2009; Dooley & Tresender 2012).

The shift in microbial communities immediately post-fire could also be explained by the variation of various key factors listed in Figure 1.7, including the C-substrate limitation in soil (Dooley & Treseder 2012). The release of organic matter from heat-sensitive microbial communities post-fire, serve as an energy source for heat-tolerant bacteria (Daiz-Ravina *et al.* 1996). Bárcenas-Moreno and Bååth (2009) studied the effect of soil heating, to simulate different fire intensities, on microbial communities and found that the bacterial respiration and growth are dependent on the availability of C. In addition, the bacterial growth after heat exposure is relatively fast. In contrast to bacteria, fungal responses are relatively slow due to the effect of heat (Bárcenas-Moreno & Bååth 2009). These findings were similar to what has been observed in the study of Barreiro *et al.* (2016) which indicated that the decrease of soil fungal biomass post-fire remains relatively low for a long period of time. A possible reason for this could be the absence of vegetation post-fire (Dooley & Treseder 2012). Bååth *et al.* (1995) found that fire destroys the roots and mycorrhizal fungi in soil, which consequently decreases the recolonisation capacity and rate in burnt areas. Furthermore, the reduction in soil fungal biomass, along with above-ground plant biomass on the soil surface layer post-fire, influence essential environmental processes such as the microbial degradation of plant biomass and the incorporation of the plant C into microbial biomass to produce CO_2 (Dooley & Tresender 2012; Lal 2004; Van Wilgen & Richardson 1985). A decrease in microbial biomass may leads to a decrease in microbial activity (Jha *et al.* 1992). This mostly depends on whether there are concomitant losses in keystone taxa which may drive microbial activity. For this reason, it may be hypothesised that the low abundance or absence of plant production post-fire might be a key component for the slow recovery of soil fungal species.

Another factor which could promote a shift in soil microbial community structure is the intensity of the fire (Reazin *et al.* 2016; Xiang *et al.* 2014). Reazin *et al.* (2016) observed that a high-intensity fire, which generally has a longer fire duration than a low-intensity fire, has a greater effect on the soil fungal communities in comparison to a low-intensity fire. This study has recorded a notable difference in the soil fungal diversity and community structure exposed

to high- and low-intensity fires (Reazin *et al.* 2016). As for the soil bacterial communities, Xiang *et al.* (2014) observed that the relative abundance of alpha-*Proteobacteria*, *Acidobacteria*, *Plantobacteria* and delta-*Proteobacteria* spp. decrease in both high- and low-intensity fires, whereas the relative abundance of *Bacteriodetes* and beta-*Proteobacteria* spp. seemed to increase in high- and low-intensity fires.

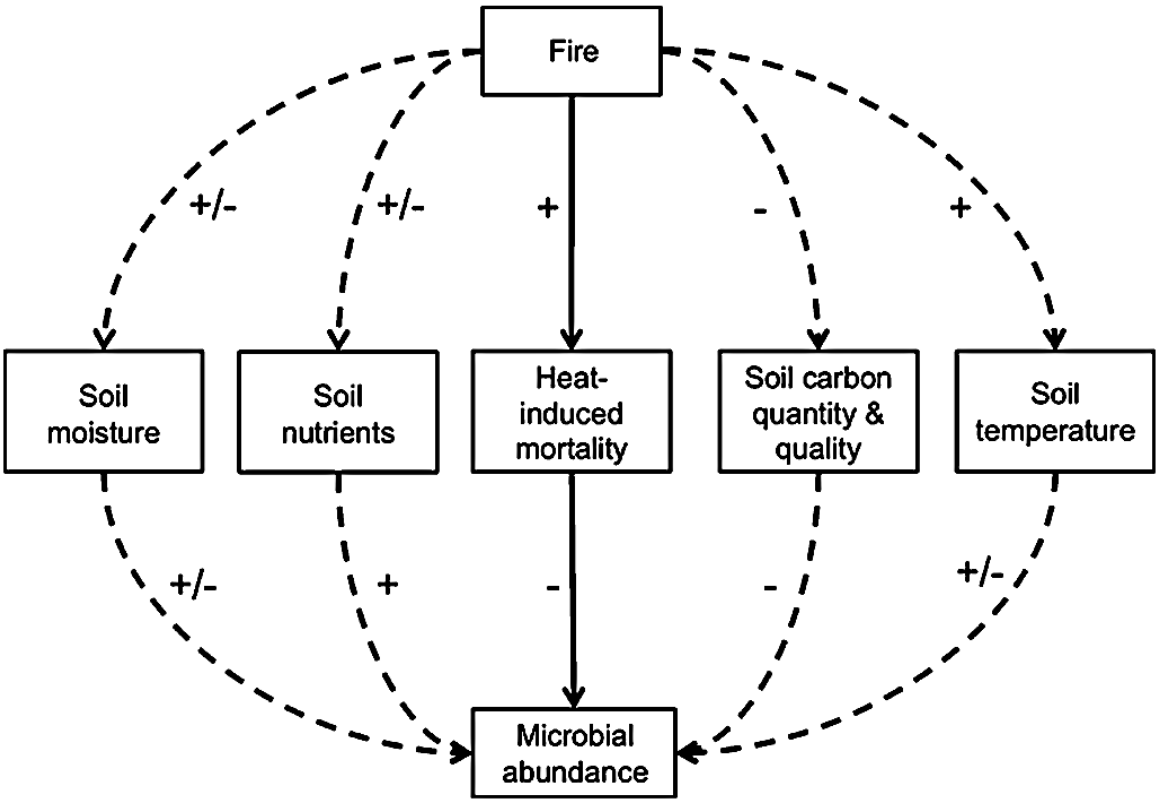


Figure 1.7: The shift in soil microbial community structure immediately post-fire could be explained by the variation of essential factors (from Dooley & Treseder 2012).

In the present study, it is hypothesised that the 'slash and burn' of invasive biomass (*Acacia and Eucalyptus* spp.) would have different fire intensities, which will promote a shift in soil microbial community structure. However, whether these soil microbial community structures will be similar or promote the recovery of fynbos vegetation, after the 'slash and burn' exposure, is unknown.

Objectives

The effect of fire on soil microbial communities differ amongst ecosystems and fire types (Dooley & Tresender 2012). No previous study has focused on the effect of 'slash and burn' of invasive biomass (*Acacia and Eucalyptus* spp.) on soil microbial (bacterial and fungal) diversity and communities in fynbos or in the riparian zones. The objectives of this study are as follows:

1. To determine the effect of 'slash and burn' of invasive biomass on the soil microbial diversity and community structure.
2. To correlate changes in the soil chemical properties to the soil microbial diversity and community structure.

CHAPTER TWO

Materials and Methods

2.1. Site description

The sites chosen for this study were fynbos riparian zones (Western Cape, South Africa) invaded by *Acacia mearnsii* or *Eucalyptus camaldulensis* (Figure 2.1). Four study sites were chosen, with different invasion statuses (Table 2.1). Before the mechanical removal of invasive species, the Bainskloof and Rawsonville sites consisted predominantly of *A. mearnsii*, with a small percentage cover of *Eucalyptus* spp. at the Rawsonville site. The Robertson and Wellington sites consisted predominantly of *E. camaldulensis*, with a small percentage cover of *Acacia* spp. at the Wellington site. The sites were all located in the riparian zones of the Berg River (Wellington), Breede River (Rawsonville and Robertson) and Wit River (Bainskloof) (Figure 2.2).

Figure 2.1: The study sites were all located in the fynbos riparian zones (Western Cape, South Africa) of the Berg River (Wellington), Breede River (Rawsonville and Robertson) and Wit River (Bainskloof).

Table 2.1: Study sites along with the dominant invasive species.

Site	River	Latitude, Longitude	Invasive species
Wellington	Berg River	-33˚ 64' S, 18˚ 96' E	*E. camaldulensis*
Rawsonville	Breede River	-33˚ 54' S, 19˚ 16' E	*A. mearnsii*
Robertson	Breede River	-33˚ 72' S, 19˚ 47' E	*E. camaldulensis*
Bainskloof	Wit River	-33˚ 84' S, 19˚ 87' E	*A. mearnsii*

(a) Berg River (Wellington)

(b) Breede River (Rawsonville)

(c) Breede River (Robertson)

(d) Wit River (Bainskloof)

Figure 2.2: The riparian zones situated next to Breede River, Berg River and Wit River of the study sites in the fynbos biome of the Western Cape.

2.2. Sample collection

Sites were selected randomly where removed invasive plant biomass was piled (stacked). Three piles were sampled at the Wellington site, of which two were located within invaded areas, while the other was surrounded by farmland. Five piles were sampled at the Rawsonville and Robertson sites, respectively. The Rawsonville site is owned by a private owner who farms with cattle and sheep as livestock and is situated at the bottom of a low mountain in the Quaggas Berg private nature reserve. The Robertson site is owned by the Langeberg Municipality. Seven piles were also sampled at the Bainskloof site which is located in the Bainskloof Pass.

Soil samples were taken from three positions inside and near the piles. The centre (C) samples were collected in the middle of the piles, while the intermediate (I) samples were collected at the area between the centre and edge of the pile. These two sampling points were used as representatives of the burnt samples. The control samples (O) were collected outside the piles (~2 m away) and serve as unburnt samples. (Figure 2.3).

Soil samples from each pile were collected at three different sample times, namely pre-fire, post-fire and a year after the burn event (Figure 2.4). Pre-fire samples were taken in May-June 2015 from undisturbed soil samples before the piles were assembled. Thereafter, each pile was built from either *Acacia* or *Eucalyptus* biomass, depending on the particular site. After the piles were assembled and burnt, post-fire samples were collected in June-July 2015 (Figure 2.5). The final sampling session took place a year after the burn event (Figure 2.6).

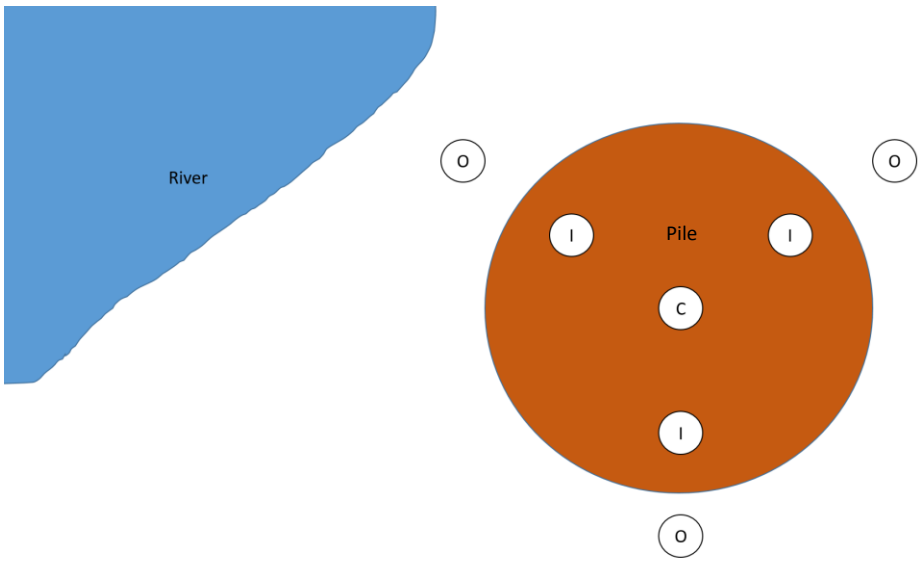


Figure 2.3: A schematic representation of the sample collection. The distance between each section depends on the size of the pile. Triplicates of each sample were taken. The centre samples are indicated as (C), intermediate as (I) and the control as (O).

(a) Pre-fire sampling

(b) A pile built from invasive biomass

(c) Burnt the pile

(d) Post-fire sampling after cooling

(e) A year after the burn event

Figure 2.4: Sampling process along with the 'slash and burn' technique at Bainskloof (Wit River).

(a) Berg river (Wellington)

(b) Breede River (Rawsonville)

Figure 2.5: The piles after 'slash and burn' of invasive biomass.

(a) Berg river (Wellington)

(b) Breede River (Rawsonville)

(c) Breede River (Robertson)

Figure 2.6: The piles of each site a year after the burn event.

2.3. Soil analysis

Up to 500 g of the surface soils (0-10 cm) were collected with a handheld spade. Samples were air dried on paper bags by spreading out each in thin layers. Thereafter, the soil was sieved through a 2 mm sieve to remove larger particles and analysed by the Soil Science Department at Stellenbosch University. Analyses included soil pH, electrical conductivity (EC), ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}) and the major cations including calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K). Ten grams of soil was mixed with 20 ml distilled water to measure the soil pH and EC. The soil pH and EC were measured by the Hanna HI 8424 meter and the Hanna HI 8733 meter, respectively (Robertson *et al.* 1999). For the NH_4^+ and NO_3^- extractions, 0.5 M potassium sulphate (K_2SO_4) solution was used (Kandeler & Gerber 1988, Page *et al.* 1992). The NH_4^+ concentration was determined according to the indophenol blue method, whereas, the NO_3^- concentration was determined according to the salicylic acid method (Anderson & Ingram 1993). Furthermore, P-Bray 2 solution was used to extract the PO_4^{3-} in soil (Bray & Kurtz 1945), while the molybdenum blue method was used to determine the PO_4^{3-} concentration in soil (Holman 1943). Genesys 20 spectrophotometer was used to measure the absorbance of NH_4^+ (at 655 nm), NO_3^- (at 410nm) and PO_4^{3-} (at 660 nm). Moreover, ammonium acetate (NH_4OAc, 1.0 M) was used to extract the major cations in soil, whereas the atomic absorption spectrometer (Varian 240 FS) was used to measure the major cations in soil (Simard 1993).

Soil samples were also sent to Bemlab in Strand (SANAS accredited testing laboratory, Western Cape) for the analyses of soil C and N. These samples were processed as described in SSSA (1996). Samples were combusted prior to measurement, with both steps carried out on a Leco Truspec® CN Analyser.

2.4. Molecular characterisation

2.4.1. DNA extraction

A ZR Soil Microbe DNA Microprep kit was used (Zymo Research USA) for DNA extractions. Two hundred and fifty milligrams (250 mg) of soil was 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 visualised under ultraviolet light.

2.4.2. PCR amplification and primers

Purified DNA samples were prepared in triplicate for polymerase chain reaction (PCR). The PCR mixture (10.0 µl) contained 0.2 µl of each 500 nM primer, 2.6 µl of MilliQ water, 5.0 µl of 2X Kapa Taq Ready Mix (KapaBiosystems, South Africa) and 2.0 µl of DNA (>100 ng/µl). The thermal cycling reaction conditions for bacterial and fungal automated ribosomal intergenic spacer analysis (ARISA) are summarised in Table 2.2 and 2.3, respectively. The primers used for bacterial and fungal ARISA are listed in Table 2.4. The GeneAmp PCR system 9700 was used to perform PCR reactions. One percent agarose gels stained with ethidium bromide were used to separate the PCR amplicons. Two microliters of each PCR product were added to the gel to be visualised using ultraviolet light. Triplicate PCR samples were pooled and sent to the DNA Sequencing Facility at the Central Analytical Facility (CAF) at Stellenbosch University for capillary electrophoresis.

Table 2.2: 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	34
Annealing	56 °C	50 s	
Extension	72 °C	70 s	
Final extension	72 °C	7 min	1
	4 °C	Hold	

Table 2.3: 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 2.4: The primers used in this study.

ARISA	Primer	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

2.4.3. Automated ribosomal intergenic spacer analysis (ARISA)

Changes in the microbial diversity and community structure were measured using ARISA fingerprinting. The PCR amplicons were separated by means of capillary electrophoresis according to different fragment sizes and fluorescent intensities. GeneMapper 5.0 software was used for the conversion of fluorescence data to electropherogram data of the separated components. A LIZZ 1200 size standard was used for bacterial and fungal ARISA with base pairs sizes between 100-1000 bps in length. Peak heights were analysed instead of peak sizes. This is because peak sizes are influenced by the variation in an area, which in turn, leads to an inconsistency in larger peaks. The bin size for ARISA profiles was 3 bps, as it shared the highest number of peaks in comparison to other bin sizes ranging from 1 to 7 bps (Slabbert *et al.* 2010). For further analysis, the data retrieved from GeneMapper 5.0 software was transferred to Microsoft Excel (Microsoft Corporation).

2.5. Soil microbial diversity

2.5.1. Diversity indices

To investigate the effect of 'slash and burn' of invasive biomass (*Acacia and Eucalyptus* spp.) on soil microbial (bacterial and fungal) communities in riparian zones of fynbos, the data was collated according to the study sites described in Table 2.1. The different fragment sizes 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). The average number of OTUs for each pile for all three sample times was determined and used to calculate the Shannon diversity (*H'*) and Simpson's complement (1-*D*) indices.

The Shannon diversity (*H'*) and Simpson's complement (1-*D*) indices were calculated to compare the soil microbial diversity profiles of ARISA of each pile at each site pre-fire, post-fire and a year after the burn event. The *H'* index (Equation 2.1) uses the proportional abundance (p_i) to describe the evenness and species abundance of each sample. Whereas the 1-*D* index (Equation 2.2) 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 are likely to be the same. 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.

The Shannon diversity (*H'*) index is defined by: **Equation 2.1.**

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

The Simpson's complement (1-*D*) index is determined by: **Equation 2.2.**

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

2.5.2. Statistical analysis

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

In order to assess the effect of 'slash and burn' of invasive biomass on the microbial diversity in invaded fynbos sites, the controls were compared to the burnt 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). In addition, the variation within and between invasion sites was analysed on the same principle.

2.6. Soil microbial communities

2.6.1. Cluster analysis

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 the soil bacterial and fungal communities within invasion sites for all three sample times. The S_w index units ranged from 0 to 1, with 0 indicating completely different communities and 1 indicating that sites share the same communities. The b_{i1} and b_{i2} variables compared the fraction of different fragment sizes of two distinct samples.

The Whittaker similarity (S_w) index was calculated by: **Equation 2.3.**

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

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

2.6.2. Analysis of Similarity

The Bray-Curtis index was applied to quantify the dissimilarity (R-value) of the soil microbial communities by using Analysis of Similarity (ANOSIM). 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, with 0 indicating that sites share the same communities and 1 indicating completely different communities.

2.6.3. Non-metrical multidimensional scaling

To observe the relatedness of microbial communities, non-metrical multidimensional scaling (NMDS) was performed to analyse the microbial community distance matrices of all three sampling times within and between invaded fynbos sites. 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.

2.7. Soil chemical properties correlated to soil microbial diversity and community structure

The correlation between soil chemical properties and microbial diversity was determined by using Pearson correlation coefficient (PCC) analysis. 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 principle component analysis (PCA) was used to determine which chemical properties may explain the variation of microbial community structure after the effect of ‘slash and burn’ of invasive biomass.

CHAPTER THREE

Results

3.1. Molecular characteristics

DNA was successfully extracted from soil samples and the concentration was >100 ng/µl for all the samples. The bacterial and fungal PCR products presented fragments ranging from 200-1500bps and 300-1200bps, respectively. The bacterial and fungal ARISA electropherograms presented peaks ranging from 100-1000bps in length as indicated by the LIZZ 1200 size standard.

