Identifying Relationships Between Soil Processes and Biodiversity to Improve Restoration of Riparian Ecotones Invaded by Exotic Acacias

Report to the
WATER RESEARCH COMMISSION

by

S Jacobs[1], M Naude[1], E Slabbert[2], O Kambaj[1], M Fourie[1], K Esler[1,3], K Jacobs[2], B Mantlana[4,7], A Rozanov[5] and D Cowan[6,8]

[1] Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University
[2] Department of Microbiology, Faculty of Science, Stellenbosch University
[3] Centre for Invasion Biology, Stellenbosch University
[4] Global Change Research Group, South African National Biodiversity Institute, Kirstenbosch Research Centre
[5] Department of Soil Science, Faculty of AgriSciences, Stellenbosch University
[6] Department of Biotechnology, University of the Western Cape
*[7] Department of Environmental Affairs**
*[8] Department of Genetics, University of Pretoria**
**New affiliations*

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Water Research Commission
Private Bag X03
Gezina, 0031

orders@wrc.org.za or download from www.wrc.org.za

EXECUTIVE SUMMARY

INTRODUCTION

Riparian zones exist at the nexus of high resource availability, and high disturbance within landscapes, however, are also centrally involved in delivering several hydrological ecosystem services. As riparian ecotones usually exist at the lowest topographical position within catchments, and they are thus receivers of material, propagules, genes and information, they are often also the first to show significant alterations that affect the catchments. As such, they are useful to monitor overall ecosystem health within catchments. In addition to natural disturbances such as floods, fires and droughts, several anthropogenic stressors can affect riparian zones. In the fynbos biome, these stressors can range from impoundments, habitat destruction, urban development, agricultural activities and alien invasive species. In the Western Cape, and also other parts of South Africa, the Australian tree *Acacia mearnsii* has displaced many native riparian plants in some upper, but many middle and lower reaches, establishing populations with high stand density, which regenerate quickly following a natural disturbance such as fire. Where stands of *A. mearnsii* have established, evapotranspiration has been shown to be higher than where native riparian scrub and forests are found, prompting a major restoration initiative, the Working for Water programme, which aims to clear the invasive species from riparian ecotones.

Thus far little information is available on the impact of invasive *Acacia* species in fynbos riparian ecotones on ecosystem function as it pertains to nutrient cycling and soil processes, some of which are central to riparian ecosystem services. Furthermore, it is unknown whether soil chemistry and processes return to pre-invasion levels after removal of the alien invasive *Acacia* species from fynbos riparian ecotones. One of the objectives of the Working for Water programme is to clear riparian zones of invasive alien trees like *Acacia* species, which takes place with the expectation that both vegetation structure and ecosystem function will recover following clearing. However, while vegetation structure recover in some instances, it remains unclear whether ecosystem function also recover. The main objective of this project, therefore was to investigate the impact of the invasive *A. mearnsii* (occasionally co-occurring with *A. longifolia* in the western Cape and *A. dealbata* in the southern and eastern Cape) on nitrogen (N), carbon (C) and phosphorus (P) stocks and cycling, as well as its impact on the soil bacterial and fungal community structure within fynbos riparian ecotones and nearby upland areas. Our sites were located mostly in upper reaches of catchments, which was dictated by the availability of reference sites. We also went further and investigated the relationship between riparian soil processes and soil and plant diversity in “pristine” (reference; natural), invaded and cleared ecotones in order to improve the strategies for riparian repair after removal of alien invasive *Acacia* species. Finally, we compared aspects of N, C and P cycling in soils at our main sites in the western Cape with measurements at sites in the southern and eastern Cape.

The objectives, in more detail, are:

1. To quantify changes in soil total C, total N, available N and available P stocks in natural, invaded and cleared riparian ecotones and associated upland fynbos.
2. To quantify soil properties, soil N mineralization and denitrification in natural, invaded and cleared riparian ecotones and associated upland fynbos.
3. To quantify soil respiration in natural, invaded and cleared riparian ecotones and associated upland fynbos.
4. To quantify soil and plant biodiversity in natural, invaded and cleared riparian ecotones and associated upland fynbos.
5. To determine the relationships between soil characteristics, soil processes and soil and plant biodiversity.

The main question we asked was:

How do soil properties, processes and biodiversity (restricted to microbial diversity) differ between different landscape positions (wet bank, dry bank and terrestrial areas) and different invasion statuses (natural, cleared and invaded riparian ecotones), and what is the trajectory after clearing of *A. mearnsii* (predominantly) from riparian ecotones?

BRIEF DESCRIPTION OF THE MAJOR RESULTS AND CONCLUSIONS

Plant functional types

As we chose invaded sites in the western Cape (primary research sites) with high *A. mearnsii* cover, both seedling and adult *A. mearnsii* cover was significantly higher in invaded (keeping in mind that the terrestrial parts of each site were not invaded to any great extent) compared to natural riparian ecotones. Cleared riparian ecotones had significantly lower *Acacia* cover, as can be expected from riparian ecotones where clearing operations are active and initial clearing has been followed up repeatedly. In contrast, grass cover (especially in dry banks) increased in cleared sites, a trend which has been observed before in other studies. The grass species that were prominent in cleared riparian ecotones were *Briza maxima, Ehrharta calycina* and *Pennisetum clandestinum*. Our objectives only pertained to structural aspects of plant diversity; hence we did not fully investigate other aspects of plant biodiversity.

Soil physicochemical properties

Results were obtained from soils sampled from the first 10 cm of the topsoil of the sites in the western Cape, hence discussions and conclusions only pertain to this soil layer. We measured several soil properties over the course of one year (seasonally), while other properties were only measured once off- or twice over the course of one year. Soils showed significant differences between sites with different invasion statuses, with lower coarse sand content in invaded areas. However, the content of finer material (silt + clay) was not significantly different between sites with different invasion statuses. As expected, gravimetric soil water content differed between different landscape positions, and seasonal interactions were apparent and prominent. The most prominent difference emerged in electrical conductivity (EC) where invaded riparian ecotones had significantly higher electrical conductivity compared to natural riparian ecotones, which is consistent with other studies where invasive alien plants (IAPs) in riparian and upland environments have been shown to promote the build-up of salts in the topsoil. Also notable was a decline in EC after clearing of IAPs, which suggests a trajectory towards the pre-invasion state. While these differences are, on the surface, relatively minor, soil properties such as particle size, soil pH and EC have been shown to have a major impact on soil processes and also influence soil microbial structure. The results obtained on soil physicochemical properties contribute to meeting objective 5.

Nitrogen cycling

One of the processes influenced by soil physical and chemical properties is N cycling. Nitrogen stocks were elevated in invaded riparian wet banks, and showed a trend towards the natural state when *Acacia* species were removed. Dry banks, in the natural state did not show any differences with wet banks and terrestrial areas, which does not support our expectation that riparian topsoil are enriched in N and other nutrients. The magnitude of the increase in N stocks in invaded riparian topsoil with invasion by N fixing trees was much lower that was found by Yelenik et al. (2004; 2007) and others in terrestrial soils, suggesting that riparian soils do not accumulate N to the same extent. The soil fine (clay+sand) fraction showed strong correlations with soil total N content (Spearman's rho = 0.663), as well as with other soil properties such as soil phosphatase activity and soil microbial structure.

One potential pathway for loss of N produced by N-fixing invasive *Acacia* species (thus reducing N loading of receiving waters) is denitrification. Denitrification rates, either in the field, measured as N_2O emissions, or in the laboratory measured as denitrification enzyme activity did not show appreciably higher rates in riparian soils affected by high *Acacia* cover. This is in line with work carried out elsewhere (e.g. Pinay et al., 1992), which showed that higher fine soil fractions are associated with higher denitrification rates. One can deduce that fynbos riparian ecotones in longitudinal zones closer to the headwaters do not support high levels of denitrification, even if N is added to the system by N-fixing *Acacia* species. An alternative pathway in riparian ecotones for reducing N loads is that annual floods and larger floods are able to mobilize most of the litter accumulating on soils of invaded dry banks, and leach some of the available N from time to time.

In cleared riparian ecotones, especially dry banks, available N remained high more than seven years after removal of invasive *Acacia* species. Available N is the only soil property that remained relatively high after removal of alien species, and at the same time, riparian dry banks also showed high grass cover, especially the grass species *B. maxima, E. calycina* and *P. clandestinum*, as noted before. Secondary invasion by alien invasive grass species into riparian zones has been previously noted in studies in the fynbos biome, and the hypothesis is that the relatively higher available nutrients in cleared riparian zones may facilitate establishment of these invasive grasses. While we were not able to show a causal relationship, the trends do support that contention that increased available N post-clearing may facilitate higher grass cover following *Acacia* removal.

Phosphorus

We measured available (Bray 2) P (P_i) in riparian soils in natural, invaded and cleared riparian environments and uplands, thus addressing our original objective, although using a different method than was originally planned (the proposed method was the Hedley fractionation method). Available P did not show significant differences when expressed by invasion status; however, wet banks had lower P_i concentrations than dry banks and terrestrial areas when the data was pooled over seasons.

While available P did show some trends, more prominent differences emerged from analysis of acid phosphatase monoesterase (APME), an enzyme that can derive from plant roots or microbes, and is involved in transformation of organic P to available P. The presence of high APME activity is associated with low soil P availability to plants and microbes, and this suggests that APME may be a useful index of P as it is known to be a limiting element in fynbos riparian soils. Thus under invasive alien acacias, we expected AMPE to be high as P plays an important role in N fixation, hence is in high demand. Following this reasoning, invasion of usually low P fynbos riparian soils by N fixing woody species, any available P is taken up quickly by *Acacia* roots. Acid phosphatases are exuded by *Acacia* roots or associated microbes to mineralize P locked up in organic matter (OM). Indeed, in dry banks invaded by alien *Acacia* species, APME activity was double that of the comparative landscape position in natural riparian ecotones, and APME activity decline upon removal of the *Acacia* individuals. This supports a contention that P cycling is relatively closed in fynbos soils, meaning little P losses. When the invasive species are removed, AMPE activity also decline, suggesting that the demand for P has dissipated.

Carbon

Parallel with N, soil total C concentrations were significantly higher in invaded dry banks, and were significantly lower in the wet bank compared to other landscape positions. Invaded dry banks also had higher soil respiration (R_S) rates compared to natural and cleared riparian ecotones. When *Acacia* individuals are removed from wet banks the R_S rate declined significantly. In fact, there were little differences in *in situ* soil respiration rates between natural and cleared riparian ecotones. To answer the question of what drives soil respiration rates, especially in invaded dry banks, we conducted a small experiment where intact blocks of soil were trenched (trenches dug around it to sever roots). Trends showed an immediate decline in R_S (first two seasons), although it later bounced back (subsequent seasons). This does suggest, although it does not prove, that root respiration may be involved in the higher R_S rates in invaded riparian ecotones. The lack of differences between trenched and control plots later on may be due to elevated root decomposition. Further support for this is found when soils, minus roots were incubated in the laboratory at constant temperature and moisture (potential soil respiration; PR_S). Few differences were found between incubated soil from sites with different invasion statuses or landscape positions, suggesting that soil microbial activity is not the major driver of the higher *in situ* R_S rates in *Acacia*-invaded riparian dry banks.

The main environmental drivers for R_S seem to differ from season to season. Though soil temperature seem to be influential, physical soil properties such as soil wetness is a major factor constraining CO_2 emissions from soils, at least during dry periods. Soil respiration peaked when soil temperature was highest and soil moisture was between 0 and 5% gravimetric soil water content (i.e. summer). This, however, may be related to plant physiology, which, based on seasonal photosynthesis dynamics for fynbos plants, should be highest in summer.

Soil microbial diversity

Overall, soil bacterial diversity (Shannon diversity index) changed with invasion by *A. mearnsii* (and associated *Acacia* species such as *A. longifolia*). The Shannon diversity index was significantly lower in the invaded wet bank zones. The effect of *A. mearnsii* on the bacterial diversity could thus only be observed within the wet bank, showing the influence of the river on structural differences between bacterial communities. No such differences was evident with fungal diversity, suggesting that fungi may be more resistant to invasion by these N-fixing, arbuscular mycorrhizal woody species, but the return of bacterial diversity to a state resembling the natural condition suggest that the bacterial community, while not resistant, are resilient, able to bounce back when the invasive species are removed.

Soil bacterial and fungal community structure also changed when invaded by *Acacia* species, however, the change was reversible. Members of the phyla *Alphaproteobacteria* (including root associated genera such as *Rhizobium*, which fixes atmospheric N in symbiosis with plant roots) was significantly overrepresented in the invaded sites, but declined relative to other biota when *Acacia* species were removed.

Soil bacterial and fungal diversity were driven by different soil properties. The soil bacterial community structure was driven by soil pH, and to a lesser extent, particle size and soil available P. It was noted during analyses for soil N dynamics that soil particle size was also important for N cycling; this suggests an important role for soil texture in riparian processes and soil microbial diversity.

Comparisons between the southern and eastern Cape and the western Cape

When comparing sites in the southern and eastern cape on the one hand, and the western Cape sites on the other, some differences emerged. We found that denitrification enzyme activity appeared to show higher rates in invaded areas, compared to the natural and cleared riparian ecotones. This difference may be the result of differences in soil properties, however, we found little difference in physicochemical soil properties when comparing the two regions. Soil respiration (*ex situ*; PR_S) showed no clear trends, while soil microbial diversity showed clear differences between natural, invaded and cleared riparian ecotones, as was the case for the western Cape sites.

CONCLUSIONS AND RECOMMENDATIONS

Our results point to changes in soil properties, soil processes and soil microbial diversity when invasive *Acacia* species establish in large numbers in fynbos riparian ecotones. Like the adjoining fynbos, topsoils of these mountain stream and mountain stream transition zone riparian ecotones are relatively nutrient poor. However, with invasion, soil total N and total C increase, though not to the same magnitude that has been found in terrestrial sites in other studies. Invasion also affects soil processes – N availability increase, soil C efflux increase and soil phosphatase activity increase. Along with these changes in soil processes, the underlying microbial diversity also change in terms of diversity and structure, though bacterial and fungal communities show different trends, and also different relationships with soil physicochemical factors.

When the *Acacia* spp. are removed, soil properties, soil processes and soil microbial diversity recovers, suggesting that ecosystem function, as it pertains to these aspects also recover. A legacy effect does exist – soil available N levels remain elevated in cleared riparian ecotones and this may provide an opportunity for nitrophilous grasses to establish and thrive in these cleared riparian ecotones. The soil microbial community seems to be relatively resistant (soil fungi) to invasive *Acacia* species and removal of these species. It also seems to be resilient (bacteria), meaning that bacterial populations recover in structure and diversity after removal of the *Acacia* individuals.

Our results support the contention, finding application in the Working for Water Programme (http://www.dwaf.gov.za/wfw/), that removal of *Acacia* species may assist in restoration of riparian ecotones. Major changes to soil functioning that take place with or after invasion of riparian ecotones with exotic *Acacia* species, and these changes may impact ecosystem service delivery. While plant structure and diversity have not been studied to a great extent in this research (it has been researched extensively in other studies), aspects of ecosystem and soil function that received attention suggest a trajectory after removal of *Acacia* species back towards the natural state, an additional motivation for removing invasive *Acacia* species from watercourses. However, more than seven years after removal of *Acacia* species, legacy effects still remain, and these legacies (e.g. high available N) need to be carefully

considered in managing clearing and follow-up activities. This may involve active treatment of certain areas to speed up removal of residual N and other resources that may be utilised by nitrophilous and other opportunistic plant species that may form persistent secondary invasions in riparian environments.
The main research findings, conclusions and recommendations will be communicated to relevant stakeholders, e.g. officials at Working for Water through research briefs, which are short, one-page summaries of topics covered in the research.

RECOMMENDATIONS FOR FUTURE RESEARCH

Several new avenues for research have been identified. The central role of soil P as a constraint on soil processes and soil microbial diversity is seen though the exudation of acid phosphatases and the relationship of available P with bacterial community structure. Given that P oversupply in riparian and aquatic environments can lead to eutrophication (Smith et al., 1999), further research on the role of P cycling in riparian environments will shed light on the risk of P loading of riparian soils and aquatic environments, especially with agricultural modification of fynbos riparian ecotones.

Soil texture seems to be an important control over soil processes, and the role of silt and clay in riparian soil processes deserves closer inspection. Soil N and P cycling were both related to the content of fine material in soils, and more empirical studies of this relationship may yield predictive models that may be useful in predicting biogeochemical consequences of disturbances on fynbos riparian ecotones.

Relatively little is known about how fluvial dynamics (which strongly influence the spatial distribution of different mineral particle sizes; Bechtold and Naiman, 2006) influence the sorting of sediments in fynbos riparian soils. We suggest that this should be further investigated as hydrogeomorphological processes play a critical role in the composition of riparian plant and soil communities (Naiman et al., 2005). Nevertheless, it can be assumed that predictable differences in particle size classes across geomorphological zones in riparian ecosystems contribute to equivalent contrasts in soil conditions.

The study was carried out in the upper reaches of fynbos rivers, hence our results may not be directly applicable to reaches further down the river (e.g. the deposition zones of wider floodplains) where more fine material is usually deposited. Nutrient dynamics in the lower reaches, usually also invaded and/or modified through agricultural or urban development may reveal additional information on how soil processes, properties and microbial dynamics are affected, and how this related to catchment biogeochemical dynamics, if at all.

Additions of N to soils through biological fixation may augment N flux rates and enhance the loss of soluble nitrate (NO_3^-) to receiving waters (e.g. Follstad Shah et al., 2010). Acacias may therefore potentially be an important new source of bioactive N in rivers and ecosystems further downstream, however, this needs to be assessed in future research. In fact, little is known about nutrient fluxes across the riparian-aquatic interface in fynbos under natural conditions, and basic research on this and the role of *Acacia* species in modifying these fluxes are necessary. There is also a risk that clearing woody invasive species may lead to eutrophication of surface water bodies and NO_3^- contamination of groundwater when N-rich litter decomposition is enhanced (Jovanovic et al., 2009). Further research is needed, especially on the relative role of different N immobilisation pathways (denitrification versus leaching).

It is important to understand the magnitude and controls of R_S at the landscape level in Mediterranean ecosystems, and while our study has revealed much new information on R_S, further unpacking of the controls on R_S will be needed, which will not only contribute to riparian ecology, but also to understanding of regional contribution to global C cycles. Equally, given our results of enhanced soil C efflux, the role of alien invasive species such as *Acacia* species in enhancing or mitigating global climate change must be investigated further.

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LIST OF ABBREVIATIONS

ANN	Artificial Neural Network
Anosim	Analysis of Similarity
ANOVA	Analysis of Variance
APME	Acid Phosphatase Monoesterase
ARISA	Automated Ribosomal Intergenic Spacer Analysis
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
C	Carbon
CC	Canopy Cover
CO_2	Carbon Dioxide
DEA	Denitrification Enzyme Activity
DNA	Deoxyribonucleic Acid
DOM	Dissolved Organic Matter
CFR	Cape Floristic Region
EC	Electrical Conductivity
GC	Gas Chromatograph
GSWC	Gravimetric Soil Water Content
IAP	Invasive Alien Plant
IRGA	Infrared Gas Analyser
ITS	Internal Transcribed Spacer
MEGAN	Metagenome Analyzer
MID	Multiplex Identifier
MTE	Mediterranean Type Ecosystem
N	Nitrogen
NCBI	National Center for Biotechnology Information
NMDS	Non-Metric Multidimensional Scaling
Ns	Unidentifiable base pairs
NH_4^+	Ammonium
NMP	Potential Anaerobic Nitrogen Mineralization
NO_3^-	Nitrate
OM	Organic Matter
OTU	Operational Taxonomic Unit
P	Phosphorus
PCR	Polymerase Chain Reaction

PFT	Plant Functional Type
Pi	Extractable Inorganic Phosphorus
PR_S	Potential Soil Respiration
PVC	Polyvinylchloride
RDP	Ribosomal Database Project
RDPII	Ribosomal Database Project II
rRNA	Ribosomal Ribonucleic Acid
R_S	Soil Respiration
spp.	Species
SWC	Soil Water Content
TC	Total Carbon
TN	Total Nitrogen
UPGMA	Unweighted Pair Group Method with Arithmetic

1. INTRODUCTION

In many temperate ecosystems riparian zones represent a sharp transition in environmental factors, ecological processes and biota (Gregory et al., 1991; Kroger et al., 2009). In Mediterranean-type environments, riparian zones are often very prominent, with higher resource availability than elsewhere in the catchments (Stella et al., 2012). Riparian zones are representative of ecotones, that is, transitional areas between environmental patches, in this case terrestrial and aquatic ecosystems. The term “critical transition zone” has recently been coined for these zones, defined as hybrid ecosystems that functions as conduits of materials and energy between clearly defined ecosystems (Wall et al., 1997; Bardgett et al., 2001; Ewel et al., 2001). Riparian zones are located at the nexus of high resource availability (water and nutrients) and variable, often unpredictable disturbance dynamics, ensuring structurally complex soils and biotic communities (Gregory et al., 1991; Malanson, 1993; Naiman et al., 2005). The geomorphological position of riparian zones at the lowest position in catchments allows gravitational movement of material, while biotic vectors utilize the abundance of resources. As such, a riparian zone integrates and reflects many processes that occurs elsewhere in the catchment, is highly sensitive to disturbances and is often the first indicator of changing environmental conditions, natural or anthropogenic (Naiman and Decamps, 1997). A multitude of anthropogenic disturbances influence riparian zones, including landuse change, habitat destruction, alien invasive species, climate change, changes in fire regimes and impoundments. These critical transitions zones have thus become the focus of much effort in terms of monitoring, restoration and conservation in support of ecosystem services, and have emerged as a major issue at the United Nations Conference on Environment and Development in Rio de Janeiro in 1992 (Bardgett et al., 2001).

Riparian soils are often considered enriched in nutrients compared to adjacent terrestrial areas due to sediment and dissolved and particulate organic and inorganic nutrients arriving from terrestrial, aquatic and atmospheric sources elsewhere in the catchment, as well as biotic nutrient returns from riparian plants and animals (Naiman and Decamps, 1997; Weathers et al., 2001; Jacobs et al., 2007). The linear nature and inherent heterogeneity and associated surface roughness of riparian zones results in them acting either as a buffer for material and energy originating in uplands, or a conduit for transient and accumulated material and energy from terrestrial and aquatic ecosystems (Strayer et al., 2003). Riparian soils play a crucial role in transmitting, transforming, absorbing or reflecting materials from uplands and rivers and streams (Naiman and Decamps, 1997; Burt and Pinay 2005; Jacobs et al., 2007). Riparian processes are mediated by soil microbial, micro-, meso and macrofauna, which convert organic material to mineralized C, N and P. This is coupled with higher uptake and storage of C, N and P by riparian plants, often higher in standing biomass than the surrounding vegetation (Balian and Naiman, 2005; Naiman et al., 2005). The trapping and storage of nutrients within the riparian corridor is a valued ecosystem service, ensuring biotic integrity of streams and rivers is maintained (Miltner and Rankin, 1998; Smith et al., 1999; Ewel et al., 2001).

Due to the proximity to water sources, both surface and subsurface, as well as the high proportion of fine soil particles and organic material, riparian soils in temperate areas are mostly moist throughout the year, though in more arid environments this depends on the nature of the river or stream (Stella et al., 2012). Coupled with higher nutrient stocks, moist conditions result in high process rates of many biogeochemical processes, and high nutrient availability for uptake by plants (Chirmo and McDonnell, 1997; Stella et al., 2012). In semiarid areas, intermittent floods result in pulsed nutrient availability for short periods (Belnap et al., 2007; Jacobson et al., 2007); however, due to the higher stature and biomass of riparian plants, nutrient stocks may also be higher, especially in depositional reaches of the river. Finer material in the riparian zone is positively correlated with biogeochemical properties such a denitrification and mineralization, while finer sediments also contain higher concentrations of P and is also associated with larger microbial biomass (Pinay et al., 1992; Bechtold and Naiman, 2005; Naiman et al., 2005).

There is strong consensus that structure and function of many ecosystems have been compromised as anthropogenic stressors such as deforestation, habitat fragmentation, invasive alien species, pollution and climate change act individually or synergistically on ecosystem processes. In South Africa, all our biomes have experienced changes to structure such as losses or increases in plant cover, changes in biodiversity, changes in soil properties, both physical and chemical (DEAT, 2006). There seem to be general agreement that changes in ecosystem function is also taking place, either as a result of changes in structure, or as a direct result of anthropogenic impacts (Chown, 2010). However, ecosystem function is often more difficult to detect and measure. A large volume of research points to changes in ecosystem structure, e.g. changes in plant density, plant height, leaf area index, plant community composition when terrestrial and riparian ecosystems are invaded by alien plant species (e.g. Richardson and van Wilgen, 2004; Aguiar et al., 2006; Richardson et al., 2007; Beater et al., 2008). At the same time relatively little evidence exist of how ecosystem functioning is affected by introduction of invasive species into ecosystems (Ehrenfeld, 2003; Holmes et al., 2008).

There seem to be some ambiguity when structure and function are reported and policy solutions designed (Jax, 2005). This ambiguity is also evident when considering riparian zones. Riparian zones in South Africa is currently receiving much attention as a combination of stressors, including alien invasive species, impoundments, development and pollution has led to changes in structure and function (Richardson et al., 2007; Esler et al., 2008). The central role of riparian and associated freshwater ecosystems in supplying provisioning, regulating, supporting and cultural ecosystem services in what is generally acknowledged as a dry country has ensured a major restoration programme, the Working for Water (WfW) programme was instituted. The major objective of WfW is ‘to restore and maintain natural resources by clearing invasive alien plants while creating jobs and economically empowering unemployed people from historically disadvantaged communities' (Marais and Wannenburg 2008). According to Esler et al. (2008) and Holmes et al. (2008), there is an unstated implication that on removal of alien invasive species, structure and function of riparian zones would 'self-repair'. While measures of structure (cover, diversity, evenness of vegetation) have been determined in numerous studies, it remains unclear what measures of function can and should be measured in order to evaluate the return of riparian zone function (Jax, 2005), and ensure that cleared riparian zones is on the right trajectory toward restoration.

Some evidence is emerging from various biomes on declines of ecosystem function (using the broadest definition of ecosystem function) when invasive species enters riverine areas in large numbers (Marchante et al., 2008; Follstad Shah et al., 2010). Some information is available for terrestrial areas in the fynbos biome (e.g. Yelenik et al., 2004), however, for riparian ecotones in the fynbos biome, very little baseline information on soil processes and nutrient stocks exist, and equally little for areas which have been invaded by *Acacia* species (van der Waal, 2009), putative ecosystem transformers that can fix N (Morris et al., 2011). It was the objective of the project we report on here to investigate ecosystem function within riparian ecotones that have been invaded with putative ecosystem transformer species, *Acacia meansiii* and other invasive Australian *Acacia* species. We used uninvaded riparian ecotones as reference sites, and we also sampled from various landscape positions (wet bank, dry bank and terrestrial area).

At the same time we carry out research at sites which have been cleared of *Acacia mearnsii* and other invasive *Acacia* species. In South Africa, a large amount of money is spent annually on restoring riparian environments by clearing *Acacia* trees, and follow-up actions are meant to keep these ecotones clear of invasive species (van Wilgen et al., 2011). However, both empirical and anecdotal evidence suggest that the mere clearing of the invasive trees may not put the ecosystem on the trajectory towards restoration (Holmes et al., 2008). In fact, secondary invasion often result, which means that alien invasive grasses replace alien invasive *Acacia* species within riparian environments, while native trees and shrubs do not recover in the short term, or at all (Milton 2004). Among resources that may facilitate grass invasion are available nutrients, especially N. In our project we compare natural, invaded and cleared riparian ecotones to investigate biogeochemical changes that may prevent native species from establishing after clearing, or facilitate increases in alien grass cover, possibly at the expense of native trees and shrubs.

Riparian ecotones are known to be hotspots for biogeochemical cycling, including N cycling through the processes of N mineralization and denitrification (McClain et al., 2004). Carbon cycling also seems to be higher in riparian ecotones (Pacific et al., 2007); this presents several biogeochemical pathways for N and C to be processed within riparian ecotones, which may not be available to terrestrial environments at the same magnitudes. In this project we measured denitrification, N mineralization, R_S as well as soil phosphatase activity, which have been demonstrated to be an indicator of P demand in the ecosystem and also change when N is added to the ecosystem (Sinsabaugh et al., 1993).

Evidence exist that microbial dynamics are also affected by invasion of alien plant species into ecosystems, and that legacies can exist for some time after removal of the alien species (Kourtev et al., 2002; Marchante et al., 2008), though, conversely it has been demonstrated that microbial communities can recover after removal of alien invasive plant species (Lankau et al., 2010). Thus, we also endeavoured to measure the impact of alien invasive acacias on the soil microbial community (the topsoil to a depth of 10cm only). We used a relatively new technique, automated ribosomal intergenic spacer analysis (ARISA) as well as a new, cutting edge sequencing technique (pyrosequencing) to understand how microbial community composition and structure is affected by alien invasive species encroaching into riparian environments, and the impact of clearing of alien invasive *Acacia* species. Using the sequencing technology we can also understand which species and taxonomic groups are responsible for the observed changes in microbial community composition and structure.

2. OBJECTIVES AND KEY QUESTIONS

We aim to investigate relationships between riparian soil processes and biodiversity (soil and plant biodiversity) in “pristine” (reference; natural), invaded and cleared ecotones in order to improve the strategies for riparian repair after removal of alien *Acacia* spp. Our objectives (and key questions) are:

1. To quantify changes in soil total C, total N, available N and available P stocks in natural, invaded and cleared riparian ecotones and associated upland fynbos.
    - Do riparian soils have higher stocks of C, N and P? (Key Question 1)
    - Are there changes with invasion by invasive alien plants (IAPs), and what are the changes after removal of IAPs? (Key Question 2)
2. To quantify soil properties, soil N mineralization and denitrification in natural, invaded and cleared riparian ecotones and associated upland fynbos.
    - How do soil N mineralization and denitrification in riparian ecotones differ from upland areas, and amongst natural, invaded and cleared riparian sites? (Key Question 1)
    - What is the role of riparian denitrification in immobilization and removal of N, especially as a removal strategy in cleared areas? (Key Question 2)
    - What are the relationships between soil biophysical, biogeochemical, and hydrological characteristics and N mineralization and denitrification? (Key Question 3).
3. To quantify soil respiration in natural, invaded and cleared riparian ecotones and associated upland fynbos.
    - How does soil respiration in riparian ecotones differ from upland areas, and amongst natural, invaded and cleared riparian sites? (Key Question 1)
    - What is the role of riparian soil respiration in regulation and return of C stocks to pre-invasion levels (Key Question 2)
    - What are the relationships between soil biophysical, biogeochemical, and hydrological characteristics and soil respiration? (Key Question 3)
4. To quantify soil and plant biodiversity in natural, invaded and cleared riparian ecotones and associated upland fynbos.
    - Do riparian ecotones harbour high levels of soil biodiversity (compared to upland areas)? (Key Question 1)
    - What are the levels of heterogeneity within the different riparian areas? (Key Question 2)
    - Do these communities show significant shifts over seasons? (Key Question 3)
    - What is the impact of invasion and removal of IAPs on soil biodiversity? (Key Question 4)
    - What are the changes over the gradient between the wet bank and the upland fynbos? (Key Question 5)

5. To determine the relationships between soil characteristics, soil processes and soil and plant biodiversity
    - What is the relationship between soil and plant biodiversity? (Key Question 1)
    - How does soil biodiversity relate to soil process rates? (Key Question 2)
    - How does invasion by and clearing of IAPs influence the relationships between soil characteristics, soil processes and soil and plant biodiversity? (Key Question 3)

3. SITE DESCRIPTION

3.1 HYDROLOGICAL, GEOLOGICAL AND ECOLOGICAL TEMPLATE

The western part of South Africa experiences a Mediterranean winter rainfall climate, more than 70% of which occurs between April and September, and with hot, dry summers (Deacon et al., 1992; Sieben, 2003). Rivers in the western part of the fynbos biome rise in mountains that can reach an elevation of about 2000 m, but the gradient is gentler in the eastern part of the CFR. The geomorphology is dominated by the Cape Fold Belt Mountains (Prins et al., 2004). Rainfall in the mountainous regions in the western Cape range between 1000 and 2000 mm per year, but may exceed 3000mm in certain areas (very high peaks) (Sieben, 2003). As a consequence, the proportion of perennial flow in rivers is higher in headwater or mountain streams for the south-western Cape compared to elsewhere in the Fynbos Biome (Galatowitch and Richardson, 2005). The riparian zones that are embedded in Mediterranean-type ecosystems such as the fynbos biome are physically, chemically, and biologically shaped by the geology, geomorphology and seasonally predictable rainfall events (Gasith and Resh, 1999; Stella et al., 2012). In the fynbos biome this produces small, narrow streams defined by strongly seasonal patterns of flow, with high-flows in winter and spring in response to precipitation, and low-flows in summer (Corbacho et al., 2003). Fynbos ecosystems are adapted to periodic fires, while the soils tend to be old, are shallow, acidic and sandy in nature with low nutrient status (Stock and Lewis, 1986; Witkowski and Mitchell, 1987; Deacon et al., 1992; Rebelo et al., 2006; Sieben et al., 2009). Riparian zones of the fynbos biome (and western Australia) are exposed to more summer flows than other MTEs (Cowling et al., 2004), hence, ecosystem processes such as recovery after fire, nutrient cycling and productivity is likely also attune to this.

Characteristics of a particular site within the river network in part depend on the geology and the derived substrate of the catchment. Basin geology, hydrology and inputs of organic/inorganic material from the surrounding catchment interact to form the geomorphological structure of a valley floor (Gregory et al., 1991). The uplift of the Cape Fold Belt Mountains and associated high degree of topographical diversity within the CFR has created a complex of diverse soils, with young and ancient soils occurring over relatively short distances (Cowling et al., 2009). The hard sandstone and granite underlying much of the geology of the fynbos biome has led to constrained river reaches in the headwaters, with strong bedrock influence on hydrology and ecohydrology (Reinecke et al., 2007). In the mountain stream zones erosion is the dominant geomorphological process, and as the elevation is relatively steep, stream power is high. These headwater stream sections of the river tend to dominate the landscape, a result of the short distance between the mountains of the Cape Fold Belt and the coast (Prins et al., 2004).

Lithologies in the western part of the fynbos biome are dominated by sandstone and quartzite, underlain by granites (Rebelo et al., 2006), with occasional shale bands. While the fynbos generally occur on sandy soils, soils may be silty in some places and are predominantly derived from weathering of Table Mountain Sandstone, Cape Supergroup shale and Cape granite (Rebelo et al., 2006), yielding relatively nutrient-poor substrate (Prins et al., 2004). Typically, the upper reaches of rivers contain sandstone and granitic soils, and this changes further down to calcareous sand and shale derived soils (Sieben et al., 2009). More often than not the soils adjacent to mountain stream sections of rivers are weakly developed, shallow and consist of a high proportion of bedrock, boulders and large cobbles. However, typically deeper soils are found in the dry bank zone further away from the water's edge (Sieben and Reinecke, 2008). As a result of these dominant geological substrates, many river floodplains in the fynbos biome are covered with deep sandy alluvium (Rebelo et al., 2006).

Fynbos vegetation of the fynbos biome has been particularly well studied ecologically (Holmes and Richardson, 1999). The fynbos biome has been recognised as biologically distinct, supporting unique vegetation types rich in endemic species (Rebelo et al., 2006). Fynbos riparian vegetation, Cape Lowland freshwater wetlands and Cape Lowland Alluvial vegetation are the three broad azonal community groups in the fynbos (Mucina et al., 2006). Riparian ecosystems with a fynbos affinity have also been described as Closed-Scrub Fynbos dominated by broadleaved woody species, mainly small, perennial trees and shrubs, including characteristic fynbos elements such as species of Ericaceae and Restionaceae (Cowling and Holmes, 1992) in addition to forbs and graminoids in the understory (Reinecke et al., 2008). However, a particularly large turnover of species (i.e. high beta diversity) occurs between different catchments (King and Schael, 2001; Reinecke et al., 2007), although there are some common species with wider distributions across catchments (e.g. *Ischyrolepis subverticillata*; *Brabejum stellatifolium*; *Brachylaena neriifolia*; *Meterosideros angustifolius*) (Reinecke et al., 2008). In steep valleys that are protected from fire, riparian ecosystems give rise to Afromontane forest, where taller tree species (e.g. *Ilex mitis*, *Rapanea melanophloeos*, *Kiggelaria africana*, *Podocarpus* species) may establish (Manders, 1990; Mucina et al., 2006; Reinecke et al., 2008).

3.2 SITE SELECTION

At the commencement of the main part of the study, eleven sites were selected, including four reference or natural sites that are relatively pristine and uninvaded (and has never been highly invaded), four moderate to highly invaded riparian sites (mainly *A. mearnsii* or a mixture of *A. mearnsii* and *A. longifolia* invaded for at least more than 10 years, or reinvaded after clearing) and three cleared riparian sites (a previously invaded site that has been cleared more than 7 years ago at the commencement of the study, with the specific IAPs listed above necessary for selection). The sites represented the main (primary) sites in the western part of the fynbos biome (hereafter western Cape) where sampling and measurements were carried out (a secondary set of sites were selected in the southern and eastern Cape where limited sampling took place), and were distributed across six perennial river systems within the western Cape (viz. the Jakkals, Sir Lowry's Pass, Eerste, Dwars, Molenaars and Wit; Figure 3.1). For our study at least three sites of each invasion status (natural, invaded, cleared) were located on different river systems in order to minimize the effects of catchment differences (geology, time since last fire, species composition, precipitation and streamflow). These sites thus represent a range of reach types, but is broadly representative of fynbos riparian zones in the Mountain Stream and Transitional longitudinal zones (Rowntree et al., 2000; King and Schael, 2001; also referred to as geomorphological zones).

Our sample sites were chosen based on the history and intensity of *Acacia* invasion, or the absence of appreciable invasion by IAPs, the management history, and where feasible, sites were selected in the same location where previous studies were carried out, e.g. the studies of Blanchard et al. (2008), Reinecke et al. (2007) and Vosse (2007). Our original sampling design called for nine sites, consisting of three natural (reference), three invaded (predominantly *A. mearnsii* or a mixture of *A. mearnsii* and *A. longifolia* invaded for at least more than 10 years) and three cleared riparian sites (a prior invaded site that has been cleared more than 7 years ago, with *A. mearnsii* as the dominant invader). However, in order to compensate for unscheduled clearing of sites or fires, one additional invaded and an additional natural site was added, thus the total number of sites was 11 sites and the number of invaded and natural sites was four each. No other cleared sites that fitted in with our required criteria were found, hence we carried out research at only three cleared sites.

Reference sites are critical in restoration and rehabilitation efforts to provide compositional and structural information on vegetation communities and on historical disturbance conditions, which provides direction for restoration goals (Blanchard and Holmes, 2008; Sieben and Reinecke, 2008). Our reference sites were chosen to be within or as close as possible to the longitudinal zones (based upon river gradient and other characteristic; Rowntree et al., 2000) where the invaded and cleared sites were also located.

Reference sites were also chosen to represent a relatively pristine condition, with minimal invasion by woody IAPs, and free of other human interference where at all possible.

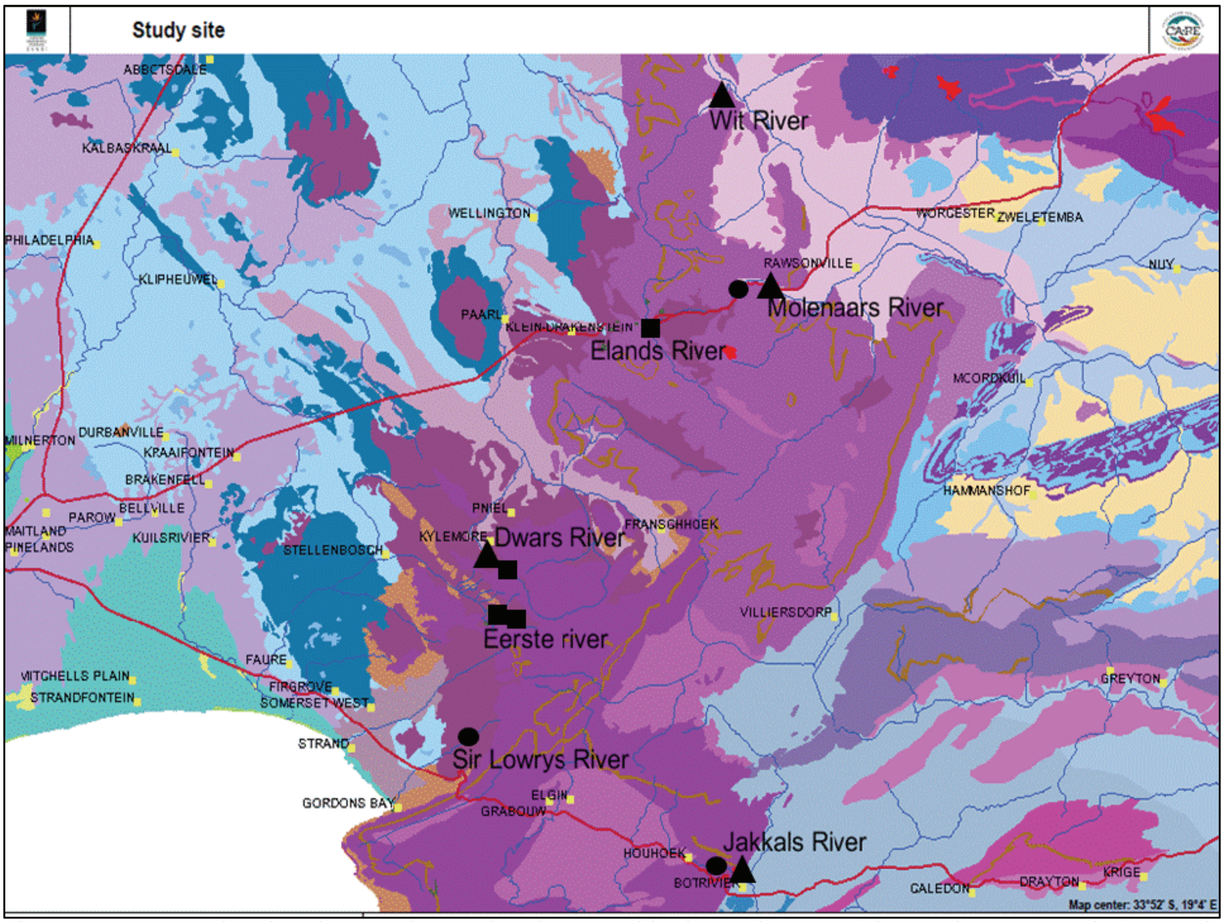

Figure 3.1: Map showing the location of the sites used in the main part of the study. Natural (reference) sites are indicated with black squares (■)symbols, invaded sites with black triangles (▲), and cleared sites with black circles (●). The map was created with spatial data provided by South African National Biodiversity Institute (SANBI) GBIS. The colours denote different vegetation types as described by Mucina and Rutherford (2006). Vegetation types associated with sites are Kogelberg sandstone fynbos (Jakkals; Eerste; Dwars), Hawequas sandstone fynbos (Wit; Molenaars), Boland granite fynbos (Sir Lowry's Pass; Eerste; Dwars), and Breede alluvium fynbos (Molenaars; Wit). Map scale: 1:750 000. (From Naude, 2012).

In order to be selected, invaded sites had to have an aerial cover of at least 50% *A. mearnsii* or a mixture of *Acacia* species. Cleared sites, on the other hand, was chosen based on history of clearing; all cleared sites were required to have been cleared 7 years prior to commencement of the study, and with regular follow-up treatment. Information on management history was gathered from WfW databases, studies such as that of Reinecke et al. (2007), Blanchard (2007) and Vosse (2007), and from landowners. All sites were subject to inspection prior to selection. These criteria applied to both the western Cape sites (the main sites) as well as the sites in the southern and eastern Cape, with one exception. One of the natural sites in the Eastern Cape was a sites restored by WfW more than 7 years prior, using plantings of native species. This site was selected as no other natural sites fitted the criteria in terms of level of invasion by *A. mearnsii*. An important factor we considered in selection of the primary sites in the western Cape sites is their relatively close proximity to Stellenbosch University, thus none of the sites were outside of a 100 km radius around Stellenbosch.

Several classification systems exist to describe ecohydrological zones parallel to the water's edge, and a new project (Brown et al., 2012) has started, which will further investigate the links between hydraulic parameters and the occurrence of riparian vegetation. Boucher (2002) identified as many as seven different zones, based on hydrology and plant community characteristics. We used three broad lateral zones parallel to the water's edge, which are described by Reinecke et al. (2007). These are the wet bank, the dry bank and the terrestrial area. Wet- and dry banks in the western part of the fynbos biome contain distinctive vegetation types, with smaller statured vegetation such as species within the families Restionaceae, Cyperaceae and shrubs most prominent in the wet bank (e.g. *Prionium serratum, Isolepis prolifera, Blechnum capense*), while the dry bank often contains trees and larger shrubs (*Brachylaena neriifolia, Metrosideros angustifolia, Brabejum stellatifolium*); the border between the two zones can be quite sharp (Sieben and Reinecke, 2008). The location and extent of wet bank zones are determined by intra-annual flows, compared to dry banks, which are determined by longer intervals between floods and also larger floods (Brown et al., 2012). In the southern and eastern Cape dry banks also harboured herbaceous species such as *P. serratum*, sedges, Restionaceae species and grasses, while larger trees such as *Virgilia oroboides* was prominent in some of the natural sites. Wet banks remain moist throughout the year in these perennial rivers, while dry banks are inundated for shorter periods, and less frequently, however, the longer lived vegetation of dry banks have deep root systems that can access groundwater. Figure 3.2 represents a cross-section through a hypothetical riverbed illustrating these different habitats. In addition to the two riparian lateral zones, we also sampled the non-riparian fynbos (upland; terrestrial area) in order to compare and determine the impact of landscape position on soil properties, soil processes and soil microbial and plant functional diversity.

