

INVESTIGATING SOIL NITROGEN DYNAMICS IN NATURAL, INVADED AND CLEARED FYNBOS RIPARIAN ECOTONES AND IMPLICATIONS FOR RIPARIAN FUNCTIONING

by

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Science in Conservation Ecology*



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This thesis reports original research carried out during 2011, 2012 and 2013, at the University of Stellenbosch, South Africa. It has not been submitted in whole or in part for a degree at any other university. Data presented here are original, and any other sources of data acquired are acknowledged.

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Summary

Riparian ecotones provide a variety of ecosystem services, however they are understudied in Mediterranean-type ecosystems compared to other types of ecosystems. Riparian ecotones occupy the space between aquatic ecosystems and terrestrial environments, and they are seen as a transitional area between these two ecosystems, which allow movement of species and resources. Riparian ecotones are threatened by invasive alien plant species (IAPs) that establish after disturbance, whether it is human-induced or natural. In South Africa, particularly the fynbos region, comprising much of the Mediterranean-type ecosystem, the most prominent invasive alien plants are from Australia and belong to the genus *Acacia*. They are nitrogen-fixers that can have a great influence on riparian ecotone functioning if left unmanaged due to the addition of nitrogen to the system; they are also profligate users of water when growing in riparian environments, and are recognised as transformer species.

The South African government started a clearing program, the “Working for Water Program” in 1995, in order to clear IAPs from riparian ecotones. Recent studies produced substantial knowledge on the effect that clearance of alien woody species have on restoration of indigenous species. However, the effect of invasion and removal of invasive species on soil processes, and potential changes in water quality in fynbos riparian ecotones have not been investigated in great detail. Even though there have been numerous investigations carried out globally in this field, we still lack relevant information for Mediterranean-type ecosystems, specifically in the Western Cape where fynbos riparian ecotones are poorly studied. This study evaluated whether invasion by *Acacia* spp. caused changes to net nitrogen mineralization, available nitrogen, nitrous oxide (N₂O) emissions and denitrification potential and whether soil properties were changed irreversibly. The study also evaluated whether the clearing of invasive species, which is widely applied as a management practice in the Western Cape, led to restoration of riparian functioning in terms of nitrogen cycling (considering that restoration includes both recovery of structure and function) or whether the invasion led to irreversible changes in riparian ecosystems.

The results from the study showed that there was no difference in the nitrogen mineralization activity from *Acacia* spp. invasion compared to natural (“reference”) sites and clearing the IAPs did not lead to changes in nitrogen mineralization. Nitrogen mineralization also did not differ between riparian zones (wet and dry banks) and upper terrestrial areas. Low C:N ratios in invaded and cleared sites implied that carbon was limiting and were responsible for the lack of differences in the nitrogen mineralization activity. There was more NH₄⁺ and NO₃⁻ available for uptake by plants and/or soil micro-organisms in the invaded sites than natural and cleared sites. There was also more NH₄⁺ and NO₃⁻ available in the dry banks than the wet banks and the terrestrial sites and NH₄⁺ became more dominant over time. Clearing of invasive alien *Acacia* spp. led to a change in the availability of nitrogen towards pre-invasion conditions as similar levels of total available nitrogen for the cleared sites (9.65µg.g⁻¹) compared to the natural sites (7.87µg.g⁻¹) were measured.

The field N₂O emissions were similar for the invaded and cleared sites compared to the natural sites and were most likely the result of the sandy soil that limited denitrification. The maximum denitrification potential was similar across different landscape positions and under different invasions statuses and the N₂O emissions from the field and laboratory incubations were in the same order of magnitude, which implied that N₂O emissions from the field is not only the product of anaerobic denitrification, but also a product of aerobic soil respiration processes such as nitrogen mineralization.

Results from the study were used to discuss implications for the “Working for Water Program” and for restoration of riparian functioning and the effects of invasion in general. The effects of invasive alien *Acacia* spp. were visible in areas where clearing efforts has not yet taken place. It is possible that soil recovery processes have taken place in the areas that were cleared from IAP’s compared to natural and invaded sites. It is very important that clearing efforts be monitored and that follow-up clearing takes place in order to prevent further negative impacts of enhanced nitrogen inputs to the soil underneath the Acacias. It is important for “Working for Water” to keep on clearing IAP’s from riparian ecotones, especially when there is the potential for restoring riparian ecotones through the natural process of denitrification.

Samevatting

Rivieroewers verskaf 'n verskeidenheid ekosisteen dienste, maar hulle is swak bestudeer in Mediterreense ekosisteme in vergelyking met ander tipes ekosisteme. Rivieroewers kom voor in die ruimte tussen akwatiese ekosisteme en terrestriële omgewings, en word gesien as 'n oorgangsones tussen die twee ekosisteme, wat die beweging van spesies en hulpbronne moontlik maak. Rivier oorgangsones word deur eksotiese indringer plante bedreig wat hulleself na versterking vestig, ongeag of die versterking menslik of natuurlik is. In Suid-Afrika, veral die fynbos streek, wat 'n groot deel van die Mediterreense ekosisteen uitmaak, kom die mees prominente eksotiese indringer plante in die algemeen van Australië af en behoort tot die genus *Akasië*. Hulle is stikstof-fikseerders wat 'n groter impak kan hê op die funksionering van rivier oorgangsones as gevolg van die toevoeging van stikstof tot die ekosisteen indien hulle nie aktief bestuur word nie; hulle is ook buitengewoon groot gebruikers van water as hulle langs die rivieroewers groei en word ook herken as omskakeling spesies.

Die Suid-Afrikaanse regering het 'n program, die "Working for Water Program", in 1995 geloods om eksotiese indringer plante langs rivieroewers te verwyder. Baie inligting is gegenereer deur onlangse studies oor die effek van die verwydering van houtagtige eksotiese spesies op die herstel van inheemse plantegroei. Die effek wat die verwydering van eksotiese indringer spesies op grondprosesse en potensiële veranderinge in water kwaliteit van fynbos rivier oorgangsones kan hê was nog nie deur vorige studies in diepte ondersoek nie. Alhoewel daar 'n verskeidenheid van ondersoeke op 'n globale skaal in dié veld uitgevoer word, is daar steeds 'n tekort aan ondersoeke in Mediterreense ekosisteme, veral in die Wes-Kaap waar fynbos rivier oorgangsones nog swak bestudeer is. Hierdie studie het bepaal of die indringing deur *Akasië* spp. veranderinge aan die stikstof mineralisasie, N_2O gas emissies en denitrifikasie potensiaal gebring het en of dit onherstelbaar verander was. Hierdie studie het ook die verwydering van eksotiese indringer spesies, wat 'n algemeen toegepaste bestuurspraktyk in die Wes-Kaap is, ge-evalueer om te bepaal of dit tot die herstel van rivieroewer funksionering lei in terme van nutriënt siklring (in gedagte dat die herstel beide strukturele en funksionele herstel insluit) en of die indringing tot onomkeerbare veranderinge in rivier ekosisteme lei.

Die resultate van die studie het bewys dat die stikstof mineralisasie aktiwiteit van die areas wat deur die indringer plante besmet is in vergelyking met natuurlike areas nie verander het nie en dat daar geen verskil is as die indringer plante verwyder word nie. Daar was ook geen verskil in die stikstof mineralisasie aktiwiteit van rivieroewers en die hoërliggende terrestriële areas nie. Lae C:N verhoudings in besmette en skoongemaakte areas impliseer dat logging van NO_3^- geskied het en dat dit die soortgelyke stikstof mineralisasie aktiwiteit kan verduidelik. Daar was meer NH_4^+ en NO_3^- beskikbaar vir opname deur plante en/of grondmikrobes in die besmetteareas as die natuurlike en skoongemaakte areas. Daar was ook meer NH_4^+ en NO_3^- beskikbaar in die droër dele van die rivieroewers as die nat dele en die hoërliggende terrestriële areas en NH_4^+ het meer dominant geraak

met die verloop van tyd. Die verwydering van die eksotiese indringer *Akasië* spp. het moontlik gelei tot 'n verandering in die beskikbaarheid van stikstof met soortgelyke vlakke as die natuurlike areas wat ondersteun word deur soortgelyke vlakke in beskikbare stikstof in skoongemaakte areas ($9.65\mu\text{g.g}^{-1}$) en natuurlike areas ($7.87\mu\text{g.g}^{-1}$). Die werklike veld N_2O emissies was dieselfde in die besmetteareas as die areas waar indringers verwyder was en kan heel moontlik wees as gevolg van die sanderige aard van die rivieroewers wat nie voordelig vir die denitrifikasie proses is nie. Die maksimum denitrifikasie potensiaal was soortgelyk in al die verskillende landskapposisies en indringer status en die N_2O emissies van die veld en laboratorium inkubasies was in dieselfde graad van orde, wat impliseer dat veld N_2O emissies nie net die produk van anaerobiese denitrifikasie is nie, maar ook van aerobiese grond respirasie prosesse soos stikstof mineralisasie.

Resultate van die studie was gebruik om implikasies vir die "Working for Water Program" en herstel van rivieroewer funksionering en die algemene impak van eksotiese indringer plante te bespreek. Die impak van eksotiese indringer *Akasië* spp. was sigbaar in areas waar verwydering van dié plante nog nie plaasgevind het nie. Dit is moontlik dat grond herstel prosesse alreeds plaasgevind het in die areas waar eksotiese indringer plante verwyder was in vergelyking met natuurlike en besmette areas. Dit is baie belangrik dat die verwyderingsproses gemoniteer word en dat opvolg verwydering van indringer plante plaasvind om verdere negatiewe impakte van stikstof insette tot die grond onder die *Akasiës* te voorkom. Dit is belangrik vir "Working for Water" om aan te hou om indringerplante van rivieroewers te verwyder, veral wanneer daar potensiaal is om die areas te herstel deur die natuurlike proses van denitrifikasie.

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CHAPTER 1:**INTRODUCTION AND LITERATURE REVIEW**

1. Riparian Ecotones

Riparian ecotones are located in the stream channel between the high and low water mark of the river and the landscape from the high water mark to the terrestrial vegetation and include all vegetation that may be influenced by the river flow (Naiman and Décamps, 1997). Riparian ecotones comprise of biotic communities that belong to a river bank or shores of streams or lakes (Naiman and Décamps, 1997; Reinecke et al., 2007). Riparian corridors are part of a naturally disturbance-mediated ecosystem that receive matter and propagule inputs from large areas and they accumulate, concentrate and exacerbate impacts and disturbances caused by humans (Reinecke et al., 2007; Esler et al., 2008). Natural riparian ecotones have very different vegetation types from the surrounding environments and they are some of the most diverse terrestrial habitats on earth (Naiman et al., 1993). Vegetation outside the riparian ecotones is not influenced by river or stream water, but may be closely linked to riparian ecosystems as they contribute organic matter, sediment and other material to these zones (Naiman and Décamps, 1997). Intact riparian vegetation provides several ecosystem services and is classified as “critical transition zones” (Ewel et al., 2001).

Riparian ecotones provide a unique habitat for a variety of biota (Naiman et al., 1993) such as aquatic plants and animals (Esler et al., 2008) and also provide important supporting ecosystem services such as nutrient recycling, and regulating services such as erosion control (Reinecke et al., 2007), while maintaining good water quality (Wantzen and Junk, 2008; Brauman et al., 2007). Riparian ecotones function as buffer zones that control the movement of nutrient pollutants such as nitrates and phosphates, suspended solids, heavy metals, and pesticides from terrestrial into aquatic systems (Hedin et al., 1998; Jacobs et al., 2007). These ecotones have the ability to reduce water pollution drastically by transforming nitrogen pollutants (nitrates and nitrites) into gasses (NO, N₂O and N₂), and by trapping and immobilizing phosphates (Mander, 2008). The riparian ecotones also have a significant effect on carbon sequestration by accumulating and degrading organic matter, and by changing organic matter into the greenhouse gas CO₂, which is emitted into the atmosphere (Wantzen and Junk, 2008). Conversely, riparian ecotones may also release organic matter and nutrients into the stream, and in this way maintain critical ecological processes such as in-stream nutrient cycling and productivity of aquatic plants (Naiman et al., 2008).

Riparian ecotones are thus important systems with the ability to regulate the link between the aquatic and terrestrial environment (Naiman and Décamps, 1997). They also have the ability to function as a nutrient and a physical buffer zone, which warrants a predetermined distance from the stream where land use activities are restricted to protect the stream from external inputs such as surface run-off containing pollutants or sediment (Naiman and Décamps, 1997; Shah et al., 2010). Riparian

ecotones also enhance landscape heterogeneity by increasing the diverse mosaic of landforms, communities and environments within the larger landscape (Naiman and Décamps, 1997). This allows these zones, and by extension, the landscape, to be a habitat for a great diversity of organisms (Naiman and Décamps, 1997; Reinecke et al., 2007), which includes micro-organisms, plants, animals (terrestrial and aquatic), invertebrates and fish. These zones are also dynamic, and allow for the mass movement of materials such as woody debris (Naiman and Décamps, 1997; Esler et al., 2008), which is able to influence movement and retention of plant propagules such as seeds and plant fragments (Naiman and Décamps, 1997) within these zones. Riparian ecotones also function as ecological corridors that allow organisms to move through a large network of areas (Shah et al., 2010). It is thus possible for riparian vegetation to be dispersed to other areas and become established, especially downstream. Riparian vegetation, including indigenous and invasive alien species, has the ability to regulate the micro-climate of the stream water, soil and air and the relative humidity (Naiman and Décamps, 1997; Richardson et al., 2007). Riparian degradation may occur if invasive alien species push out indigenous species and dominate these zones.

1.1. Riparian degradation

Most of the riparian vegetation in the higher reaches where the mountain streams occur is more or less in a natural state (Sieben and Reinecke, 2008). Riparian ecotones are easily invaded by invasive alien plant populations due to natural disturbances or disturbances caused by humans, the dynamic hydrological nature of rivers, the ability to easily transport propagules along the river and the role of stream banks as a reservoir for propagules from indigenous and alien species, which makes it easy for them to establish in the riverine ecosystems (Blanchard and Holmes, 2008; Vosse et al., 2008).

These invasive alien species cause degradation to riparian habitats such as suppressing or replacing indigenous species (Blanchard and Holmes, 2008), they alter nutrient recycling processes (Jovanovic et al., 2009; Roura-Pascual et al., 2009; Meek et al., 2010), reduce the colonisation ability of indigenous species (Vosse et al., 2008), in-stream nutrient cycling and productivity of aquatic plants (Naiman et al., 2008), which ultimately leads to a decrease in water quality, they increase soil erosion and cause propagules to be lost that were stored in the soil by indigenous species (Vosse et al., 2008).

Invasive alien species are a major cause for riparian degradation, and also work in synergy with direct human-induced changes to natural ecosystems, e.g. physical alterations to increase the negative effects that IAPs have on these areas (Brooks, 2007; Stella et al., 2012). Urbanisation (Holly and Margaret, 2006; Brooks, 2007) and development (Gardiner and Perala-Gardiner, 1997), herbivore overgrazing (Gardiner and Perala-Gardiner, 1997; Van der Zanden et al., 2006), impoundments (Dudgeon, 2010; Naiman and Dudgeon, 2011), cropping agriculture (Brooks, 2007), plantation silviculture (Brooks, 2007) and forest clear-cutting (Gardiner and Perala-Gardiner, 1997) are all

human-induced disturbances that cause degradation of these areas, dependent on ecosystem management practices (Brooks, 2007).

Urbanisation leads to a loss of habitat (Czech, 2004) and all the biodiversity along with it (Urban et al., 2006). Urbanisation also impedes the ability of natural soil forming processes to occur, which will eventually lead to a loss of suitable substrate for vegetation establishment. Overgrazing by large herbivores, especially domesticated animals such as livestock, is also responsible for the disturbance of riparian ecotones (Van der Zanden et al., 2006). Large herbivores have a trampling effect on the vegetation (Girel and Pautou, 1997) and may cause secondary invasions of invasive alien grasses (Loo et al., 2008). This may ultimately lead to a decline in community richness (Stella et al., 2012).

Large herbivores also have the ability to enhance soil nutrients in riparian zones (Tolsma et al., 1987) by excreting nitrogen (Abdalla et al., 2009), which may have knock-on effects such as eutrophication of water bodies if there are too many nutrients e.g. nitrate that moves through the soil into the rivers (Martin et al., 1999). Impoundments such as damming of rivers to regulate stream flow (Stella et al., 2012) change the flow regime (Haycock et al., 1997). Reduced or altered flow rates of rivers may also cause a change in the vegetation structure and composition as well as increased abundance of IAPs, which may ultimately affect the functioning of riparian ecotones (Stella et al., 2012).

Disturbed conditions as a result of lower water availability in these areas may favour invasive alien plants that are more drought-tolerant than indigenous species (Foroughbakhch et al., 2001), which would give the alien species a competitive advantage in these areas. Cropping agricultural activities may cause a loss in riparian areas due to cultivation where indigenous species is physically removed and agricultural crop species take over the space of the indigenous species (Brooks, 2007). Forestry activities may also have a negative impact due to invasive alien trees that are planted too close to riparian zones, because they use more water than indigenous species (Le Maitre et al., 2000) and take up the space of indigenous species' habitat.

2. Riparian zones of Mediterranean-type ecosystems

Mediterranean-type ecosystems other than the south-western Cape, are often comprised of three basic biome types, namely Mediterranean forests and woodlands, shrublands and grasslands (Medail, 2008) with a characteristic sclerophyllous vegetation-type (Yelenik et al., 2004) that is associated with small, leathery, leaves with an outer waxy layer to retain moisture in dry summer months (WWF, 2012). These ecosystems are located along the western edges of five relatively small eco-regions, namely the Mediterranean Basin near Europe and Asia, the central Chilean matorral, the California chaparral and woodlands eco-region of California and the Baja California Peninsula, the Cape Province in the Western-Cape of South Africa and south-western and southern Australia (Medail, 2008; WWF, 2012; Figure 1.1.) between the 30° and 40° parallels in both the northern and southern hemispheres (Rutherford et al., 2006; Medail, 2008).

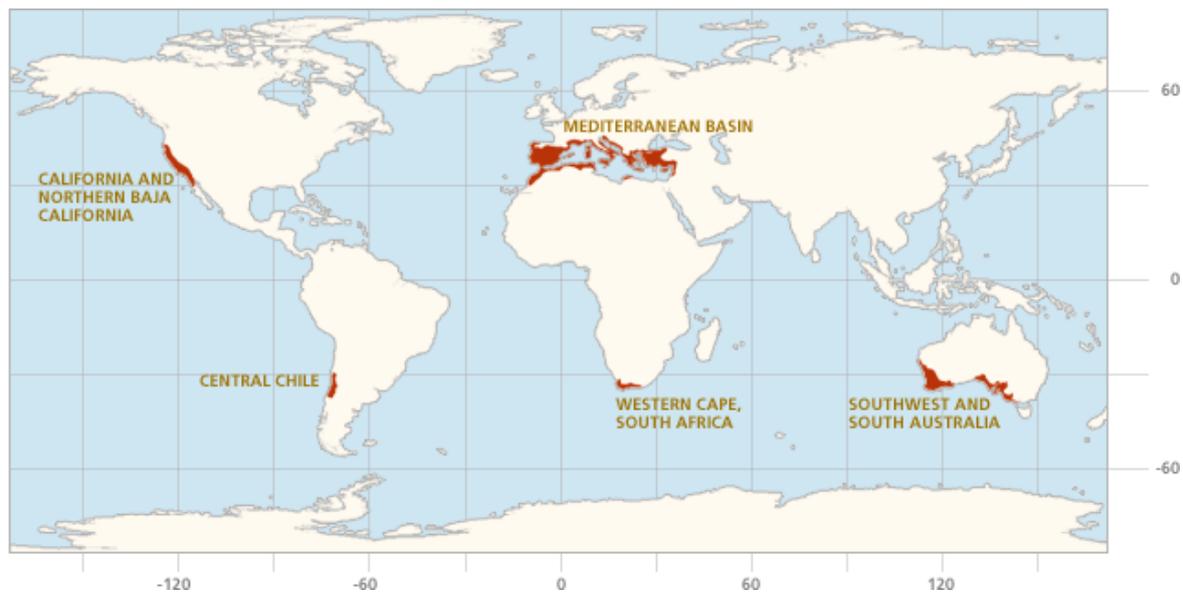


Figure 1.1. The world regions where the Mediterranean-type ecosystems occur. (Photo credit: http://www.mednscience.org/mediterranean_ecosystem)

Globally this ecosystem type comprise of only 5 relatively small areas, but they still maintain high levels of biodiversity (Rebelo et al., 2006; Stella et al., 2012), especially for plants and animals. The biota have adapted in various ways to maintain a presence in stressful conditions (Stella et al., 2012) such as long hot summers with little rain (WWF, 2012). These areas are also known as Mediterranean climate eco-regions and each one of them is distinctive; collectively they contribute 10% (WWF, 2012) to 20% (Rutherford et al., 2006) of the earth's vascular plant species, although they only cover approximately 5% of the earth's land area (Rutherford et al., 2006).

It is common for riparian zones of Mediterranean-type ecosystems to form a "mosaic habitat" within the landscape where the different vegetation types are interlinked with each other through complex patterns that resulted due to variations in soil (Goldblatt and Manning, 2002; Rebelo et al., 2006), topography (Goldblatt and Manning, 2002), exposure to wind (Holmes et al., 2005; Reinecke et al., 2007) sun, and fire history (Bowman, 1998; Reinecke et al., 2007). These areas also tend to have a high degree of endemism for both plant and animal species (Decamps, 2011) due to small and often clustered geographical ranges that frequently occur in isolated or topographically variable regions such as islands, mountains and peninsulas (MEA, 2005). This led to convergent evolution, which created similarities in plants where evergreen shrublands dominate each of the different regions (Verdu et al., 2003).

The 5 different eco-regions all have a distinctive Mediterranean-type climate where cool, wet winters and dry hot summers prevail (Gasith and Resh, 1999; Medail, 2008; Meek et al., 2010) due to cold offshore ocean currents that moderate the climate (Goldblatt and Manning, 2002, Medail, 2008). These areas are prone to a series of regular and often extreme flooding events that is followed by drying periods (Gasith and Resh, 1999). Floods play a key role to maintain populations of plant species that are typically adapted to flood disturbances in riparian habitats (Holmes et al., 2008;

Bendix and Cowell, 2010). Flooding events can have a stabilising impact on the species composition in riparian zones of Mediterranean-type ecosystems (Holmes et al., 2008; Bendix and Cowell, 2010). In literature it is suggested that sites that are experiencing frequent floods may not necessarily be influenced by a specific flood event, but rather by repeated flood events (Bendix and Cowell, 2010). These repeat flood events may cause the vegetation composition to stabilise over decades or even over longer time periods (Bendix and Cowell, 2010). Flood events can also assist in the recolonisation of burned areas due to the delivery of seeds and vegetative propagules to riparian zones (Dwire and Kauffman, 2003). Some indigenous riparian tree species, such as *Brabejum stellatifolium*, have also adapted to the drier cycles by being more drought resistant than other indigenous riparian plant species (Crous et al., 2012). It was also proven that IAPs are more drought resistant than the indigenous species (Crous et al., 2012). IAPs may have negative impacts on the soils of riparian zones in the Mediterranean-type ecosystems if they are able to persist in drier conditions.

In terms of soils, phosphorus (P) levels and total N also tend to be low in Mediterranean ecosystems. Mineralization rates tend to be low in Mediterranean-type ecosystems due to allelopathic compounds that leach from plants and the quality of the sclerophyllous leaves associated with such shrublands (Castaldi and Aragosa, 2002). This can cause available mineral N to be low in the soil and further limit soil microbial processes such as nitrification and denitrification (Castaldi and Aragosa, 2002). South African and Australian Mediterranean-type ecosystems are especially limited in nutrients, nitrogen and phosphorus (Goldblatt and Manning, 2002; Medail, 2008). In many areas the vegetation sits on stabilised sand dunes that is characterised by poor nutrient and water retention, which further enhances soil aridity in summer and limits soil water content in the wetter months (Castaldi and Aragosa, 2002). The capacity of soils to retain water can be exacerbated by disturbance events such as fires (Castaldi and Aragosa, 2002), but not all the Mediterranean-type ecosystems are driven by fire, such as Chile for example (SCRLC, 2012). Fire causes both the water holding capacity and the resistance to decrease, which lead to an increase in evaporation (Castaldi and Aragosa, 2002).

The riparian ecotones of Mediterranean-type ecosystems are considered to be resource rich habitats (Sheley et al., 2006) that occur in large landscapes limited by water (Stella et al., 2012). There are three general characteristics of these areas that can be used to distinguish them from other ecosystem types: (1) they have high levels of plant biodiversity, structural complexity, and species are regularly introduced in the same eco-regions, (2) there are strong physical controls on plant demographics and community structure and (3) humans are intensely impacting these zones (Stella et al., 2012). Many of the areas have a vegetation type that is associated with the rivers and distinctly different to other vegetation types of the other eco-regions. Many riparian biota are well-adapted to various stress factors that are associated with these areas such as dynamic flooding events, sediment regimes, seasonal water shortage (summer droughts), and fire (Gasith and Resh, 1999; Stella et al., 2012). Riparian ecotones of Mediterranean-type ecosystems are different in many ways when compared to their mesic temperate and tropical counterparts (Stella et al., 2012). These ecosystems

are able to support a dense and productive closed canopy in comparison to the surrounding landscape by providing optimum conditions of sunlight, nutrients and water. This generally causes biogeochemical fluxes and nutrient cycle rates to be higher in these areas than surrounding semi-arid landscape (Naude, 2012; Stella et al., 2012).