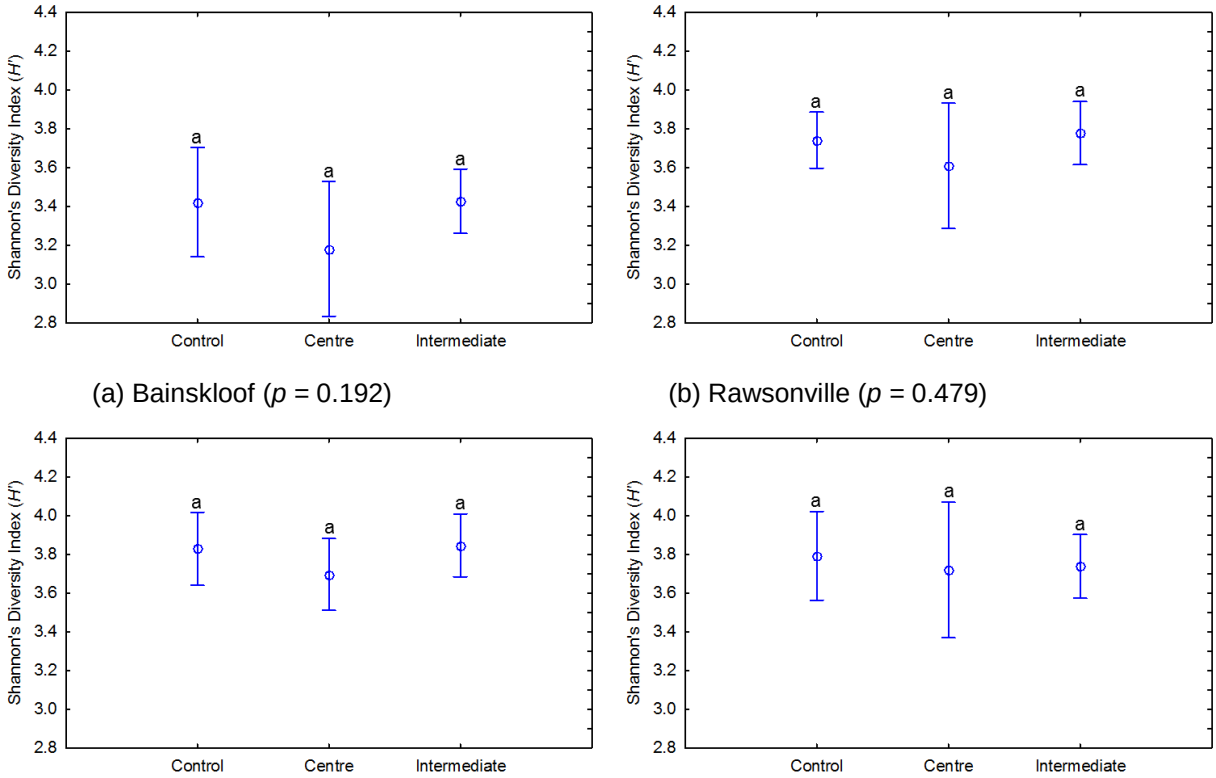
3.2. Soil bacterial diversity

3.2.1. Variation within sites pre-fire

The bacterial richness and diversity profiles of ARISA for each respective study site are shown in Table 3.1. The pre-fire bacterial diversity (*H'*), in the respective study sites, was similar (not significantly different, $p > 0.05$) at all the different sampling points, i.e. control, centre and intermediate samples (Figure 3.1). Based on these results, the control, centre and intermediate samples taken from each pile were pooled to determine whether the pre-fire bacterial diversity (*H'*) was similar in all the piles at a particular study site. Results from the one-way ANOVA in Figure 3.2 indicated that the pre-fire bacterial diversity (*H'*) was similar in all the soil samples for all the study sites, apart from the Wellington site ($p = 0.003$). Here, the pre-fire bacterial diversity (*H'*) in the samples of pile 3 was significantly lower in comparison to pile 1 ($p = 0.003$) and 2 ($p = 0.011$) (Figure 3.2d). The variation in pre-fire bacterial diversity (*H'*) between piles may have resulted because the piles were located in different areas within the Wellington site. Piles 1 and 2 were located towards the centre of the area where biomass burning was practised, while pile 3 were located on the edge of the area, surrounded on one side by vineyards and conceivably could have been affected by agricultural activities.

Table 3.1: The bacterial richness and diversity profiles of ARISA of the four study sites during all three sample times. The diversity was determined by means of the Shannon diversity (*H'*) and Simpson's complement (1-*D*) indices. The standard deviation is indicated in brackets.

	Pre-fire		**Post-fire**		**A year after burn event**	
	Control	Centre and intermediate	Control	Centre and intermediate	Control	Centre and intermediate
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)



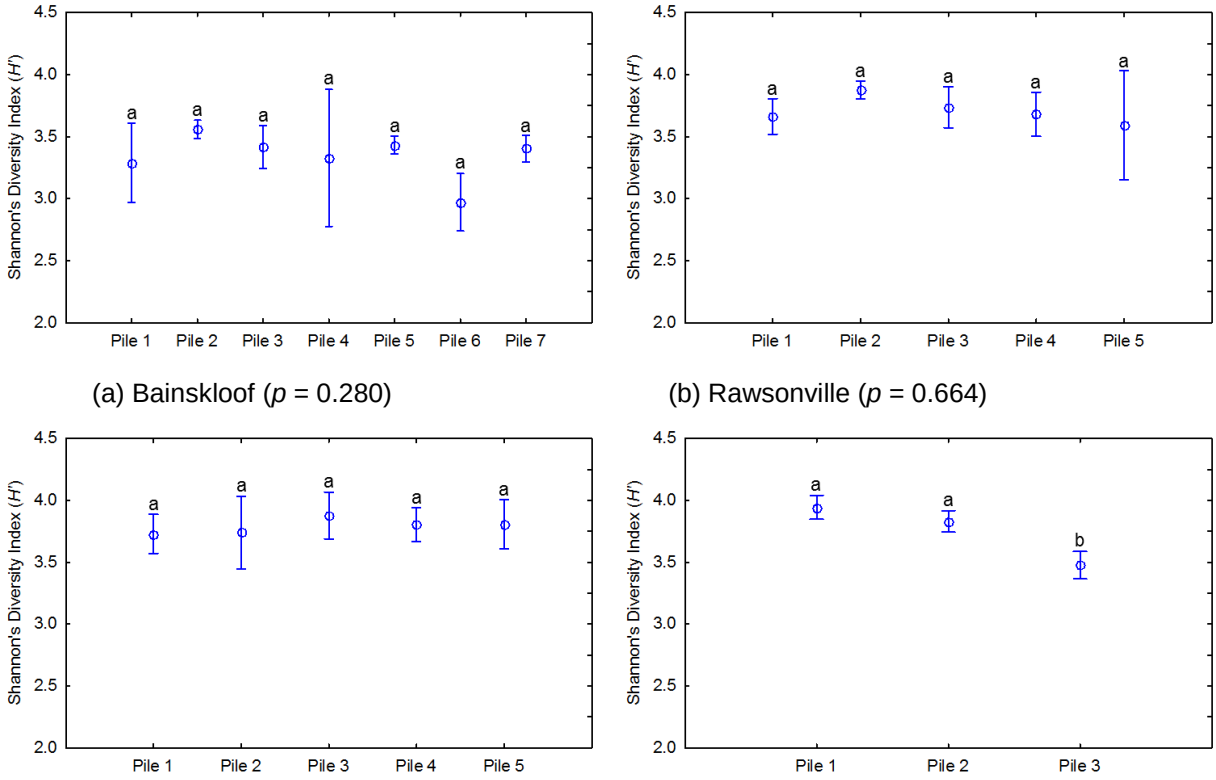
(a) Bainskloof (p = 0.192)

(b) Rawsonville (p = 0.479)

(c) Robertson (p = 0.384)

(d) Wellington (p = 0.940)

Figure 3.1: The pre-fire bacterial diversity (*H'*) at different sampling points (control, centre and intermediate samples), in respective study sites. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).



(a) Bainskloof (p = 0.280)

(b) Rawsonville (p = 0.664)

(c) Robertson (p = 0.895)

(d) Wellington (***p* = 0.003**)

Figure 3.2: The pre-fire bacterial diversity (*H'*) in all the soil samples (control, centre and intermediate samples) of each pile. The *p*-values are indicated in brackets and the significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

3.2.2. Variation between sites pre-fire

The bacterial diversity (*H'*) in the pre-fire soil samples ranged from 3.30 to 3.77 across the four study sites (Table 3.1). These values represent the mean bacterial diversity (*H'*) of the centre and intermediate samples. The Robertson site had the highest bacterial diversity score (*H'* = 3.77), followed by Wellington (*H'* = 3.73), Rawsonville (*H'* = 3.69) and then Bainskloof (*H'* = 3.30). When the pre-fire bacterial diversity (*H'*) in the centre and intermediate samples (combined) of all the study sites was compared to one another, a significant difference was recorded ($p < 0.001$) (Figure 3.3). This comparison was performed to evaluate whether the pre-fire bacterial diversity (*H'*) was similar in all the sites, in the areas to be burnt. The one-way ANOVA post-hoc (Tukey Honest Significant Difference (HSD)) test in Figure 3.3 revealed that the pre-fire bacterial diversity (*H'*) in the Bainskloof site was significantly lower ($p < 0.05$) in comparison to the other study sites, i.e. Rawsonville, Robertson and Wellington. Before the mechanical removal, the Bainskloof and Rawsonville sites consisted predominantly of *A. mearnsii*. Nonetheless, there was a small percentage cover of *Eucalyptus spp.* at the Rawsonville site. The Robertson and Wellington sites consisted predominantly of *E. camaldulensis*, with a small percentage cover of *Acacia spp.* at the Wellington site.

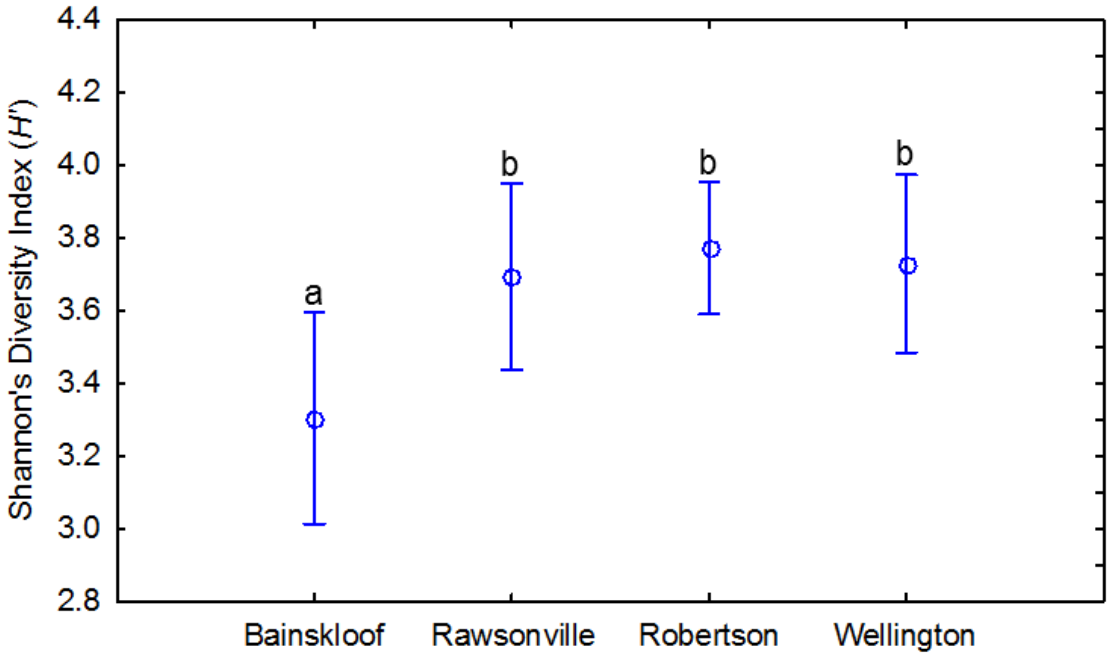
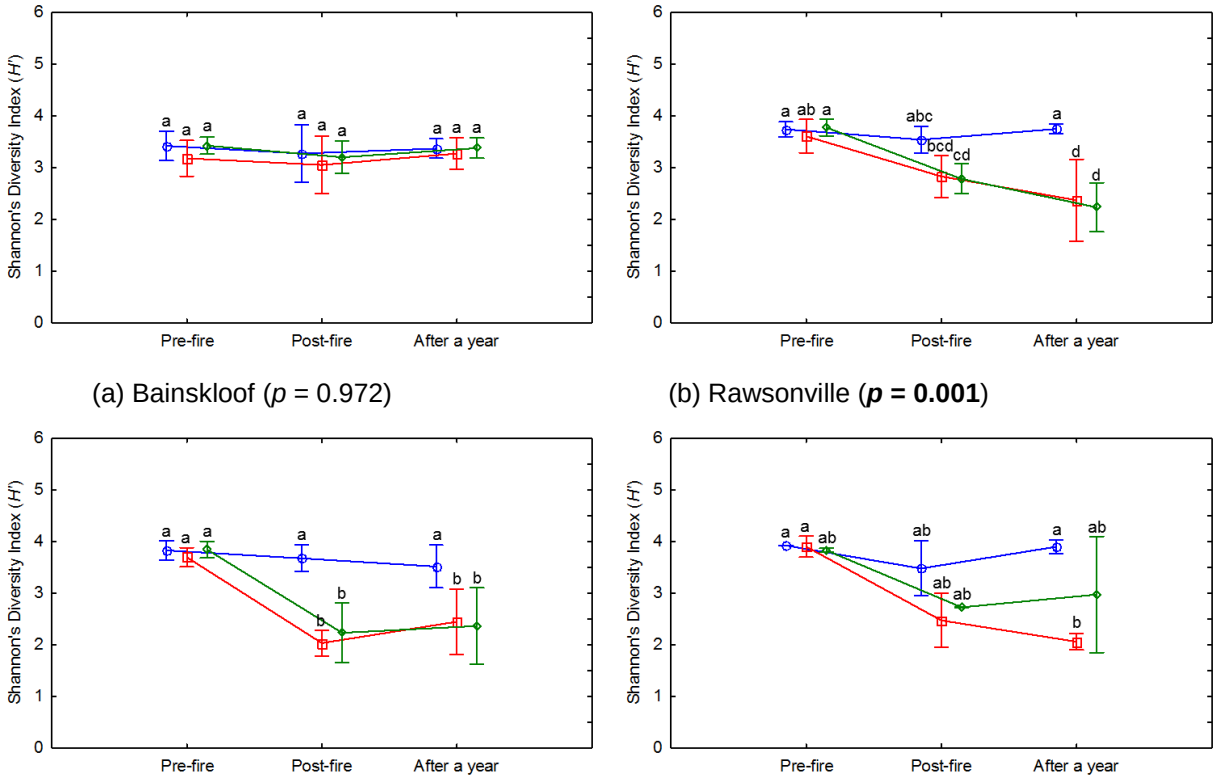


Figure 3.3: The pre-fire bacterial diversity (*H'*) in respective study sites (***p* <0.001**). Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

3.2.3. Effect of 'slash and burn' of invasive biomass on soil bacterial diversity

After the burning of the invasive biomass (post-fire), the bacterial diversity (*H'*) and Simpson's diversity (1-*D*) index from the centre and intermediate samples (burnt samples) were similar for all the respective sites (Figures 3.4; 3.5). Based on these results, the centre and intermediate samples (burnt samples) were pooled for further analysis.

Post-fire, the bacterial diversity (*H'*) in the burnt samples showed two different trends (Table 3.1). In the Bainskloof site, the pre-fire bacterial diversity (*H'*) 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, however, indicated a decrease in bacterial diversity (*H'*) immediately post-fire which remained low a year after the burn event (Table 3.1).



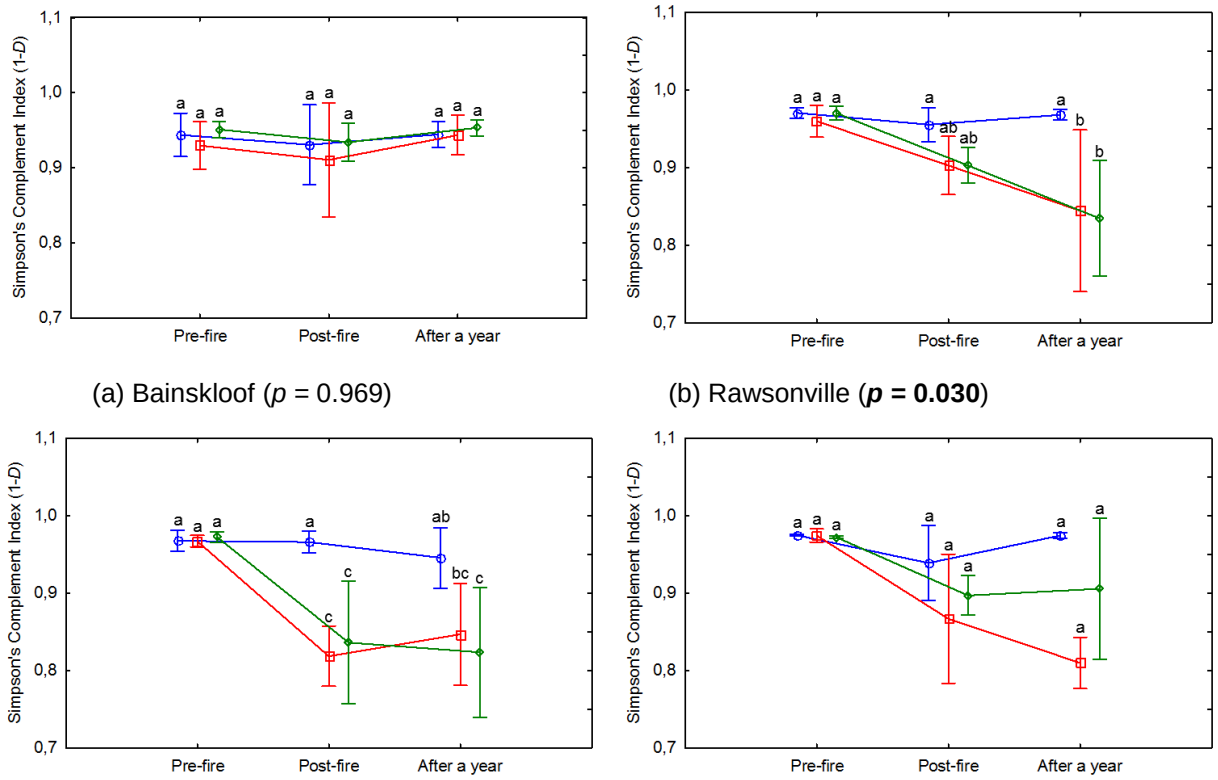
(a) Bainskloof (p = 0.972)

(b) Rawsonville (***p* = 0.001**)

(c) Robertson (***p* = 0.002**)

(d) Wellington (p = 0.178)

Figure 3.4: The effect of 'slash and burn' of invasive biomass on the bacterial diversity (*H'*) in the four study sites. The blue represents the bacterial diversity (*H'*) of the control samples, whereas the red (centre) and green (intermediate) represent the bacterial diversity (*H'*) in the soil samples underneath the piles (or burnt samples). Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).



(a) Bainskloof (p = 0.969)

(b) Rawsonville (***p* = 0.030**)

(c) Robertson (***p* = 0.007**)

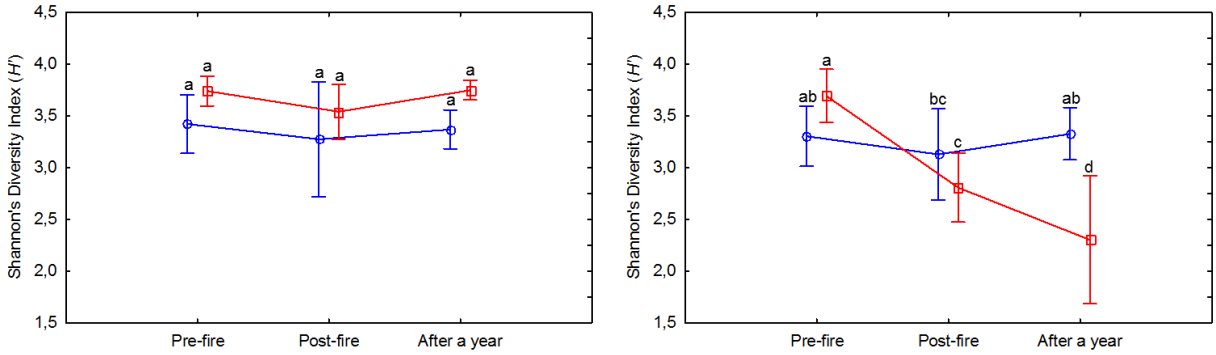
(d) Wellington (p = 0.255)

Figure 3.5: The effect of 'slash and burn' of invasive biomass on the bacterial diversity (1-*D*) in the four study sites. The blue represents the bacterial diversity (1-*D*) of the control samples, whereas the red (centre) and green (intermediate) represent the bacterial diversity (1-*D*) in the soil samples underneath the piles (or burnt samples). Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

3.2.4. Variation within invasion sites for all three sample times

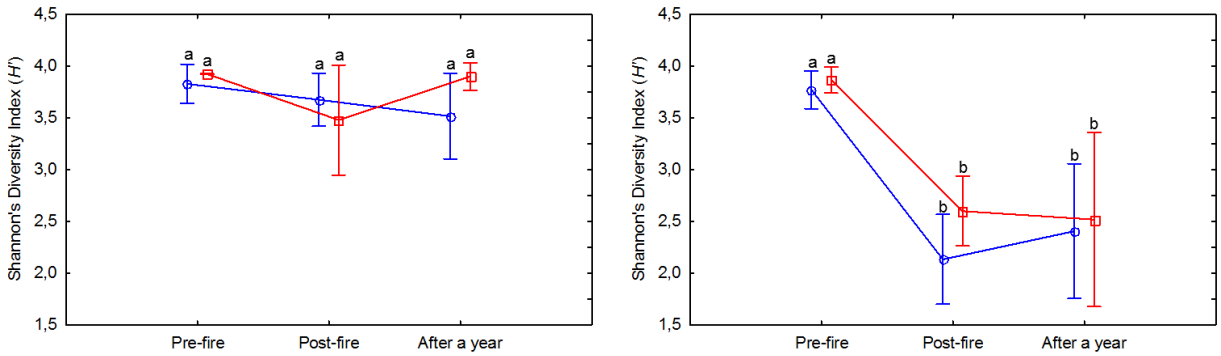
At the *Acacia* sites, the bacterial diversity (*H'*) in the control samples (unburnt samples) of the Bainskloof and Rawsonville sites was similar throughout the study (Figure 3.6a). However, when the bacterial diversity (*H'*) in the burnt samples of the Bainskloof and Rawsonville sites was compared for all three sample times, a significant difference was observed ($p < 0.001$) (Figure 3.6b). The factorial ANOVA post-hoc (Tukey HSD) test confirmed this observation and indicated that the significant difference was found a year after the burn event ($p < 0.001$) (Figure 3.6b). Based on the variation of bacterial diversity (*H'*) between *Acacia* sites, before (Figure 3.3) and after (Figure 3.6b) the 'slash and burn', the Bainskloof and Rawsonville sites were separated for further analysis.