Within each site, 4-5 plots were selected from each zone (wet bank, dry bank, and upland zones) giving a total of 12-15 sampling plots per site, and four to five transects with one sampling plot of each landscape position per transect. Wet and dry banks were distinguished based upon geomorphological and vegetation characteristics. All individual student projects used the same sample sites for analysis.

Terrestrial sampling plots were located at least 15 m from the outer boundary of the dry bank zones and each transect (consisting of one sampling plot in the dry bank, one in the wet bank and one from the upland) were a minimum of 10m from each other. Each sampling plot was marked and the coordinates of sites located with a GPS.

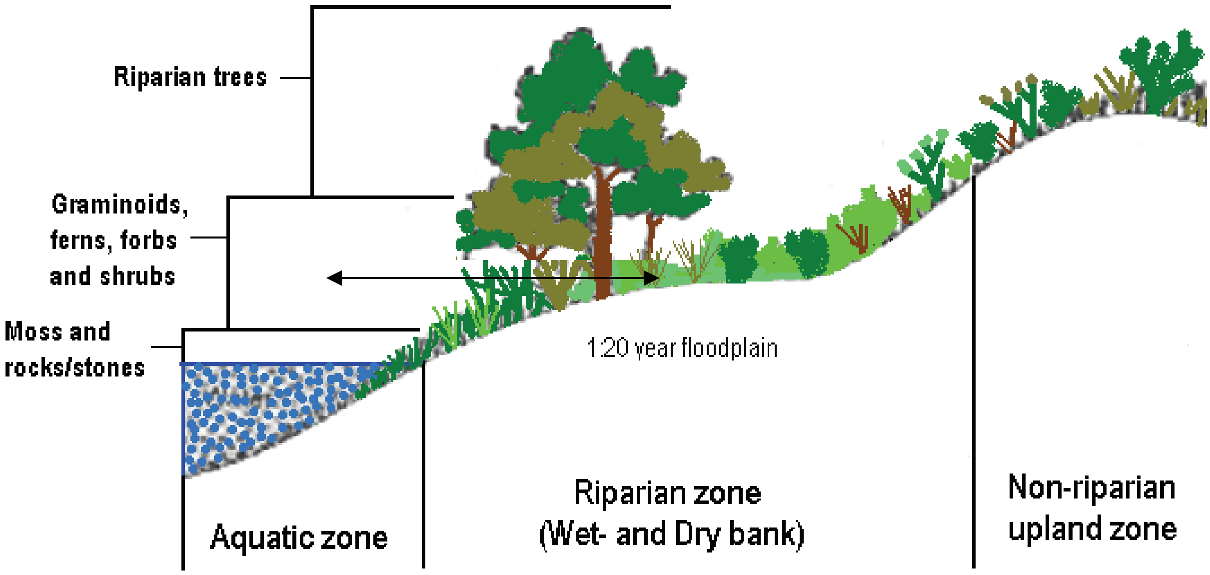

Figure 3.2: Conceptual diagram of zonation patterns in fynbos riparian ecosystems. The riparian tree zone and the upper part of the graminoid zone roughly represented the dry bank in our study, while the wet bank is represented by the zones indicated by mosses, as well as the lower part of the graminoid zone. Modified from Sieben (2003).

A short summary of some pertinent information for each site is displayed in Table 3.1. Fire is a major disturbance in the fynbos, and this is also apparent in the site history; however, this disturbance is uncontrollable as fires affecting the sites often originate off the sites we used. One such fire, originating in the southern part of the Limietberg Nature Reserve affected the Wit River site in February 2012 and destroyed all the permanently installed equipment on site (soil moisture probes with loggers; collars for soil respiration), and destroyed the *Acacia* stand where experimentation was carried out. Earlier, at the end of summer in 2011, the Lower Molenaars River site was burned in a fire following an unscheduled clearing in February 2011 (by the farm owner without our knowledge), while a fire swept through the Upper Molenaars River site at the same time. This later fire originated further south and burned part of the Limietberg Nature Reserve (much of the rest later burned in the 2012 fire described above).

A number of the chosen sites were affected by disturbances such as fire and unscheduled clearing during the sampling period. Molenaars Lower was cleared towards the end of the sampling period, while Molenaars Upper burned in a low intensity fire in autumn 2011, though only a few patches in the upland area of the latter site were affected. As a rule, we continued to carry out measurement and sampling at the sites affected by fire and/or unscheduled clearing. We consider this part of the natural variation within the sites, and which cannot be necessarily controlled. Given the apparent importance of fire in fynbos riparian ecology (natural, invaded and cleared sites), continued monitoring may well provide further opportunity to uncover the ecological responses of fynbos riparian ecotones to disturbance.

Table 3.1: Geormorphological characteristics and site-specific information on each of the study sites within the western Cape region. Information was gathered from local conservation agencies, farm managers and owners (from Jacobs et al., 2009; Naude, 2012).

Sites	Geology	Invasion Status	Coordinates	Landowners	History of invasion	History of clearing	Fire History	Gradient	Longitudinal zone
Upper Eerste River	Sandstone/Granite	Natural	33.9537 S; 18.9788 E	CapeNature	No evidence	None	Burned in March 2009	0.038	Transitional
Lower Eerste River	Sandstone/Granite	Natural	33.9895 S; 18.9675 E	CapeNature	No evidence	None	Burned in March 2009	0.058	Mountain Stream
Upper Dwars River	Sandstone/Granite	Natural	33.9544 S; 18.9799 E	CapeNature	No evidence	None	Burned in March 2009	0.120	Mountain Headwater Stream
Wit River	Sandstone/Granite	Invaded	33.5384 S; 19.0154 E	Mount Bain Development	Invaded for ≥ 15 years *Acacia mearnsii*	No evidence	No evidence of fire at the start of the field work, however, burned in March 2012	0.022	Transitional
Lower Molenaars	Sandstone	Invaded	33.7047 S; 19.2332 E	CapeNature	Invaded for > 10 years A. *mearnsii* */A. Longifolia*	Cleared in summer 2011	Burned in March 2011	0.020	Transitional
Lower Jakkals River	Sandstone	Invaded	33.2088 S; 19.1774 E	Beaumont Wine Farm, Bot River	Invaded for > 15 years A. *mearnsii* */A. Longifolia*	Yes, some evidence	No evidence of recent fire	0.050	Mountain Stream
Lower Dwars River	Sandstone/Granite	Invaded	33.9481 S; 19.9698 E	CapeNature	Invaded for > 10 *years A. mearnsii* */A. Longifolia*	No evidence	No evidence of recent fire	0.050	Mountain Stream
Sir Lowry's Pass River	Sandstone/Granite	Cleared	34.0948 S; 18.9444 E	Wedderville Estate, Sir Lowry Village	Mixed invasions: *Acacia spp.*+ *Pinus* spp.>8	Cleared in 2002 and annual followed up by	No evidence of recent fire	0.068	Mountain Stream

Sites	Geology	Invasion Status	Coordinates	Landowners	History of invasion	History of clearing	Fire History	Gradient	Longitudinal zone
					years ago	landowner: fell (aboveground, stump remained in place) and burn			
Upper Molenaars	Sandstone	Cleared	33.7107 S; 19.1970 E	CapeNature	Invaded for >7 years mostly *A. mearnsii*	Yes. Initial treatment: 2002-2003 follow-up treatments clearing treatment: fell (aboveground, stump remained in place) and remove	No evidence of fire at the start of the field work, however, burned in March 2011	0.044	Mountain Stream
Upper Jakkals	Sandstone	Cleared	33.2169 S; 19.2072 E	CapeNature	*A. mearnsii*/*A. longifolia* > 12 years	Initial clearance: 1996-97. Two follow-up treatments. Treatment: fell and burn	Burned in January 2010	0.040	Mountain Stream

3.3 SITE DESCRIPTION

Two sets of sites were chosen where soils were sampled and measurements taken. The first set was confined to the western part of the fynbos, around Stellenbosch, and was intensively sampled. These sites were the primary sample sites. The second set was located in the southern and eastern Cape around the towns of Kareedouw and Haarlem, and were used primarily to ascertain whether the trends observed in the western Cape sites were repeatable.

3.3.1 Eerste River system and sites

The Eerste River is a perennial river, relatively short in length (~40km), and has its source at an altitude of 1320 m above sea level in the Dwarsberg Mountains, which forms part of the Jonkershoek Nature Reserve, and contains relatively pristine fynbos and riparian environments (Rebelo et al., 2006). The river flows in a north-westerly direction through the town of Stellenbosch, where it becomes canalized and dominated by woody non-native trees, along with native riparian species (Meek et al., 2010).

The vegetation in the upper half of the river (inside Jonkershoek Nature Reserve) is near pristine and comprises predominantly indigenous fynbos and riparian communities (Vosse, 2007; Figure 3.3), though a few *Acacia* individuals have been removed in the past. The catchment consists of Cape Granite and Table Mountain Group Sandstone along with some Malmesbury Shale bands. The upland vegetation in the area is mainly Kogelberg Sandstone fynbos and Boland Granite Fynbos, while the riparian zone is classified as Fynbos Riparian Vegetation (Rebelo et al., 2006).

Figure 3.3: (A) Lower Eerste River natural site, with *Prionium serratum* a prominent component of wet banks and (B) upper Eerste River natural sites, with dry banks consisting of *Brabejum stellatifolium* and *Metrosideros angustifolia*.

Two natural sites are located in the Mountain Stream and Transitional longitudinal zones above and below Swartbrug. At both sites wet and dry banks and upland sample sites were chosen where sampling and measurements were carried out. Both sites burned during March 2009, prior to commencement of most of the sampling.

3.3.2 Dwars River system and sites

The Dwars River is a tributary of the Berg River, and originates in the Dwarsberg mountains; its catchment runs parallel to the Jonkershoek catchment. The Dwars River runs past the town of Kylemore in a northerly and then in a north-easterly direction. Similar to the Eerste River catchment, the geology consists of Table Mountain Group Sandstones, Cape Granite, Malmesbury Group shales, with Table Mountain Sandstones more prominent in the upper reaches of the river. Due to the steep gradients in many places, soils tend to be shallow and rocky in terrestrial areas. The vegetation is classified as a combination of Boland Granite Fynbos and Kogelberg Sandstone Fynbos (Rebelo et al., 2006). According to the measured gradient (Table 3.1) the longitudinal zone at the Upper Dwars site (natural) and the lower Dwars (invaded) can be classified as Mountain Headwater Stream and Mountain Stream respectively.

At the Dwars River Upper site wet bank sampling plots could not be established, mostly due to the narrowness of the riparian zone and the rocky nature of the riverbed and extremely shallow soils. There was evidence of some light invasion by *A. mearnsii and A. longifolia* in the past, however, this was deemed sufficiently close to a pristine condition to establish a natural site (Figure 3.4A). The invaded site was established approximately 1 km downstream, on the southern side of the river, on land belonging to CapeNature. On the northern side of the river, IAPs have been cleared by the private landowner in 2009, however the southern site contains thick stands of *A. longifolia* and *A. mearnsii* (Figure 3.4B). A fire swept through the valley in March 2009, but was confined to the upper part of the catchment, and the higher-lying areas; the fire affected the Dwars River Upper sites, but not the Dwars River Lower site.

Figure 3.4: The Dwars River sites. (A) The Dwars River Upper site (natural), (B) the Dwars River Lower site (invaded with a mixture of *A. mearnsii* and *A. longifolia*, and (C) riverbank erosion at the invaded site.

3.3.3 The Wit River system and site

The Wit River is the main river flowing through the Bainskloof Pass (the Haweques mountain range) east of the town of Wellington, and is a tributary of the Breede River. The Wit River is flanked by the Slanghoek Mountains and the Obiekwa Mountains (Brown et al., 2004; Reinecke et al., 2007; Vosse, 2007) and has an estimated length of 11-12 km. The study site was located in the Transitional section of the river where the channel has widened to 5-10 m). According to Brown et al. (2004) the geology of the Wit River catchment consists of mainly sandstone of the Peninsula formation (part of the Table Mountain Group Sandstones), and it supports the Hawequas Sandstone Fynbos vegetation type (Rebelo et al., 2006). The Wit River site is located on the southern side of the river, on private land where some efforts have been made by the landowner to clear areas along the river, however, no recent clearing has taken place where our site is located.

At the site, both the wet and dry bank lateral zone is heavily invaded by closed-stands of *A. mearnsii* trees (Figure 3.5), and only a few scattered individuals of *A. longifolia* and also *B. stellatifolium* were evident underneath the *A. mearnsii* canopies. The wet bank nearest the water's edge support a narrow band of palmiet (*P. serratum*) and also includes some *B. stellatifolium*, *M. angustifolia* and *Morella serrata*. On the northern bank invasive alien species, including *A. mearnsii* were cleared some time ago (Reinecke et al., 2007). In its natural state, the species assemblages within the riparian zone of the Wit River would have contained both characteristic riparian genera that do not have a terrestrial affinity, as well as riparian scrub species with a fynbos affiliation (Pretorius et al., 2008). According to Campbell (1985) the Wit River riparian community included common species: *M. angustifolia*, *B. stellatifolium*, *B. neriifolia*, *Erica caffra* and *Elegia capensis* (see Figure 3.5). This riparian vegetation community is common to the western and southern interior of the Cape Fold Belt Mountains (Blanchard, 2007).

Figure 3.5: **The Wit River site, which is highly invaded by *A. mearnsii*, but with some native herbaceous species and trees such as M. angustifolia evident.**

3.3.4 Sir Lowry's Pass River system and site

The Sir Lowry's Pass River site is located to the south of Stellenbosch, on the Sir Lowry's Pass River, which arises near Somerset West in the Hottentots Holland Mountain range and flows in a south-easterly direction, with its estuary on False Bay. The site is located on the Wedderwille Estate, which is currently grazed by game under low stocking density. The geology of the area consists of Quaternary sediments with granite of the Stellenbosch Batholith occurring upstream, and the river follows a faultline between shale and granite terraces. According to Rebelo et al. (2006) the vegetation type of the area is Boland Granite fynbos.

The site is the most disturbed compared to any of the other cleared sites, with large erosional scars, as well as the burn scars which remained after the burning of slash piles (Figure 3.6). Closed-stand invasion consisting of 30 year old stands that had supported mixed invasions of *A. mearnsii, A. saligna, A. longifolia* and *Pinus pinaster,* was clearfelled in 2000 and 2002 and the alien debris was burnt in stacks (Reinecke et al., 2007) on higher ground away from riparian zone. These scars in the landscape are still evident (Figure 3.6C), an indication of high temperatures during burning.

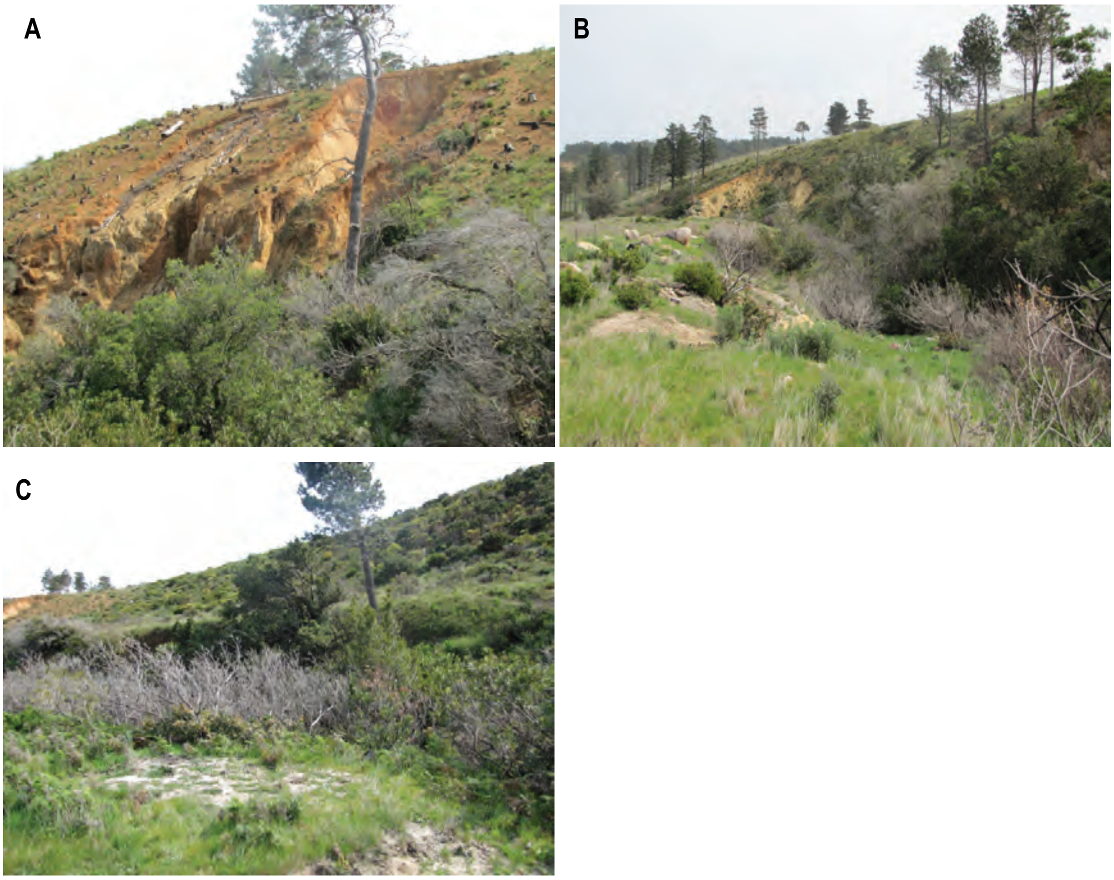

Figure 3.6: Sir Lowry's Pass River site (cleared). (A) river bank erosion; (B) riparian zones supporting high grass cover and (C) scars present in terrestrial zones caused by slash that was burned in piles.

The areas inside scars were generally avoided during sampling and measurement. Cover generally remained low on burned patches; therefore our sampling plots were carefully selected in terrestrial upland zones so as to avoid these highly disturbed areas. Visual inspection revealed also that the study site contains high grass cover in both riparian and terrestrial areas (Figure 3.5B), the latter a remnant of prior landuse (cultivation). This site is relative free of alien invasive trees, and follow-up clearance is carried out on a regular basis by the landowner.

3.3.5 Jakkals River system and sites

The Jakkals River is a tributary of the Bot River, and is the main river flowing through the Houwhoek Pass. The region has a complex geology and consists of the Skurweberg, Goudini, Cederberg, and Rietvlei formations. The vegetation type is mostly Kogelberg Sandstone Fynbos (Rebelo et al., 2006).

Two sites were located along the Jakkals River, one invaded and one cleared (Figure 3.7). Both invaded and cleared sites are characterized as Transitional longitudinal zones. The cleared site was first cleared in 1996 and 1997 and occurs upstream of the invaded site. Alien species on the now cleared site consisted of several *Acacia* species, although *A. mearnsii* was the dominant species, and still is the dominant species in the remaining non-cleared riparian area below the cleared site (Figure 3.7C). Alien trees were felled and burned and two follow-up treatments were recorded yearly after the initial clearing (Blanchard, 2007). The terrestrial sampling plots are situated along the mountain slope and the soils are rocky and fairly shallow. At the cleared site, a fire swept through the riparian and terrestrial zones during January 2010. Localized erosion with exposed riverbanks was noted at this site (Figure 3.7A, B). Subsequent to this fire, only a few partially burned riparian shrubs were observed in the riparian zone with a number of emerging seedlings of *A. longifolia* and *A. mearnsii*.

The invaded site is located further downstream and is heavily invaded by mature stands of *A. mearnsii* trees and a few individuals of *Eucalyptus* species. No sign of a recent fire was evident within the riparian or terrestrial areas at the invaded sites. As the river is somewhat incised, the terrestrial sampling plots were located some 10 m above the riparian sites.

Figure 3.7: The Jakkals River cleared site with (A) exposed riverbanks and (B) localized erosion (indicated by the arrow) and emerging seedlings of *A. mearnsii* and *A. longifolia*. The invaded site is shown in (C) and contains mostly *A. mearnsii*.

3.3.6 Molenaars River system and sites

The Molenaars River originates in the Klein Drakenstein Mountains in the Du Toit's Kloof Pass and runs through the town of Rawsonville before joining the larger Breede river. The Molenaars River site is surrounded by high peaks where several tributaries of the Molenaars River originate; one of these tributaries, the Elands River was included as an additional natural site, though due to difficulty for access for equipment, measurement of soil processes were not carried out at this site. The river drains the northern slopes of the Du Toits Kloof Mountains, the north-eastern part of the Klein Drakenstein Mountains, and the south-eastern part of the Slanghoek Mountains (Brown et al., 2004). The main river channel is somewhat braided in certain areas forming islands close to either side of the river. The geology consists of Peninsula and Wellington pluton granite outcrops and recent Quaternary deposits. Further downstream the alluvial deposits make up most of the riverbanks (Rebelo et al., 2006).

The major vegetation types within the catchment valley are Hawequas Sandstone Fynbos with Breede Alluvium Fynbos (Rebelo et al., 2006).

Figure 3.8: (A) The Molenaars River cleared site, (B) the invaded site (mostly *A. mearnsii*) and (C) The Elands River site (natural).

Two sites are located on the Molenaars River, one cleared (Molenaars River Upper; Figure 3.8) and one invaded (Molenaars River Lower). Based on the river gradient, the two sites are classified as Mountain Stream and Transitional longitudinal zones, respectively. The invaded site was cleared in an unscheduled clearing in 2011, however, some sampling and measurements were still carried out subsequent to this event. The Molenaars cleared site occurs approximately two kilometres upstream from the invaded site, and has been used before by Blanchard (2007), Reinecke et al. (2007) and Vosse (2007). Records indicate that *A. mearnsii* was the dominant alien species targeted in the riparian areas. For the initial clearing treatment, alien trees were felled and slash was removed from the riparian zone in 2002 and 2003. Blanchard (2007) recorded that other alien species were also present, namely: *A. longifolia*, *A. saligna* and *Rubus* species. Woody riparian trees, *B. stellatifolium* and *M. angustifolia*, were prominent along the riparian corridor during the sampling period (Figure 3.8A). Above the wet bank, the gradient flattens and the substrate is soft sand overlaying cobbles and boulders. A fire occurred at the site in March 2012.

The Lower Molenaars site was moderately to highly invaded with *A. mearnsii* (Figure 3.7B). Here the river is also braided in sections and erosion has scoured the river channel, which was particularly evident between the wet- and dry bank lateral zones. Scattered shrubs of *B. stellatifolium* and *M. angustifolia* occurred in the upper wet and dry bank zones, and palmiet (*P. serratum*) was prominent along the active channel. The invaded site was cleared during February 2011, where *A. mearnsii* stands were felled and some of the slash removed. The riparian zone and adjacent terrestrial fynbos burned during March 2011, however, most of the soil sampling was already done at this site.

A natural site is located on the Elands River southwest of the two Molenaars sites (Figure 3.7C). The Elands River is a tributary of the Molenaars River system (Reinecke et al., 2007) and it flows in a north-westerly direction where it joins the Molenaars River close to the Huguenot Tunnel. The site is characterized by steep mountain slopes and flanks of intermontane valleys. Riparian woody shrubs and trees are relatively sparse compared to the Eerste and Dwars River natural sites (Figure 8C). Vegetation is classified as Hawequas Sandstone Fynbos and has dominant restioid, asteraceous and ericoid fynbos components (Rebelo et al., 2006). Characteristic riparian scrub species observed included *B. stellatifolium, B. neriifolia, M. angustifolia, Isolepis prolifer, P. serrata* and *Todea barbara.* Shrubs, ferns and graminoids that are common in the dry bank zone included *Elegia capensis, Blechnum capense, Pteridium aquilinum* and *Askidiosperma chartaceum*. The geology of the area consists of acidic lithosol soils derived from Ordovician sandstones of the Table Mountain Group. The site is relatively pristine and undisturbed by human impacts, except for a hiking trail that separates the terrestrial from the riparian sampling plots. The terrestrial section of the site burned during March 2011, but there was no evidence of recent fire before or during sampling and vegetation surveys.

A short summary of the sites used in the southern and eastern Cape is given in Table 3.2. The criteria for selecting the sites were similar to that of the main sites (described in detail in previous sections). However, one exception was allowed, and that was for the natural site in the eastern Cape, the Baviaans River site. This site was restored by WfW more than 7 years prior, using plantings of native species. This site was selected as no other natural sites fitted the criteria in terms of level of invasion by *A. mearnsii* and other *Acacia* species.

Table 3.2: Site information for each of the sites in the southern and eastern Cape which formed part of a secondary set of sites. Information was gathered from landowners and restoration ecologists active at these sites.

Sites	Coordinates	Invasion status	Vegetation
Baviaans River	33.8117 S; 24.4305 E	Restored- active restoration	Fynbos
Voeght's River Upper	33.7749 S; 23.1561 E	Natural	Fynbos,
Voeght's River Lower	33.7749 S; 23.1561 E	Natural	Fynbos
Witteklip River Upper	33.8297 S; 24.4489 E	Invaded	*A. mearnsii*,
Kammanassie River	33.7049 S; 23.1604 E	Invaded	*A. mearnsii/A. dealbata*

Sites	**Coordinates**	**Invasion status**	**Vegetation**
Groot River	33.7232 S; 23.3737 E	Invaded	*A. mearnsii*
Witteklip River Lower	33.8285 S; 24.4450 E	Cleared	Cleared of *A. mearnsii*
De Hoop River	33.7010 S; 23.1564 E	Cleared	Cleared of *A. mearnsii*, *A. dealbata*
Groot River	33.7232 S; 23.3737 E	Cleared	Cleared of *A. mearnsii*

The Baviaans River and the Witteklip sites are all in the Eastern Cape Province, northeast of the town of Kareedouw, and the rest of the sites near the town of Haarlem in the Langkloof valley of the Western Cape Province. The two natural sites here, Voeght's River Upper and Lower are on the southern slopes of the northern peaks of the Langkloof Mountains at the start of the Prince Alfred's Pass.

4. SOIL PHYSICAL AND CHEMICAL PROPERTIES IN NATURAL, *ACACIA*-INVADED AND CLEARED RIPARIAN ECOTONES IN THE WESTERN CAPE

4.1 INTRODUCTION

Riparian corridors are are linear, dynamic and complex biophysical systems embedded in terrestrial areas, but also an essential element in the landscape that contributes more to overall species and functional diversity than its modest size suggest (Naiman and Décamps, 1997; Hood and Naiman, 2000; Décamps et al., 2004). As riparian systems are intimately linked to the hydrological characteristics of streams they are highly dynamic in the short term, however, over longer timespans and if not altered by anthropogenic factors, they demonstrate substantial stability (Ewel et al., 2001). Biotic properties, hydrological processes and physical and chemical characteristics are expressed differently within riparian corridors compared to the surrounding landscape, and have specific and often idiosyncratic connections with both terrestrial and aquatic environments (Tickner et al., 2001; Décamps et al., 2004).

Little work has been carried out on biophysical properties of fynbos riparian ecotones, and how that relates to vegetation characteristics. However, it has been suggested that that water availability and susceptibility to fires are the main environmental determinants of vegetation structure (Sieben, 2003; Reinecke et al., 2008). However, from a theoretical point of view it is likely that soil physical and chemical properties (such as soil pH and texture) contribute to the establishment of different vegetation communities (Cowling and Holmes, 1992). Soil particle size and bulk density within the riparian corridor has a close connection to riverine dynamics, and in turn may impact on plant establishment and survival through its impact on other soil properties (Naiman et al., 2005). Indeed soil texture influences a variety of soil physical, chemical and biological properties, such as accumulation of organic material, and soil hydrology and the rates of microbiological processes (Pinay et al., 1995; Chapin et al., 2002). Soil pH may also play an important role in microbial diversity, soil-plant interactions, nutrient uptake and soil processing (Naiman et al., 2005; Gutknecht et al., 2006). Alien invasive plant species e.g. *Acacia* species have been found to change sediment dynamics such as deposition mobilization and erosion rates (Tickner et al., 2001), and may affect soil chemical properties directly and indirectly (Gaertner et al., 2011). Riparian stands of invasive alien plants have been shown to have higher rates of evapotranspiration compared to native riparian communities (e.g. Dye et al., 2001; Dye and Jarmain, 2004).

In this part of the study, we compared soil properties (physical and chemical) in fynbos riparian zones with associated non-riparian upland fynbos (comparison of lateral zones) and riparian zones invaded by, and cleared of *Acacia* species (comparison of invasion status). We aimed to quantify soil physical and chemical properties in natural, invaded and cleared riparian ecotones and associated upland fynbos. The research reported here relates to objective 2 (To quantify soil properties, soil N mineralization and denitrification in natural, invaded and cleared riparian ecotones and associated upland fynbos).

4.2 METHODS

For all soil sampling we used a stainless-steel tube-type sampler (5 cm diameter) to collect six soil samples from the upper 10cm (top soil or A horizon) of the soil profile after loose litter was removed. Where soils were too rocky and/or shallow a hand trowel was used. The following parameters were assessed on each sample:

- pH (seasonally)
- gravimetric soil water content (GSWC) (seasonally)
- electrical conductivity (EC) (seasonally)
- soil particle size (twice)
- bulk density (once)

In the laboratory, were sieved samples using a 2 mm sieve to remove roots and large pieces of organic debris. We used a modified version of the rapid method by Kettler et al. (2001) to determine particle size. Sand fractions were divided in 2 classes: medium and fine sand (0.053-0.425 mm) and coarse sand (0.425-2.0 mm). Silt and clay fractions (<0.053 mm) were not separated.

Soil pH was measured electrometrically using a Hanna 211 pH meter after mixing a 1:2 (w/v, soil:deionised water) slurry (Robertson et al., 1999). Electrical conductivity, a measure of dissolved materials in aqueous solution, was determined on 10 g of air-dried soil material. Samples were shaken in 40ml deionized water for approximately 60 min and allowed to stand for 30 min to settle before measurements were taken using a Corning 441 conductivity meter. Bulk density was determined during spring as oven-dry mass per volume (based on volume of the sampling tube).

4.3 RESULTS

No significant seasonal interactions were found for any of the soil particle size distribution classes we measured, viz. course sand, medium to fine sand and silt and clay combined (Table 4.1). We therefore combined the data from the two seasonal sampling dates. Medium to fine sand particles were found to be significantly greater ($p<0.001$) and coarse sand significantly reduced ($p<0.05$) under invaded stands compared to that of natural riparian fynbos respectively. Natural and cleared sites tended towards higher combined silt and clay content, however this was not significant. On the other hand, riparian soils were dominated by medium to fine sand, which was found to contribute more than 60% to topsoils, while course sand was most prominent in the wet banks; this differed significantly from the upland soils ($p<0.05$; Table 4.1). Silt and clay content differed significantly ($p<0.05$) between landscape positions and the lowest mean contents were found in wet banks (3.93%), followed by higher contents in dry bank (7.1%) and upland (13.7%) areas.

Table 4.1: Soil particle size class distribution between invasion statuses and landscape positions. No significant seasonal interactions were observed for any of the particle size distribution classes (both invasion statuses and landscape positions [#]), therefore were combined the data of the two sampling dates and mean ± SE are based on pooled data for autumn and spring. For each class, letters (distributed horizontally) denote significant differences based on a one-way ANOVA with Tukey's post-hoc test ($p<0.05$): invasion status (^{a},^{b},^{c}) and landscape position (^{x},^{y},^{z}).

Particle size distribution classes	**Invasion Status**			**Landscape Position**		
	Natural	**Invaded**	**Cleared**	**Wet bank**	**Dry bank**	**Terrestrial**
Coarse sand (0.425-2.0 mm)	29.88±1.76^{a}	20.09±2.19^{b}	29.78±2.32^{a}	36.00 ±2.09^{x}	29.88±1.76^{y}	28.29±1.56^{y}
Medium to fine sand (0.053-0.425 mm)	63.00±2.00^{b}	69.99±2.81^{a}	63.06±2.72^{ab}	60.07±2.07^{xy}	63.00±2.00^{x}	57.98±1.33^{y}
Silt and clay (<0.053mm)	7.13±0.63^{a}	9.92±1.37^{a}	7.15±1.06^{a}	3.92±0.30^{z}	7.13±0.63^{y}	13.73±0.68^{x}

[#] *Interactions between invasion statuses X season: coarse sand (p=0.905), medium to fine sand (p =0.714) and silt and clay (p =0.127). Interactions among landscape positions X season: coarse sand (p =0.743), medium to fine sand (p =0.791) and silt and clay (p =0.133).*

Gravimetric soil water content (GSWC) varied significantly throughout the year (one-way ANOVA; $p<0.001$; Table 4.2; Figure 4.1A), with a peak in winter (12.49%) and was lowest in summer (5.03%). No significant differences were evident when comparing invasion statuses, and interactions with seasons were insignificant (Table 4.2). Averaged over the year, GSWC showed highly significant differences between landscape positions ($p<0.001$; Table 4.2). Soil moisture averaged around 14.9% for wet banks, 8.9% for dry banks and 6.6% for upland areas. Wet bank soils had significantly higher moisture content compared to the other landscape positions across seasons, with exception of winter. Differences in GSWC between dry banks and terrestrial areas were less apparent, and dry banks were only significantly different from upland terrestrial zones during winter ($p<0.01$).

Table 4.2: F-values for selected physical and chemical properties. Three different ANOVAs were computed as indicated by the different shades of grey. Invasion status and invasion status X season (and the same for landscape position) indicate significant differences for main and interaction effects based on ANOVAs. Significance levels are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001). Overall differences between seasons were tested with a one-way ANOVA, based on all data, irrespective of invasion status and landscape position.

ANOVA	df	GSWC	pH	EC
Landscape position	2	32.82***	07.29***	01.10
Landscape position X Season	6	01.62	02.24*	00.64
Invasion status	2	01.89	01.98	20.31***
Invasion status X Season	6	00.22	03.07**	02.23*
Season (all data)$^{\#}$	3	35.42***	50.98***	13.23***

Electrical conductivity (EC) of soils, a measure of dissolved material in aqueous solutions, differed significantly between seasons (p<0.001; Table 4.2) with highest mean values observed during summer (46.98 µs/cm; Figure 4.1B) and the lowest during winter (14.55 µs/cm). Electrical conductivity showed significant interactions between invasion statuses and season (p<0.05). The highest mean concentrations of dissolved materials were observed in invaded sites (51.05 µs/cm), followed by the cleared sites (40.57 µs/cm) and natural sites (21.45 µs/cm), and the invaded sites had consistently and significantly higher EC compared to the natural sites, with the cleared sites falling in between. No differences were observed between landscape positions; however wet bank zones had the lowest mean EC values, at 19.63 µs/cm.

Soil bulk densities differed significantly between invasion status (p<0.05; Figure 4.1C) and landscape positions ($F_{2, 42}$ =5.23, p<0.01; results not shown). Soils sampled from underneath *Acacia* stands had significantly lower bulk densities (0.93g/cm^3) compared to natural and cleared (p<0.05) sites. However, no differences between natural and cleared sites were not significant (p=0.88). Wet banks (1.24 g/cm^3) had significantly higher bulk densities (i.e. heavier soils) compared to dry banks (1.11 g/cm^3; p<0.05) and upland (1.06 g/cm^3; p<0.01) areas.

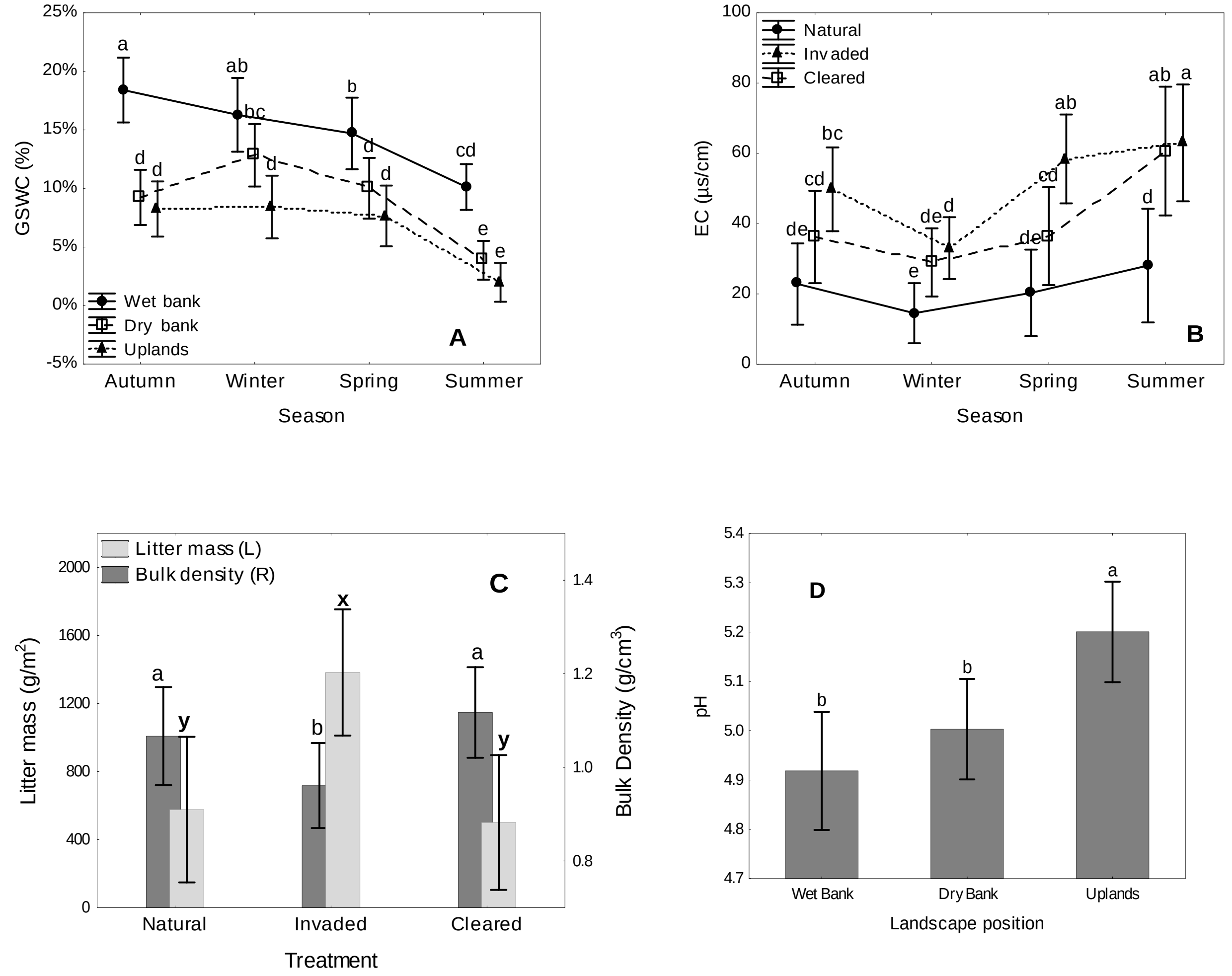

Figure 4.1: Soil physical, chemical and biological (litter mass) properties. (A) Gravimetric soil water content (GSWC) for landscape positions across seasons; (B) Electric Conductivity (EC) for invasion statuses across seasons; (C) litter dry mass and bulk density for invasion statuses; and (D) soil pH (water) for landscape positions. Point symbols (Figure 4.1 A, B) and bars (Figure 4.1 C, D) indicate means and whiskers indicate ± 95% confidence intervals. (Figure 4.1A) Landscape positions X seasons (p =0.147) and (Figure 4.1B) invasion status X season (p =0.049) indicate significant differences (Tukey tests; p<0.05) for interaction effects based on repeated measures ANOVAs. Different letters represent significant differences (Tukey tests, p<0.05) based on one-way ANOVAs: Figure 4.1C [litter mass (^{x},^{y}) and bulk density (^{a},^{b})] and Figure 4.1D [soil pH (^{a},^{b})]. Statistics for litter mass was computed on log transformed data to meet normality assumptions.

Soil pH was acidic (Figure 4.1D), and mean pH in water varied from 4.92 (wet banks) to 5.2 (uplands); wet bank and dry bank soils were significantly more acidic than upland soils. Soil pH decreased significantly as the dry season progressed (one-way ANOVA: p<0.001). Soils were significantly more acidic in spring (4.90) and summer (4.82) compared to autumn (5.29) and winter (5.25). Soil pH showed no statistical significances between invasion statuses (Table 4.2) however, invaded sites trended towards lower soil acidity compared to natural sites. We observed a significant interaction between seasons and landscape ($p<0.05$; Table 4.2).

4.4 DISCUSSION

Soil texture tended to be very sandy, a reflection of the products of weathering of Table Mountain Sandstone, the dominant lithology observed across the sites sampled, although hydraulic factors and geomorphic characteristics of the river also have an important influence on sediment sorting (Heydorn and Grindley, 1982; Pinay et al., 1995). Overall soils with higher proportion of finer material were found on higher topographic positions in the landscape (upland areas), reflecting the influence of the stream on mobilization of clay from soils. Silt and clay contents progressively increased away from the river channel, while coarsely textured materials (0.425-2.0 mm) declined. Wet banks had low concentrations (<4%) of fine materials, and dry banks had soils with values intermediate between wet bank and upland lateral zones. Several factors interact to determine these trends. Vegetation decreases water velocity further away from the active channel, and dry banks are also less prone to stream influences, hence lighter and finer material is deposited here, whereas the heavier, coarser sand fractions are found closer to the channel (Adair et al., 2004; Brown et al., 2004). Though not directly comparable, Manders (1990) also showed that Afromontane forest patches associated with streams in the fynbos biome tended to have higher fine material compared to fynbos. Being inundated for most of the year, wet banks experience constant erosion and net loss of clay and silt in these lateral zones, whereas terrestrial areas experience less severe hydraulic disturbance from water movement, thus is able to retain more fine material compared to riparian areas. Fluvial dynamics is therefore the most important factor that determines the differences observed between landscape positions with regards to soil textural classes.

Fine-textured soils are associated with higher OM levels and larger microbial biomass, which may allow faster nutrient cycling and greater nutrient retention (Austin et al., 2004). On can thus expect that differences in soil particle size distribution within riparian zones and compared to uplands may also give rise to different patterns of resource distribution and cycling, such as for N (Bechtold and Naiman, 2006). Low silt and clay content and hydraulic disturbance in wet bank soils, may explain why soils closely associated with the river channel have a lower capacity to store nutrients (soil available P and N; total N and C, see subsequent chapters). Consistent with Bechtold and Naiman (2006) available inorganic N, total N, total C, and EC were significantly positively correlated to soil silt and clay contents in our study. Thus, the variation in silt and clay contents with landscape position is a significant control on ecosystem processes and properties, and impacts of alien invasive species and other disturbances may have consequences for riparian functioning. As such, soil texture, soil moisture content and bulk density can be a particularly good predictor of many ecosystem properties (Chapin et al., 2002), and our study confirms this applies also to fynbos riparian ecotones.

Indeed, soils under *Acacia*-invaded stands had significantly lower contents of coarse sand fractions and higher contents of silt and clay, compared to soil under natural vegetation. This is consistent with the findings of DeCant (2008) who found finer textured soils under cottonwood trees in riparian zones with subcanopy N_2-fixer, *Elaeagnus angustifolia,* compared to native cottonwood trees alone. It has been suggested that this may be due to altered stem density, as well as modified litter dynamics. However, Rice et al. (2004) did not find any differences in particle size distribution between native pine oak ecosystems and N_2-fixing *Robinia pseudoacacia* (black locust) stands. When seen together, this may suggest that these differences are context specific and may depend on local factors such as geomorphology, longitudinal zone involved and soil type. A catchment effect may be at work, as Brown et al. (2004) found that soils under invaded and natural vegetation differ significantly between rivers in different catchments with regard to the direction of differences in soil texture and pH, if at all. However, increased fine grains associated with invasion of *Acacia* species into riparian environments can have major implications for nutrient cycling as clay and silt show a positive correlation with cycling of N and other nutrients (Pinay et al., 1992). Therefore, the mechanism and context under which such changes occurs deserves to be investigated further.

In summer GSWC tend to be low, which may lead to increased concentrations of minerals in the soil (Gasith and Resh, 1999) and to higher EC values observed in summer. Both soil moisture and silt and clay contents (which showed a significant negative and positive correlation with EC respectively) strongly affect the concentration of dissolved materials in the soil (Chapin et al., 2002). Similarly, biophysical factors, such as soil moisture, OM distribution and acid and base-forming ions in soils interact (Chapin et al., 2002) to influence seasonal trends in soil pH and therefore possibly explain the decline in pH observed in spring and summer. Similar to our results, Cramer (2010) also found soil pH was significantly lower in fynbos riparian zones (both wet- and dry banks) compared to upland terrestrial fynbos.

Changes in soil pH with invasion have been reported in several other ecosystems, and also in the fynbos. Soil chemistry under *Acacia* species differed from those under indigenous species in that they had significantly higher concentrations of dissolved minerals in soil solution. However, we could not find any significant differences between sites with different invasion statuses. Though not in riparian environments, both Marchante et al. (2008) and Malcolm et al. (2008) failed to show significant differences in soil pH in sites invaded by *A. longifolia* (>20 years) and *Robinia pseudoacacia* respectively, compared to non-invaded sites. Witkowski (1991) also did not show significant differences between areas invaded by *A. saligna* and *A. longifolia* in sand fynbos and strandveld ecosystems respectively, compared to native sites. However, Caldwell (2006) found higher soil pH with invasion by *Cytisus scoparius*, a perennial herbaceous legume.