These above differences may be the result of various physical drivers that play a role in modifying the structure and function of riparian ecotones. Stella et al. (2012) identified five principal categories into which physical drivers of Mediterranean riparian ecotones can be divided, namely (1) flood magnitude and hydraulics; (2) flood timing; (3) stream flow and water table dynamics; (4) sediment dynamics and texture; and (5) fire. Floods create disturbance events that alter plant communities by dispersing propagules, they initiate the recruitment of pioneer species on cleared, moist substrate and they kill vulnerable plants. Plant mortality is linked to the energy that is associated with floods, which scours the roots and breaks the stems of plants (Stella et al., 2012). Plant size and flexibility, root and substrate characteristics and the relative location to the distribution of energy within the stream flow determine the vulnerability of the plant. Flood-adapted species may start to dominate in areas that experience high-energy flow. Seedlings are especially vulnerable to high-energy flows due to their small size and limited root development and their recruitment is influenced by the right seedbed conditions with seed fall when floods scour and/or deposit moist substrate after rainfall events (Pettit and Froend, 2001; Stella et al., 2012).

The substrate may affect sediment dynamics, which include the influences of bar and floodplain creation on vegetation colonization, plant mortality by burial, and the control of texture on water availability (Stella et al., 2012). Geomorphic surfaces are created in active alluvial rivers where floodplain migration is regulated by the spatial and temporal distribution of plant communities, which allows the riparian vegetation to colonise. Unfortunately, sedimentation deposits may also have a negative impact on the mortality of plants by burying them, especially if they are buried too deep and the plants can't resprout from the epicormic buds (Stella et al., 2012). Sediment processes are important in order to determine the characteristics of soil resources such as texture, which further impacts the availability of nutrients and water. This has a further influence on plant germination, survival and growth (Stella et al., 2012) as well as riparian biogeochemistry (Pinay et al 1993; Pinay et al, 2000).

Rainfall runoff and erosion processes (especially subsequent to fires) can cause land-derived sediment and nutrients to be transported from the slopes of mountain catchments into adjacent riparian ecotones and then cause it to eventually end up in the ocean. Short-lived climate events such as floods, rainfall and erosion processes cause a loss of sediment and dissolved nutrients and may have an effect on the productivity of the catchment (Bolton et al., 1991; Tesi et al., 2013). A loss in nutrients and sediment may even lead to further loss in vegetation and increased erosion. The water that flows through catchments does not only transport nutrients and sediment, but it may also transport organic matter or even contribute excess nutrients to riparian ecotones (Bolton et al., 1991).

The mobilisation and transport of sediments to and through the river system and the potential for nutrient uptake by the sediment from the water column is dependent on the nutrient content of the suspended sediment, which in turn is affected by the catchment soil types and land use, the selectivity of erosion and sediment delivery processes (Walling et al., 2000).

3. Riparian zones of the fynbos biome

The fynbos biome is a member of the global Mediterranean biome (Rebelo et al., 2006) and is situated at the south-western tip of the African continent between the 31 and 34° latitudes in the southern hemisphere (Goldblatt and Manning, 2002; Figure 1.2.). This biome (and all other biomes) is defined on the basis of climate, similar life-form patterns and major natural disturbances (Rebelo et al., 2006) such as fires (Goldblatt and Manning, 2002), for example in this specific biome. The biome derived its name from the Afrikaans word “fynbos”, which is the dominant vegetation type that mainly occurs in the south-western Cape region of South Africa (Rebelo et al., 2006). Although the fynbos biome is geographically well defined, it actually comprises of three distinct, naturally fragmented vegetation types, namely fynbos, renosterveld and strandveld, that occur in both summer- and winter-rainfall areas of which all are dominated by small leaved evergreen shrubs with a fire adapted regeneration strategy (Rebelo et al., 2006). The fynbos biome is also endemic to South Africa, and although similar types of vegetation may be found in other Mediterranean-type ecosystems on other continents such as North America, South America, Asia, Europe and parts of West and South Australia (Gasith and Resh, 1999), they are structurally and functionally significantly different from fynbos.

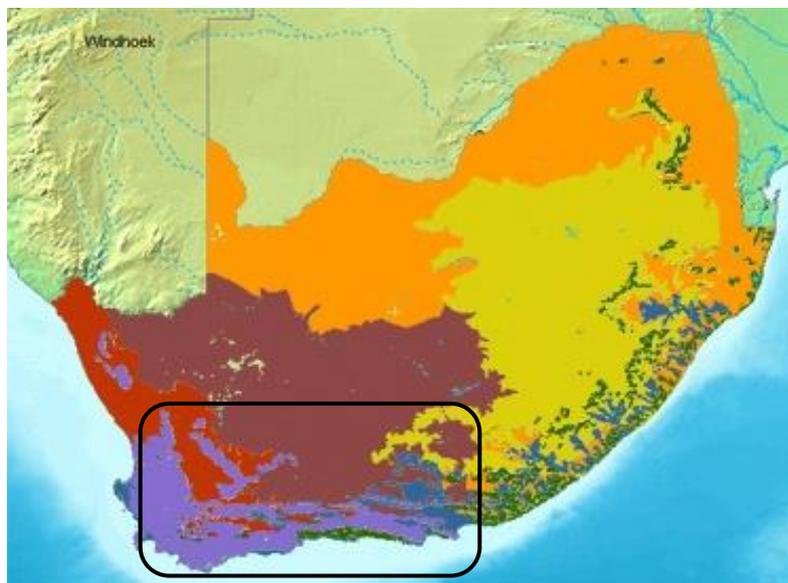


Figure 1.2. The fynbos biome of South Africa indicated by purple in the black box. (Photo credit: <http://www.ekapa.ioisa.org.za/biomes/intro.htm>)

The fynbos biome can be found along the Cape Fold Belt, which includes the north-south and east-west mountain chains as well as the wetter valleys, and the adjacent lowlands between the mountains and the Atlantic Ocean in the west and south, and between the mountains and the Indian Ocean in

the south (Rebelo et al., 2006). There is a mosaic of various geological substrates, which leads to evolutionary processes that are responsible for a high diversity of taxa and vegetation types found in the fynbos biome (Rebelo et al., 2006; Reinecke et al., 2007). The sediments of the region are typically derived from sandstone, quartzite, granite, gneiss, shale and young limestone (Rebelo et al., 2006). The most dominant landscape type of the fynbos biome is quartzite mountain chains and sandstone peaks, which form part of the Cape Supergroup rocks and is extremely poor in nutrients (Rebelo et al., 2006). Soil formation processes will lead to the formation of soils with very low nutrient contents, which have already been noted in the current fynbos soils (Goldblatt and Manning, 2002; Medail, 2008). Differences in the intensity of the Permo-Triassic mountain-building events of the Cape Supergroup caused sandstones to be found as flat-lying or gently dipping layers (e.g. Cape Peninsula and Cederberg) or as tightly folded, vertical and even overthrust layers (e.g. Langeberg and Swartberg ranges) (Rebelo et al., 2006). The extent and hardwearing nature of these formations determine the morphology of the landscape in the fynbos biome (Rebelo et al., 2006).

The structural and functional features of rivers and streams are strongly influenced by climate and geomorphology (Gasith and Resh, 1999). The rainfall pattern normally dictates the discharge regime of rivers (Gasith and Resh, 1999). The fynbos biome is characterised by tall mountains close to the coast (the Cape Supergroup), which leads to relatively high gradients of rivers, and subsequently high stream power (Sieben et al., 2009). The summer months are normally responsible for a drying and declining flow, which ends abruptly in autumn or early winter when the next rainfall season begins (Gasith and Resh, 1999). This causes a seasonal pattern of high discharge that corresponds to cool temperatures and is followed by warm or hot temperatures responsible for low discharge (Gasith and Resh, 1999). Aquifers may store large amounts of water, which moderates seasonal fluctuations in flow (Gasith and Resh, 1999). However, in contrast to other Mediterranean areas, fynbos (and Western Australia) have more, shorter rainfall events, and significant rainfall that occur during summer (Cowling et al., 2005), in contrast to the Mediterranean region in California. This allows riparian plants, including seedlings that otherwise may have perished, to persist. Drought years may occur if low rainfall is experienced, which reduces floods and extends the drying periods (Gasith and Resh, 1999). Brief and intense rainstorms may cause streams to flood and reach peak discharges after which they will decline to a normal seasonal flow shortly after the storm ends, especially if the area is characterised by high gradient topography, with constrained channels and saturated soils (Gasith and Resh, 1999). Perennial rivers will occur in the wetter areas and they usually maintain permanent flow or hold surface water throughout the year (Gasith and Resh, 1999).

The fynbos biome is maintained by fire and it is one of the factors that drives evolutionary and ecological processes (Rebelo et al., 2006) such as the diversity, productivity, and stability of the ecosystem (SCRLC, 2012). The frequency of fires varies among regions (SCRLC, 2012); for example there are only two types of vegetation units in South Africa that are not exclusively driven by fire, namely the Namaqualand Sand Fynbos and the Stikfontein Quartzite Fynbos (Rebelo et al., 2006). Fire is very important to maintain the high levels of biodiversity and endemism as most of the

fynbos plants have adapted to recover after a fire (Goldblatt and Manning, 2002; Stella et al., 2012). Fires are part of a natural process (SCRLC, 2012) that occurs in the fynbos biome on a rotation rate with at least one fire occurring every 5-50 years and normally during late summer and early autumn at the end of the dry season (Rebelo et al., 2006). Fires are naturally started due to rock fall events and lightning, but the frequency of man-made fires has increased due to an increase in population sizes (Rebelo et al., 2006). Fire regimes are primarily determined by nutrients and climate (Rebelo et al., 2006), but they may also control nutrient cycles and energy pathways (SCRLC, 2012). Long, dry summers accompanied by heat waves are enhanced by global warming, which may increase the frequency and intensity of wildfires, flash floods and flooding in the Mediterranean area (IPCC, 2007; Ginzburg and Steinberger, 2012).

There is little recorded about the fire frequency within fynbos riparian zones, however, most riparian plants recover well following fires, either as basal or epicormic shoots, suggesting that fires are a factor in the ecology of these zones (Dwire and Kauffman, 2003; Swift et al., 2010; Verkaik et al., 2012). This is similar to other Mediterranean regions where fire is a significant factor in riparian zones, and resprouting a major life-history trait for plants occurring in Mediterranean riparian environments in California (Bendix and Cowell, 2010). Where Afromontane forests have established along river courses, fire is usually excluded, unless windthrow or other factors caused a gap where fire can penetrate (van Wilgen et al., 1992). Some of the other impacts of fires on riparian characteristics have to do with the release of nutrients from organic material - fire may increase the amount of N that is available for plant uptake, which may shorten the establishment time of plants if they use these increased nutrients.

4. Fynbos Riparian Ecotones

“Riparian” generally refers to biological communities and environments on the shores/banks of rivers, lakes, ponds and wetlands (Naiman and Décamps, 1997; Reinecke et al., 2007) and ecotones generally refer to the transition zone between one ecosystem and another (Ewel et al., 2001) such as separating an aquatic ecosystem (e.g. a river) from a terrestrial ecosystem (e.g. mountain fynbos). For the purpose of this study, fynbos riparian ecotones refer to a perennial river that splits one vegetated fynbos area in the fynbos biome into two vegetated fynbos areas adjacent to the opposite river banks. These riparian ecotones occupy a small area in comparison to the greater catchment, but they have a great influence on the surrounding landscape, especially through their vegetation (Reinecke et al., 2007). It is easy to see where the boundary lays that separates the riparian and terrestrial area in arid areas, which is a band of green vegetation (riparian community) against a much drier and less densely vegetated terrestrial area higher up the slopes (Reinecke et al., 2007). This is not the case with fynbos riparian ecotones, where the boundary is obscure by just as densely vegetated neighbouring areas due to changes in rainfall and temperature (Reinecke et al., 2007).

Riparian ecotones are naturally disturbed by seasonal floods (Reinecke et al., 2007), which normally occur during intense rainfall events, especially at the onset of the winter rainfall season. These floods

are able to create and remove patches of vegetation habitat, which causes riparian ecotones to be vulnerable to invasion by alien plants (Reinecke et al., 2007), especially alien Australian woody invasive vegetation such as *Acacia mearnsii*, *Acacia longifolia* and *Acacia saligna* that need to be actively managed in the areas where they occur (Reinecke et al., 2007, Blanchard and Holmes 2008). The vegetation in these transitional zones is one of the factors that affects the flow of the river, inputs and recycling of litter upstream to downstream areas of the river (aquatic food), buffering against sediments and other matter that drains downhill through the catchment (Reinecke et al., 2007). Riparian vegetation such as palmiet (*Prionium serratum*) is particularly crucial to regulate the intensity and direction of stream flow, trapping of sediment and stabilizing the riverbed and riverbank (m'Afrika Xaba, 2005).

Riparian ecotones are particularly vulnerable to human disturbances (Esler et al., 2008) in the flow of energy and materials through the landscape (Reinecke et al., 2007). Disturbances that are also responsible for local disturbance include impounding of rivers such as the Buffalo river in the Eastern Cape and the Palmiet river (Palmer, 1991), Dwars river, Eerste river (Bredenhand and Samways, 2009) and Elands river in the south-western Cape. Impoundments may cause changes in water temperature, suspended organic matter, chlorophyll a, inorganic material, water quality and they regulate the flow (Palmer, 1991). Changes in the annual water temperatures of the receiving stream may be lower during summer and higher in winter (Palmer, 1991). Impoundments also significantly affect pH, conductivity and ammonium in downstream reaches (Palmer, 1991). Impoundments such as dams thus disrupt the natural continuum of physical, chemical and biotic changes further down the river. Water abstraction is also responsible for local disturbance (Palmer, 1991; Hughes, 2001; Holmes, 2007) and may influence the natural flow of rivers (Hughes, 2001); ultimately this may prevent restoration of natural hydrological functioning (Holmes, 2007).

Riparian ecotones vary widely in terms of structure and range from broad, forested floodplains to narrow, intermittent streams in incised valleys (Reinecke et al., 2007). Riparian ecotones form linear and narrow features in the landscape if floodplain development is restricted (Reinecke et al., 2007), which can be seen in most fynbos riparian ecotones of the south-western Cape, especially near the headwaters (Reinecke et al., 2007). Headwater streams may have small riparian ecotones that are embedded in forest and are wider in mid-sized rivers (Reinecke et al., 2007). The vegetation communities of these riparian ecotones are completely different from drier (higher) terrestrial areas in terms of species composition and growth form (Reinecke et al., 2007). There are also generally sharp gradients in environmental and ecological processes that result in a mosaic of environmental patches and variation in species richness and composition (Reinecke et al., 2007). Different landscape elements in natural rivers determine their ecological nature and function, which in turn is influenced by the size of the river, position in the catchment's drainage network, the hydrologic regime and local geology and geomorphology (Reinecke et al., 2007).

Seasonal flooding and lateral migration of matter in these ecosystems leads to the development of different vegetation communities and soils with different redox conditions depending on elevation above the active channel (Reinecke et al., 2007). The different vegetation communities are responsible for the longitudinal, vertical and lateral differences where longitudinal refers to the river from source to mouth, vertical refers to the bank, river bed and fluvial aquifers and lateral refers to the floodplain. There are also differences in plant communities from the water's edge to the outer edge of land that are influenced by flooding (Reinecke et al., 2007). These zones are: (1) the 'wet bank zone' and (2) the 'dry bank zone'. The wet bank zone contains surface water most of the year and submerged plants are rooted beneath the surface although their leaves do not float at the surface (Reinecke et al., 2007); this zone typically experiences flow during flood events of all magnitudes. The dry bank zone supports trees and shrubs with their roots submerged for at least some part of the year while their leaves and stems are above the water surface (Reinecke et al., 2007). The dry bank zone is dry for most of the year, and may become wet during high flow events (Reinecke et al., 2007). There are several other zones outside the wet and dry banks, depending on the classification scheme, and have different terms associated with them (Reinecke et al., 2007).

5. Invasive alien plants in fynbos riparian ecotones

Invasion by alien species is one of the major drivers of global change and loss of biodiversity (Evans et al., 2001). The riparian ecotones of the Western Cape are one of the areas in South Africa that is most heavily invaded by invasive alien plants (Le Maitre et al., 2000). The Western Cape covers approximately 33% of the total area of the estimated 10.1 million ha of South Africa and Lesotho (Le Maitre et al., 2000) invaded by IAPs, mostly trees and woody shrubs (Le Maitre et al., 2000; Reinecke et al., 2008). Stands of invasive alien trees can cause an increase in evaporation when compared to natural vegetation (Marais and Wannenburg, 2008). The alien invading plants have a total incremental water use that is estimated at 3 300 million m³ of water per year (Le Maitre et al., 2000). Alien invaders in the Western Cape are held accountable for approximately one third of the estimated total water use by volume (Le Maitre et al., 2000). They have an increasing effect on the water resources, which have an effect on water availability in the catchment areas (Le Maitre et al., 2000).

Many catchment areas and riparian ecotones in South Africa are being cleared of alien woody invasive vegetation that were introduced from Australia, such as *Acacia cyclops* (Rooikrans), *Acacia saligna* (Port Jackson Willow) and *Acacia mearnsii* (Black wattle), commonly found in the Western Cape (Holmes et al., 2005; Jovanovic et al., 2009). The genus *Acacia* is the most actively managed genus by Working for Water (WfW) because it has a reputation for being a major water user (Marais and Wannenburg, 2008). All the invasive *Acacia* species are putative nitrogen-fixers, which makes them even more successful as invading trees (Yelenik et al., 2004), because they can take up atmospheric nitrogen that was fixed by plant-microbe symbiosis as well as soil organic nitrogen that was transformed by soil microbes into nitrates and ammonium (Yelenik et al., 2004). This allows them to be more effective competitors (Le Maitre et al., 2004), which is why *Acacia mearnsii* is thought of as one of the worst invaders in riparian ecotones (Le Maitre et al., 2000). Invasive *Acacia*

mearnsii uses the most water (estimated 576.58 million m³ / year in South Africa) of all the *Acacia* species (Le Maitre et al., 2000).

The *Acacia* spp. are being cleared in order to reduce water loss, preserve stream flow and aid ecosystem restoration (Jovanovic et al., 2009). The economic impact of *Acacia mearnsii* in South Africa is currently a net cost of \$1.4 billion (Le Maitre et al., 2004). The WfW program is also attempting to mitigate some of the negative impacts such as suppressing or replacing indigenous species and altering ecosystem functioning (Blanchard and Holmes, 2008), colonization ability, soil erosion and the loss of propagules stored in the soil by indigenous species (Vosse et al., 2008). Studies have shown that stream flow increased by 12m³/ha/day immediately after clearing *Acacia mearnsii* in Mpumalanga and also by 9, 10 and 12m³.ha⁻¹.day⁻¹ in Du Toitskloof (Paarl), Oaklands (Wellington) and Somerset West in the Western Cape after clearing Australian *Acacia* and *Eucalyptus* species from riparian areas (Marais and Wannenburg, 2008). It is also possible that some endemic species may recover after alien plants have been cleared from a river system and this can have a positive effect on the biodiversity in the area (Marais and Wannenburg, 2008). An example of such a study is by Samways and Taylor (2004) who found that several populations of dragonfly (Odonata) endemic to South Africa have recovered due to the clearing of dense stands of *Acacia mearnsii* in riparian ecotones.

Other Mediterranean regions than the Western Cape are also influenced by invasive *Acacia* spp. such as the coastal areas of Portugal (Marchante et al., 2009) and Israel (Marchante et al., 2008) that have been invaded by *Acacia longifolia* and *Acacia saligna* due to an initial introduction to stabilize the dunes and prevent sand erosion (Marchante et al., 2008; Marchante et al., 2009). Areas in Portugal that were invaded for more than 20 years had a decrease in basal soil respiration, microbial biomass, nitrogen mineralization, potential nitrification, carbon and nitrogen content (Marchante et al., 2009). It takes a few years for these soil nutrients and processes to return to the pre-invasion state after an area has been cleared of an N₂-fixing invasive tree, but the process of ecosystem recovery is hypothesized to take place faster if the N-rich litter layer is removed (Marchante et al., 2009).

6. Impacts of invasive alien plants on the riparian nitrogen cycle

6.1. Nitrogen inputs

Alien nitrogen-fixing woody invasive Acacias (legumes) have the ability to fix atmospheric N and convert it into inorganic N (e.g. NH₄⁺ or NO₃⁻) (Tye and Drake, 2012), which adds N to the ecosystem. This changes the limitation that N as a nutrient has on the system and may affect productivity, composition, dynamics and diversity (Vitousek et al., 2002). Leguminous N-fixers not only fix atmospheric nitrogen, but they also produce thick litter layers with carbon that binds to organic N in microbial pools that has long lasting effects on the soil N pool (Rascher et al., 2011). There are many diverse and widely spread organisms with the ability to fix N (Vitousek et al., 2002). Some of the soil micro-organisms that are able to assist in the conversion of atmospheric N to inorganic N are *Rhizobium* spp. (Bala and Giller, 2001) or *Bradyrhizobium* spp. (Postgate, 1982). Nitrogen fixation

may also be carried out by free-living soil micro-organisms who rely on the existing soil N pool (Houlton et al., 2008) or other micro-organisms to supply them with oxidised organic molecules if they are not able to photosynthesise and make their own sugars. Free-living micro-organisms obtain these molecules once other micro-organisms have died and their cell contents are released to the soil.

Some of the variables that have an influence on nitrogen fixation are soil moisture and temperature (Pinay et al., 1993; Castaldi and Aragosa, 2002; Machefert et al., 2002; Vourlitis et al., 2009). An increase in soil moisture levels leads to an increase in microbial activity and a decrease in oxygen, which causes facultative anaerobes (denitrifiers) to start using NO_3^- as an electron acceptor and reduces the efficiency of these processes to take place (Roach and Grimm, 2011). Soil temperatures that are too low may have a retarding effect on the soil microbial activity and their metabolism might not be active enough for these processes to take place. Soil temperature also plays a role in the molecular structures of micro-organisms and may denature key metabolites such as ATP if the temperatures are too high (Goberna et al., 2012). The processes of the N cycle (Figure 1.3.) are ubiquitous and controlled at a broader scale by differences in climates, management and soil type due to increased N deposition, which will have different effects in different parts of the world (Adams et al., 2004). The focus on the N cycle for this study is in the fynbos biome of the south-western Cape Province of South Africa.

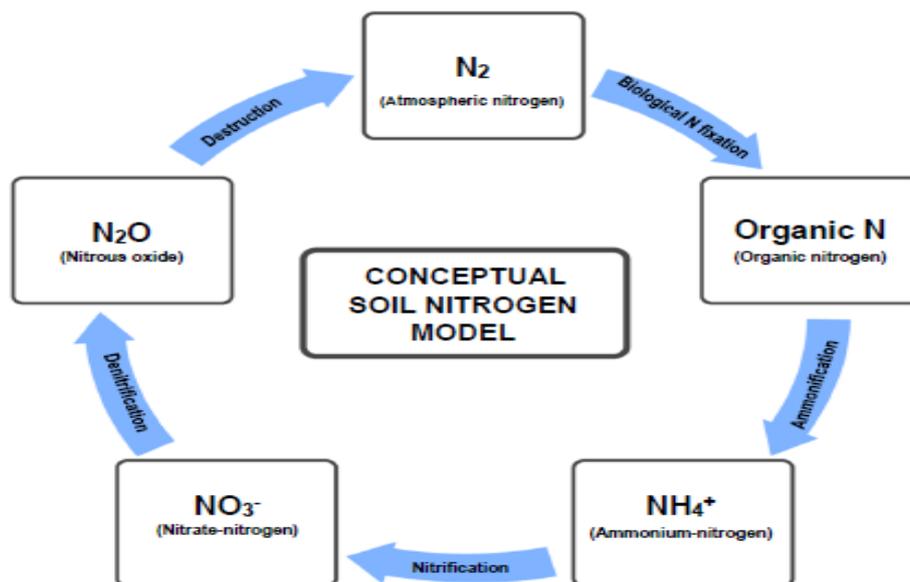


Figure 1.3. Conceptual soil nitrogen model developed during the study for the different forms of N that occurs in the soil and/or the atmosphere and the processes that are responsible for a change in structure and availability.

6.1.1. Biological fixation of atmospheric nitrogen (N_2)

Biological nitrogen fixation is the conversion of atmospheric nitrogen to inorganic nitrogen that is immediately available for plant uptake (Wagner, 2012). This can be done naturally by lightning or in association with nitrogen-fixing bacteria that live in the root nodules of a host plant; this latter process is intimately connected to many invasive alien *Acacia* spp. worldwide. The bacterium gets sugars as

an energy source that is the result of carbon capture by photosynthesis by the plant and in return the plant obtains fixed nitrogen that can be utilised immediately for growth (Wagner, 2012).

Biological N fixers have an enormous capacity to convert N_2 to organic N and the rate of N fixation may even exceed $100 \text{ kg ha}^{-1} \text{ y}^{-1}$ where symbiotic N fixers are abundant, which is more than enough to sustain N pools in ecosystems and to replenish N losses (Vitousek et al., 2002; Tye and Drake, 2012). The process is able to drive the accumulation of N on long time scales and bring the N supply close to equilibrium with other potentially limiting resources such as phosphorous (Vitousek et al., 2002). This equilibration may be reached relatively rapidly in some systems (Vitousek et al., 2002). Human-induced changes to the nitrogen cycle may significantly change the systems where N is naturally limited, which may result in great N losses (Vitousek et al., 2002).

Nitrogen fixation is relatively energy-intensive and nitrogenase enzymes are inactivated by O_2 , which means micro-organisms must maintain a fine balance between using O_2 efficiently as an electron acceptor and the inactivation of nitrogenase (Vitousek et al., 2002). This necessitates free-living photosynthetic N fixers to segregate the O_2 they produce from their nitrogenase system (Vitousek et al., 2002). Nitrogen fixers may also need more P, Fe, and/or other nutrients than other organisms (Vitousek et al., 2002). High levels of nitrogen inhibit the synthesis and/or activity of nitrogenase in most N-fixing micro-organisms (Vitousek et al., 2002).