At the *Eucalyptus* sites, the bacterial diversity (*H'*) in the control samples of the Robertson and Wellington sites was similar throughout the study (Figure 3.7a). In addition, when the bacterial diversity (*H'*) in the burnt samples of the Robertson and Wellington sites was compared for all three sample times, no significant difference was recorded ($p = 0.586$) (Figure 3.7b). The bacterial diversity (*H'*) in the Robertson and Wellington sites followed a similar trend after the 'slash and burn' of *Eucalyptus* biomass (Figure 3.7b). Based on the similarities in bacterial diversity (*H'*) within *Eucalyptus* sites, the Robertson and Wellington sites were grouped for further analysis.



(a) Control (p = 0.909) (b) Burnt samples (**p <0.001**)

Figure 3.6: The effect of 'slash and burn' of *Acacia* biomass on the bacterial diversity (*H'*) in Bainskloof (blue) and Rawsonville (red). Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

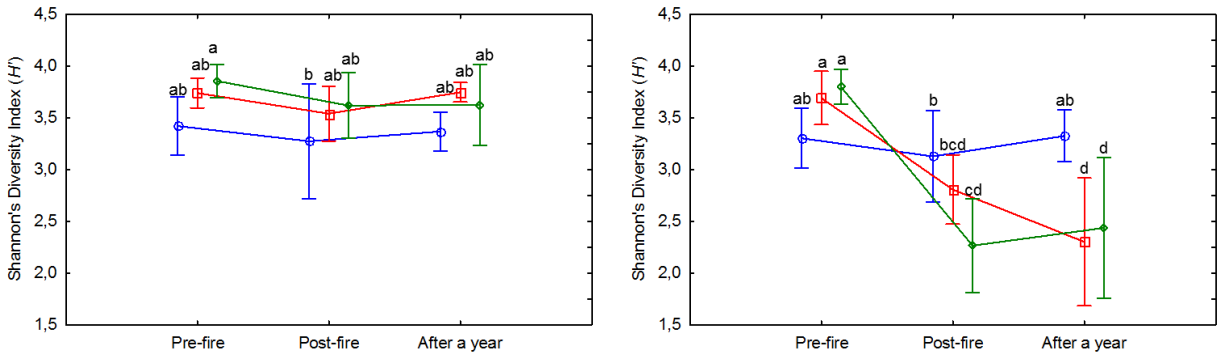


(a) Control (p = 0.304) (b) Burnt samples (p = 0.586)

Figure 3.7: The effect of 'slash and burn' of *Eucalyptus* biomass on the bacterial diversity (*H'*) in Robertson (blue) and Wellington (red). Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

3.2.5. Variation between invasion sites for all three sample times

The two grouped *Eucalyptus* sites (Robertson and Wellington) were compared to the two separate *Acacia* sites (Bainskloof and Rawsonville) to evaluate whether a significant difference existed between *Eucalyptus* and *Acacia* invasion. The pre-fire bacterial diversity (*H'*) in the *Eucalyptus* sites was higher than the *Acacia* sites, nonetheless, this comparison was not significant ($p = 0.881$) (Figure 3.8). Post-fire, the bacterial diversity (*H'*) in the burnt samples of the *Eucalyptus* and Rawsonville sites followed a similar trend, and no significant difference was recorded between these two study sites ($p > 0.05$) (Figure 3.8b). This result was in contrast to the comparison between the *Eucalyptus* and Bainskloof sites (Figure 3.8b). The factorial ANOVA post-hoc (Tukey HSD) test of the comparison between the *Eucalyptus* and Bainskloof sites recorded a significant difference post-fire and a year after the burn event ($p < 0.001$), respectively.



(a) Control ($p = 0.881$) (b) Burnt samples (**$p < 0.001$**)

Figure 3.8: The effect of 'slash and burn' of *Eucalyptus* biomass on the bacterial diversity (*H'*) compared to the effect of 'slash and burn' of *Acacia* biomass on the bacterial diversity (*H'*). The blue indicates the bacterial diversity (*H'*) in Bainskloof, red in Rawsonville and green in *Eucalyptus* sites (Robertson and Wellington). Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means ($p < 0.05$).

3.3. Soil bacterial community structure

3.3.1. Variation within invasion sites for all three sample times

Three distinct clusters separated by different sample times were observed in the centre and intermediate samples for each respective study site based on the ANOSIM results in Table 3.2. The cluster analysis illustrates that the bacterial communities within invasion sites pre-fire are site-specific, i.e. each site showed unique communities (Figure 3.9). The bacterial communities in the *Acacia* sites (Bainskloof and Rawsonville) followed two different trends post-fire (Figure 3.9a). The bacterial communities in the Bainskloof site were different from the bacterial communities in the Rawsonville site throughout the study (Table 3.3). Based on these results, the Bainskloof and Rawsonville sites were separated for further analysis. As for the bacterial communities in *Eucalyptus* sites (Robertson and Wellington), a similar trend was observed post-fire (Figure 3.9b). Table 3.3 showed that the Robertson and Wellington sites shared similar bacterial communities throughout the study. Based on these results, the Robertson and Wellington sites were grouped for further analysis.

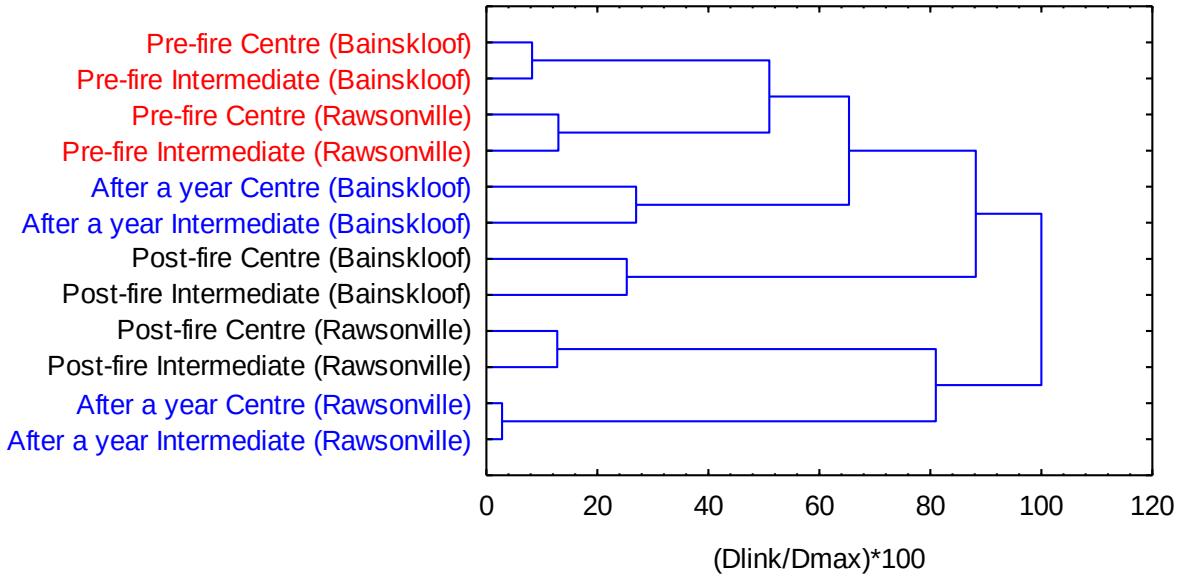
Table 3.4 and Figure 3.10 indicate that the 'slash and burn' of *Acacia* and *Eucalyptus* biomass resulted in a shift in bacterial community structure. All the sites showed that the bacterial community structure, a year after the burn event, was different from the community structure post-fire (Figure 3.10). Moreover, the Bainskloof site showed no differentiation between the bacterial community structures of the control, centre and intermediate samples (burnt samples) a year after the burn event (Table 3.4). This was in contrast to the results found in the *Eucalyptus* and Rawsonville sites. Here, the bacterial community structures in the centre and intermediate samples were different from the bacterial community structure in the control samples a year after the burn event (Table 3.4).

3.3.2. Variation between invasion sites for all three sample times

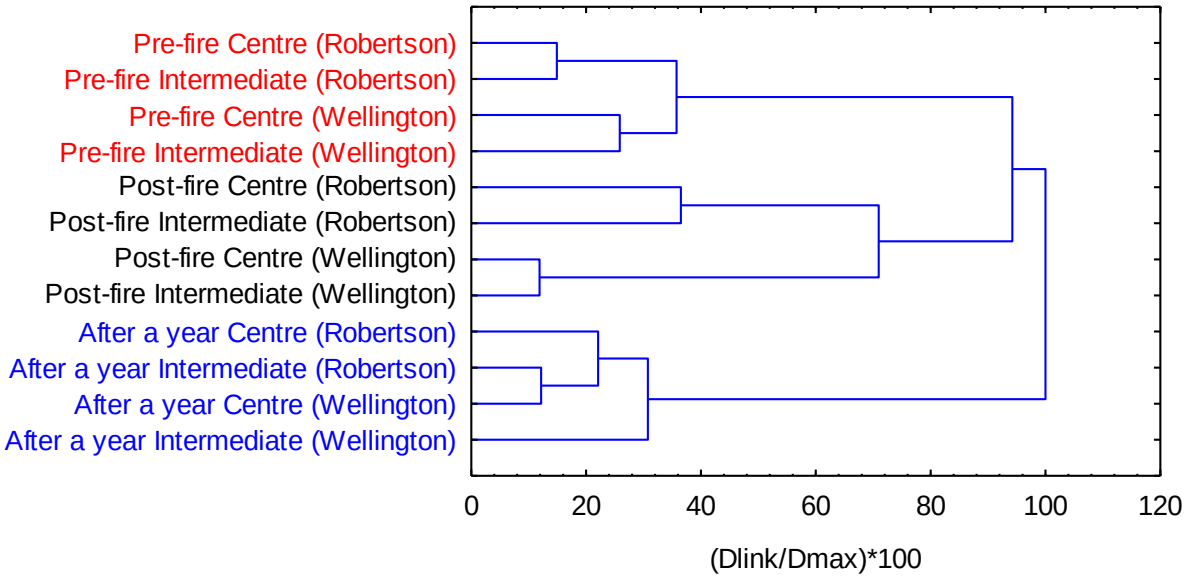
When the bacterial community structure of the two grouped *Eucalyptus* sites (Robertson and Wellington) were compared to the two separated *Acacia* sites (Bainskloof and Rawsonville), three distinct bacterial community structures were displayed pre-fire (Figure 3.11a). The *Eucalyptus* and Rawsonville sites showed an R-value of R = 0.565 and R = 0.240, respectively compared to the Bainskloof site pre-fire, while an R = 0.240 existed between the *Eucalyptus* and Rawsonville sites (Table 3.3; 3.5). Post-fire and a year after the burn event, the centre and intermediate samples (burnt samples) of all the study sites presented two distinct bacterial community structures (Figure 3.11b). One bacterial community cluster represented the community structure of the Bainskloof site and the other bacterial community cluster 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 3.5).

Table 3.2: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) was used to compare the bacterial communities in the centre and intermediate samples of all three sample times in respective study sites. The *p*-values are indicated in brackets and significant results are indicated in bold.

Sites	**Pre-fire vs. Post-fire**	**Pre-fire vs. After a year**	**Post-fire vs. After a year**
Bainskloof	0.728 (**0.001**)	0.465 (**0.001**)	0.619 (**0.002**)
Rawsonville	0.883 (**0.001**)	1.000 (**0.001**)	0.922 (**0.001**)
Robertson	0.717 (**0.001**)	0.615 (**0.001**)	0.427 (**0.003**)
Wellington	1.000 (**0.035**)	0.750 (**0.024**)	0.688 (**0.027**)



(a) *Acacia* sites



(b) *Eucalyptus* sites

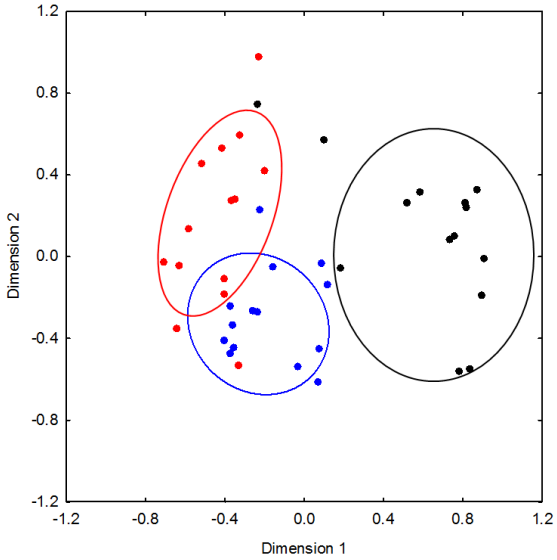
Figure 3.9: The Whittaker similarity index along with the cluster analysis was used to compare the bacterial communities in the centre and intermediate samples of all three sample times in the respective invasive sites.

Table 3.3: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) was used to compare the bacterial communities in the centre and intermediate samples of all three sample times within invasion sites. The *p*-values are indicated in brackets and significant results are indicated in bold.

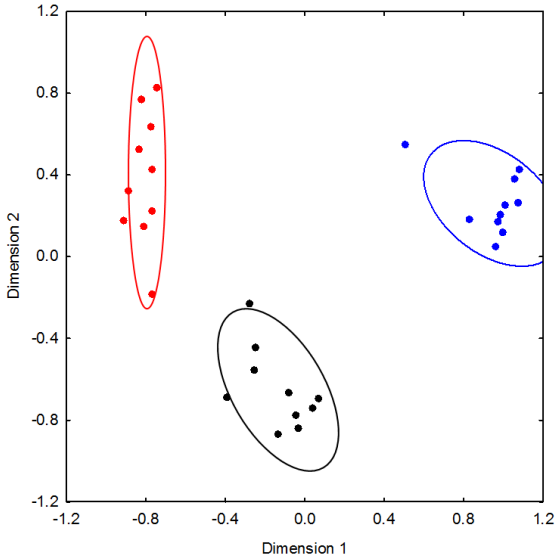
Invasion sites	**Pre-fire**	**Post-fire**	**After a year**
Bainskloof compared to Rawsonville	0.240 (**0.001**)	0.786 (**0.001**)	0.898 (**0.001**)
Robertson compared to Wellington	0.071 (0.323)	0.212 (0.132)	0.120 (0.738)

Table 3.4: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-value) was used to compare the bacterial communities between the control samples and the centre and intermediate samples (combined) for all three sample times in respective sites. Robertson and Wellington are grouped as *Eucalyptus* sites. The *p*-values are indicated in brackets and significant results are indicated in bold.

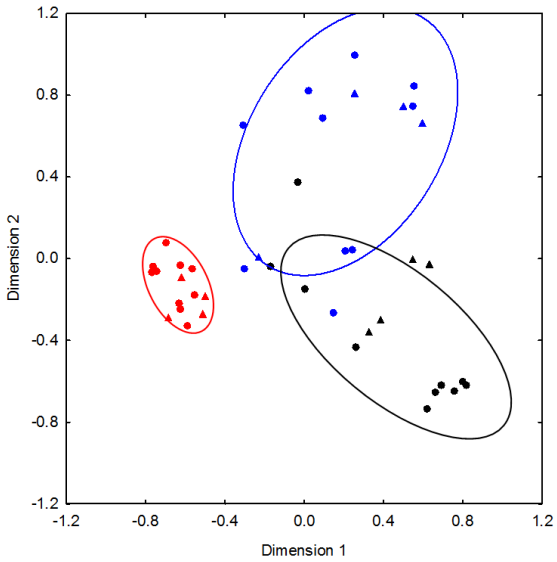
Sites	**Pre-fire**	**Post-fire**	**After a year**
Bainskloof	0.137 (0.955)	**0.456 (0.001)**	0.001 (0.462)
Rawsonville	0.112 (0.196)	**0.821 (0.001)**	**0.963 (0.001)**
Eucalyptus sites	0.004 (0.440)	**0.550 (0.001)**	**0.368 (0.003)**



(a) Bainskloof (9D, stress value = 0.051)

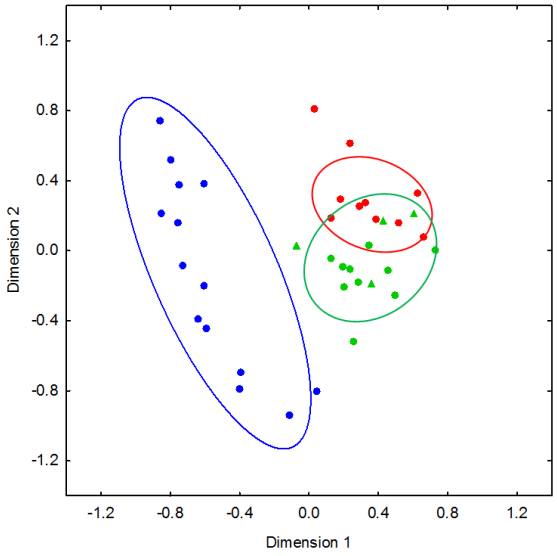


(b) Rawsonville (4D, stress value = 0.054)

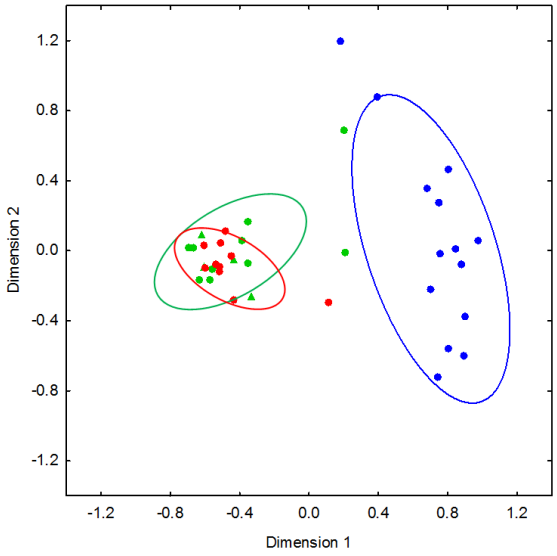


(c) *Eucalyptus* sites (9D, stress value = 0.043)

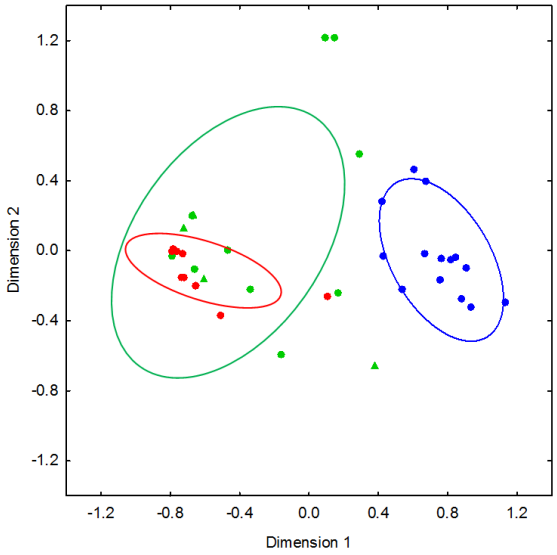
Figure 3.10: Non-metrical multidimensional scaling (NMDS) of bacterial community structure in the centre and intermediate samples (circles) for three sample times in respective study sites. Red presents the bacterial community structure pre-fire, black presents post-fire and blue presents a year after the burn event. For the *Eucalyptus* sites, the bacterial community structure in Robertson is indicated as circles, whereas the bacterial community structure in Wellington is indicated as triangles. Ellipse represent 75% confidence levels.



(a) Pre-fire (9D, stress value = 0.049)



(b) Post-fire (8D, stress value = 0.041)



(c) A year after the burn event (7D, stress value = 0.054)

Figure 3.11: Non-metrical multidimensional scaling (NMDS) of bacterial community structure in the centre and intermediate samples (circles) of (a) pre-fire, (b) post-fire and (c) a year after the burn event sample times. The blue indicates the bacterial community structure in Bainskloof, red in Rawsonville and green in *Eucalyptus* sites. For the *Eucalyptus* sites, the bacterial community structure in Robertson is indicated as circles, whereas the bacterial community structure in Wellington is indicated as triangles. Ellipse represent 75% confidence levels.