5. PLANT FUNCTIONAL TYPES (PFTS) AND OTHER ECOSYSTEM COMPONENTS

5.1 INTRODUCTION

The proliferation of invasive species is increasing worldwide, and this contributes significantly to global environmental change. Invasive species are regarded as one of the major threats to the earth's biodiversity (Scherer-Lorenzen et al., 2007; Liao et al., 2008); invasive species are also considered one of the most serious threats to structure and function of riparian ecosystems (Hood and Naiman, 2000; Esler et al., 2008). Due to its position in the catchment, its relatively open structure and its particular disturbance regime, riparian ecosystems have been invaded disproportionately more than other ecosystems worldwide (Hood and Naiman, 2000). This has fundamentally altered both riparian structure, as well as its function, thus affecting ecosystem services associated with riparian ecotones (Naiman and Decamps, 1997). The impact of plant invasions on riparian ecosystems are such that ecosystem stability has been affected (Richardson et al., 2007; Liao et al., 2008; Gaertner et al., 2009; Marchante et al., 2010; Hellmann et al., 2011). This is especially the case in Mediterranean ecosystems where woody alien species has heavily affected fire regimes and intensity of fires (van Wilgen et al., 2011). Biodiversity is also heavily affected, especially in Mediterranean regions, which are all classified as hotspots of biodiversity (Myers et al., 2000), with endemic and specialized plant communities (Hellmann et al., 2011). Several local studies have investigated the role of woody IAPs in changing riparian biodiversity, however, less attention has been paid to functional components when riparian zones are invaded, and ecosystem changes in restoration trajectories when invasive acacias are cleared.

Clearing stands of exotic plants is a major restoration activity in order to repair ecosystem function to rivers (Galatowitch and Richardson, 2005). Restoration efforts have been conducted throughout the fynbos biome, however, with mixed results (Holmes and Cowling, 1997; Yelenik et al., 2004; Galatowitch and Richardson, 2005; Holmes et al., 2008; Reinecke et al., 2008). In some cases, especially where stands of woody invasive alien species are very dense, recovery have been found to be slower compared to those cleared of less dense infestations (Richardson et al., 1989; Holmes and Cowling, 1997). The clearing method matters – where the fell and remove method is applied (even in some dense stands), natural vegetation returns to dry banks in most cases (Blanchard and Holmes, 2008). Also, where riparian vegetation have been invaded for several decades the ecosystem may not rapidly recover following alien clearing operations and inadequate restoration may over time result in profound changes to river channel morphology. Furthermore, in riparian zones cleared of IAPs, soil erosion, poor water quality, depletion of the seedbank, re-invasion by the same species of suite of alien plant species, or secondary invasion by other nitrophilous species may pose serious threats to recovery of native riparian plant assemblages over the long term (Holmes, 2001; Boucher, 2002; Yelenik et al., 2004; Galatowitch and Richardson, 2005). As such, plant functional types (PFTs) in different stages of invasion and clearing can give some information on the existence of biogeochemical legacies following clearing, and on whether structure and function recovers, however, this has not been explored in any great detail. In this part of the project we used plant functional types to compare natural, invaded and cleared riparian zones, and we also investigated plant diversity and abundance by focusing on specific groups such as grasses. The work carried out relates to objective 4.

5.2 METHODS

While plant density is a sensitive measure to monitor change in plant communities, ecosystem function is most likely driven by changes in vegetation cover, and it can be used as a surrogate measure for biomass (Holmes et al., 2000). Major efforts are underway to classify plant species into different functional groups as it has been recognized that changes in functional groups rather than species assemblages can give more pertinent information on how ecosystem function may be altered by anthropogenic disturbances such as IAPs. Thus classifying species with similar life history or physiological traits into functional groups and the associated changes in plant cover for a particular growth form may be a good indicator of ecosystem function (Holmes et al., 2000; Yelenik et al., 2004), especially in our study where we investigated changes amongst invasion statuses and between landscape positions. Vegetation structure also provides a measure of community recovery following clearing and restoration (Reinecke et al., 2008).

Table 5.1: Growth forms classes and other ecosystem components used in this study, with their respective descriptions. Growth form descriptions (1-5) follow Goldblatt and Manning (2000).

Growth form classes	**Description**
1. Forb	A broad-leaved herbaceous plant other than graminoids.
2. Grasses (graminoid)	Plants in the family Poaceae.
3. Other Graminoids	Plants in the family Juncaceae, Cyperaceae, and Restionaceae.
4. Shrub (<2m)	A low or medium sized woody perennial plant often with multiple stems.
5. Adult shrub/tree (>2 m)	A large woody perennial plant usually with multiple stems or with a main trunk.
6. Tree seedling (<1 m)	Seedlings of the above mentioned growth form class 5
7. Tree juvenile (1-2 m)	Juveniles of the above mentioned growth form class 5
8. Ferns	Both woody and herbaceous
9. Moss	Both on hard soil surfaces or rocks/stones.
Additional classes	**Description (where applicable)**
10. Stones and/or rocks	Either loose or fixed
11. Bare ground	Surface area not covered by any other class
12. Litter	Twigs, leaves, seeds, and small branches
13. Dead standing	Any standing senesced vegetation
14. Tree basal cover	GC of the base of juvenile and adult indigenous and *Acacia* shrubs and trees

The PFT approach that we used embraces broad morphological life-form characteristics (i.e. growth form types; Goldblatt and Manning, 2000). In order to determine differences between invasion statuses (natural, invaded, and cleared) and landscape positions (wet, dry bank, and uplands), we measured the cover of a number of growth form types and other ecosystem attributes (Table 5.1). The four broad growth form classes we used were forbs (herbaceous dicotyledonous plants), graminoids, shrubs and trees, and plants were assigned to growth forms based on morphology and maximum height reached, as described by Goldblatt and Manning (2000). The narrow growth form classes comprised forbs, graminoids (restioids, sedges, rushes and grasses), shrubs and trees (Goldblatt and Manning, 2000; Table 5.1). Trees (native and invasive) were divided into 3 height classes: seedlings (<1m); juveniles (1-2 m) and adults (>2 m). Ferns were assigned to their own class, since they are very common in riparian as well as terrestrial communities (Reinecke et al., 2007). Restioids, sedges and rushes were separately recorded from grasses, since secondary invasion by weedy grass species has been reported after clearing operations.

For phytosociological studies in fynbos vegetation, 10 X 5 m sized survey plots are generally used, however, these dimensions are often not possible in narrow linear bands of riparian vegetation, especially the wet bank in mountain and upper foothill segments of rivers (Reinecke et al., 2007). Thus, for our study, vegetation survey plots measuring 10 x 2 m (similar to plots used for soil sample collection) were set up in the riparian (wet- and dry bank) and terrestrial zone with the long edge parallel to the river. We recorded canopy cover as it is easily measured, and provides the researcher with information of relative dominance in addition to the influence of plants on soil temperature and rainfall interception (Hanley, 1978). Both Ground Cover (GC) and Canopy Cover (CC) were recorded in this study. Canopy cover generally involves a visual estimation of the percentage cover of ground included (i.e. area of influence) in the natural spread of foliage of the growth form (modified from the usual measure by species; Hanley, 1978). The measure of GC used in this study is the estimated percentage cover for a number of classes (litter, rocks and/or stones, bare ground, smaller plants, and moss) that cover the soil surface. Within each of the plots, a wooden frame (20 X 50 cm; 0.1 m^2) was placed at six fixed intervals (every 1.5 meters) along the tape measure and the cover of classes present within or overhanging the frame was recorded, in a manner similar to the study by Daubenmire (1959). Percentage CC (>2 m) of larger shrubs and trees were estimated from a photo taken at a fixed distance from the ground at an angle of 90°, using the timer on the camera. For any given area, total ground- and/or canopy cover classes almost always exceeds 100%, due to vegetation overlap, except for areas that have a very simple structure (i.e. only GC).

In spring 2012 we also recorded the species, cover and abundance of individual species in 2.5 x 1 m survey plots positioned over the centre of each sampling plot. This was done to understand trends in individual species per landscape position and per invasion status, especially regarding changes in herbaceous species.

5.3 RESULTS

Visual assessment of cover types translated into significant differences in the cover of selected plant growth form types and other ecosystem attributes between sites with different invasion statuses and also between landscape positions (Table 5.2). Natural sites were structurally the most complex, with a good combination of cover of different plant growth form types. In natural sites the four prominent functional types, which also differed significantly from both invaded and cleared sites, were: trees/shrubs (>2m); grasses; graminoids (excluding grasses); and shrubs. Compared to both natural ($p<0.001$) and cleared ($p<0.001$) sites, litter cover was significantly higher in invaded areas. Invaded sites had the lowest ground cover of other ecosystem components (Table 5.2); the canopy cover of grasses, shrubs, bare ground, rocks/stones, forbs, ferns, and graminoids under the canopy of invaded sites were significantly different from natural sites (Table 5.2). Invaded riparian dry bank zones were structurally and compositionally the least complex, as litter- and *Acacia* trees (<2m) cover dominated the understorey (74.7%; Table 5.2) and *Acacia* trees (>2m) formed the greater part of the overstorey (61.3%; Figure 5.2), respectively. Cleared sites were more open, with more bare ground (39.6%) while cover of grasses was highest here (36.52%). A visual assessment showed that mostly alien grass species, such as *B. maxima* and *P. clandestinum* were detected at the cleared sites.

The cover of PFTs and other components also differed according to landscape position (Table 5.2) with significant differences recorded for grasses, moss, litter, graminoids, shrubs and forbs. The cover of grasses was 8.3%, for graminoids 18.7% and for litter 23.6%, and were higher in dry banks compared to wet bank and upland zones. Moss cover was 8.08% in the wet banks, and was significantly lower in dry bank and terrestrial zones ($p<0.001$), while shrubs were more prominent in uplands areas (18.2%; $p<0.01$).

Table 5.2: Percentage canopy- and ground cover for plant functional types and other ecosystem attributes. The data is means ± SE based on percentage cover for all classes. For each class, letters (distributed horizontally) denote significant differences based on a Kruskal–Wallis multiple comparisons test ($p<0.05$): invasion status (^{a},^{b},^{c}) and landscape position (^{x},^{y},^{z}). Invasion statuses are based on dry bank data and landscape position on natural site data only. GC: ground cover; and CC: canopy cover.

Cover classes	INVASION STATUS			LANDSCAPE POSITION		
	Natural	Invaded	Cleared	Wet Bank	Dry Bank	Uplands
Litter (GC)	23.58±2.62 ^{b}	74.75±5.49 ^{a}	7.29±1.43 ^{c}	8.24±2.2 ^{y}	23.58±2.6 ^{x}	18.21±2.5 ^{x}
Bare ground (GC)	27.92±2.81 ^{a}	20.11±5.24 ^{a}	39.61±8.08 ^{a}	37.63±4.7 ^{x}	27.92±2.8 ^{x}	39.66±4.2 ^{x}
Rocks/stones (GC)	9.11±1.74 ^{a}	1.52±1.07 ^{b}	4.15±2.42 ^{b}	22.31±5.4 ^{x}	9.10±1.7 ^{x}	14.28±2.7 ^{x}
Moss (GC)	0.71±0.26 ^{a}	0.05±0.04 ^{a}	0.24±0.24 ^{a}	8.08±1.6 ^{x}	0.71±0.3 ^{y}	0.59±0.3 ^{y}
Grasses (CC)	14.70±2.77 ^{b}	0.41±0.37 ^{c}	36.52±8.07 ^{a}	4.68±1.5 ^{y}	14.70±2.8 ^{x}	8.28±1.9 ^{xy}
Graminoids	18.65±2.83 ^{a}	1.39±0.80 ^{b}	3.03±1.35 ^{b}	8.51±2.2 ^{y}	18.65±2.8 ^{x}	8.73±2.3 ^{y}

Geophytes (CC)	0.71±0.26 ^{a}	0.00±0.00 ^{a}	0.04±0.04 ^{a}	1.40±0.4 ^{x}	0.71±0.3 ^{x}	2.33±0.7 ^{x}
Shrubs (CC)	9.25±1.74 ^{a}	0.62±0.30 ^{b}	1.87±0.77 ^{b}	2.89±1.3 ^{y}	9.25±1.7 ^{xy}	18.18±2.9 ^{x}
Forbs (CC)	3.74±1.10 ^{a}	0.01±0.01 ^{b}	1.77±0.44 ^{a}	1.56±1.0 ^{y}	3.74±1.1 ^{xy}	4.71±1.1 ^{x}
Ferns (CC)	7.25±1.44 ^{a}	0.23±0.15 ^{b}	0.57±0.31 ^{b}	13.86±4.4 ^{x}	7.25±1.4 ^{x}	9.59±3.5 ^{x}

Adult and seedling cover was appreciably higher in wet banks compared to dry bank zones in natural sites, and we did not record any *Acacia* individuals (Figure 5.1). In comparison, the mean cover of *Acacia* individuals was highest in the dry banks of the invaded sites, (61.3%) compared to the wet bank (35.7%). In contrast, we found that both seedlings and adults of native species were largely absent from dry and wet banks of invaded sites (Figure 5.1). Clearing did not lead to a recovery of native species as cover remained lower (17.7%) in wet banks compared to natural sites where 53.9% canopy cover was recorded.

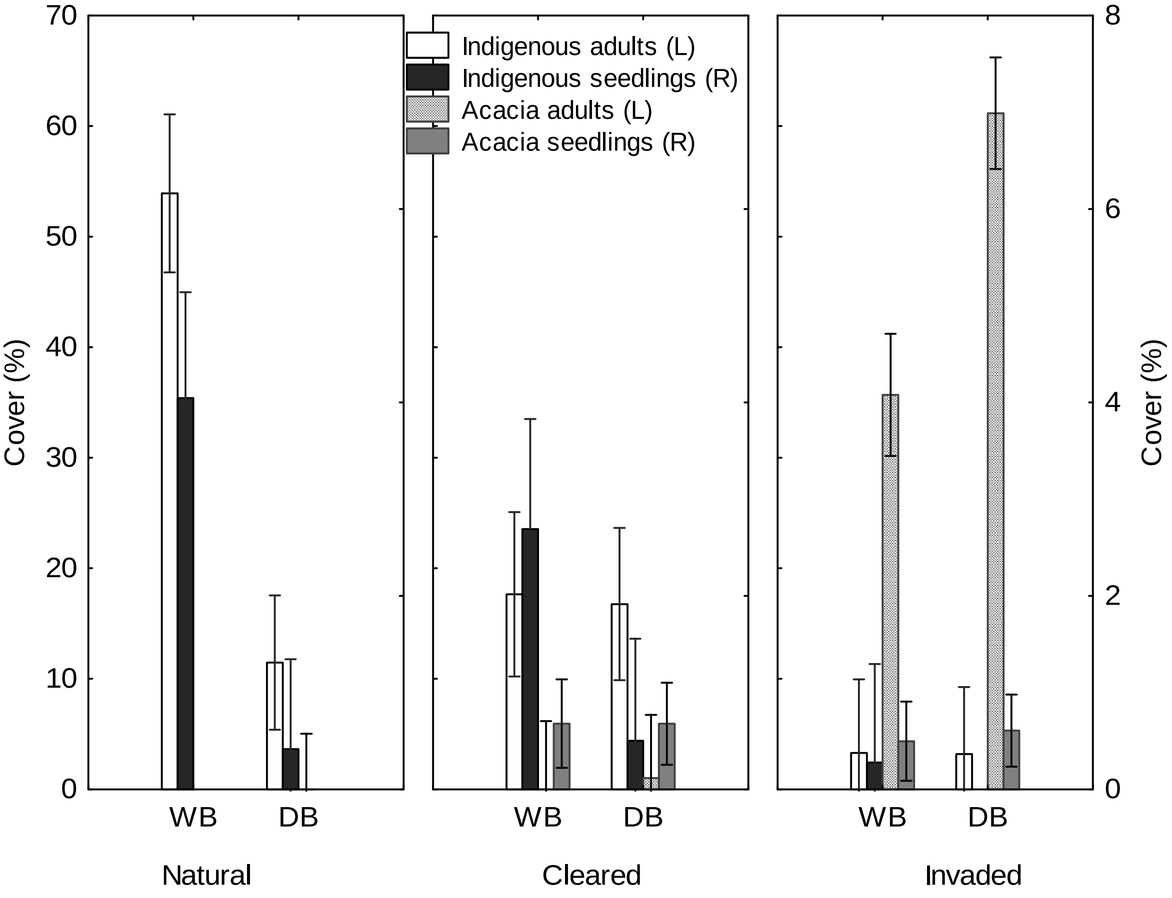

Figure 5.1: Canopy cover (%) of indigenous- versus invasive *Acacia* adult (>2m) trees/shrubs and seedlings (<1m) between invasion statuses (natural, cleared, invaded) and landscape positions (wet- and dry banks). Bars indicate means ± SE. Only data for dry and wet bank are shown since *Acacia* species were absent in

upland terrestrial survey plots. Wherever data are not shown, cover is 0%. WB: wet bank; DB: dry bank, L: Left Y-axis; and R: Right Y-axis.

We found more different grass species present in the dry bank, wet banks and upland areas of cleared sites than either the natural or invaded sites, and the abundance of grass individuals was also much higher compared to the cleared sites (Table 5.3). This is consistent with earlier visual assessment where we found that species such as *B. maxima, Ehrharta calycina* and *P. clandestinum* are present in great numbers in certain riparian and upland environments.

Table 5.3: Grass species recorded and the number of individuals per species for nine sites where sampling and field measurements were carried out. (A) Wet bank, (B) Dry bank, and (C) Upland area

A

Grass species	**Upper Eerste River (Natural)**	**Lower Eerste River (Natural)**	**Dwars River Upper (Natural)**	**Wit River (Invaded)**	**Lower Jakkals River (Invaded)**	**Lower Dwars River (Invaded)**	**Upper Molenaars River (Cleared)**	**Sir Lowry's Pass River (Cleared)**	**Upper Jakkals River (Cleared)**
Pennisetum clandestinum					6			4	
Pentaschistis patula					1				
Ehrharta calycina								3	
Lolium perenne								3	
Sorghum bicolor					2			26	
Dactylus glomerata								1	

B

Grass species	**Upper Eerste River (Natural)**	**Lower Eerste River (Natural)**	**Dwars River Upper (Natural)**	**Wit River (Invaded)**	**Lower Jakkals River (Invaded)**	**Lower Dwars River (Invaded)**	**Upper Molenaars River (Cleared)**	**Sir Lowry's Pass River (Cleared)**	**Upper Jakkals River (Cleared)**
Cymbopogon marginatus								2	1
Pennisetum clandestinum	6						3		
Pentaschistis patula							1	2	2
Unknown grass spp.							1		
Ehrharta calycina					4		1	9	9
Lolium perenne								5	5
Sorghum bicolor								9	8
Briza maxima								22	22
Cynodon dactylon			2						
Pentameris thuarii		3							

C

Grass species	**Upper Eerste River (Natural)**	**Lower Eerste River (Natural)**	**Dwars River Upper (Natural)**	**Wit River (Invaded)**	**Lower Jakkals River (Invaded)**	**Lower Dwars River (Invaded)**	**Upper Molenaars River (Cleared)**	**Sir Lowry's Pass River (Cleared)**	**Upper Jakkals River (Cleared)**
Cymbopogon marginatus		7							
Pentaschistis curvifolia	11	1				14			11
Cynodon dactylon		1						4	
Cymbopogon plurinoides					4	15		16	
Pentaschistis patula									
Stenotaphrum secundatum							26	11	
Ehrharta calycina	6						14	86	6
Ehrharta ramosa	31						15	23	
Lolium perenne					1				
Aristida junciformis								15	
Pentaschistis pallida								3	
Briza minor								5	
Tribolium uniolae								14	
Pennisetum clandestinum								16	31
Briza maxima	10							100+	11

5.4 DISCUSSION

High density of invasive alien *Acacia* species, of which *A. mearnsii* was the most prominent in dry banks and wet banks of invaded sites, lead to significant changes in functional type cover and composition. *Acacia mearnsii*, and to a lesser extent (at least in the sites we selected, *A. longifolia*) invasion is most prominent in dry bank zones, this is consistent with similar observations by Boucher (2002). A possible reason for these findings is that physical stresses associated with frequent flooding, which causes erosion and periodic inundation, is limited in this part of the channel (Gregory et al., 1991), and that *A. mearnsii* is unable to cope with these physical stresses. However, as visual assessment showed, *A. mearnsii* forms a novel structural layer in both wet- and dry bank zones where present in high stem densities, and they are able to grow to a height of 10 m or more, overtopping most fynbos riparian tree species). Furthermore, invasion by *Acacia* species resulted in lower structural complexity in terms of the diversity of different functional types present. *Acacia* litter dominated the ground cover, while other lower statured functional groups such as grasses and forbs are to a large degree absent. This could be ascribed to the competitive advantage of *Acacia* species, which is able to fix N, which leads to faster growth rate compared to native species (Yelenik et al., 2004; Marchante et al., 2008; Werner et al., 2010; Morris et al., 2011). Alien invasive species has also been shown to be more effective competitors for limited resources including moisture, light and space (Funk and Vitousek, 2007; Werner et al., 2010; Gaertner et al., 2011; Morris et al., 2011).

Our results show that acacias dominated the canopy, reducing light penetrability and suppressing the growth and cover of indigenous trees/shrubs, tree seedlings and herbaceous species. Any native woody vegetation that was able to persist in *Acacia*-invaded ecosystems mostly occurred along the outside fringe of the invaded area along the wet bank zones, and existed within the *Acacia* stands only in very low numbers. Our findings are consistent with other studies that reported a decrease in cover of indigenous species and a shift in species assemblages beneath woody invasive canopies as a result of a canopy-level shift to invasive acacias in terrestrial fynbos (Richardson et al., 1989; Musil, 1993; Holmes and Cowling, 1997; Gaertner et al., 2011) and riparian ecotones (Blanchard and Holmes, 2008; Reinecke et al., 2008) in the fynbos and elsewhere, such as Portuguese dune ecosystems (Hellmann et al., 2011; Racher et al., 2011). It also reflects findings where other N-fixing species have replaced native species, such as finding by Vitousek and Walker (1989) that introduced *Myrica faya* displaces native Hawaiian forest species by outcompeting the dominant native trees for nutrients and light.

The shade tolerance of native riparian species in the fynbos is largely unknown, however, in terrestrial fynbos communities, where overstory shrubs/trees forms a dense canopy, understory plant species richness tend to be reduced, which suggest that certain fynbos species are shade intolerant (Holmes and Cowling, 1997; Brown et al., 2004). Native cover is relatively high in natural wet and dry banks (>50% in wet bank zones), thus one may expect that riparian species would be relatively shade tolerant. However, the structural complexity (vertical and horizontal) present in native riparian ecotones allows significant light to penetrate the canopy, thus allowing species that may be shade intolerant to thrive. However, in invaded wet and dry banks, the structural complexity is replaced by more uniform canopy with high leaf area index, which leaves little opportunity for native species to survive underneath the canopy. In addition, heavy litter layers may also play a role in preventing seeds that germinated from establishing and persisting in *Acacia*-invaded ecosystems.

As mentioned earlier, several other ecosystem attributes changes with *Acacia* invasion, and may play a role in competitive interaction between the invaded and the native species (Malcolm et al., 2008). For instance, soils became more saline; this may play a role in terms of the ability of native plants to form associations with microbes in the soil, while changes in physical attributes of soil such as soil particle size may influence nutrient availability. These factors may individually or collectively modify the environment such that native seedlings will not be able to establish as soils may be less suitable for native plants (Witkowski and Mitchell, 1987). Nitrogen-fixing invasive plants in other ecosystems have been shown to alter vegetation community composition by enhancing soil available N underneath the invaded canopy (Vitousek and Walker, 1989; Maron and Connors, 1996; Hughes and Denslow, 2005). Invasion by acacias may therefore decrease functional heterogeneity by dominating functional diversity in fynbos riparian ecotones, hence limiting the establishment and growth of native species. Perhaps no one factor is responsible for ecosystem changes relating to invasion and rather multiple and interactive ecosystem effects may cause changes in community composition (Eviner and Chapin, 2003).

Clearing of riparian zones previously invaded by *Acacia* species did not lead to recovery of growth form composition, despite more than seven years having elapsed since first clearing, and several follow-up clearings in the interim. Instead, cleared areas showed sparse woody cover and significant cover of alien grasses, including *B. maxima* and *P. clandestinum*. There was also limited regeneration of woody riparian shrubs and trees, which is consistent with findings reported in several other studies in fynbos riparian ecotones (e.g. Galatowitsch and Richardson, 2005; Blanchard and Holmes, 2008; Reinecke et al., 2008). In fynbos terrestrial ecosystems, Musil (1993) and Holmes and Cowling (1997) also found poor recovery of fynbos elements after clearing. However, opposite trend were also found, such as reported by Blanchard and Holmes (2008), who found a general trend towards increased vegetation cover and recovery in older cleared sites when compared to younger cleared sites, which represented a progression towards the pre-invasion state. Galatowitch and Richardson (2005) also reported that some sites cleared of *Acacia* species returned to the pre-invasion state without appreciable management interventions, whilst other sites failed to recover naturally on their own. In contrast to these diverging trends with acacias, Reinecke et al. (2008) reported that growth form composition of fynbos riparian vegetation recovered rapidly recovered in areas where *Pinus pinaster* was cleared. It was suggested that species identity has an important role to play in determining the outcomes of recovery post-clearing in fynbos riparian zones, and that the impact of the species on ecosystem structure and function and the density of the invasive species are major factors that influence the outcome. Furthermore, lack of recovery following clearing may be linked to seedbanks, such as when native species are absent from seed banks due to prolonged invasion, or that mature native trees simply did not survive invasion (Galatowitch and Richardson, 2005; Reinecke et al., 2008). It has further been suggested that alterations to microclimatic conditions after alien clearing (e.g. soil temperatures) and soil properties as a result of invasion (e.g. elevated N levels) might also impede germination or establishment of native seedling, as noted elsewhere (Yelenik et al., 2004; Hellmann et al., 2011; Rascher et al., 2011).

It has been suggested that secondary invasion by nitrophilous and other opportunistic species, such as many alien grasses may result where resource availability remain high in post-clearing environments (Milton and Hall, 1981; Milton, 2004; Yelenik, 2004; Holmes et al., 2005; Reinecke et al., 2008) and these species may also benefit from disturbances caused by control operations (Le Maitre et al., 2011). In our study we found that the abundance and cover of known nitrophilous species such as *B. maxima* and *E. calycina* remain high in riparian ecotones that have been cleared, even more than seven years subsequent to the clearing. More grass species were also present in cleared dry banks compared to native and invaded dry banks. In at least one case (Sir Lowry’s Pass River), prior landuse may have played a role in determining which grass species have established in riparian zones (e.g. grasses that may originate from pastures), however, the number of grass species at upper Jakkals and at Upper Molenaars have remained high, despite no appreciable landuse changes recorded. This suggests that soil conditions post clearing may have played a role in establishment of grass species, and possibly in the lack of recruitment of native seedlings, which also remained low in the post-clearing environment.

The growth and spread of alien grasses can also be facilitated by long-distance transport and other disturbances, as suggested by Milton (2004) or by the absence of riparian shrub and tree cover (Reinecke et al., 2008). Gaertner et al. (2011) recorded high abundances of *P. clandestinum* (an alien grass species also recorded in this study) was not associated with significant alterations in soil nutrient concentrations compared to native terrestrial fynbos areas. They suggested that the low native cover and species richness in a kikuyu field (*P. clandestinum*) could indicate a competitive advantage of this grass species over fynbos species. Given that cleared areas in our study differed significantly from natural areas with regard to plant functional type cover, and that areas previously invaded by *Acacia* species had been cleared more than seven years ago, it is likely that these areas may not recover as time progresses as established grass species outcompete native species. However, a mechanistic understanding of fynbos riparian recovery after clearing remains elusive, as the success of re-establishment may depend on complex interaction and feedback cycles between plants and their physical environment.

6. NITROGEN CYCLING IN NATURAL, *ACACIA*-INVADED AND CLEARED RIPARIAN ECOTONES IN THE WESTERN CAPE

6.1 INTRODUCTION

Nitrogen is frequently in short supply to terrestrial and aquatic ecosystems (Chapin et al., 2002), limiting productivity; this also play a major role in structuring plant ecosystems. Riparian zones are instrumental in modulating flow of N within and between landscapes (Decamps et al., 2004) by, *inter alia*, transforming organic N and immobilization of excess N emanating from natural and anthropogenic sources. However, different redox conditions are needed for different processes (e.g. nitrification, an aerobic process, versus denitrification, and anaerobic process), therefore riparian environments, with its fluctuating hydrological gradients are ideal buffers. Aerobic and anaerobic conditions may exist in riparian soils, sediments and groundwater over short distances and may alternate over short periods of time (ranging from days to seasons) (Naiman et al., 2005). In fynbos riparian environments, however the environmental conditions for mineralization and denitrification (temperature and soil moisture) may be optimal in an asynchronous way, as Mediterranean ecosystems have typically warm, dry summers and wet cool winters, thus temporally separating optimal conditions for N cycling in riparian zones (Bernal et al., 2007).

In this chapter we report on seasonal measurements of N cycling in natural, invaded and cleared riparian environments, separated into two lateral zones (wet banks and dry banks) and also compared to nearby upland areas. We selected sites invaded by *Acacia* species, which are widespread invaders in riparian zones in the western Cape sites. As invasive *Acacia* species such as *A. mearnsii* and *A. longifolia* are legumes, and putative N-fixers, we expect N cycling to be higher in invaded riparian zones compared to natural riparian zones. On the other hand, it has been shown that N cycling decline substantially after removal of the invasive legume in fynbos soils. Moreover, in riparian zones, seasonal and inter-seasonal floods and processes typically elevated in riparian zones, such as denitrification may quickly reduce available and total N stocks. Thus we expect lower rates of N cycling in cleared riparian ecotones, and for the general trend to return to the pre-invasion condition.

The work carried out is in response to objectives 1 and 2 of the original proposal. Nitrogen stocks were measured twice in 2010, *ex situ* N mineralization seasonally in 2010 and 2011, *in situ* N mineralization and denitrification measured seasonally in 2011 and 2012. Denitrification enzyme activity was measured once off in the western Cape and the southern and eastern Cape, but the latter is only reported on in Chapter 10.

6.2 METHODS

6.2.1 Nitrogen stocks

Samples were taken in autumn 2010 and spring 2010. Six composite soil samples were taken using a stainless-steel tube-type sampler (5 cm inner diameter) from the top 10cm (top soil or A horizon) of the soil profile after loose litter was removed. As riparian soils are typically heterogeneous, a hand trowel was used in places where soils were too rocky and/or shallow. Samples were sieved using a 2 mm sieve to remove roots and organic debris. Samples were analysed for total N (and total C) at the Soil Science Department at Stellenbosch University using a dry combustion elemental analyser method (Soil Science Department, Stellenbosch University), the result of which soil C/N ratios were computed.

6.2.2 *In situ* N mineralization rates

Two thin-walled tubes (schedule 40 PVC (Robertson et al., 1999) with a diameter of 3.8 cm and 15 cm long (Sasser and Binkley, 1989) per plot were knocked into the soil to a depth of 10cm. The remaining top part was covered with a piece of square, black, thin plastic (8 cm x 8 cm) that was tied to the PVC tube with nylon string. This prevented the plastic from the top part of the PVC tube to be uncovered and minimized moisture influences. Two small holes were cut with a scissors on the opposite ends of the plastic that covered the PVC tube, which allowed gas exchange to take place. Soil samples were collected, extracted and analysed for available N (NH_4-N and NO_3-N) and moisture content for the same day the PVC pipes were inserted into the soil, which had an initial value at time zero. The PVC pipes were left for approximately 7 days in the field and then removed (with the soil intact in the PVC tube) and placed in marked sealed plastic bags and then transported to the laboratory. The soil samples were extracted and analysed for available N (NH_4-N and NO_3-N) and moisture content for the same day the PVC pipes were taken out, which presented the end values for the time period (T_7). This allowed us to determine how available N and moisture content changed during that specific time period. All samples were analysed according to the 7-day anaerobic incubation method.

Nitrate were analysed using an adapted method from Cataldo et al. (1975). Sodium hydroxide (4 M) and salicylic acid (5 %) was used as reagents in the determination of nitrates in the soil extractions. The absorbance was read on a spectrophotometer set at 410 nm. A standard curve was drawn up with the absorbance values against the standards. The same procedure was followed for the unknown samples. The available NO_3-N per millilitre concentrations was calculated from the unknown samples by solving X in the standard curve. The corrected available NO_3-N concentrations (in micrograms per gram dry soil) was calculated by multiplying the value by 25 (the extractant volume) and then divided by the dry mass of the soil. Dilutions were made for the very dark samples that did not give a reading on the spectrophotometer. Determination of NH_4-N was done using the method of Page et al. (1992), and is described in section 6.2.3.

The net N mineralization rates (N min) were determined by the difference in available N at the start (T_0) and the end of the incubation period (T_7), divided by the gravimetric soil water content of the dry soil and then divided by the amount of days incubated in the field, which gave a value in μg N g^{-1} dry $soil^{-1}$ day^{-1}.

6.2.3 *Ex situ* N mineralization rates

The 7-day anaerobic incubation method, which has been used widely as an index of available N (Schomberg et al., 2009) and for detecting differences in N cycling in different areas and sites (Binkley and Vitousek, 1989; Anderson and Ingram, 1993), was used in this study. The method measures NH_4-N released under anaerobic conditions, which prevents the oxidation of NH_4^+ to NO_3^- (which takes place only in aerobic conditions). Only NH_4^+ is analysed, as the concentration of NO_3-N is usually insignificant after anaerobic incubation and most NO_3-N is lost through denitrification (Binkley and Vitousek, 1989; Anderson and Ingram, 1993; Schomberg et al., 2009). While anaerobic incubation conditions do not necessarily closely mimic environmental conditions, the results obtained mostly correlate well with plant uptake and foliar nutrient concentrations (Binkley and Vitousek, 1989). This method thus a valid measure of potential anaerobic N mineralization rates (Anderson and Ingram, 1993; Chapin et al., 2002).

We determined seasonal measurements of potential anaerobic N mineralization (NMP) rates for each sampling plot by adding 20 ml of distilled water to 10 g of a field moist subsample which was then incubated for 7 days (T_7) at 27°C, following the method by Keeney and Bremmer (1966). In order to prevent the exchange of gases such as oxygen, we fastly tightened the caps of the centrifuge tubes we used. We measured initial concentrations (T_0) of NH_4–N for each sample (from each plot), and extracted a 10g subsample of fresh soil with 25 ml of 0.5M K_2SO_4, shaken at medium speed for 1 hour, which was then filtered. The concentrations for each sample was determined using a spectrophotometer set at 540 nm (Page et al., 1992) and a standard equation used to calculate NH_4–N concentrations after the volume of the solution, the mass of the soil, and soil moisture content were considered. After 7 days of incubation, 2M K_2SO_4 were added to stop microbial activity, which brought the solution to a concentration of 0.5 M K_2SO_4. Concentrations of NH_4-N (T_7) underwent a similar procedure as for the determination of for NH_4-N (T_0). NMP rates were calculated from the difference in the final incubated concentration minus the initial concentration (T_7- T_0; in units μg g^{-1} dry $soil^{-1}$ day^{-1}) for each soil sample divided by the incubation interval.

6.2.4 Field N_2O emissions

The chamber method (Fisher, 2006) was used to measure the actual N_2O emission from soils in the field. PVC tubes that were 10 cm in diameter, 25 cm in length and, 5 mm thick and sealed off with end caps were used as gas collection chambers. They were knocked into the soil to a depth of 5 cm at least 6 days before sampling took place. This allowed the soil to settle and not be influenced by human disturbance. The chambers were sealed by folding the extended rubber tubing at the top of the chamber over itself and kept in place with a metal binder clip. A 10 ml plastic disposable syringe was used to take air samples from the chambers at zero, one, two and three hours. The syringes were sealed with rubber stoppers and then transported back to the laboratory for analysis that took place within six hours. The N_2O concentrations were measured in parts per billion (ppb) with a gas chromatograph (SRI 8610C Compact GC) that was fitted with a ^{63}Ni electron capture detector (ECD). Field N_2O measurements were taken from four chambers (provided that there were enough chambers) at each landscape position along one transect.

6.2.5 Denitrification enzyme activity

The effect of available N and organic C on the maximum potential denitrification activity was determined and compared to reference conditions once off during spring 2012 for the western Cape and during spring 2012 for the southern and eastern Cape. This was done by a denitrification enzyme assay carried out in the laboratory with fresh field soil samples. The process involved the inhibition of N_2O conversion to N_2 in the presence of acetylene under anaerobic conditions. It is important to note that this assay actually measured the concentration of functional denitrifying enzymes in the sample at the time of collection and not the denitrifying activity of the natural sample itself. However the denitrifying enzyme concentration of the field sample does reflect the environmental history of the study site, which meant that this method can be used successfully (Tiedje et al., 1989).

Collected fresh field soil samples were analysed at zero, one, two and three hours to determine the maximum potential denitrification enzyme activity. The denitrification enzyme assay (DEA) method that was used was described by Tiedje et al. in 1989. It is an acetylene inhibition method that inhibited the conversion of N_2O to N_2. 15 g of fresh field soil was added to a 100 ml Erlenmeyer flask that contained a 15 ml mixture of a 1 mM solution of potassium nitrate (1 mM KNO_3) and sodium succinate (1mM $Na_2C_4H_4O_4.6H_2O$). The flask was sealed with a rubber stopper and flushed with high purity nitrogen for approximately 2 min to make sure the flask was under an anaerobic condition. 10 ml of acetylene gas (C_2H_2) was added to each flask (15% of headspace). The flask was shaken for 30 seconds and then vented with a needle to bring the headspace to atmospheric pressure. The flasks were incubated at 30°C in a temperature controlled incubation room.

Samples of the headspace gas were drawn from each flask at zero, one, two and three hours. It was manually injected into a gas chromatograph (SRI 8610C Compact GC) fitted with a ^{63}Ni electron capture detector (ECD) to measure the N_2O concentration as the maximum potential denitrification activity. The two stainless steel columns (one pre-column and one main column) was 2 mm thick and 3 m in length and packed with Porapak Q, 80/100 mesh. The column and the GC ECD oven temperatures were set at 70 and 300°C respectively and high purity nitrogen was used as the carrier gas with a flow rate of 29 ml min^{-1} (Mosier and Mack, 1980). Atmospheric air samples were used to draw a standard curve, which incorporated the area of the different peaks for the standard air samples as well as the DEA headspace samples and was used to determine the actual concentrations. The production of N_2O was calculated as a volumetric unit in parts per billion (ppb) per 3 hour sample period. The production of N_2O gas represented the maximum potential denitrification activity.

Three different treatments were used to determine the effect of an available nitrogen and carbon source on the maximum potential denitrification activity and also the combined effect of the two. This was compared to a control in distilled water where no nitrogen or carbon was added. A 1 mM KNO_3 solution was used as the nitrogen source, a 1 mM $Na_2C_4H_4O_4.6H_2O$ solution was used as the carbon source and distilled water was used as the control. This was measured once off during Spring 2012 for the Eastern- and Southern Cape and also once off during spring 2012 for the Western Cape.

6.2.6 Statistical analyses

Soil N stocks were statistically analysed using one-way ANOVA for both invasion status and landscape position, followed by Tukey's post hoc tests. Net N mineralization (*in situ* N min) was analysed using one-way ANOVAs for each individual season for both invasion status and landscape position, followed by Tukey's post hoc tests. For net N mineralization rates one way ANOVA for both invasion status and landscape position, followed by Tukey's post hoc tests was used.

For NMP rates, net N mineralization and N_2O emissions, repeated measures analysis of variance (RMANOVA) was applied to test for significant interactive effects between invasion statuses across seasons and landscape positions across seasons. When seasons were compared, one-way ANOVAs were carried out on all data collected, irrespective of invasion status or position in the landscape. To test the effect of soil NMP rate between invasion status (natural, invaded, cleared) and landscape position (wet- and dry bank, uplands) across seasons, a one way ANOVA was applied. When significant differences between the main (landscape position) and interaction effects (landscape position X seasons) were tested, only data from the four natural sites were applied with the intention of excluding possible effect of invasion and clearing on soil properties. As sampling plots selected in terrestrial upland areas at invaded sites were not invaded by *Acacia* species they were excluded from statistical analyses for the purpose of comparing different effects between invasion statuses (for NMP only). Similarly, only measurements from dry bank zones of each site were used to indicate significant differences between invasion status and interaction (invasion status X season) effects, since invasion is most prominent in the dry bank zone.

Spearman rank correlation coefficients were used to determine relationships between soil N processes and soil properties.

6.3 RESULTS

6.3.1 Nitrogen stocks

We determined total N on two occasions, autumn 2011 and spring 2011. The results are displayed in Figure 6.1 (A and B), and the data from the two seasons are combined.

No significant interactions between invasion statuses and seasons were found soil total N ($p = 0.36$), however we found significant differences between invasion statuses for total N (one-way ANOVA: $p<0.01$) when we pooled all the data regardless of season (Figure 6.1a). Total N content was significantly higher in soils collected from under *Acacia* stands compared to natural ($p<0.01$) and cleared ($p<0.01$) areas, with no differences between the latter two invasion statuses ($p = 0.78$). No differences were evident when the landscape positions were compared.

6.3.2 Available N

There was a significant interaction between available N (NH_4^+ and NO_3^-) and seasons (two-way ANOVA: $p<0.001$; Figure 6.2A). Ammonium had significantly higher concentrations in summer (4.03 $\mu g\ g^{-1}$) relative to the other seasons (autumn, 1.43 $\mu g\ g^{-1}$; winter 0.94 $\mu g\ g^{-1}$; spring (1.36 $\mu g\ g^{-1}$), and was also significantly than NO_3^- in summer, but lower overall. Nitrate concentrations peaked in autumn (2.99 $\mu g\ g^{-1}$) and showed significantly lower concentrations in winter (1.93 $\mu g\ g^{-1}$; Figure 6.2A). Significant overall interaction ($p<0.01$) showed that NO_3^- (mean: 2.49 $\mu g\ g^{-1}$) is the dominant form of available inorganic N in the topsoil.

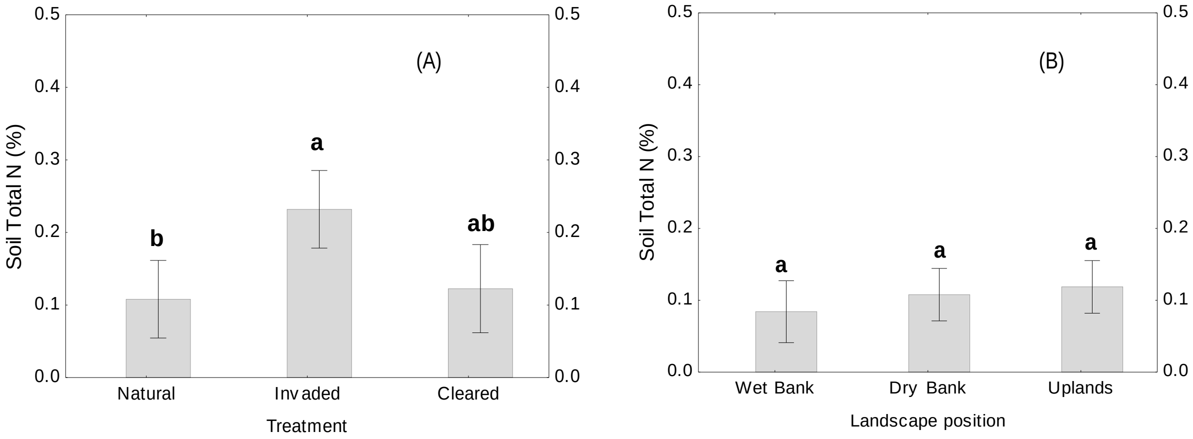

Figure 6.1: Soil total N (seasonal data combined) for (A) invasion statuses, and (B) landscape positions. Bars represent means and whiskers represent ± 95% confidence intervals for percentages. Significance levels (Tukey's post hoc test; $p<0.05$) are indicated by different letters for one-way ANOVAs for invasion statuses and landscape positions. All statistical analyses were computed on log-transformed data to meet the assumptions for ANOVA.

On average, soils sampled from *Acacia*-invaded and cleared sites had 1.7 and 2 times as much NO_3^- and 2.2 and 1.8 times as much NH_4^+ as soils from natural riparian areas respectively (Figure 6.2B). Mean annual total available N ($NO_3^-$and NH_4^+) was significantly greater in both *Acacia*-invaded soils (5.9 µg g^{-1}; $p<0.001$) and cleared riparian soils (6.1 µg g^{-1}; $p<0.001$) compared to those sampled from natural fynbos (3.62 µg g^{-1}; Figure 6.2B). When all data across seasons were pooled, mean concentrations of NH_4^+ and NO_3^- differed significantly between landscape positions (Figure 6.2C). Overall, we recorded that NH_4^+ concentrations in wet banks differed from dry banks ($p<0.001$) and terrestrial ($p<0.001$) areas, while NO_3^- concentrations did show differences between riparian wet and dry bank zones ($p =0.45$). However, both NH_4^+ and NO_3^- concentrations were significantly lower than terrestrial upland areas (Figure 6.2C). When totalled, available N (NO_3^- and NH_4^+) showed significant differences between landscape positions ($F_{[2, 92]}$ =7.195, $p<0.01$; Figure 6.2C) with wet banks (2.49 µg g^{-1}) lower than dry banks (3.63 µg g^{-1}; $p<0.01$) and uplands (3.96 µg g^{-1}; $p<0.001$) and no differences between dry bank and uplands ($p =0.36$).