The most important contributors of fixed N in most ecosystems are symbiotic N fixers, free-living cyanobacteria, and heterotrophs (Vitousek et al., 2002). Woody legumes are the most common symbiosis with nitrogen-fixing bacteria, but herbaceous legumes and actinorhizal (*Frankia*-based) symbioses are also widespread (Vitousek et al., 2002). A lack of suitable plants to form symbioses with and a slow rate of biomass accumulation may have a retarding effect on the ability of these micro-organisms to fix N. When the plants have established and they produce biomass there is initially no available N for uptake by these micro-organisms and N loss is uncontrollable in addition to the excess available N (Vitousek et al., 2002). Micro-organisms are forced to actively fix N at a higher cost to them and only excess available N would be lost to the system (Vitousek et al., 2002).

Nutrient availability such as P and N also plays a role in the N-fixing ability of micro-organisms (Vitousek et al., 2002). An addition of P (and a loss of N without losing P) may cause N to become limited to non-fixing micro-organisms and N-fixing micro-organisms may gain a competitive advantage (Vitousek et al., 2002). The N:P ratio may be influenced if symbiotic N-fixers systematically require more P or are less efficient at acquiring this source than non-fixers, which mean they will be limited by P and non-fixers limited by N (Vitousek et al., 2002). A greater disparity in the requirements for P between fixers and non-fixers will create disequilibrium in the supply of N versus P (Vitousek et al., 2002). N-fixers use more P for the building and/or maintenance of the fixation process itself than non-fixers (Vitousek et al., 2002).

6.1.2. Nitrogen mineralization

Approximately 95% of the total nitrogen in natural, undisturbed soils occurs in the organic form of nitrogen (Walworth, 2013). This nitrogen is made up of compounds that are found in the tissues of plants, animals and micro-organisms, which include amino acids, nucleic acids and proteins that can be released when their waste or dead bodies are deposited and decomposed in the soil (Walworth, 2013). The decomposition of these products may affect nitrogen availability to other animals, plants and micro-organisms, but also that of carbon input (Walworth, 2013). The inputs of carbon to soils are affected by litterfall, exudates and mycorrhiza, which may determine whether the soil nitrogen is immobilised as a result of a high carbon input rate or mineralised as a result of a low carbon input rate (Nasholm, 1998). It is generally believed that a high carbon to nitrogen ratio (e.g. >25:1) will have less than 10% leaching of NO_3^- and cause nitrogen to be mineralised by soil micro-organisms (Emmett et al., 1998). Conversely a low carbon to nitrogen ratio (e.g. <24:1) may stimulate the occurrence of leaching and a reduction in nitrogen mineralization (Emmett et al., 1998).

Nitrogen mineralization is a two-step biological process whereby organic nitrogen is converted to inorganic nitrogen and made available to plants (Al-Ismaily and Walworth, 2008), especially when there is an excess of nitrogen (Walworth, 2013). Nitrogen mineralization involves the processes of ammonification and nitrification (Van Wesemael, 1993). Ammonification is a two-step biological process whereby organic nitrogen compounds are converted firstly to ammonia in the absence of water and then to ammonium in the presence of water (Walworth, 2013). Ammonification occurs under both aerobic and anaerobic conditions (Pinay et al., 2007; Naude, 2012), while nitrification occurs under aerobic conditions (Barton and Atwater, 2002; Pinay et al., 2007; Naude, 2012). Ammonification will generally occur at a higher rate than nitrification when there is more organic nitrogen than ammonium in the soil, but also due to a greater soil microbial community to perform ammonification. Ammonification precedes nitrification and provides the substrate for nitrification to occur (Dannenmann et al., 2011). Ammonium may alternatively be immobilised into the soil microbial biomass (Dannenmann et al., 2011), especially when there is a shortage of organic nitrogen (Walworth, 2013). High levels of ammonium in the soil are believed to inhibit the nitrification process (Al-Ismaily and Walworth, 2008).

Nitrification is a two-step biological process whereby ammonia and ammonium is firstly converted to nitrite and then secondly nitrite is rapidly converted to nitrate (Walworth, 2013). Nitrification will generally occur at a higher rate than ammonification when there is more ammonium than organic nitrogen. The reactions are generally coupled and they proceed rapidly to the nitrate form, which is why nitrite is a relatively rare form of inorganic soil nitrogen (Walworth, 2013). Nitrification will generally occur more regularly than denitrification when there is more ammonium than nitrates in the soil where gas exchange is not limited (Al-Ismaily and Walworth, 2008). After nitrification, nitrate may also be immobilised by soil micro-organisms (Walworth, 2013), or be converted back to ammonium through dissimilatory nitrate reduction under anaerobic or waterlogged conditions (Silver et al., 2001), especially when there is less ammonium than nitrates in the soil. Nitrogen mineralization can

increase beyond the N requirements of other biota if the invasive alien species is removed (Jovanovic et al., 2009). There is a distinct risk for nitrate contamination of groundwater and eutrophication, due to an excess of nitrate (Martin et al., 1999) after alien putative nitrogen-fixing plants have been cleared from an area (Jovanovic et al., 2009).

6.1.2.1. *The impact of fire on nitrogen mineralization (pyro mineralization)*

Fire may also determine the amount of available N that is released (Casals et al., 2005). Fire has a high potential to alter ecosystem N stocks and N cycling (Dannenmann et al., 2011). It is expected that fire frequencies may increase in the future due to regional predictions of an increase in air temperatures and droughts as a result of climate change (Dannenmann et al., 2011). This may have a further effect on the interaction between soil micro-organism and the N cycle such as retaining or releasing N through gaseous and hydrological pathways (Dannenmann et al., 2011). Soil microbial N cycling may have further effects on atmospheric chemistry, climate change, water quality, and plant nutrient availability (Dannenmann et al., 2011).

Fires generally tend to decrease microbial biomass, which may increase soil N cycling processes such as ammonification, nitrification and denitrification and reduce microbial immobilisation (Dannenmann et al., 2011). Burned vegetation provides organic substrates and mineral N, which may be responsible for an increase in microbial N turnover (Dannenmann et al., 2011). Fires also potentially remove competition from plants for mineral N (Dannenmann et al., 2011) as they are either burned to ash or damaged beyond recovery. The competition between plants and soil micro-organisms for mineral nitrogen uptake is released by fire when the plants' allelopathic compounds are destroyed and microbial N turnover is not inhibited anymore (Castaldi and Aragosa, 2002; Dannenmann et al., 2011). Soil micro-organisms can either consume inorganic N through immobilisation or produce inorganic N through dieback during drought events (Dannenmann et al., 2011).

Fires also have the ability to influence the structure and function of soil micro-organisms by exposing them to extreme temperatures that change their physical and chemical environment (Goberna et al., 2012), which is the soil in which they occur. Some of the biochemical processes of the soil micro-organisms may change when temperatures reach 100°C such as changes to the structure of bio-molecules, the breakdown of key metabolites such as ATP and an increase in the exchangeability of solutes and gasses, which further compromises the integrity of the micro-organisms' cell contents (Goberna et al., 2012). Micro-organisms respond to these unfavourable conditions through molecular mechanisms that increase the stability of bio-molecules and membranes to heating or repair damaged cell components and by developing heat resistant structures (Goberna et al., 2012). Some of these structures include spores and thick-walled aggregated hyphae (sclerotia) that are produced by fungi and able to withstand temperatures up to 80°C. Many soil bacteria are also able to produce similar resistant structures such as spores, cysts and akinetes that are able to withstand desiccation and slow temperature increases of up to 50–60°C (Goberna et al., 2012). Endospores are different

structures that are very resistant to extreme conditions such as radiation, physical disruption and temperatures as high as 120°C for 20 min (Goberna et al., 2012).

Fires may also cause changes to the physical and chemical soil properties, but this is determined by the severity of the fire and soil type (Goberna et al., 2012). Burning may cause a pulse of nutrients, which increases the resources for soil micro-organisms temporarily through the mineralization of organic substances (Goberna et al., 2012). Cations that are released to the soil solution may cause the pH to increase, which has an influence on microbial community structure (Goberna et al., 2012). Soils with high carbonate content will not be influenced by the increase in cations due to the buffering effect that carbonate has on soil pH (Goberna et al., 2012). The temperatures must be above 1 000°C for carbonate to start burning (Goberna et al., 2012). Some chemicals that are released by organic matter such as hydrophobic hydrocarbons in association with the ash layers may limit the soil's water infiltration capacity and its moisture content (Goberna et al., 2012). The soil structure as a whole may collapse if the organic and inorganic components are burned very severely, which causes a reduction in porosity, and eventually limits the movement of air, water and other dissolved substances through the soil matrix (Goberna et al., 2012). These changes may ultimately determine the community composition of the three soil micro-organism groups, namely archaea, bacteria and fungi (Goberna et al., 2012).

6.2. Nitrogen Outputs

6.2.1. Leaching

Nitrate is a highly mobile form of N in the soil, which means it can easily move through soil with the soil water (Vitousek et al., 2002; Abaas et al., 2012) due to its solubility and limited soil anion exchange sites. Ammonium is a cation, which means it has a positive charge that is attracted to cation exchange sites in soils with 2:1 clays such as vermiculite, talc and montmorillonite. The movement of ammonium is almost completely prevented due to microbial immobilisation (Abaas et al., 2012), but also locked into the cation exchange sites (Naude, 2012). Ammonium is not available to plants for uptake. Leaching is a hydrological pathway responsible for the reduction of biologically available N in ecosystems due to the mobility of nitrate N (Vitousek et al., 2002). The “excess available N” that remains in the soil after plants and microbes cannot take it up anymore may be lost through this hydrological pathway in the form of nitrate (Vitousek et al., 2002; Abdalla et al., 2009).

6.2.2. Denitrification

Denitrification can be defined as a sequence of microbial respiration processes in the soil where facultative heterotrophic bacteria transform nitrate (NO_3^-) or nitrite (NO_2^-) into gaseous forms of nitrogen such as nitric oxide (NO), nitrous oxide (N_2O) and eventually to nitrogen gas (N_2) (Knowles, 1982), a gas that is abundant in the earth's atmosphere (Martin et al., 1999). The sequence of the change in nitrogen can be viewed as follows:



There are three main factors that control this process, which are 1. facultative heterotrophic bacteria from one of over 50 genera (e. g. *Pseudomonas* spp., *Neisseria* spp. and *Bacillus* spp.) must be present in order for the process to be initiated (Throback, 2006), 2. there must be an available nitrogen (e.g. NO_3^-) and carbon source (e.g. glucose) to use as an energy source by the denitrifying bacteria, and 3. anaerobic conditions or low oxygen levels must occur (Naiman and Décamps, 1997; WPC, 2009). The reaction rate of denitrification is influenced by other factors such as the soil temperature, moisture and type of carbon source (Payne, 1981; Naiman and Décamps, 1997). pH has an influence on whether N_2O or N_2 is produced (Naiman and Décamps, 1997), which normally occurs optimally at a pH ranging from 7.0 – 8.5 (WPC, 2009). Soil texture also has an influence on denitrification, because optimal conditions normally occur in fine-textured soils, which include anoxic conditions and an available carbon source (Ullah et al., 2005).

Spatially distributed global models of denitrification suggest that terrestrial soils account for 22% of total global denitrification (Seitzinger et al., 2006). Denitrification may cause nitrate to be immobilized by microbes or bio-assimilated by plants and can eventually return the nitrogen to the landscape through mineralization (Martin et al., 1999). Chronic N inputs will not cause this sink to become saturated with nitrogen (Martin et al., 1999). The ecosystem can thus not become saturated with N and it will not lose its ability to be an effective filter for nitrate from external sources if the process of denitrification occurs, because it removes nitrate from the system (Martin et al., 1999).

Nitrate can persist in groundwater for decades and may accumulate to high levels if more nitrogen is applied to the land surface each year, which causes the control of nitrate in freshwater systems to be very important (Martin et al., 1999). Nitrate can be reduced in subsurface water flow by buffer zones (i.e. riparian ecotones) to low levels in natural catchments (Correll, 1997; Martin et al., 1999). Plant uptake and microbial immobilization are able to retain nitrate in riparian ecotones, while bacterial denitrification removes nitrate from riparian ecotones and prevents groundwater contamination (Correll, 1997; Martin et al., 1999). Bacterial denitrification occurs when denitrifying organisms do not have sufficient oxygen levels available to use as a terminal electron acceptor (Martin et al., 1999). Bacteria are forced to use ionic nitrogen oxides to gain energy by coupling their reduction to electron transport phosphorylation (Martin et al., 1999). They transform soil nitrogen oxides (NO_3^- and NO_2^-) to gaseous oxides (NO ; N_2O) which may be further reduced to nitrogen gas (N_2), a gas that is abundant in the earth's atmosphere (Martin et al., 1999).

Nitrous oxide (N_2O) is a greenhouse gas that causes destruction of the stratospheric ozone (Robertson et al., 1999; Rees et al., 2006; IPCC, 2007), which in turn contributes to global climate change. The reduction of NO_3^- to N_2O may cause a shift in environmental pollution where groundwater pollution of NO_3^- is converted to atmospheric pollution if high emissions occur and if N_2O is not converted to N_2 in the atmosphere (Dhondt et al., 2004). The “excess available N” that remains in the soil after plants and microbes cannot take it up anymore may be lost through denitrification that

can continue even in areas where and when N is limiting other biological processes in the ecosystem (Vitousek et al., 2002).

6.3. *Nutrient buffering by riparian ecotones*

Nitrogen in the form of organic N is one of the elements that can enter and move through riparian ecotones by being transported in particulate matter. Ammonium and NO_3^- are two of the inorganic elements that can enter riparian ecotones in the form of a dissolved nutrient (Groffman, 1997; Tesi et al., 2013). Ammonium and NO_3^- are transported in surface runoff, groundwater or particulate matter (Groffman, 1997). Contaminated groundwater may also contribute to high levels of dissolved nitrogen in the form of NO_3^- that is transported in suspended material during low flow events (Tesi et al., 2013). Nitrogen is available to plants primarily in the form of NH_4^+ and NO_3^- (Chapman, 2006; Naude, 2012). Ammonium is the preferred form of inorganic N over NO_3^- that is taken up by plants and micro-organisms (Silver et al., 2001; Chapman, 2006), but they can also take up an organic form of nitrogen (Nasholm et al., 1998; Chapman, 2006; Miller et al., 2007). Organic compounds that contain nitrogen can be processed as a by-product as a result of the carbon in the compounds that was used by micro-organisms as an energy source (Groffman, 1997). Nitrogen in the organic form may be taken up from the environment, but this will depend on the nitrogen content of the compound being degraded and the nitrogen demands of the micro-organisms (Groffman, 1997).

Nitrogen is considered to be one of the main pollution sources in the Mediterranean Basin (CEPF, 2010). It can be retained in riparian buffer zones by restricting its movement in the sediment if there are vegetative filter strips (Gilliam et al., 1997). Nitrate, one of the forms in which nitrogen is available for plant uptake and a pollutant in high concentrations, can be reduced in shallow groundwater by flowing through riparian buffers (Gilliam et al., 1997). This causes an improvement of water quality (Gilliam et al., 1997) and prevents NO_3^- from moving through the soil profile to deeper layers where it may potentially pollute the groundwater or move into the stream itself and be transported downstream.

Invasive alien vegetation can alter patterns of nutrient cycling in soils (Jovanovic et al., 2009) and they may also change nitrogen cycling regimes in ecosystems from low to high nitrogen cycling (Jovanovic et al., 2009; Marchante et al., 2008), which may create a large sink for nitrogen when the trees are taking up inorganic nitrogen in the growing season. Many processes are responsible for making nitrogen unavailable for further uptake by plants and micro-organisms and may include immobilisation and denitrification (Groffman, 1997).

Trees with symbiotic nitrogen-fixing bacteria are able to retain inorganic nitrogen through uptake by supplying carbon to the soil micro-organisms in the form of glucose that can be taken up by the soil micro-organisms (Nasholm, 1998). Nitrogen may be immobilised in plant biomass and decomposing litter (Vitousek et al., 2002). Immobilisation is the process by which NO_3^- may be removed in these ecotones through uptake as a nutrient that supplies energy to one particular group of micro-

organisms, namely denitrifying bacteria (Groffman, 1997). The continuous uptake of NO_3^- by evergreen sclerophyllous Mediterranean shrubs may also remove NO_3^- and cause it to be a serious limiting factor in denitrification (Castaldi and Aragosa, 2002). Nitrogen immobilisation may also be influenced by the input rate of carbon into the soil through litterfall and exudates (Nasholm, 1998).

Micro-organisms also play a very important role in the process of removing nutrient pollutants from riparian ecotones by utilising them as energy sources and nutrients for growth or electron acceptors in respiration (Groffman, 1997). Micro-organisms are also responsible for the conversion of NO_3^- into nitrogen gases (e.g. N_2O and atmospheric N_2) through denitrification under anaerobic conditions (Groffman, 1997). Nitrate serves as the electron acceptor for a diverse group of facultative anaerobic bacteria (Groffman, 1997) of which denitrifiers are responsible for the process of denitrification being carried out.

Nitrogen may also be retained in the soil under anaerobic conditions that rapidly transform NO_3^- first to NO_2^- and then to NH_4^+ by fermentative bacteria, generally found in flooded environments, through the process of nitrate dissimilatory reduction (Silver et al., 2001). Plants and micro-organisms generally take up more NH_4^+ than NO_3^- due to energy costs associated with nitrate reduction in tissues (Silver et al., 2001). Nitrate dissimilatory reduction may increase the NH_4^+ pool and enhances the availability and uptake of NH_4^+ , which contributes to nitrogen retention when it is taken up by plants or soil micro-organisms (Silver et al., 2001). This process is absent from areas that do not experience periodic inundation due to the absence of a highly reducing environment able to maintain sustained anaerobic metabolism of the fermentative bacteria (Silver et al., 2001). This process seems to be primarily limited by the availability of NO_3^- when compared to carbon or oxygen and is thought to be favoured by a high ratio of available carbon to electron acceptors (Silver et al., 2001).

6.4. Invasion and clearing impacts on the N cycle

Invasive alien nitrogen fixing vegetation such as legumes threatens indigenous ecosystems by replacing indigenous species (Tye and Drake, 2012). They not only affect the structure of ecosystem services (Beater et al., 2008; Le Maitre et al., 2011), but they may also have an influence on the function of regulatory ecosystem services such as nutrient cycling (Yelenik et al., 2007; Le Maitre et al., 2011), especially nitrogen cycling. They have the ability to fix atmospheric N_2 and convert it into inorganic nitrogen (i.e. NH_4^+ and/or NO_3^-) through biological nitrogen fixation (Vitousek et al., 2002; Yelenik et al., 2004). The nitrogen cycle may be affected in a negative manner due to an enhanced nitrogen level in the system (Vitousek et al., 2002; Tye and Drake, 2012).

Enhanced nitrogen levels change the limitation that nitrogen as a nutrient has on the system, which may also affect productivity, composition, dynamics and diversity (Vitousek et al., 2002) of especially indigenous evergreen fynbos shrub species that are adapted to the low N status of the soils (Stock and Lewis, 1984). Indigenous evergreen fynbos shrub species may be outcompeted by invasive alien vegetation (Le Maitre et al., 2011), for especially nitrate nitrogen, during the growing season due to

the low uptake rates, poor NO_3^- assimilation rates and slow growing habit of fynbos shrubs (Stock and Lewis, 1984). Invasive alien vegetation also produces thick litter layers with carbon that binds to organic N in microbial pools that has long lasting effects on the soil N pool (Rascher et al., 2011). These thick litter layers of the invasive alien vegetation may also contribute to N inputs into the system that will favour uptake by invasive alien vegetation and also contributes to the limitation that N has on the system.

The impact that invasive nitrogen fixers have on N dynamics in the ecosystem will depend largely on the indigenous plant species assemblages and the physiological characteristics of both indigenous and invasive species (Tye and Drake, 2012). Invasive N-fixers may have a greater impact on nitrogen inputs in ecosystems where there is a naturally low abundance of N-fixing species, because these ecosystems will have low base level nitrogen fixation inputs (Tye and Drake, 2012). This may further lead to a positive paradoxical feedback between N availability and individual plant N-fixation where invading legumes can both benefit from and worsen nitrogen pollution (Tye and Drake, 2012). In this manner they are able to facilitate their own invasion, which contributes to their success as alien invading vegetation (Le Maitre et al., 2004). This may lead to an increase in aboveground biomass and litter (Beater et al., 2008) that may increase the occurrence of fires and affect the N cycle even further. N losses could occur after fires, which may be offset by the introduction of fixed atmospheric N (Casals et al., 2005). These invaders use high levels of mineralized N that is abundant after fires (Casals et al., 2005), which causes a decrease in this N source for uptake by indigenous species. They thus cause N to be a limited nutrient in the soil where they occur. Nitrogen fixation is very costly to these plants and they will rather take up available N in the soil and fix N only when it becomes limited (Casals et al., 2005). Nitrogen fixation becomes more feasible than plant uptake in favourable conditions such as an open canopy (after clearing), roots are well spread in the soil, soil inorganic N concentrations are low and other resources such as P and water are readily available (Casals et al., 2005). N-fixers are also able to respond rapidly in growth and activity to conditions following fires, where there is more light available (Casals et al., 2005).

Our national invasive vegetation clearing program, the "Working for Water Program", has put in many efforts to clear invasive alien vegetation, since the project was implemented in 1995 (Holmes et al., 2005; Beater et al., 2008) in order to reduce the negative impacts that invasive alien vegetation has on the environment. The clearing efforts up to date have unfortunately had some disturbance to the area due to the clearing method where the slash (plant litter) is either left on the soil, removed or burned in stack piles (Holmes et al., 2008). Disturbances caused by clearing may lead to reinvasion by the same or secondary alien species (Blanchard and Holmes, 2008) and cause the negative impacts on the nitrogen cycle to be continued.

7. Research Rationale

7.1. Problem identification

Fynbos riparian ecotones occupy a small area in comparison to the greater catchment (Reinecke et al., 2007). They may have a great influence on the surrounding landscape, especially through their vegetation (Reinecke et al., 2007). Natural riparian ecotones have very different vegetation types from the surrounding environments and they are one of the most diverse terrestrial habitats on earth (Naiman et al., 1993). These riparian ecotones are prone to natural disturbances such as seasonal floods that create and remove patches of vegetation, which causes them to be vulnerable to invasion by alien plants (Reinecke et al., 2007). In the fynbos biome, riparian zones are especially vulnerable to invasion by Australian woody invasive alien vegetation such as *Acacia mearnsii*, *Acacia longifolia*, *Acacia saligna* and *Acacia cyclops* (Reinecke et al., 2007, Blanchard and Holmes, 2008).

These invasive alien species cause degradation to riparian habitats by suppressing or replacing indigenous species (Blanchard and Holmes, 2008) and altering nutrient recycling processes (Jovanovic et al., 2009; Roura-Pascual et al., 2009; Meek et al., 2010). They may change the structure of riparian vegetation by over-shadowing herbaceous species and preventing them from establishing and preventing succession of riparian species (Reinecke et al., 2008). Invasive alien vegetation may also release allelopathic compounds that inhibit the growth of other riparian species and interfere with their metabolic processes (Castaldi and Aragosa, 2002). They also influence the ability of riparian ecotones to provide ecosystem services such as nutrient cycling (Roura-Pascual et al., 2009; Meek et al., 2010).

A national invasive vegetation clearing program, the Working for Water Program (WFW), was implemented in SA in 1995 in order to address changes in riparian structure and function (Van Wilgen et al., 1998). There is an assumption that riparian ecotones will spontaneously repair, both structurally and functionally once the stress (woody invasive alien species) is removed (Galatowitsch and Richardson, 2005; Holmes, 2007; DWAF, 2008). Recent studies produced substantial knowledge on the effect of clearance of woody alien species on restoration of indigenous species (e.g. Pretorius et al., 2008; Blanchard and Holmes, 2008; Esler et al., 2008). The effect of removal of invasive species on soil processes, and potential changes in water quality in fynbos riparian ecotones have not been determined by previous studies. For example, it is known that invasive alien *Acacia* spp. are able to fix nitrogen (Tye and Drake, 2012), which can change the amount of nitrates that leach into the rivers (Yelenik et al., 2004). It is not known if the invasion by alien *Acacia* spp. can lead to irreversible changes in soil properties. It is also not known if these properties can be restored (i.e. microbial conversion of excess nitrogen released to the atmosphere as nitrogen gas) by clearing the invasive vegetation.

Even though there are numerous investigations carried out globally in this field, investigations for Mediterranean-type ecosystems are still lacking, specifically in the Western Cape. Fynbos riparian ecotones are poorly studied in this regard. A novelty of this study is that it is conducted in the Fynbos

Biome, with nutrient-poor soils, and a distinctive climate characterized by summer droughts and winter rains.

7.2. Research Aims

This project aims to investigate the impact of (1) invasion by *Acacia mearnsii* on net nitrogen mineralization, nitrous oxide emission and the potential for denitrification enzyme activity in fynbos riparian environments and (2) the effect of clearing, in both cases using indigenous riparian zones (near pristine areas) as reference sites. The potential for water pollution in fynbos riparian environments is also investigated.

7.3. Objectives

The objectives of this study are to investigate:

1. the effect of invasive *Acacia* spp. on net nitrogen mineralization and nitrous oxide emission in fynbos riparian environments;
2. how the clearing of invasive vegetation modify net nitrogen mineralization and nitrous oxide emission in fynbos riparian environments;
3. whether riparian functioning in terms of N cycling is repaired after clearing of invasive alien *Acacia* spp. from riparian ecotones.