Table 3.5: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) was used to compare the bacterial communities in the centre and intermediate samples of all three sample times between invasion sites. The *p*-values are indicated in brackets and significant results are indicated in bold.

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

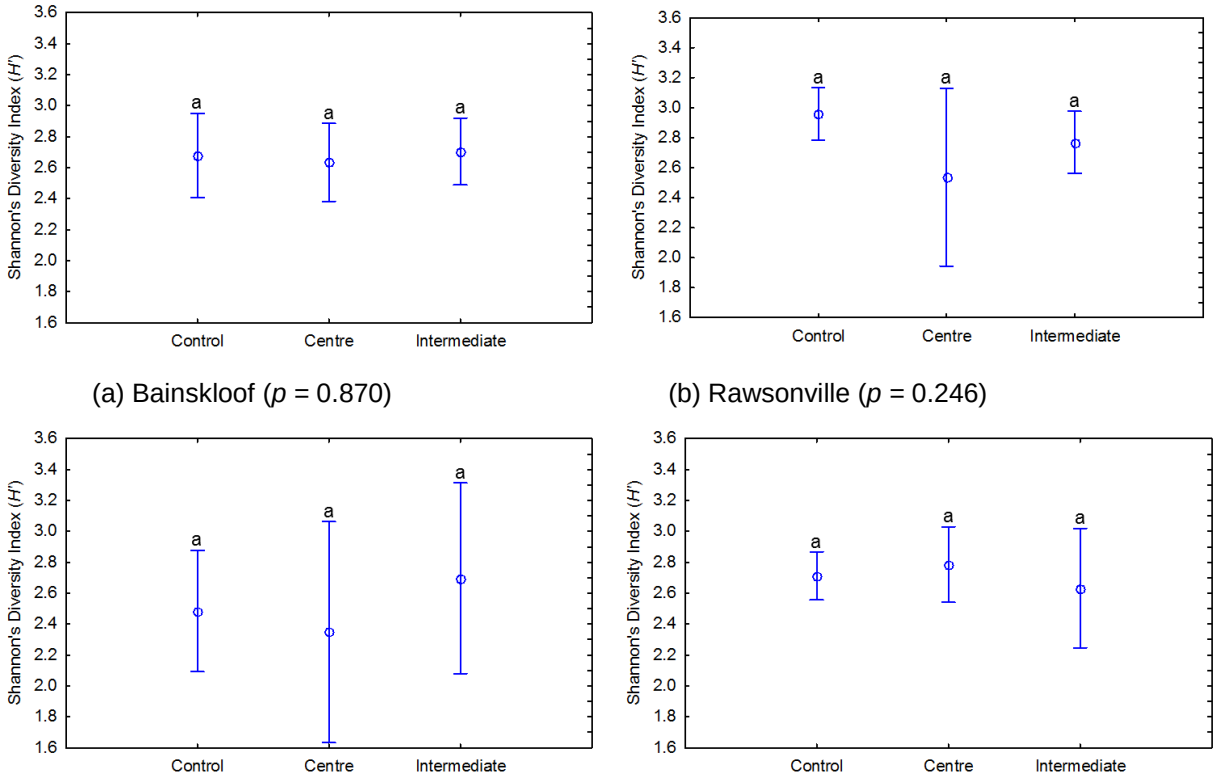
3.4. Soil fungal diversity

3.4.1. Variation within sites pre-fire

Table 3.6 summarises the fungal richness and diversity profiles of ARISA for four study sites. All the study sites indicated that the pre-fire fungal diversity (*H'*) was similar (not significantly different, $p > 0.05$) at all the different sampling points, i.e. control, centre and intermediate samples (Figure 3.12). Based on these results, all the pre-fire samples (control, centre and intermediate samples) taken from each pile were pooled to determine whether the pre-fire fungal diversity (*H'*) was similar in all the piles at a particular study site. Figure 3.13 revealed that the pre-fire fungal diversity (*H'*) was similar in all the soil samples, apart from the Bainskloof ($p = 0.004$) and Wellington ($p = 0.028$) sites (Figure 3.13). The one-way ANOVA post-hoc (Tukey Honest Significant Difference (HSD)) test of these respective sites, indicated that the pre-fire fungal diversity (*H'*) in the soil samples of pile 3 was significantly lower in comparison to the other piles ($p < 0.05$) (Figure 3.13). Based on these results, pile 3 in the Bainskloof and Wellington sites were excluded from further analysis.

Table 3.6: The fungal richness and diversity profiles of ARISA of the four study sites during all three sample times. The diversity was determined by means of the Shannon diversity (*H'*) and Simpson's complement (1-*D*) indices. The standard deviation is indicated in brackets.

	Pre-fire		**Post-fire**		**A year after burn event**	
	Control	Centre and intermediate	Control	Centre and intermediate	Control	Centre and intermediate
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)



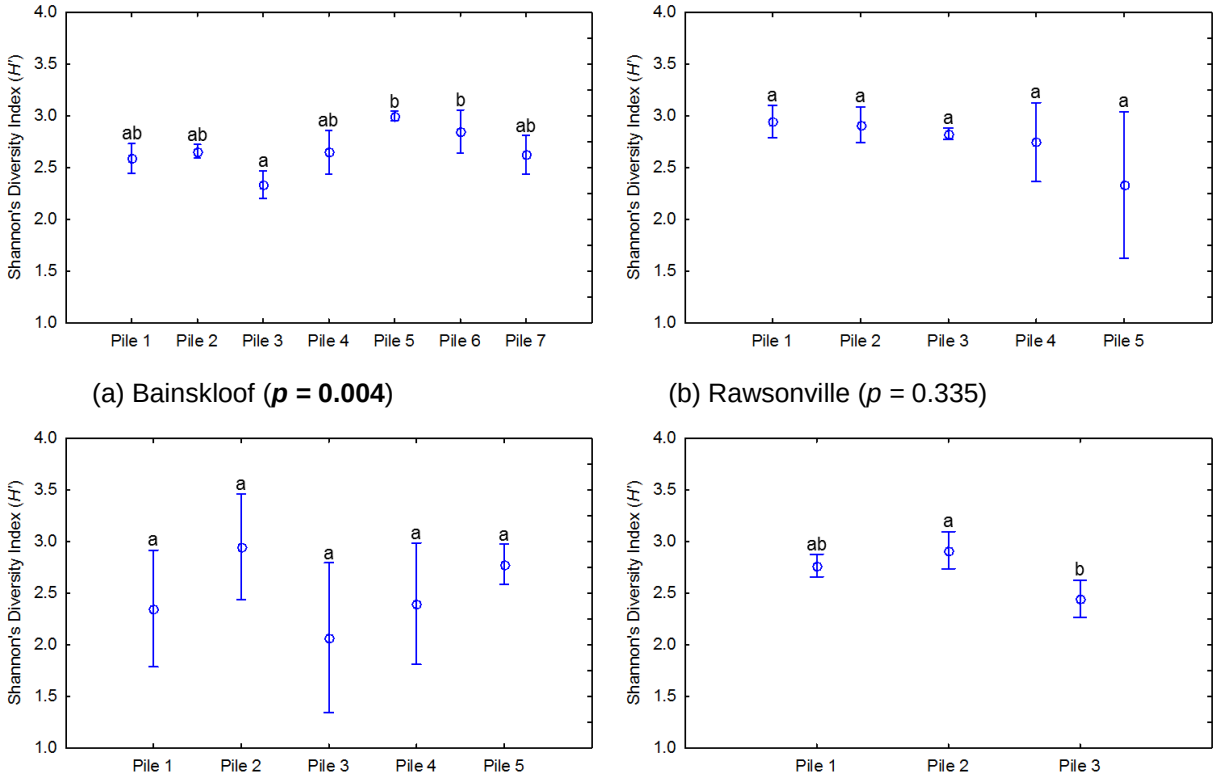
(a) Bainskloof (p = 0.870)

(b) Rawsonville (p = 0.246)

(c) Robertson (p = 0.653)

(d) Wellington (p = 0.805)

Figure 3.12: The pre-fire fungal diversity (*H'*) at different sampling points (control, centre and intermediate samples), in the respective study sites. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).



(a) Bainskloof (***p* = 0.004**)

(b) Rawsonville (*p* = 0.335)

(c) Robertson (*p* = 0.351)

(d) Wellington (***p* = 0.028**)

Figure 3.13: The pre-fire fungal diversity (*H'*) in all the soil samples (control, centre and intermediate samples) of each pile. Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means ($p < 0.05$).

3.4.2. Variation between sites pre-fire

The mean fungal diversity (*H'*) in the pre-fire centre and intermediate samples (combined) ranged from 2.52 to 2.71 in all the study sites (Table 3.6). The Wellington site had the highest fungal diversity score (*H'* = 2.71), followed by Bainskloof (*H'* = 2.67), Rawsonville (*H'* = 2.65) and then Robertson (*H'* = 2.52). Furthermore, no significant difference was recorded when the pre-fire fungal diversity (*H'*) in the centre and intermediate samples (combined) of all the study sites was compared to one another (*p* = 0.347) (Figure 3.14).

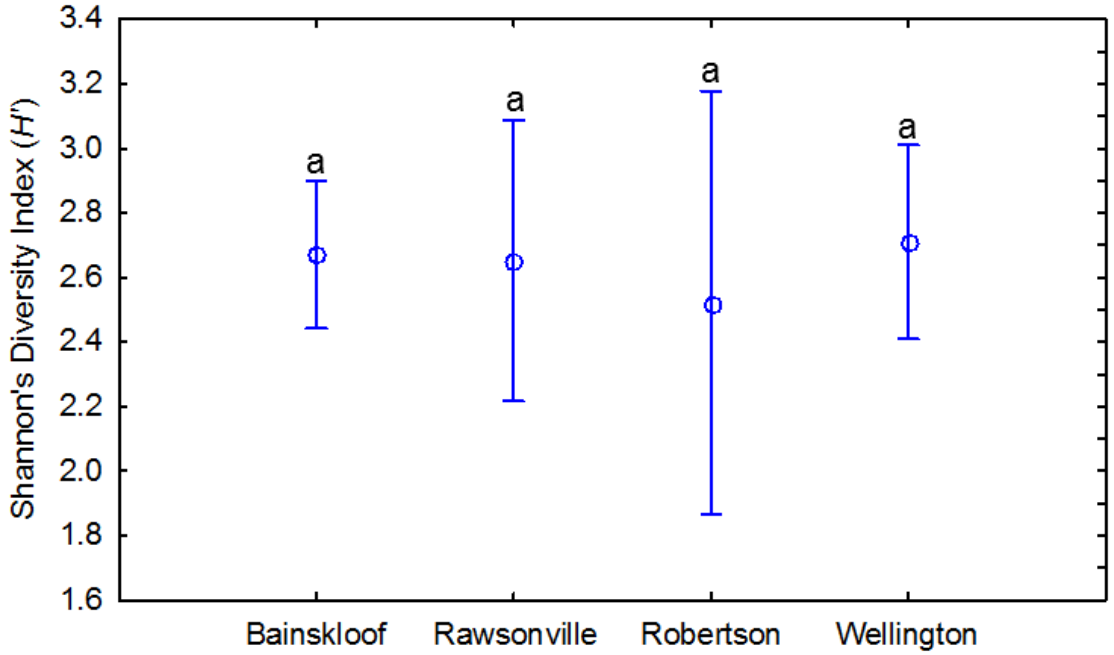
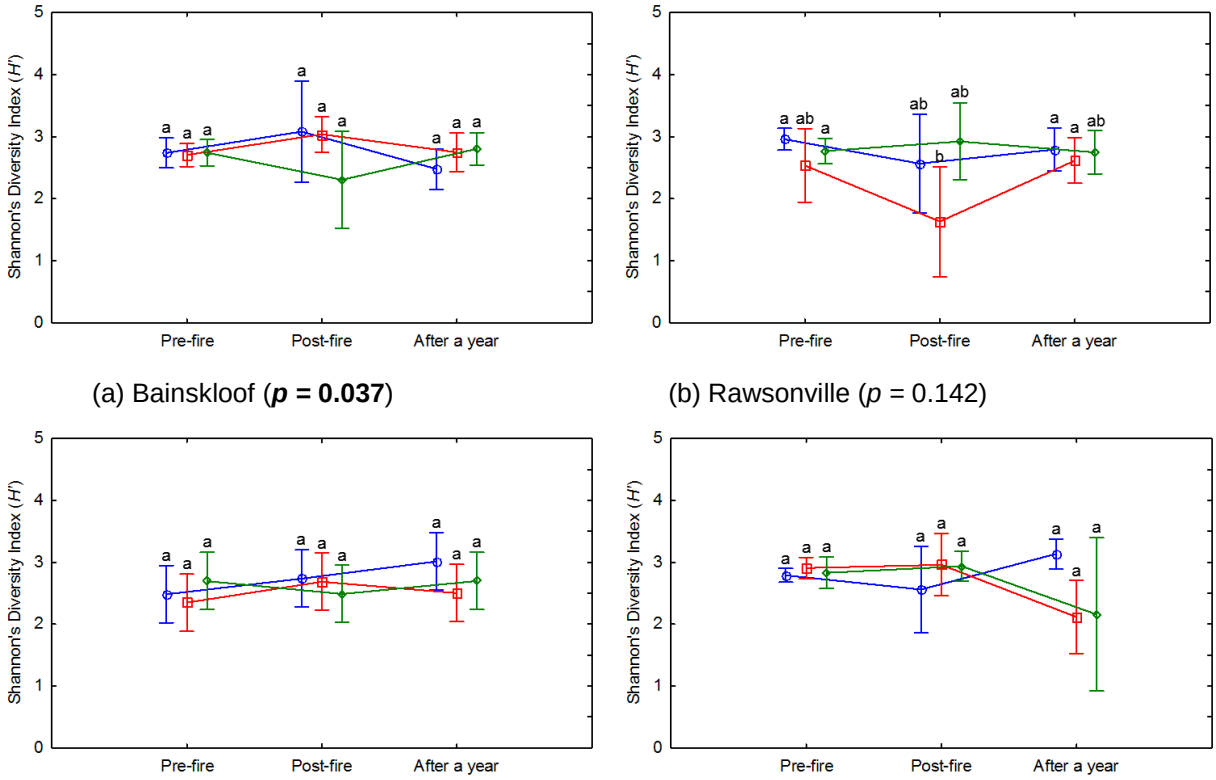


Figure 3.14: The fungal diversity (*H'*) pre-fire in respective study sites (*p* = 0.347). Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (*p* <0.05).

3.4.3. Effect of 'slash and burn' of invasive biomass on soil fungal diversity

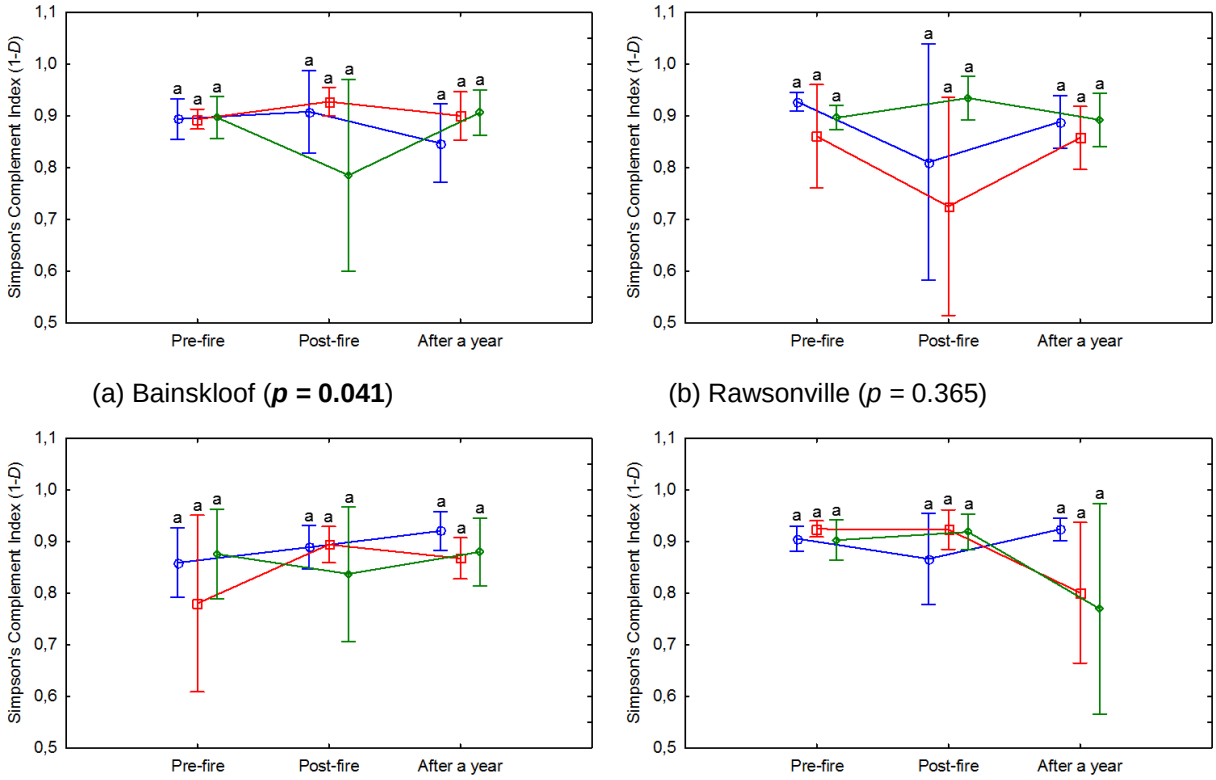
After the 'slash and burn' of invasive biomass (post-fire), the fungal diversity (*H'*) and Simpson's diversity (1-*D*) index from the centre and intermediate samples (burnt samples) were similar for all the study sites (Figures 3.15; 3.16). In addition, all the study sites showed that the 'slash and burn' of *Acacia* and *Eucalyptus* biomass had no effect on the fungal diversity (*H'* and 1-*D*) and that the pre-fire fungal diversity (*H'* and 1-*D*) remained similar post-fire and a year after the burn event. Nonetheless, the one-way ANOVA of the Bainskloof site recorded a significant difference (Figure 3.15a; 3.16a). In Figure 3.15a and 3.16a, the factorial ANOVA post-hoc (Tukey HSD) revealed no significant variation between the mean of variables throughout the study ($p > 0.05$). Based on the fungal diversity (*H'*) similarities between the burnt samples, the centre and intermediate samples were pooled for further analysis.



(a) Bainskloof (***p* = 0.037**) (b) Rawsonville (p = 0.142)

(c) Robertson (p = 0.590) (d) Wellington (p = 0.403)

Figure 3.15: The effect of 'slash and burn' of invasive biomass on the fungal diversity (*H'*) in the four study sites. The blue represents the fungal diversity (*H'*) of the control samples, whereas the red (centre) and green (intermediate) represent fungal diversity (*H'*) in the soil samples underneath the piles (or burnt samples). Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).