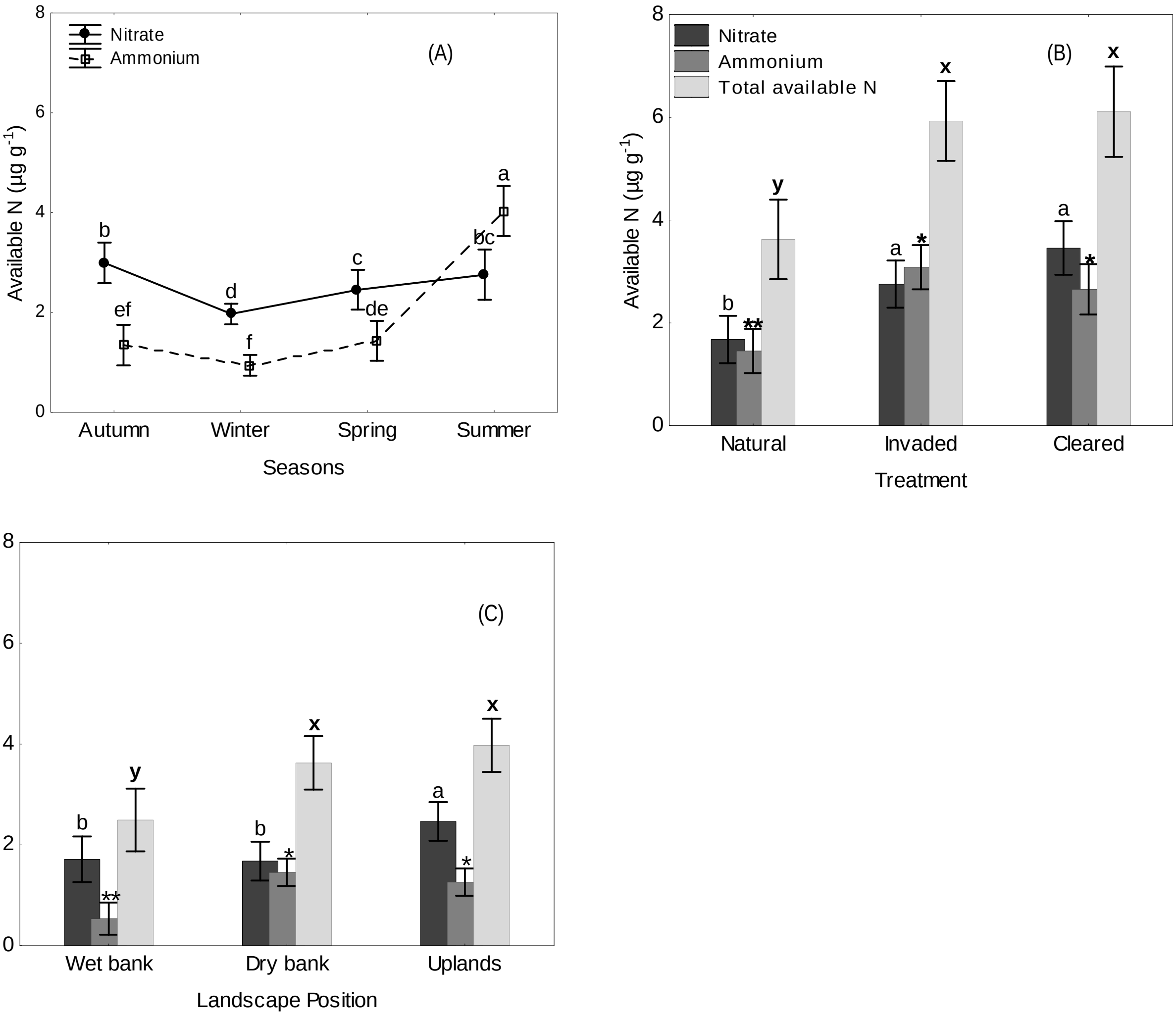

Figure 6.2: (A) Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in soils across seasons. Letters denote significant differences (Tukey tests, $p<0.05$) based on a two-way repeated measures ANOVA ($p<0.001$) using all the data collected over the year, and irrespective of invasion status or position in the landscape. Mean values are indicated by solid symbols (NO_3^-) and squares (NH_4^+), and whiskers represent the 95% confidence interval. Available inorganic N in the form of ammonium (NH_4^+), nitrate (NO_3^-), and total available inorganic N ($NH_4^+ + NO_3^-$) for (Figure 6.2B) natural, invaded and cleared sites (invasion statuses), and (Figure 6.2C) wet- and dry bank and uplands lateral zones (landscape position). Mean values are indicated by bars and whiskers represent ± 95% confidence interval. Different letters and symbols [NO_3^- ($^{a;b}$); NH_4^+ (*;**); and total available N($^{x;y}$)] represent significant differences (Tukey tests, $p<0.05$) based on one-way ANOVAs: invasion statuses (NH_4^+: $p<0.001$; NO_3^-: $p<0.001$; total available N: $p<0.001$) and landscape positions (NH_4^+: $p<0.001$; NO_3^-: $p<0.01$; total available N: $p<0.01$).

6.3.3 *In situ* nitrogen mineralization

In situ N mineralization (net N mineralization) measurement were carried out in autumn 2011, Spring 2011, autumn 2012 and winter 2012, and the results are displayed in Figure 6.3 (A and B), where seasonal measurements were pooled and means expressed by invasion status and landscape position. Overall, *in situ* N mineralization rates were low, and during several sampling occasions, net immobilization occurred.

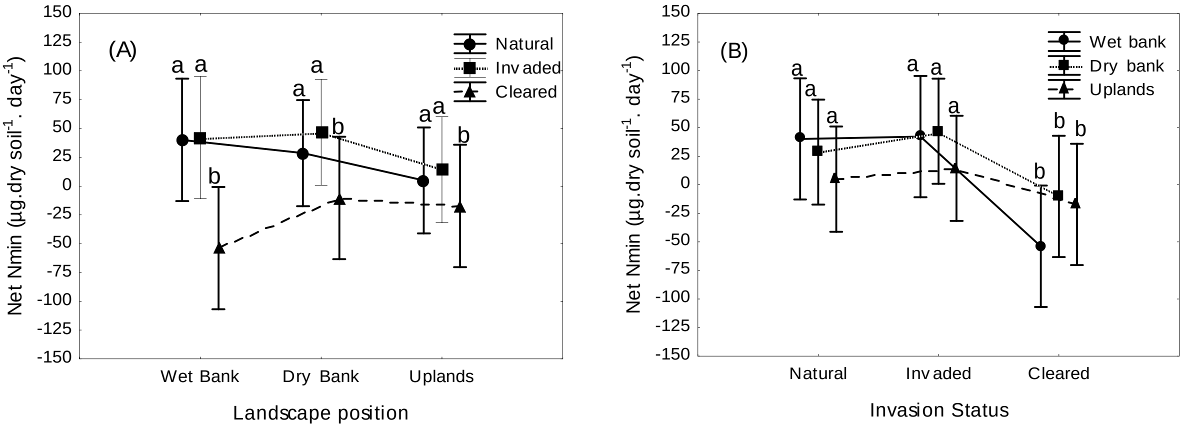

Figure 6.3: (A) Mean net N mineralisation arranged by invasion statuses (natural, invaded and cleared) and (B) arranged by landscape position (wet bank, dry bank and terrestrial) pooled across different seasons. This was done as no significant seasonal interactions were obtained. The points are mean values and the whiskers represent ± 95% confidence interval. Letters denote significant differences determined by RMANOVA's for invasion status x season and landscape position x season followed by post hoc Bonferroni tests at three different significant levels ($p < 0.05$; $p < 0.01$; $p < 0.001$).

Little interaction was apparent between seasons and invasion status (p =0.66) and season and landscape positions (p = 0.54). When all data across seasons were pooled, cleared riparian sites showed significantly lower net N mineralization compared to natural and invaded sites; the latter were not significantly different from each other.

6.3.4 *Ex situ* nitrogen mineralization

When we incubated soils in the laboratory we found that the highest rates was for samples taken in spring, declining towards summer (apart from invaded sites, where NMP rates were highest in summer), and low but measurable rates in autumn and winter (Figure 6.4A). It is noticeable that immobilization was not as prominent when compared with net N mineralization from *in situ* incubations.

Rates of NMP did not show significant seasonal interactions with invasion statuses (p =0.28; Figure 6.4A) or landscape positions (p =0.15; Figure 6.4C). However we found mean NMP rates differed significantly between seasons (one-way ANOVA: p<0.001), between invasion statuses (one-way ANOVA: p<0.05; Figure 6.4B) and landscape positions (one-way ANOVA: p<0.001); Figure 6.4D). Compared to wet banks, mean NMP rates of soils from dry banks were 195% higher, and from terrestrial areas, 199% higher when taken across all seasons (Figure 6.4D).

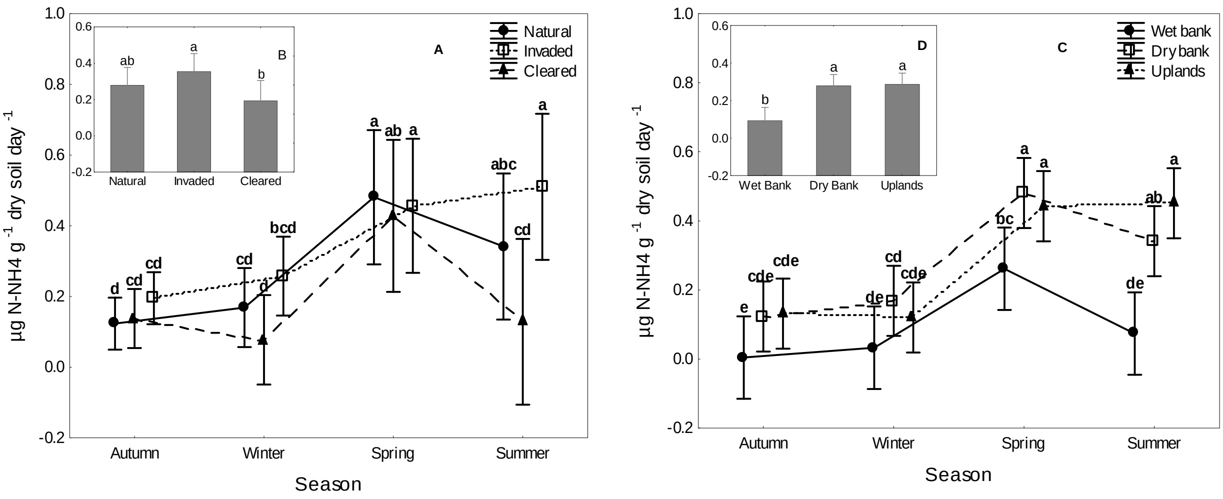

Figure 6.4: (A) Potential anaerobic N mineralization (NMP) rates for invasion statuses (natural, invaded and cleared) and (C) landscape positions (wet bank, dry bank, and uplands) across seasons. Mean values indicated by different symbols, and whiskers represent ± 95% confidence interval. Letters denote significant differences using repeated measures ANOVAs: landscape position x seasons and invasion status x seasons, followed by post hoc Tukey tests (p<0.05). Average seasonal measurements for NMP rates taken over 1 year are depicted in the embedded bar graph for invasion status (B) and landscape position (D). Mean values indicated by bars, and whiskers ± 95% confidence interval. Different letters indicate statistical differences using one-way ANOVAs followed by Tukey's post hoc tests (p<0.05).

NMP rates under *Acacia* stands were significantly higher (p<0.05) when compared with cleared areas, but did not differ from natural sites (p>0.05: Figure 6.4B). As noted earlier, on average net N mineralization was evident throughout the year, however microbial immobilization occurred in some sampling locations, apparently more so in the wet banks (Figure 6.4C).

6.3.5 N_2O emissions

Field N_2O emissions were measured and used as an indication of denitrification, however, this should be seen in conjunction with denitrification enzyme activity results (Section 6.3.6) to ascertain more accurately the role of N_2O emissions and denitrification in fynbos riparian zones and its relationship to invasion of *Acacia* species and clearing thereof.

Figure 6.5 show the results of field N_2O emission measurements in (A) natural, invaded and cleared riparian zones and (C) wet banks, dry banks and terrestrial (upland) areas. The data is averaged across seasons, and showed little trends in terms of rates of N_2O emissions, expressed either by invasion status or by landscape position. However, spring appeared to be the season where absolute values of soil N_2O emissions were highest.

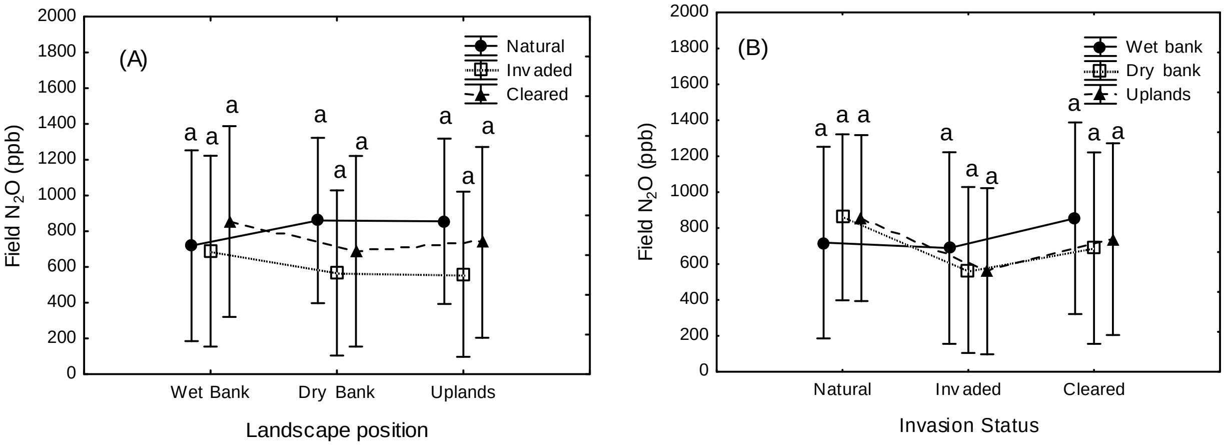

Figure 6.5: (A) field N_2O emission arranged by invasion statuses (natural, invaded and cleared) and (B) arranged by landscape position (wet banks, dry banks and terrestrial areas) pooled across different seasons. This was done as no significant seasonal interactions were obtained. The points are mean values and the whiskers represent ± 95% confidence interval. Letters denote significant differences determined by RMANOVA's for invasion status x season and landscape position x season followed by post hoc Bonferroni tests at three different significant levels ($p < 0.05$; $p < 0.01$; $p < 0.001$).

6.3.6 Denitrification enzyme activity

Laboratory N_2O emission was determined on incubated soils sampled in spring 2012 from the western Cape sites as an indication of the maximum potential denitrification enzyme activity and analysed for interacting effects on invasion status (natural, invaded and cleared) and landscape position (wet bank, dry bank and terrestrial) (Figure 6.6). One way ANOVAs followed by *post hoc* Bonferroni tests were used to determine any significant differences within these interacting effects. Values were considered to be significant at three different levels, namely $p < 0.05$, $p < 0.01$ and $p < 0.001$.

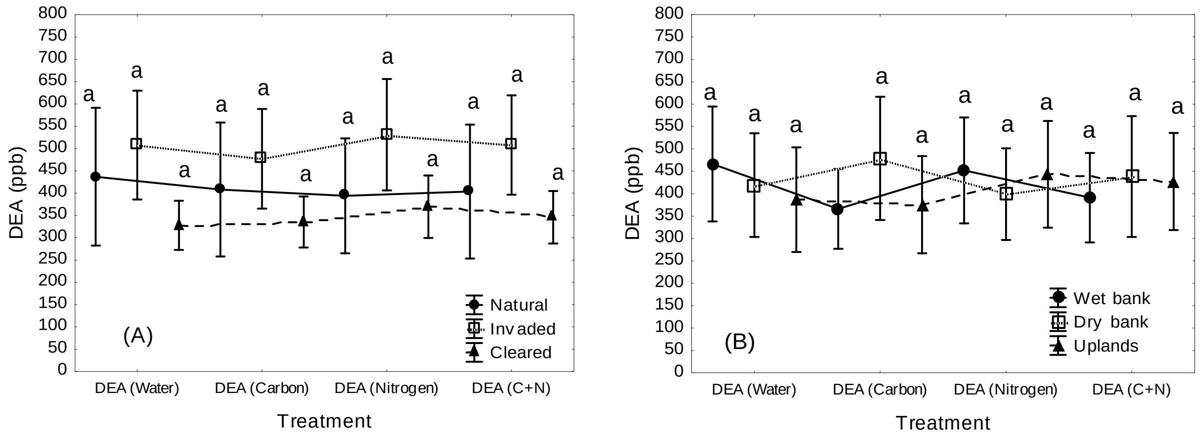

Figure 6.6: (A) Denitrification enzyme activity for the different treatments arranged by invasion statuses (natural, invaded and cleared) and (B) arranged by landscape position (wet bank, dry bank and terrestrial). Mean values are indicated by different symbols and whiskers represent the standard error. Letters denote significant differences determined by one-way ANOVAs for invasion status, landscape position and DEA treatment followed by post hoc Bonferroni tests at three different significant levels ($p < 0.05$; $p < 0.01$; $p < 0.001$).

There were no significant interacting effects between DEA and invasion status ($F_{[6, 62]} = 0.75$; $p = 0.02$; Figure 6.5A) or landscape position ($F_{[6, 62]} = 0.79$; p = 0. 576; Figure 6.5B). Densely invaded areas showed greater potential for denitrification but also greater variability (higher standard error) than cleared areas. The wet and dry banks seemed to be responding differently to the availability of N and C as an energy source, where N seemed to limit potential denitrification in the wet banks and C seemed to limit potential denitrification in the dry banks.

6.3.7 Drivers of nitrogen cycling

Pinay et al. (1992; 1995) suggest that soil physical properties such as particle size, soil nutrient contents (N, C) and environmental properties such as soil water content all have an influence on N cycling. Table 6.1 lists selected soil properties and its relationships to N (total and available).

Table 6.1: Spearman's correlation coefficient ρ (rho) for selected soil physical and chemical properties. Significant relationships ($p<0.05$) are indicated by an asterisk (*). Correlations were calculated from all available data irrespective of invasion status or landscape position, however, different variables differed in the regularity of sampling: seasonally (NO_3^-, NH_4^+, EC and pH); biannually (soil total N) and once of during spring (bulk density). Correlation values represent only instances where comparisons could be made. N/A = Not applicable.

Soil Properties	**GSWC**	**Silt and clay**	**Bray-2 P_i**	**NO_3^-**	**NH_4^+**	**Soil TN**
NO_3^-	-0.258*	0.501*	0.380*			
NH_4^+	-0.486*	0.559*	0.442*	0.535*		
Soil TN	0.110	0.663*	0.345*	0.419*	0.565*	
EC	-0.208*	0.404*	N/A	0.395*	0.661*	0.439*
Bulk density	N/A	-0.355*	-0.037	-0.355*	-0.435*	-0.494*
pH	N/A	0.379*	-0.043	-0.162	0.091	N/A

Silt and clay content was significantly correlated with soil nutrients (N, P and C), bulk density and other chemical properties (pH and EC), regardless of invasion status or landscape position. Soil bulk density showed a significant negative relationship with available N (NO_3^- and NH_4^+) concentrations and soil TN (Table 6.1). Bray-2 P_i concentrations showed a significant positive relationship with soil total and available N (NO_3^- and NH_4^+). A significant negative correlation occurred between soil moisture content (GSWC) and available nutrient (N and P) concentrations (Table 6.1). Furthermore, available N concentrations, particularly NH_4^+, were strongly related to soil total N. Both N (Table 6.1) and P cycling was also correlated to soil physical properties such as soil pH, soil bulk density and gravimetric soil water content (Table 6.2).

Data collected during 2011 and 2012, which focussed on net N mineralization and denitrification, when compared to physicochemical properties showed that GSWC was positively correlated to NH_4^+ ($R = 0.26$; $p < 0.01$; Table 6.2) total available inorganic N ($R = 0.25$; $p< 0.01$; Table 6.2) and volumetric soil water content (VSWC) ($R = 0.43$; $p < 0.001$). Volumetric soil water content was negatively correlated to NH_4^+ ($R = -0.301$; $p < 0.05$), total available inorganic N ($R = -0.29$; $p < 0.05$), and the temperature at 1cm ($R = -0.57$; $p < 0.001$), and 6cm ($R = -0.58$; $p < 0.001$). The temperature at 1 and 6cm was positively correlated to NH_4^+ (1cm: $R = 0.33$; $p < 0.01$; 6cm: $R = 0.34$; $p < 0.01$) and total available inorganic N (1cm: $R = 0.33$; $p < 0.01$; 6cm: $R = 0.34$; $p < 0.001$). The temperature at 1 and 6cm was positively correlated to each other ($R = 0.98$; $p < 0.001$). The temperature at 1cm is positively correlated to pH (water) ($R = 0.21$; $p < 0.05$). NH_4^+ was positively correlated to total available inorganic N ($R = 0.99$; $p < 0.001$) and NO_3^- ($R = 0.31$; $p < 0.01$), but negatively correlated to net N mineralisation ($R = -0.27$; $p < 0.01$). NO_3^- is positively correlated to total available inorganic N ($R = 0.31$; $p < 0.001$).

6.4 DISCUSSION

6.4.1 Total and available N

Several recent and older studies recorded the impact of IAPs, particularly *Acacia* species, on terrestrial soil N stocks in the south-western Cape (e.g. Witkowski, 1991; Musil, 1993; Stock et al., 1995; Yelenik et al., 2004, 2007); N was generally increased in invaded areas. We recorded significantly enhanced N in soils under *Acacia*- invaded and cleared riparian zones relative to natural areas. Increases in soil N were expected, as *A. mearnsii* is a N-fixer (Forrester et al., 2007; Tye and Drake, 2011) and has been shown to have enriched litter that adds to soil stocks once shed (Yelenik et al., 2004). Our findings are thus in line with other studies that investigated the impacts of N_2-fixers on soil N dynamics. Yelenik et al. (2004, 2007) found that total annual available N (NH_4^+ and NO_3^-) was greater in *A. saligna*-invaded and cleared (Yelenik et al., 2004) areas compared to native terrestrial fynbos), suggesting a significant lag, or legacy effect of invasive acacias. This also concurs with meta-analyses by Ehrenfeld (2003) and Liao et al. (2008), who reported consistent increases in soil total- and available N with invasion by leguminous wood species. There is also evidence, ranging from arid to temperate ecosystems, that N-fixing species, such as invasion by *A. longifolia* in Portugal dune sand ecosystems (Marchante et al., 2008; Hellmann et al., 2011), *Elaeagnus angustifolia* in New Mexico, USA (DeCant, 2008) and *Robinia pseudoacacia* in pine-oak ecosystems (Rice et al., 2004) increased available inorganic N relative to their non-invaded native environments. Increases in available N by factors up to 100-fold was reported by Hughes and Denslow (2005) when comparing *Falcataria*-invaded stands and native forests in Hawaii

.

Table 6.2: Spearman's rank correlation coefficient ρ (rho) for labile N variables and some of their drivers. Asterisks indicates significant relationships (* $p < 0.05$; **$p < 0.01$; *** $p < 0.001$). Correlations were calculated from all available data irrespective of invasion status, landscape position or season, although there are seasonal differences for some of the variables.

	NH_4^+	NO_3^-	Total available N	Net N min	Field N_2O	Gravimetric Soil Water Content	Volumetric Soil Water	Temp 1cm	Temp 6cm
NO_3^-	0.308**								
Total available N	0.987***	0.310***							
Net N min	-0.271**	-0.005	-0.272**						
Field N_2O	0.030	0.000	0.031	-0.135					
GSWC	0.258**	0.040	0.245**	0.164	-0.059				
VSWC	-0.301*	0.122	0.299**	0.059	0.067	0.428***			
Temp 1cm	0.326**	-0.179	0.328**	-0.029	-0.144	-0.191	-0.565***		
Temp 6cm	0.336**	-0.196	0.337***	-0.032	-0.116	-0.187	-0.575***	0.981***	
pH (water)	0.149	0.152	0.152	-0.182	0.140	0.013	0.216	0.205*	0.149

However, not all woody *Acacia* species increase available N after invasion, for instance the study by Stock et al. (1995) recorded that compared to adjacent native ecosystems, available N was significantly higher in *A. cyclops*-invaded strandveld ecosystems, but not in *A. saligna*-invaded low nutrient, acid sand fynbos. They suggested that differences in these effects may be attributable to differences in leaf chemistry or soil properties between sites between invasive and native species. Indeed, Dassonville et al. (2008), in a continent-wide study, showed that IAPs increased site nutrient pools only in sites with initially low soil nutrient levels. Due to site-specific effects, as mentioned above, each particular ecosystem should be considered, investigated and managed independently (Ehrenfeld, 2003).

Variation in post-invasion changes in ecosystem N stocks may be associated with the physiological and physical characteristic of the invasive species (Ehrenfeld, 2004). Some of the factors that might contribute to the significantly greater total and available N observed under *Acacia* stands may be due to biological traits which differ from native riparian communities (Morris et al., 2011), e.g. higher growth and photosynthetic rates, larger size (Ehrenfeld, 2004; Liao et al., 2008; Marchante et al., 2011; Morris et al., 2011) and higher inputs of N-rich litter with rapid decomposition rates, resulting faster return of N to the soil (Musil and Midgley, 1990; Witkowski, 1991; Ehrenfeld, 2004; Yelenik et al., 2004; Marchante et al., 2008). Our finding of increases in soil total N content associated with *Acacia*-invaded fynbos riparian ecotones are in line with other reported results with invasion of N-fixers into native fynbos communities, such as those of Stock et al. (1995) and Yelenik et al. (2004, 2007) and other ecosystems around the world (e.g. Rice et al., 2004; Allison et al., 2006; Caldwell, 2006; DeCant, 2008; Marchante et al., 2008; Follstad Shah et al., 2010). Slow decomposition of fynbos litter, which is ascribed to high concentrations of recalcitrant compounds and high C/N ratios (Witkowskii, 1991; Stock and Allsopp, 1992), is consistent with the low nutrient availability found in natural riparian zones, suggesting that native riparian litter may also decompose relatively slowly.

The studies of Witkowski (1991) and Stock et al. (1995) both showed that the increase in soil N, in response to invasion by acacias, were very different between two invaded ecosystems (higher fertility strandveld versus lower fertility acid sand fynbos ecosystems) under similar climatic conditions, thus also suggesting context-specificity of N accumulation following *Acacia* invasion. In general, we recorded lower magnitudes of change in N stocks in fynbos riparian ecotones. Sandy soils in riparian zones, contain low amounts of OM and clay (particularly wet banks) and may be subjected to rapid leaching and hence may be incapable of storing large quantities of N (Vitousek and Walker 1989; Chapin et al., 2002). Our results from previous chapter show that riparian ecosystems generally have higher sand fractions relative to associated terrestrial uplands, thus is predisposed to leaching; this, combined with suspension and leaching of OM and available nutrients during seasonal floods (including the loss of N through denitrification, etc.) in riparian habitats, may explain the less dramatic effect of *Acacia* invasion on these systems compared to those reported in terrestrial fynbos ecosystems invaded by other *Acacia* species (e.g. Yelenik et al., 2004). Denitrification is a process often associated with riparian environments (Naiman and Décamps, 1997; Naiman et al., 2005; Jacobs et al., 2007), and may be an important factor in N loss in fynbos riparian zones, even though rates may be low, as found in our study. Denitrification by soil and sediment-bound microbes will quickly reduce soil NO_3^- concentrations, given the right conditions.

6.4.2 NMP and *in situ* mineralization rates

Temporal variability in plant productivity, which may vary inter-seasonally and inter-annually, between adjacent habitats, may affect nutrient fluxes such as N-cycling (Chapin et al., 2002; Ballinger and Lake, 2006). We found high seasonal variation in N mineralization rates, which is not inconsistent with other studies, such as those of Maron and Jefferies (1999), thought this was less prominent in net N mineralization rates. A peak in spring and lower rates in winter was exhibited by all sites when soils were incubated and subjected to optimal conditions for N mineralization, regardless of invasion status or position in the landscape. These trends may be explained by competition for resources between plants and microbes, which is expected to be greatest during optimal growing conditions (spring) and low in winter when plant photosynthesis rates are low. In spring and early summer a combination of soil moisture (residual soil water after seasonal flooding events) and favourable temperature may stimulate mineralization (Bardgett, 2005). Generally, N mineralization occurs at higher rates under moist rather than dry conditions, and has been shown to slow down significantly at low temperatures, even if moisture conditions remain favourable (Chapin et al., 2002), as was observed in this study. Rice et al. (2004) found that mean net N mineralization and nitrification rates were enhanced during the growing season, and declined in winter. At the end of summer, the annual summer drought conditions associated with Mediterranean climates then leads to a decline in N mineralization rates, although temperatures may remain near optimal.

We found that river hydrogeomorphology (lateral zones) had a definite impact on N mineralization, though not under field conditions. Pinay et al. (1995) suggested that hydrogeomorphology controls the sorting of sediments on the basis of particle size in a riparian floodplain (Pinay et al., 1995). Low-lying wet bank zones are exposed to high energy and water velocity during flooding so that coarsely-textured suspended matter, low in its capacity to retain OM inputs is deposited (Rhoades et al., 2001). The accumulation of C, N and OM is slower in wet banks, and N cycling may further be impacted by leaching of NO_3^- (Prescott et al., 2000). Thus different microclimatic conditions and hydrogeomorphological processes associated with wet banks may slow decomposition rates, such that mineralization rates measured throughout the year remained consistently lower than that of dry bank and upland areas. Indeed, *in situ* measurement of N mineralization showed lower rates, and in many cases net immobilization of N was recorded, suggesting high levels of competition between plants and microbes for scarce resources, in this case, N. In addition, pools of labile soil organic N in wet bank zones becomes depleted (i.e. leached) with high rainfall events, and as a consequence, reduce the substrate (soil OM) necessary for N transformations (Chapin et al., 2002). Dry bank zones, on the other hand, are not influenced to the same extent by within-year flooding events and, accordingly, OM accretion is enhanced. Mean NMP rates between landscape positions and amongst invasion statuses were all above zero, indicating net mineralization (compared to *in situ* N mineralization rates, which showed immobilization during certain times of the year). This suggests that microbial immobilization was lower than mineralization such that plants and/or microbes were never completely deprived of N.

Litter produced by native species adapted to low-nutrient ecosystems of the fynbos decomposes relatively slowly, because of high C/N ratios and high contents of lignin, tannins and other toxic or recalcitrant compounds (Chapin et al., 2002; Yelenik et al., 2004). Litter quality of N-fixers are frequently better than the mostly sclerophyllous litter of fynbos plants, and as a results N-fixing species strongly influence available N pools and enhance N mineralization rates (e.g. Stock et al., 1995; Yelenik et al., 2004, 2007; see also Rice et al., 2004 and Malcolm et al., 2008). However, neither NMP rates not net N mineralization rates under *Acacia* stands differed from natural sites, hence, higher litter inputs and N pools (e.g. TN in this study) in invaded sites does not necessarily lead to higher mineralization rates. Instead, interactions between plants' requirements for N and available soil resources (e.g. dissolved organic and inorganic N) may limit the rate of N mineralization. It is also possible that mycorrhizal fungi associated with *Acacia* species absorb amino acids and therefor may not greatly depend on N mineralization to meet their N needs in infertile ecosystems due to absorption of dissolved organic matter (DOM). Since there are several environmental factors (temperature, soil moisture), biological (microbial activity and substrate quality), and soil physical and chemical properties that affect N mineralization rates, it is difficult to draw further conclusions from these results. Thus, future research should address in detail the link between N-cycling and soil physical, chemical and biological factors affecting these processes.

Overall NMP rates in cleared riparian ecotones were not significantly different from natural sites, suggesting that N-cycling post-clearing has approached background levels. Upon removal of the invasive plants from the ecosystem, N that otherwise would have been absorbed by plants may be immobilized by soil microbes or leached from the soil, resulting in low or negative net N mineralization values (Schmidt et al., 1999). Also, as riparian zones are frequently flooded and hence more susceptible to nutrient leaching and resuspension of litter, they may recover more rapidly compared to terrestrial environments by increasing the rate at which available and organic nutrient pools that accumulated as a result of invasion, is lost. However, it remains unclear how long the biogeochemical legacies in the soil may persist after alien clearing activities, and it is suggested that long-term research be undertaken to monitor N, C and P concentrations and cycles directly following the eradication of *Acacia* species from riparian zones.

Malcolm et al. (2009) recently investigated biogeochemical legacies in areas cleared of N_2-fixing invaders. The removal the N_2-fixing species *R. pseudoacacia* rapidly diminished the mineralisable supply of N in soils. Marchante et al. (2009) reported significant decreases in microbial soil parameters related to N-cycling four and half years after removal of *A. longifolia*, hence the soil microbial parameters declined faster than soil chemical pools such as N and C pools. However, in areas cleared from *A. saligna* stands in terrestrial fynbos ecosystems (Yelenik et al., 2004) and removal of *Lupinus arboreus* in coastal prairies (Maron and Jefferies, 1999) did not result in N mineralization rates different from the invaded state. The latter two studies were conducted a few years post-clearing (1-4 years), and longer term studies may have revealed different trends. However, long-term success of restoration may be inhibited by high rates of N availability that affect plant community interactions and alter successional trajectories (Rice et al., 2004); this concurs with our findings.

6.4.3 N_2O emission rates and denitrification enzyme activity

Denitrification is influenced by the redox state (presence or absence of oxygen) of the soil environment (Bailey and Beauchamp, 1973; Sutton-Grier et al., 2012). Denitrifying organisms would only switch to denitrification, which is an anaerobic process, once oxygen becomes limited or is not available to be used as an energy source (Skiba, 2008). Suitable conditions for denitrification to take place most likely occurred at the start of the spring 2011 season when temperatures started to increase and the rainy season ended. It allowed the soil to be saturated with water, which created waterlogged conditions that is characteristic of an oxygen-deprived state and conducive for denitrification to take place in the long-term (Pinay et al., 2000). We found higher rates of N_2O emissions in spring, when soils are still wet. However, little consistent differences are apparent when expressing the results by invasion status, suggesting that invaded riparian ecotones are not emitting more N_2O that either natural and cleared riparian ecotones.

Denitrification is also influenced by the availability of a N and C source (Skiba, 2008), which can only practically be carried out by incubation of soils at constant temperature and moisture. One of the N sources that may be utilized by denitrifying organisms is NO_3^-, an inorganic N form, as a result of the end-product of nitrification carried out by nitrifying soil organisms. It is evident that there was a N source available for denitrification to take place, which was most likely NO_3^- when considering the general positive N mineralization rates found in the study. Little increase was found when NO_3^- was added to soil slurries in the laboratory, hence, denitrification for these sites may not be limited by NO_3^-. When looking more specifically at the effect of invasion and clearing on these soil processes and their products, it is clear that invasion by *Acacia* species such as *Acacia mearnsii* and *Acacia longifolia* did not lead to an increase in soil N_2O emissions, nor did a decrease in N_2O appear after alien clearing has taken place. From these results it appears that denitrification is not a major avenue of N loss in fynbos riparian zones invaded by *Acacia* species, nor does it appear important when *Acacia* species are removed from riparian ecotones. The reason that denitrification may seem less important here than in other temperate riparian ecotones, may be seated in two other finding. The first is that the soils were almost uniformly sandy, with little clay content, which may not be conducive to high rates of denitrification, even if all substrate and environmental conditions have been met (see for instance Pinay et al., 1992). On the other hand, as noted earlier, the optimal conditions for denitrification may be temporally separated, with the wettest (hence possible anaerobic conditions) in winter when temperatures are lowest (Bernal et al., 2007).

6.4.4 Drivers of nitrogen cycling

It is clear that soil bulk density and soil particles size play a major role in controlling soil available N in riparian soils, while the C/N ratio of substrates, microbial growth- and N-use efficiency regulate N mineralization and immobilization rates (Hart et al., 1994; Austin et al., 2004). There exist a close relationship between total N and C, thus soil C/N ratios may play an essential role in controlling the rate of OM decomposition and consequentially, regulating the rate at which nutrients are released and cycled (DeBano, 1990). The negative, but significant relationship between NMP rate and soil C/N shows that soils with low C/N ratios have higher potential mineralization rates (Bengtsson et al., 2003). Numerous studies have shown that litter C/N correlates with decomposition and N mineralization rates, also in fynbos soils (e.g. Yelenik et al., 2007), and in many ecosystems, also fynbos, rates of microbial decomposition can be predicted fairly well from soil TN content and soil C/N ratios (Stock et al., 1995). A soil C/N below which net N mineralization readily occurs is in the range of 25-30 (Prescott et al., 2000). However, Eviner and Chapin (2003) suggested that soil the C/N ratio which controls the threshold between N mineralization versus immobilization is variable and often differs between species. Even if soil C/N ratio and soil available N are proximate predictors of site specific variations of N transformations, other, more stochastic factors such as temporal variation in soil moisture and temperature may influence N decomposition and mineralization more than the spatial variation of the soil C/N (Bengtsson et al., 2003; Erhenfeld, 2003).

We uncovered a positive relationship between soil silt and clay contents and soil biogeochemical processes such as NMP rate (and APME activity, see in subsequent chapters). Soils that have high contents of fine materials can accommodate higher levels of microbial biomass and OM , allowing greater retention of nutrients (Austin et al., 2004). It can therefore also be deduced that soil particle size influences the rates of microbiological processes involved in nutrient cycling (Pinay et al., 1995). Furthermore, we found that soil bulk density, which is a good indicator of OM content (Robertson et al., 1999; Chapin et al., 2002), was significantly correlated with APME rates, hence enzyme activity, and may also influences NMP rates by controlling dynamics of soil water and nutrients in soils (Chapin et al., 2002).

In general, relationships found here differed between landscape positions and invasion statuses. The position within the landscape appears related to activity of NMP rates in fynbos riparian soils, perhaps as a result of different soil properties and ecohydrological factors related to each lateral zone. Available N was also positively related to soil moisture, suggesting that the lower positions in the landscape may contain higher soil available N because of higher soil moisture. However, this does not hold for the wet banks, which are constantly leached, and also show immobilization rather than net N mineralization.

Correlations between silt and clay and NMP rates measured in this study and also other studies, e.g. Bechtold and Naiman (2006) suggest important linkages between soil particle size and resource availability. Fine-textured soils correlate well with soil N mineralization rates, soil fertility, and soil microbial biomass (Pinay et al., 1992; Chapin et al., 2002; Eviner and Chapin, 2003; Naiman et al., 2005), has higher labile pools of N and C as well as water-holding capacity compared to coarsely-textured soils, and also frequently show a much higher flush of N mineralization (Austin et al., 2004). Differences in soil NMP rates observed between invasion statuses and landscape positions in our study could also be due to microbial utilization of organic N compounds, which may vary in concentration (quantity), and in ease of utilization by, or accessibility to, microorganisms (quality) (Fyles et al., 1990). Wet banks and dry banks differ substantially in important ecohydrological characteristics, and erosion in wet bank zones during floods likely hampers the accumulation of OM, which may explain their low soil total N and available inorganic N and process rates compared to dry bank zones, and also the propensity towards immobilization. Well-defined pedological and ecohydrological differences at short distances between lateral (wet and dry bank) zones are typical for steep, river-influenced zones within catchments (Gregory et al., 1991; Ettema et al., 1999), of which the upper reaches of fynbos riparian ecotones is a good example. These spatial differences may strongly influence the effectiveness of nutrient removal associated with various distances from the river (Ettema et al., 1999). The gradient of soil moisture and soil particle distributions from riparian to upland exerts some control over nutrient cycling.

7. CARBON CYCLING IN NATURAL, *ACACIA*-INVADED AND CLEARED RIPARIAN ECOTONES IN THE WESTERN CAPE

7.1 INTRODUCTION

According to Paulo et al. (2009) soil respiration is the result of plant litter decomposition, soil OM decomposition (microbial or heterotrophic respiration) and also root respiration (autotrophic respiration), and is proportional to net primary productivity of the ecosystem. Faunal activities also forms a small part of overall soil respiration. Soil respiration is a major process that controls C exchange between soil stocks and atmospheric C stocks, and the spatial and temporal variation in soil respiration (R_S) rates are thus of great interest to ecologists. Moreover, changes in R_S rates (also sometimes referred to as soil CO_2 efflux) induced by anthropogenic stressors may play an important role in global C dynamics, which may impact ecosystems in terms of small and large climatic shifts (Luo and Zhou, 2006). At a landscape scale, R_S rate is influenced by environmental factors such as temperature, soil moisture, and ecosystem properties such as root biomass, soil C stocks and plant physiological dynamics (e.g. plant respiration) (Lloyd and Taylor, 1994; Tufekcioglu et al., 2001; Wiseman and Seiler, 2004). In MTE ecosystems, soil water content is a major factor that influences soil CO_2 efflux, thus usually constraining CO_2 efflux during periods of drought stress (Inglima et al., 2008). Increasing temperatures, concomitant with drought in MTEs, also influences R_S rates. Soil respiration is generally low in dry conditions (late summer in MTEs) and increases to a maximum at intermediate moisture levels, and then declines again when moisture content excludes oxygen (Saiz et al., 2006). On the other hand, plant physiological dynamics have been shown to influence R_S rates on an hour-to-day scale through root CO_2 exchange (autotrophic R_S).

One of the areas most heavily invaded in the fynbos biome are riparian ecotones, with *Acacia* species some of the most prevalent woody invasive species (Dye et al., 2004; Le Maitre et al., 2011). Morris et al. (2011) suggested that invasive *Acacia* species are more productive and grow taller compared to native species, especially in riparian ecotones. Where IAPs occur and management intervention has not been applied, invasive acacias tend to establish and regenerate quickly to form dense, sometimes monoculture stands, entirely displacing native species (Holmes et al., 2007; Richardson et al., 2007). Litter produced by *Acacia* species tend to have lower C/N ratios than native species (the latter mostly tending to be sclerophyllous), and thus one would expect decomposition of *Acacia* litter to be more rapid, releasing C, N and other nutrients quickly into the soil (Le Maitre et al., 2011; Naude, 2012). Root density of these invasive plants tend also to be higher than native plants (Morris et al., 2011), especially where dense stands of invasive trees have established in riparian zones, soils of which tend to be sandy and porous.

We aimed to measure soil respiration (*in situ*) at three different landscape position, namely the wet bank zone, the dry bank zone and the adjoining terrestrial area, where soil moisture conditions and soil properties have been shown to be somewhat different (Naude, 2012). Our expectation is that R_S will be highest where intermediate soil moisture conditions exist (this may change on a seasonal basis), though soil temperature will also be a major driver of $R_{S.}$ Further, we also measured R_S in natural, invaded and cleared riparian ecotones, and expects that R_S will be higher in invaded riparian ecotones due to the propensity of *Acacia* stands to have higher biomass, more dense stands, more litter production. We also took samples for incubation of soils under constant conditions in the laboratory (*ex situ*), and conducted trenching experiments to determine the influence of roots on total RS. This part of the project relates to objective 3.

7.2 METHODS

7.2.1 Soil C stocks

We used the same procedures for sampling soil C stocks that has been described for soil N stocks in Chapter 6.

7.2.2 *In situ* soil respiration

Twenty-four hours before soil respiration measurements were taken, 15 polyvinylchloride (PVC) soil collars with an internal diameter of 10 cm and a length of 5 cm were installed in the riparian and upland sampling plots (one per plot) leaving a 1 cm rim extending above the soil surface. The soil chamber of the infrared gas analyser (IRGA) is held by the collar during measurements. Collars were used to avoid disturbing the soil within the soil chamber each time a measurement was being made. Soil respiration was measured using a LI-8100 portable carbon dioxide infrared gas analyser (Li-Cor Inc., Lincoln, Biosciences, USA), that had an 8100-101 soil chamber attachment. Soil CO_2 efflux was measured twice per day by placing a 10.7 cm chamber on the collars and measuring the rate of increase of CO_2 concentration over 2 min periods with a portable IRGA connected to a laptop. Measurements were generally taken at mid-morning and mid-afternoon, once a season at each plot, thus 15 (collars) x 11 (sites).

Sample locations were of three types: the upland sites, with no riverine influence, the dry bank (inundated several times during the wet season, but mostly dry at the soil surface) and the wet bank, closest to the stream, and wet throughout. Every time a collar was sampled, two replicate soil respiration measurements were also taken at that collar. Any plants growing inside the collars were cut off at the ground surface level to prevent any new root growth which might influence R_S measurements. Further, the soil collars and the soil chamber were opaque, so no photosynthesis was expected to take place during soil respiration measurements. We measured soil respiration rates as CO_2 flux µmol m^{-2} s^{-1}.

At the natural sites dominant vegetation was by fynbos plants and riparian trees. The most prominent families are Ericaceae, Proteaceae, Iridaceae and Rutaceae. Some typical fynbos genera *Aspalathus, Agathosma, Crassula, Erica* and *Pelargonium* were found in the area (Boshoff et al., 2000). The trees grew in the dry banks while the stands fynbos were located furthest apart from the river in the upland areas. Fire had passed through the area (Jonkershoek and Dwarsberg) in March 2009, but the regeneration of trees and grass vegetation was already taking place when the 2010 R_S fieldwork took place.

Measurements at *Acacia*-invaded sites were done along a gradient within the site (wet bank nearest the active channel, dry bank and terrestrial area), within a space of 50 to 100 m, depending on the invasion status and site gradient. The riparian area was densely covered with alien plants, mostly *A. mearnsii* and occasionally *A. longifolia*. Some native riparian vegetation includes species of Restionaceae, Cyperaceae and some woody elements.

At the cleared sites, soil CO_2 efflux measurements were made at riparian locations (wet and dry banks) and adjacent hillslope sites (terrestrial area) over a distance of between 50 to 100 m. Often the surrounding area was bare, while native and invasive graminoids often dominated.

At each plot, environmental measurements were collected parallel to R_S measurements on every day of data collection from autumn 2010 to spring 2011. While measuring soil respiration rates, we simultaneously recorded soil temperature at 5 cm depth (TS, °C) and volumetric soil moisture within the 12-20 cm depth (SWC, %). With each CO_2 efflux measurements, soil temperature was recorded using an incorporated soil temperature probe placed near the collar at 5 cm depth, and this temperature was recorded. Similarly, a soil moisture probe (Hydrosense, Campbell Scientific Inc., Australia) was used to measured volumetric soil water content of top 12 and 20 cm of soil nearby each collar. During the seven intensive sampling campaigns, soil was sampled using a metallic soil tube at the 5 cm depth for gravimetric determination of water content. Initial mass of soil samples collected was measured in the laboratory with digital scale and samples were dried in an oven at 105 °C in the lab, followed by measuring of dry mass on the same scale.

At all three treatments (natural, invaded, and cleared riparian ecotones) from June 2010, hourly measurements of soil volumetric water contents (m^3 m^{-3}) were obtained with ECH2O-TE sensors, interfaced with data loggers (EM-50, Decagon Devices Inc. USA), buried 20 cm beneath the soil surface in natural invaded, and cleared riparian ecotones. These devices were not available for the earlier campaigns, and soil water content was determined gravimetrically close to each collar (and also for each campaign as basic soil moisture data). Volumetric water content (m^3 m^{-3}) was calculated by assuming a soil particle density of 2.65 g/cm^3 and converted into gravimetric soil water content.