7.4. Research questions

The following specific research questions were asked to address the objectives:

1. Does invasive *Acacia* spp. increase available nitrogen beyond the requirement for mineralization?
2. Does the invasion by alien *Acacia* spp. provide more nitrogen than can be transformed to nitrous oxide?
3. How is the mineralization process affected by invasion and clearing?
4. What is the effect of selected soil properties on mineralization and nitrous oxide emission?
5. What is the effect of selected soil properties on denitrification enzyme activity?
6. How is the potential denitrification enzyme activity affected by invasion and clearing?
7. Can riparian functioning be repaired by removal of nitrogen from the system as a volatile gas (nitrous oxide) after clearing of invasive alien *Acacia* spp. from riparian ecotones?

8. Outcomes of the study

This study will evaluate whether invasion by *Acacia* spp. cause changes to net nitrogen mineralization, nitrous oxide emissions and denitrification potential and whether soil properties were changed irreversibly. This study will also evaluate whether the clearing of invasive species, which is widely applied as a management practice in the Western Cape, leads to restoration of riparian functioning in terms of nitrogen cycling (considering that restoration included recovery of both structure and function) or whether the invasion leads to irreversible changes in riparian ecosystems. The study will significantly enhance our understanding of nutrient cycling in riparian ecotones, in

particular whether nitrous oxide emission and denitrification enzyme activity in riparian ecotones reduce nitrogen loads in riparian ecotones and ultimately nitrogen release to lotic systems. The results of this study will also contribute to conservation management planning for maintaining ecosystem services in riparian ecotones, particularly in fynbos environments that are currently understudied.

9. Study sites

9.1. Western Cape

Seven perennial river systems (Dwars River, Eerste River, Elands River, Jakkals River, Molenaars River, Sir Lowry's River, and the Wit River) were chosen within the mountain catchment areas surrounding Stellenbosch (Figure 1.4.). They were classified into similar categories with reach type, invasion, clearing and fire history as the main criteria for the selection of these sites. The study focused on the upper reaches (mountain streams) and foothill segments of the rivers where human impacts were minimal and where invasion statuses could be separated in "natural" (near pristine or reference sites) sites, densely invaded (> 70% canopy cover; not cleared 10-15 years prior to the study) and cleared sites (cleared 7 years prior to the commencement of the study). Sites of different invasion statuses (natural, invaded and cleared) were selected in the same river system where it was possible in order to reduce environmental variation due to differences in climate or geology.

The sites were also selected in the same locations of previous studies where it was possible (e.g. Blanchard, 2007; Reinecke et al., 2007; Vosse, 2007), which facilitated the process of getting permission and access to sites and in order to make cross-study comparisons. Reinecke et al. (2007) provided valuable insights into the selection of the positions within the landscape (wet bank, dry bank, and terrestrial fynbos), which was selected to minimize the influence of soil moisture and different vegetation types. The fire history is also different for many of the sites and it plays a role in the structure and function of riparian ecotones. The categories used for the site selection are summarized in Table 1.1.

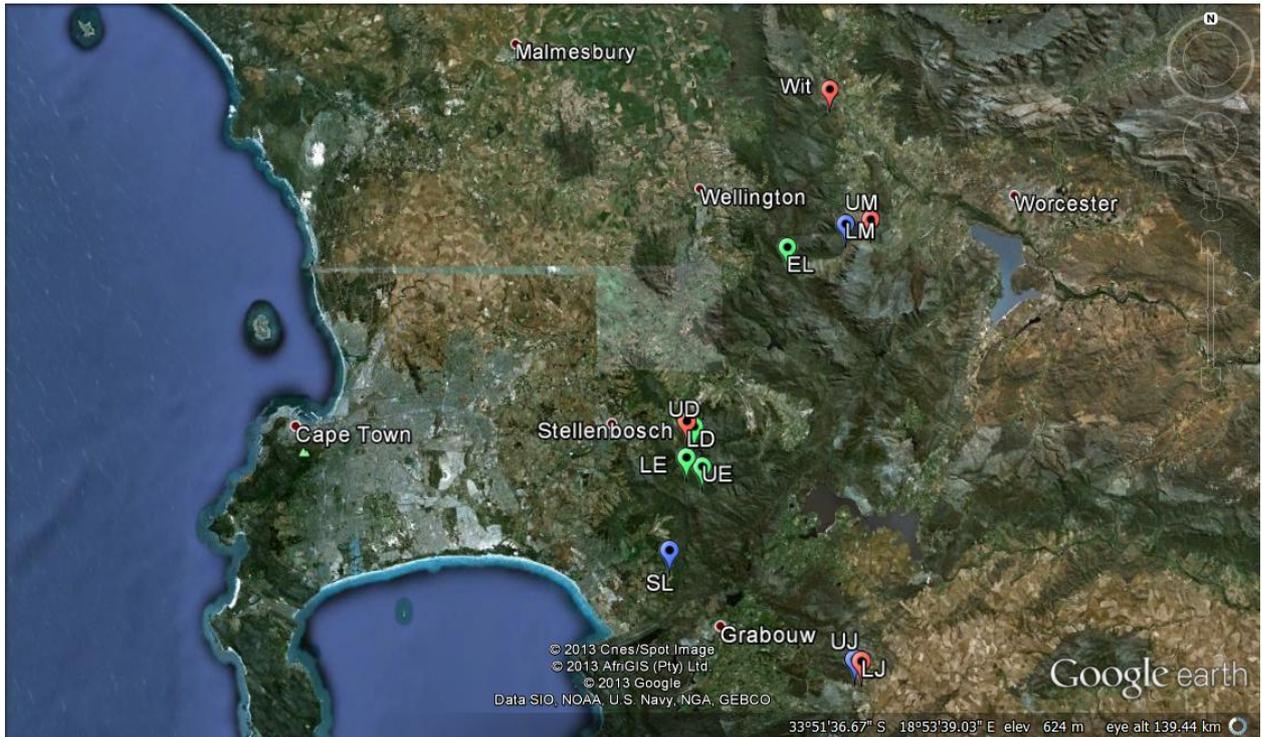


Figure 1.4. Study sites with the compliments of Google earth within the mountain catchment areas surrounding Stellenbosch University represented by symbols with different colours: green (“natural” sites); UE: Upper Eerste River, LE: Lower Eerste River, UD: Upper Dwars River, EL: Elands River, red (densely invaded sites); LD: Lower Dwars River, WIT: Wit River, LM: Lower Molenaars River, LJ: Lower Jakkals River and blue (cleared sites); SL: Sir Lowry’s River, UM: Upper Molenaars River, UJ: Upper Jakkals River.

Table 1.1. Summary of the geographical characteristics and site history of the study sites (Naude, 2012).

Sites	Geology	Coordinates	Landowners	History of invasion	History of clearance	Fire History	Longitudinal zone
Upper Eerste River (Natural)	Sandstone and/or Granite	34°00'00.34"S; 18°59'22.55"E	CapeNature	None	None	March 2009	Mountain Stream
Lower Eerste River (Natural)	Sandstone and/or Granite	33°59'22.56"S; 18°58'05.36"E	CapeNature	None	None	March 2009	Mountain Stream
Upper Dwars River (Natural)	Sandstone and/or Granite	33°57'14.38"S; 18°58'44.69"E	CapeNature	None	None	March 2009	Mountain Headwater Stream
Elands River (Natural)	Sandstone	33 44'23.46"S; 19 06'47.29"E	CapeNature	None	None	March 2011	Mountain Stream transitional
Lower Dwars River (Invaded)	Sandstone and/or Granite	33°56'51.45"S; 19°58'11.25"E	CapeNature	Invaded for >10 years (<i>A. mearnsii</i> and <i>A. longifolia</i>)	No clear evidence	No evidence of recent fire	Mountain Stream
Wit River (Invaded)	Sandstone	33°32'21.35"S; 19° 10'50.06"E	Mount Bain Development	Invaded for >15 years (<i>A. mearnsii</i>)	No clear evidence	No evidence of recent fire	Foothill
Lower Molenaars River (Invaded)	Sandstone	33 42'16.95"S; 19 13'59.42"E	CapeNature	Invaded for >10 years (moderately invaded with <i>A. mearnsii</i>)	Cleared Jan/Feb 2011.	March 2011	Upper Foothill
Lower Jakkals River (Invaded)	Sandstone	33°13'02.40"S; 19°12'08.54"E	Beaumont Wine Farm, Bot River	Invaded for >15 years (<i>A. mearnsii</i> and some <i>A. longifolia</i>)	Some evidence of clearing, with piles on site	No evidence of recent fire	Mountain Stream transitional
Upper Jakkals River (Cleared)	Sandstone	34°12'55.08"S; 19°11'35.62"E	CapeNature	Invasion with mostly <i>Acacia species</i> >10 years ago	Initial clearance: 1996-1997. 2 follow-up treatments. Clearing treatment: Fell and burn	January 2010	Mountain Stream transitional
Sir Lowry's River (Cleared)	Sandstone and/or Granite	34°05'41.50"S; 18°56'39.70"E	Wedderville Estate, Sir Lowry's Village	Mixed invasions: mostly <i>Acacia species</i> (also <i>Pinus species</i>) >8 years ago	Initial clearance: 2002 followed up annually by landowner. Clearing treatment: fell and burn	No evidence of recent fire	Mountain Stream
Upper Molenaars River (Cleared)	Sandstone	33°42'38.56"S; 19°11'49.24"E	Boundary of CapeNature and Rainbow's End Farm	Invasion with <i>Acacia mearnsii</i> dominant species >7 years ago	Initial treatment: 2002-2003. 2 follow-up treatments. Clearing treatment: fell and remove	No evidence of recent fire	Upper foothill

Table 1.1 displays the various study sites with their invasion statuses as briefly explained in the title. It also gives the geographical location in degrees, minutes and seconds format of the various sites with the landowners and invasion, clearing and fire history as well as where along the longitudinal zone the site fits in.

9.2. Southern- and Eastern Cape

Study sites were selected from six river systems (Baviaans River, Voeght's River, Witteklip River, Kammannassie River, Groot River, De Hoop River) within the mountain catchment areas surrounding Kareedouw and Haarlem (Figure 1.5.). They were classified into similar categories with invasion status, location and vegetation type as the main criteria for the selection of these sites. The study focused on the segments of the rivers where human impacts were minimal and where invasion statuses could be separated in "natural" (near pristine or reference sites) sites, densely invaded (> 70% canopy cover) and cleared sites (cleared 7 years prior to the commencement of the study). Sites of different invasion statuses (natural, invaded and cleared) were selected in the same river system where it was possible in order to reduce environmental variation due to differences in climate or geology. The categories used for the site selection are summarized in Table 1.2.

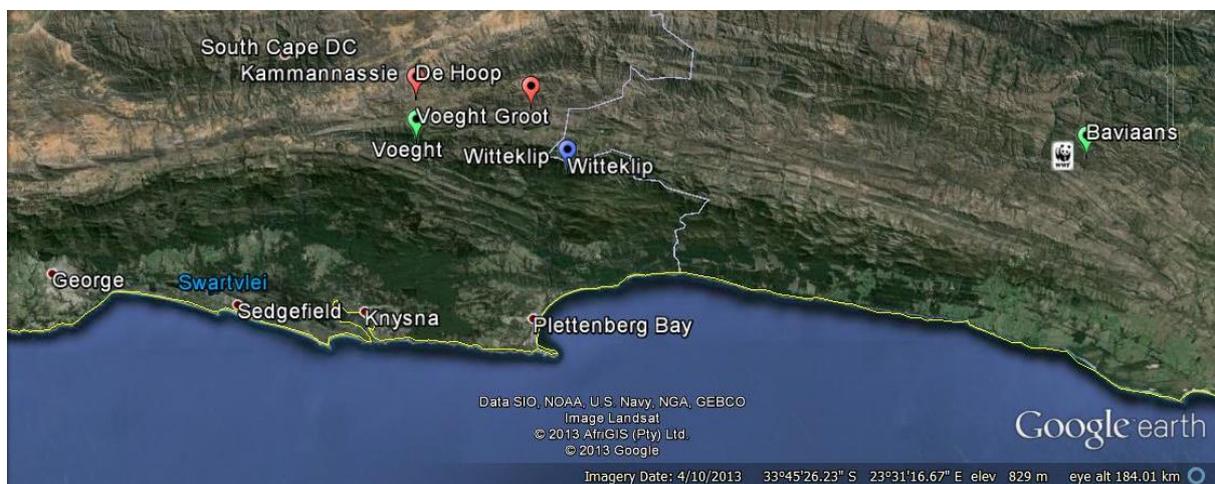


Figure 1.5. Study sites with the compliments of google earth within the mountain catchment areas surrounding Kareedouw and Haarlem represented by symbols with different colours: green ("natural" sites); Upper and lower Voeght River (densely invaded sites); Upper Witteklip River, Kammannassie River, Groot River and blue (cleared sites); Lower Witteklip River and De Hoop River.

Table 1.2. Summary of the geographical characteristics and invasion status of the study sites (Adapted from Kambol, 2013).

Sites	Invasion status	Geology	Coordinates	Location	Vegetation
Baviaans River	Restored	Quartzitic Sandstone	33°48'7.06"S 24°25'8.32"E	Kouga Mountain	Fynbos
Voeght's River	Natural	Quartzitic Sandstone	33°46'4.96"S 23°09'3.68"E	Langkloof	Fynbos, Restionaceae
Voeght's River	Natural	Quartzitic Sandstone	33°46'4.96"S 23°09'3.68"E	Langkloof	Fynbos, Restionaceae
Witteklip River	Invaded	Quartzitic Sandstone	33°49'7.82"S 24°26'9.32"E	Kouga Mountain	<i>A. mearnsii</i> , Restionaceae, forbs and grasses
Kammanassie River	Invaded	Quartzitic Sandstone	33°42'2.96"S 23°09'6.23"E	Langkloof	<i>A. mearnsii/A. dealbata</i>
Groot River	Invaded	Quartzitic Sandstone	33°43'3.92"S 23°22'4.20"E	Langkloof	<i>A. mearnsii</i> , <i>A. dealbata</i> , <i>A. longifolia</i> ,
Witteklip River	Cleared	Quartzitic Sandstone	33°49'7.13"S 24°26'6.99"E	Kouga Mountain	<i>A. mearnsii</i> ,
De Hoop River	Cleared	Quartzitic Sandstone	33°42'0.60"S 23°09'3.83"E	Langkloof	<i>A. mearnsii</i> , <i>A. dealbata</i> , <i>A. longifolia</i> ,

Table 1.2 displays the various study sites with their invasion statuses as briefly explained in the title. It also gives the geographical location in degrees, minutes and seconds format of the various sites with the geology, location and the dominant vegetation type of the area.

10. Thesis structure

10.1. Chapter 1

This chapter gives an introduction into some of the literature that is available on the effect of invasive alien plants on net nitrogen mineralization, nitrous oxide emissions and the maximum denitrification potential in invaded and cleared fynbos riparian ecotones and their associated non-riparian terrestrial areas under field and laboratory conditions. It also briefly introduces the study sites and summarises the importance of the research by stating the research aims, objectives, hypotheses, and questions and concludes what the research study will contribute. This chapter does not contribute to any of the objectives, but is purely aimed at giving the scientific literature supporting this study.

10.2. Chapter 2

This chapter investigates labile nitrogen in natural, invaded and cleared fynbos riparian ecotones and their associated non-riparian terrestrial areas under field conditions and the various forms in which it is retained and/or released in the soil by micro-organisms and/ or plants. It also reports on some of the soil properties that drive changes in labile nitrogen. This chapter addresses objective 1 and 2 by asking questions 1, 2, 3 and 4.

10.3. Chapter 3

Chapter 3 investigates the maximum denitrification potential through enzyme activity of soil micro-organisms under laboratory conditions as well as some of the factors that control the process. In this chapter objective 3 is addressed by asking questions 5, 6 and 7 mentioned above.

10.4. Chapter 4

This final chapter gives a brief summary of the main findings and recommendations for management and implications of invasive alien *Acacia* spp. invasion and the clearing thereof in fynbos riparian ecotones and identifies future research requirements.

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CHAPTER 2:
AVAILABLE NITROGEN AND N₂O EMISSIONS FOR NATURAL, INVADED AND CLEARED
FYNBOS RIPARIAN ECOTONES UNDER FIELD CONDITIONS

Abstract

Riparian ecotones provide a unique habitat for a variety of plants and animals, recycle nutrients, control erosion, maintain good water quality and act as a buffer against nutrient pollutants. Unfortunately in the Western Cape they are heavily invaded by Australian invasive alien *Acacia* species. These are nitrogen-fixers with the ability to alter nutrient cycling processes and they may also change nitrogen cycling regimes in ecosystems from low to high nitrogen cycling. The aim of this chapter was to investigate the impact of invasive alien *Acacias* on nitrogen mineralization, nitrogen availability and actual field N₂O emissions and how clearing modified nitrogen mineralization and actual field N₂O emissions compared to reference sites (near pristine areas). It was also determined whether riparian functioning in terms of nitrogen cycling was repaired after clearing of invasive alien *Acacia* spp. from riparian ecotones.

Nitrogen mineralization in the fynbos riparian ecotones of the study was not different across the seasons and it was similar for invaded and cleared sites compared to the reference sites with no differences in the riparian ecotones (wet and dry banks) compared to the upper terrestrial areas. Nitrogen mineralization was comparable to other Mediterranean-type ecosystems (MTEs), but the field nitrogen mineralization of the current study was not comparable to nitrogen mineralization carried out in the laboratory for the same study sites, which means laboratory measurements must always be validated in the field where optimal conditions do not necessarily always occur.

Nitrogen mineralization is driven by the C:N ratio that was less than 24 in the current study. A decrease in nitrogen mineralization was induced by the C:N ratio of less than 24. The measured levels of NH₄⁺ were different across seasons and increased over time in especially the dry banks of the invaded and cleared sites. Invasion by *Acacia* spp. thus has an effect on nitrogen input to the system in the form of NH₄⁺ and legacy effects of IAPs are still visible. Invasion of this nitrogen-fixing tree species also adds nitrogen in the form of NO₃⁻ to the system, while reference sites were similar; recovery of cleared sites is taking place. The position in the landscape did not have an effect on available NO₃⁻ when riparian ecotones were compared to the upper terrestrial areas. Total available nitrogen is driven by NO₃⁻ although NH₄⁺ becomes more dominant over time, especially in the winter when the ratio of NH₄⁺ to NO₃⁻ is higher in the dry banks.

No clear trends were observed in the actual field N₂O emissions across the different seasons, invasion statuses and landscape positions. Similar soil microbial communities are producing N₂O through aerobic nitrogen mineralization and not denitrification. Denitrification is limited by low levels of NO₃⁻ and very low silt and clay content (<5%), which creates a well-drained soil with low nutrient

and water retention. The surface field N₂O emissions are driven by processes lower in the soil profile in winter and groundwater denitrification may be overriding the effect of invasion in surface soils. The lack of differences in field N₂O emissions means that denitrification as a pathway to reduce NO₃⁻ in riparian ecotones have been overestimated, because there are various other pathways that reduce nitrate from aquatic ecosystems, which include dissimilatory nitrate reduction to NH₄⁺ and anaerobic ammonium oxidation. It is important to monitor and manage NO₃⁻ in riparian ecotones or wetlands, because they can become sources of N₂O and contribute to global climate change. While apparently invaded fynbos riparian ecotones may not contribute significantly more N₂O, they are very significant in terms of their contributions to atmospheric C levels.

KEYWORDS: Nitrogen mineralization, available N, field N₂O emissions, invasive *Acacia* spp.

1. Introduction

Riparian ecotones consist of biological communities adjacent to a river bank or shores of streams or lakes (Naiman and Décamps, 1997; Reinecke et al., 2007). They form corridors that are part of an ecosystem shaped by natural disturbances (Esler et al., 2008). These ecotones receive matter and propagule inputs from elsewhere in the catchment and they have the ability to accumulate, concentrate and exacerbate impacts and disturbances caused by humans (Esler et al., 2008). Disturbances include natural disturbances such as floods, fire, and drought as well as anthropogenic disturbances such as impoundments, destruction of riparian zones through development, land use change, and invasive alien species (Naiman and Décamps, 1997).

Riparian ecotones have been extensively studied in the past (Hubbard, 1977; Swanson 1982; Peterjohn and Correll, 1984) and the number of studies are increasing at a tremendous rate (Stella et al., 2012) due to the importance of the various ecological services that they provide. They provide a unique habitat for a variety of biota (Naiman et al., 1993), recycle nutrients and control erosion (Reinecke et al., 2007), maintain good water quality (Wantzen and Junk, 2008) and act as a buffer against nutrient pollutants such as nitrates and phosphates, suspended solids, heavy metals, and pesticides (Jacobs et al., 2007). They also have the ability to move organisms through a large network of sites (Shah et al., 2010), which makes it possible for riparian vegetation to be dispersed to other sites and become established downstream. Unfortunately riparian vegetation may also include invasive alien trees with the ability to alter the micro-climate of stream water, soil and air relative humidity (Naiman and Décamps, 1997; Richardson et al., 2007). Invasive alien plants may thus have a negative effect on the structure and function of riparian ecotones.

The Western Cape of South Africa is part of the fynbos biome and is heavily invaded by invasive alien plants (Le Maitre et al., 2000), especially Australian *Acacia* species (Holmes et al., 2005) that are nitrogen-fixers (Tye and Drake, 2012). They have the ability to alter nutrient cycling processes (Jovanovic et al., 2009) and they may also change N-cycling regimes in ecosystems from low to high N-cycling (Jovanovic et al., 2009; Marchante et al., 2008). One of the ways in which they add

inorganic N (e.g. NH_4^+ or NO_3^-) to the system is through biological N-fixation (Yelenik et al., 2004; Tye and Drake, 2012). These unique ecotones also produce thick litter layers with low quality carbon that binds to organic N in microbial pools that has legacy effects on the soil N pool (Rascher et al., 2011). Biological N fixation can be carried out by symbiotic N-fixers (Vitousek et al., 2002).

Symbiotic N-fixers may have an impact on nitrogen mineralization (Van Wesemael, 1993), a process that only occurs in the presence of free dissolved oxygen (aerobic conditions) due to the fact that the nitrifying bacteria that carry out this process are strict aerobes (Simek, 2000). Ammonification is a hydrolysis process whereby organic nitrogen is converted to ammonia and/or ammonium in the presence of water. Ammonification precedes nitrification and provides the substrate for the latter process to occur. Nitrification is a two-step biological process whereby ammonia and ammonium is firstly converted to nitrite and then secondly nitrite is rapidly converted to nitrate. The reactions are generally coupled and they proceed rapidly to the nitrate form, which is why nitrite levels at any given time are usually low. Nitrogen mineralization can increase beyond the N requirements of other biota if this sink (invasive alien species) is removed (Jovanovic et al., 2009).

Many conservation efforts are focussed on the clearing of these invasive alien plants, which threaten our water supplies and the local fynbos vegetation (Le Maitre et al., 2000). A classic South African example of such a project that was developed is the "Working for Water Program" (WfW), which was implemented in 1995 in order to address changes in riparian structure and function (Van Wilgen et al., 1998). There is an assumption that riparian ecotones will spontaneously repair, both structurally and functionally once the stress (woody invasive alien species) is removed (Nilsson et al., 2005; Esler et al., 2008; Ruwanza, 2012). Recent studies produced substantial knowledge on the effect of clearance of woody alien species on restoration of indigenous species (e.g. Pretorius et al., 2008; Blanchard and Holmes, 2008; Esler et al., 2008). The effect of removal of invasive species on soil processes, and potential changes in water quality in fynbos riparian ecotones have not been determined by previous studies. For example, as mentioned previously, it is known that invasive alien *Acacia* spp. are able to fix nitrogen, which can change the amount of nitrates that leach into the rivers (Yelenik et al., 2004). Nitrate can persist in groundwater for decades and may accumulate to high levels if more nitrogen is applied to the land surface each year, which causes the control of nitrate in freshwater systems to be very important (Martin et al., 1999).

However, it is not known whether invasion by alien *Acacia* spp. into riparian zones leads to irreversible changes in soil properties such as enhanced available inorganic N, lower levels of soil moisture, changes in pH and bulk density, etc. It is unclear if these properties can be restored by clearing the invasive vegetation such as reducing N cycling to levels before sites became invaded. Even though there are numerous investigations carried out globally in this field, it is still lacking investigations for Mediterranean-type ecosystems (MTEs), specifically in the Western Cape. Fynbos riparian ecotones are poorly studied in this regard, which is why the study investigated the impact of invasive vegetation on denitrification and nitrogen mineralization and potential for water pollution in

fynbos riparian environments. A novelty of this study is that it is conducted in the fynbos biome, with nutrient-poor soils, and a distinctive climate characterized by summer droughts and winter rains.

The main objectives of this chapter is to investigate the effect of invasive *Acacia* spp. on net nitrogen mineralization and nitrous oxide emission and how the clearing of this invasive species modifies net nitrogen mineralization and nitrous oxide emission in fynbos riparian environments compared to reference sites. Invaded sites are expected to have higher nitrogen mineralization rates, more nitrogen available in the form of ammonium and higher N₂O emissions compared to reference sites. Sites cleared of invasive *Acacia* spp. are expected to have lower nitrogen mineralization rates, more nitrogen available in the forms of ammonium and higher N₂O emissions compared to invaded sites and reference sites.

2. Material and methods

2.1. Soil sampling procedure

Soil samples were taken at 15m intervals along three transects of 60m in length parallel to the river (i.e. wet banks, dry banks and upper terrestrial area) in Spring 2011, Autumn 2012 and Winter 2012 for the Western Cape (Figure 1.4. and Table 1.1.). Six random soil samples were taken using a stainless steel soil-core type sampler (5 cm diameter) from the top 10 cm (topsoil or A-horizon) of the soil profile after loose litter (organic matter) was removed as this part of the soil profile is thought to be the most biologically active (Gold and Kellog, 1997). A hand trowel was sometimes used in places with very dry and sandy or rocky and/or shallow soils. The samples were pooled and homogenized within polyethylene bags (Ziploc bags) that contained the sampling position, date and site name on the outside of the bag, which was pre-marked with a permanent marker. Most of the air was pushed out of the bag and sealed airtight, which prevented contamination by atmospheric nitrogen deposition. Samples were stored at a temperature of 4°C for no longer than 3 days in cases where soil extractions could not take place immediately. Samples were sieved with a 2 mm stainless steel sieve to remove roots and organic debris. Samples were analysed for organic carbon and the sand, silt and clay content were determined by the Western Cape Department of Agriculture.