(a) Bainskloof (***p* = 0.041**)

(b) Rawsonville (*p* = 0.365)

(c) Robertson (*p* = 0.425)

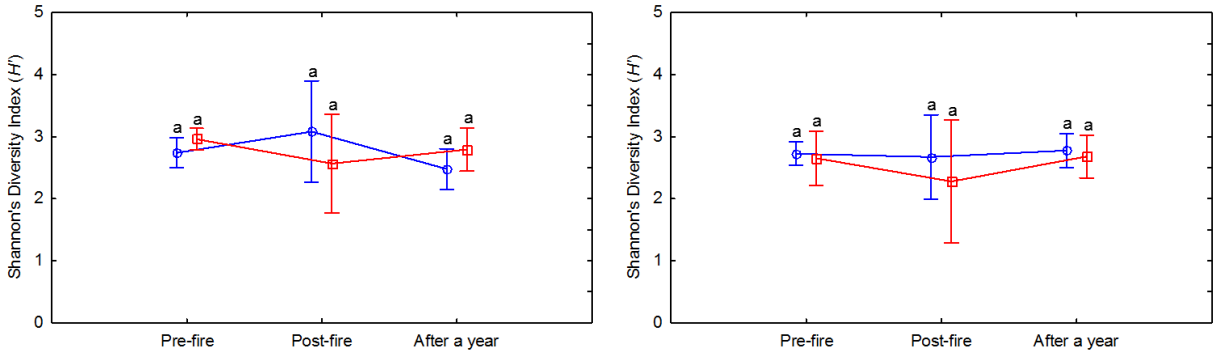
(d) Wellington (*p* = 0.532)

Figure 3.16: The effect of 'slash and burn' of invasive biomass on the fungal diversity (1-*D*) in the four study sites. The blue represents the fungal diversity (1-*D*) of the control samples, whereas the red (centre) and green (intermediate) represent fungal diversity (1-*D*) in the soil samples underneath the piles (or burnt samples). Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

3.4.4. Variation within invasion sites for all three sample times

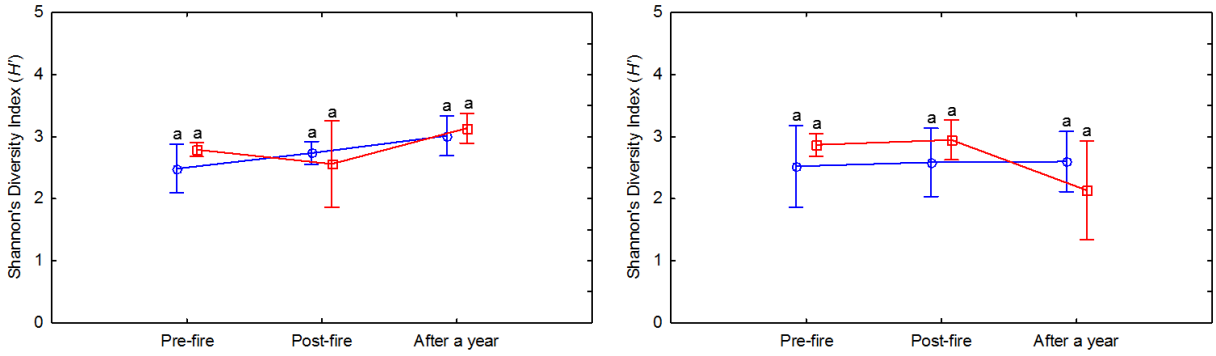
At the *Acacia* sites, the fungal diversity (*H'*) in the control samples (unburnt samples) of the Bainskloof and Rawsonville sites was similar throughout the study (Figure 3.17a). In addition, when the fungal diversity (*H'*) in the burnt samples of the Bainskloof and Rawsonville sites was compared for all three sample times, no significant difference was recorded ($p = 0.559$) (Figure 3.17b). Based on these results, the fungal diversity (*H'*) of the *Acacia* sites (Bainskloof and Rawsonville) was pooled for further analysis.

At the *Eucalyptus* sites, the fungal diversity (*H'*) in the control samples of the Robertson and Wellington sites was similar throughout the study (Figure 3.18a). Additionally, when the fungal diversity (*H'*) in the burnt samples of the Robertson and Wellington sites was compared for all three sample times, no significant difference was recorded ($p = 0.139$) (Figure 3.18b). Based on the fungal diversity (*H'*) similarities post-fire, the *Eucalyptus* sites (Robertson and Wellington) were grouped for further analysis.



(a) Control (p = 0.145) (b) Burnt (p = 0.559)

Figure 3.17: The effect of 'slash and burn' of *Acacia* biomass on the fungal diversity (*H'*) in Bainskloof (blue) and Rawsonville (red). Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

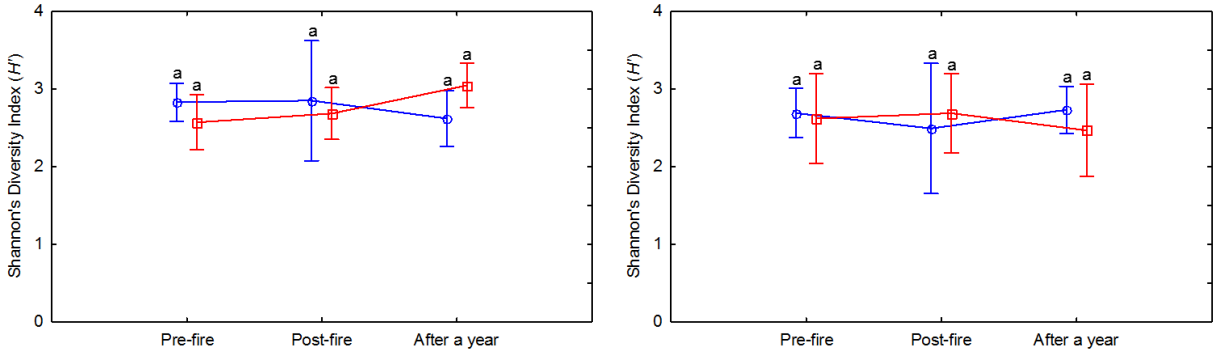


(a) Control (p = 0.496) (b) Burnt samples (p = 0.139)

Figure 3.18: The effect of 'slash and burn' of *Eucalyptus* biomass on the fungal diversity (*H'*) in Robertson (blue) and Wellington (red). Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

3.4.5. Variation between invasion sites for all three sample times

The two grouped *Eucalyptus* sites (Robertson and Wellington) were compared to the two grouped *Acacia* sites (Bainskloof and Rawsonville) to evaluate whether a significant difference existed between *Eucalyptus* and *Acacia* invasion. The pre-fire fungal diversity (H') in the *Acacia* sites was higher than the *Eucalyptus* sites, nonetheless, this comparison was not significant ($p = 0.074$) (Figure 3.19a). Post-fire, the pre-fire fungal diversity (*H'*) in the centre and intermediate samples (combined) of the *Eucalyptus* and *Acacia* invaded sites remained similar (Figure 3.19b). This, in turn, resulted in no variation of fungal diversity (H') between invasion sites before and after the 'slash and burn' event.



(a) Control ($p = 0.074$) (b) Burnt samples ($p = 0.226$)

Figure 3.19: The effect of 'slash and burn' of *Eucalyptus* biomass on the fungal diversity (*H'*) compared to the effect of 'slash and burn' of *Acacia* biomass on the fungal diversity (*H'*). The blue indicates the fungal diversity (*H'*) in the *Acacia* sites (Bainskloof and Rawsonville) and the red indicates the fungal diversity (*H'*) in the *Eucalyptus* sites (Robertson and Wellington). Significant results are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means ($p < 0.05$).

3.5. Soil fungal community structure

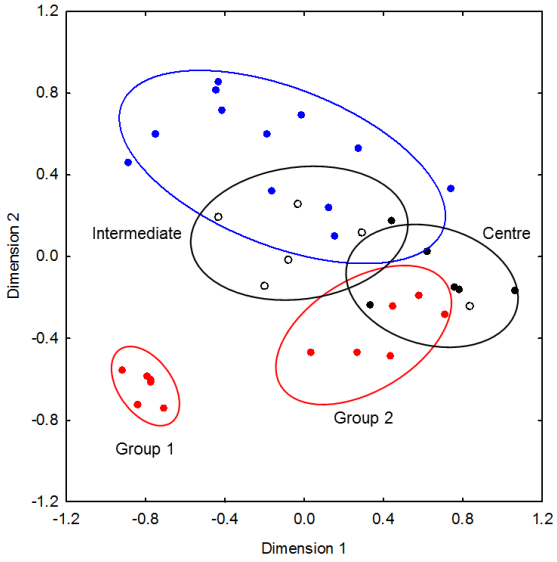
3.5.1. Variation within invasion sites for all three sample times

The NMDS plots in Figure 3.20a indicated two distinct fungal community structures in the Bainskloof site pre-fire and post-fire. According to Table 3.7 and Figure 3.21, it appears that the pre-fire fungal communities underneath piles 1, 2 and 4 (group 1) are different from the fungal communities underneath piles 5, 6 and 7 (group 2) ($R = 0.989$, $p = 0.002$). Furthermore, the

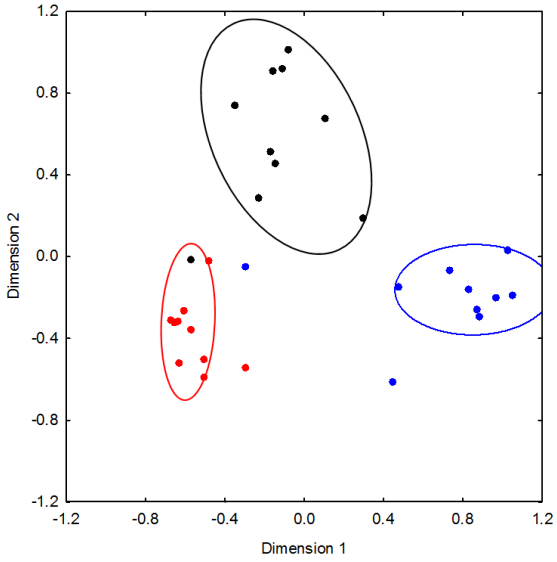
fungal community structures post-fire are due to the dissimilarities between the centre and intermediate samples (R = 0.246, p = 0.038) (Table 3.8). A year after the burn event, no separate fungal community structures in the Bainskloof site were formed between the centre and intermediate samples (R = 0.048, p = 0.573). As for the Rawsonville and Wellington sites, three distinct fungal community structures separated by different sample times were observed in the centre and intermediate samples for each respective site (Table 3.9; Figure 3.20). At the Robertson site, however, two distinct fungal community structures were observed in the centre and intermediate samples throughout the study (Table 3.9). One fungal community cluster represented the pre-fire community structure and the other fungal community cluster was a combination of the samples from post-fire and a year after the burn (Table 3.9; Figure 3.20c).

Post-fire, all the sites indicated a shift in fungal community structures (Table 3.10). The cluster analysis in Figure 3.22a illustrated that the fungal communities in the *Acacia* sites (Bainskloof and Rawsonville) followed two different trends post-fire. The fungal communities in the Bainskloof site were different from the fungal communities in the Rawsonville site throughout the study (Table 3.7; 3.8; 3.11). As for the *Eucalyptus* sites (Robertson and Wellington), the fungal communities followed a similar trend post-fire (Figure 3.22b). Pre-fire, the fungal communities in the Robertson site were different from the fungal communities in the Wellington site (R = 0.772; p = 0.009) (Table 3.7). After the burning of *Eucalyptus* biomass, the Robertson and Wellington sites shared similar fungal communities (Table 3.8; 3.11).

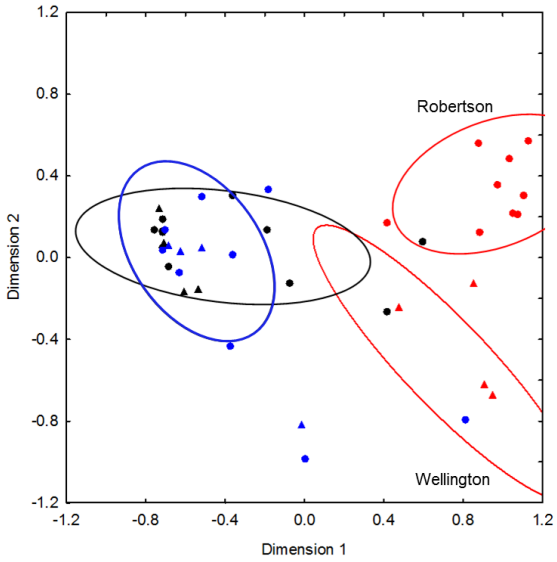
A year after the burn event, the Bainskloof site showed no differentiation between the fungal community structures of the control, centre and intermediate samples (burnt samples) (Table 3.10). This was in contrast to the results found in the *Eucalyptus* and Rawsonville sites. Here, the fungal community structures in the centre and intermediate samples were different from the fungal community structure in the control samples a year after the burn event (Table 3.10).



(a) Bainskloof (7D, stress value = 0.053)



(b) Rawsonville (7D, stress value = 0.051)



(c) *Eucalyptus* sites (7D, stress value = 0.050)

Figure 3.20: Non-metrical multidimensional scaling (NMDS) of fungal community structure in the centre and intermediate samples (circles) for all three sample times in respective study sites. Red presents the fungal community structure pre-fire, black presents post-fire and blue presents a year after the burn event. Group 1 of the Bainskloof site (pre-fire) represents the fungal communities underneath piles 1, 2 and 4, whereas group 2 represents the fungal communities underneath piles 5, 6 and 7. For the *Eucalyptus* sites, the fungal community structure in Robertson is indicated as circles, whereas the fungal community structure in Wellington is indicated as triangles. Ellipse represent 75% confidence levels.

Table 3.7: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) were used to compare the pre-fire fungal communities of all the sites in the centre and intermediate samples. Group 1 of the Bainskloof site (pre-fire) represents the fungal communities underneath piles 1, 2 and 4, whereas group 2 represents the fungal communities underneath piles 5, 6 and 7. The *p*-values are indicated in brackets and significant results are indicated in bold.

Sites	**Bainskloof Group 1**	**Bainskloof Group 2**	**Rawsonville**	**Robertson**	**Wellington**
Bainskloof Gr 1		0.989 (**0.002**)	0.444 (**0.002**)	0.206 (0.058)	0.772 (**0.009**)
Bainskloof Gr 2	0.989 (**0.002**)		0.887 (**0.001**)	0.592 (**0.001**)	1.000 (**0.009**)
Rawsonville	0.444 (**0.002**)	0.887 (**0.001**)		0.384 (**0.001**)	0.411 (**0.021**)
Robertson	0.206 (0.058)	0.592 (**0.001**)	0.384 (**0.001**)		0.353 (**0.023**)
Wellington	0.772 (**0.009**)	1.000 (**0.009**)	0.411 (**0.021**)	0.353 (**0.023**)	

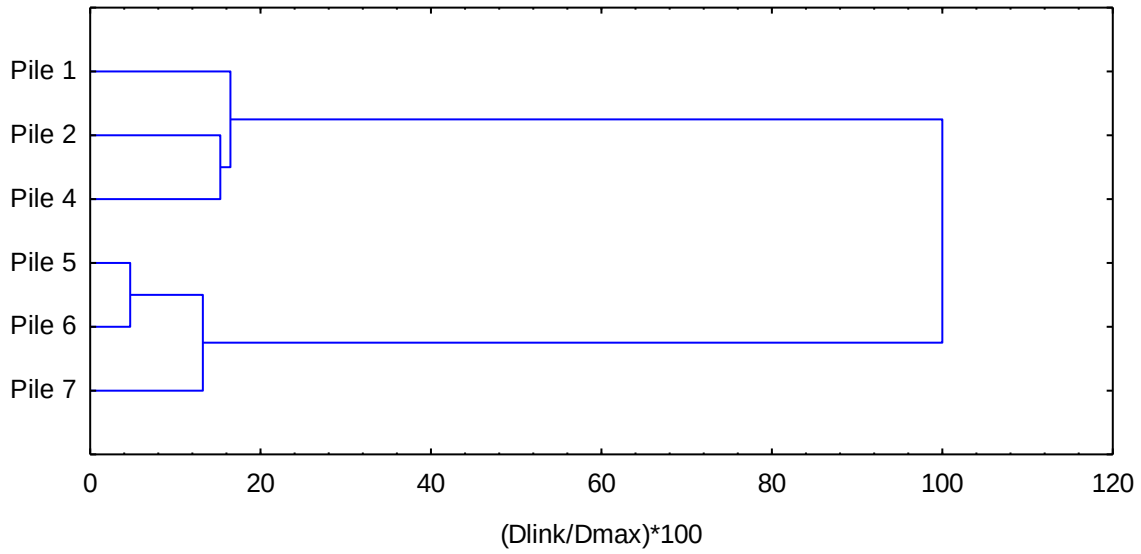


Figure 3.21: The fungal communities in the centre and intermediate (combined) samples underneath respective piles pre-fire in the Bainskloof site.

Table 3.8: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) were used to compare the post-fire fungal communities of all the sites in the centre and intermediate samples. The *p*-values are indicated in brackets and significant results are indicated in bold.

Sites	**Bainskloof Centre**	**Bainskloof Intermediate**	**Rawsonville**	**Robertson**	**Wellington**
Bainskloof (C)		0.246 (**0.038**)	0.608 (**0.001**)	0.007 (0.366)	0.036 (0.366)
Bainskloof (I)	0.246 (**0.038**)		0.597 (**0.001**)	0.448 (**0.004**)	0.458 (**0.029**)
Rawsonville	0.608 (**0.001**)	0.597 (**0.001**)		0.628 (**0.001**)	0.425 (**0.002**)
Robertson	0.007 (0.366)	0.448 (**0.004**)	0.628 (**0.001**)		0.107 (0.694)
Wellington	0.036 (0.366)	0.458 (**0.029**)	0.425 (**0.002**)	0.107 (0.694)	

Table 3.9: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) were used to compare the fungal communities in the centre and intermediate samples of all three sample times in respective study sites. The *p*-values are indicated in brackets and significant results are indicated in bold.

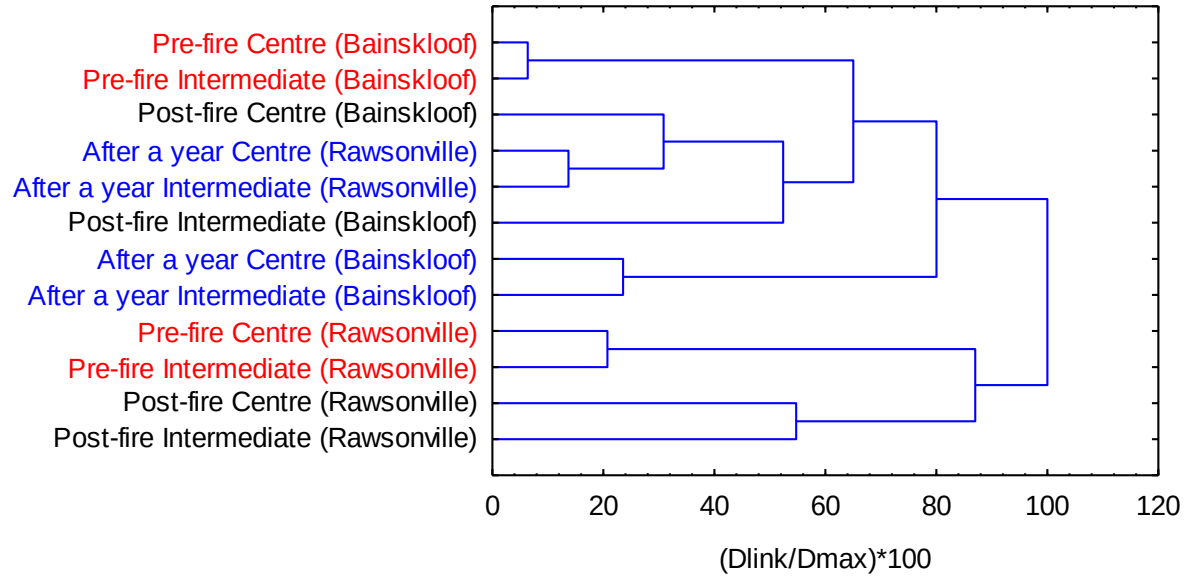
Sites	**Pre-fire vs. Post-fire**	**Pre-fire vs. After a year**	**Post-fire vs. After a year**
Bainskloof	n/a	n/a	n/a
Rawsonville	0.551 (**0.001**)	0.862 (**0.001**)	0.658 (**0.001**)
Robertson	0.645 (**0.001**)	0.651 (**0.001**)	0.063 (0.080)
Wellington	1.000 (**0.025**)	0.958 (**0.033**)	0.406 (**0.019**)

n/a - not applicable due to two distinct fungal communities in the Bainskloof site pre-fire and post-fire.

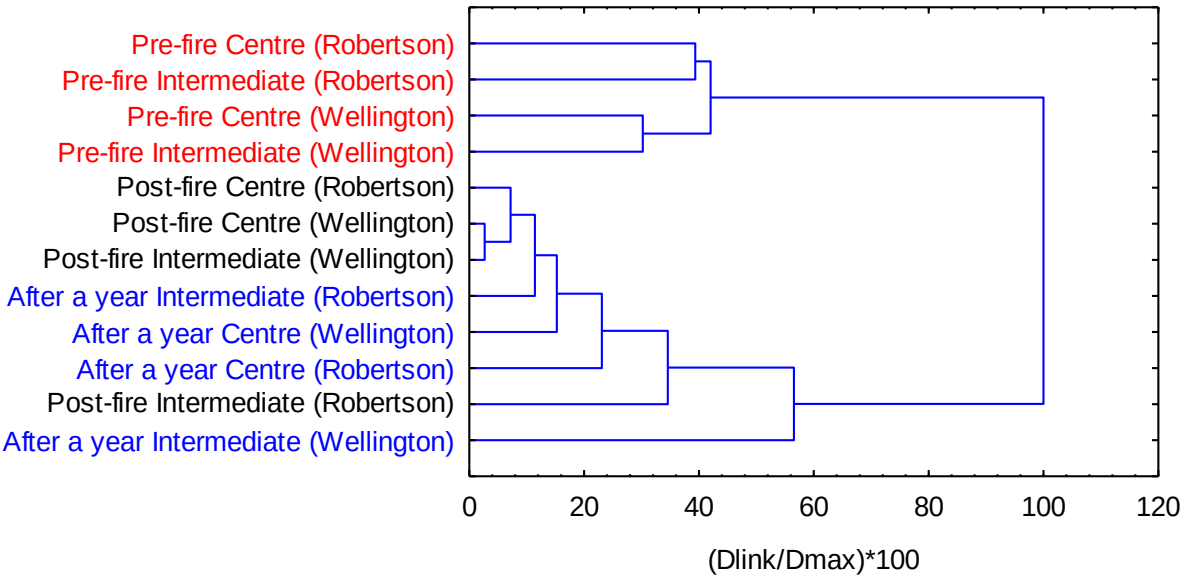
Table 3.10: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-value) was used to compare the fungal communities between the control samples and the centre and intermediate samples (combined) for all three sample times in respective sites. Robertson and Wellington are grouped as *Eucalyptus* sites. The *p*-values are indicated in brackets and significant results are indicated in bold. The letters indicate two distinct fungal communities in a particular site, at the same sampling time.