7.2.3 *Ex situ* soil respiration

In June 2011 soils from natural, *Acacia*-invaded, and cleared riparian zones were sampled to investigate the effect of invasion and clearing on R_S, using an incubation or microcosm methods to measure potential soil respiration (PR_S). Soil samples weighing 800 g were placed into sealed PVC tubes for incubation. After the initiation of the experiment by adding water, the whole soil was incubated in the dark at 30 ^{0}C and maintained at 60% WHC by periodic weighing and addition of small amounts (10-20 ml) of distilled water. Using a method modified from Robertson et al. (1999), potential soil respiration was measured at day 1 and subsequently every seven days for 28 days after initiation of the experiment using an IRGA (Li 8100, Li-Cor Biosciences).

The IRGA response to CO_2 emission was calibrated against prepared standards of 380 and 630 µmol mol^{-1} CO_2. Two IRGAs were available in case one needed to be calibrated, and measurements were routinely compared in the laboratory to ensure validity. The instrument was allowed a 30-minute warm-up time, after which it remained on for the duration of measurements. Potential CO_2 measurements required 2 - 5 min circulation time before stabilized were recorded. Two empty PVC tubes were used as blanks. The soil inside the microcosm was representative of the soil collar in the field, leaving a 1 cm perimeter extending below the microcosm's rim, allowing air to flow between the bulked soils and the chamber for CO_2 emission during measurement. Measurements were carried out before midday to minimize temperature variation during CO_2 measurement in the laboratory. It took approximately five hours to complete the first cycle. To avoid pulses of CO_2 due to pressure fluctuations created by opening and closing the lid, the measurement interval was confined to that with minimal pressure fluctuations. The short time used to measure the increase in CO_2 within the microcosm headspace (60-120 s) reduced diffusion artefacts that may have affected the flux estimates (Pumpanen et al., 2004). The soil moisture measurements were taken before the wet-up amendment (distilled water) and after CO_2 emission measurement using a portable probe (Hydrosense: Campbell Scientific, Australia). Soil temperature was measured continuously at 10 cm depth using temperature probe attached to the IRGA, despite the 30 ^{0}C constant temperature of the incubator room.

Composited soil cores were collected from 16 to 30 June 2011. By this date, rain at the sites had ceased for two to four days, the grass was partially dried and trees were still photosynthesizing (green and with mostly full canopies). In riparian and upland areas, ten soil cores were collected using a stainless steel tube soil sampler from the upper part of the soil profile (0-10 cm). Before soil collection, the upper-most layer of litter with visible under-decomposed materials was removed. Collected soil samples were kept separately in labelled plastic bags and transported to the laboratory in a cooler box. Soil samples were air-dried inside the laboratory room by spreading it on the benches for about 72 hours. Soil lumps were gently crushed and gravel, roots and coarse debris were removed by hand and the soil finally sieved through a 2 mm sieve mesh. This was to ensure that it was free of any large pieces of root or other organic material. Subsample soils for chemical and physical analysis were stored in a refrigerator at 4°C prior to the sampling process. To insure that soils were well settled before measurement, the microcosms were left equilibrated for one day before taking the first CO_2 flux measurements and the incubation course started.

Soil moisture was determined gravimetrically as the ratio of the weight of water and the weight of dry soil is expressed as a percentage. The weight of water was determined as the difference between the weight of the sample and its weight after oven drying at 110°C for 24 hours.

Soil pH and electrical conductivity (EC) were measured in a 1:2 aqueous extract. Soil particle size was analysed by a rapid method according to Kettler et al. (2001). This method was followed as described, except that clay and silt were sieved into 350 ml beakers and rinsed with 250 ml deionized water. Litter mass from three different riparian ecosystems were collected using a 25 m^2 frame and then placed in paper bags. In the laboratory, the litter mass was weighed and oven dried at 70°C. Soil bulk density of the upper 10 cm soil was measured with 4.5 cm inner diameter cylindrical sampler .Soil root density was measured from soil samples collected from the upper 10 cm of soil using a hand auger (4.5 cm inner diameter). The samples were dried (at 60°C), and the roots manually collected, while finer roots were collected after washing and sieving, dried, and weighed in the laboratory. Litter mass, root density was carried out twice over the study period (inclusive of both the *in situ* and *ex situ* experiment), while soil particle size was carried out once.

7.3 RESULTS

7.3.1 Soil carbon stocks

We found no significant interactions between invasion statuses and seasons for soil total C ($p = 0.67$), but significant differences between invasion statuses for total C (one-way ANOVA: $p < 0.05$) were apparent when seasons were pooled (Figure 7.1A), but not for landscape position (Figure 7.1B). Natural sites were not different from invaded sites ($p = 0.09$) with regard to total C (TC) content, but cleared areas had a significantly lower TC content ($p < 0.01$) relative to invaded sites.

We found an effect of season on soil C/N however this was only apparent at cleared sites ($p < 0.001$); Figure 7.1C). Soil mean C/N was lower (6.4%) in soils from underneath acacias compared to native stands (spring), however this was not significant. Cleared sites showed high soil C/N ratios which differed significantly from invaded ($p < 0.05$), however did not differ from natural ($p = 0.22$) sites (when all data were pooled). Soil total C differed significantly between landscape positions with upland and dry bank soils showing higher TC compared to wet banks (Figure 7.1B). We recorded significant differences between landscape positions were for soil C/N (one-way ANOVA: $F_{[2, 95]} = 4.03$, $p < 0.05$). Wet bank zones were characterized by high mean C/N ratios (31.5) and differed significantly from upland (25.8; p <0.01), but not dry bank (20.7; Figure 7.1D) zones.

7.3.2 *In situ* soil respiration

Soil temperature (TS) and gravimetric soil water content (GSWC) were significantly different between seasons (p<0.05). Maximum GSWC coincided with minimum TS during wet season campaigns whereas minimum SWC were measured in summer when TS were high (Figure 7.2).

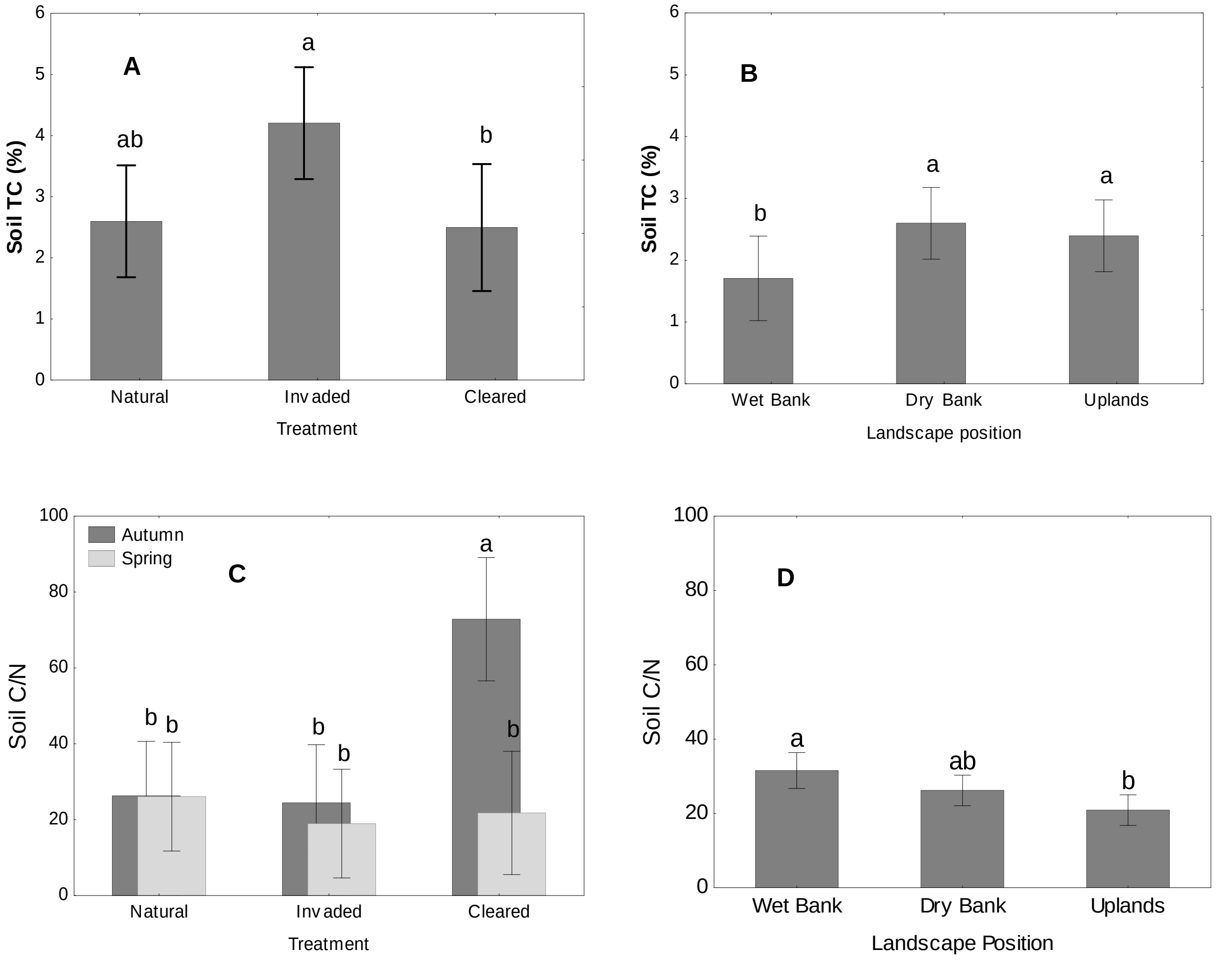

Figure 7.1: Soil total C for (A) invasion statuses and (B) landscape positions. Soil C/N for (C) invasion status across seasons and (D) landscape positions. Bars represent means and whiskers represent ± 95% confidence intervals for percentages (soil TC) and ratios (C/N). Significance levels (Tukey's post hoc test**;**

p<0.05) are indicated by different letters for one-way ANOVAs. All statistical analyses were computed on log-transformed data to meet the assumptions for ANOVA.

Soil temperature varied from 10-18°C during the wet season campaigns and 20-30°C during the dry season campaign (Figure 7.2). Soil temperature declined by ~ 5°C by the beginning of May (autumn) at most all sites. A sharp increase occurred by end of spring, early summer coincided with warm weather. Winter was the period with the highest measured GSWC, with soil sometimes reaching saturation at riparian zones but remaining lower in the terrestrial areas. High GSWC in winter corresponded to recent rain events before CO_2 efflux measurement which peaked in the end of May and decreased over September (spring), with lowest values (3 - 6.5%) in January and February in the terrestrial areas.

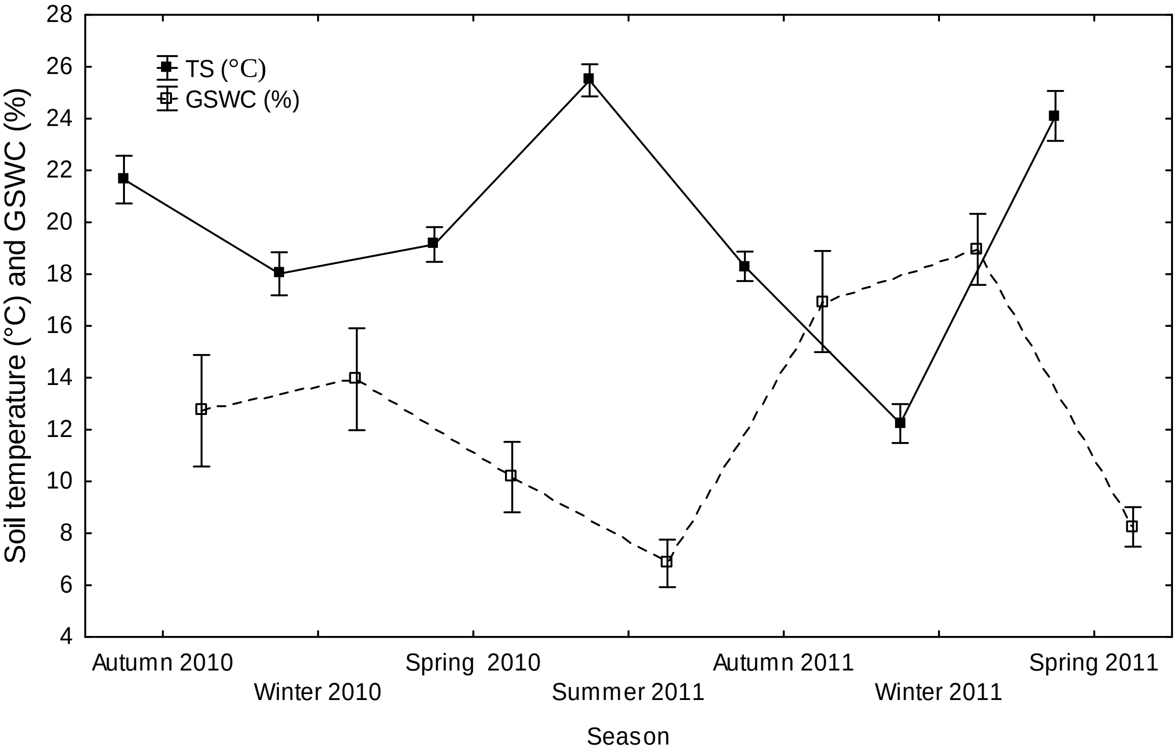

Figure 7.2: Seasonal variability of soil temperature (TS °C) and gravimetric soil water content (GSWC %) during seven field sampling campaigns at natural, invaded and cleared ecotones. Data are means and standard errors.

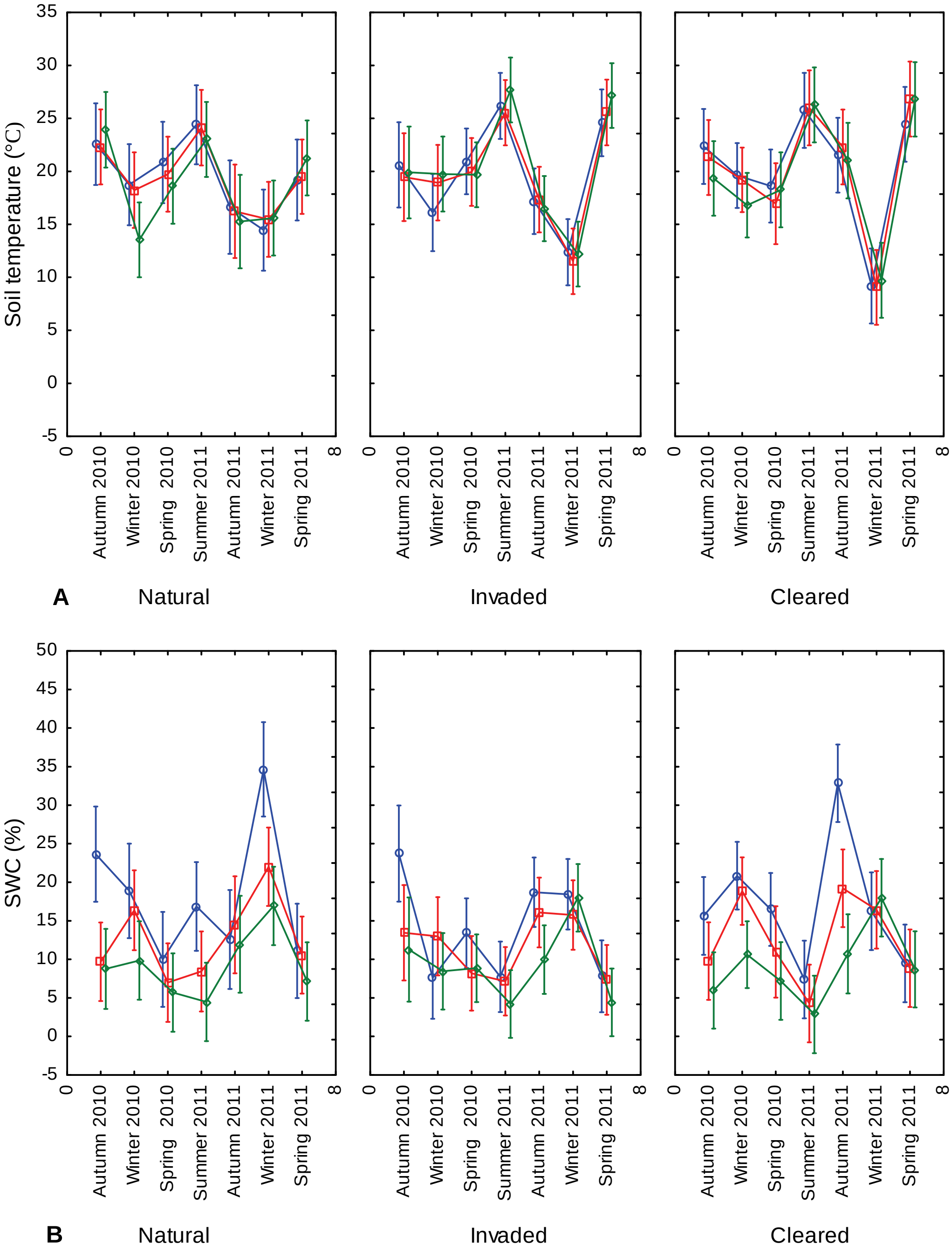

Figure 7.3: Measured soil temperature (°C) at 0-5 cm depth (A) and gravimetric soil water content (SWC %) at 0-5 cm depth (B) in riparian ecosystems across seasons. Data are means and standard errors for seven campaigns and are categorized by landscape and invasion status. The blue colour represents wet banks, red dry banks, and green colour terrestrial sampling sites.

Overall, during the study period, mean soil temperature was significantly higher in natural and cleared sites than in the invaded sites ($p < 0.05$; Figure 7.3A). There were no significant differences in soil temperatures across landscape position (means including all seasons), with wet banks at 19.82°C (± 0.39, standard error), dry banks at 19.78°C (± 0.39) and 19.63°C (± 0.38) in the terrestrial upland areas (p=0.76) (Figure 7.3A). The same results for TS was also observed across and between treatments (p=0.61; means including all seasons).

Soil temperature showed a similar trend among the three treatments during the study period (Figure 7.3A), increasing dramatically from spring until summer to reach a maximum in January 2011 (25.44°C), and falling through winter to reach the lowest value in June 2010 and 2011 (12.17°C) . There were significant differences between seasons for both years (p<0.05). Soil temperatures were slightly cooler under tree canopies (riparian) than out in the adjacent upland, but this was not statistically significant. Soil temperature (0-5 cm) was not significantly different across treatments and within riparian and terrestrial landscape areas (Figure 7.4A). Average TS at different landscape position were within 19.82°C (± 0.40) of each other across seasons.

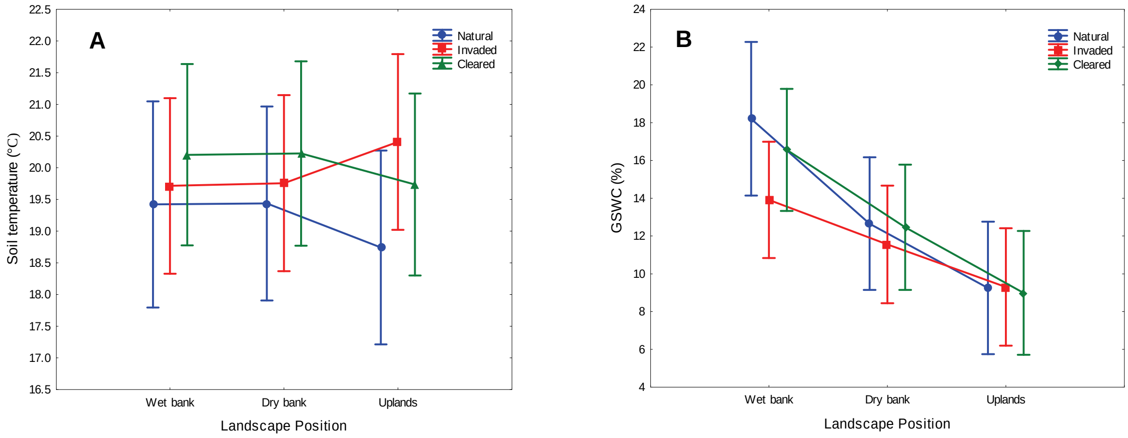

Figure 7.4: Measured soil temperature at 0-5 cm depth (TS °C), gravimetric soil water content (GSWC %) at 0-5 cm depth. Data are means and standards errors for seven seasons and categorised according to invasion status (A) and landscape position (B).

Mean gravimetric soil water content (expressed by invasion status; across all seasons) at 0-5 cm depth varied from 13.37 (natural), 11.59 (*Acacia*-invaded) and 12.91% (cleared sites), and was not significantly different (p=0.54) (Figure 7.3B). GSWC, expressed by landscape position (across all seasons), were highest in the riparian zones, with wet banks averaging 16.37 (± 0.94), dry banks 12.27 (± 0.89) and terrestrial areas 9.23 (± 0.89%), and differences were statistically significant (p<0.05) (Figure 7.3B; 7.4B). There were bigger differences between the dry bank and the wet bank (p=0.00) than there were between the dry bank and the terrestrial area. GSWC was above 10% from late autumn to late winter (end of August), except for wet banks because of its proximity to the river channels (Figure 7.3B). In addition, GSWC in the *Acacia*-invaded sites was consistently lower overall compared to the other treatments throughout the study period and no significant differences were found between natural and cleared riparian sites, but significant differences was observed between riparian (wet and dry banks combined) and terrestrial areas (Table 7.1). There were significant differences in GSWC between riparian and upland areas.

Table 7.1: Analysis of variance (ANOVA) statistics for riparian zones and upland areas versus treatment, season, soil temperature (TS °C), gravimetric soil water content (GSWC %) and soil CO_2 efflux µmol m^{-2} s^{-1} (0-5cm) from autumn 2010 to spring 2011. (DF: Degrees of freedom, F: Statistics, P: Probability F value at P < 0.05.

	Source	**DF**	**F**	**P-value**	**Significance**
Treatment X Landscape position	R_S	4	6.11	0.00	**
	TS	4	1.67	0.20	n.s.
	GSWC	4	0.76	0.05	***
Treatment X Season	R_S	12	3.87	0.00	***
	TS	12	1.85	0.07	n.s.
	GSWC	12	3.51	0.00	*
Landscape position X Season	R_S	12	1.22	0.25	***
	TS	12	2.32	0.00	n.s.
	GSWC	12	2.97	0.00	***
Treatment Landscape position X Season	R_S	24	1.25	0.18	**
	TS	24	1.54	0.04	**
	GSWC	24	2.60	0.00	***

*n.s. indicates non significance, **: significant and ***: indicates highly significant.*

Soil respiration rates in the natural, *Acacia*-invaded and cleared riparian ecotones were significantly different across treatments (p=0.01) and seasons. Highest rates of R_S were measured in the *Acacia*-invaded compared to riparian sites within the other treatments (Figure 7.5A). All the invaded sites (Wit River, Lower Molenaars, Lower Dwars and Lower Jakkals) had significantly higher R_S compared to natural and cleared sites (Figure 7.5B).

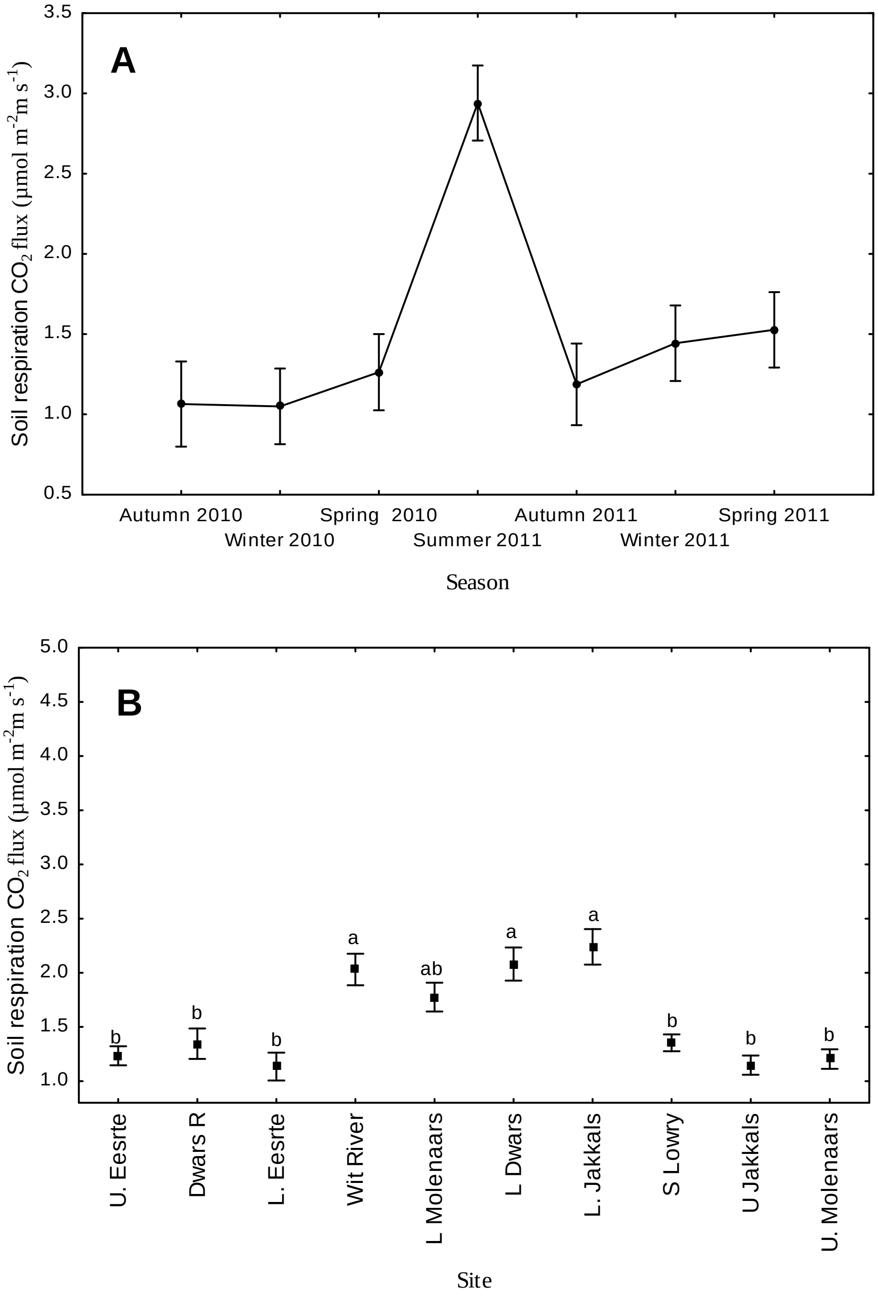

Figure 7.5: (A) Mean (±SE) seasonal soil respiration rates (µmol m^{-2} s^{-1}) averaged over the period of measurement and (B) mean (± SE) soil respiration at each of the sites over the duration of the fieldwork (seven seasons). U = Upper; L = Lower. Different letters denote significant difference at $p < 0.05$ analysed using Tukey's test after a two-way ANOVA.

Different invasion statuses showed differences in soil CO_2 efflux between and within seasons and across riparian and upland areas (Table 7.1; Figure 7.6). Soil respiration was significant different at different landscape position ($p=0.01$) lowest rates of soil respiration were observed during all campaigns in the upland areas under wet and dry conditions. Soil respiration was significant different in the wet banks and dry banks ($p<0.05$). Overall, mean values of soil CO_2 efflux ranged from 0.47 - 5.65 (μmol m^{-2} s^{-1}) for invaded sites, from 0.31 to 2.89 μmol (m^{-2} s^{-1}) for the natural riparian sites and from 0.22 to the 2.53 μmol m^{-2} s^{-1} for the cleared treatment. The soil CO_2 efflux increased during summer in all treatments when soil temperature varied from 12 to 35 °C. All treatments experienced low values when soil temperature was less than 15°C. Differences in R_S increased with soil temperature in spring and summer and were the highest when soil temperature exceeded 25 °C.

During the low temperature period of May to July, the R_S rate was not significantly different between treatments. From the late September until the end of summer experiment, the treatment effect was highly significant ($p<0.001$). Soil respiration increased steadily during spring and summer following increases in soil temperature in March, until soil respiration reached a maximum of 7.34 μmol m^{-2} s^{-1} in summer 2010/2011.

7.3.3 Drivers of *in situ* soil respiration

When all R_S, TS and GSWC data was viewed together, some trends emerged, notably that R_S is positively related to ST, and negatively to GSWC (Figure 7.7 A and B). However, these relationships were not significant. Very prominent, however, is the ranges where maximum R_S takes place – R_S rates were highest at a GSWC of between 0 and 20%, with maximum values between 0 and 5%.

When values were averaged for seasons and invasion statuses and landscape positions, more clear relationships emerged, with highest R_S at >20°C and below 12% GSWC (Figure 7.7 C and D).

Variation in R_S throughout seasons was due to changes in GSWC during the wet season in autumn and winter but shifted in spring and summer when changes in soil temperature became more influential. Soil respiration increased markedly in summer 2011 following increased soil temperature, and reached a highest means CO_2 flux of 5.65 (± 0.33) in invaded sites, 3.37 (± 0.36) in natural and 2.53 μmol m^{-2} s^{-1} (± 0.13) in the cleared sites, this continued until early autumn. R_S then declined in autumn and winter of both years and the effect of soil temperature become apparent as soil water content increased and soil temperature fell below 15°C.

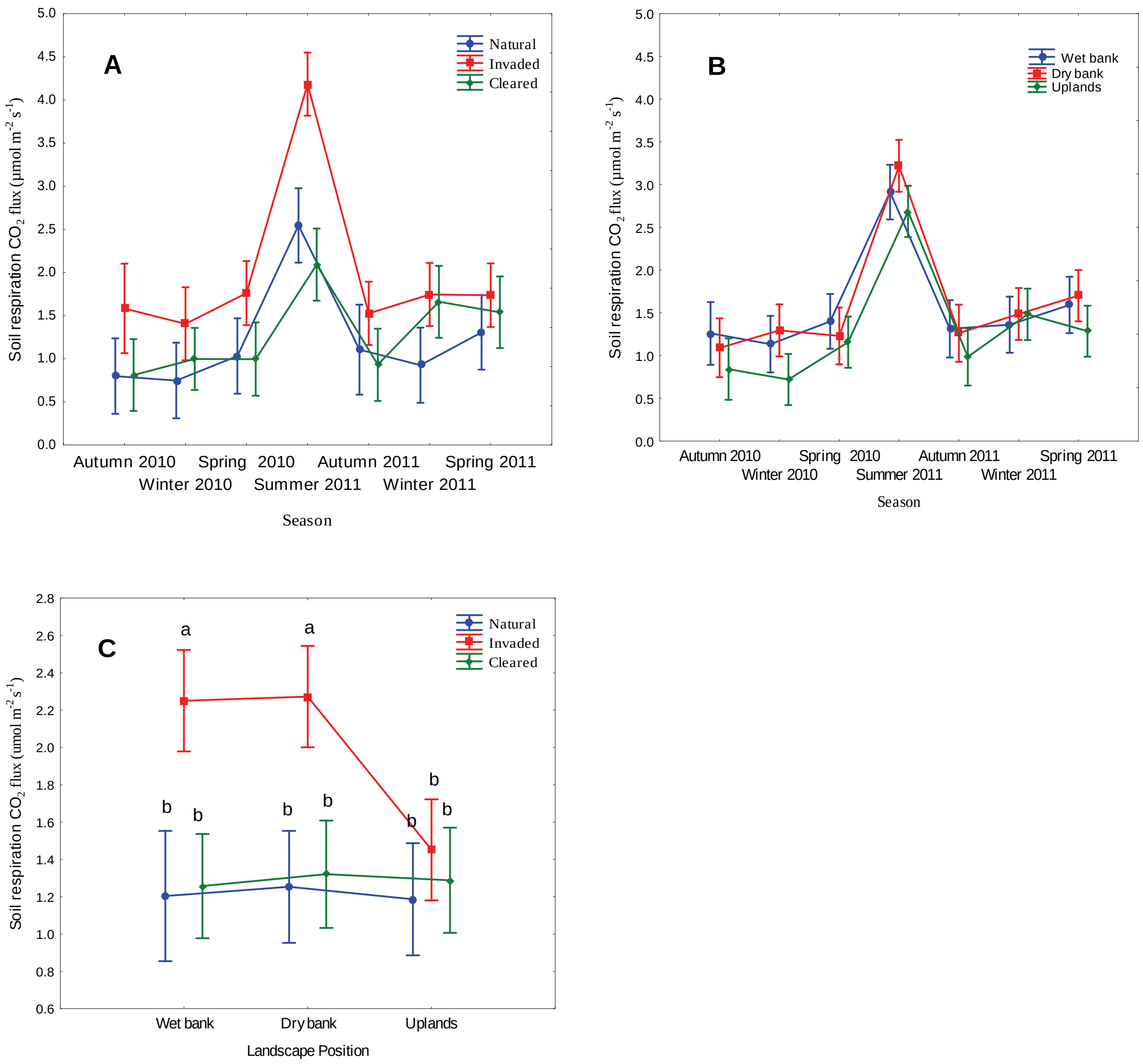

Figure 7.6: Mean (± SE) seasonal soil respiration across season arranged by invasion status (A) and landscape positions within season during seven campaigns (B), and differences between natural, invaded, and cleared riparian sites across landscape positions (wet, dry banks and terrestrial areas) (C). Different letters denote significant difference at $p < 0.05$ analysed using Tukey's test after a two-way ANOVA. The symbols are the means and the whiskers standard errors.

It emerged that TS may be the limiting factor for soil respiration in fynbos MTEs as the maximum R_S occurred when temperature reached a range of between 28 and 30°C. This may explain the limiting effect of soil temperature on soil respiration in fynbos riparian ecotones as CO_2 efflux increased quickly in response to warm and dry condition compared to the wet and cold season occurring during winter. Mean values of soil respiration were low, less than 1 µmol m^{-2} s^{-1}, or close to zero during autumn and winter 2010 campaigns and increased dramatically in late January and early February 2011 (summer) and declined from autumn until the end of the wet season.

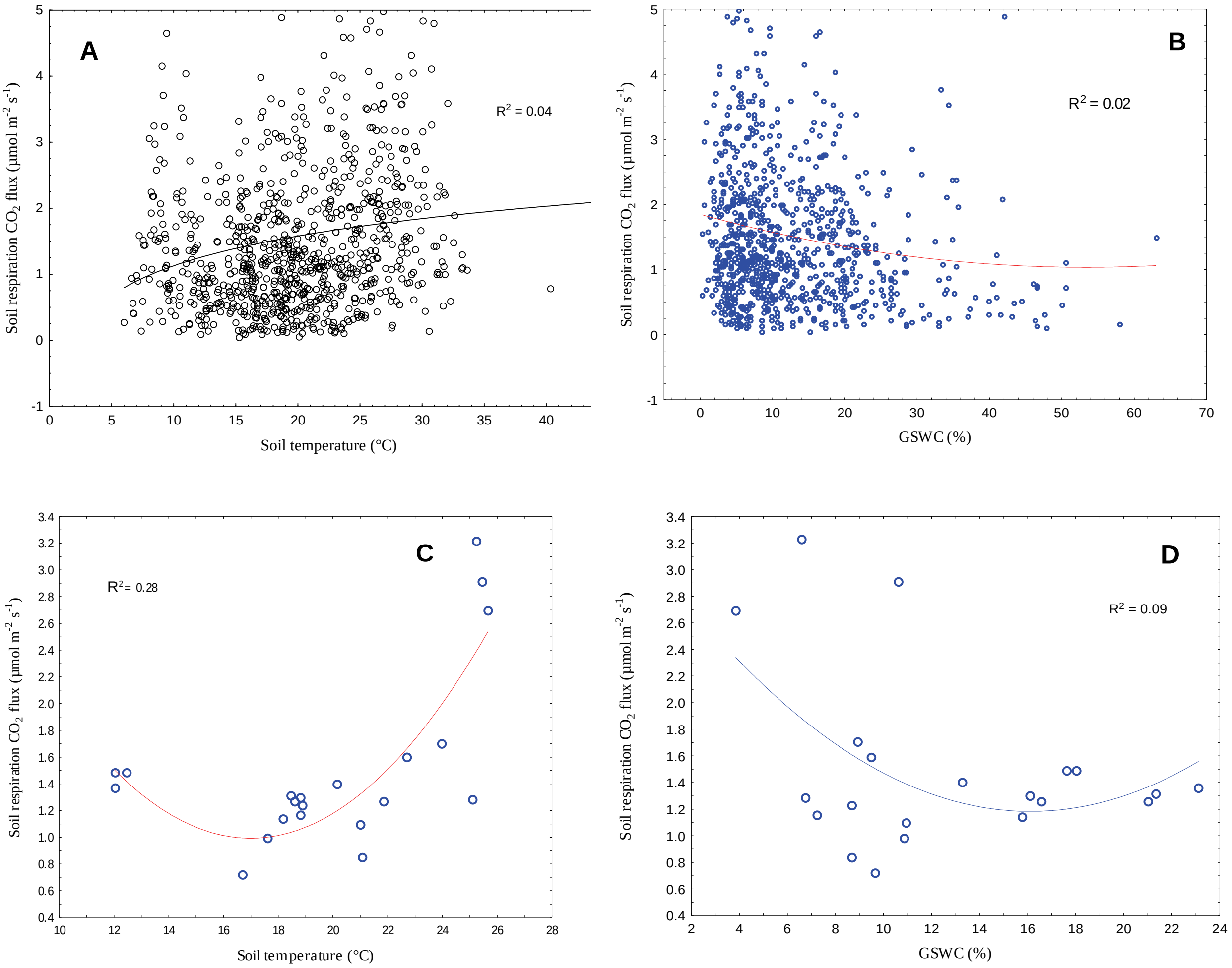

Figure 7.7: Effect of soil temperature on the rate of (A) soil respiration during seven campaigns from autumn 2010 to spring 2011 in the natural, *Acacia*-invaded, and cleared riparian sites and (B) interaction between soil respiration CO_2 efflux and soil water content (%) within across season and within riparian ecosystems. The dots represent actual data and the solid line represents the fitted regressions. C and D: relationship between soil respiration against soil temperature at 0-5 cm depth (C) and soil respiration against gravimetric soil water content at 0-5cm depth (D).

7.3.4 *Ex situ* soil respiration

Soil respiration was also determined in the laboratory by incubating soil at constant soil moisture and temperature and the rate of CO_2 emission determined after 21 to 28 days, starting on day 1, and measuring on a weekly basis. Figure 7.8 shows the results of the western Cape sites after 1, 7, 14, 21 and 28 days of incubation at 30°C and 60% water holding capacity.

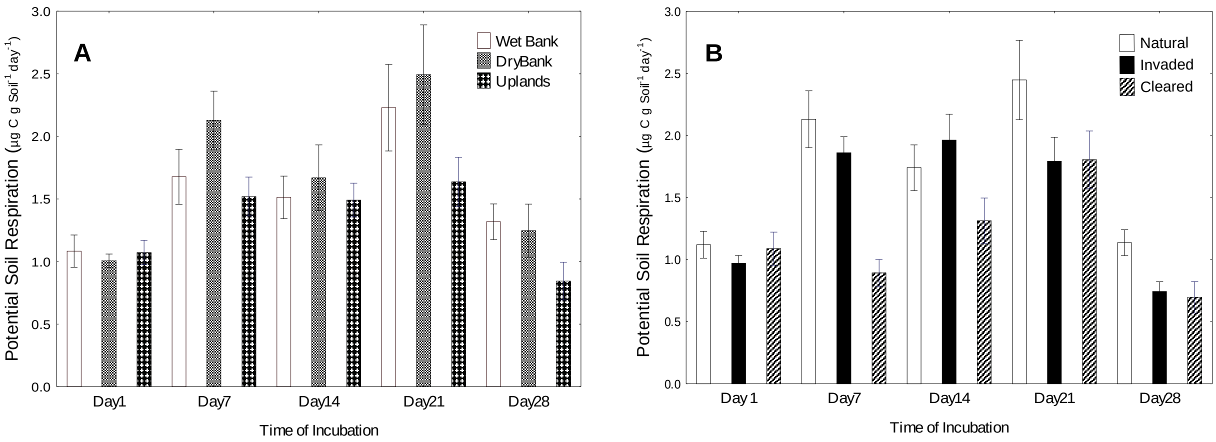

Figure 7.8: *Soil respiration rates in incubated soils of natural, invaded and cleared riparian ecotones and associated upland areas. (A) data arranged by landscape positions, and (B) data arranged by invasion status. The bars represent the means and the whisker is the standard error.*

Incubated soils did not show any particular or consistent pattern in terms of R_S rates when categorised according to landscape positions (Figure 7.8). Soil respiration increases from day one to day 7, declined and increased again at day 21. However, when expressed according to invasion status, natural and invaded sites had slightly higher R_S rates after days 7-14, and natural sites after day 21.

7.3.5 Trenching experiment

Autotrophic soil respiration can be separated from heterotrophic soil respiration by trenching of soils (Tang et al., 2005). This can be done by digging a trench around a block of soil from where all plants are excluded, and from where roots from surrounds plants are excluded by using plastic sheets (Figure 7.9A and B). We elected to carry out trenching only at three of the four invaded sites as these sites had the thickest soils, and were relatively stone-free, which did not impede the digging of trenches. The results of soil respiration measurements carried out on the trenched soils are displayed in Figure 7.9C.

The results of the trenching experiment show different trends on the medium to the short term in that immediately following trenching, soil respiration rates in the trenched plots fell significantly compared to control plots (no trenching). Soil respiration showed seasonal differences in control and trenched plots and was highest in summer in control plots compared to the root exclusion plot (trenching) and decreased in autumn in both trenched and control plots with second peak of soil respiration CO_2 efflux emerged in autumn and spring (Figure 7.9). Overall, seasonal soil respiration was highest in control plot compared in trenched plots (P=0.01). Soil respiration in trenched plots was lowest during summer, 0.50 (±0.24 SE) μmol m^{-2} s^{-1} and autumn, 0.51 (±0.24 SE) μmol m^{-2} s^{-1} (P=0.00). Conversely, soil respiration did not differ between treatments during winter and spring with peak in trenched plots of 2.13 (±0.24 SE). Thus, after several months, R_S picked up in the trenched plots until differences were again non-significant.

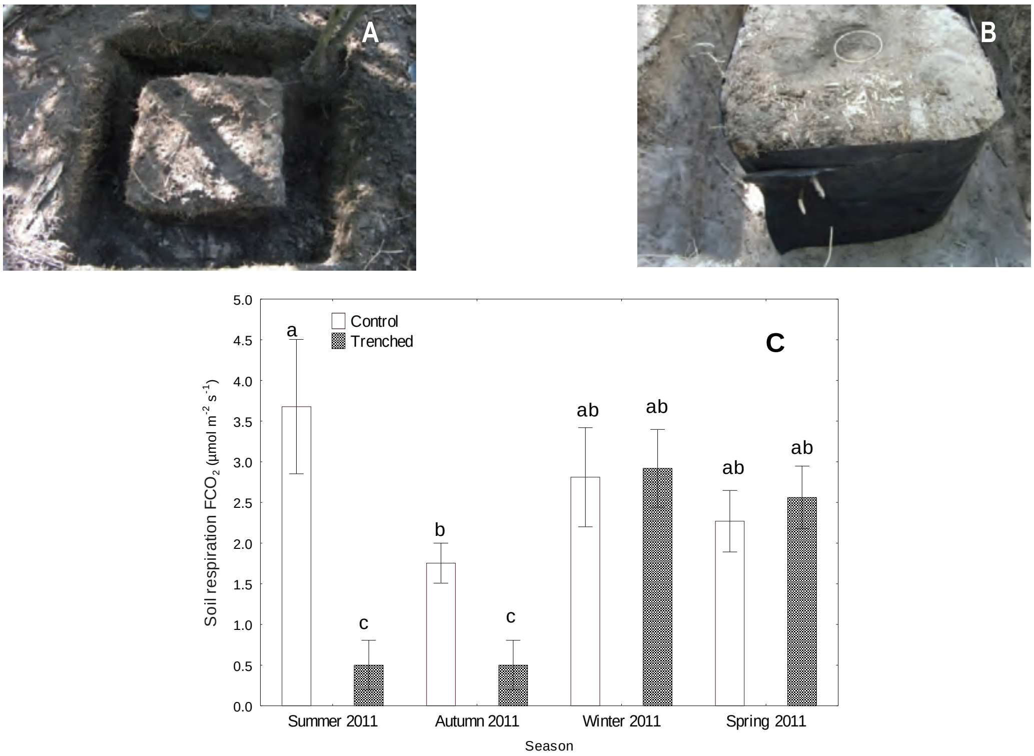

Figure 7.9: Photos of trenches dug in the invaded dry banks at the Wit River site. The first photo (A) is of a trench, showing the high root densities, and the second photo (B) shows the plastic sheeting used to exclude roots from the central soil block. (C) Soil respiration in dry banks of three of the four invaded sites. The data displayed represents areas where soil respiration was carried normally (control), and where roots were excluded by trenching (trenched). The bars represent the means and the whisker is the standard errors. Different letters denote significant difference at $p < 0.05$ analysed using Tukey's test after a one-way ANOVA.

7.4 DISCUSSION

Soil respiration rates are consistently greater in invaded sites than in natural and cleared riparian sites, which is consistent with the elevated levels of C in *Acacia*-invaded riparian ecotones, especially dry banks. Soil respiration was 43% higher on average in invaded wet and dry banks, suggesting that riparian conversion to *Acacia*-invasion would stimulate soil CO_2 emissions to the atmosphere. The biggest difference in R_S was in summer when soil respiration in invaded dry banks was more than twice the rates found in natural and cleared riparian ecotones. This does not explain the reasons for the difference, but physiological and structural differences between natural, invaded and cleared riparian ecosystems are likely involved, e.g. root density and biomass and higher decomposition rates as suggested by Yelenik et al. (2004; 2007).