2.2. Inorganic nitrogen extractions

A 0.5 molar solution of potassium sulphate (0.5 M K₂SO₄) was made up by adding 87 g of potassium sulphate salt to 1 L distilled water. The solution was stirred mechanically until all the salt was dissolved. Ten grams of fresh field soil (sieved) was measured off with a digital scale accurate up to two decimals and placed into a 50 ml plastic centrifuge tube that contained 25 ml of this solution (the extractant). The tube was first shaken by hand and then placed on a mechanical shaker at medium speed for 60 minutes after which the solution was filtered. This allowed the soil to separate from the extractant, which contained available nitrogen in the inorganic forms (NH₄⁺) and (NO₃⁻) as well as ammonia (NH₃). The clear extracted solution was used to determine the NH₄⁺ and NO₃⁻ concentrations colorimetrically. This process was repeated in order to create backup extractions for future use.

2.3. *In situ* nitrogen mineralization

Four 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 transect were knocked into the soil to a depth of 10 cm. The remaining top part was covered with a piece of square black 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 external moisture influences. Excessive soil moisture (>60% of water holding capacity) in the soil creates anoxic conditions that reduce nitrogen mineralization activity (Robertson et al., 1999). Very low soil moisture has a negative impact on the metabolic processes of soil micro-organisms. Two small holes were cut with a scissors on the opposite ends of the plastic that covered the PVC tube, which allowed natural gas exchange to take place. Soil samples were collected, extracted (as described in the previous section) and analysed for available nitrogen (NH_4^+ and NO_3^-) and moisture content for the same day the PVC tubes were inserted into the soil, which had an initial value at time zero. This process was used to correlate the impact that environmental conditions may have had on the nitrogen that was measured in the system. The PVC tubes were left in the field for 7 days, which depended on weather conditions, and was then removed with the soil intact inside the PVC tube. The PVC tubes were collected only if it did not rain the day before or on the day of collection and at the same time whenever it was possible. The plastic tubes were placed in marked sealed plastic bags and then transported back to the lab. The soil samples were extracted and analysed for available nitrogen (NH_4^+ and NO_3^-) and moisture content for the same day the PVC tubes were removed, which had an end value for the time period (T_7). This allowed the study to determine how available nitrogen changed during that specific time period (nitrogen mineralization rate).

Ammonium was analysed using the colorimetric method known as the “indophenol method” or the “phenate method” (Solorzano, 1969). It was based on the reaction of ammonia in an alkaline solution with phenate, which produced a blue colour in the presence of a strong oxidizing reagent such as hypochlorite (commercial bleach). The absorbance was read on a spectrophotometer set at 630 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 ammonium nitrogen (NH_4^+) per millilitre concentrations was calculated from the unknown samples by solving X in one standard curve. The corrected available NH_4^+ concentrations (in $\mu\text{g g}^{-1}$ dry soil⁻¹) was calculated by multiplying the value by 25 (the extractant volume) and then divided by the dry mass of the soil (in $\mu\text{g g}^{-1}$ dry soil⁻¹).

Nitrate was analysed using an adapted colorimetric 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 nitrate nitrogen (NO_3^-) per millilitre concentrations was calculated from the unknown samples by solving X in one standard curve. The corrected available

NO_3^- concentrations (in $\mu\text{g g}^{-1}$ dry soil⁻¹) was calculated by multiplying the value by 25 (the extractant volume) and then divided by the dry mass of the soil (in $\mu\text{g g}^{-1}$ dry soil⁻¹).

Nitrogen mineralization was determined by the difference in available nitrate and ammonium at the start (nitrate₀ and ammonium₀) and the end of the incubation period (nitrate_f and ammonium_f), divided by the gravimetric soil water content of the dry soil and then divided by the amount of days incubated in the field (T_{days}), which gave a value in micro gram nitrogen per gram dry soil per day ($\mu\text{g N g}^{-1}$ dry soil⁻¹ day⁻¹). The rates were standardised by dividing the difference in available nitrogen by the short-term incubation period of seven days, which allowed the measured rates of nitrogen mineralization to be comparable to other studies where the same process was measured, but under different environmental conditions. The equation that was used in this analysis was based on the methodology by Robertson et al (1999): Nitrogen mineralization = [(nitrate_f + ammonium_f) - (nitrate₀ + ammonium₀)] / T_{days} . Total available nitrogen was determined by simply adding the initial NH_4^+ and NO_3^- concentrations together.

2.4. Actual field N_2O emissions

An adapted chamber method (Mosier and Mack, 1980) was used to measure actual N_2O emission from soils in the field. PVC tubes that were 15cm in diameter, 25cm long, 5mm thick and sealed off with end caps, that could be opened or closed by hand, were used as gas collection chambers. They were knocked into the soil to a depth of 5cm 6 days before sampling took place and the end cap was opened. This allowed the soil to settle and not be influenced by human disturbance (the 'wounding effect'). The end caps of the chambers were sealed prior to collecting an air sample. A 10ml 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) fitted with a ⁶³Ni electron capture detector (ECD). The two stainless steel columns (one pre-column, that absorbed impurities in the air sample, and one main column, that absorbed purified N_2O gas from the air sample) was 2mm thick and 3m in length and packed with Porapak Q, 80/100 mesh. The column and the GC ECD oven temperatures were 70°C and high purity nitrogen was used as the carrier gas with a flow rate of 29 ml min⁻¹ (Mosier and Mack, 1980).

Field N_2O measurements were taken from four chambers (provided that there were enough chambers) at each landscape position along one transect during Spring 2011, Autumn 2012 and Winter 2012. Field sampling took place at the same time (in the mornings from 10:00-13:00) and on days with no cloud cover, whenever it was possible, as temperature and cloud cover may have affected soil microbial activity.

2.5. Soil physical properties

2.5.1. Gravimetric Soil Water Content (GSWC)

GSWC were determined by the methodology of Robertson et al (1999). Ten grams of each sieved fresh soil sample was measured off with a digital scale and then placed in an oven at 100°C for twelve hours. The dry mass of the soil sample was then weighed after it has been allowed to cool off for 5 minutes. The dry soil weight was then subtracted from the wet soil weight. That value was divided by the dry soil weight and then multiplied by 100 in order to determine the gravimetric soil water content as a percentage. The formula that was used to determine the gravimetric soil water content was: $GSWC = [(wet\ soil\ mass - oven\ dried\ soil\ mass) / dry\ soil\ mass] \times 100$.

2.5.2. Volumetric soil moisture (VSM)

The volumetric soil water content was determined with a portable soil moisture meter (Hydrosense CS620) by simply inserting the probe into the soil to a depth of 12cm and writing down the reading. The units were expressed on a volumetric basis as a percentage of the volume of water (cm³) per total volume of the soil (cm³) (Gold and Kellog, 1997; Robertson et al., 1999).

2.5.3. Soil temperature

The soil temperature was determined with a portable soil temperature meter by simply inserting the probe into the soil to a depth of 1cm and 6cm and recording the reading. The units were expressed in degrees Celsius.

2.5.4. pH (water)

Soil pH was determined in distilled water based on the methodology of Robertson et al (1999). A 1:2.5 (w:v, soil:distilled water) soil slurry was prepared by adding 10g sieved fresh field soil to 25ml distilled water. The soil solution was first shaken by hand and then placed in a mechanical shaker for an hour. The soil solution was left to settle for 5 minutes after which the pH was measured with a pH meter (Hanna 211 Microprocessor) by inserting the pH probe in the supernatant solution and taking a stabilised reading.

2.5.5. Electrical conductivity (EC)

Electrical conductivity was conducted in the same manner as pH. A 1:2.5 (w:v, soil:distilled water) soil slurry was prepared by adding 10g sieved fresh field soil to 25ml distilled water. The soil solution was first shaken by hand and then placed in a mechanical shaker for an hour. The soil solution was left to settle for 5 minutes after which the EC was measured with an EC meter (Corning Conductivity meter 441) by inserting the EC probe in the supernatant solution and taking a stabilised reading.

2.5.6. Bulk Density

Bulk density (BD) was determined by a small PVC tube (cylinder) with a known volume (V) that was assumed to be equal to the volume of the soil. The volume of the PVC tube was determined by making use of the following formula: $V = \pi r^2 h$, where $\pi \approx 3.1416$ (one standard value of pi), r = the

radius of the PVC tube (in cm) and h = the height of the PVC tube (in cm). The radius was determined by the distance from the middle point of the PVC tube to the inside edge of the PVC tube. The height was determined by the distance on the side of the PVC tube from the top to the bottom. The PVC tube was pushed into the soil and a soil sample was taken for which the mass of the oven dried (at 105°C for 24 hours) soil was determined (in grams). The bulk density was then calculated with the formula: $BD = \text{mass of oven dried soil (g)}/\text{volume of the soil (cm}^3\text{)}$.

2.5.7. Soil particle size

The soil particle size content of three fractions (sand, silt and clay percentage) and percentage organic carbon content was analysed by the Western Cape Department of Agriculture.

2.5.8. Total carbon and total nitrogen

The total carbon and total nitrogen percentage was analysed by the Soil Science Department at Stellenbosch University through dry combustion elemental analysis. The carbon to nitrogen ratio was worked out from these values by simply dividing carbon by nitrogen.

2.6. Statistical Analyses

All statistical analyses were carried out with Statistica, a statistical program that was developed by Statsoft (Statsoft Inc., 2011). A repeated measures ANOVA was used to determine the main effects of invasion status (included only the wet banks and dry banks) across seasons and landscape position (only the natural sites) across seasons, where season was the within group repeated factor and invasion status and landscape position the between group factors. For invasion status, only the wet and dry banks were compared, and for landscape position, only the natural sites were compared. This was because invasion was limited to wet and dry banks, and in most cases the uplands were not invaded at all. One-way ANOVAS were carried out on pooled data for the variables that were measured once-off. The comparisons between seasons were determined by all the data collected, irrespective of invasion status or landscape position. *Post-hoc* Fisher LSD tests were carried out if ANOVAS displayed significant results to determine whether there were differences between the means of invasion status and landscape position. Spearman rank order correlation values (r^2) were calculated to determine whether there were any correlations between the various soil physical and chemical properties that were believed to have an influence on labile nitrogen and if so how strongly they interacted with one another. This was analysed across seasons irrespective of invasion status or landscape position.

3. Results

3.1. Nitrogen mineralization

The potential for ammonium and nitrate production through the process of mineralization was determined under field conditions and analysed for the effect of invasion status (natural, invaded and cleared) and landscape position (wet bank, dry bank, terrestrial) across seasons: Spring 2011, Autumn 2012 and Winter 2012.

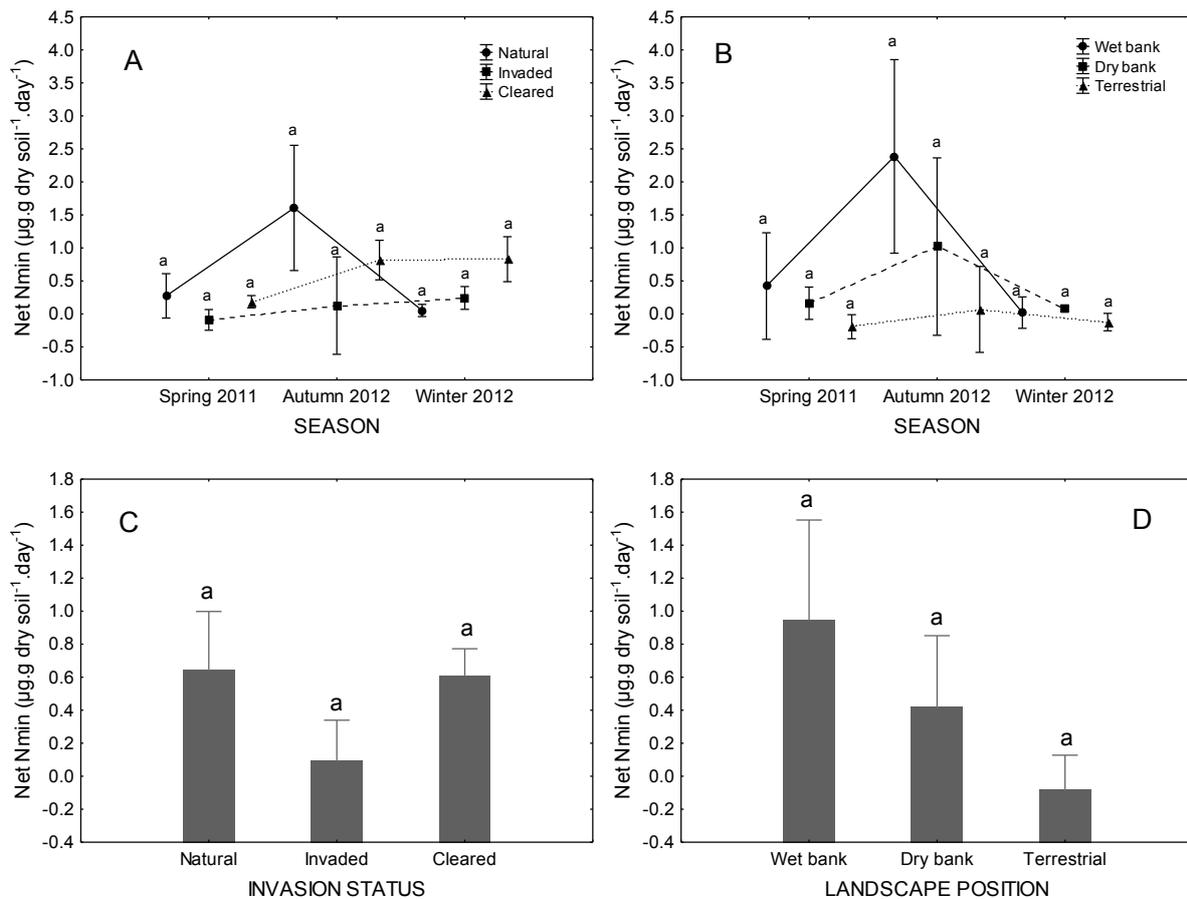


Figure 2.1. Net nitrogen mineralization rate for **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) across seasons. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences determined by repeated measures ANOVA followed by *post hoc* Fisher LSD tests ($p < 0.05$).

The seasons did not have a significant effect on the nitrogen mineralization process of natural, invaded and cleared sites ($F_{2, 34} = 1.7633$; $p = 0.1868$, Figure 2.1. A) and there was also not a seasonal effect on the nitrogen mineralization process of the wet banks, dry banks and upper terrestrial sites ($F_{2, 16} = 2.1095$, $p = 0.1538$; Figure 2.1. B). There was a tendency for nitrogen mineralization to increase from spring to autumn and then to decrease in winter to levels similar for spring, although this tendency was not significant. There was no effect of invasion status on the nitrogen mineralization process ($F_{2, 17} = 1.4964$; $p = 0.2520$; Figure 2.1. C) and no difference between the natural sites and invaded sites (Fisher LSD = 0.1373). There was also no difference when the natural sites were compared to cleared sites (Fisher LSD = 0.9273) and when invaded sites were compared to cleared sites (Fisher LSD = 0.1778). There was a tendency for nitrogen mineralization to decrease in the invaded sites and increase in the cleared sites to that of the natural sites, although this tendency was not significant (Figure 2.1. C). The landscape position did not have an effect on the nitrogen mineralization process ($F_{2, 8} = 1.8208$; $p = 0.2230$; Figure 2.1. D) and there was no difference between the wet banks and dry banks (Fisher LSD = 0.3603). There was also not a

difference between the wet banks and the terrestrial sites (Fisher LSD = 0.0935) and the dry banks and terrestrial sites (Fisher LSD = 0.3433). There was however a trend for nitrogen mineralization to decrease from the wet banks to the dry banks to the upper terrestrial sites.

3.2. Carbon to nitrogen ratio

The carbon to nitrogen ratio was determined and the effect of invasion status (natural, invaded, cleared) and landscape position (wet bank, dry bank, terrestrial) was analysed.

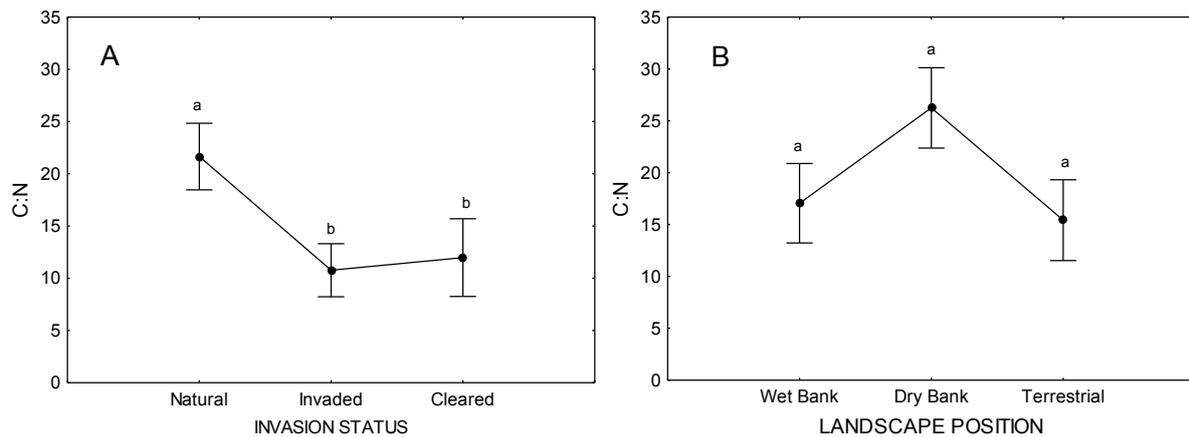


Figure 2.2. Carbon to nitrogen ratio for **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial). Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences determined by repeated measures ANOVA followed by *post hoc* Fisher LSD tests ($p < 0.05$).

The carbon to nitrogen ratio was significantly higher in natural sites compared to invaded and cleared sites ($F_{2, 15} = 3.5095$; $p = 0.0562$; Figure 2.2. A), but the carbon to nitrogen ratio was not affected by the position in the landscape and the ratios were the same ($F_{2, 6} = 2.2790$; $p = 0.1835$; Figure 2.2. B).

3.3. Ammonium

Ammonium was determined under field conditions and analysed the effect of invasion status (natural, invaded, cleared) and landscape position (wet bank, dry bank, terrestrial) across three different seasons, Spring 2011, Autumn 2012 and Winter 2012.

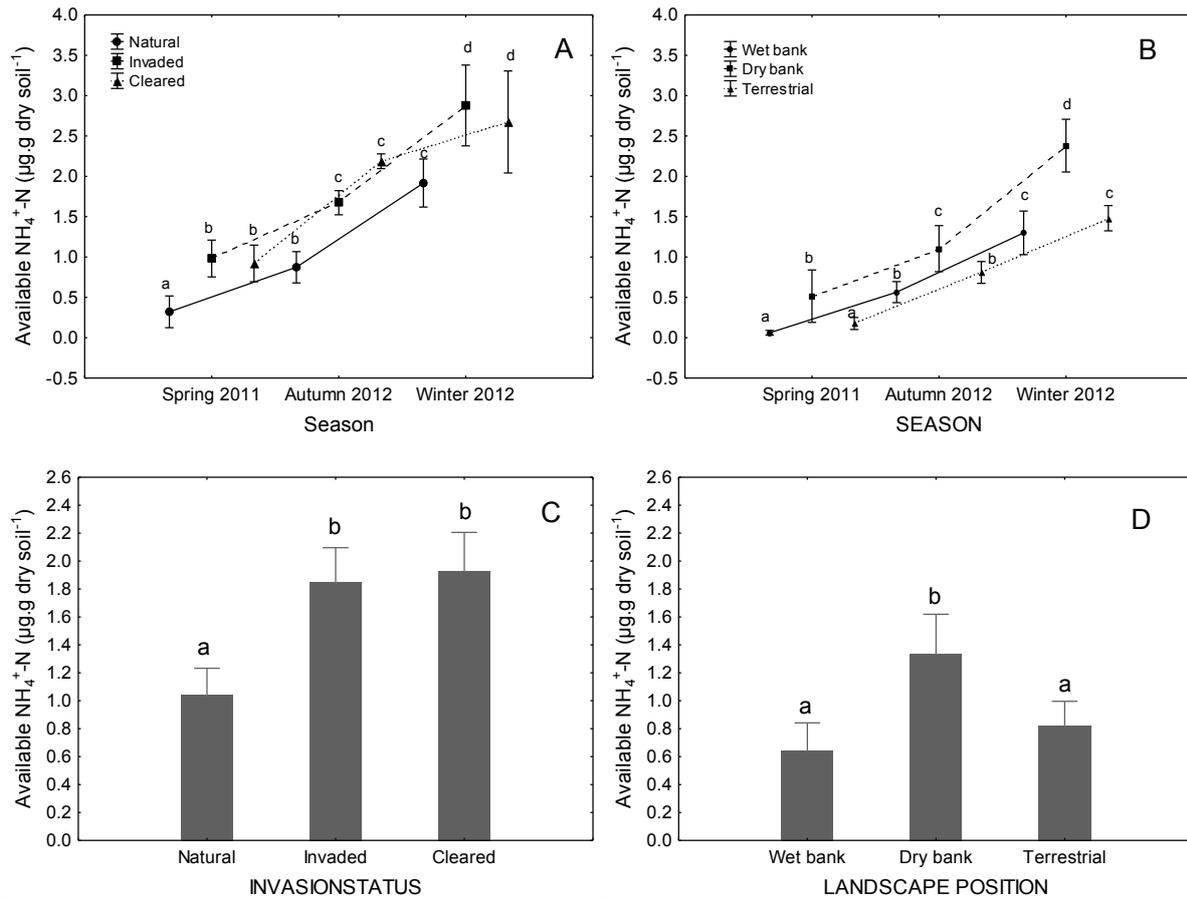


Figure 2.3. Ammonium for **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) across seasons. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences determined by repeated measures ANOVA followed by *post hoc* Fisher LSD tests ($p < 0.05$).

Invasion status had a significant effect on NH_4^+ across the different seasons with a linear increase from spring to autumn to winter ($F_{2, 34} = 22.7663$; $p = 0.0000$; Figure 2.3. A). Similarly the landscape position also had a significant impact on the nitrogen mineralization rate where there was also a linear increase from spring to autumn to winter ($F_{2, 16} = 22.2344$; $p = 0.0000$; Figure 2.3. B). There was less NH_4^+ available in the natural sites than the invaded and cleared sites ($F_{2, 17} = 7.1848$; $p = 0.0055$; Figure 2.3. C). The dry banks had more NH_4^+ available than the wet banks and the upper terrestrial sites ($F_{2, 8} = 29.0300$; Figure 2.3. D).

3.4. Available nitrate

Available NO_3^- was determined under field conditions and analysed for the effect of invasion status (natural, invaded, cleared) and landscape position (wet bank, dry bank, terrestrial) across three different seasons: Spring 2011, Autumn 2012 and Winter 2012.

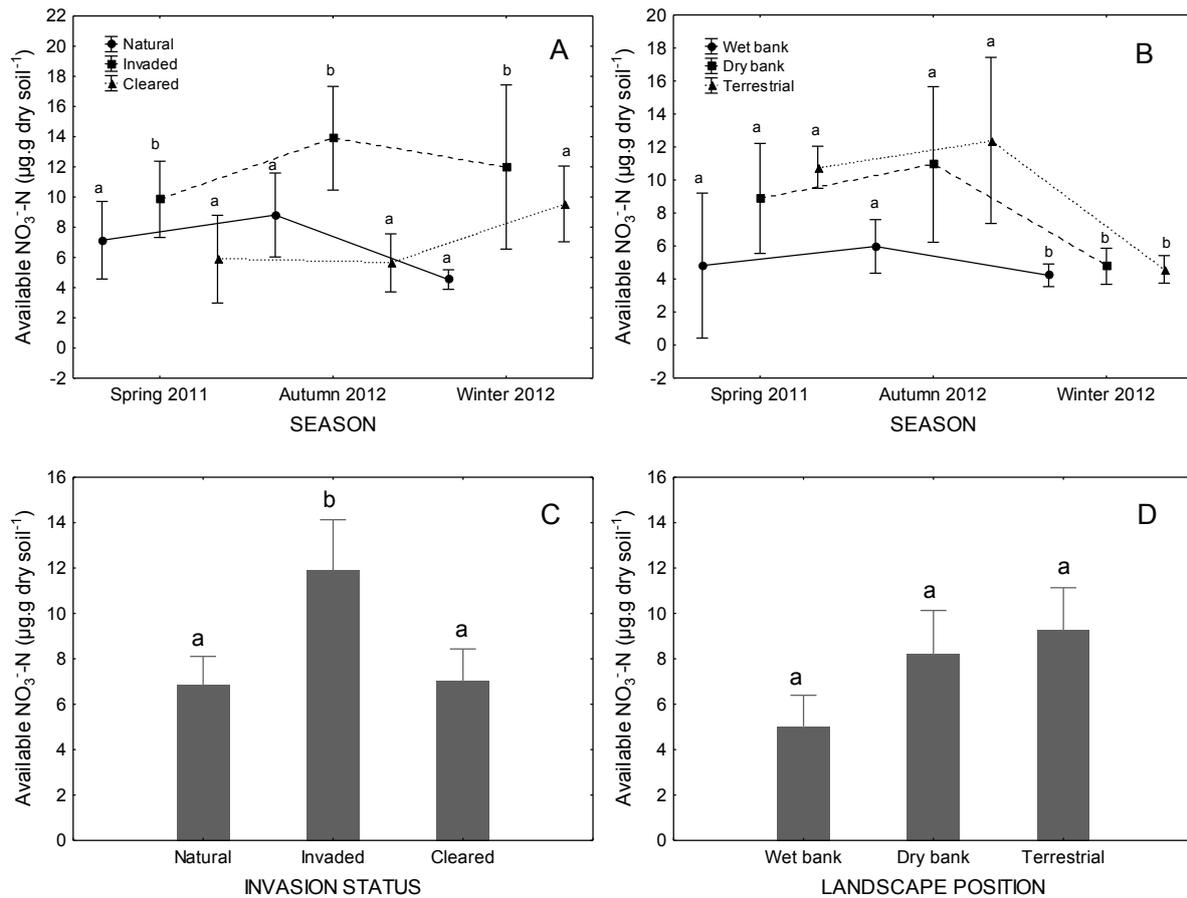


Figure 2.4. Available nitrate for **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) across seasons. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences determined by repeated measures ANOVA followed by *post hoc* Fisher LSD tests ($p < 0.05$).