Sites	**Pre-fire**	**Post-fire**	**After a year**
Bainskloof	x	z	0.160 (0.070)
Rawsonville	0.034 (0.575)	**0.335 (0.014)**	**0.743 (0.001)**
Eucalyptus sites	y	**0.458 (0.003)**	**0.615 (0.001)**

x - Group 1 (R = 0.157, *p* = 0.776); Group 2 (R = 0.102, *p* = 0.283)
y - Robertson (R = 0.173, *p* = 0.116); Wellington (R = 0.214, *p* = 0.733)
z - Centre (**R = 0.392, *p* = 0.007**); Intermediate (**R = 0.310, *p* = 0.013**)



(a) *Acacia* sites



(b) *Eucalyptus* sites

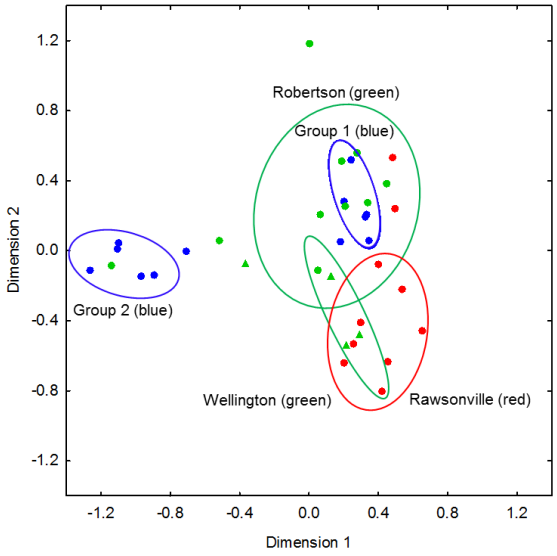
Figure 3.22: The Whittaker similarity index along with the cluster analysis was used to compare the fungal communities in the centre and intermediate samples of all three sample times in respective invasive sites.

Table 3.11: The Bray-Curtis index along with the analysis of similarity (ANOSIM) (R-values) were used to compare the fungal communities, a year after the burn event, of all the sites in the centre and intermediate samples. The *p*-values are indicated in brackets and significant results are indicated in bold.

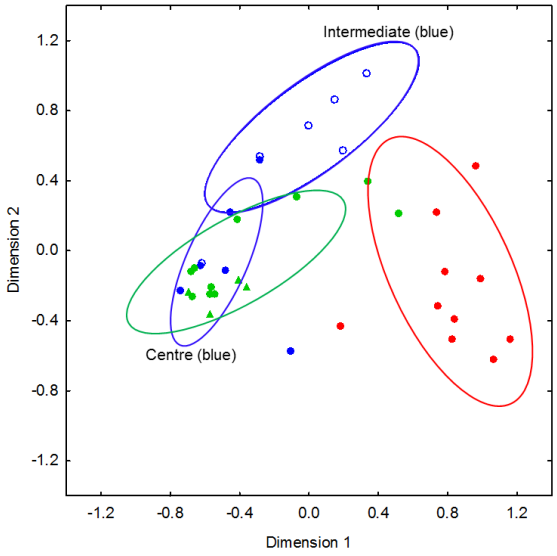
Sites	Bainskloof	Rawsonville	Robertson	Wellington
Bainskloof		0.615 (**0.001**)	0.533 (**0.001**)	0.585 (**0.002**)
Rawsonville	0.615 (**0.001**)		0.012 (0.341)	0.180 (0.163)
Robertson	0.533 (**0.001**)	0.012 (0.341)		0.085 (0.641)
Wellington	0.585 (**0.002**)	0.180 (0.163)	0.085 (0.641)	

3.5.2. Variation between invasion sites for all three sample times

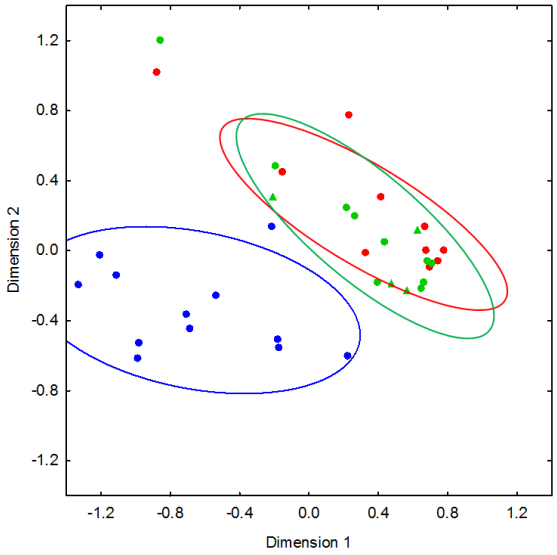
When the fungal community structure of all the study sites was compared for all three sample times four fungal community structures were revealed pre-fire (Figure 3.23a); three fungal community structures were revealed post-fire (Figure 3.23b) and two fungal community structures were revealed a year after the burn event (Figure 3.23c). Pre-fire, the fungal community structure of the Robertson site (*Eucalyptus* site) was similar to group 1 (piles 1, 2 and 4) of the Bainskloof site (R = 0.206, p = 0.058) (Table 3.7, Figure 3.23a). Whereas, the Rawsonville, Wellington and group 2 (piles 5, 6 and 7) of the Bainskloof sites formed three separate fungal community structures (Table 3.7, Figure 3.23a). 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), while the fungal community structure of the Rawsonville site and the intermediate samples of the Bainskloof site formed two distinct fungal community structures (Table 3.8, Figure 3.23b). A year after the burn event, the fungal community structure of both the *Eucalyptus* and Rawsonville sites was similar (R = 0.038, p = 0.213), whereas the Bainskloof site formed a distinct fungal community structure (Table 3.11, Figure 3.23c).



(a) Pre-fire (8D, stress value = 0.052)



(b) Post-fire (7D, stress value = 0.044)



(c) A year after the burn event (7D, stress = 0.054)

Figure 3.23: Non-metrical multidimensional scaling (NMDS) of fungal community structure in the centre and intermediate (circles) samples of (a) pre-fire, (b) post-fire and (c) a year after the burn event sample times. Blue indicates the fungal community structure in Bainskloof, red in Rawsonville and green in *Eucalyptus* sites. Group 1 of the Bainskloof site (pre-fire) represents the fungal communities underneath piles 1, 2 and 4, whereas group 2 represents the fungal communities underneath piles 5, 6 and 7. For the *Eucalyptus* sites, the fungal community structure in Robertson is indicated as circles, whereas the fungal community structure in Wellington is indicated as triangles. Ellipse represent 75% confidence levels.

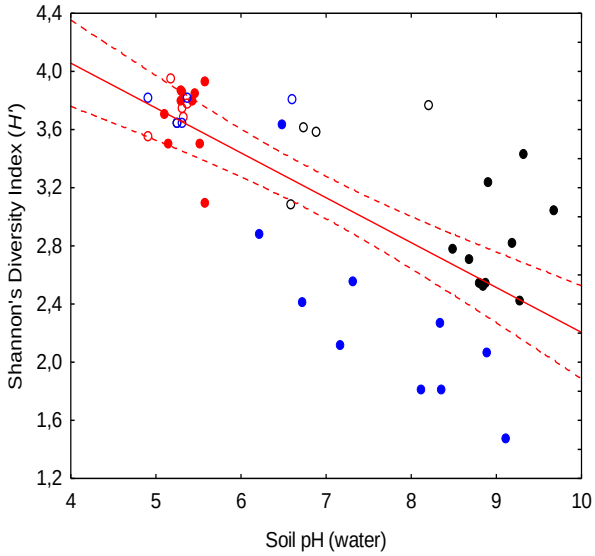
3.6. Soil chemical properties correlated to soil microbial diversity and community structure

3.6.1. Pearson correlation coefficient (PCC) analysis

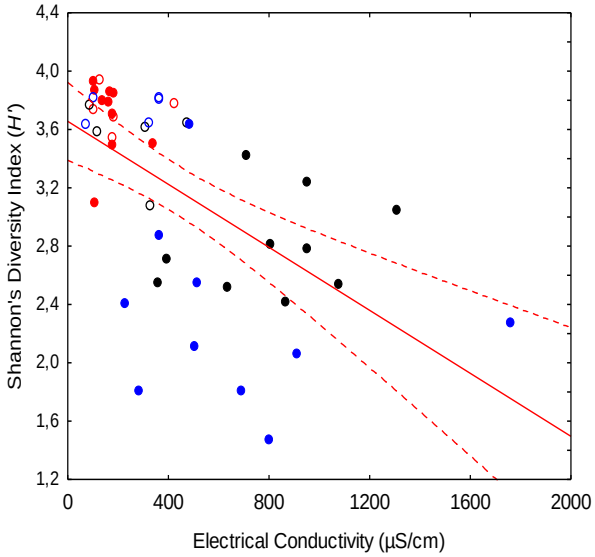
No significant correlation between the chemical properties and bacterial diversity (*H'*) was recorded at the Bainskloof site (Table 3.12). Here, the chemical properties and the bacterial diversity remained similar after the 'slash and burn' of *Acacia* biomass, apart from soil pH that increased immediately post-fire and decreases gradually after the burn event (Table 3.1; 3.13). As for the *Eucalyptus* (Robertson and Wellington) and Rawsonville sites, the soil pH, electrical conductivity (EC) and phosphate (PO_4) concentration, respectively, indicated a negative correlation to the bacterial diversity (*H'*) (Table 3.12). Furthermore, the soil pH showed the strongest correlation coefficient in the *Eucalyptus* and Rawsonville sites with r = -0.746 and r = -0.721, respectively. Figure 3.24 illustrates an overview of the significant correlations between the chemical properties and bacterial diversity (*H'*). Moreover, no significant correlation was recorded between the chemical properties and fungal diversity (*H'*) (Table 3.14).

Table 3.12: Pearson correlation coefficient (PCC) analysis between soil chemical properties (i.e. soil pH, EC, available nitrogen (N) and PO_4, respectively) and bacterial diversity (*H'*). The available N is the ammonium (NH_4) and nitrate (NO_3) concentrations combine. Robertson and Wellington are grouped as *Eucalyptus* sites. The *p*-values are indicated in brackets and the significant results are indicated in bold.

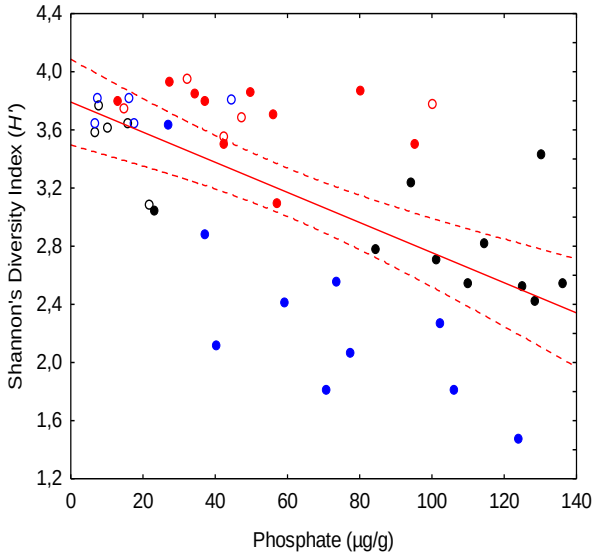
	Bainskloof	**Rawsonville**	***Eucalyptus* sites**
Soil pH (water)	-0.115 (0.370)	-0.721 (**<0.001**)	-0.746 (**<0.001**)
EC (µS/cm)	0.018 (0.888)	-0.579 (**<0.001**)	-0.604 (**<0.001**)
Available N	-0.091 (0.194)	-0.030 (0.843)	0.246 (0.052)
PO_4 (µg/g)	0.194 (0.128)	-0.610 (**<0.001**)	-0.446 (**<0.001**)



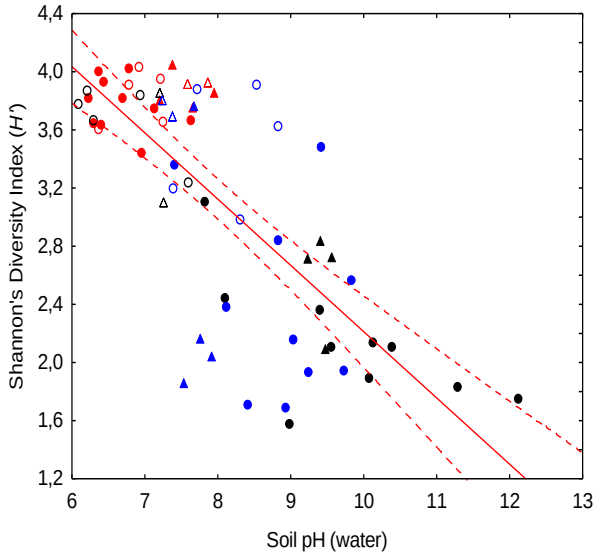
(a) Rawsonville (Soil pH, r = -0.721)



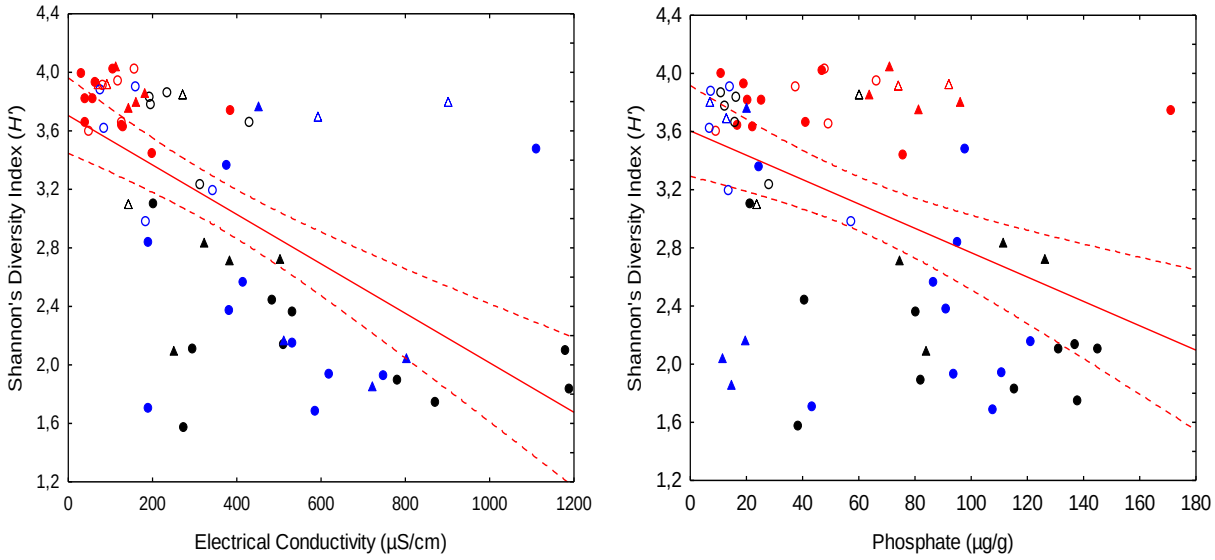
(b) Rawsonville (EC, r = -0.579)



(c) Rawsonville (PO_4, r = -0.610**)**



(e) *Eucalyptus* sites (Soil pH, r = -0.746)



(f) *Eucalyptus* sites (EC, r = -0.604) (g) *Eucalyptus* sites (PO_4, r = -0.446)

Figure 3.24: Pearson correlation coefficient (PCC) analysis between soil chemical properties and bacterial diversity (*H'*) of all the study sites pre-fire (red), post-fire (black) and a year after the burn event (blue). The control samples are indicated as empty circles while the centre and intermediate samples are indicated as filled circles. As for the *Eucalyptus* sites, Robertson is indicated the same as aforementioned, whereas the control samples of Wellington are indicated as empty triangles, and the centre and intermediate samples as filled triangles. Regression bands denote 90% confidence levels.

Table 3.13: The soil chemical properties of all the study sites of all three sample times. Significant differences (p <0.05) of the factorial ANOVA (Tukey HSD) test between the control and burnt samples, at particular sample time, are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

	Pre-fire		Post-fire		A year after burn event	
	Control	**Centre and intermediate**	**Control**	**Centre and intermediate**	**Control**	**Centre and intermediate**
Bainskloof						
pH (water)**	4.36^{ac}	4.57^{ab}	**4.93^{a}**	**6.44^{d}**	3.69^{c}	4.11^{bc}
EC (µS/cm)	118.69^{a}	90.73^{a}	75.43^{a}	80.42^{a}	304.86^{b}	185.00^{ab}
Avail N (µg/g)	37.26^{a}	34.73^{a}	31.53^{ab}	35.94^{a}	19.48^{b}	19.04^{b}
PO_4 (µg/g)	1.82^{ab}	1.28^{a}	9.15^{bc}	12.62^{c}	4.55^{ab}	6.80^{abc}
Rawsonville						
pH (water)***	5.22^{a}	5.37^{a}	**6.73^{bc}**	**9.00^{d}**	**5.48^{ab}**	**7.67^{c}**
EC (µS/cm)*	202.13^{a}	164.29^{a}	**261.40^{ab}**	**803.60^{d}**	242.00^{ac}	651.80^{bcd}
Avail N (µg/g)	55.65^{a}	55.56^{a}	45.90^{a}	44.22^{a}	42.86^{a}	95.14^{a}
PO_4 (µg/g)***	47.32^{ac}	49.19^{ac}	**12.40^{a}**	**104.66^{b}**	**18.33^{a}**	**71.71^{bc}**
***Eucalyptus* sites (Robertson and Wellington)**						
pH (water)***	7.14^{a}	6.93^{a}	**6.79^{a}**	**9.68^{b}**	7.91^{ac}	8.55^{c}
EC (µS/cm)	97.85^{a}	126.03^{a}	252.71^{ab}	554.21^{b}	333.27^{ab}	544.43^{b}
Avail N (µg/g)	53.49^{a}	45.79^{a}	71.89^{a}	35.63^{a}	20.07^{b}	23.67^{b}
PO_4 (µg/g)*	53.59^{ab}	54.24^{ab}	**23.70^{a}**	**94.49^{b}**	16.87^{a}	66.84^{ab}

No indication p >0.05
* p <0.05
** p <0.01
*** p <0.001

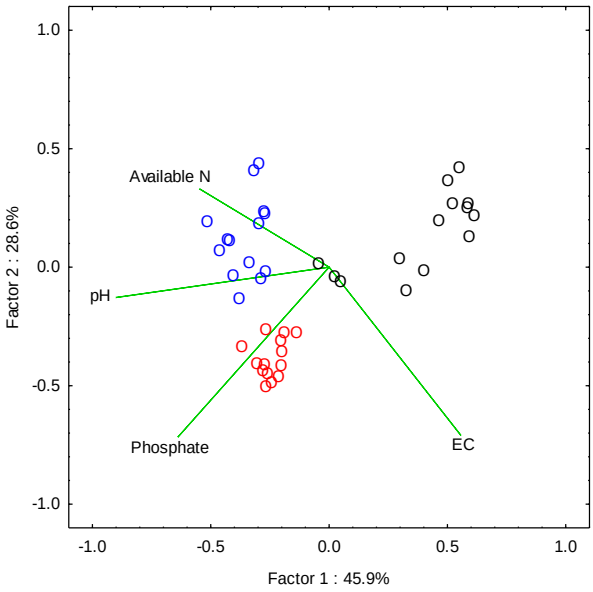
Table 3.14: Pearson correlation coefficient (PCC) analysis between soil chemical properties (i.e. soil pH, EC, available N (NH_4-N and NO_3-N) and PO_4, respectively) and fungal diversity (H'). Robertson and Wellington are grouped as *Eucalyptus* sites. The p-values are indicated in brackets and the significant results are indicated in bold.