7.4.1 Drivers of soil respiration

Great variation in CO_2 efflux, TS and GSWC were observed at all scales covered in this survey, between treatments and landscape position at the given sites, seasonally from March 2010 to October 2011 and conducted at approximately similar periods of the year. Generally, variables such TS and GSWC were the most important controls (other relationships were also explored but is not reported here). Numerous studies consider TS and SWC as two of the most important parameters controlling the variation in R_S (Fang and Moncrieff, 1999; Kirschbaum, 2000). The seasonal variations of soil CO_2 efflux recorded in this study generally supported these controls by temperature and soil moisture as seen previously in semiarid conditions or Mediterranean environments (Casals et al., 2000; Rey et al., 2002; Joffre et al., 2003; Ma et al., 2005; Scott et al., 2009; Matias et al., 2012). When SWC remains continuously and relatively high, temperature is the only parameter related to soil respiration variations (Ohashi et al., 1999; Thuille et al., 2000).

At all the eleven sites, soil respiration seems to be temperature limited, however, it is also clear that high soil moisture impeded soil CO_2 efflux. The increase in R_S during the summer of 2010 clearly demonstrates this. Moreover, increases in soil respiration are observed with progressive drying, even for the wet banks and dry banks riparian areas. The lowest soil CO_2 effluxes were associated with the wettest soils particularly in winter, when biogeochemical interaction between soil, root and microbes activities may be curtailed. Laboratory studies have shown that higher soil water content can disturb the diffusion of CO_2 efflux in the soil (Skopp et al., 1990), on the other hand, low SWC can decrease microbial activity and root respiration (Luo and Qi, 2001; Curiel Yuste et al., 2003). Variations in precipitation may alter root and microbial activities either by limiting aeration, inhibiting CO_2 flux and air diffusivity when high or by stressing microbial communities and root respiration when low (Davidson et al., 1998; Xu and Qi, 2001; Rey et al., 2002). According to these authors, the optimum SWC is usually somewhere near field capacity, when soil pores, are mostly air filled, thus facilitating the diffusion of soluble substrates. Thus, a more likely explanation is that CO_2 diffusion is reduced in wet soils (Luo and Zhou, 2006), hence the low R_S rates in winter, while in summer, CO_2 can diffuse without this impediment (Mantlana, 2008). CO_2 dissolves in water, hence residence times in wet soils are higher than in dry soils.

In our study the seasonal pattern of R_S followed TS for only part of the year (spring and summer), contrary to some other studies reported in the Mediterranean ecosystems (Rey et al, 2002; Joffre et al., 2003; Ma et al., 2009). Soil temperature in different treatments of natural, invaded and cleared sites varied from season to season. Overall, the highest temperature sensitivity values seem to be in the cleared sites, where the seasonal and daily temperature variation is higher than in the other habitats, with the lowest in *Acacia*-invaded sites and especially in dry banks. In addition to this high sensitivity to temperature, a positive relationship was found between temperatures and R_S, indicating that higher temperatures did increased soil respiration rates. Thus, in MTE's climate zones, a moderate increase of temperature as expected for the coming decades is likely to induce a greater soil respiration.

As hypothesized, results indicated that *Acacia* species changed soil physical and chemical properties and soil respiration varied across treatments within riparian sites and adjacent upland areas, which was potentially influenced by differences in ecohydrological properties, plant attributes, and associated spatial patterns of both soil water content and soil temperature. Seasonal variation of R_S can also be driven by changes in photosynthate production, in addition to soil moisture and temperature. The main controlling factors of seasonal variation of soil respiration may depend on the type of ecosystems and climate. Large differences observed between riparian sites in several CO_2 efflux measurement campaigns reflect the different riparian sites types. High soil respiration in *Acacia*-invaded sites may result from greater fine root biomass than in cleared sites, with the latter probably having lower roots biomass. Root respiration may contribute relatively less to soil respiration than heterotrophic respiration during the dry season (Hanson et al., 2000). Microbes have different sensitivity to changes in the biophysical environment, thus heterotrophic respiration may be more important than root respiration when photosynthesis is constrained by soil moisture (Singh and Gupta 1977). Thus, soil moisture is a critical biophysical factor controlling the magnitude and pattern of soil respiration in Mediterranean ecosystems.

7.4.2 Soil respiration and invasion by *Acacia* species, and clearing of riparian zones

Our findings indicate that invasive alien plants, the N_2-fixing species investigated in this study, *A. mearnsii* and *A. longifolia* alter R_S rates in the low-nutrient soils characteristically associated with the riparian fynbos vegetation at the western Cape sites. Invaded riparian sites had the highest R_S rates, which declined when *Acacia* species were removed from riparian zones. The invaded sites also showed the highest soil respiration rates, regardless of season, though the differences are most prominent during summer. As was reported by Yelenik et al. (2004; 2007), and also in riparian environments by Naude (2012), *Acacia* species have a propensity to increase soil C stocks (also soil N stocks) in riparian environments, and litter produced also show high decomposition rates (Morris et al., 2011). It is likely that invaded riparian zones have high C stocks that decompose relatively faster than native riparian zones, thus reflecting higher R_S rates.

Clearing of riparian zones caused soil respiration to decline. The research of Naude (2012) (see also Chapter 6) showed that soil C and N stocks decline when invaders are removed, and native plants return (to a degree). However, it is unclear what causes the decline in R_S associated with clearing of invasive *Acacia* species. The main environmental drivers of R_S appears to be soil temperature (positive) and soil moisture (negative), and while GSWC showed trends more reflective of natural sites, soil temperature of cleared sites were more closely related to invaded sites. In both cases the differences where minor. More likely, the differences related to the physiological effects of *Acacia* species manifested through root density and root exudates (thus the autotrophic component of RS) (Luo and Zhou, 2006). This is consistent with the major reduction in R_S when root are severed during trenching. Though R_S bounce back after some months, this is most likely due to decomposition of fine roots rather than root respiration.

7.4.3 Soil respiration at different landscape positions

Soil respiration is different between landscape positions and this confirms the importance of vegetation, roots, and microbial communities in understanding the mechanism of R_S variation. More fine roots in *Acacia*-invaded wet banks may result in higher R_S compared to that in terrestrial areas. However, when comparing cleared and natural riparian zones, little differences emerged in terms of landscape positions. It is important to note that terrestrial areas associated with invaded riparian zones was not invaded themselves by *Acacia* species (apart from some scattered invasive trees), thus the similarity in R_S rates between the terrestrial areas of all invasion statuses. Thus apart from high R_S in invaded riparian (dry and wet banks, little other trends are discernible when comparing R_S rates in this way.

The lack of differences when comparing landscape positions is in some ways surprising as environmental parameters are significantly different. It is in contrast to the results of Pacific et al. (2008), who found significantly higher R_S in riparian environments. In our study, soil moisture, especially, is higher in wet banks than terrestrial areas, which is consistent with the work of Pacific et al. (2008). As mentioned above, high soil moisture levels may impede soil CO_2 efflux from soils, hence the lack of major differences may reflect different soil diffusivity rather that decomposition rates or soil microbial activity. This is somewhat analogous to what Pacific et al. (2008) found in that their highest riparian R_S was during the dry season, although it was still higher than adjoining hillslopes.

7.4.4 *Ex situ* soil respiration and soil trenching

In addition to *in situ* measurements of R_S that was carried out over seven seasons, we also carried out *ex situ* measurements of R_S where the effect of roots was excluded. We did the latter by incubating soils from each of the sites, inclusive of the different landscape positions and the different invasion statuses at a constant temperature and moisture level (PR_S). We also sampled soils from the same sites in the western Cape where the *in situ* measurement has been carried out, but also from a series of secondary sites (the southern and eastern Cape, results in Chapter 10). The objective was to determine the role that microbial respiration played in overall R_S, and to ascertain whether general trends emerged.

Our results show that there were, on average little significant differences emerged between PR_S values obtained from soils sampled from different invasion status. Although we found differences over time i.e. a fast rise in PR_S rate, which later declined), this is likely the results of different types of C (labile vs. recalcitrant; Bechtold and Naiman 2006). Booi (2010) found differences in PR_S when soils from succulent karoo, sampled on and off heuweltjies (similar to 'mima-like' mounds) were incubated at constant temperature and moisture levels. Heuweltjies have significantly different soils and vegetation. The difference that emerged was attributed to inherently different soil microbial biomass, and which was expressed in different rates of PR_S. Therefore, one can make the assumption that at a broad level, differences in microbial biomass did not drive differences in PR_S of sites with different invasion status observed in the field.

In addition, in the field, most of the differences emerge in summer, with hardly any differences between the different sites in the other seasons. This suggests that another factor, other than microbial populations may be at play. From work by Miller et al. (1983), it is clear that fynbos species are predominantly photosynthetically active during early summer and that stomatal conductance, and hence C capture declines later as the dry season progresses. Activity is much higher than in the winter and autumn. For *A. mearnsii*, Dye et al. (2001) found that evapotranspiration and sapflow is highest in summer, but again, this declines later when the seasonal drought progresses. *Acacia* species were found to be more profligate water users, and this may be at the core of its ability to outcompete native species in the fynbos. It is likely that the phenology and its associated physiological dynamics play a role in the observed responses we measured in the field. Högberg and Read (2006) suggested that photosynthate captured by trees are rapidly converted to C respired by the roots, and that this may happen on a timespan of hours. Thus, while plant ecophysiology fell outside of the scope of this thesis, it likely played a more significant role in driving R_S in summer than microbial respiration.

We measured higher root mass (measured twice over the course of one year) in invaded riparian dry banks compared to other landscapes, and to the natural and the cleared sites (Kambol Kambaj, O., unpublished data, 2010-2011). Invaded dry banks showed higher root mass. While on its own this does not suggest a causal link between R_S and roots, combined with our contention that plant physiology play a significant role in overall R_S rates, higher root density in *Acacia*-invaded sites (see also Morris et al., 2011) may also contribute to the elevated R_S rates in invaded sites.

We attempted to isolate the role of roots in invaded sites by trenching areas within *Acacia*-invaded sites which lend themselves to digging trenches. After leaving trenched areas to equilibrate, we measured a 40% decline in R_S in these plots compared to non-trenched controls. The decline we found also suggested a large role for root respiration, however, when the plots were monitored over time, we found that the initial difference between the trenched and control plots disappeared, and indeed subsequently, the trenched plots showed higher R_S rates. This result is puzzling, however, it may be attributed to decomposition of OM and severed roots. We showed that soil moisture was higher inside the trenched areas when R_S rates were higher during later sampling times, which suggest this may be faster decomposition rates due to more moist conditions, and this may be a relic of the method we used (heavy plastic sheeting, which may have retained moisture), rather than a true reflection of root respiration. It is unlikely that fine roots grew into the trenched plots from outside and we did not notice any fine roots below 40cm soil depth when the trenches were dug.

Jauhiainen et al. (2012), working in *Acacia* plantations found that there was a decline in R_S rate after trenching, which allowed them to work out a contribution of root respiration to overall R_S, which amounted to between 56 and 21% contribution of root respiration, depending on treatment. The contribution of root respiration to CO_2 efflux was found to be 21% on average along transects in mature *Acacia* tree stands. Significantly higher root respiration occurred very close to *Acacia* trees, but was negligible within treatment (distances between the measurement transects), indicating that measured CO_2 effluxes well away from trees were free of any contribution from root respiration and represented heterotrophic (i.e. microbial) respiration only (Jauhiainen et al., 2012). Similarly, our results support the important role of root respiration in overall soil respiration.

8 PHOSPHORUS IN NATURAL, *ACACIA*-INVADED AND CLEARED RIPARIAN ECOTONES IN THE WESTERN CAPE

8.1 INTRODUCTION

Of the macronutrients, P appear to be the most limiting nutrient in the fynbos biome, and plants have developed several strategies to cope with low soil P levels, e.g. proteoid roots or participation in symbiotic relationships such as between *Erica* species and ericoid mycorrhiza (Cowling and Holmes, 1992). Hence the spatial distribution of P in fynbos soils influences many soil processes and even plant community composition and properties (Power et al., 2010). Even though riparian environments are often considered to be enriched in nutrients due to its low relief within catchments (Jacobs et al., 2007), the scant information on fynbos riparian zones suggest a more complex picture. Manders (1990) suggested that fynbos riparian zones are neither consistently enriched in P, nor other nutrients.

Phosphorus plays a major role in the process of N-fixation by legumes (Vitousek et al., 2002). Thus it can be expected that where soil P is in short supply plants, especially legumes, may employ strategies to obtain more soil P which may not be otherwise easily obtained. Some of these strategies may include exuding acid and/or alkaline phosphatases, which is involved in the conversion of unavailable organic P to available P (Orlander and Vitousek, 2000). As legumes are generally underrepresented in native fynbos communities, except perhaps after fires, and lack of soil P has been suggested to be the reason for this (Power et al., 2010), one can expect the highly successful invasive *Acacia* species to be able to use strategies to efficiently obtain P. Thus, one can expect soil phosphatases to be elevated in soils where invasive *Acacia* species dominates. Alternatively, as it is not clear that soil P are elevated in fynbos riparian soils, *Acacia* species may, in fact not need to exude phosphatases, instead able to obtain enough P due to elevated soil P availability, thus contributing to the success of these invasive woody plants in fynbos riparian environments.

This chapter related to objective 1. Sampling was carried out concurrently with that for available N and total N as well as *ex situ* soil N mineralization (NMP) rates. We investigate whether fynbos soils (dry bank and wet banks) are enriched in available P relative to terrestrial areas, and whether soil in areas where invasive *Acacia* species dominates are enriched in available P (referred to as P_i) or soil phosphatases, and we did this on a seasonal basis in 2010.

8.2 METHODS

8.2.1 Available phosphorus stocks

Bray-2 extractable inorganic P (P_i) is a measure of plant available P (Witkowski and Mitchell, 1987) and was measured in fresh soil (collected within 2-3 days; soils stored at 4°C) by a method adapted from, Bray and Kurtz (1945). Concentrations of Bray-2 P_i were determined colorimetrically using a spectrophotometer (Genesys 20, Thermo Scientific).

8.2.2 Soil phosphatase activity

The activity of extracellular phosphatase was determined each sampling season with the exception of winter, using field-moist soil samples (2-3 days after collection; soils stored at 4°C). We used field-moist soils as this is likely be more representative of enzyme activity under field conditions; phosphatase activity is markedly altered when air-dried soils have been used. Studies have shown that acid phosphatase predominates in acidic soils such as those of fynbos, hence only acid phosphatases were analysed, using the method suggested by Tabatabai (1982). The procedure for the assay of acid phosphomonoesterase (PME) activities involves colorimetric estimation of the p-nitrophenylphosphate (p-NPP) released by phosphatase activity when soil is incubated at constant temperature with buffered pH 6.5 sodium p-nitrophenylphosphate solution and toluene (Tabatabai, 1994).

Four milliliters of 0.1 M maleate buffer (pH 6.5) and 1.0 ml 0.115 M *p*-nitrophenylphosphate (PNP) were added to 2 g soil sample. After stoppering the flask, it was placed in an incubator at 37°C. After one hour, the reaction was terminated by the addition of 4.0 ml of 0.5 M NaOH and 1.0 ml 0.5 M $CaCl_2$ were added to allow the dispersion of clay particles. Controls were prepared as ascribed for the assay procedure, however the substrate (p-NPP) was added before the addition of $CaCl_2$ and NaOH in order to assess the colour precipitation not coming from the enzymes (Tabatabai, 1982). All samples were filtered and the yellow colour intensity of calibration standards, samples and controls were measured with a spectrophotometer, a Spectronic model Genesys 20 at 402 nm against the reagent blank. Enzyme activities were expressed as µg *p*-NP g^{-1} dry soil h^{-1}. It is not possible to differentiate among extracellular, abiontic, or released intracellular enzymes using this method.

8.3 RESULTS

8.3.1 Available phosphorus stocks

Higher overall concentrations of available Pi were recorded in summer (15.9 µg g^{-1}) and autumn (16.3 µg g^{-1}), and these seasonal differences were statistically significant ($p<0.001$; Figure 8.1A). Phosphorus did not differ between invasion statuses (one-way ANOVA: $p=0.27$). There was, however, a general trend towards enhanced available P_i concentrations in soils under *Acacia* canopies (mean: 17.42 µg g^{-1}; Figure 8.1B). Interactions amongst landscape positions and season were insignificant ($p=0.409$), however, when data was pooled over all seasons, available P_i showed significant differences between landscape positions (one-way ANOVA: $p<0.001$; Figure 8.1D). Mean Bray-2 P_i concentrations in soils of wet bank zones (5.63 µg g^{-1}) were significantly lower compared to dry banks (12.47 µg g^{-1}; $p<0.001$) and upland (15.76 µg g^{-1}; $p<0.001$) areas, however no differences were observed between dry banks and upland terrestrial zones ($p=0.95$; Figure 8.1D) and dry banks and wet uplands areas followed similar trends across seasons (Figure 8.1C).

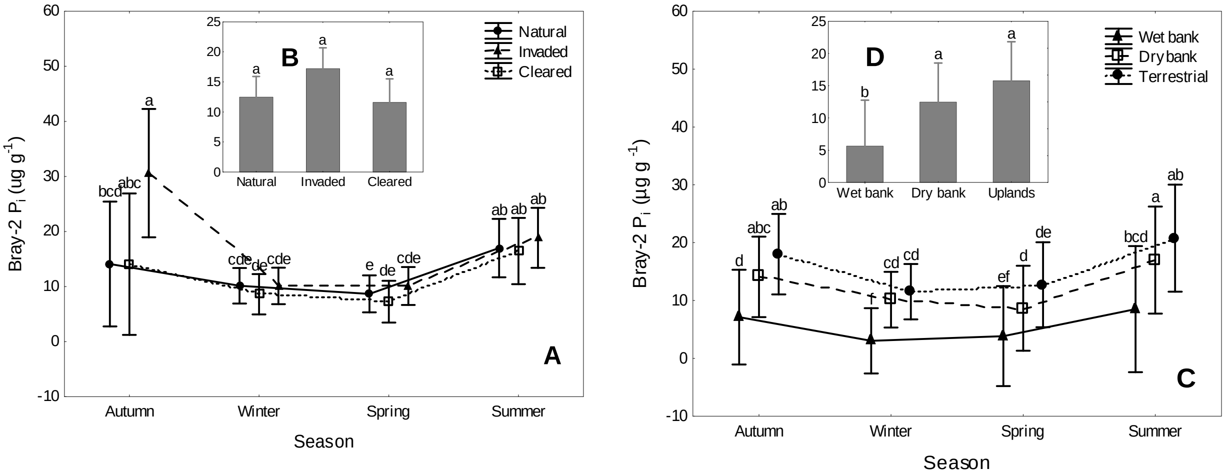

Figure 8.1: (A) Bray-2 available P_i for invasion statuses and (C) landscape positions across seasons. Mean values are indicated by different point symbols and whiskers indicate ± 95% confidence interval. Letters indicate significant differences (Tukey tests, $p<0.05$) for interaction effects based on repeated measures ANOVAs: Invasion statuses X seasons (Figure 8.1A: $p=0.499$) and landscape position X season (Figure 8.1C: $p=0.409$). Mean seasonal measurements for Bray-2 P_i are depicted in embedded bar graphs for (Figure 8.1B) invasion statuses and (Figure 8.1D) landscape positions. In the embedded graphs letters represent significant differences (Tukey tests, $p<0.05$) based on one-way ANOVAs: invasion statuses ($p=0.268$) and landscape positions ($p<0.001$). All statistics were computed on log-transformed data to meet the assumptions of ANOVA.

8.3.2 Soil phosphatase activity

Acid phosphatases, which release inorganic phosphate from organic monophosphate esters, showed significant interactive effects between landscape positions and seasons and the same was also true for invasion statuses (Figure 8.2A and C). Enzyme activity was highest in summer at each lateral zone and invasion status (Figure 8.2A and C). APME was significantly lower in wet bank zones during spring and summer compared to both dry bank and uplands (Figure 8.2C). Upland areas, on the other hand, were significantly different from both wet- and dry banks during autumn and spring. Overall mean acid phosphatases showed low activity in wet bank soils (196 µg *p*-NP g^{-1} h^{-1}), intermediate activity in dry banks (265 µg *p*-NP g^{-1} h^{-1}) and high activity in upland zones (337 µg *p*-NP g^{-1} h^{-1}; Figure 8.2D). Soils under *Acacia* species exhibited significantly greater enzyme activity across all seasons, compared to both natural and cleared sites, whereas natural sites showed APME activities similar to cleared riparian zones across all seasons (Figure 8.2B). Acid phosphatases were on average 2.3 and 2.4 times higher in *Acacia*-invaded riparian zones than natural and cleared ones respectively.

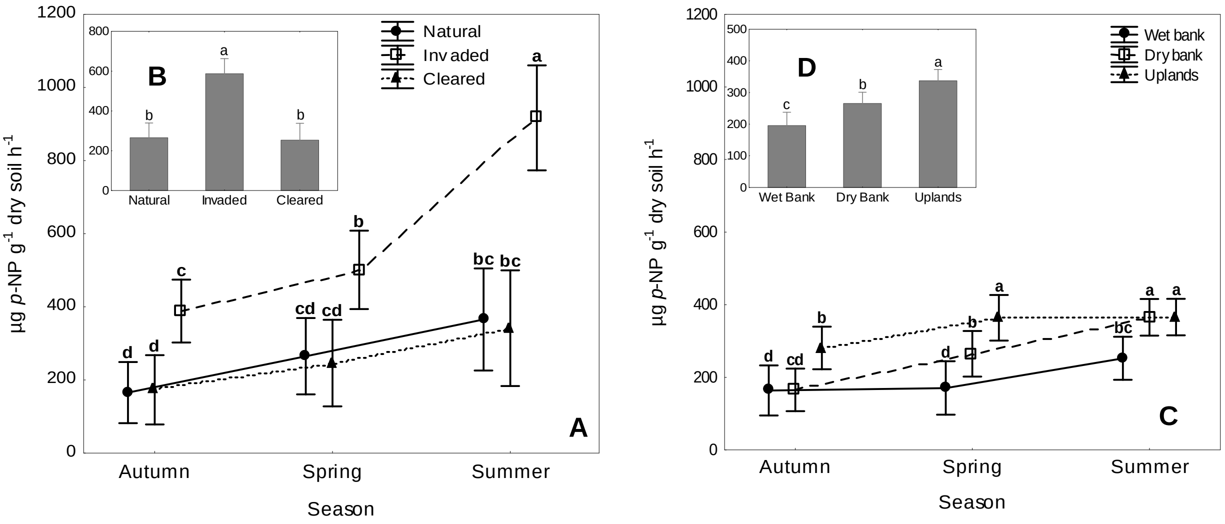

Figure 8.2: (A) Acid phosphatase monoesterase (APME) activity across seasons for landscape positions (wet bank, dry bank, and uplands) and (C) invasion statuses (natural, invaded and cleared). Mean values indicated by different symbols, and whiskers represent ± 95% confidence interval. Letters represent significant differences ($p<0.05$; Tukey tests) for repeated measures ANOVAs: landscape positions X seasons ($p<0.01$) and invasion status X season ($p<0.001$). Mean APME activity averaged across seasons are depicted in the embedded graph for (D) landscape positions and (C) invasion statuses. Mean values indicated by bars, and whiskers ± 95% confidence interval. Letters denote significant differences ($p<0.05$; Tukey tests) between one-way ANOVAs: landscape position ($p<0.001$) and invasion status ($p<0.001$).

The strongest linear relationship of the AMPE rate was with soil bulk density and soil C/N ratio. Both these relationships were negative, and were statistically significant, as was the correlation with GSWC (Table 8.1).

Table 8.1. Spearman's correlation coefficient ρ (rho) for P process rates (APME) and selected soil physical and chemical properties. Significant relationships ($p<0.05$) are indicated by an asterisk (*). Correlations were calculated from all available data irrespective of invasion status or landscape position, however, different variables differed in the regularity of sampling: seasonally (GSWC); biannually (Soil C/N) and once of during spring (bulk density). Correlation values represent only instances where comparisons could be made.

Soil Properties	APME rate
pH	-0.129
GSWC	-0.204
Bray-2 P_i	-0.039
Bulk density	-0.433
Soil C/N	-0.262

8.4 DISCUSSION

Acid phosphatases showed a seasonal pattern with maximum activity during the hot and dry summer season, and more so in drier landscape positions. Seasonal variations in enzyme activity were less prominent in wet banks relative to dry bank and upland areas. Temperature and to a degree soil moisture are drivers of soil phosphatase activity, and the more open-canopied dry bank and upland areas receive more solar radiation (Chapter 5), hence experience higher increases in temperature and soil moisture during warmer seasons compared to wet bank zones. The activities of phosphatases are also related to the physiological response of plant and soil communities to seasonal changes in soil moisture and temperature as well as presence of certain plant functional types, e.g. legumes (Grierson and Adams, 2000; Kramer and Green, 2000). Accordingly, spatial and seasonal heterogeneity in AMPE in soils may be affected by plant species composition, which in turn influences spatial root and microbial activity (Grierson and Adams, 2000). Although plant roots are major producers of acid phosphatases in the soil (Amador et al., 1999; Kramer and Green, 2000), microbes can also produce and release large quantities of extracellular phosphatases due to their high metabolic activity, large combined biomass, and short lifecycles (Tabatabai, 1994; Kramer and Green 2000).

Higher soil temperatures can promote microbial activity and metabolism, enhancing the turnover of nutrients and their availability (Chapin et al., 2002; Sardans et al., 2006). As mentioned earlier, the increase in acid phosphatases observed in spring and summer coincides with warmer temperatures. This is consistent with the results recorded by Sardans et al. (2006) in a Mediterranean shrubland during summer where warming significantly enhanced the activities of acid phosphatases. Moreover, the growth response of fynbos plants reaches their peak during spring and summer, and likely microbial activity is also high during this time of the year, hence this stimulates mining for P, which may become more deficient as a result of competition for resources. Temperature and soil moisture are important factors that govern the rate of chemical reactions and the activity and growth of organisms in the soil (Bardgett, 2005). The buildup of phosphatases released into the soil from lysing of cells of micro-organisms may also explain the peak activity found in summer (Kramer and Green, 2000). These seasonal variations in APME activity and NMP rate (Chapter 7), re-emphasize the importance of careful sampling across seasons for ecological studies on biogeochemical cycling (Grierson and Adams, 2000).

McClain et al. (2003) suggested that riparian zones and topographical depressions (e.g. riparian zones, wetlands) are hotspots for biogeochemical activity; however, this appear not to apply to fynbos riparian ecotones we looked at, which are all in longitudinal zones more associated with sediment suspension rather than sediment deposition zones . The results presented so far are contrary to our hypotheses; compared to terrestrial uplands, biogeochemical cycles were not enhanced within riparian zones, but for some soil stocks and processes revealed lower process rates compared to uplands. Wet bank zones had significantly lower NMP (Chapter 7) and APME rates compared to those higher up in the landscape, whereas dry banks only differed from uplands with regard to phosphatase activity. However, this does not necessarily relate to all fynbos riparian zones and process rates may well be in line with our hypothesis for riparian zones closer to the estuary, i.e. floodplain sections of river.

We hypothesized that *Acacia* invasion will enhance the activity of APME compared to both natural and cleared sites. One of the mechanisms by which N-fixing invasive species affect P biogeochemistry may include changing the components and chemical nature of soil OM and soil enzyme activity in addition to the dynamics of soil biogeochemical reactions through litter inputs (Zou et al., 1995). By enhancing the supply of soil C and N and litter inputs, in addition to possible changes in litter quality (Yelenik et al., 2004; Marchante et al., 2008), *Acacia* species may stimulate P demand and provide raw materials for the synthesis of extracellular acid phosphatases. Indeed, a negative correlation between APME activity and C/N ratio was evident in our results, meaning more N relative to C would enhance APME activities. Allison et al. (2006) reported that higher inputs of litter and enhanced C and N pools in *Falcataria*-invaded

Hawaiian ecosystems and this lead to high enzyme activity with significant increases in acid phosphatases relative to native forests (Allison et al., 2006). This suggests that enhanced N availability in *Acacia* invaded fynbos riparian zones will increase the demand for P by *Acacia* species by increasing phosphatase activity. This is the most likely mechanism for increasing the supply of P (Allison et al., 2006). Houlton et al. (2008) reported that the release of available P_i by phosphatase activity appears to be of direct benefit to N-fixing plants species, which apparently was the source of the elevated soil phosphatase levels.

Acacia-invaded fynbos riparian ecotones support higher fine-root biomass compared to natural ones (Kambol Kambaj, O., unpublished data, 2012), suggesting that root activity likely played a significant role in enhanced enzymatic activity observed at invaded sites. Furthermore, APME activity may also be enhanced due to increased fungal activity associated with invaded sites (Grierson and Adams, 2000; Chapter 9). The activity of extracellular enzymes, such as phosphatases, associated with different plant species (e.g. natural versus invasive N-fixers), is a function of the morphological and physiological attributes of root type (Grierson and Adams, 2000). Molecular studies have showed that the production of extracellular APME is closely related to the P status of plants so that the production of these enzymes might to be a specific response to P deficiency (Gress et al., 2007). Greater release of these enzymes provides a mechanism for scavenging P from organic material (McGill and Cole, 1981).

9 MICROBIAL COMMUNITY STRUCTURE AND COMPOSITION IN NATURAL, *ACACIA* INVADED AND CLEARED RIPARIAN ECOTONES IN THE WESTERN CAPE

9.1 INTRODUCTION

Microbes mediate many processes in N, C and P cycling, and have been shown to be crucial to ensuring that ecosystem services are delivered (Wall et al., 2001). The abundance, diversity and function of soil microbes interact in a multitude of ways with plants in the ecosystem (Bohlen et al., 2001; Sullivan et al., 2004; Bonfante and Anca, 2009; Hartmann et al., 2009;). For example, according to Schnitzer et al. (2011) plant root pathogens play a significant role in controlling densities of plant species and the alpha diversity in an area.

Soils that are frequently inundated such as those found in riparian zones and wetlands seem to have high diversity of fauna and microflora, which supports the underlying soil processes that riparian zones are commonly associated with (Ettema et al., 1999, 2000; Hartman et al., 2008). Due to the unique set of hydrological, biogeochemical and geomorphological factors in riparian zones and resulting soil conditions, microbial and faunal community composition, abundance and biomass of the mineral and organic horizons may be different from surrounding areas, and from other ecosystems. However, many gaps exist in our understanding of soil microbial dynamics in riparian and wetland soils (Ettema et al., 1999; Gutknecht et al., 2006; Hartman et al., 2008). Broadly, though, in richer soils bacteria seem to dominate (e.g. wetland soils), while in poorer soils fungal species seem to be prominent, and play an important role in nutrient uptake via e.g. root symbioses (mycorrhiza) (Wardle et al., 2004).

Recent results show that microbial interactions may contribute to shaping plant community structure and may partially control riparian ecosystem functioning (Gutknecht et al., 2006). In riparian zones microbes are involved as plant pathogens, mutualists or general soil saprophytes (Callaway et al., 2004; Ball et al., 2009; Harner et al., 2010), or have specific roles in soil processes such as denitrifyers and methanogens, sulfide reducers and fermenters (Gutknecht et al., 2006; Hartman et al., 2008). Furthermore, some plant species, e.g. *Trapa natans* seem to play an unexpectedly large role, along with associated rhizosphere and soil microbes to control processes such as denitrification (Tall et al., 2011). It has been shown that invasive plants change soil microbial structure and composition, however, that microbial populations can recover quickly following removal of the invasive plant species (Kourtev et al., 2002). As few studies exist regarding the impact of invasive *Acacia* species, our research aims to discover soil microbial structure in riparian and upland soils.

This research addresses objective 4 and key questions associated with this objective. Sampling was carried out in 2010 and 2011.

9.2 METHODS

9.2.1 Soil sampling and DNA extraction

Soil samples were collected during March 2010 during the end of the dry autumn season. The sampling protocol was repeated in August 2010 at the end of the rainy season and during January 2011 during the dry summer season. This was done to consider temporal and seasonal variations in the community composition (although no sampling was carried out in winter). Sampling plots were positioned to form transects of three plots, located in every lateral zone. Soil core samples were taken from each plot using a 25 mm diameter steel cylinder. This process was repeated for all the sites and every lateral zone. The sub-samples were homogenised and DNA extracted within 48h of sampling. DNA from every sample was extracted using 0.35 g of soil with the ZR Soil Microbe DNA kit (Zymo Research, California, USA) and the presence of genomic DNA was checked on a 1% agarose gel, stained with ethidium bromide. Samples from the natural, cleared and invaded sites were first characterized using automated ribosomal intergenic spacer analysis (ARISA). Appropriate representative samples from each lateral zone and every invasive status were selected for pyrosequencing.

9.2.2 PCR amplification, purification and pyrosequencing of bacteria

The hyper-variable regions of V1 to V3 in the bacterial 16S rRNA gene were amplified from extracted community DNA from all nine samples using the universal bacterial primers 27F and 340R. PCR mixtures of 50 µl volume was prepared with 25 µl KAPA HiFi™ HotStart ReadyMix (Kapa Biosystems, South Africa), 1 µl of each primer (50 pmol), 1 µl of the template DNA and 22 µl sterile MilliQ water. PCR reactions were performed using GeneAmp PCR system 9700 (Applied Biosystems, South Africa). The initial PCR was performed under the following conditions: initial denaturation (94°C; 5 min), 25 cycles of denaturation (98°C; 20 sec), annealing (60°C; 15 sec), extension (72°C; 1 min), and a final extension (72°C; 7 min). The PCR products were purified using the DNA Clean and concentrator kit (Zymo Research, USA) which served as template for the second round of PCRs.

The PCR reaction mix in the second round of PCR was the same as for the first reaction. However, in this reaction the reverse primer 340RA was used, and contained the sequencing primer A, an identification key, a unique multiplex identifier (MID) and the universal bacterial primer 340F. The forward 27FB primer contained the sequencing primer B, an identification key, and the universal bacterial primer 27FB. PCR conditions were the same as for the first reaction. The PCR amplicons were gel purified using the BioSpin Gel Extraction Kit (BioFlux, Japan).

Equal molar concentrations of the PCR amplicons with the different sample-specific barcode sequences (MIDs) were pooled and submitted for pyrosequencing (Inqaba Biotec, South Africa) using the 454 GS FLX Titanium Sequencing System (Roche).

9.2.3 DNA extraction, PCR amplification, purification and pyrosequencing of fungi

DNA from every sample was extracted using 0.35 g of soil with the ZR Soil Microbe DNA kit (Zymo Research, California, USA) and the presence of genomic DNA was checked on a 1% agarose gel, stained with ethidium bromide. Samples from the natural, cleared and invaded sites were first characterized using automated ribosomal intergenic spacer analysis (ARISA). Appropriate representative samples from each lateral zone and with every invasive status were selected for pyrosequencing.

The ITS1 and ITS2 region in the fungal rRNA gene were amplified from extracted community DNA from all nine samples using the universal fungal primers: ITSF and ITS5. PCR mixtures of 50 µl volume was prepared with 25 µl KAPA HiFi™ HotStart ReadyMix (Kapa Biosystems, South Africa), 1 µl of each primer (50 pmol), 1 µl of the template DNA and 22 µl sterile MilliQ water. PCR reactions were performed using GeneAmp PCR system 9700 (Applied Biosystems, South Africa). Initial PCR was performed under the following conditions: initial denaturation (94°C; 5 min), 25 cycles of denaturation (98°C; 20 sec), annealing (60°C; 15 sec), extension (72°C; 1 min), and a final extension (72°C; 7 min). The PCR products were purified using the DNA Clean and concentrator -5 kit (Zymo Research, USA) which served as template for the second round of PCR.

The PCR reaction mix in the second round of PCR was unchanged from the first reaction. The reverse primer ITS1FA contained the sequencing primer A, an identification key, a unique multiplex identifier (MID) and the universal fungal primer ITSF. The forward primer contained the sequencing primer B, an identification key, and the universal fungal primer ITS5. PCR conditions were the same as the first reaction. The PCR amplicons were gel purified using the BioSpin Gel Extraction Kit (BioFlux, Japan).

Equal molar concentrations of the PCR amplicons with the different sample-specific barcode sequences (MIDs) were pooled and subjected to pyrosequencing. Pyrosequencing was performed by Inqaba Biotec (South Africa) using the 454 GS FLX Titanium Sequencing System (Roche).

9.2.4 Pyrosequencing data analysis

Sequences were filter and separated according to the MID sequences. Reads with no Ns and length >50 bp were retained. The MID sequence, key and the reverse and forward primers were trimmed prior to further analysis. Sequences less than 100 bp after trimming were discarded in the analysis. The 16S rRNA gene fragments were phylogenetically assigned using RDP Naive Bayesian rRNA Classifier Version 2.2 from the ribosomal database project (RDPII) database (Cole et al., 2007). RDP training set 6 was used, based on nomenclatural taxonomy of Berkley’s Manual using a confidence threshold of 50%.

Multiple sequence alignment was done using the Infernal Aligner (Nawrocki & Eddy 2007). Based on the alignments, complete linkage clustering was performed using the RDPII pyro-sequencing pipeline. The defined dissimilarities of the clusters ranged from 0% to 20% dissimilarity. The accepted cut-off points for accurate estimation of different taxonomic hierarchies are 3% for species level, 5% for genera and 20% at a phylum level. The cluster based on 0%, 3%, 5% and 20% dissimilarity was used as Operational Taxonomic Units (OTUs) to generate rarefaction curves of every sample. The clusters were also used to calculate the Shannon diversity index and applied with the non-metric Choa1 richness estimator (Chao and Bunge 2002).

9.2.5 Non-metric multi-dimensional scaling and UPGMA cluster analysis for bacteria

The 16S rRNA sequence reads from all 27 aligned samples were used to generate clusters based on the sequence similarity. The similarity clusters were used to pairwise compare the different samples based on the Sorenson index. The pairwise comparisons used use Chao abundance for abundance correction. These pairwise comparisons were then used to generate a distance matrix of all the samples.

Unweighted Pair Group Method with Arithmetic (UPGMA) cluster analysis was used to determine the structural differences observed between the samples. The Sorenson distance matrix was additionally used to analyse samples on a non-metric multi-dimensional scale. Cluster analysis and multi-dimensional scaling was performed to test whether the qualitative environmental factors are important for structuring the bacterial community. Samples were grouped *priori* based on their invasive status, lateral zone and site location. The comparisons was visualised using UPGMA to construct a cladogram as well as a NMDS plot. The significance of the comparisons was tested by ANOSIM analysis using 10000 iterations. The first comparisons were made to investigate the effect of the different lateral zones of the riparian ecotones on the bacterial community structure. The influence on bacterial community composition was investigated by comparing the dry bank and wet bank zones while taking the invasion status of the zones into consideration.

9.2.6 Phylogenetic assignment of the ITS reads with MEGAN for fungi

The individual read files from the different samples were used to generate BLAST assignment files using NCBI BLAST against the GenBank database. The BLAST search was limited to exclude environmental samples to reduce the possibility of incorrect assignment. A maximum of top ten sequences per BLAST search was selected for taxonomic classification. The ITS sequences were phylogenetically assigned using MEGAN v. 4.0.2 (MetaGenome Analyzer, Centre for Bioinformatics, Tübingen, Germany) (Huson et al., 2007). MEGAN provides unique names and IDs for taxa from the NCBI taxonomic database. All parameters of MEGAN were kept at default values, except for the minimum number of sequence reads that must be assigned to a taxon, which was set to 10 (Wu & Eisen, 2008). The diversity of the samples were determined and compared based on the number of fungal genera detected. A rarefaction curve was generated based on the genera, class and order classifications.

9.2.7 Unifrac cluster analysis for fungi

The Unifrac programme was used to assess the significance of the differences observed in the different fungal communities form the various sites by calculating the Unifrac distances (Lozupone et al., 2006). This was done to determine the influence of environmental factors on the composition of the fungal community structure. The environmental factors in this case was qualitative and include the identity of the lateral zone and as well as its invasive status. Multiple sequence alignment was done using MAFFT v. 6.24 (Katoh and Toh 2008). The alignments were clustered at 97% similarity using the UPGMA algorithm. The cluster files were used to generate cladograms using Fasttree (Price et al., 2009). Communities were then compared in a pair-wise approach by generating UniFrac distance matrices. This was done to quantify the proportion of the branch length on the tree unique to each community in each pair. The UniFrac distances were used to generate a matrix of all distances between the fungal communities of the samples. Cluster analysis and multi-dimensional scaling was performed to test whether the qualitative environmental factors are important for structuring the fungal community. Samples were grouped *priori* based on their invasive status, lateral zone and site location. The three sampling sites thus resulted in nine lateral zones with different invasive statuses. The comparisons was visualised using UPGMA to construct a cladogram as well as a NMDS plot. The significance of the comparisons were tested by ANOSIM analysis using 10000 iterations. The first comparisons were made to investigate the effect of the different lateral zones of the riparian ecotones on the bacterial community structure. The influence on bacterial community composition was investigated by comparing the dry bank and wet bank zone in context of the invasion status of the zones.

9.3 RESULTS

9.3.1 The number of bacterial OTUs

Our results show definite downward trends in OTUs when riparian zones are invaded by *Acacia* species, with the wet banks the most responsive, followed by the dry banks. However, when the *Acacia* individuals are removed, bacterial OTUs responded by increasing, resembling that of the natural sites (Figure 9.1). This shows a shift in richness, and the pyrosequencing data shows which taxonomic units responded most (Figure 9.3).

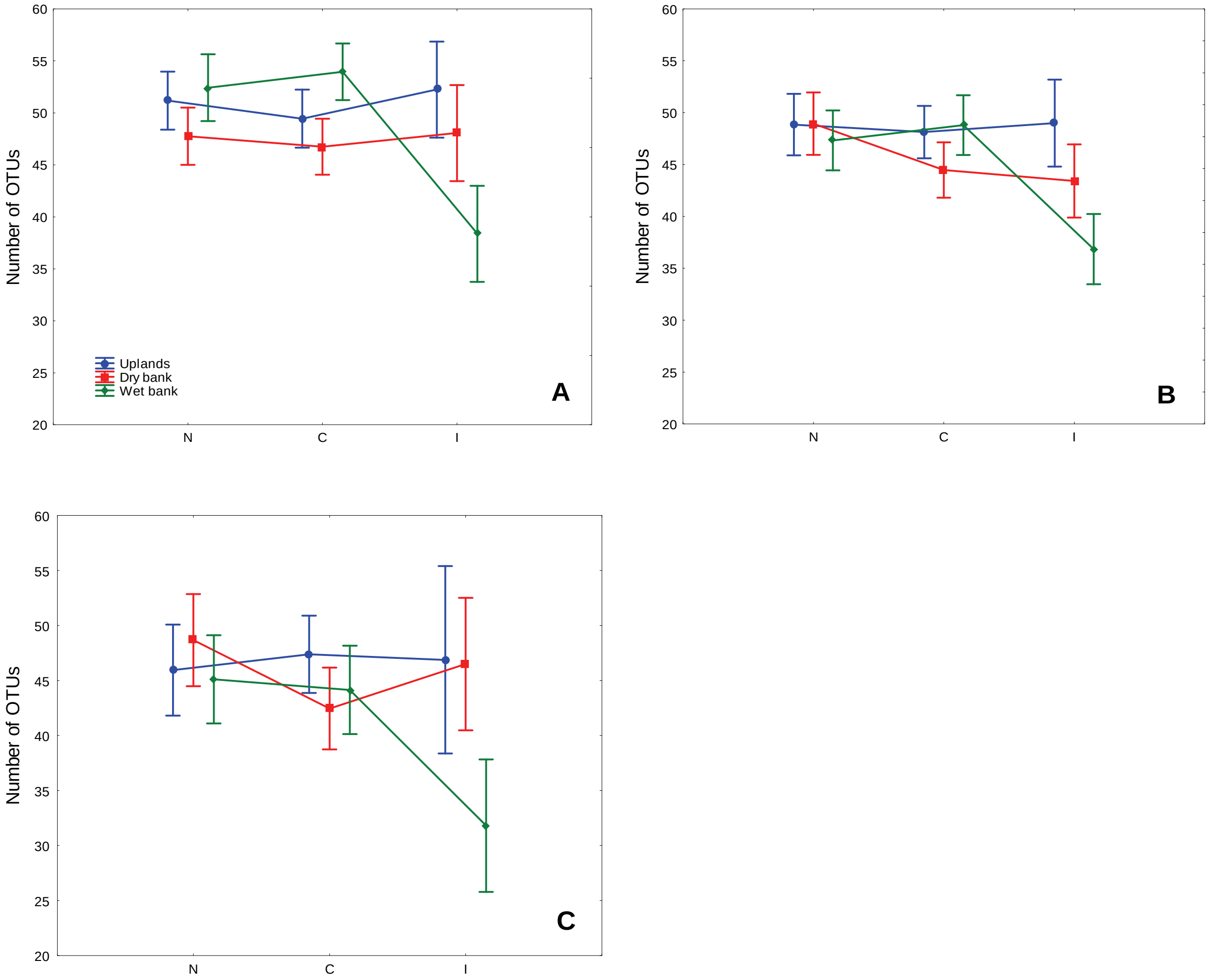

Figure 9.1: Whisker plot of the average number of bacterial operational taxonomic units based on the ARISA profiles (B-ARISAs) comparing natural, invaded and cleared riparian ecotones in season 1, autumn 2010 (A), season 2, winter 2010 (B) and season 3, summer 2010/2011 (C). The points represent the means and the whisker is the SE. N = Natural; C = Cleared, and I = Invaded.

9.3.2 Bacterial OTU richness

The number of reads obtained from the samples varied between 764 and 12727 (Table 9.2) and were sufficient to compare the relative abundance and diversity of the bacterial communities from the different samples. The number of clusters ranged from 431 to 2826 at a 3% dissimilarity level and between 294 and 1727 at a 5% dissimilarity level (Table 9.2).

Table 9.1: Summary of sequencing information and diversity data of all the samples over the three season sampled. The seasons are: season 1, autumn 2010; season 2, winter 2010, and season 3, summer 2010/2011.