Available NO_3^- did not display a seasonal difference in invasion status ($F_{2, 30} = 4.8794$; $p = 0.0146$; Figure 2.4. A), but there was a significant effect of winter on the wet banks, dry banks and terrestrial sites (Fisher LSD = 0.0394; Figure 2.4. B), and the measured levels of NO_3^- were higher in the invaded sites than the natural and cleared sites ($F_{2, 15} = 4.8794$; $p = 0.0146$; Figure 2.4. C). There was also not a difference between the wet banks, dry banks and the upper terrestrial sites ($F_{2, 8} = 1.1467$; $p = 0.3649$; Figure 2.4. D), but there was however a tendency for NO_3^- to decrease from the upper terrestrial sites to the dry banks to the wet banks.

3.5. Total available N

Total available nitrogen was determined for Spring 2011, Autumn 2012 and Winter 2011 and tested for the effect of invasion status (natural, invaded, cleared) and landscape position (wet bank, dry bank, terrestrial) across the seasons.

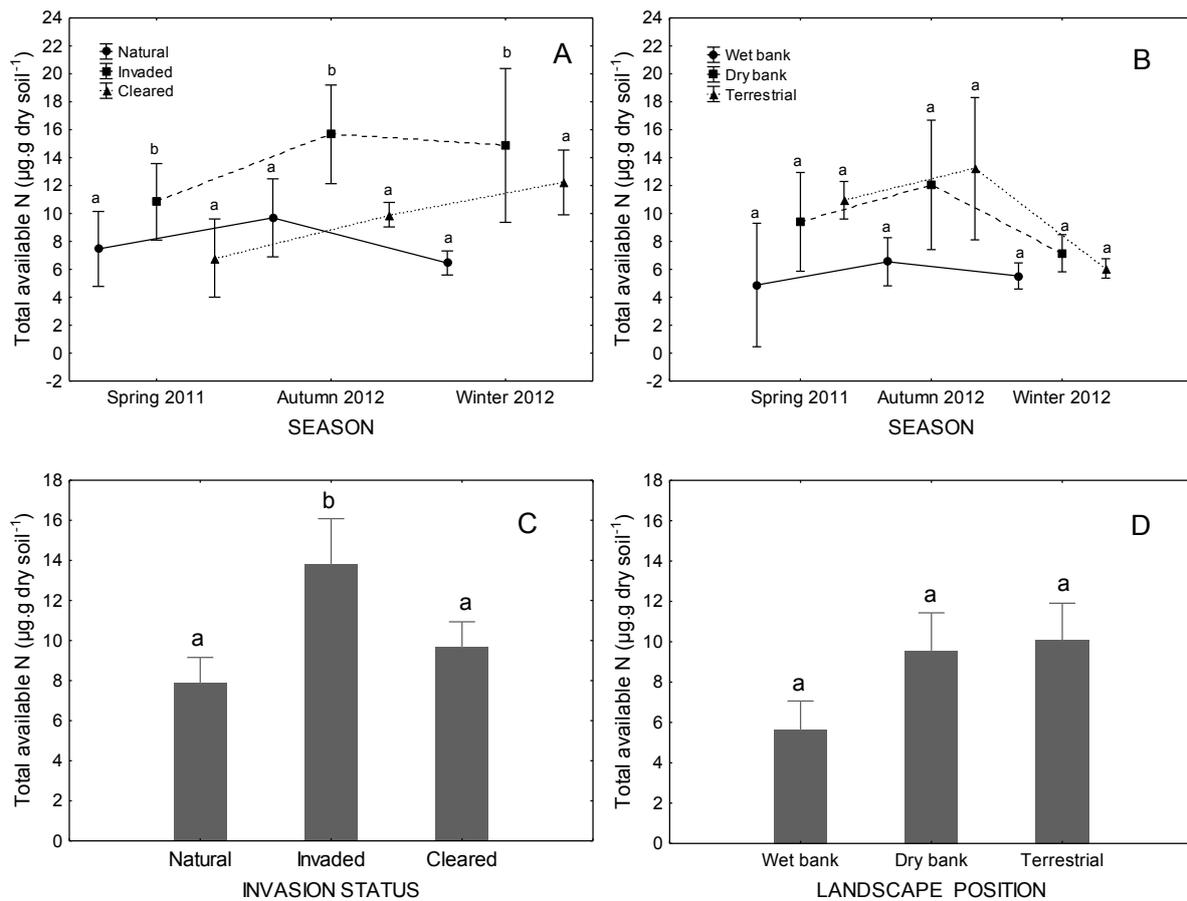


Figure 2.5. Total available nitrogen for **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) across seasons. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences determined by repeated measures ANOVA followed by *post hoc* Fisher LSD tests ($p < 0.05$).

There were no seasonal differences for invasion status ($F_{2, 30} = 1.5168$; $p = 0.2512$; Figure 2.5. A) or landscape position ($F_{2, 16} = 1.4615$; $p = 0.2612$; Figure 2.5. B). Total available nitrogen displayed similar trends to that of available NO_3^- with invaded sites having higher nitrogen availability than natural and cleared sites ($F_{2, 15} = 5.6439$, $p = 0.0083$; Figure 2.5. C) and there was also not a difference in the wet banks, dry banks and terrestrial sites ($F_{2, 8} = 1.3148$; $p = 0.3208$; Figure 2.5. D).

3.6. Ammonium to nitrate ratio ($\text{NH}_4^+:\text{NO}_3^-$)

The ammonium to nitrate ratio was determined for the different seasons and the effect of invasion status (natural, invaded, cleared) and landscape position (wet bank, dry bank, terrestrial) was tested.

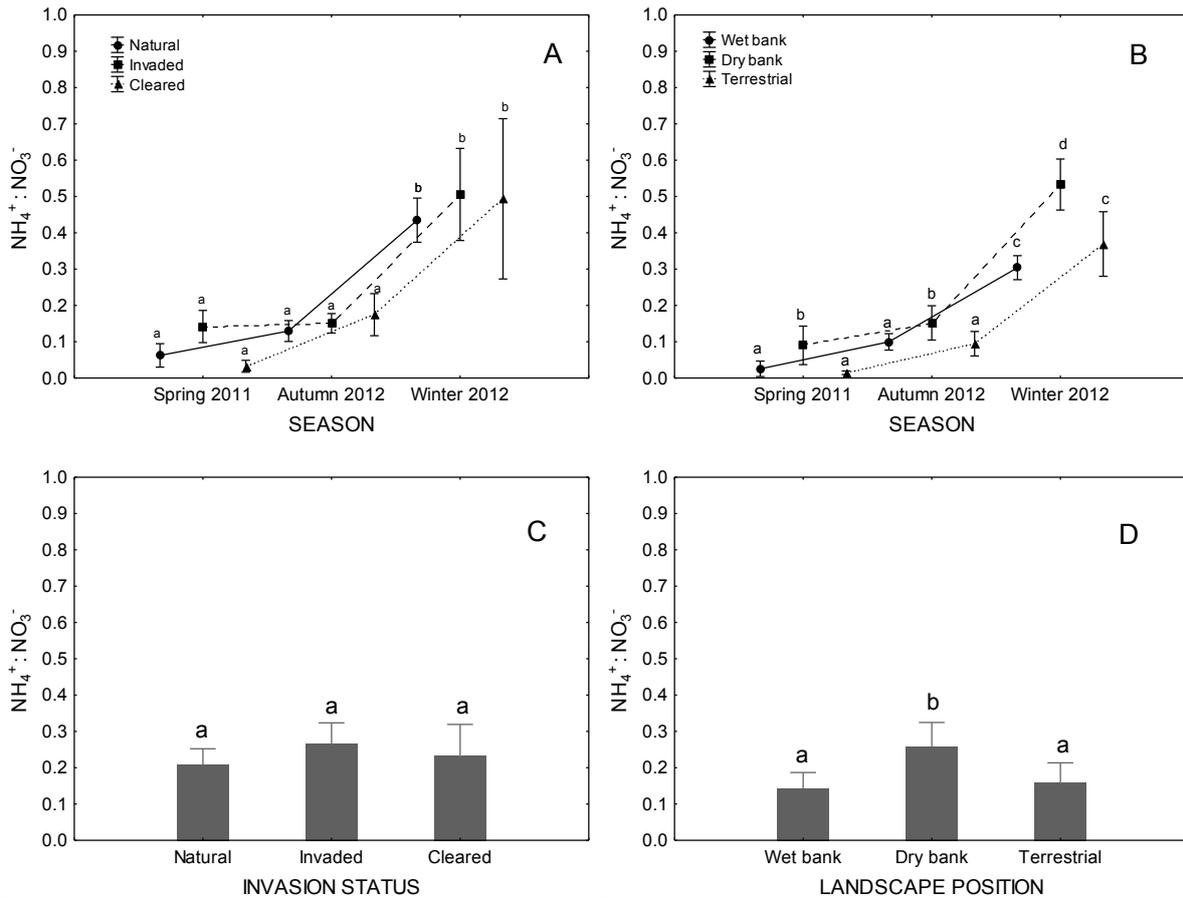


Figure 2.6. Ammonium to nitrate ratio for **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) across seasons. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences determined by repeated measures ANOVA followed by *post hoc* Fisher LSD tests ($p < 0.05$).

There was a significant difference in the ratio of NH_4^+ to NO_3^- in winter compared to the other seasons ($F_{2,34} = 18.3417$; $p = 0.0000$; Figure 2.6. A and B) as well as an increase in the ratio of NH_4^+ to NO_3^- in the dry banks ($F_{2,16} = 8.4454$; $p = 0.0107$; Figure 2.6. D). There was no difference in the ratio of NH_4^+ to NO_3^- when invaded sites were compared to natural and cleared sites ($F_{2,34} = 0.3168$; $p = 0.7327$; Figure 2.6. C).

3.7. Field N_2O

Field N_2O emissions were measured during the time period Spring 2011 to Winter 2012 and tested for the effect of invasion status (natural, invaded, cleared) and landscape position (wet bank, dry bank, terrestrial).

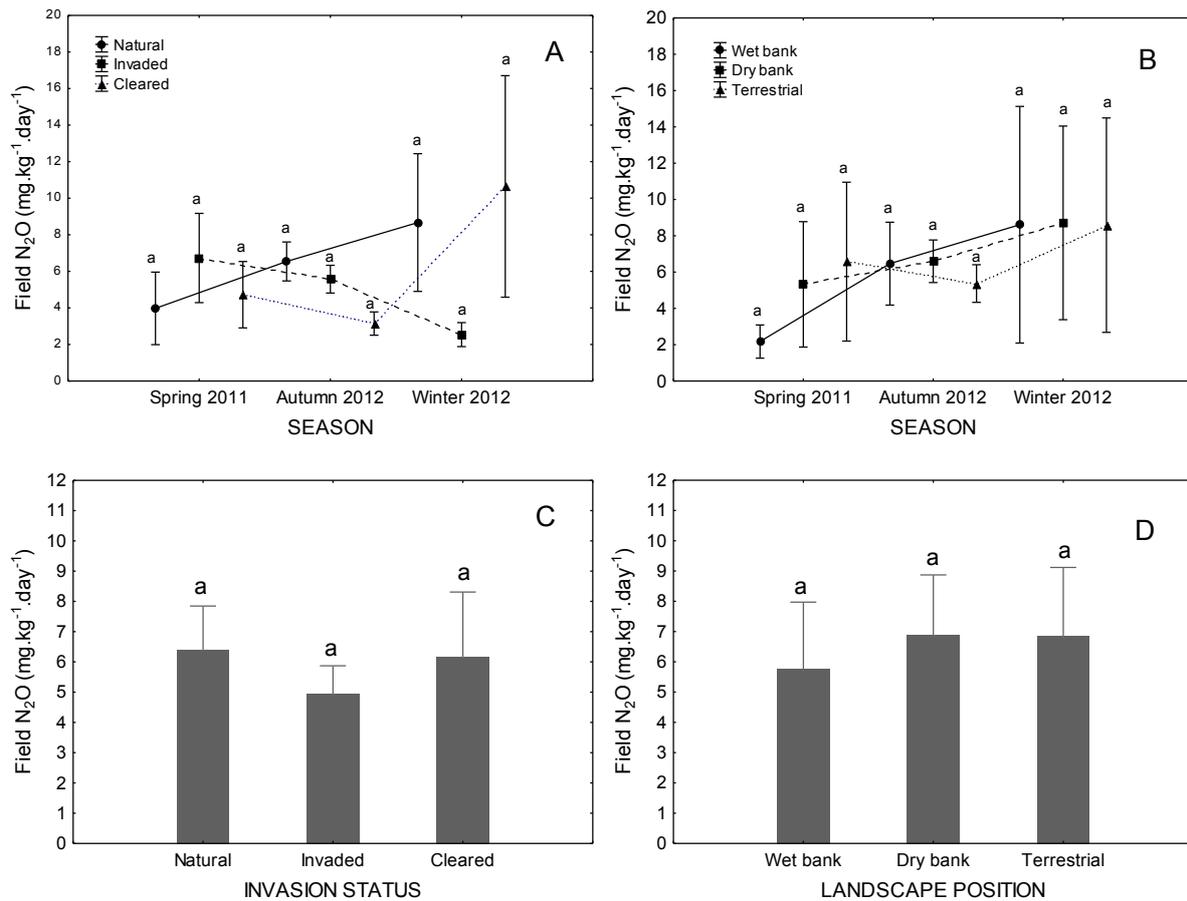


Figure 2.7. Field N₂O for **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) across seasons. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences determined by repeated measures ANOVA followed by *post hoc* Fisher LSD tests ($p < 0.05$).

Seasonality did not have a significant effect on the field N₂O emissions expressed by invasion status ($F_{2, 34} = 0.6423$; $p = 0.5323$; Figure 2.7. A) and landscape position ($F_{2, 16} = 0.6119$; $p = 0.5545$; Figure 2.7. B). There was no difference in the field N₂O emissions for the natural, invaded and cleared sites ($F_{2, 17} = 0.3239$; $p = 0.7277$; Figure 2.7. C) as well as no difference in field N₂O emissions for wet banks, dry banks and terrestrial sites ($F_{2, 8} = 0.0984$; $p = 0.9074$; Figure 2.7. D).

3.8. Relationships of N processes to environmental factors (correlations)

Spearman rank order correlations (r^2) were determined for all the measured soil physical and chemical properties regardless of invasion status or landscape to determine whether there were any relationships between nitrogen processes and environmental factors. Significant correlations at $p < 0.05$ are indicated with an asterisk (Table 2.1.).

Table 2.1. Spearman rank order correlations (r^2) for various soil physical and chemical properties. Significant correlations are indicated by an asterisk (* $p < 0.05$). Correlations were calculated from all available data irrespective of invasion status or landscape position.

	GSWC (%)	VSM (%)	Temp 1cm (°C)	Temp 6cm (°C)	pH	Sand (%)	Silt (%)	Clay (%)	EC	BD	OC	Nmin	NH ₄ ⁺ (µg/g)	NO ₃ ⁻ (µg/g)	Available N (µg/g)	Total C (µg/g)	Total N (µg/g)	C:N
VSM (%)	0.870*																	
Temp 1cm (°C)	-0.580*	-0.474*																
Temp 6cm (°C)	-0.507*	-0.509*	0.924*															
pH	-0.196	-0.314	0.397*	0.349*														
Sand (%)	0.140	0.324	-0.449*	-0.490*	-0.562*													
Silt (%)	-0.116	-0.284	0.434*	0.465*	0.567*	-0.979*												
Clay (%)	-0.217	-0.367	0.399*	0.453*	0.443*	-0.879*	0.778*											
EC	0.012	-0.191	0.184	0.231	0.644*	-0.533*	0.583*	0.304										
BD	-0.331	-0.287	0.128	0.293	0.138*	-0.015	-0.026	0.111	-0.086									
OC	0.190	-0.033	0.112	0.131	0.423*	-0.722*	0.729*	0.574*	0.642*	-0.351								
Nmin	-0.059	-0.055	0.165	0.041	-0.018	-0.005	0.050	-0.130	0.212	-0.100	0.064							
NH₄⁺ (µg/g)	-0.104	-0.245	0.378	0.403	0.416*	-0.485*	0.551*	0.213	0.656*	-0.044	0.576*	0.381*						
NO₃ (µg/g)	0.249	-0.090	-0.172	-0.079	0.507*	-0.055	0.121	-0.002	0.554*	0.211	0.397	-0.090	0.434					
Available N (µg/g)	-0.078	-0.327	0.334	0.390	0.510*	-0.517*	0.555*	0.324	0.723*	0.080	0.538*	0.366	0.858*	0.889*				
Total C (µg/g)	0.056	-0.131	0.209	0.161	0.484*	-0.645*	0.683*	0.457*	0.607*	-0.388*	0.853*	0.205	0.596*	0.265	0.519*			
Total N (µg/g)	-0.010	-0.158	-0.111	-0.205	0.404*	-0.162	0.159	0.226	0.200	-0.107	0.360	-0.054	0.163	0.302	0.140*	0.516*		
C:N	0.132	0.038	0.253	0.290	-0.112	-0.359	0.380	0.157	0.283	-0.297	0.392*	0.316	0.261	-0.261	0.206	0.390*	-0.468*	
Field N₂O	-0.286	-0.302	0.379	0.518*	0.176	0.064	-0.073	-0.036	0.195	0.587*	-0.144	-0.189	0.206	0.552*	0.319	-0.269	-0.141	-0.210

There was not a significant relationship between the nitrogen mineralization process and environmental factors although the carbon to nitrogen ratio was found to be influenced by the organic carbon in the soil ($r^2 = 0.392$). Ammonium had a positive influence on pH ($r^2 = 0.416$) and EC ($r^2 = 0.656$) and was affected by the organic carbon content ($r^2 = 0.576$) and the sand content ($r^2 = -0.485$) and silt content ($r^2 = 0.551$), which provided the substrate for nitrification to take place. Nitrate had an influence on pH ($r^2 = 0.507$), EC ($r^2 = 0.554$) and field N_2O ($r^2 = 0.552$) and total available nitrogen was influenced by the organic carbon content ($r^2 = 0.538$), sand content ($r^2 = -0.517$), silt content ($r^2 = 0.555$), total carbon ($r^2 = 0.519$) and total nitrogen ($r^2 = 0.140$). Field N_2O was influenced by bulk density ($r^2 = 0.587$) and nitrate availability ($r^2 = 0.552$). There was a negative relationship between the soil temperature at 1cm and the gravimetric soil water content ($r^2 = -0.580$) and between the soil temperature at 6cm and the gravimetric soil water content ($r^2 = -0.507$).

pH is one of the soil properties that has an influence on many of the forms of nitrogen and processes associated with nitrogen cycling and is indirectly affected by some of the other soil physical properties such as temperature, bulk density, electrical conductivity and the silt and clay content (see Table 2.3). The sand content in the soil is one of the properties that has many negative correlations with the other soil physical and chemical properties and can be the main reason for all the trends that were observed and one of the drivers of the nitrogen processes. The silt and clay contents and the organic and total carbon have an effect on many of the nitrogen processes and soil properties such as pH and temperature. The nitrogen mineralization process is influenced by carbon. Available nitrogen is influenced by pH, the particle size content of silt and clay, the electrical conductivity and the nitrogen mineralization process. Field N_2O emissions were influenced by the temperature beneath the soil surface, bulk density and the availability of NO_3^- .

Selected soil physical properties were measured across seasons for the Western Cape and are displayed in Table 2.2.

Table 2.2. Summary of the mean \pm SE for selected soil physical properties expressed by invasion status across seasons for the Western Cape

		Natural	Invaded	Cleared
GSWC (%)	Spring 2011	11.22 \pm 1.66	7.01 \pm 1.71	9.86 \pm 1.81
	Autumn 2012	7.65 \pm 1.47	9.35 \pm 1.07	8.08 \pm 1.25
	Winter 2012	19.58 \pm 4.91	16.26 \pm 1.99	12.44 \pm 2.60
VSM (%)	Spring 2011	16.20 \pm 4.07	11.18 \pm 3.11	15.36 \pm 2.32
	Autumn 2012	9.84 \pm 1.76	9.04 \pm 1.13	11.58 \pm 3.55
	Winter 2012	16.80 \pm 1.73	17.95 \pm 3.22	22.39 \pm 4.64
Temp 1 cm (°C)	Spring 2011	14.87 \pm 3.03	19.23 \pm 3.33	20.98 \pm 1.49
	Autumn 2012	18.69 \pm 2.39	19.51 \pm 1.19	19.23 \pm 1.12
	Winter 2012	12.20 \pm 0.39	11.25 \pm 0.75	11.68 \pm 0.80
Temp 6 cm (°C)	Spring 2011	14.94 \pm 2.36	15.33 \pm 2.79	17.84 \pm 11.32
	Autumn 2012	18.10 \pm 2.12	17.71 \pm 1.08	17.44 \pm 0.74
	Winter 2012	11.77 \pm 0.31	10.70 \pm 0.64	11.45 \pm 0.85
pH	Spring 2011	4.05 \pm 0.46	3.76 \pm 0.66	5.14 \pm 0.11
	Autumn 2012	4.73 \pm 0.16	4.89 \pm 0.10	4.88 \pm 0.12
	Winter 2012	4.96 \pm 0.12	5.13 \pm 0.15	5.02 \pm 0.15

There was more soil moisture available in the natural sites during winter and autumn had the highest soil temperature at both depths of 1cm and 6cm. The measured pH for the fynbos riparian soils was generally below 5, which means that the soils are acidic.

Selected soil properties were measured once-off to describe and understand the soils better, because these properties are believed not to change in the short-term.

Table 2.3. Description of a once-off measurement of soil physical properties expressed by invasion status for the Western Cape

	Natural	Invaded	Cleared
EC ($\mu\text{S/m}$)	24.40 \pm 2.63	26.90 \pm 4.99	42.09 \pm 7.84
Bulk density (g/cm^3)	0.85 \pm 0.06	1.06 \pm 0.05	1.04 \pm 0.06
Sand (%)	93.05 \pm 0.93	94.10 \pm 0.77	92.34 \pm 1.13
Silt (%)	3.00 \pm 0.59	2.24 \pm 0.39	3.66 \pm 0.69
Clay (%)	3.95 \pm 0.32	3.66 \pm 0.28	4.00 \pm 0.38
Organic C (%)	1.60 \pm 0.28	1.41 \pm 0.51	1.99 \pm 0.41
Total C (%)	2.38 \pm 0.41	2.80 \pm 1.13	2.91 \pm 0.57
Total N (%)	0.13 \pm 0.02	0.25 \pm 0.09	0.87 \pm 0.50
C:N	18.62 \pm 2.76	11.60 \pm 2.19	16.64 \pm 5.25

The electrical conductivity was higher in cleared sites than natural sites (Fisher LSD = 0.022) and there was no difference in electrical conductivity between natural and invaded sites (Fisher LSD = 0.750). There was significant bulk density differences between the natural, invaded and cleared sites ($F_{2, 26} = 4.288$; $p = 0.025$). The soils of natural sites had higher bulk densities than invaded sites (Fisher LSD = 0.017) and cleared sites (Fisher LSD = 0.023), which means natural sites are more compacted and there are less pore spaces in these soils compared to the invaded sites and cleared sites. There was no difference in the soil particle size content of sand in natural invaded and cleared sites ($F_{2, 26} = 0.750$; $p = 0.481$). There was also no difference in the soil particle size content of silt in natural, invaded and cleared sites ($F_{2, 26} = 1.258$; $p = 0.301$). Once again, there is no difference in the soil particle size content of clay in natural invaded and cleared sites ($F_{2, 26} = 0.226$; $p = 0.817$). There is however a significantly higher sand content than silt and clay content in soils under all the invasion statuses (natural: $F_{2, 30} = 6\ 155.965$; $p = 0.000$, invaded: $F_{2, 21} = 10\ 150.410$; $p = 0.000$ and cleared: $F_{2, 27} = 4\ 137.747$; $p = 0.000$). There was no difference for organic carbon ($F_{2, 21} = 0.559$; $p = 0.580$) and total carbon ($F_{2, 21} = 0.199$; $p = 0.821$) under the different invasion statuses. Total nitrogen and the carbon to nitrogen ratio did not differ significantly when natural, invaded and cleared sites were compared ($F_{2, 21} = 1.575$; $p = 0.231$ and $F_{2, 21} = 0.712$; $p = 0.502$).