	Bainskloof	**Rawsonville**	***Eucalyptus* sites**
Soil pH (water)	0.189 (0.172)	-0.290 (0.054)	0.127 (0.321)
EC (µS/cm)	0.066 (0.636)	-0.145 (0.341)	0.080 (0.532)
Available N (µg/g)	0.059 (0.672)	0.108 (0.482)	-0.190 (0.136)
PO_4 (µg/g)	0.235 (0.087)	0.209 (0.167)	0.203 (0.111)

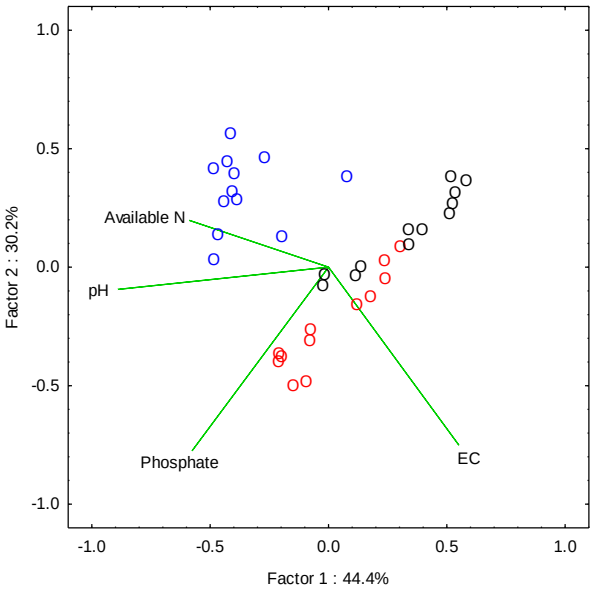
3.6.2. Principle component analysis (PCA)

The first principle component (Factor 1) of the Bainskloof site was determined by soil pH (Figure 3.25a-b). The second principle components (Factor 2) were determined by EC and PO_4 concentration (Figure 3.25a-b). The first and second principle components (combined) of the Bainskloof site explained 74.5% and 74.6% of the variation in the bacterial and fungal communities, respectively. According to Figure 3.25a-b, the microbial (bacterial and fungal) communities post-fire could be differentiated from the microbial communities pre-fire and a year after the burn event along the first principle component in the Bainskloof site. This is primarily due to the differences in soil pH. Here, the soil pH increased significantly post-fire and decreases gradually after the burn event (Table 3.13). However, the microbial communities pre-fire and a year after the burn event could also be differentiated from one another along the second principle component. This appears to be mainly due to the variation in EC and PO_4 concentration (Figure 3.25a-b). Here, no variation in EC and PO_4 concentration was recorded immediately post-fire (Table 3.13). However, EC and PO4 concentration a year after the burn event was relatively higher compared to the conditions pre-fire.

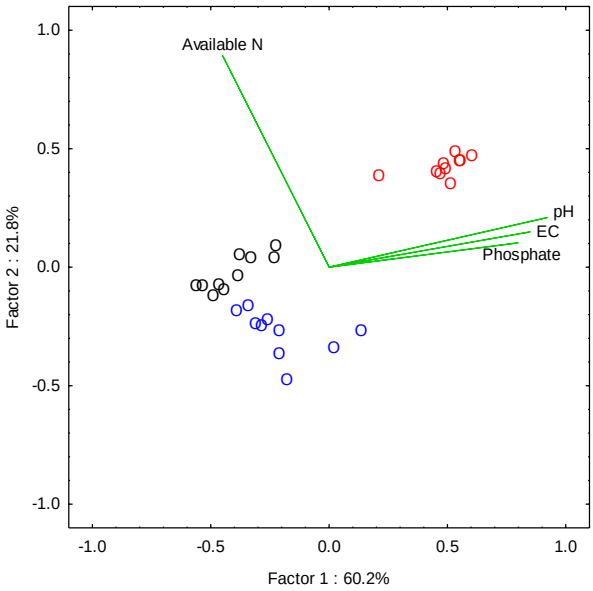
The first principle components of the *Eucalyptus* (Robertson and Wellington) and Rawsonville sites were determined by soil pH, EC and PO_4 concentration (Figure 3.25c-f). The second principle component was determined by the available N concentration (NH_4 and NO_3 concentrations combined) (Figure 3.25c-f). The first (62.6%) and second (18.2%) principle components of the *Eucalyptus* sites explained 80.8% of the variation in the microbial communities. Furthermore, the first (60.2%) and second (21.8%) principle components of the Rawsonville site encompassed 82.0% of the variation in the microbial communities. The burnt samples, in the *Eucalyptus* and Rawsonville sites, illustrated a clear separation between the microbial communities pre-fire, and the microbial communities post-fire and a year after the burn event along the first principle component (Figure 3.26c-f). This is due to the variation in soil pH, EC and PO_4 concentration. Here, the soil pH, EC and PO_4 concentration showed a steep increase immediately post-fire which remained relatively high a year after the burn event (Table 3.13). Furthermore, the microbial communities post-fire and a year after the burn event, however, were closely related and clustered in the same quadrant.



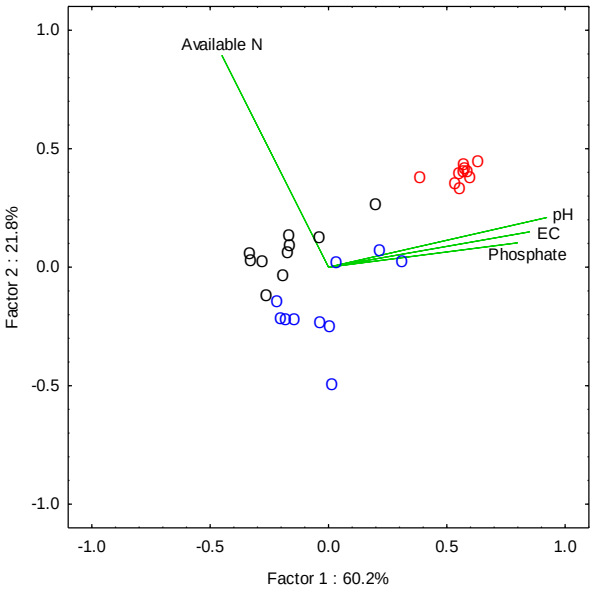
(a) Bainskloof - Bacterial communities



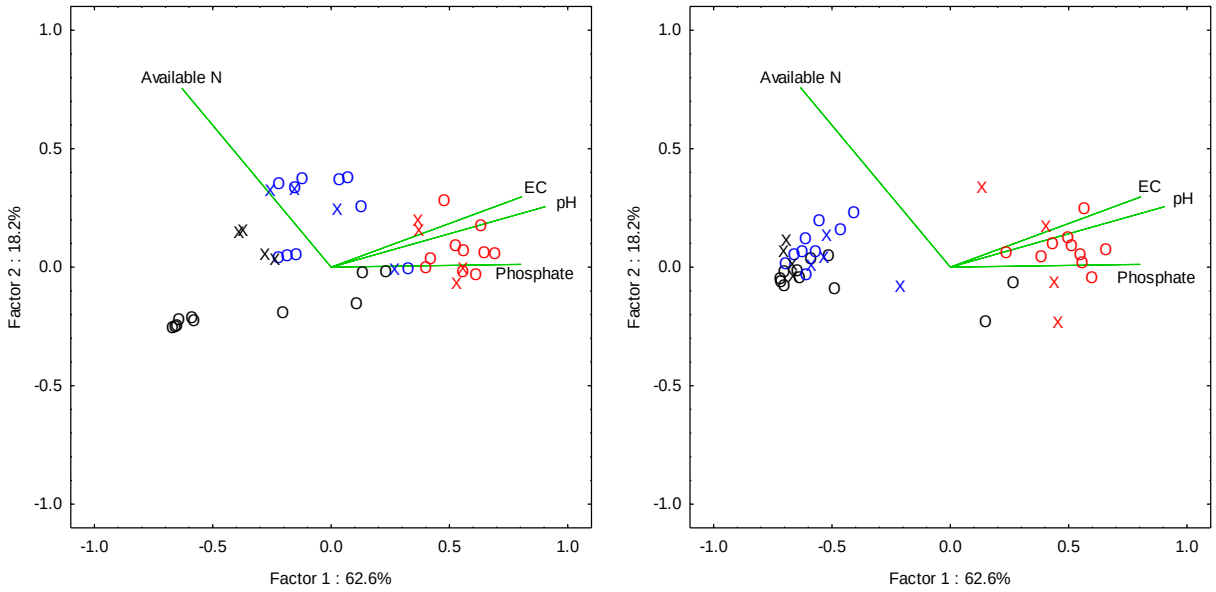
(b) Bainskloof - Fungal communities



(c) Rawsonville - Bacterial communities



(d) Rawsonville - Fungal communities



(e) *Eucalyptus* sites - Bacterial communities (f) *Eucalyptus* sites - Fungal communities

Figure 3.25: Principle component analysis (PCA) between soil chemical properties and microbial community structures of the centre and intermediate samples (indicated as o) in all the study sites pre-fire (red), post-fire (black) and a year after the burn event (blue). For the *Eucalyptus* sites, the microbial community structure in Robertson is indicated as (o), whereas the microbial community structure in Wellington is indicated as (x).

CHAPTER FOUR

Discussion

4.1. Introduction

Alien plant invasion poses a major threat to fynbos (Moran & Hoffman 2012; Myers *et al.* 2000). Alien plant invasion in fynbos vegetation has led to a decrease in the abundance of fynbos species and soil microbial diversity (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. This transition has a significant effect on the natural patterns and processes of the soil environment, soil physicochemical properties and soil microbial communities (Morris *et al.* 2011; Stock *et al.* 1995; Witkoski 1991). Invasive species, as listed in Table 1.1 (Chapter 1), have been removed in many parts of fynbos by means of mechanical management strategies for commercial purposes such as firewood, poles, structural and mining timber, and shelter-breaks (Allsopp & Cherry 2004). However, the remaining bark, branches, leaves, stems and twigs of these invasive species (i.e. dead plant biomass) after the mechanical removal of invasive species becomes a major problem (Holmes 2001). This is due to the dead plant biomass that might ignite a fire under the right conditions. In addition, the remaining dead plant biomass of invasive species may also inhibit the natural recovery of fynbos vegetation in cleared areas. One cost-effective and efficient management strategy to remove the remaining dead plant biomass of *Acacia* and *Eucalyptus* spp., after the mechanical removal, is the use of fire (the 'slash and burn' technique). The 'slash and burn' technique uses felling of invasive stands, which is then stacked to build a pile from dead plant biomass and burnt. In this study, the emphasis was on the effect of 'slash and burn' of *Acacia* and *Eucalyptus* biomass on soil microbial (bacterial and fungal) diversity and community structure in riparian zones of fynbos.

4.2. Soil bacterial diversity

4.2.1. Pre-fire

There was a significant difference in bacterial diversity *(H')* between invasion sites after the mechanical removal of invasive biomass (pre-fire) in the riparian zones of fynbos (Figure 3.3, Chapter 3). The bacterial diversity (*H'*) in the Bainskloof site was significantly lower (p <0.05) in comparison to the Rawsonville site. Before the mechanical removal of invasive species, the Bainskloof and Rawsonville sites consisted predominantly of *A. mearnsii*. Nonetheless, there

was a small percentage cover of *Eucalyptus* spp. at the Rawsonville site. Furthermore, the bacterial diversity (*H'*) in the Bainskloof site was significantly lower in comparison to the *Eucalyptus* sites (Robertson and Wellington). The Robertson and Wellington sites consisted predominantly of *E. camaldulensis*, with a small percentage of *Acacia spp.* cover at the Wellington site. Moreover, the bacterial diversity (*H'*) in the Rawsonville site was similar to the *Eucalyptus* sites. Therefore, these findings suggest that the bacterial diversity (*H'*) in *Acacia* invaded sites in fynbos are not only significantly lower in comparison to the native fynbos vegetation as found by Slabbert *et al.* (2014), but it is also significantly lower in comparison to the *Eucalyptus* invaded sites in fynbos. At present, the bacterial diversity (*H'*) in the native fynbos vegetation compared to that in *Eucalyptus* invaded sites in fynbos is unknown.

4.2.2. Effect of 'slash and burn' of invasive biomass on soil bacterial diversity

After the 'slash and burn' of invasive biomass (post-fire), the bacterial diversity (*H'*) and Simpson's diversity (1-*D*) index from the centre and intermediate samples (burnt samples) were similar for all the study sites, regardless of the dominant invasive species before clearing and burning (Figures 3.4; 3.5, Chapter 3). This implies that the bacterial diversity (*H'*) in the burnt samples followed a similar trend immediately after the 'slash and burn'.

Post-fire, the bacterial diversity (*H'*) in the burnt samples showed two different trends (Table 3.1, Chapter 3). The data indicated that the 'slash and burn' of *Acacia* biomass (Bainskloof) did not affect the bacterial diversity (*H'*) post-fire. Here, the pre-fire bacterial diversity (*H'*) remained similar post-fire and a year after the burn event. To the contrary, the 'slash and burn' of *Eucalyptus* biomass (Robertson and Wellington; also Rawsonville, where some *Eucalyptus* biomass was present in the piles) led to a steep decrease in bacterial diversity (*H'*) immediately post-fire, which remained relatively low a year after the burn event. Furthermore, Table 3.1 (Chapter 3) indicated that the 'slash and burn' of *Eucalyptus* biomass also resulted in a decrease in bacterial richness. These results suggest that the 'slash and burn' of *Eucalyptus* biomass might have achieved greater heat release and soil heating compared to the 'slash and burn' of *Acacia* biomass. It is also possible that the 'slash and burn' of *Eucalyptus* biomass could have led to soil bacterial mortality of some members of the community (i.e. some bacteria were more heat

resistant than others) (Egidi *et al.* 2016; González-Pérez *et al.* 2004; Hart *et al.* 2005), which may have resulted in a significant decrease in bacterial richness and diversity (*H'*).

4.3. Soil bacterial community structure

4.3.1. Pre-fire

Results from the NMDS plots showed that the pre-fire bacterial community structures are to a large degree site specific (Figure 3.11, Chapter 3), which is similar to what was found by Slabbert *et al.* (2010). This could be due to the study sites having different invasion statuses, before the mechanical removal of invasive species (pre-fire) (see Section 4.2.1). Another reason for this could be the recent recovery of fynbos species, after the mechanical removal of invasive biomass, in the invaded sites. All these study sites appear to fall into different natural fynbos vegetation types, which are exposed to different fynbos species (Midoko-Iponga *et al.* 2005; Mokotjomela *et al.* 2013; Moll & McKenzie 1994; Moll *et al.* 1984; Ruwanza *et al.* 2012). This also extends to riparian species, with plant assemblages unique to each site (Reinecke *et al*. 2008).

4.3.2. Effect of 'slash and burn' of invasive biomass on soil bacterial community structure

Figure 3.9 (Chapter 3) showed that at each study site, the burnt samples (i.e. centre and intermediate samples) presented three distinct bacterial community structures, separated by different sampling times. This suggests that the 'slash and burn' of *Acacia* and *Eucalyptus* biomass resulted in a shift in bacterial community structure, as previously pointed out by others (Dooley & Treseder 2012; Ferrenberg *et al.* 2013; Neary *et al.* 1995; Reazin *et al.* 2016). Previous studies stated that the shift may be explained by the heat impact of fire, which is greater in the top two centimetres of soil where the microbial communities are in abundance (Dooley & Treseder 2012; Hart *et al.* 2005; Sun *et al.* 2008).

When the bacterial community structure of the two grouped *Eucalyptus* sites (Robertson and Wellington) were compared to the two separated *Acacia* sites (Bainskloof and Rawsonville), the data recorded that the *Eucalyptus* and Rawsonville sites shared similar bacterial communities post-fire (Table 3.5, Chapter 3). This finding was rather surprising seeing as the piles at the

Rawsonville site were predominantly built from *Acacia* biomass, with a small percentage of *Eucalyptus* biomass. This finding supports the previous suggestion that the 'slash and burn' of *Eucalyptus* biomass had a greater impact on the microbial communities compared to the 'slash and burn' of *Acacia* biomass. This might be explained by the essential oils found in the biomass of *Acacia* and *Eucalyptus* spp. that are known to have antimicrobial properties (Batish *et al.* 2008; Jelassi *et al.* 2017; Silva *et al.* 2016). The mechanical removal of invasive species happened a year before the actual pre-fire sampling, which could have allowed the large wood logs and leaves of *Eucalyptus* spp. to have higher concentration of essential oils compare to the small branches of *Acacia* spp. before the fire. Based on this, it is possible that the residues that remained after the burning of *Eucalyptus* biomass could have left behind more concentrated chemical footprints than *Acacia* biomass post-fire. The toxic residues post-fire could possibly explain why the 'slash and burn' of *Eucalyptus* biomass had a greater impact on the bacterial communities compared to the *Acacia* biomass and why the Rawsonville site seemed to follow a similar trend with regards to the bacterial diversity and community structure as the *Eucalyptus* sites, although the piles were predominantly built from *Acacia* biomass with a small percentage of *Eucalyptus* biomass.

The bacterial community structure in the *Eucalyptus* and Rawsonville sites remained similar a year after the burn event (Table 3.5 and Figure 3.11, Chapter 3). However, the post-fire bacterial community structure in these sites was different from the community structure a year after the burn event (Table 3.2 and Figure 3.9, Chapter 3). The unique bacterial community structures in the *Eucalyptus* and Rawsonville sites, a year after the burn event, are likely due to the successional changes of bacterial communities after the 'slash and burn' (Ferrenberg *et al.* 2013).

A year after the burn event, the Bainskloof site (*Acacia* site) experienced a flood, which disturbed the piles (burnt areas). According to Table 3.4 (Chapter 3), no differentiation between the bacterial community structures of the control, centre and intermediate samples (burnt samples) was evident. Therefore, no concrete conclusion can be drawn about the impact of 'slash and burn' of *Acacia* biomass on the bacterial community structure, a year after the burn event, at the Bainskloof site. Moreover, the unique bacterial community structure in the Bainskloof site, a year after the burn event, could be due to the disturbance of a flood that disturbed the burnt areas and led to the re-establishment of *A. mearnsii* in the burnt areas (Figure 2.4e, Chapter 2).

4.4. Soil fungal diversity

4.4.1. Pre-fire

Little is known about the fungal communities in the riparian zones of fynbos. Nonetheless, Slabbert *et al.* (2010) have stated that the fungal communities in fynbos are unique and are closely associated with the above-ground flora. According to the data in this study, the pre-fire fungal diversity (*H'*) was similar between invasion sites (Figure 3.14, Chapter 3). This implies that the fungal diversity (*H'*), after the mechanical removal of invasive biomass (*Acacia* and *Eucalyptus* spp.), was similar in both types of invaded fynbos sites, while the fungal communities within invasion sites pre-fire were site-specific, i.e. each site showed unique communities (Figure 3.23, Chapter 3).

4.4.2. Effect of 'slash and burn' of invasive biomass on soil fungal diversity

The 'slash and burn' of *Acacia* and *Eucalyptus* biomass had no effect on the fungal diversity (*H'* and 1-*D*) (Figures 3.15; 3.16, Chapter 3). This, in turn, resulted in little variation of fungal diversity (*H'*) within and between invasion sites before and after the 'slash and burn' event (Figures 3.17, 3.18; 3.19, Chapter 3). This study, along with previous studies, recorded no significant difference in fungal diversity post-fire (Jonsson *et al.* 1999; Longo *et al.* (2000). In contrast, Reazin *et al.* (2016) recorded a significant decrease in fungal diversity and richness immediately post-fire. The fire treatments in Reazin *et al.* (2016) lasted for ~6 hours with soil surface temperatures reaching up to 700 °C. In the present study, however, the 'slash and burn' of *Acacia* biomass lasted for ~2 hours with the soil remaining heated for ~5 hours afterwards contributed by ash and steaming. Here, small smouldering branches were present after fire and the soils cooled down after 5 hours (Figure 2.4d, Chapter 2). Comparatively, the 'slash and burn' of *Eucalyptus* biomass lasted for >3 hours with large smouldering logs that kept the soil heated for up to 9 days (Figure 2.5, Chapter 2). Based on the classification reported by Reazin *et al.* (2016) that low-intensity fire generally burns for a shorter period of time, whereas high-intensity fire burns for a longer duration, it can be assumed that the fire treatment in the study of Reazin *et al.* (2016) had a greater fire intensity than the 'slash and burn' in the present study. This might explain why the effect of fire in Reazin *et al.* (2016) has led to a decrease in fungal diversity and

the 'slash and burn' treatment not. Furthermore, it can also be assumed that the fire intensity of *Eucalyptus* biomass was greater than the fire intensity of *Acacia* biomass. The *Eucalyptus* biomass had larger wood logs than *Acacia* biomass and consequently formed larger piles. This, in turn, could have resulted in *Eucalyptus* biomass burning longer than the *Acacia* biomass and also achieving a greater fire intensity. In spite of this, none of the treatments affected the fungal diversity (*H'*) post-fire.

4.5. Soil fungal community structure

4.5.1. Pre-fire

Table 3.7 and Figure 3.23a (Chapter 3) showed that each respective study site presented a unique pre-fire fungal community structure. These observations were similar to what was reported by Slabbert *et al.* (2010) who found that the fungal communities in fynbos are site specific. The unique pre-fire fungal community structure at each site could be due to remains of dead plants (i.e. bark, branches, leaves, stems and twigs) on soil surface layer, after the mechanical removal of invasive species (pre-fire). Other possibilities may include different plant assemblages (including native and invasive species) in different sites.