							Predicted OTUs			
			Number of OTUs		Shannon diversity		Distance level 3%		Distance level 5%	
Sample	Season	Reads	dissimilarity 3%	dissimilarity 5%	Distance level 3%	Distance level 5%	Rarefaction	Choa1	Rarefaction	Choa1
Eerste River Upper, Terrestrial	1	4029	905	629	6.14	5.69	1 469.27	1 137.57	1238.44	737.37
	2	1125	636	484	6.05	5.62	913.3	1 353.41	838.4	899.53
	3	1080	554	400	5.97	5.50	865.64	1 150.36	783.86	689.46
Eerste River Upper, Dry Bank	1	12 727	2826	1727	5.25	4.95	3 442.15	4 368.97	2 568.84	2 388.01
	2	1161	520	373	6.96	6.37	393.44	1 078.26	355.52	621.56
	3	1640	683	472	6.03	5.56	874.28	1 277.01	752.43	733.42
Eerste River Upper, Wet Bank	1	466	416	336	5.32	5.01	994.18	667.04	901.37	347.67
	2	746	367	283	6.92	6.31	438.54	885.75	397.60	489.72
	3	1 479	782	591	6.27	5.91	993.48	1 962.54	885.57	1 003.72
Molenaars River Upper, Terrestrial	1	4 479	1463	926	6.35	5.94	1826.0	2 548.56	1 477.28	1 325.57
	2	1 926	911	663	6.61	6.0044	1 203.06	1 781.94	1 043.27	1 114.36
	3	913	497	358	5.82	5.37	652.42	1 256.89	600.50	572.05
Molenaars River Upper, Dry Bank	1	9 384	2531	1574	6.15	5.71	2903.39	3 744.03	2095.62	2,062.19
	2	1 297	700	515	7.05	6.38	857.49	1 555.73	763.57	901.40
	3	1 458	750	542	6.21	5.74	961.99	1,591.77	817.19	892.03
Molenaars River Upper, Wet Bank	1	6 341	1913	1164	5.05	4.58	2 139.86	3 265.68	1 541.29	1 652.54
	2	523	257	191	6.88	6.25	348.46	572.98	313.53	382.76
	3	925	482	377	5.71	5.35	576.51	1 259.48	519.58	707.19

							Predicted OTUs			
			Number of OTUs		Shannon diversity		Distance level 3%		Distance level 5%	
Sample	**Season**	**Reads**	**dissimilarity 3%**	**dissimilarity 5%**	**Distance level 3%**	**Distance level 5%**	**Rarefaction**	**Choa1**	**Rarefaction**	**Choa1**
Wit River, Terrestrial	1	726	522	406	5.79	5.37	666.64	552.73	594.66	320.72
	2	785	449	342	6.34	5.73	573.41	1 051.56	511.50	596.03
	3	861	392	298	5.72	5.37	626.46	802.55	547.56	528.00
Wit River, Dry Bank	1	3423	1079	660	5.77	5.27	1 250.37	1 839.78	978.55	951.70
	2	570	442	358	5.43	4.96	534.46	1 141.79	475.54	743.02
	3	814	431	331	5.80	5.50	656.48	894.89	568.57	550.38
Wit River, Wet Bank	1	1711.5	539.50	330	4.98	4.60	708.01	1 471.20	618.20	760.80
	2	411	404	294	4.56	4.18	604.82	365.28	263.53	235.75
	3	764	592	466	4.53	4.36	1 371.16	1551.77	1 193.30	938.65

The Shannon diversity was significantly lower in invaded wet bank samples compared to all other samples according to one-way ANOVA (p<0) (Figure 9.2).

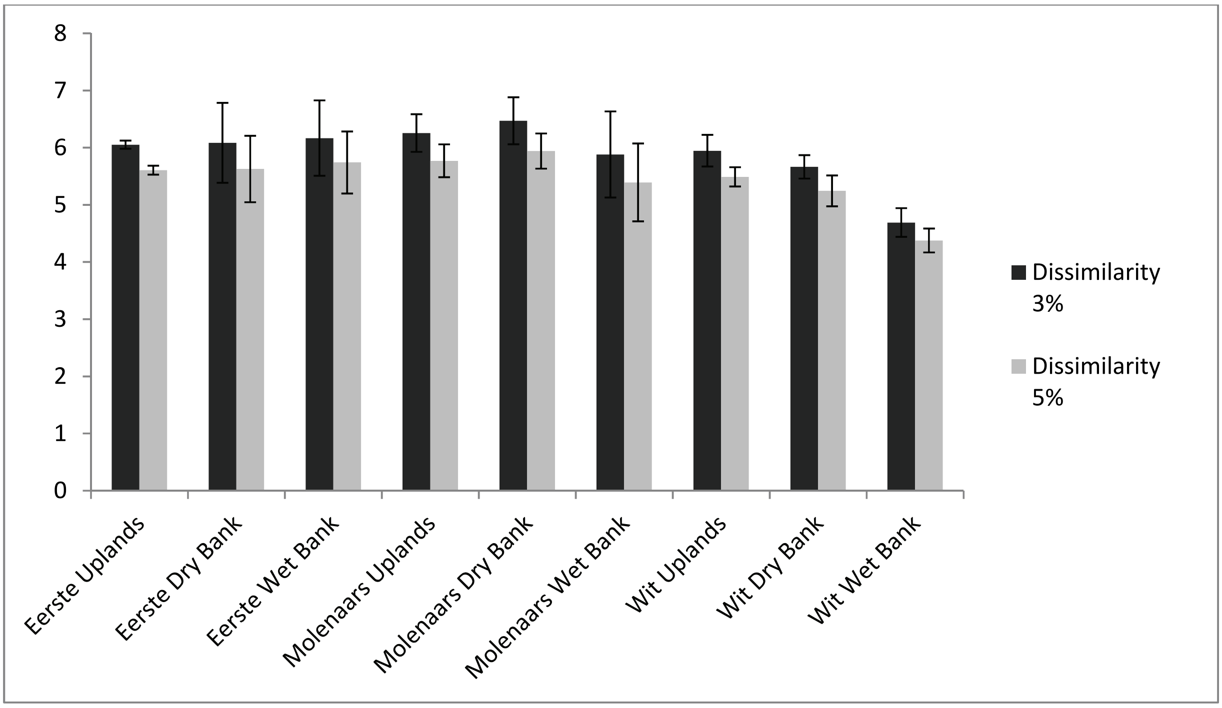

Figure 9.2: The Shannon diversity data of all the samples from the different sites and lateral zones over the three seasons. The Shannon diversity was compared at 3% and 5% read similarity.

The Chao1 estimation model predicated 552 to 4368 OTUs at a dissimilarity level of 3% between the different samples. These values correspond to the estimated number of species in the samples. The Chao1 estimation model also predicated between 263 and 2388 OTUs at a dissimilarity level of 5% between the different samples. These values correspond to the estimated number of genera in the samples.

9.3.3 Bacterial OTU classification and composition

The Naive Bayesian rRNA Classifier identified eighteen different bacterial phyla in all the samples with an average of 12 phyla identified in individual samples. Only eight of the 18 phyla occurred at levels higher than 1% of the total fraction of reads (Figure 9.3). These phyla include the *Actinobacteria, Firmicutes, Bacteroidetes, Proteobacteria, Acidobacteria, Planctomycetes, Cyanobacteria* and *Bacteria_incertae_sedis*.

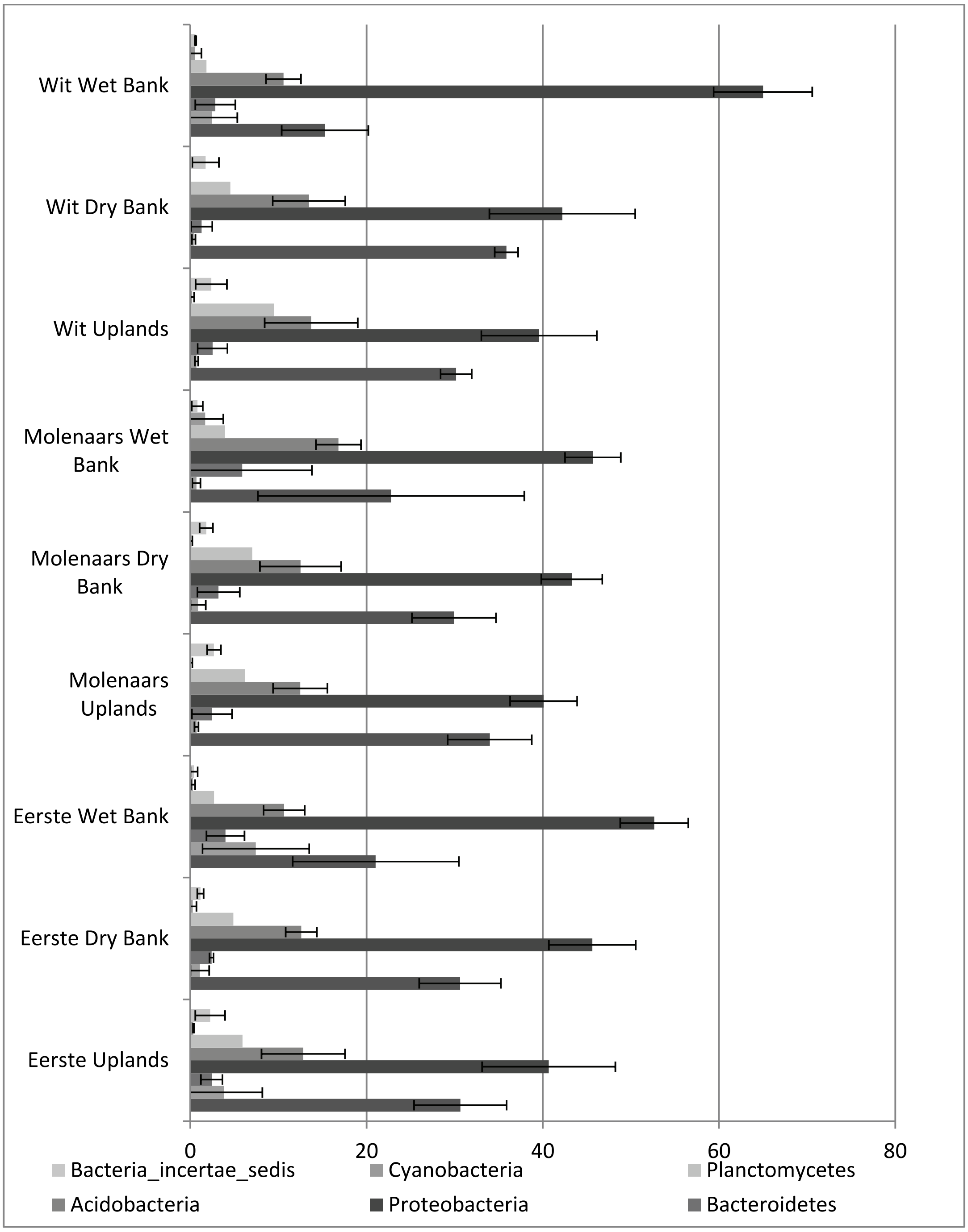

Figure 9.3: Summary of the distribution frequency of bacterial phyla between natural, cleared and invaded lateral zones. Only phyla occurring at levels higher than 1% of the total reads are shown.

Data from all sites suggest that the Alphaproteobacteria was the dominant group of soil bacteria followed by the Actinobacteria. When comparing the distribution of phyla (Figure 9.3) a significant higher proportion of Alphaproteobacteria was observed in the samples from the invaded site. The proportion of Actinobacteria represented in the samples from the invaded site were, however, lower. When these are broken down according to the invasion status, a clear shift in the community composition was observed. In the invaded site, the genus *Bradyrhizobium,* which form N-fixing associations with plant roots, occurred at lower frequencies compared to the pristine and cleared sites.

The genus *Methylocapsa,* an obligatory methanotrophic gram-negative bacterium, also occurred at lower frequencies in the invaded sites. The trend was similar in the case of the phototrophic genus *Rhodopila* which also occurred in lower levels in the invaded sites. On the other hand, some groups were present at higher levels in invaded soils, compared to pristine and cleared soils. These include the genera *Microvirga* and *Rhizobium,* root nodulation formers and *Methylosinus, Methylobacterium*, obligatory methanotrophic gram-negative bacterium as well as *Acidicaldus*, a moderately acidophilic thermophile.

It seems there were differences between sites (Figure 9.3), and that the Proteobacteria changed upon invasion, but recovers to a state resembling the pre-invasion state after clearing of the *Acacia* trees from the riparian areas. Actinobacteria is the next most responsive taxonomic group, followed by the Firmicutes. The phylum Actinobacteria was represented to a lesser extent in all the wet bank samples. The genera in the phylum which was consistently underrepresented in comparison to other lateral zones include *Conexibacter, Mycobacterium* and *Blastococcus* which is a common soil aerobe.

9.3.4 The number of fungal OTUs

Fungi respond unlike Bacteria, and showed little difference between the invasion statuses. Little differences were also evident in terms of wet banks, dry bank and terrestrial areas (Figure 9.4).

Summer data showed more variation in the number of OTUs measured than either autumn or winter (Figure 9.4).

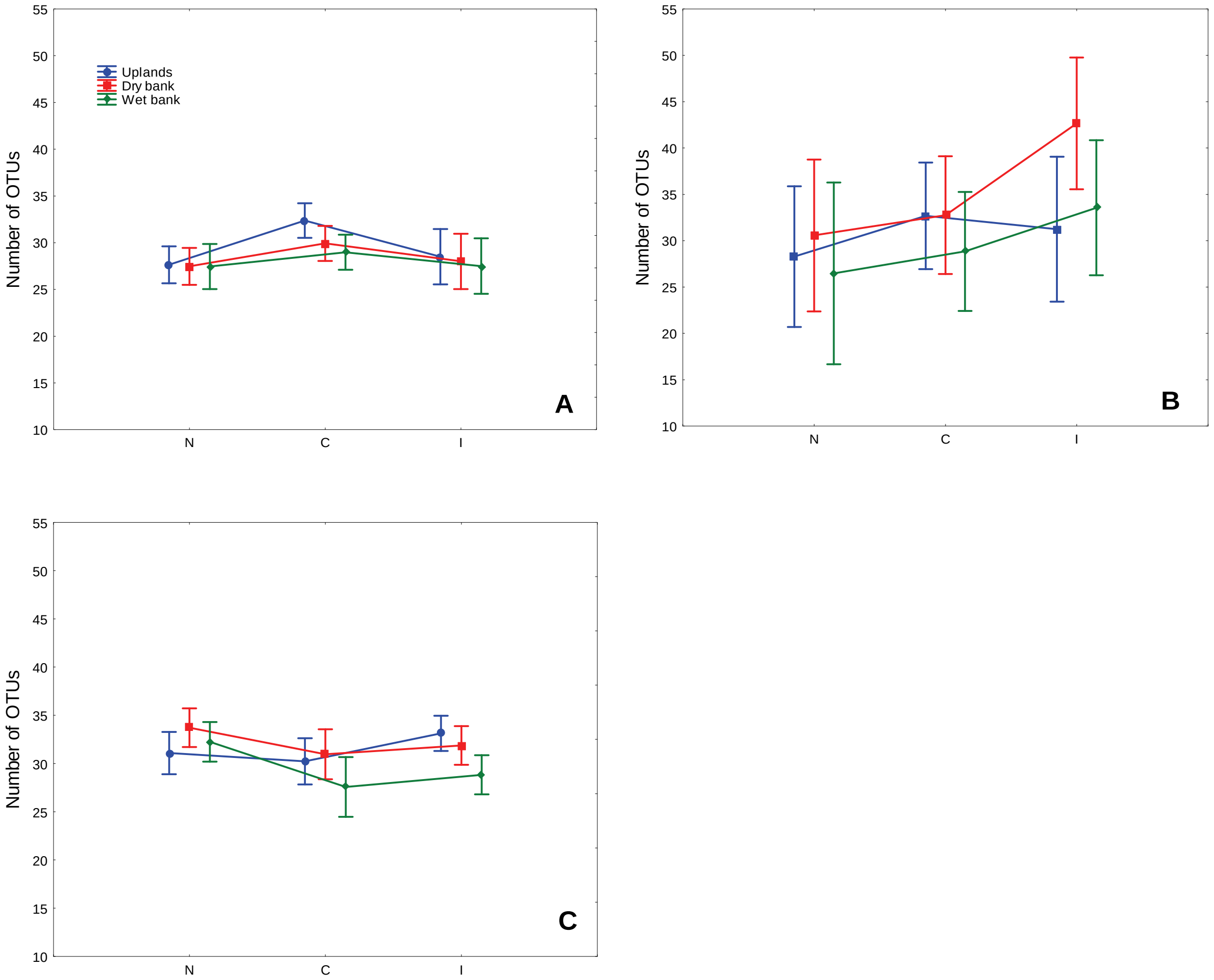

Figure 9.4: Whisker plot of the average number of fungal operational taxonomic units based on the ARISA profiles (f-ARISAs) comparing natural, invaded and cleared riparian ecotones in season 1, autumn 2010 (A), winter 2010 (B) and summer 2010/2011 (C). The points represent the means and the whisker is the SE.

9.3.5 Fungal OTU richness

The Shannon diversity of the fungal communities in the samples were not significantly lower compared to all the other samples and shown with repeated measures ANOVA ($p>0.05$) (Table 9.2).

Table 9.2: The Shannon diversity index of fungal genera based on the MEGAN analysis classification. E = Eerste (Natural); m = Molenaars (Cleared); W = Wit (Invaded); T = Terrestrial; R = Dry bank; W = Wet bank

Sample	autumn 2010	winter 2010	summer 2011
1ET	2.6333	2.4184	2.1615
1ER	2.7805	2.0007	N/A
1EW	2.5685	N/A	2.252
1MT	2.8349	2.3472	2.1204
1MR	2.01	2.258	2.7616
1MW	2.5982	2.2111	2.5634
1WT	2.4617	2.5079	3.0368
1WR	2.5508	1.9713	2.3737
1WW	2.7881	2.1029	2.1434

9.3.6 Fungal OTU classification and composition

Sequences generated from the analysis showed that 224 fungal genera, 86 orders and 34 classes were detected from all the samples (Figure 9.5). The sequences from the genus *Penicillium* were the most dominant group detected in the soil followed by those of *Cryptococcus* (Figure 9.5). These were the only two fungal groups that were found in every sample. The other common genera occurring in most of the samples included *Fusarium, Oidiodendron, Cheatonium, Lyctosphora* and *Mortierella* (Figure 9.5). The Shannon diversity of the fungal communities in the samples were not significantly lower compared to all the other samples as shown with RMANOVA ($p > 0.05$).

9.3.7 Interactions

When all the microbial and selected environmental data gathered are put together, it is possible to use principle component analyses to discover the relationships between the different factors (Figure 9.6A and B).

Bacteria and Fungi seem to be driven by different drivers. As with soil biogeochemical processes (see Chapter 6 - 8), silt and clay percentage emerged as a major influence on bacterial genera, but it did not emerge as a major driver of the fungal genera. However, for the bacterial genera, the more important soil properties were available P, pH and the C/N ratio. These properties have been noted before to change with invasion of *Acacia* species into the fynbos biome (Yelenik 2004; 2007). These drivers were, however, less important in structuring the fungal genera.

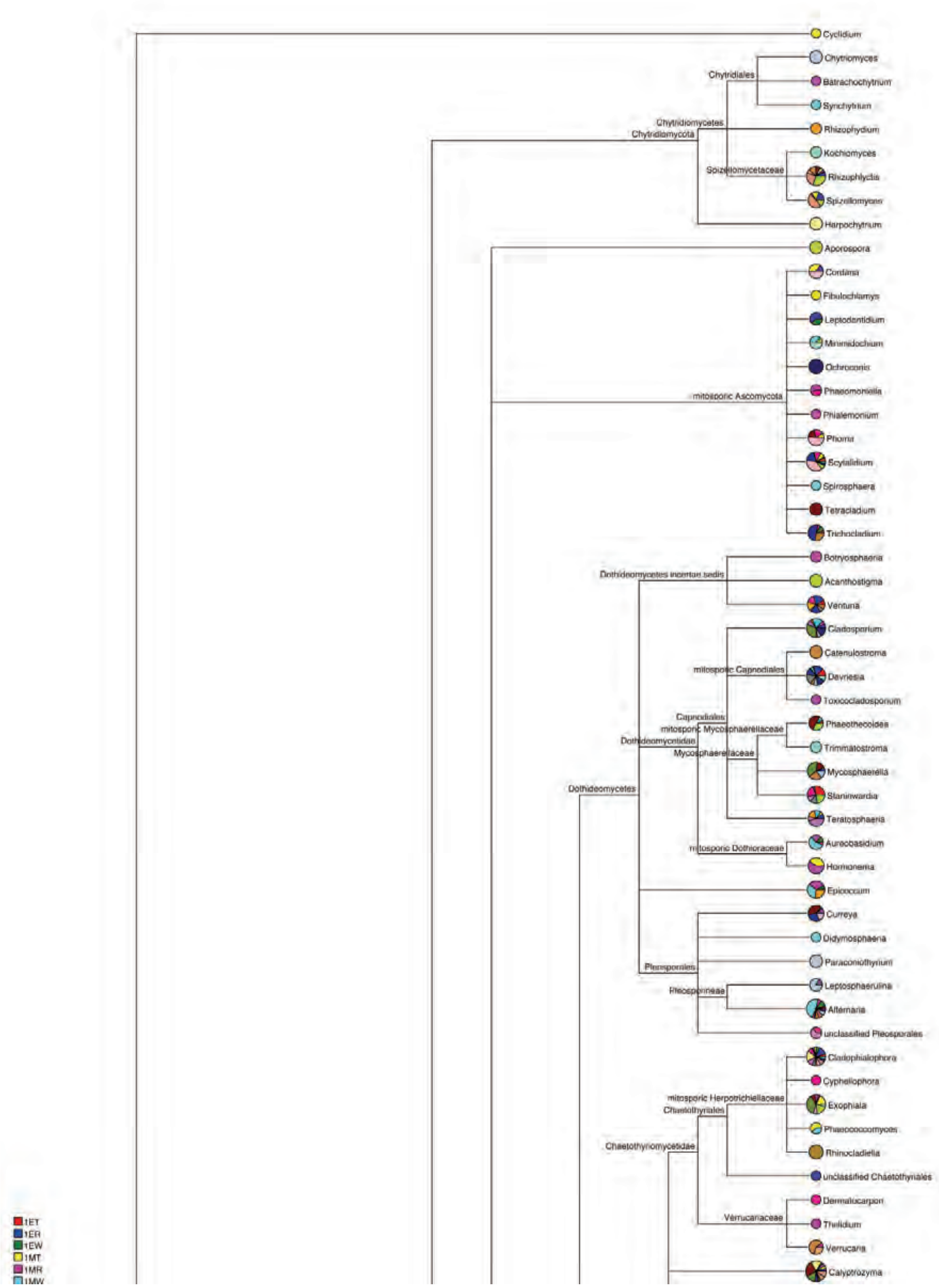

Figure 9.5: *MEGAN classification of sequences from all the sites to genus level.*

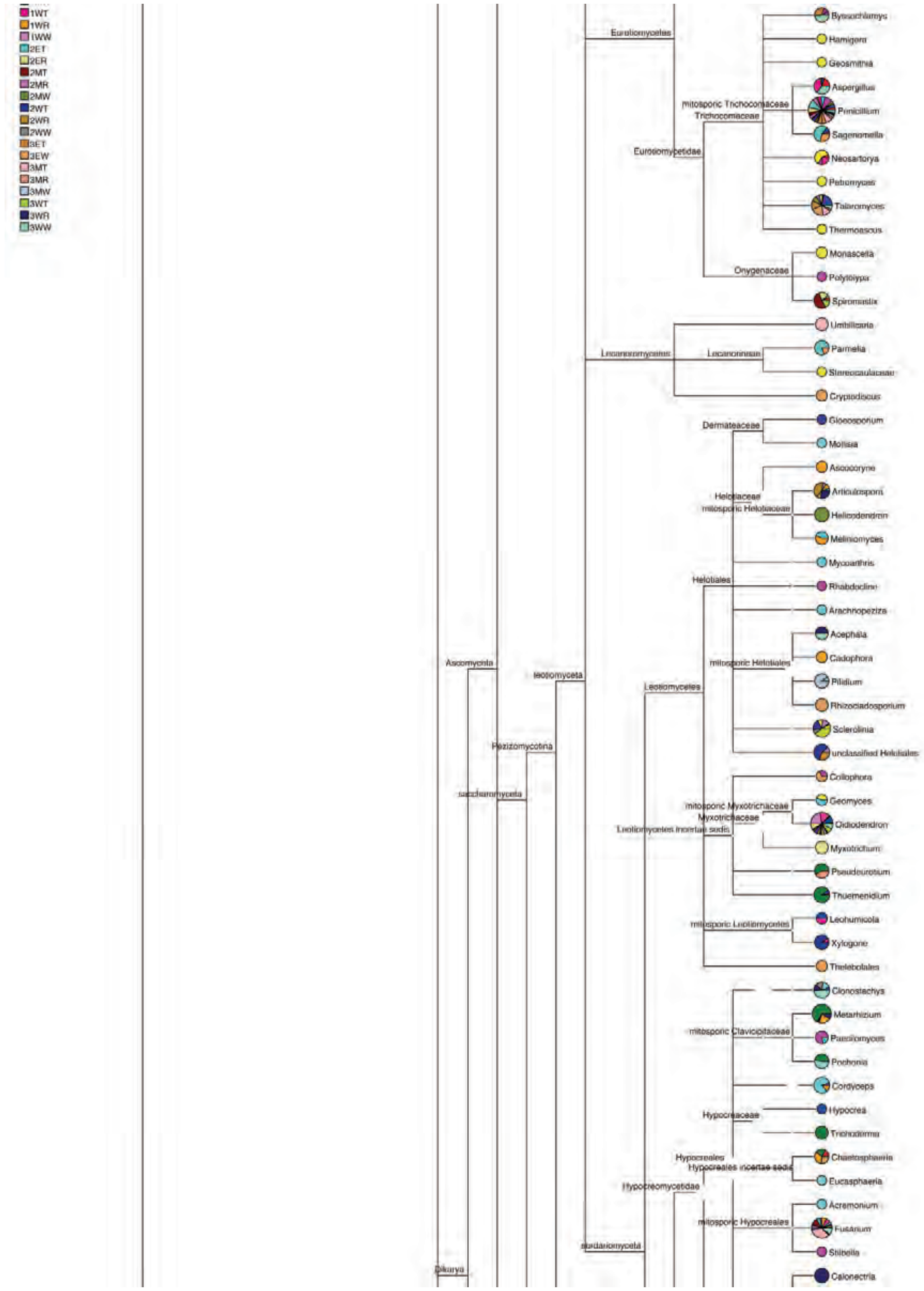

Figure 9.5 (Continued): *MEGAN classification of sequences from all the sites to genus level.*

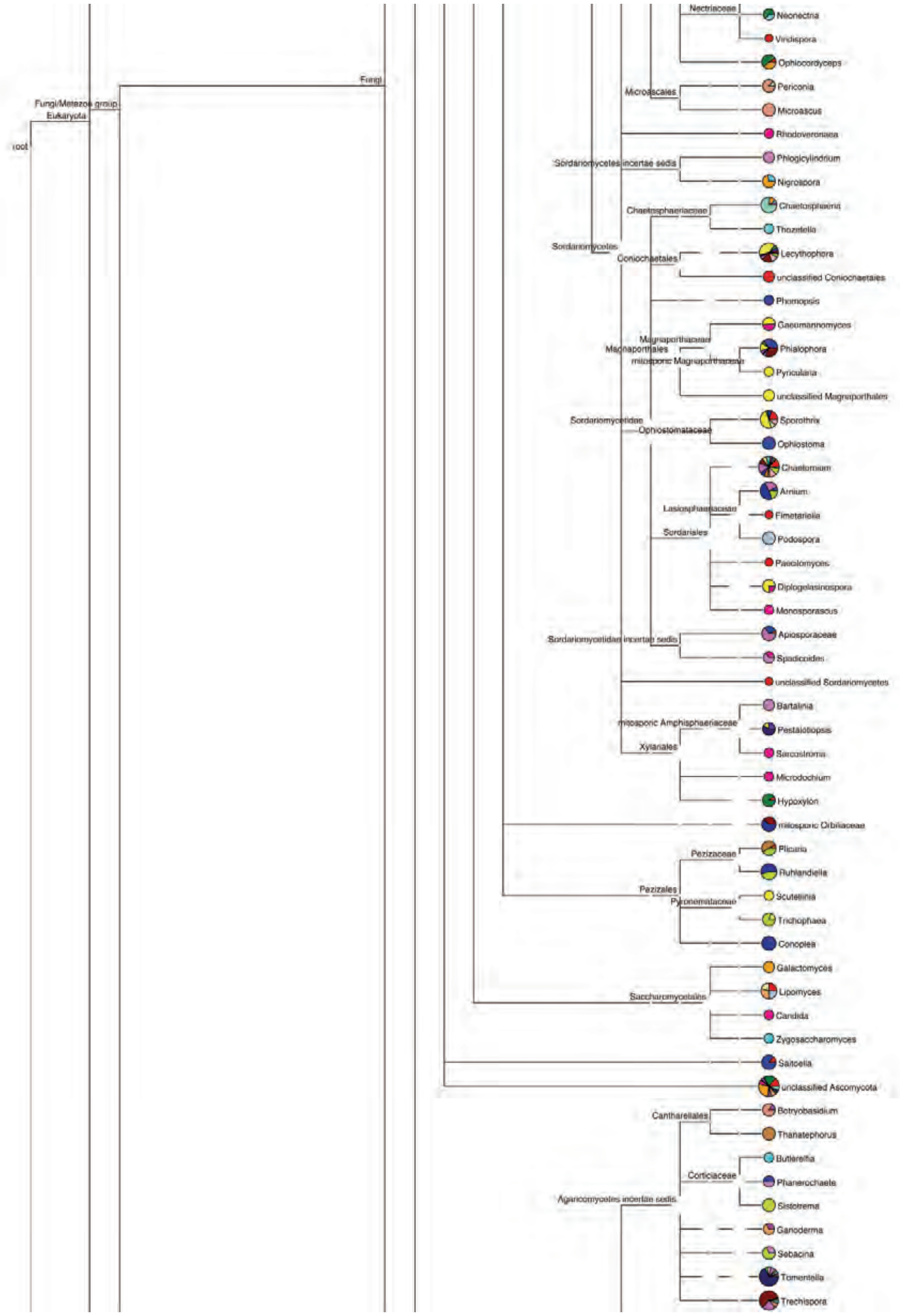

Figure 9.5 (Continued): *MEGAN classification of sequences from all the sites to genus level.*

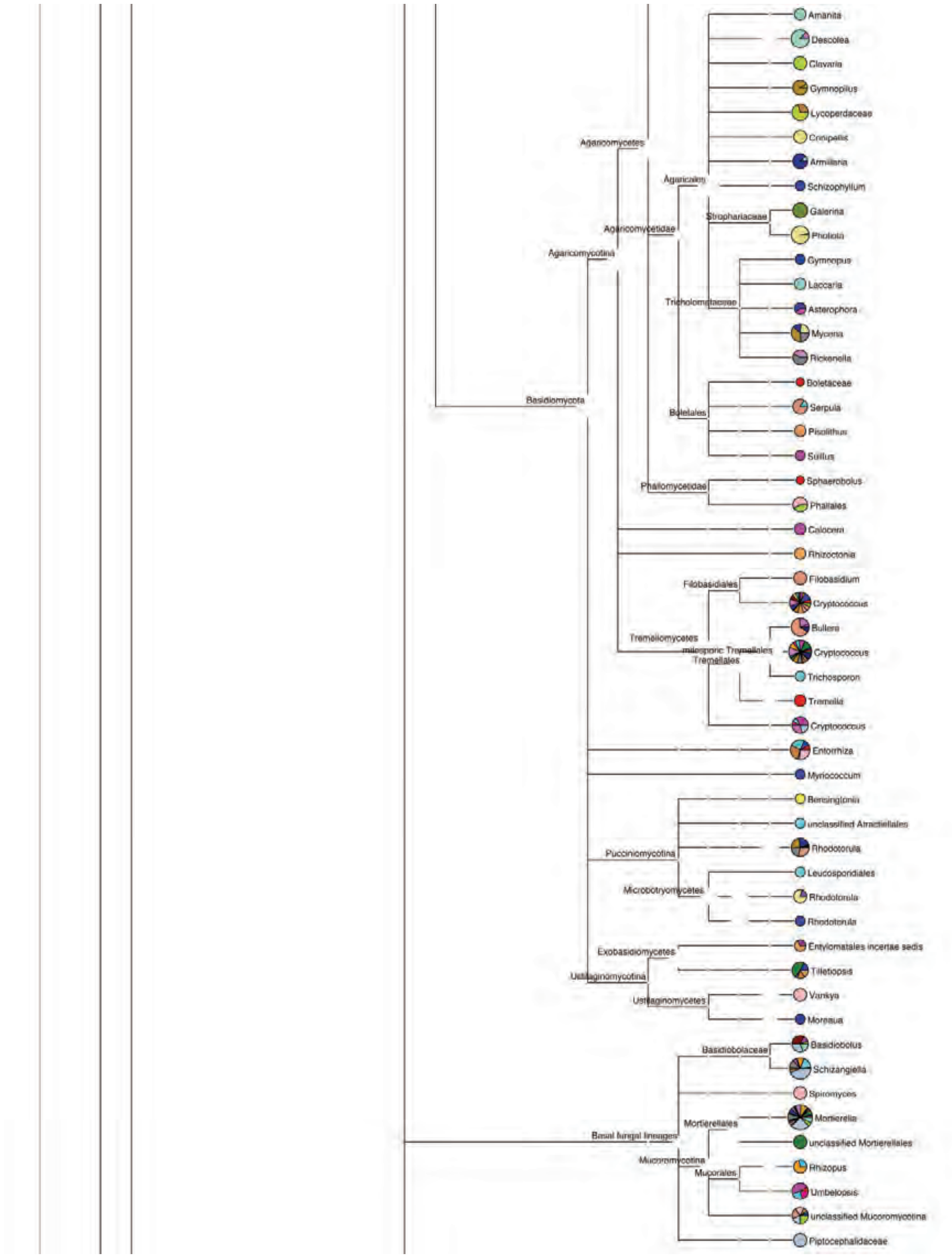

Figure 9.5 (Continued): *MEGAN classification of sequences from all the sites to genus level.*

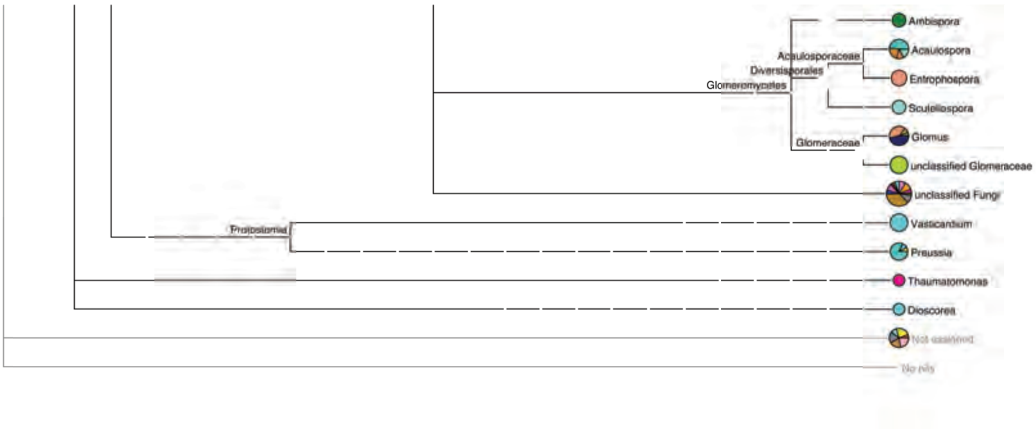

Figure 9.5 (Continued): *MEGAN classification of sequences from all the sites to genus level.*

9.4 DISCUSSION

From this study it was clear that invasion by *A. mearnsii* (and associated *Acacia* species such as *A. longifolia*) had a significant effect on the bacterial communities in soils of the riparian zone. The Shannon diversity was significantly lower in the invaded wet bank zones. The effect of *A. mearnsii* on the bacterial diversity could thus only be observed within the wet bank, showing the influence of the river on structural differences between bacterial communities. The reduction of bacterial diversity in the invaded wet bank zones is consistent during all three sampling seasons. The lower number of 5% dissimilar OTUs per number of reads, when compared to the other samples indicate a dominance of certain bacterial groups within the invaded wet bank zones.

In addition to a lower diversity, the structure of these communities is significantly impacted. The classification analysis showed that members of the phylum Alphaproteobacteria was dominant in all the soil samples. Alphaproteobacteria however, was significantly overrepresented in the invaded sites. Although function cannot precisely be inferred on bacterial OTUs when observing bacteria genera higher frequencies of N-fixing root associated Alphaproteobacteria bacterial genera was observed in invaded wet bank samples (Bontemps et al., 2010; Weir, 2004). Roots with nodulating N-fixing bacteria are commonly associated with *Acacia* invasion (Andam and Parker, 2008; Rodríguez-Echeverría et al., 2011). This is consistent with acacia's ability to fix additional N in the soil, which has been suggested to play a role in its invasive ability (Yelenik et al., 2007). The genera showed to be abundant in the invaded sites also occurred in the natural and cleared sites, although at lower levels. The soil bacterial communities of fynbos riparian soil may already be suitable to support the establishment of invasive *Acacia* species, without the co-invasion of N-fixing bacterial species specific to native Australian ecosystems.

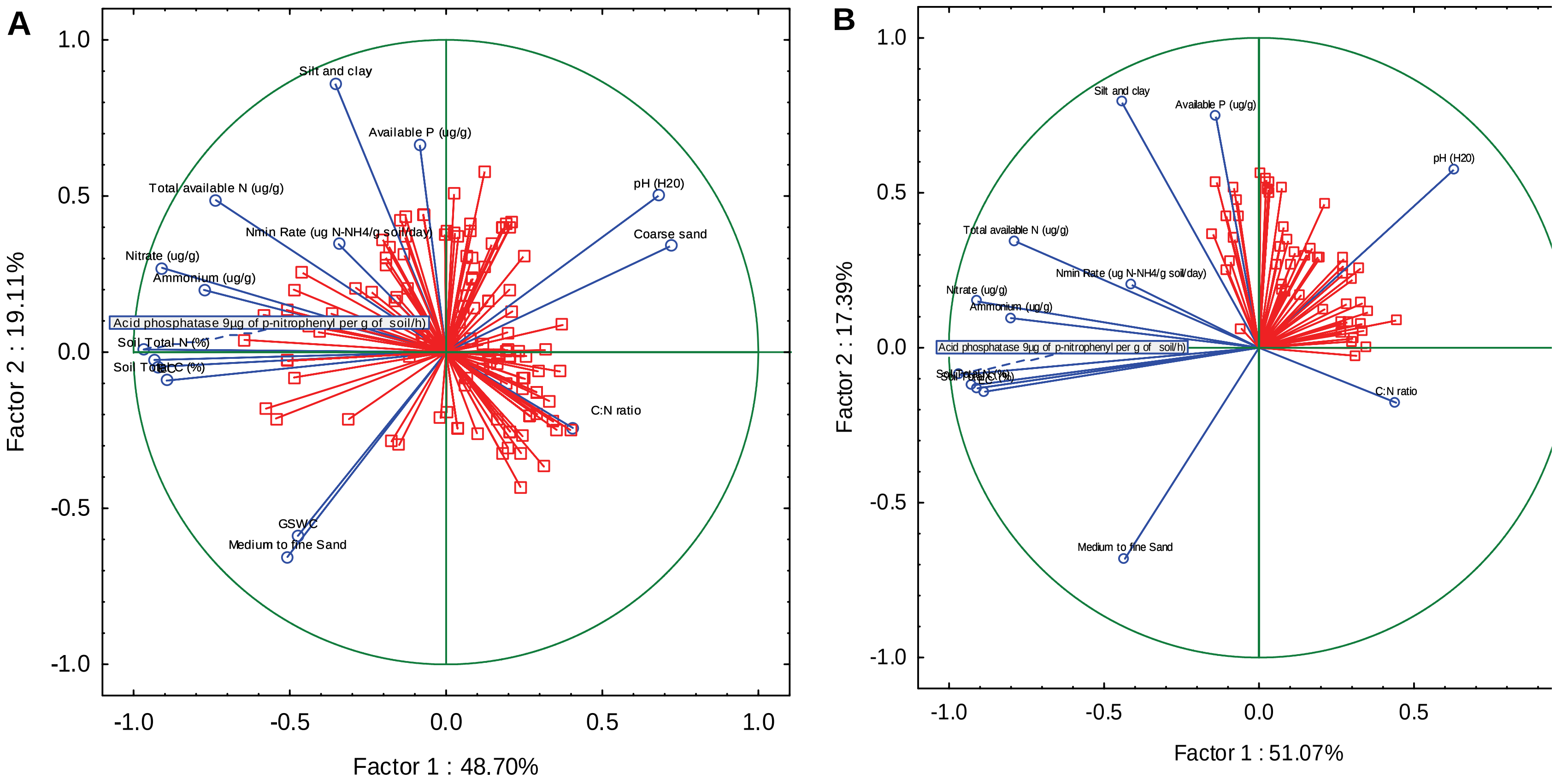

Figure 9.6: Principle component analysis of the measured environmental variables (circles) with the fungal genera plotted as supplementary values (squares) (A). (B) PCA of the measured environmental variables (circles) with the bacterial genera plotted as supplementary values (squares).

The most commonly associated bacteria associated with *Acacia* invasion belong the genus *Bradyrhizobium,* which supports the findings of Rodríguez –Echeverría et al. (2011). This genus occurred at high levels in all the samples including the natural sites. The lower frequency of representatives from the phylum Actinobacteria was also expected when considering the hydrological properties of the wet bank. The wet banks are generally less well aerated and phylum *Actinobacteria* consists of a substantial number of obligatory aerobic bacteria.

The effect of location of the sampling sites in context of its hydrology was determined to have an overriding effect on the structure of the bacterial community. The difference observed when observing the communities from the three lateral zones within every individual site was thus due to local environmental differences. These may include the influence of the plant community, hydrology or soil properties differences. The environmental factors structuring the communities were shown to be consistent over the three seasons, because bacterial community structure was also unchanged. The terrestrial, dry bank and wet bank zones from the different sampling sites did not form significant groupings when performing non-metric multi-dimensional scaling and cluster analysis (data not shown). This indicates the bacterial communities between similar lateral zones from different sites were different in structure. This may be due to differences in environmental variables, which may be site specific. The influence of spatial separation may also have an influence on the way bacterial communities are structured in similar riparian lateral zones.

From the result it appeared that unlike the bacterial communities, invasion by *Acacia* has little effect on the fungal communities in the fynbos. The taxonomic classification at all levels of taxonomic hierarchy showed that no specific fungal groups could be associated with the invasive status or the hydrology of the riparian zones. This was confirmed with Unifrac analysis, which indicated a random distribution of fungal genera. *Penicillium* and *Cryptococcus* species were, however, consistently detected and the most dominant in all the samples. This is an indication that these fungal genera are cosmopolitan in the fynbos soil. The community composition appeared to be relatively random between all the samples. This significant variation was also seen in samples from the same sites between the different seasons. This huge variation in the community composition between all the samples may be an indication of a high degree of community turnover within a site or over time. The distributions of fungi may thus be very heterogeneous within a site or the fungal community may have changed significantly between seasons. Both cases would indicate that the fungal community composition may be highly sensitive to soil environmental conditions. The spatial and temporal scale at which samples were taken proved to be insufficient to accurately assess the fungal taxonomic diversity across the sites. Due to the high diversity and variability of the fungal community composition between samples it was not possible to determine if any seasonal variation occurred. The fungal community structure could thus not be explained by the invasive status or the lateral zones of the sites due to this high variability.

10 SOIL PROPERTIES, SOIL PROCESSES AND PLANT AND MICROBIAL DIVERSITY IN NATURAL, *ACACIA*-INVADED AND CLEARED RIPARIAN ECOTONES IN THE EASTERN AND SOUTHERN CAPE: COMPARISONS WITH THE WESTERN CAPE

10.1 INTRODUCTION

The fynbos biome is incorporated into the smallest floral kingdom the Cape Floral Kingdom, of which only 6 exist worldwide (Cowling, 1992). At the most eastern extent, the Baviaanskloof and Gamtoos catchments contain several types of fynbos, and are, like the western part of the fynbos biome, heavily invaded with *Acacia* species and other invasive woody species within the genera *Eucalyptus* and *Pinus*. *Acacia mearnsii* is one of the main invaders, and riparian zones invaded by *A. mearnsii* in the Eastern Cape have been the subject of several studies (e.g. Fourie, 2008, van der Waal, 2009).

Van der Waal (2009) studied soil properties of invaded and cleared (‘restored’) riparian ecotones in the Gamtoos River catchment. He found that riparian soils in invaded areas had higher contents of N, potassium and manganese, and that soil pH also differed from natural areas. These differences persist in cleared areas, but differences in pH became smaller (i.e. the cleared areas appeared to be on a trajectory back to resembling the natural areas). Other nutrients, e.g. N, potassium and manganese also decline when *A. mearnsii* was removed from riparian zones. Not known, however, was how soil processes and soil microbial diversity differed. This research partially addresses objective 5, and a subsequent decision to compare results for the western Cape on the one hand with those of the southern and eastern Cape. The data presented was gathered during the course of 2011 and 2012, during which several field trips were undertaken to the southern and eastern Cape.