4. Discussion

4.1. Trends in nitrogen mineralization, C:N ratio, and available N

The nitrogen mineralization rates of the fynbos riparian ecotones that were measured under field conditions in the study were comparable to those of a Mediterranean shrubland in Italy that was measured by Dannenmann et al. (2011). The study by Dannenmann et al. (2011) used a different

approach to analyse the nitrogen mineralization rates of soils by incubating intact soil cores at a set temperature of 10 °C that was amended with ammonium and nitrate separately and labelled with a nitrogen tracer. The average nitrogen mineralization rates of Dannenmann et al. (2011) were similar to our study, even though the methodology allowed them to determine the potential for nitrogen mineralization to occur. Knoepp and Swank (1998) determined nitrogen mineralization rates through field incubations under oak-pine, mixed oak and cove hardwood vegetation in the southern Appalachians and found that the nitrogen mineralization rates were two orders of magnitude higher than those of the wet and dry banks of especially the reference sites and cleared sites of the current study. The nitrogen mineralization rates of the terrestrial sites and the invaded sites of the field incubations of the current study were two orders of magnitude lower than the study by Knoepp and Swank (1998), which implies that more favourable conditions for nitrogen mineralization occurs under the forest vegetation than fynbos vegetation of the current study. An explanation for the higher nitrogen mineralization rates in the southern Appalachian forests is due to higher litter biomass that is richer in nitrogen that decomposes easier than fynbos vegetation with low nitrogen content. These differences of nitrogen mineralization rates for soils under different vegetation types indicates that there is variability in space and that this spatial variability has an effect on the nitrogen cycling process carried out by soil micro-organisms. The current study did not find any seasonal differences in the field incubated nitrogen mineralization rates, which was contradictory to the results by Knoepp and Swank (1998) who found seasonal differences in the field incubated nitrogen mineralization rates. Their result was as a result of an altitudinal difference that caused differences in temperature. The Appalachian sites reached temperatures that were below the threshold of high nitrogen mineralization during winter. Another factor could have been the enhanced nitrogen inputs from nitrogen rich litter released by Appalachian forests compared to fynbos. The lack of seasonal differences in the nitrogen mineralization rates of the current study proves that temporal variation does not have an effect on the soil micro-organisms, but that other factors play a more important role in the nitrogen mineralization processes of fynbos riparian ecotones.

The nitrogen mineralization rate across seasons, under different invasion statuses and different landscape positions in the study, did not differ significantly. At the same sites, Naude (2012) found that seasons, invasion status and landscape position had a significant effect on the nitrogen mineralization potential. The study by Naude (2012) focussed on the nitrogen mineralization potential in the laboratory where optimal conditions for nitrogen mineralization were created. Naude (2012) found that there was a higher nitrogen mineralization potential under areas invaded by *Acacia* spp., which was apparently contradictory to our study where invaded sites were not significantly different from natural sites, which served as our reference sites. This was also in contrast to the results of Yelenik et al. (2004), who found higher net nitrogen mineralization in invaded (terrestrial) sites compared to reference sites. These apparent contradictions can be explained by the fact that the study by Naude (2012) was done under ideal conditions, while under natural conditions in the field, at any time, one or more of the controlling factors of N mineralization may be in short supply or suboptimal (e.g. water, temperature or the microbial population may be less than ideal). There was

also not a significant difference between cleared sites and either invaded or reference sites in the current study. This suggests that there may be one or two factors common between the various sites that may have been constraining N mineralization in these seasons, and it can be assumed from Naude (2012) and Yelenik et al. (2004)'s studies that should microbial populations be presented with optimal resources, differences may be observable, and that invaded sites may have higher N mineralization. Furthermore, the seasons observed in the current study tended to be wet seasons (see Table 2.2) soil moisture and low nutrient retention of sandy soils may have been another factor affecting the available N during the course of the field incubation.

The relationship in nitrogen mineralization expressed according to landscape position in the current study was different from that of Naude (2012), who found an increase in nitrogen mineralization potential in the dry banks and terrestrial sites, which again demonstrates that laboratory studies under ideal conditions must be verified under field conditions, because different results and trends may be obtained from similar process under different environmental conditions. Again, the likelihood of more soil moisture closer to the stream (wet banks) may influence results obtained under field conditions. There is a difference in nitrogen mineralization under field conditions and laboratory conditions, because one of the trajectories observed by Naude (2012) and Pinay et al. (1993) was a higher nitrogen mineralization potential in the upper terrestrial sites compared to the dry banks and the wet banks, which was different to the trend in the current study where higher actual nitrogen mineralization rates were observed in the wet banks than the dry banks and terrestrial sites, although the trend was not significant.

The ratio of carbon to nitrogen (C:N) is a driver of nitrogen mineralization where a ratio of 24 is the suggested threshold for predicting nitrogen retention in British forests and heath lands (Emmett et al., 1998). Emmet et al. (1998) suggested that a C:N ratio of less than 24 will result in a decrease in nitrogen mineralization due to too much total nitrogen in relation to total carbon available to the soil micro-organisms. An increase in the nitrogen mineralization process can be predicted at a ratio above 24, because at the C:N ratio > 24 nitrogen in the form of NO_3^- becomes less limiting to the soil micro-organisms. Nitrate is less limited at a ratio greater than 24, because nitrate leaching represents less than 10% of NO_3^- inputs. However, in general, the lower the ratio, the more likely N losses will occur. In the current study it was found that all the carbon to nitrogen ratios were below 24 for the natural, invaded and cleared sites where the natural sites had a significantly higher ratio than the invaded and cleared sites (see Table 2.3.). Similar C:N ratios were calculated by Naude (2012) in spring, that was also below the 24 C:N ratio threshold of likely increased nitrogen mineralization rates. The C:N ratio of 24 might be specific to Mediterranean soils of low nutrient status, compared to the British forest and heath land sites where Emmett et al. (1998) did their study. One would expect more carbon to accumulate and remain in the soils of forests and heath lands compared to MTEs where much of the carbon gets volatilized due to fire. However, the general trend and significance of C:N ratio may also be applicable to MTEs as lower C:N ratios means there is more N in relation to C. Hence one would expect nitrogen mineralization potential to decrease significantly in the invaded and

cleared sites, which was not the case in the current study. However, the relationship holds well for a situation where all resources are available for N mineralization (Naude, 2012).

The range for the NH_4^+ concentrations that was measured during the study (0.7-2.5 $\mu\text{g.g dry soil}^{-1}$) was comparable to a study by Stock et al. (1995), where they measured a range of 1.5-3.0 $\mu\text{g.g dry soil}^{-1}$. The highest values of NH_4^+ in the current study were observed in the invaded dry banks, which are the areas where the invasion by the alien *Acacia* spp. was the most prominent. There were also higher NH_4^+ concentrations in the invaded and cleared sites than the reference sites, which were also found by Yelenik et al. (2004). Ammonium is not as mobile as NO_3^- and does not leach as easily (Templer et al., 2008) and may stay in the soil for longer time periods due to sorption to cation exchange sites that limits movement (Abaas et al., 2012). This can be a possible reason for the high levels of NH_4^+ observed in the invaded and cleared sites. Another possible explanation for the higher levels of NH_4^+ in invaded sites is through the process of atmospheric nitrogen fixation by alien symbiotic bacteria such as *Rhizobium* spp. (Bala and Giller, 2001) and/or *Bradyrhizobium* spp. (Postgate, 1982) that increases the input of NH_4^+ in densely invaded areas (Crisostomo et al., 2013). An increase in NH_4^+ in the invaded sites can also be due to an increase in litter fall with very high levels of tissue nitrogen with fast decomposition rates by invasive alien *Acacia* spp. that contributes to an increase in organic nitrogen (Yelenik et al., 2004), which is the substrate for ammonification to take place.

The dry banks contained significantly higher NH_4^+ than the wet banks and the terrestrial sites, which was contradictory to what Naude (2012) found where the NH_4^+ concentrations in the lab incubations were higher in the dry banks and terrestrial sites than the wet banks. The landscape position thus influence the availability of NH_4^+ in fynbos riparian ecotones, with higher values where most of the invasive alien *Acacia* spp. can be found. Pinay et al. (1993) found similar results where the levels of NH_4^+ increased along the catena (from the upper terrestrial sites to the lower wet banks). The higher availability of NH_4^+ in the dry bank landscape position may be attributed to the fact that the terrestrial sites are not invaded by the invasive alien trees. Dry banks also had high litter cover and thick litter layers (Kambol, 2013). Cover by Acacias is lower in wet banks, and litter produced by these trees more patchily distributed, which might explain the lower NH_4^+ in the wet banks. There was a linear increase in the measured NH_4^+ concentrations across the seasons from spring to autumn to winter, which was similar to what Naude (2012) reported. The increase in NH_4^+ from spring to autumn to winter can be explained by the soil water content that is higher during the winter months through enhanced rainfall and plant uptake of NH_4^+ during these times are limited, because it is not the growing season.

The trend of significantly lower available NO_3^- concentrations that were measured during winter of this study was also found by Naude (2012), where there was also significantly less NO_3^- available during winter. The low levels of NO_3^- during winter can be explained by the leaching of NO_3^- , an ion that is highly mobile in wet soils (e.g. during winter), and that is easily leached (Abaas et al., 2012). The

impact of the invasive alien *Acacia* spp. can clearly be seen in the higher NO_3^- concentrations that were measured in the sites where they occur, which is also supported by results from Stock et al. (1995), Yelenik et al. (2004), Jovanovic et al. (2009) and Naude (2012), who have all found higher available NO_3^- levels and increased litter biomass content in invaded areas compared to natural fynbos. The availability of NO_3^- ranged from $7.17 \mu\text{g.g dry soil}^{-1}$ for natural sites to $11.91 \mu\text{g.g dry soil}^{-1}$ for invaded sites and $7.44 \mu\text{g.g dry soil}^{-1}$ for cleared sites. The availability of NO_3^- under field conditions was higher in the invaded sites of the study compared to the concentrations found by Naude (2012) under laboratory conditions. It is possible that the impacts of IAPs as well as secondary invasion by alien grasses on the availability of NO_3^- are increasing the nitrogen cycling in the system, but there is a decrease in NO_3^- availability in cleared sites of the study. The high levels of NO_3^- in the invaded sites can also be explained by the substrate for nitrification, NH_4^+ that was also higher in invaded sites than the natural and cleared sites. Lastly, the higher production of NO_3^- in invaded sites can also be explained by the absence of evergreen sclerophyllous Mediterranean shrubs from the invaded sites that continuously take up NO_3^- (Castaldi and Aragosa, 2002).

Total available nitrogen follows a similar trend as NO_3^- with higher levels of total available nitrogen in invaded sites and cleared sites than natural sites, which suggests that the availability of nitrogen is driven by the inorganic nitrogen form of NO_3^- more than NH_4^+ . In general NO_3^- is more available than NH_4^+ in especially the invaded sites that are associated with increased nitrogen deposition through litterfall (Yelenik et al., 2004; Naude, 2012) and higher conversion rates of NH_4^+ to NO_3^- (Stock et al., 1995; Yelenik, 2004). It is possible that historic legacy effects play a small role in the total available nitrogen that was measured in the cleared sites and that those soils have not yet fully recovered from the effects of the invasive alien Acacias.

Total available nitrogen is driven by the availability of NO_3^- through the process of nitrification and NH_4^+ is the more limiting form of available inorganic nitrogen in nutrient poor soils of fynbos riparian ecotones. The invasion by alien *Acacia* spp. cause increased levels of available nitrogen in the soil. Ehrenfeld (2003) reported similar results in a mini review on the effects of nitrogen-fixing alien plant invasions on soil nutrient cycling processes where extractable inorganic nitrogen (NO_3^- and NH_4^+) increased as a result of increased nitrification in response to invasion. Other studies on *Acacia cyclops*, *Acacia saligna* and *Acacia mearnsii* invasion in the Western Cape also reported similar changes in soil nutrient cycling processes and availability under alien plant invasions (Stock et al., 1995; Yelenik et al., 2004; Yelenik et al., 2007; Jovanovic et al., 2009; Naude, 2012).

An interesting trend was found with the ratio of NH_4^+ to NO_3^- that increases over seasons, which means that NH_4^+ becomes more dominant over time. This may be because of the tendency of NO_3^- that is lower in the winter months, which is a result of leaching through the soil profile and short residence time of NO_3^- in the soil. Nitrate is also more mobile and dissolves easier in soil water than NH_4^+ , which means nitrate can easily leach through the soil profile to deeper layers (Abaas et al., 2012). The increase in the NH_4^+ to NO_3^- ratio corresponds to the seasonal increase in NH_4^+ that was

observed in the study from spring to winter. In this period an increase in soil moisture occurs (see Table 2.3.), which indicates that the process of ammonification is likely driven by soil moisture and that NH_4^+ has a longer residence time in the soil than NO_3^- . It does seem that there is a tendency for increasing nitrogen mineralization rates over time, which means that nitrogen becomes available for uptake by soil micro-organisms and indigenous riparian vegetation, especially in the dry banks. The higher availability of NH_4^+ that becomes dominant over time can also provide a form of nitrogen that may have a longer residence time in the soil, which can reduce nitrogen losses in the system and be more readily available for uptake by indigenous riparian vegetation and soil micro-organisms responsible for nitrogen cycling by sustaining their metabolic activity, which may assist in the establishment of indigenous vegetation in these fynbos riparian ecotones after clearing efforts took place and may ultimately lead to an improvement in the structure and function of these fynbos riparian ecotones.

4.2. Field N_2O emissions

The actual N_2O emissions that were measured under field conditions during this study were one order of magnitude higher than the *in situ* denitrification measurements of studies by Pinay et al. (2000) and Bernal et al. (2007) in other MTEs. The actual N_2O emissions under field conditions for this study ranged from $213.32\text{ng.g}^{-1}.\text{h}^{-1}$ to $283.17\text{ng.g}^{-1}.\text{h}^{-1}$ while the *in situ* denitrification measurements for Pinay et al. (2007) ranged from $0.00\text{-}20.00\text{ ng.g}^{-1}.\text{h}^{-1}$. The *in situ* denitrification measurements for Bernal et al. (2007) ranged from $0.00\text{-}0.80\text{ mg.kg}^{-1}.\text{h}^{-1}$ and the actual field N_2O emissions for the current study ranged from $5.16\text{-}7.12\text{ mg.kg}^{-1}.\text{h}^{-1}$. Pinay et al. (2007) and Bernal et al. (2007) did however have a slightly different approach to determine field N_2O emissions; intact soil core samples were collected and incubated in the laboratory at the field temperature, which meant environmental conditions were consistent. The different approaches in determining field N_2O emissions indicate that the field N_2O that was produced in the current study was the product of aerobic nitrogen mineralization processes and not just the result of denitrification.

This suggestion that field N_2O production can originate from other sources than denitrification in this study is supported by the lack of consistent trends with field N_2O emissions, especially where no significant differences in the field N_2O measurements, expressed by invasion status and landscape position were observed during the study. The low consistency in trends that were observed with field N_2O emissions during the study were contradictory to expected higher levels of field N_2O emissions in the invaded areas and particularly the wet banks where more ideal conditions, sufficient levels of soil moisture to create anaerobic conditions and a higher availability of NO_3^- , the substrate needed for the release of N_2O , were thought to occur.

One possible explanation for the lack of differences in field N_2O emissions is that similar soil microbial communities are producing N_2O through the process of nitrification and not denitrification, which is supported by similar nitrogen mineralization activities in natural, invaded and cleared sites as well as in wet banks, dry banks and upper terrestrial sites. Another possible explanation for the lack of

differences in field N_2O emissions is C:N ratios lower than 25, which implies that NO_3^- leaching is occurring in the whole study area and that NO_3^- is not available as the substrate for denitrification to occur. The sandy nature of the soils contributes to the limitation of NO_3^- and organic carbon (see Table 2.3.) in the soil due to the poor nutrient retention of sandy soils and the fact that NH_4^+ becomes more dominant over time, which is supported by the seasonal increase in the $\text{NH}_4^+:\text{NO}_3^-$ ratio. The low clay content in the sandy soils of the fynbos riparian ecotones (<5%) is the most feasible explanation for the limited field N_2O emissions in the current study. This tendency is supported by Pinay et al. (2000, 2007) that suggests that denitrification becomes a factor at a clay content of 65%. The low clay and silt content implies that soil moisture retention is fairly low (see Table 2.3.), and that soils are well drained, which limits the anaerobic conditions necessary for denitrification to take place, but makes conditions more favourable for N_2O formation through aerobic nitrogen mineralization.

Another possibility is that N_2O emissions at the soil surface are driven by processes taking place lower down the soil profile. It is noticeable for instance that N_2O emissions do not have a significant correlation with surface temperature, but with temperature at 6 cm (see Table 2.1.). It is more than likely that NO_3^- leached through the soil profile may end up in groundwater, which in the winter may be near the soil surface in riparian areas, and that groundwater denitrification may be overriding the effect of invasion in surface soils. The scope of the study did not include the deeper soil layer, nor groundwater, however, it is likely that nitrogen processes associated with these deeper layers may also be significant.

The difference in soil processes in different parts of the river reach was also not included in the scope of the study, but similarities in the geology of the mountain catchments and the sandy nature of the soils of the fynbos riparian ecotones led to similarities in some soil processes. Denitrification was one of the measured soil process that was similar under different invasion statuses and landscape positions. There may however be a different effect of denitrification on removing excess nitrogen from lower parts of the river (foothill streams for example) where differences in particle sizes may be expected. There may be an increase in denitrification in these lower parts of the river, because higher levels of sand and silt are expected as you move down the river. This increase in silt and clay content further down a river is supported by the river continuum concept of Vannote et al. (1980).

In summary, the impact of invasive alien vegetation and the clearing thereof in comparison to reference sites are not significantly affecting the field N_2O emissions of the fynbos riparian ecotones, which means that respiratory denitrification as a pathway to reduce NO_3^- in riparian ecotones, have been overestimated, because there are various other pathways with the ability to reduce nitrate from aquatic ecosystems. A possible reason for the lack of differences in the ability of NO_3^- to be reduced in the studied riparian ecotones through the process of denitrification can be due to high soil moisture that does not occur for long enough time periods in the soil to create favourable conditions for denitrification, which is once again why we should consider alternative microbial pathways that are responsible for the removal of NO_3^- . One of these alternative indigenous microbial pathways to reduce NO_3^- is dissimilatory nitrate reduction to NH_4^+ through iron or sulphur oxidation; NO_3^- is

reduced to NH_4^+ in the presence of iron or sulphate as an electron acceptor and under ideal conditions the NH_4^+ is then converted back to nitrate via nitrification and NH_4^+ can further be incorporated into plant or microbial biomass (Burgin and Hamilton, 2007). Anaerobic ammonium oxidation from soils with a deeper underlying water table is another underestimated NO_3^- reduction pathway.

The reduction of NO_3^- in areas associated with high levels of soil moisture such as riparian ecotones or wetlands can become sources of N_2O and contribute to global climate change if NO_3^- is left unmonitored and unmanaged in these areas due to N_2O being a potent greenhouse gas with a very long residence time in the atmosphere (119 years) and a destructive impact on the stratosphere (IPCC, 2007; Schlesinger, 2013). Even though no differences in measured field N_2O emissions were observed, the low levels of N_2O released to the atmosphere can have a great influence on climate change, because the global warming potential for N_2O is 310, the constant used to convert N_2O as greenhouse gas to mass CO_2 equivalents (IPCC, 2007). The lack of differences in the field N_2O emissions of invaded and cleared sites compared to reference areas, and similar field N_2O emissions in wet banks and dry banks compared to upper terrestrial areas, was contradictory to enhanced soil respiration activity (actual field CO_2 emissions) that was found by Kambol (2013) in the same studied fynbos riparian ecotones. Thus, while apparently invaded fynbos riparian ecotones may not contribute significantly more N_2O , they are very significant in terms of their contributions to atmospheric C levels.

5. Conclusion

The rates of nitrogen mineralization in the current study were comparable to other MTEs, although there was no difference in the mineralization activity when invasion status and landscape position was investigated across seasons. The similarities in nitrogen mineralization activity between natural, invaded and cleared sites and similarities between wet banks, dry banks and upper terrestrial sites may be explained by the main factor of high sand content in the soil that limits N mineralization under natural conditions in the field due to a short supply of factors such as water or low nutrient retention or the microbial population may be less than ideal in coarse soils. There is however a tendency for N mineralization activity to be higher in the riparian zones than the upper terrestrial areas. One of the factors in short supply in especially the invaded and cleared sites is reduced nitrogen in the system through possible leaching, because NH_4^+ becomes more available over time in the riparian zones due to higher litterfall rates and higher nitrogen content of the litter. Available N is significantly higher in invaded sites compared to cleared areas due to higher levels of NH_4^+ in the invaded sites and potential leaching of NO_3^- in the cleared sites. Nitrate is the driver for available nitrogen in fynbos riparian ecotones, although NH_4^+ becomes more dominant over time. The measured field N_2O emissions are most likely the result of nitrification more so than denitrification, which means denitrification is overestimated as a pathway to reduce NO_3^- in fynbos riparian ecotones.

These different dynamics of nitrogen cycling under different invasion statuses and landscape positions can be used to evaluate the impacts of invasion and clearing on riparian functioning and

make recommendations from the results obtained in this study. The impact of invasive alien *Acacia* spp. is not significant on the N mineralization and denitrification processes across seasons, although there was more NH_4^+ and NO_3^- available in the invaded dry banks. The lack of difference in N mineralization activity may prove that soil micro-organisms are not influenced by invasive alien Acacias, but that there is still the potential for groundwater or surface water to be polluted by higher levels of NO_3^- compared to reference sites. It appears that soil recovery processes in the form of N_2O release to the atmosphere has not yet taken place and that riparian zones do not produce more N_2O than upper terrestrial areas, which means riparian zones are not necessarily contributing towards climate change.

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CHAPTER 3:
THE MAXIMUM DENITRIFICATION POTENTIAL FOR NATURAL, INVADED AND CLEARED
FYNBOS RIPARIAN ECOTONES UNDER LABORATORY CONDITIONS

Abstract

Many studies on invasive alien *Acacia* spp. in Mediterranean-type ecosystems (MTEs) provided proof that they have an effect on nutrient cycling, especially nitrogen cycling when they are a nitrogen-fixing tree species. Nitrogen-fixing trees may add nitrogen to the system, which then becomes available in the form of NO_3^- that may cause contamination of groundwater or surface waters if they are in excess and the nitrogen-fixing trees are cleared from an area. Fortunately there are processes in the buffer zones (i.e. riparian ecotones) that may constantly reduce NO_3^- to low levels of which biological denitrification is thought to be the main mechanism. The process of biological denitrification will only occur under environmental conditions conducive to denitrifying soil microbial populations.

The aim of this chapter was to determine what the effect is of invasive alien *Acacia* spp. on the maximum denitrification potential under different invasion statuses and landscape positions and whether there are any limitations in nitrogen or carbon when favourable conditions for denitrification to take place were supplied in the laboratory and what the impact of clearing was on soil recovery from excess nitrogen in the system.

Field soil samples were taken from the riparian ecotones of the Western Cape and the Eastern- and Southern Cape and analysed for various soil physical properties as well as available nitrogen. The collected field soil samples were incubated for three hours under anaerobic conditions and included a control sample, one amended with nitrogen and carbon separately and a combination of nitrogen and carbon in a temperature controlled room that was set at 30°C. Hourly headspace samples were taken and analysed for N_2O gas emissions, which was expressed as a rate of mg N_2O per kg dry soil per day. This rate of N_2O gas emitted from the incubations was used as an indicator of the maximum denitrification potential of the soil micro-organisms under ideal environmental conditions.

The maximum denitrification potential was comparable to other Mediterranean-type ecosystems (MTEs) and summer values were one order of magnitude lower than spring in the Eastern- and Southern Cape, which is explained by the lack of sufficient soil moisture to create anaerobic soil conditions and leaching of NO_3^- from the previous rainy season. The maximum denitrification potential in the Western Cape did not differ significantly when expressed by invasion status or landscape position and the amendment of nitrogen and carbon did also not affect the denitrification potential, which meant that the high sand particle size content and low silt and clay particle size content were responsible for the lack of differences in denitrification potential.

There was a higher maximum denitrification potential in the Eastern- and Southern Cape riparian ecotones (wet and dry banks) compared to the upper terrestrial sites, which may be explained by the

presence of indigenous *Virgilia* spp. in the riparian ecotones that also fix nitrogen and contributes to NO_3^- , the substrate for denitrification to take place. The presence of *Virgilia* spp. enhanced the denitrifying microbial community and higher silt and clay contents also contributed to enhanced denitrification potential in the Eastern- and Southern Cape. The general environmental factors that played a role in the denitrification potential included the sand, silt and clay content as well as the organic carbon content and the electrical conductivity.

KEYWORDS: Denitrification potential, soil recovery, Western Cape, Eastern- and Southern Cape

1. Introduction

It is clear from Mediterranean studies of the past and the present that invasive alien plants have an effect on nutrient cycling where nitrogen is generally limited (Vitousek and Melillo, 1979; Yelenik et al., 2004; Marchante et al., 2008; Jovanovic et al., 2009). The impact of IAPs may be exacerbated if they are *Acacia* spp., nitrogen-fixing tree species from Australia with the ability to add atmospheric nitrogen to the system (Tye and Drake, 2012). These nitrogen-fixing tree species may further cause nitrogen cycling processes to be enhanced beyond the requirement of soil micro-organisms or plants if clearing of invasive alien vegetation takes place (Jovanovic et al., 2009). Invasive alien Clearing efforts in invaded stands of invasive alien *Acacia* spp. may also lead to contamination of groundwater if the excess available nitrogen in the form of NO_3^- is not released to the atmosphere as N_2O through the process of biological denitrification (Martin et al., 1999). The problem with high levels of NO_3^- in riparian ecotones is the long-term persistence (decades) of NO_3^- if it ends up in the groundwater, especially if nitrogen is continuously added to the system (Martin et al., 1999). Fortunately there are processes for NO_3^- to be consistently reduced to low levels through subsurface water flow through buffer zones (i.e. riparian zones) in natural catchments (Martin et al., 1999). Some of the processes that are able to reduce nitrate in riparian ecotones include plant uptake, microbial immobilization and bacterial (biological) denitrification (Martin et al., 1999) of which biological denitrification is thought of as the main mechanism by which nitrate is reduced in especially groundwater (Martin et al., 1999).