According to the NMDS plots (Figure 3.20a, Chapter 3), cluster analysis (Figure 3.21, Chapter 3) and ANOSIM results ($R = 0.989$, $p = 0.002$), the Bainskloof site indicated two distinct fungal communities pre-fire. Further analysis showed that the pre-fire fungal communities underneath piles 1, 2 and 4 (group 1) are different from the fungal communities underneath piles 5, 6 and 7 (group 2) (Figure 3.21, Chapter 3). This suggests that fungal communities in the soil samples underneath the piles were not homogeneously distributed. The spatial distribution of fungal communities could have been influenced by soil moisture patterns and texture, the presence and absence of plant species at particular areas on the same site and the availability of remaining dead plant biomass of *Acacia* spp. (after mechanical removal) on the soil surface layer mostly at group 1 (Ettema & Wardle 2002; Morris 1999; Morris & Boerner 1999; Unbanová *et al.* 2015).

The fungal communities in group 1 of the Bainskloof site and the Robertson site were similar pre-fire ($R = 0.206$, $p = 0.058$) (Table 3.7 and Figure 3.23a, Chapter 3). This finding was rather surprising, seeing that the Bainskloof site was predominantly invaded by *Acacia spp.*, whereas

the Robertson site was predominantly invaded by *Eucalyptus spp.* The similarity between the fungal communities in group 1 of the Bainskloof site and the Robertson site could be explained by site history in terms of secondary invasion. It is important to note that the mechanical removal of invasive species in the Bainskloof and Robertson sites happened a year before the actual pre-fire sampling. This has allowed the fynbos vegetation to recover and *Acacia* spp. to re-establish. During pre-fire sampling, a small percentage cover of *Acacia* spp. was present in the Bainskloof and Robertson sites. This means, after the mechanical removal of *Eucalyptus* spp. (primary invasion) in the Robertson site, *Acacia* spp. (secondary invasion), which were not dominant before the mechanical removal, had the competitive advantage over the fynbos species to compete for essential resources such as light, nutrients, space and water. This competitive advantage could have increased the abundance of *Acacia* spp. in the riparian zones of the Robertson site. In addition, previous studies have reported that fungal communities are more host-specific towards plant roots than bacterial communities (Barreiro *et al.* 2016; Unbanová *et al.* 2015). Based on these findings, it is possible that the re-establishment of *Acacia spp.*, at these sites, could have promoted the similarity in fungal communities pre-fire by selecting for certain groups.

4.5.2. Effect of 'slash and burn' of invasive biomass on soil fungal community structure

The 'slash and burn' of *Acacia* and *Eucalyptus* biomass resulted in a shift in fungal community structure (Table 3.9; 3.10, Chapter 3). Nonetheless, the 'slash and burn' of *Acacia* biomass in the Bainskloof site led to two distinct fungal communities post-fire, which were separated by the centre and intermediate samples (Table 3.8 and Figure 3.20a, Chapter 3). According to the analyses of soil chemical properties, the soil pH in the centre samples was significantly higher compared to the intermediate samples (Table 1, Appendix). This result was not evident in the *Eucalyptus* and Rawsonville sites (Table 1, Appendix). Based on these findings, it is possible that the soil pH could serve as a key factor which promoted the two distinct fungal communities post-fire. Studies that focused on the effect of fire on microbial communities have found that soil pH is a crucial environmental variable which explained the most variation of fungal communities post-fire (Hamman *et al.* 2007; Prendergast-Miller *et al.* 2017).

The post-fire fungal communities in the centre samples of the Bainskloof site and the *Eucalyptus* site were similar (R = 0.072, p = 0.267) (Table 3.8 and Figure 3.23b, Chapter 3). This result was rather surprising, as these study sites were exposed to: (i) the 'slash and burn' of different invasive biomass (*Acacia and Eucalyptus* spp.); (ii) different durations of the respective fires and (iii) different soil pH and EC post-fire (Table 2, Appendix). At present, no concrete conclusion can be drawn. Nonetheless, seeing that fire has a major effect on the soil composition (Raison 1979), there may be other factors that could have promoted these similarities that were not covered in this study. These factors might include water relations, microhabitats, structure, porosity, temperature and moisture in soil as well as the introduction of fungal spores by air immediately after the soils have cooled down (Batten *et al.* 2006; Fayos 1997; Górny *et al.* 2001; Neary *et al.* 1999; Pasanen *et al.* 1991).

After the 'slash and burn' event, the Bainskloof site experienced a flood which disturbed the piles. Flooding in riparian zones is a common disturbance that results in the water-aided dispersal of *Acacia* seeds (Teraria *et al.* 2013; Vosse *et al.* 2008). The effect of flooding may have caused the fungal community variation between the centre and intermediate samples in the Bainskloof site, post-fire, to be similar a year after the burn event. Additionally, *Acacia* growth was visible in the burnt areas (Figure 2.4e, Chapter 2) and no separation of fungal community structures was evident between the control, centre and intermediate samples (Table 3.10, Chapter 3). Furthermore, the fungal communities in the Bainskloof site, a year after the burn event, showed a community structure that was significantly different from those presented pre-fire (Figure 3.20a, Chapter 3). The fungal community variation, pre-fire and a year after the burn event in the Bainskloof site, could be due to the absence or dead plant biomass of *Acacia spp.* (after mechanical removal) and the presence of recovered fynbos species pre-fire. Comparatively, a year after the burn event, the Bainskloof site was dominated by *A. mearnsii*, with a small percentage cover of fynbos species.

The fungal communities in the *Eucalyptus* (Robertson and Wellington) and Rawsonville sites, a year after the burn event, were similar (R = 0.038, p = 0.213) (Table 3.11 and Figure 3.23c, Chapter 3). A possible reason for this finding was explained by Reazin *et al.* (2016). They stated that the post-fire dominant fungal species - beneath the soil surface layer where fire occurred or

from adjacent areas around the burnt piles - have the capacity to disperse into the burnt areas by means of mycelial expansion from deeper to surface soil profiles or from the margins of the burnt piles into the burnt areas (Reazin *et al.* 2016). The adjacent areas around the burnt areas in the Rawsonville, Robertson and Wellington sites a year after the burn event, showed plant growth, whereas within the burnt areas no plant growth was observed (Figure 2.6, Chapter 2). This might be due to the 'slash and burn' of *Eucalyptus* biomass, which could have destroyed the roots and mycorrhizal fungi in the soil that consequently decreased the recolonisation capacity and rate in burnt areas (Bååth *et al.* 1995).

A year after the burn event, the control areas in the Robertson site were predominantly covered by *Acacia* spp. (Figure 1, Appendix). This finding supports the previous suggestion that after the mechanical removal of *Eucalyptus* spp. (primary invasion), *Acacia* spp. (secondary invasion) had the competitive advantage over the fynbos species to compete for essential resources such as light, nutrients, space and water (see Section 4.5.1). In addition, previous studies reported the persistence of invasive *Acacia* spp. in fynbos is partially attributed to their capacity to release seeds immediately after maturation, the rapid accumulation of seed banks in the soil, their rapid growth rate, and stimulation of germination by fire (Holmes & Cowling 1997b; Tucker & Richardson 1995; Werner *et al.* 2008). This, along with the different seed dispersal mechanisms (e.g. ant, birds etc.) and essential resources (i.e. light, nutrients, space and water), could have resulted in *Acacia* spp. becoming more abundant in the riparian zones of the Robertson site (Figure 1, Appendix) (D'Antonio & Vitousek 1992; Gordon 1998; Holmes & Cowling 1997a; Knight & MacDondald 1991; Rajcan & Swanton 2001; Tucker & Richardson 1995).

4.6. Soil chemical properties correlated to soil microbial diversity and community structure

Soil pH is a crucial factor for fynbos vegetation and is known to have a significant effect on microbial communities (Fierer & Jackson 2006; Kim *et al.* 2014; Lauber *et al.* 2009; Osborne *et al.* 2011; Prins *et al.* 2004). The present study, along with previous studies, found that fire increases the soil pH (Table 3.13, Chapter 3) (Barreiro *et al.* 2016; Jensen *et al.* 2001; Maubane 2016). The increase of soil pH post-fire could be attributed to the alkaline nature of ashes (Bååth

& Arnebrant 1994; Hernández *et al.* 1997). The increased soil pH post-fire could change the availability of soil nutrients, which in turn, could affect the restoration of plant communities (Pereira *et al.* 2017).

The sites exposed to the 'slash and burn' of *Eucalyptus* biomass showed that soil pH served as the strongest soil physicochemical indicator for bacterial diversity (*H'*) (Table 3.12, Chapter 3). This finding was not evident in the Bainskloof site, which was exposed to the 'slash and burn' of *Acacia* biomass. A possible reason for this might be the 'slash and burn' of biomass from different invasive species; *Acacia* sites may respond differently to sites that have supported *Eucalyptus* spp. In this study, all the sites showed that the 'slash and burn' of *Acacia* and *Eucalyptus* biomass leads to an increase in soil pH (Table 3.13, Chapter 3). However, the bacterial diversity (*H'*) showed different trends between invasion sites post-fire (Figure 3.4, Chapter 3). As explained in Section 4.2.2, the 'slash and burn' of *Eucalyptus* biomass (Robertson and Wellington; also Rawsonville, where some *Eucalyptus* biomass was present in the piles) led to a steep decrease in bacterial diversity (*H'*) immediately post-fire (Figure 3.4b-d, Chapter 3). In contrast, the 'slash and burn' of *Acacia* biomass (Bainskloof) had no effect on the bacterial diversity (*H'*) (Figure 3.4a, Chapter 3). As for the fungal communities, no soil physicochemical property served as a useful indicator for the fungal diversity (*H'*) (Table 3.14, Chapter 3).

Apart from soil pH - which showed a significant correlation with the bacterial diversity (*H'*) in the study sites exposed to the 'slash and burn' of *Eucalyptus* biomass - other soil chemical properties, i.e. EC and PO_4 concentration, showed a negative correlation with bacterial diversity (*H'*) (Figure 3.24, Chapter 3). In addition, the soil pH, EC and PO_4 concentration explained the most variation of microbial (bacterial and fungal) communities in the sites exposed to the 'slash and burn' of *Eucalyptus* biomass (Figure 3.26, Chapter 3). These trends were not observed after the 'slash and burn' of *Acacia* biomass at the Bainskloof site. At Bainskloof, no variation in EC and PO_4 concentration was recorded immediately post-fire (Table 3.13, Chapter 3). However, EC and PO_4 concentration a year after the burn event was relatively higher compared to the conditions pre-fire. As for the sites exposed to the 'slash and burn' of *Eucalyptus* biomass, the soil pH, EC and PO_4 concentration showed a steep increase immediately post-fire which remained relatively high a year after the burn event (Table 3.13, Chapter 3).

The EC, which measures the soluble cations (i.e. calcium (Ca), potassium (K) and magnesium (Mg)) in soil, increased post-fire (Kutiel & Naveh 1987; Pereira *et al.* 2017). The increase of EC is due to the combustion of organic matter, which promotes the concentration of soluble cations (Hernández *et al.* 1997). This study, along with previous studies, found that fire significantly increases the Ca and Mg concentrations (Table 3, Appendix), whereas, the sodium (Na) and K concentrations were not affected by fire (Pereira *et al.* 2017; Ponder *et al.* 2009; Rhoades *et al.* 2004). Nonetheless, the EC and the Ca and Mg concentrations decrease gradually post-fire, likely due to the fixation of the salt, precipitation and leaching (Hernández *et al.* 1997; Kutiel & Naveh 1987).

The PO_4 concentration increase immediately after the 'slash and burn' of *Eucalyptus* biomass (Table 3.13, Chapter 3). The increase in PO_4 concentration post-fire is due to the soil heating and the mineralisation of P that promotes the production of polyphosphate (soluble forms) which is deposited in ash (DeBano & Conrad 1978; Khanna & Raison 1986). The P concentration increase immediately post-fire, as it has been reported that the pre-fire P stocks in plants and litter are found in ash or the charred plant remains post-fire (DeBano & Conrad 1978; Muñoz-Rojas *et al.* 2016). In this study, the PO_4 concentration decreases gradually after the burn event. Nonetheless, the PO_4 concentration a year after the burn event was still greater than the pre-fire condition. This could possibly be due to the ash and charred plant remains which is still present on top of the soil surface profile a year after the burn event (Figure 2.6, Chapter 2).

The soil pH and PO_4 concentration in the burnt samples of the Rawsonville site, a year after the burn event, was significantly higher compared to the control samples (Table 3.13, Chapter 3). These results were not evident in the *Eucalyptus* sites. A possible explanation for this could be due to the thick ash layer underneath the top two centimetres of the soil surface profile, at the Rawsonville site. As for the *Eucalyptus* sites, the ash was on top of the soil surface profile (Figure 2.6, Chapter 2).

Microbial communities can be influenced by the alteration of soil carbon (C) and N (Hui *et al.* 2017; Sun *et al.* 2017). The cycling of nutrients (such as soil C and N), which are retained by

soil organic matter (SOM), functions to maintain soil structure that retains water for plant use and helps microbial communities to recover more rapidly after a disturbance (Alexrood *et al.* 2002). In this study, the 'slash and burn' had no effect on soil C and N, as there was no significant difference recorded between the control and burnt samples (Table 3, Appendix). Based on these results, soil C and N do not serve as key factors which promoted the shift in microbial communities post-fire.

CHAPTER FIVE

Conclusion and future research

In this study, the ARISA fingerprinting technique was effectively used to assess the soil microbial diversity and community structure in invaded fynbos areas affected by fire. By using ARISA, the trends of microbial diversity and community structure after the exposure to the 'slash and burn' of different invasive biomass (*Acacia and Eucalyptus* spp.) were observed. Investigations into the effect of 'slash and burn' of *Acacia* and *Eucalyptus* biomass on microbial (bacterial and fungal) diversity and communities in riparian zones of fynbos have shown contrasting results.

This study found that the pre-fire bacterial diversity (*H'*) in *Acacia* invaded sites in fynbos is significantly lower in comparison to the *Eucalyptus* invaded sites in fynbos. The fungal diversity (*H'*), however, was similar in both types of invaded fynbos sites after the mechanical removal of invasive species. As for the microbial community structure, each site showed different communities, pre-fire. Furthermore, the study also showed that the 'slash and burn' of *Eucalyptus* biomass had a greater impact on the microbial communities compared to the 'slash and burn' of *Acacia* biomass.

'Slash and burn' of *Eucalyptus* biomass left a scar where the fynbos vegetation did not recover. It is possible that the 'slash and burn' of *Eucalyptus* biomass may have destroyed the roots and mycorrhizal fungi in the soil that consequently decreased the recolonisation capacity and rate in burnt areas (Bååth *et al.* 1995). For future research, it will be useful to investigate the effect of 'slash and burn' of invasive biomass on specific functional groups (i.e. mycorrhizal fungi, ammonifiers and N-fixers) in the riparian zones of fynbos. It will also be of value to evaluate the recovery of these functional groups (if possible) post-fire and to determine what it means for the restoration of fynbos vegetation. Furthermore, it will also be of worth to investigate the residues that remain after the burning, seeing that the *Eucalyptus* spp. have unique oils containing antimicrobial properties that could possibly leave behind different chemical footprints that may impact the microbial communities (Delaquis *et al.* 2002). This suggestion raises the following questions: what effects do these oils have on the soil, and did the heat extract these oils?

'Slash and burn' of *Acacia* biomass, however, is unclear due to the interference of the flood at the Bainskloof site. The flood disturbed the burnt areas and led to the re-establishment of

A. mearnsii. Therefore, for future research, an observational study may be considered to assess whether fynbos vegetation will recover after 'slash and burn' of *Acacia* biomass.

In recent past, the dominant soil bacterial species in the *Acacia* invaded sites in fynbos have been identified (Slabbert *et al*. 2014), but no previous studies attempt to identify the soil bacterial species in the *Eucalyptus* invaded sites in fynbos. For future research, it might be of value to investigate the microbial species that are present in both *Acacia* and *Eucalyptus* invaded sites in fynbos. These findings may present knowledge regarding the possible contribution of microbial species towards: (i) the effect of clearing of *Acacia* and *Eucalyptus* spp. on microbial communities and how the resulting shift in microbial communities help to promote the recovery of fynbos vegetation and (ii) the difference in microbial community structure between *Acacia* and *Eucalyptus* invaded sites in fynbos pre-fire and post-fire, and (iii) the impact of seasonal changes on the succession of microbial communities post-fire and what it means for the restoration of fynbos vegetation. It should also be recommended that temperature probes are buried in the soil (say at 2 cm depth) to determine if the piles burn at different heat – this is important between invasive species, but also between sites (e.g. sun exposure and moist content of the biomass) as well as time of day and recent rainfall events.

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APPENDIX

Appendix

Table 1: The post-fire soil chemical properties in the centre (C) and intermediate (I) soil samples of all the sites were compared to one another. Robertson and Wellington are grouped as *Eucalyptus* sites. The available N is the NH_4 and NO_3 concentrations combine. The *p* indicates the *p*-value. Significant results are indicated in bold.

	Bainskloof			**Rawsonville**			***Eucalyptus sites***		
	C	**I**	***p***	**C**	**I**	***p***	**C**	**I**	***p***
pH (water)	7.14	5.77	(**0.024**)	9.15	8.85	(0.206)	10.2	9.13	(0.062)
EC (µS/cm)	82.3	82.5	(0.994)	725.2	882.0	(0.432)	661.4	447.0	(0.236)
Avail N (µg/g)	36.6	36.4	(0.934)	43.3	45.2	(0.886)	35.7	36.2	(0.428)
PO_4 (µg/g)	13.8	8.93	(0.328)	98.5	110.8	(0.590)	114.9	74.1	(0.057)

Table 2: The post-fire soil chemical properties in the centre samples of the Bainskloof site compared to that in the centre and intermediate samples (combined) of the *Eucalyptus* sites (Robertson and Wellington). The available N is the NH_4 and NO_3 concentrations combine. Significant results are indicated in bold.

	Bainskloof (centre)	***Eucalyptus* sites**	***p*-value**
pH (water)	7.14	9.68	(**<0.001**)
EC (µS/cm)	82.3	554.2	(**0.001**)
Avail N (µg/g)	36.6	35.6	(0.991)
PO_4 (µg/g)	13.8	94.5	(0.201)

Figure 1: A year after the burn event, the control areas in the Robertson site were predominantly covered by *Acacia* spp.

Table 3: The soil C and N, and the major cations (i.e. Ca, Mg, Na and K) of Rawsonville and Wellington of all three sample times. Significant differences (p <0.05) of the factorial ANOVA (Tukey HSD) test between the control and burnt samples, at particular sample time, are indicated in bold. Different letters, as determined by the post-hoc (Tukey HSD) test, show the significant differences between means (p <0.05).

	Pre-fire		**Post-fire**		**A year after burn event**	
	Control	Centre and intermediate	Control	Centre and intermediate	Control	Centre and intermediate
Rawsonville						
C%	2,82^{a}	4,07^{a}	2,10^{a}	1,35^{a}	1,65^{a}	1,24^{a}
N%	0,22^{ab}	0,31^{a}	0,16^{ab}	0,07^{b}	0,11^{ab}	0,11^{ab}
Ca (mg/l)*	44,84^{a}	68,94^{a}	**41,46^{a}**	**231,14^{b}**	52,96^{a}	144,70^{ab}
Mg (mg/l)*	15,94^{a}	23,96^{a}	**14,22^{a}**	**112,44^{b}**	16,18^{a}	34,34^{a}
Na (mg/l)	6,62^{a}	7,54^{a}	5,48^{a}	39,28^{a}	5,86^{a}	11,16^{a}
K (mg/l)	4,98^{a}	7,84^{a}	9,68^{a}	92,82^{a}	9,08^{a}	11,40^{a}
Wellington						
C%	5,42^{a}	2,56^{a}	2,41^{a}	0,70^{a}	2,60^{a}	2,32^{a}
N%	0,08^{a}	0,06^{a}	0,06^{a}	0,05^{a}	0,06^{a}	0,06^{a}
Ca (mg/l)**	224,00^{ac}	285,05^{a}	**76,67^{c}**	**298,05^{a}**	32,72^{bc}	40,03^{bc}
Mg (mg/l)	19,61^{abc}	24,32^{ac}	**8,56^{ab}**	**31,28^{c}**	5,74^{ab}	6,90^{b}
Na (mg/l)	8,03^{a}	12,85^{a}	6,63^{a}	9,10^{a}	5,60^{a}	5,87^{a}
K (mg/l)	31,45^{a}	39,84^{a}	6,96^{a}	29,26^{a}	8,34^{a}	7,32^{a}

No indication p >0.05
* p <0.05
** p <0.01
*** p <0.001