10.2 SITES AND METHODS

10.2.1 Sites

The study took place in riparian and upland areas situated in the Kouga Mountain Fynbos Complex south of Uniondale and the Baviaanskloof Mountain Fynbos towards the eastern boundary of the Baviaanskloof Wilderness area of the Western and Eastern Cape Provinces, South Africa. It incorporates several mountain ranges, notably the Outeniqua Mountains and some of the Kouga Mountains and Langkloof Mountains that define the southern end of the conservation planning domain in the Baviaanskloof region. The two geographical locations selected could be characterized as relatively cool and rainy during winter, but warm and dry during summer. The Eastern Cape sites lie approximately 80 km west of Port Elizabeth and 30km north-west of the town called Kareedouw, and form part of the Cape Folded Mountains. More specifically, the following natural sites were included: Baviaans River, Voeght's River Upper and Lower. The invaded sites were: the Witteklip River Upper, the Kammanassie River, and the Groot River Lower. The cleared sites were: the De Hoop River, the Groot River Upper, and the Witteklip River Lower. More information on the study sites were given in Chapter 3. An exception to our site selection criteria was that the Baviaans River was part of an active restoration trial where native tree, shrub and graminoid species were hand-planted in order to restore the riparian ecotones. This was deemed necessary as no other suitable natural sites were found. The same site was also used by van der Waal (2009).

The topography is characterized by several parallel mountain chains, with moderately steep slopes (20^0) (Rebelo et al., 2006). The Groot River drains a large part of the Kouga Mountains to the west and flows into the Baviaanskloof where it joins the Kouga River coming from the Karoo, forming the Gamtoos River, which eventually flows into the Indian Ocean. The study area falls within a non-seasonal rainfall area (Cowling and Holmes, 1992). It receives between 300 – 600 mm per annum (Boshoff et al., 2000), and has a bimodal peak in autumn and spring (Pietersen, 2009). On the south facing coastal mountains, southerly winds in autumn and spring bring orographic rain, associated with frontal systems (Rebelo et al., 2006). As in the western part of the fynbos biome, the summer is the driest season (especially the end of summer), however, two of the sites are situated on the southern slopes of the Langkloof Mountains, with a southern aspect, and thus receives rain throughout the year (though much lower in summer), as most of the Knysna forest complex. Precipitation ranges from drizzle to rainstorms, and frost can occur for 10-40 days per year at the more inland sites (Rebelo et al., 2006).

The three sites in the Eastern Cape (Baviaans and the two Witteklip River sites) are mainly shallow acidic lithosol soils that are derived from quartzitic sandstones of the Witteberg Group (Nardouw Subgroup) and also contain sandstones of the Table Mountain Group as described by Rebelo et al. (2006). The six sites in the southern Cape (on the Kammanassie, Groot, de Hoop and Voeght Rivers) are situated on geology ranging from Table Mountain sandstone to shales of the Bokkeveld group (Kotze, 2001).

10.2.2 Methods

The following analyses were carried out:

- Cover of plant functional types
- pH
- Electrical conductivity

- Nitrogen mineralization (*ex situ*; NMP)
- Denitrification enzyme activity
- Soil respiration
- Soil microbial structure

All methods used are as described before (Chapters 3-9).

We used ANOVAs where data was normal, and log-transformations where data was not, followed by the Tukey's post hoc test.

10.3 RESULTS

10.3.1 Plant functional type cover and soil physicochemical properties

The data presented for plant functional types, soil pH, electrical conductivity, soil respiration and soil microbial structure covers all the sites sampled, however, those for denitrification enzyme activity and for N mineralization covers only part of the sites. The data for plant functional type cover is shown in Figure 10.1.

Invaded sites showed marginally the highest *Acacia* cover in the dry banks (Figure 10.1B), while virtually no *Acacia* cover was evident in the terrestrial sites. The cover was similar to that if western Cape sites, however, the invasive species differ; in the western Cape sites *A. mearnsii* dominated *Acacia* cover, with *A. longifolia* the other prominent woody invasive species, while in the southern and eastern Cape *A. mearnsii* was by far the most prominent, followed by *A. dealbata*, which is closely related to *A. mearnsii*.

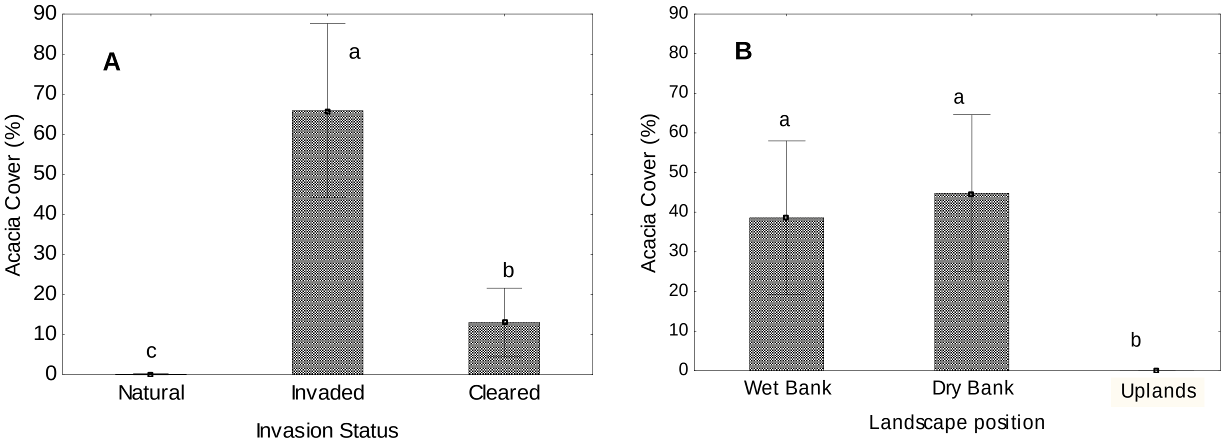

Figure 10.1: Plant cover of alien invasive *Acacia* species (A. mearnsii and A. dealbata) arranged by (A) invasion status and (B) landscape position for sites in the southern and eastern Cape. Bars indicate means and whiskers indicate ± 95% confidence interval. Letters denote significant differences determined using a one-way ANOVA followed by post hoc Tukey tests.

pH and electrical conductivity (Figure 10.2) followed the same trends evident of soils from the western Cape sites, that is, an increase in pH (water) and electrical conductivity with invasion and a downward trend when *Acacia* trees have been cleared. However, these differences were not significant.

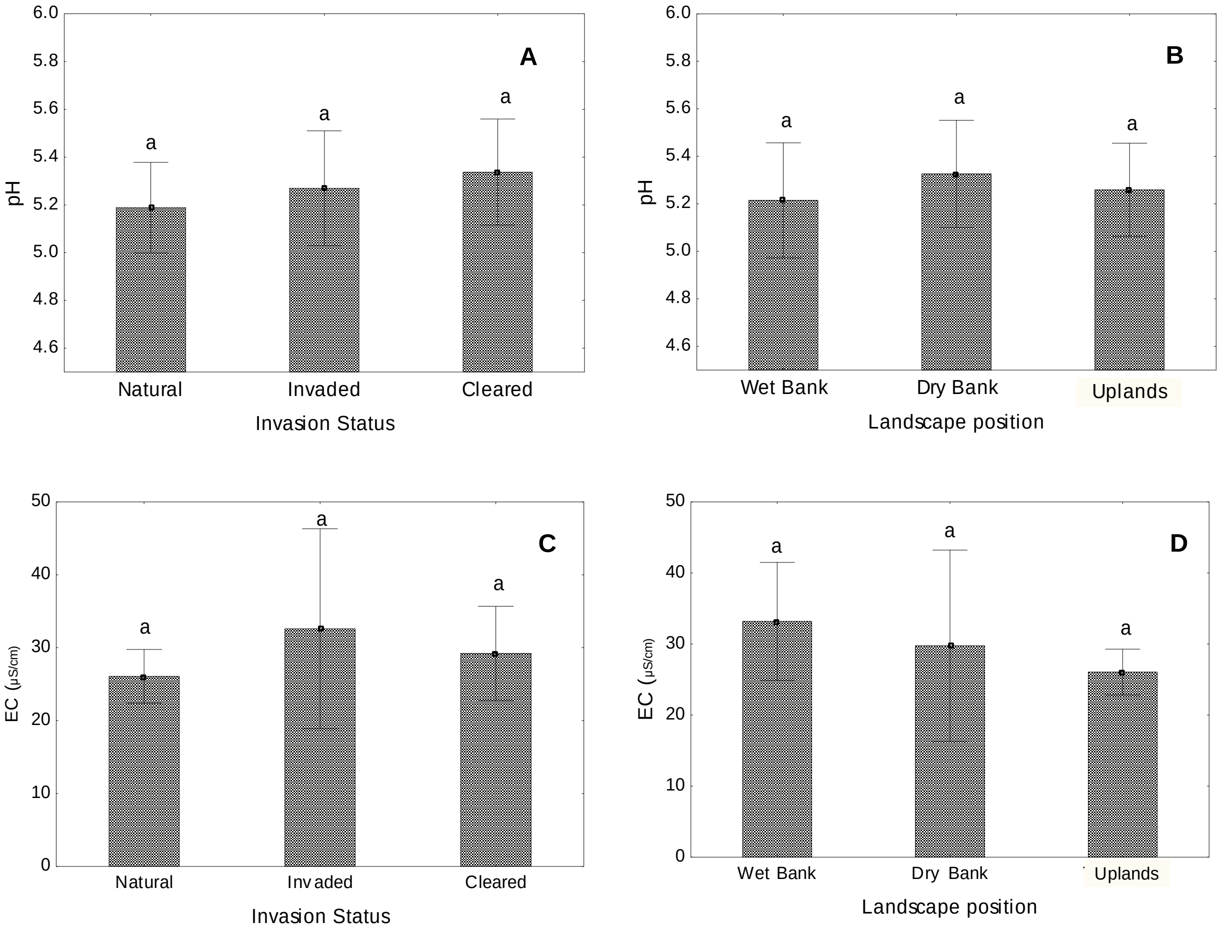

Figure 10.2: Soil pH (A and B) and electrical conductivity (C and D) arranged by (A and C) invasion status and (B and D) landscape position for sites in the southern and eastern Cape. Bars indicate means and whiskers indicate ± 95% confidence interval. Letters denote significant differences determined using a one-way ANOVA followed by post hoc Tukey tests.

10.3.2 Nitrogen cycling

Potential N mineralization rates were highest in the invaded riparian areas (Figure 10.3), though the rates were not different from that of the cleared riparian ecotones. Little differences were evident when the results are viewed by landscape position.

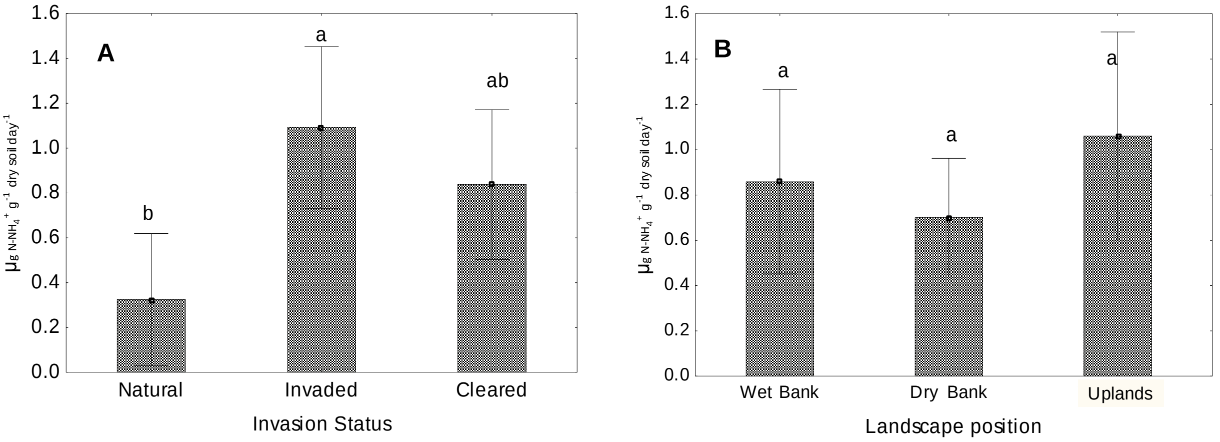

Figure 10.3: Soil N mineralization (*ex situ*) arranged by (A) invasion status and (B) landscape position for sites in the southern and eastern Cape. Bars indicate means and whiskers indicate ± 95% confidence interval. Letters denote significant differences determined using a one-way ANOVA followed by post hoc Tukey tests.

The effect of available N and organic C on the maximum potential denitrification activity was determined and compared to reference conditions once off during summer 2011/2012 for the southern and eastern Cape (Figure 10.4). This was done by a denitrification enzyme assay carried out in the laboratory with fresh field soil samples. The process involved the inhibition of N_2O conversion to N_2 in the presence of acetylene under anaerobic conditions. The denitrifying enzyme concentration of the field sample reflects the environmental history of the study site, which means this method can be used as an index of denitrification.

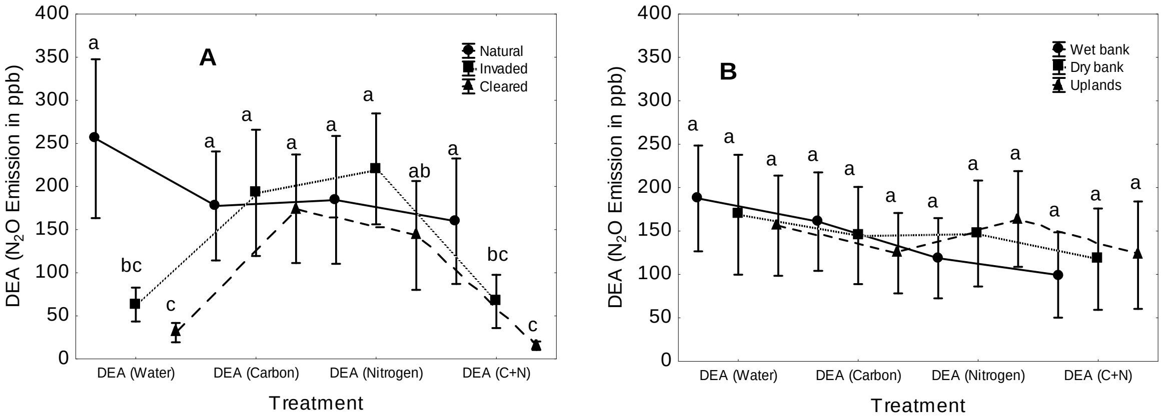

Figure 10.4. (A) Maximum potential denitrification enzyme activity for the different denitrification enzyme assay treatments arranged by invasion statuses (natural, invaded and cleared) and (B) maximum potential denitrification enzyme activity for the different denitrification enzyme assay treatments arranged by landscape position (wet bank, dry bank and terrestrial). Mean values are indicated by different symbols and whiskers represent the standard error. Letters denote significant differences determined by one-way ANOVAS for invasion status, landscape position and DEA treatment followed by post hoc Bonferroni tests.

There is no evidence from this study that available N (in the form of NO_3^--N) or organic C (in the form of sodium succinate) is a limiting resource in the maximum potential denitrification activity of soil micro-organisms in natural (Kruskal-Wallis test: p=0.29; Figure 10.4), invaded (p=0.42) and cleared (p=0.09) riparian zones. Similarly, no evidence was found comparing the wet bank (Kruskal-Wallis test: p=0.26), dry bank (p=0.67) and terrestrial (p=0.97) landscape positions. When all the data for the different treatments (reference condition, amended N and C source) were combined, there was a statistical difference between invasion status (Kruskal-Wallis test: p=0.01), and more specifically between the natural and cleared (Kruskal-Wallis test: p = 0.01) and invaded and cleared (Kruskal-Wallis test: p = 0.01) riparian zones.

10.3.3 Soil respiration

We also incubated soils from our southern and eastern Cape sites, and used an infrared gas analyser to measure soil CO_2 emissions. The method was described in detail in Chapter 6, and will not be repeated here. The results are displayed in Figure 10.5.

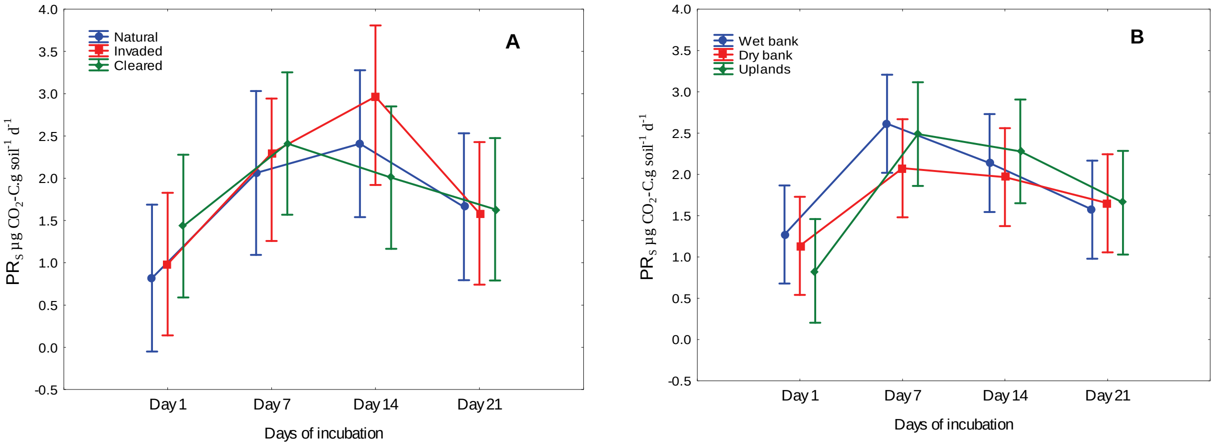

Figure 10.5: Soil potential soil respiration (*ex situ*) arranged by (A) invasion status and (B) landscape position for sites in the southern and eastern Cape. Symbols indicate means and whiskers indicate ± 95% confidence interval. PR_S = Potential soil respiration.

Initially soils from the invaded sites emitted more CO_2, however, this later dropped off, and by day 14 of the incubation, the natural sites has the highest potential soil respiration values. Little differences exists between the natural, invaded and cleared sites, though trends show higher values in invaded sites after 1 and 7 days, and lower potential soil respiration when *Acacia* species were cleared from riparian ecotones. Overall, the values obtained at each of the sampling days resemble that in of the samples from the western Cape sites, both in terms of the absolute rates of CO_2 emissions and the trends observed over the timeperiod of the incubations.

10.3.4 Fungal diversity

The fungal diversity and OTU richness of samples from the southern and eastern Cape did not differ significantly when comparing natural, invaded and cleared lateral zones (Figure 10.6 A and B).

The diversity of the landscape positions also showed no statistical differences. The fungal diversity of the southern and eastern Cape samples was however significantly higher when compared to western Cape samples.

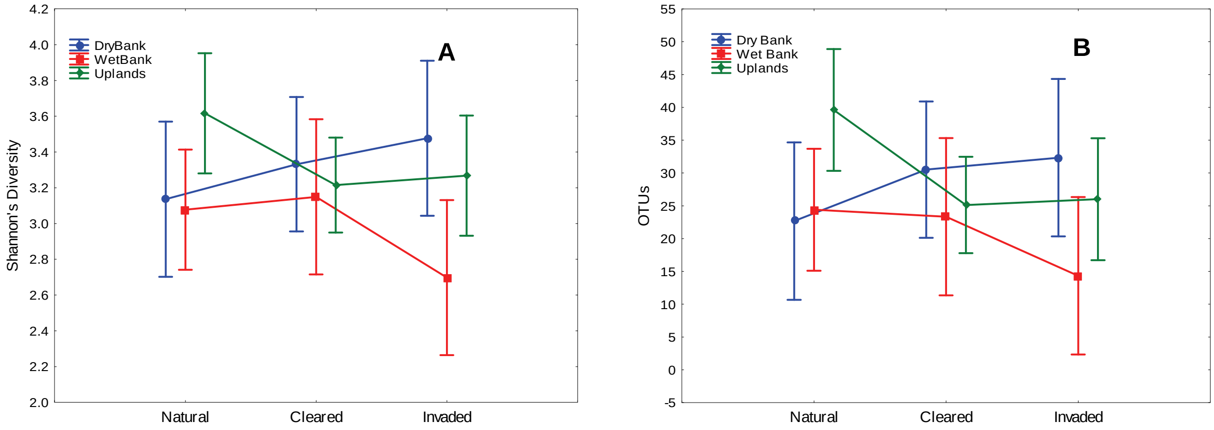

Figure 10.6: Shannon's Diversity Index (A) and OTU richness (B) of the fungal community of southern and eastern Cape samples. Symbols indicate means and whiskers indicate ± 95% confidence interval.

10.3.5 Bacterial diversity

The bacterial diversity and OTU richness of samples from the southern and eastern Cape did not differ significantly when comparing natural, cleared and invaded lateral zones (Figure 10.7 A and B). The diversity of the lateral zones also showed no statistical differences.

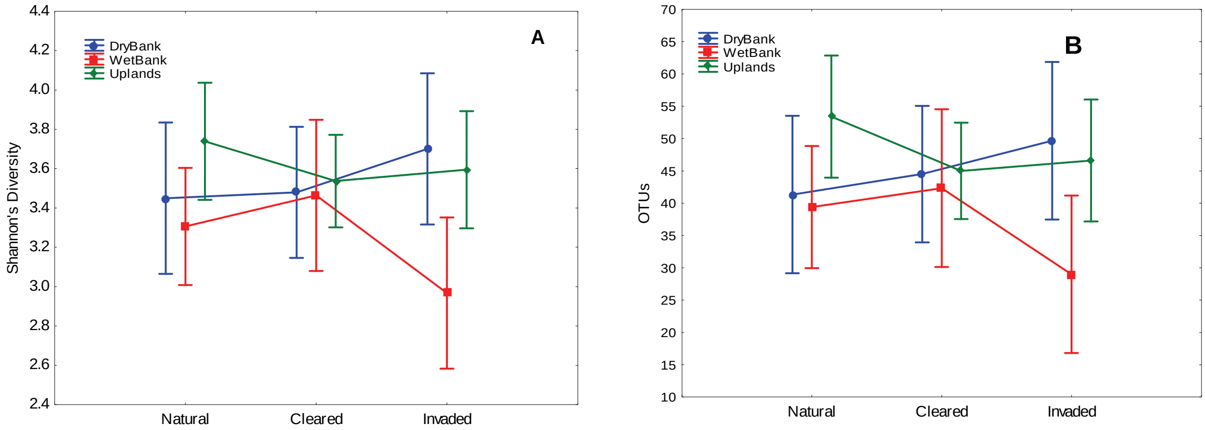

Figure 10.7: Shannon's Diversity Index (A) and OTU richness (B) of the bacterial community of southern and eastern Cape samples. Symbols indicate means and whiskers indicate ± 95% confidence interval.

In contrast to the fungal diversity, bacterial diversity of the southern and eastern Cape samples was similar compared to western Cape samples with no significant differences observed. The wet bank samples from the southern and eastern Cape did not show a significant reduction in diversity which was seen in the western Cape samples.

10.4 DISCUSSION

Most of the results suggest that soil properties from the southern and eastern Cape are broadly similar to what was found for the western Cape sites. While the invades sites had higher cover compared to the western Cape, soil pH was within the same range as that of the western Cape, and electrical conductivity did not show clear differences between natural, invaded and cleared riparian zones. However, as these measurements was done as a once-off sampling, this is not unexpected.

Similar to the western Cape sites, incubation of soils at constant temperature and moisture did not reveal clear differences between soils from sites with natural vegetation, those invaded by *Acacia* species and those where clearing have taken place. This suggests that soil microbial activity is not that different, again supporting the results obtained in the western Cape. However, N mineralization was slightly elevated in invaded riparian ecotones, and N mineralization also appeared to remain slightly elevated in cleared riparian ecotones. This may, however, been due to an interaction with landscape position.

Denitrification enzyme activity again showed slightly elevated rates in the invaded as opposed to the natural and cleared riparian ecotones, however, this was not a significant difference. A correlation exist, however, between N mineralization and denitrification, suggesting that invaded sites have accelerated N cycling, and that clearing decelerates N cycling, which is broadly in accordance with what was found at the western Cape sites. Further, the once-off sampling of soil for soil microbial diversity measurements did not show any differences between natural, invaded and cleared riparian zones, suggesting resistance to invasion for both bacterial and fungal biota at these sites. As was noted for sites in the western Cape, denitrification enzyme activity remained low, and it seems that denitrification is not the major pathway of loss of N in invaded riparian ecotones, possibly because of the low clay and silt content of fynbos soils, or as a result of the asynchronicity of soil moisture and temperature that are optimal for denitrification (Pinay et al., 1992).

Incubation of soils in microcosms showed, like in the western Cape, that inherently, soil microbial respiration was not majorly different between sites with different invasion status. Overall, the cleared riparian ecotones showed the lowest PR_S rates when comparing invasion status, and the wet banks the lowest when comparing landscape positions. However, this was only slightly lower than other invasion status and landscape positions, and differences were generally not significant. This suggests, again, an important role for root respiration, which was also shown by Juahainen et al. (2012).

11 ABOVEGROUND-BELOWGROUND INTERACTIONS IN NATURAL, *ACACIA*-INVADED AND CLEARED RIPARIAN ECOTONES

11.1 INTRODUCTION

Invasive species, especially those that associate with root symbionts and can add to or remove resources from ecosystems, can affect ecosystems functioning through above and belowground interactions (Bardgett and Wardle, 2010). *Acacia* species associate with *Rhizobium* species, and has been shown to add N and C to soils, thus changing ecosystem stoichiometry and may also affect soil biodiversity. The aim of this chapter is to explore the top-down effects of invasive *Acacia* species on soil processes and soil microbial biodiversity in riparian ecotones in the fynbos biome. We use the results generated through our research over three years to investigate how changes in functional types aboveground (increase and active removal of *Acacia* species affect belowground properties. We also consider the role that landscape position play in above and belowground interactions, and interactions between landscape position and invasion status.

Witkowski (1991), Musil (1993), Stock et al. (1995) and Yelenik et al. (2004; 2007) all showed clearly how increasing cover of invasive *Acacia* species impact nutrient stocks, and in a limited fashion, nutrient cycling (N). However, what is less clear is how other processes (denitrification, P cycling, C cycling) and soil biodiversity are affected. Based on results from other temperate regions, e.g. continental USA, it may be postulated that both the N and C cycles will be affected (e.g. DeCant, 2008; Follstad Shah et al., 2010). Even less clear is how soil process and microbial diversity can recover if the influence of the invader is removed. Do legacy affects remain, for instance in the form of elevated available nutrients and microbial biodiversity in the soil? Is there a trajectory towards restoration of ecosystem function, as assumed by agencies involved in removal of alien invasive species from riparian ecotones in South Africa?

11.2 METHODS

We developed an artificial neural network (ANN) model to understand the link between invasion by *Acacia* species in riparian zones and modification to the soil microbial structure. It was possible to develop such a model for fungi, as some biota within the community seem to react predictably in invaded riparian ecotones (even though overall soil fungal community structure did not change appreciably with invasion, see Chapter 9). It was not possible to develop a model for bacteria due to a lack of suitable data.

An ANN was constructed to model the relationship between fynbos soil fungal communities and the presence or absence of *Acacia* invasion. The community data included OTU data from 9 fynbos sites from 3 seasons (autumn, winter and spring). The data used for in the input layer include OTU data from ARISA profiles and 454 sequencing. The algorithms used for modeling included a multi-layer perceptron structure (MLF). The MLP model uses the identity function of the input layer as the activation function and a softmax algorithm as output function. The algorithm structure was trained over a maximum of 200 cycles with a learning rate of 0.1. The number of neurons in the hidden layer was heuristically determined by the software between two and a 1000 with 100 networks trained. The model used back-propagation learning function to calculate the error function while adjusting the weights of the network. The model further used cross entropy as error function. Data used for a training set constituted a random sampling set of 70% of the entire data set, 15% of the data set was used to test the model and 15% to validate the results. The number of neurons in the neural network was chosen based on the best performing models. The performance of the ANN was assessed by the measure of similarity between the observed values and the output thus determining the percentage correct predictions. Additionally the predicted and the observed values were plotted and the linear regression value determined.

11.3 RESULTS

The architecture of the best performing ANN contained a single layer with 8 neurons (Table 11.1; Figure 11.1). The training, test and validation performance were at 100% correct when compared with the expected output. Thus the classification ANN performed well and could accurately predict whether acacias occurred on a site based on the fungal community structure. The ANN could also make the predictions with a confidence level of at least 96%. The relationship between the categorical variables and the fungal community structure was thus meaningful to such a degree that it could be used to predict the invasive status of a sample. This was an indication that the fungal community is influenced by the dominance of acacias in a definite and predictable manner, at least at the level of genera, if not for the entire community structure (Chapter 9). Based on the weights of the ANN the fungal genera were the most distinctive biota of invaded and non-invaded samples. The genera showed to occur exclusively or at high levels at invaded sites were *Tomentella*, *Fusarium* and *Sclerotinia*. The genera occurring at high levels or exclusively in natural samples were, *Lecythophora*, *Talaromyces*, *Calyptrozyma*, *Chaetomium*, *Cladophialophora*, *Sporothrix* and *Entorrhiza*. No genera are available that could reliably predict whether samples originate from cleared sites.

Table 11.1: Table summarising the performance of the artificial neural network model, based on percentage correct predictions and confidence values of predictions

	Number of samples	Correct	Confidence levels (Correct)	Confidence levels (Incorrect)
Train (N)	40	100%	0.99	0.04
Train (I)	11	100%	0.96 - 1	0 - 0.01
Test(N)	13	100%	1.00	0.00
Test(I)	5	100%	1.00	0.00
Validation (N)	6	100%	1.00	0 - 0.04
Validation (I)	5	100%	0.96 - 1	0.00

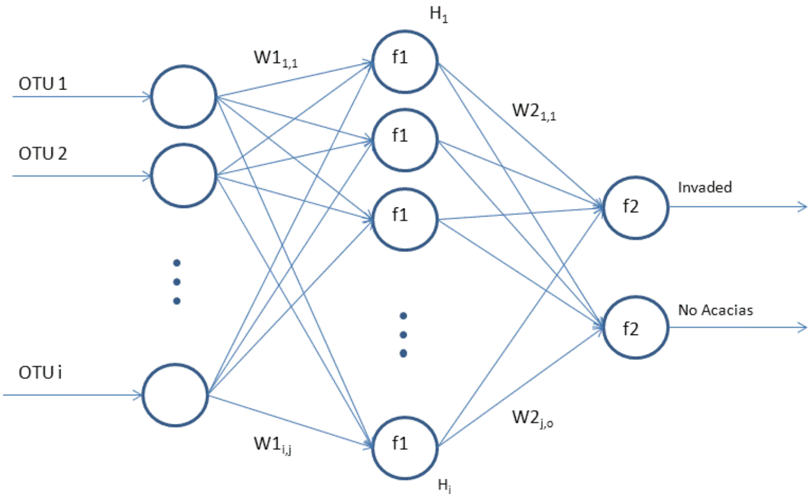

Figure 11.1: Diagrammatical depiction the structure of the artificial neural network developed to model the relationship between fynbos soil fungal communities and the presence or absence of *Acacia* invasion

11.4 DISCUSSION

In Chapter 9 we linked microbial community structure with several soil properties, among others silt and clay percentage, soil available P and the C/N ratio. All of these soil properties change with invasion of riparian zones by *Acacia* species. It was also suggested that differences are evident in the way the soil bacterial and fungal community responded to the presence of invasive *Acacia* species The soil fungal community appear to show some resistance in that soil fungal diversity (and community structure) did not change, however, the individual biota did show some predictable changes, and while this is not necessarily influential enough to change overall community structure, the presence of invasive *Acacia* species could be reliably predicted using the following biota in an artificial neural network: *Tomentella*, *Fusarium* and

Sclerotinia, occurred only in the invaded sites, while others such as *Lecythophora*, *Talaromyces*, *Calyptrozyma*, *Chaetomium*, *Cladophialophora*, *Sporothrix* and *Entorrhiza* occurred only at natural sites.. In contrast, while bacterial diversity did change upon invasion by *Acacia* species, it could be reversed by removal of the invasive *Acacia* species However, it was not possible for a neural network to be constructed using the bacterial data, suggesting that changes in community structure, where they occur are less predictable.

12 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

12.1 SUMMARY

We set out to test several key questions with the overall research question being: How does soil properties, processes and biodiversity (restricted to microbial diversity) differ between different landscape positions (wet bank, dry bank and terrestrial areas), different invasion statuses (natural, cleared and invaded riparian ecotones), and what is the trajectory after clearing of riparian ecotones?

We chose sites invaded by *A. mearnsii* (sometime mixed with other *Acacia* species e.g. *A. longifolia*), with high invasive cover, thus we also found that cover of size classes of *Acacia* trees (seedlings, juveniles and adults) were significantly higher in invaded riparian ecotones (keeping in mind that terrestrial ecotones were usually not invaded). Cleared riparian ecotones had significantly lower *Acacia* cover, as can be expected from riparian ecotones where clearing operations are active and initial clearing has been followed up. In contrast, cover of exotic grasses (especially in dry banks) increased in cleared sites, a trend which has been observed before in other studies (e.g. Maron and Connors, 1996; Yelenik et al., 2004).

Results for soil properties showed differences between invasion statuses, with lower course sand in invaded areas, however, finer material was not different (silt + clay). As expected, gravimetric soil water content differed, and seasonal interactions were apparent and prominent. The most prominent difference emerged in electrical conductivity where invaded and cleared riparian ecotones had significantly higher EC, which is consistent with other studies where invasive species in riparian and upland environments have been shown to promote the build-up of salts in the topsoil. While these differences are, on the surface, relatively minor, soil properties such as particle size, soil pH and EC have been shown to be major controls on soil processes and also influence soil microbial structure.

One of the processes influenced by soil physical and chemical properties is N cycling. There were generally positive correlations between fine particle content and soil chemical properties, including N. Nitrogen stocks was elevated in invaded riparian wet banks, and showed a trend towards the natural state when *Acacia* species was removed. Dry banks, in the natural state did not show any differences in N (or other nutrients) with wet banks and terrestrial areas, which does not support our expectation that riparian topsoil are enriched in N and other nutrients. The magnitude of the increase in N stocks in invaded riparian topsoil with invasion by N-fixing trees were much lower that was found by Yelenik et al. (2004; 2007) and others in terrestrial soils.

One potential pathway for N loss (thus reducing N loading of receiving waters) is denitrification. There were higher available N levels in invaded riparian ecotones, including NO_3^-; this anion is the substrate for denitrifying bacteria. There were also measurable N_2O fluxes coming from soils in riparian environments, however, trends that existed were generally not significant. Also, trends in denitrification enzyme activity (thus denitrifyer activity) were generally not significant. In all likelihood, denitrification is not a major consumer of NO_3^-, which may be related to the relatively low silt and clay contents of fynbos riparian soils in the higher longitudinal zones, as suggested by Pinay et al. (1992). An alternative pathway in riparian ecotones for reducing N loads is that annual floods and larger floods are able to mobilize most of the litter accumulating on soils of invaded dry banks, and leach some of the available N when riparian soils are inundated. However, the relative importance of these N loss pathways remains to be tested.

In cleared riparian ecotones, available N remained high 7+ years after removal of *Acacia* species. Available N is the only soil property that remained relatively unaffected after removal of alien species, and at the same time, these riparian dry banks also show high grass cover. Secondary invasion by alien invasive grass species (e.g. *B. maxima*) into riparian zones have been noted before in studies in the fynbos biome, and the hypothesis is that the relatively higher available nutrients in riparian zones may facilitate establishment of these invasive grasses. While we were not able to show a causal relationship, the trends do support the contention that more available N may facilitate higher grass cover flowing *Acacia* removal.

Parallel with N, soil total C was elevated in invaded dry banks, but did not differ significantly between dry banks and upland areas. Invaded dry banks also had higher soil respiration rates compared to natural and cleared riparian ecotones. When *Acacia* species are removed from wet banks the spike in soil respiration declined. In fact, there were little differences in *in situ* soil respiration rates between natural and cleared riparian ecotones. To answer the question of what drives soil respiration rates, especially in invaded dry banks, we conducted a small experiment where blocks of soil were trenched (i.e. trenches dug around it). Trends showed an immediate decline in soil respiration, although it later bounced back, possibly due to decomposition of fine and course roots. This does suggest, though it does not prove that root respiration may be involved in the higher soil respiration rates in invaded riparian ecotones. Further support for this is found when soils, minus roots are incubated in the laboratory at constant temperature and moisture. Little differences were found between different invasion statuses or landscape position, suggesting that soil microbial activity is not driving the higher soil respiration rates in *Acacia* invaded riparian dry banks. The higher rates of CO_2 efflux from invaded dry banks suggest that invaded riparian areas may be a source of CO_2, at least during some periods during the year, which has significant implications for regional C fluxes.

The main drivers for soil respiration seem to differ from season to season. Though soil temperature seem to be influential, it is appears that physical soil properties such as soil wetness is a major factor constraining CO_2 emissions from soils. Soil respiration is highest when soil temperature is highest and soil moisture is between 0 and 5% gravimetric soil water content (i.e. summer). This, however, may be related to plant physiology, which, based on seasonal photosynthesis dynamics for fynbos plants, should be highest in summer. Given the implications of our findings for regional C dynamics, the controls on R_S also need to be further investigated.

Available soil P did not show significant differences when expressed by invasion status; however, wet banks had higher available P than dry banks, but not compared to terrestrial areas. While available P did show some trends, more prominent differences emerged from analysis of acid phosphatase, a soil enzyme that can derive from root or microbes, and is involved in transformation of organic P to available P. This suggests that P may be a limiting element, which is consistent with the important role that P play in N-fixation. Following this reasoning, invasion of usually low-P fynbos riparian soils by N-fixing woody species, soil phosphatases is exuded to mineralize P locked up in OM . Any available P is taken up quickly by *Acacia* roots. This thus supports a contention that P cycling is relatively closed in fynbos soils. When the invasive species are removed, soil phosphatase activity also decline, suggesting that the demand for P has declined. While plant-soil interactions in terrestrial fynbos show how limiting P is in fynbos soils (Cowling, 1992), thus far no equivalent information exist for P in fynbos riparian ecotones.

Overall, soil bacterial diversity (Shannon diversity index) changed with invasion by *A. mearnsii* (and associated *Acacia* species such as *A. longifolia*). The Shannon diversity was significantly lower in the invaded wet bank zones. The effect of *A. mearnsii* on the bacterial diversity could thus only be observed within the wet bank, showing the influence of the river on structural differences between bacterial communities. No such differences was evident with fungal diversity, suggesting that the Fungi may be more resistant to invasion by these N-fixing, arbuscular mycorrhizal woody species, but that return of bacterial diversity to a state resembling the natural condition suggest that the bacterial community, while not resistant, are resilient, able to bounce back when the invasive species are removed. Soil bacterial and fungal community structure also changed when invaded by *Acacia* species, however, the change was reversible. Members of the phyla *Alphaproteobacteria* were significantly overrepresented in the invaded sites, but declined relative to other biota when *Acacia* species were removed. Based on the presence of individual fungal genera, we were able to construct a simple neural network model to predict the presence of invasive acacias, which shows the value of microbial data. This also shows the potential of using microbial communities as indicators of rehabilitation potential.

Soil bacterial and fungal diversity was driven by different soil properties. Soil bacterial community structure was driven by soil particle size, soil pH, and soil available P. It was noted during analyses for soil N dynamics that soil particle size was also important for N cycling, and also correlate to N cycling and acid phosphatase activity, hence, this suggest an important role for soil texture in riparian processes and soil microbial diversity. Another important soil physical property is bulk density, which showed good correlations with soil phosphatase activities, while available P correlated with soil bacterial structure. These interactions suggest that a thorough understanding of soil physical and chemical properties may add significant value to understanding soil processes and plant and microbial community structure in fynbos riparian ecotones.

We conducted no formal statistical comparisons between results for the western Cape and the southern and eastern Cape, however, in general, similar trends were observed at the secondary sites compared to the primary research sites. Soil respiration (*ex situ*) showed no clear trends between invasion statuses and between landscape positions, again suggesting an important role for root respiration, while soil microbial diversity showed clear differences between natural, invaded and cleared riparian ecotones, as was the case for the western Cape. Little can be concluded from the denitrification enzyme activity data, and overall, the soil physical data showed similar trends compared to the western Cape sites.

12.2 MANAGEMEMENT IMPLICATIONS

Currently little evidence exist on the impact of invasive species on soil functioning in the fynbos biome, other than that directly relating to nutrient levels (e.g. Yelenik et al., 2004). Along with the research of Yelenik et al. (2004) as well as the research reported here, it is clear that invasion by *Acacia* species can modify the soil environment, especially relating to N and C. As native fynbos species are adapted to low nutrient soils, this may hold implications for managers of land and water resources, especially where native fynbos species are growing in conjunction with exotic *Acacia* species along water courses, and where conservation of native fynbos is a management objective. Based on elevated soil phosphatase levels found in our study, *Acacia* species are apparently able to also modify the soil phosphorus cycle. Soil microbial structure also changes with invasion, though the impact on microbially-driven processes and ecosystem services is more challenging to quantify, and will likely require systematic follow-up studies.

Clearing of alien *Acacia* species from riparian zones restored some soil functions such as nutrient cycling, however, legacies remained. These legacies include available nitrogen, which has been associated with increases of exotic nitrophilous grasses after removal of *Acacia* species in the fynbos biome (Yelenik et al., 2004). Likewise, in the cleared sites we investigated, exotic grasses such as *E. calycina* and *B. maxima* became prominent cover types in especially the dry bank. At the same time, few seedlings of native tree species were observed. Although we could not show a causal relationship, elevated N has been shown to encourage establishment of nitrophilous grasses in temperate ecosystems where invasive legumes have been removed (Maron and Jeffries, 1999). Management may require interventions such as the use of fire, or active restoration by sowing native species.

The main research findings, conclusions and recommendations will be communicated to relevant stakeholders, e.g. officials at Working for Water through the mediums of research briefs, which are short, one-page summaries of topics covered in the research.

12.3 CONCLUSIONS

Our results point to changes in soil properties, soils processes and soil microbial diversity when invasive *Acacia* species establish in numbers in fynbos riparian ecotones. Like the adjoining fynbos, topsoils of these mountain stream zone riparian ecotones are relatively nutrient poor. However, with invasion, soil total and available N and total C increase, though not dramatically so. Invasion also affects soil processes – N availability increase, soil CO_2 efflux increase and soil phosphatase activity increase. Along with these changes in soil processes, the underlying microbial diversity also changes in terms of richness and structure, though bacterial and fungal communities show different trends.

When the *Acacia* stands are removed, soil properties, soil processes and soil microbial diversity recovers, suggesting that ecosystem function, as it pertains to these aspects also recover. A legacy effect does exist – soil available N levels remain elevated, which may provide an opportunity for nitrophilous grasses to establish and thrive in these cleared riparian ecotones. The soil microbial community is also resistant (soil Fungi) to invasive *Acacia* species and removal of these species, and resilient (Bacteria), meaning that bacterial populations recover in structure and diversity after removal of the *Acacia* individuals.

12.4 RECOMMENDATIONS FOR FUTURE RESEARCH

Several new avenues for research have been identified. Phosphorus plays a central role as a constraint on soil processes and soil microbial diversity, as we found that acid phosphatases has a close relationship with available P and bacterial community structure. Given that P oversupply in riparian and aquatic environments can lead to eutrophication (Smith et al., 1999, Maleri, 2011), further research on the role of P cycling in riparian environments will shed light on the risk of P loading of riparian soils and aquatic environments, especially with agricultural modification of fynbos riparian ecotones.

Soil texture seems to be an important control over soil processes, and the role of silt and clay in soil processes deserves closer inspection. Soil N and P cycling were both related to the content of fine material in soils, and more empirical investigation of this relationship may yield predictive models that may be useful in predicting biogeochemical consequences of disturbances on fynbos riparian ecotones. It is also apparent from our results that invasion of *Acacia* species into riparian zones in the fynbos alters soil particle size of topsoils. The mechanisms and generality for these changes should be investigated further.

Relatively little is known about how fluvial dynamics (which strongly influences the spatial distribution of different mineral particle sizes; Bechtold and Naiman, 2006) influence the sorting of sediments in fynbos riparian soils. We suggest that this should be further investigated as hydrogeomorphological processes play a vital role in the composition of riparian plant and soil communities (Naiman et al., 2005).

Nevertheless, it can be assumed that predictable differences in particle size classes across longitudinal zones in riparian ecosystems contribute to equivalent contrasts in soil conditions.

The study was carried out in the upper reaches of fynbos rivers, hence our results may not be directly applicable to reaches further down the river (e.g. the deposition zones of wider floodplains where cover of invasive acacias are often higher) where more fine material is usually deposited. Nutrient dynamics the lower reaches, usually also invaded and/or modified through agricultural or urban development may reveal additional information on how soil processes, properties and microbial dynamics are affected.

Additions of N to soils through biological fixation may augment N flux rates and enhance the loss of soluble nitrate to receiving waters (Follstad Shah et al., 2010). Acacias may therefore potentially be an important new source of bioactive N in rivers and ecosystems further downstream (Tye and Drake, 2011). There is also a risk that clearing woody invasive species may lead to eutrophication of surface water bodies and nitrate contamination of groundwater (Jovanovic et al., 2009). Further research is need, especially on the relative role of different N immobilisation pathways (denitrification versus leaching).

An important next step is to investigate the C balance of invaded riparian ecotones, and indeed also terrestrial areas that have been invaded by *Acacia* species. As part of the assessment of C balance, a closer look at the relative contributions of autotrophic and heterotrophic respiration and how this varies at local and regional scales is crucial. It has been suggested that a ‘positive’ result of invasion of South African ecosystems with woody alien invasive plants is that these plants grow fast, and hence sequester C, with may counteract increasing global CO_2 emissions in a small way. We showed that CO_2 emissions increase tremendously in invaded riparian ecotones, however, the overall balance of C in these areas remain unclear. If the increase in R_S seen here is universal, then this provide more motivation for clearing riparian ecotones and upland areas, however, this remains to be investigated.

It may be of further value to investigate the potential of identifying indicator microbial species in riparian soils, as well as other fynbos soils, which may be used as indicators of restoration potential. This most probably will not be a single species, but a consortium or even a functional group. It may greatly aid in decisions to allocated resources to areas with high restoration potential.

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