The process of biological denitrification will only occur under environmental conditions conducive to denitrifying soil microbial populations, which includes a lack of sufficient oxygen available to be used as a terminal electron acceptor, a sufficient organic carbon source that can be used as an energy source, sufficient substrate availability (NO_3^-) and the presence of a denitrifying soil microbial community (Knowles, 1982; Naiman and Décamps, 1997). If denitrifying soil micro-organisms are present in the soil they are forced to use ionic nitrogen oxides (NO_3^- and NO_2^-) to gain energy by coupling their reduction to electron transport phosphorylation. These micro-organisms then transform the nitrogen oxides to gaseous oxides (NO ; N_2O), which may then rapidly be converted to nitrogen gas (N_2), a gas that is abundant in the earth's atmosphere (Martin et al., 1999). Nitrous oxide (N_2O) is a greenhouse gas that causes destruction of the stratospheric ozone (Robertson et al., 1999; Rees et al., 2006; IPCC, 2007) and contributes to global climate change by being one of the natural drivers of climate change. Nitrous oxide is also a long-lived greenhouse gas that is chemically stable and can

persist in the atmosphere for decades to centuries, which may become well mixed in the atmosphere faster than it can be removed and ultimately has a long-term effect on climate (IPCC, 2007). There was a global increase in atmospheric nitrous oxide concentration from a pre-industrial value of 270ppb to 319ppb in 2005 due to a constant growth rate since 1980 with the majority of all the nitrous oxide emissions that arose from anthropogenic sources that are primarily due to agriculture (IPCC, 2007). The potential for denitrification to occur in fynbos riparian ecotones may contribute very little towards global N₂O emissions due to the majority of the N₂O emissions that originate from agriculture, even though N₂O concentrations increased linearly by 0.8ppb.y⁻¹ over the past decades (IPCC, 2007). The reaction rate of denitrification may be influenced by physical factors such as soil temperature, moisture and type of carbon source (Payne, 1981; Naiman and Décamps, 1997) and the pH has an influence on whether N₂O or N₂ is produced (Naiman and Décamps, 1997). Nitrous oxide production occurs optimally at a pH of 5.5 and becomes undetectable at a pH above 6.5 (Barton and Atwater, 2002). Soil texture also has an influence on denitrification, because optimal conditions normally occur in fine-textured soils, which include anoxic conditions and an available carbon source (Ullah et al., 2005). Nitrate immobilized by microbes or bio-assimilated by plants can eventually return the nitrogen to the landscape through mineralization (Martin et al., 1999). Denitrification is considered an ecological process that leads to the recovery of soils whereby excess nitrogen that was added to the system is released back to the atmosphere in the same form and closes the N cycle.

The main objectives of this chapter are to investigate the effect of invasive *Acacia* spp. on the maximum denitrification potential of denitrifying soil micro-organisms under ideal conditions in the laboratory and how the clearing of the invasive *Acacia* spp. modifies the denitrification potential in fynbos riparian environments compared to reference sites. Invaded sites are expected to have a greater denitrification potential compared to reference sites and sites cleared of invasive *Acacia* spp. are expected to have a greater denitrification potential compared to invaded sites and reference sites. It is important to note that due to the structure of the thesis there is some repetition in especially the methods, where similar soil properties that were thought to affect the denitrification potential were measured and reported on.

2. Material and methods

2.1. Soil sampling procedure

Soil samples were taken at 15m intervals along three transects of 60m in length parallel to the river (i.e. wet banks, dry banks and upper terrestrial area) in Spring 2012 for the Western Cape (Figure 1.4. and Table 1.1.) and also in Summer 2011 and Spring 2012 for the Eastern- and Southern Cape (Figure 1.5 and Table 1.2). Six random soil samples were taken using a stainless steel soil-core type sampler (5 cm diameter) from the top 10 cm (topsoil or A-horizon) of the soil profile after loose litter (organic matter) was removed as this part of the soil profile is thought to be the most biologically active (Gold and Kellog, 1997). A hand trowel was sometimes used in places with very dry and sandy or too rocky and/or shallow soils. The samples were pooled and homogenized within polyethylene bags (Ziploc bags) that contained the sampling position, date and site name on the outside of the bag,

which was pre-marked with a permanent marker. Most of the air was pushed out of the bag and sealed airtight, which prevented contamination by atmospheric nitrogen. Samples were stored at a temperature of approximately 4°C or less for no longer than 3 days if soil extractions could not take place immediately. Samples were sieved with a 2 mm stainless steel sieve in order to remove roots and organic debris.

2.2. *Maximum denitrification potential*

The effect of available N and organic C on the maximum denitrification potential was determined once off and compared to reference conditions during Spring 2012 for the Western Cape and also during Spring 2012 for the Eastern- and Southern Cape. The denitrification enzyme assay (DEA) that was developed by Tiedje et al. (1989) was used in this study. 15g of fresh field soil was added to 4 different treatments: 1 (DEA): 15ml of distilled water, 2. (DEAC): 15ml sodium succinate (1mM $\text{Na}_2\text{C}_4\text{H}_4\text{O}_4 \cdot 6\text{H}_2\text{O}$), 3. (DEAN): 15ml potassium nitrate (1mM KNO_3) and 4. (DEA C+N): a combined solution of 7.5ml potassium nitrate and 7.5ml sodium succinate. The 200ml flasks were sealed with a rubber stopper and flushed with high purity nitrogen for 2 minutes to make sure the flask was under an anaerobic condition. Acetylene gas (C_2H_2) was purified by a sulphuric acid trap and 30ml was added to each flask (15% of headspace) to prevent the conversion of N_2O to N_2 . The flasks were shaken for 30 seconds and then vented with a needle to bring the headspace to atmospheric pressure and 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. They were 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 an indication of the maximum denitrification potential. The two stainless steel columns (one pre-column and one main column) were 2mm thick and 3m 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 (Mosier and Mack 1980). Atmospheric air samples were used to draw a standard curve, which incorporated the area of the different peaks for one standard air sample 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 incubation period. The production of N_2O gas was used as an indicator of the maximum denitrification potential.

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 (Tiedje et al., 1989).

2.3. Soil physical properties

2.3.1. Gravimetric Soil Water Content (GSWC)

GSWC were determined by the methodology of Robertson et al. (1999). Ten grams of each sieved fresh soil sample was measured off with a digital scale and then placed in an oven at 105°C for 24 hours. The dry mass of the soil sample was then weighed after it has been allowed to cool off for 5 minutes. The dry soil weight was then subtracted from the wet soil weight. That value was divided by the dry soil weight and then multiplied by 100 in order to determine the gravimetric soil water content as a percentage. The formula that was used to determine the gravimetric soil water content was as follows: $GSWC = [(wet\ soil\ mass - oven\ dried\ soil\ mass) / dry\ soil\ mass] \times 100$.

2.3.2. pH

Soil pH was determined in distilled water based on the methodology of Robertson et al. (1999). A 1:2.5 (w:v, soil:distilled water) soil slurry was prepared by adding 10g sieved fresh field soil to 25ml distilled water. The soil solution was first shaken by hand and then placed in a mechanical shaker for an hour. The soil solution was left to settle for 5 minutes after which the pH was measured with a pH meter (Hanna 211 Microprocessor) by inserting the pH probe in the supernatant solution and taking a stabilised reading.

2.3.3. EC

Electrical conductivity was measured in the same way as pH in a 1:2.5 (w:v, soil:distilled water) soil slurry. It was prepared by adding 10g sieved fresh field soil to 25ml distilled water. The soil solution was first shaken by hand and then placed in a mechanical shaker for an hour. The soil solution was left to settle for approximately 5 minutes after which the EC was measured with an EC meter (Corning Conductivity meter 441) by inserting the EC probe in the supernatant solution and taking a stabilised reading.

2.3.4. Bulk Density

Bulk density (BD) was determined by a small PVC tube (cylinder) with a known volume (V) that was assumed to be equal to the volume of the soil. The volume of the PVC tube was determined by making use of the following formula: $V = \pi r^2 h$, where $\pi \approx 3.1416$ (one standard value of pi), r = the radius of the PVC tube (in cm) and h = the height of the PVC tube (in cm). The radius was determined by the distance from the middle point of the PVC tube to the inside edge of the PVC tube. The height was determined by the distance on the side of the PVC tube from the top to the bottom. The PVC tube was pushed into the soil and an intact soil core sample was taken wherever possible, for which the mass of the oven dried (at 105°C for 24 hours) soil was determined (in grams). The bulk density was then calculated with the formula: $BD = \text{mass of oven dried soil (g)} / \text{volume of the soil (cm}^3\text{)}$.

2.3.5. *Soil particle size*

The soil particle size content of three fractions (sand, silt and clay percentage) and percentage organic carbon content was analysed by the Western Cape Department of Agriculture.

2.4. *Statistical Analyses*

All statistical analyses were carried out with Statistica version 10, a statistical program that was developed by Statsoft (Statsoft Inc., 2011). A repeated measures ANOVA was used for the Western Cape as well as for the Eastern- and Southern Cape to determine the main effects of invasion status and landscape position on the different DEA treatments, where DEA treatment was the within group repeated factor and invasion status and landscape position the between group factors. The comparisons between different DEA treatments were determined for all the data collected, irrespective of invasion status or landscape position. Post-hoc Fisher LSD tests were followed by ANOVAS to determine whether there were differences between the means of DEA treatment, invasion status and landscape position. Spearman rank correlation coefficient values (r^2) were calculated to determine whether there were any correlations between the various soil physical and chemical properties that were believed to have an influence on the denitrification potential and if so how strongly they interacted. This was analysed on all data collected irrespective of invasion status or landscape position.

3. Results

3.1. *Maximum denitrification potential (DEA)*

The maximum denitrification potential was determined during Summer 2011 for the Eastern- and Southern Cape and analysed for the main effects of invasion status (natural, invaded and cleared) and landscape position (wet bank, dry bank and terrestrial) on the four different treatments (DEA: control, DEAN: nitrogen amended, DEAC: carbon amended, DEA C+N (carbon + nitrogen amended) by means of anaerobic soil incubations at a constant temperature (25°C) and moisture level (100%).

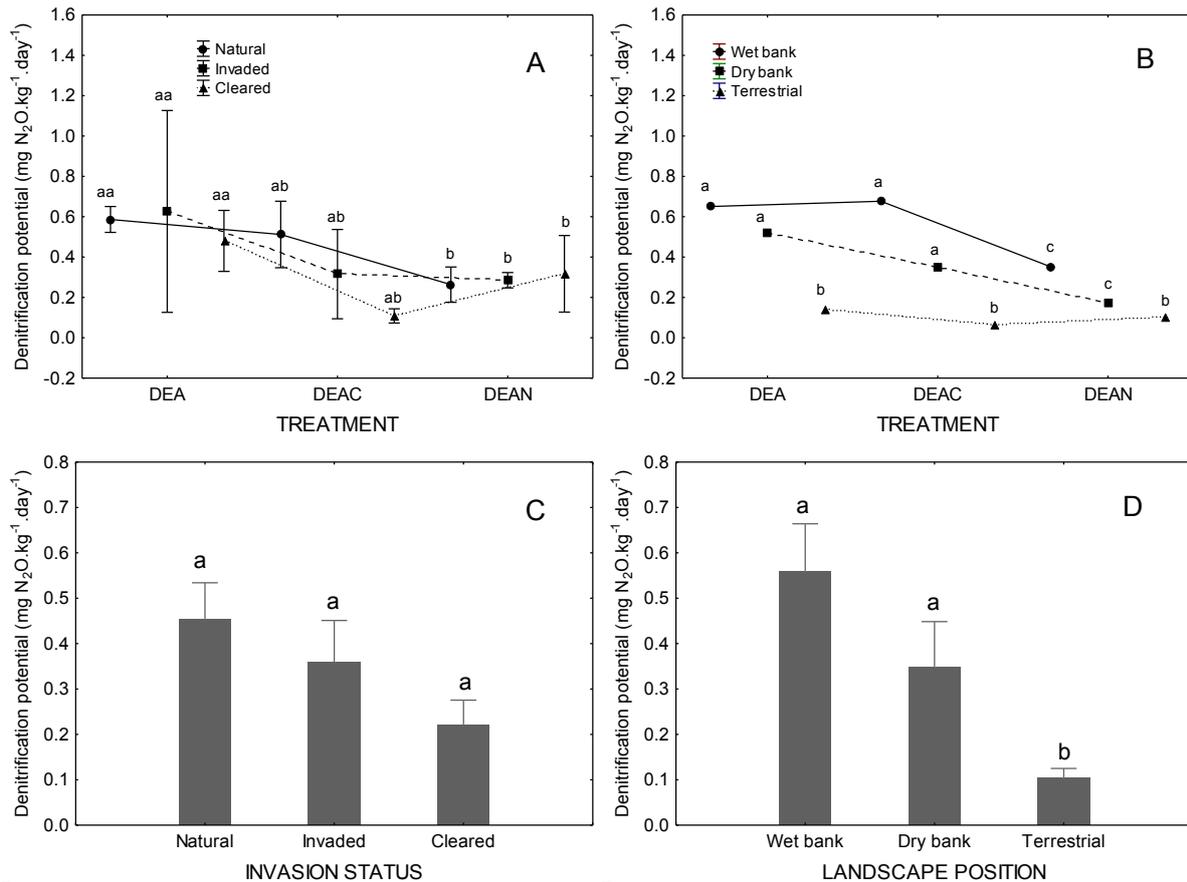


Figure 3.1. Maximum denitrification potential for the Eastern- and Southern Cape for Summer 2011 expressed by **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) for the four different treatments. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences for invasion status and landscape position ($p < 0.05$).

The maximum denitrification potential was significantly affected by nitrogen amended treatment that had a significantly lower denitrification potential compared to the control ($F_{2, 14} = 3.0468$; $p = 0.0797$; Fisher LSD = 0.0328; Figure 3.1. A). The effect of the nitrogen amended treatment can also be seen on the landscape position, because the denitrification potential of this treatment is lower than the control and the carbon amended treatment ($F_{2, 4} = 13.4165$; $p = 0.0168$; Figure 3.1. B). Invasion status did not display a difference in the maximum denitrification potential when all the collected data was combined ($F_{2,7} = 0.9861$; $p = 0.4195$; Figure 3.1. C). The terrestrial sites were significantly lower for the denitrification potential than the wet banks and dry banks ($F_{2,6} = 7.2977$; $p = 0.0247$; Figure 3.1. D).

The maximum denitrification potential determined during Spring 2012 for the Eastern- and Southern Cape were also analysed for the main effects of invasion status (natural, invaded and cleared) and landscape position (wet bank, dry bank and terrestrial) on the four different treatments (DEA: control, DEAN: nitrogen amended, DEAC: carbon amended, DEA C+N (carbon + nitrogen amended) by means of anaerobic soil incubations at a constant temperature (25°C) and moisture level (100%).

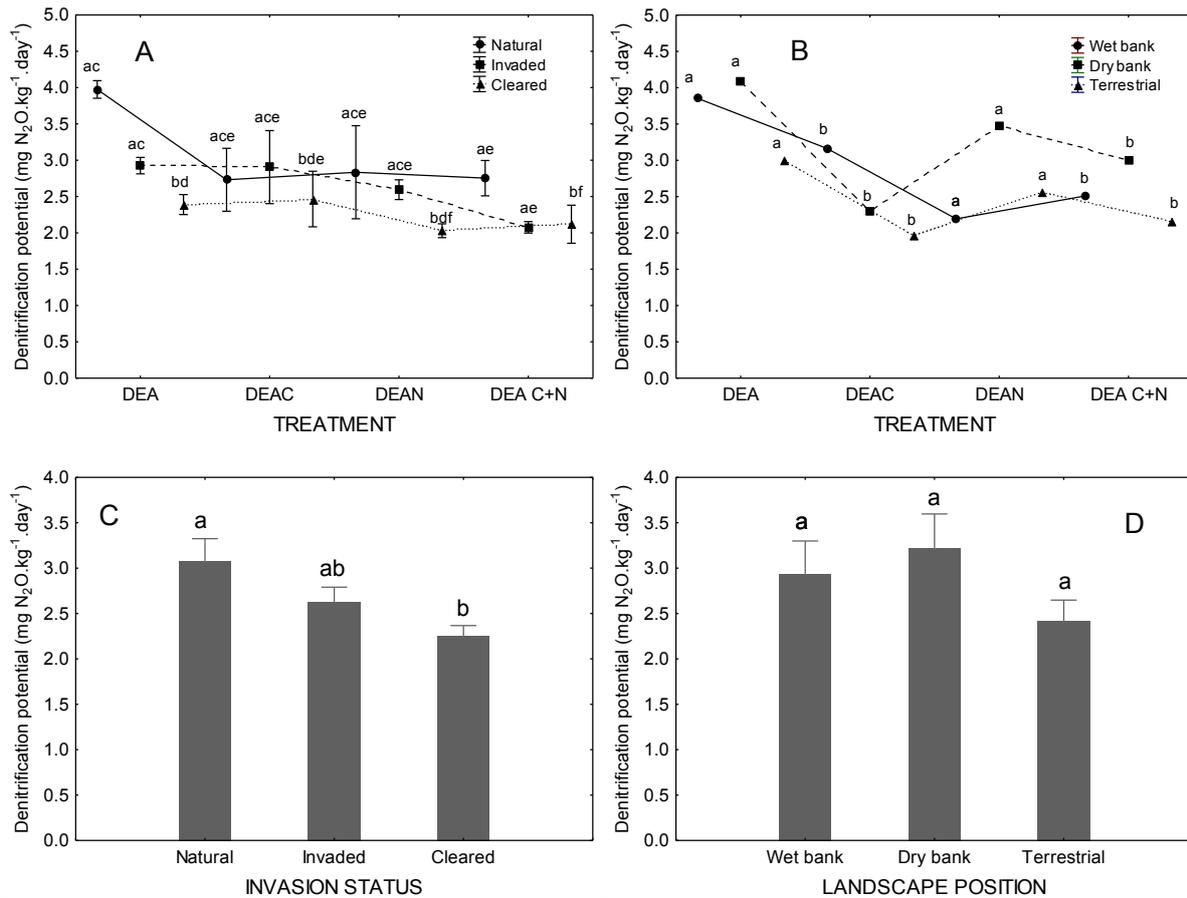


Figure 3.2. Maximum denitrification potential for the Eastern- and Southern Cape for Spring 2012 expressed by **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) for the four different treatments. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant for invasion status and landscape position ($p < 0.05$).

There was no difference in the maximum denitrification potential for the different treatments when expressed by invasion status ($F_{3,9} = 2.9740$; $p = 0.0893$; Figure 3.2. A) or landscape position ($F_{3,6} = 3.8895$; $p = 0.0739$; Figure 3.2. B). There was however significant differences between the natural and cleared sites (Fisher LSD = 0.0173), but no difference between the invaded and natural sites (Fisher LSD = 0.0796), or the invaded and cleared sites (Fisher LSD = 0.1168). The position in the landscape did not have an effect on the maximum denitrification potential ($F_{2,9} = 1.4730$; $p = 0.2796$; Figure 3.2. D).

The maximum denitrification potential was also determined during Spring 2012 for the Western Cape and analysed for the main effects of invasion status (natural, invaded and cleared) and landscape position (wet bank, dry bank and terrestrial) on four different treatments (DEA: control, DEAN: nitrogen amended, DEAC: carbon amended, DEA C+N (carbon + nitrogen amended) by means of anaerobic soil incubations at a constant temperature (25°C) and moisture level (100%).

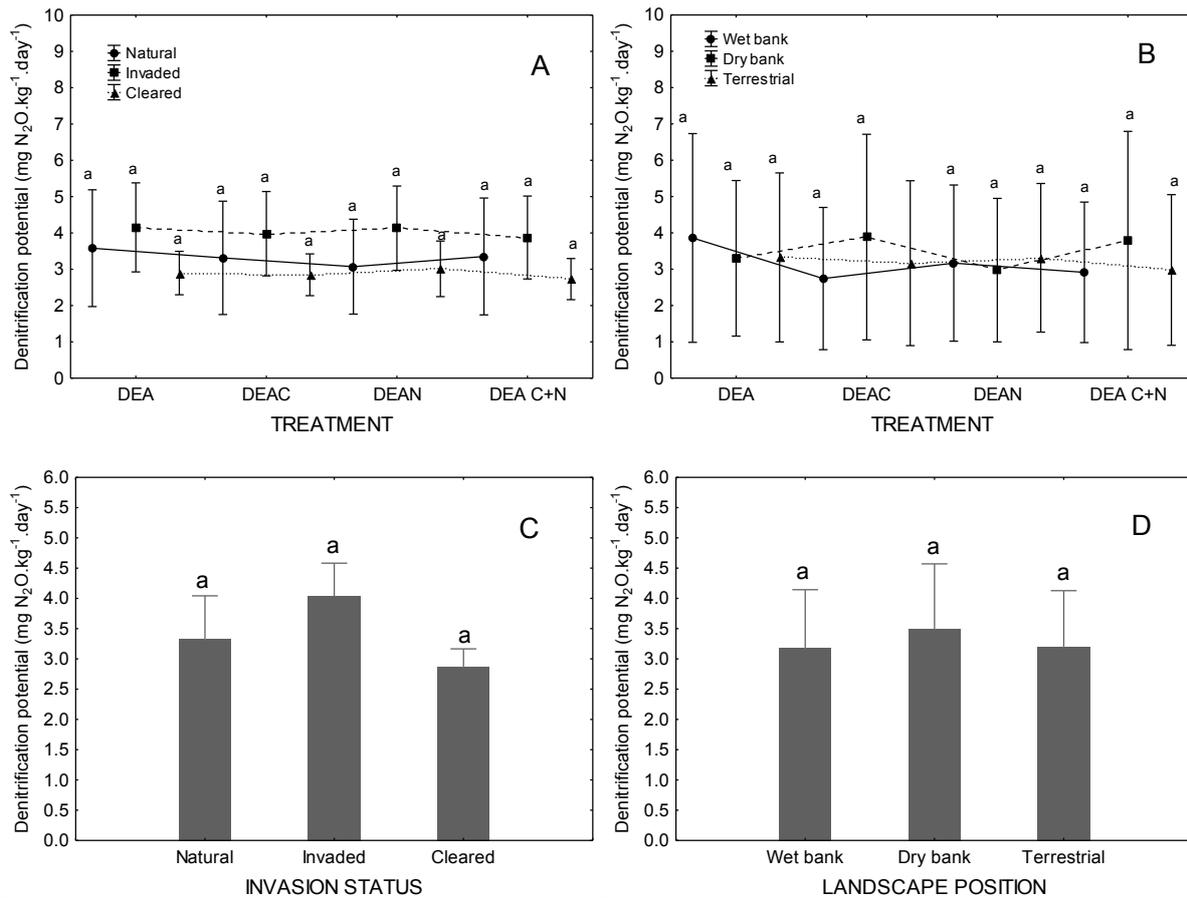


Figure 3.3. Maximum denitrification potential for the Western Cape for Spring 2012 expressed by **A.** invasion status (natural, invaded and cleared) and **B.** landscape position (wet bank, dry bank and terrestrial) for the four different treatments. The mean values of all data collected irrespective of invasion status or landscape position are depicted in graphs **C.** and **D.** Mean values are indicated by different symbols and whiskers represent one standard error. Letters denote significant differences for invasion status and landscape position ($p < 0.05$).

There was no significant difference between the denitrification potential treatments that were supplied with a carbon and nitrogen source separately or with a combination of the two compared to the control when expressed by invasion status ($F_{3, 45} = 0.2515$; $p = 0.8599$; Figure 3.3. A) or landscape position ($F_{3, 18} = 0.4022$; $p = 0.7531$; Figure 3.3. B). The effect of invasion status on the potential to convert NO_3^- to N_2O was negligible in the study ($F_{2, 15} = 0.2668$; $p = 0.7694$; Figure 3.3. C) and there was also no difference in the position within the landscape ($F_{2, 6} = 0.0058$; $p = 0.9942$; Figure 3.3. D).

3.2. Correlations between DEA and the environment

Spearman's rank correlation coefficient (r^2) values were determined for the different treatments of the maximum denitrification potential (DEA) and some of the various soil physical and chemical properties thought to affect the denitrification potential regardless of invasion status or landscape position for Spring 2012 for the Eastern- and Southern Cape. Significant correlations are indicated with an asterisk ($p < 0.05$).

Table 3.1. Spearman's rank correlation coefficient (r^2) for the different treatments of the maximum denitrification potential (DEA) and some of their drivers for Spring 2012 for the Eastern- and Southern Cape. Significant correlations are indicated by an asterisk (* $p < 0.05$). Correlations were calculated from all available data irrespective of invasion status or landscape position.

	TREATMENT			
	DEA	DEAC	DEAN	DEA C+N
NH_4^+	0.367	0.283	0.667*	0.617
NO_3^-	0.500	0.429	0.393	0.143
pH (water)	-0.367	0.367	-0.417	-0.633
EC	0.267	0.583	0.300	-0.350
Organic C	0.700*	-0.100	0.617	0.533
Sand	-0.700*	0.283	-0.650	-0.350
Silt	0.767*	-0.167	0.600	0.383
Clay	0.527	-0.259	0.837*	0.326

There was a positive correlation between the control denitrification treatment and the organic carbon content ($r^2 = 0.700$) as well as the silt content ($r^2 = 0.767$), while there was a negative correlation between the control denitrification treatment and the sand content ($r^2 = -0.700$). There was also a positive correlation between the nitrogen amended denitrification treatment and ammonium ($r^2 = 0.667$) and the clay content ($r^2 = 0.837$).

Spearman's rank correlation coefficient (r^2) values were determined for the different treatments of the maximum denitrification potential (DEA) and some of the various soil physical and chemical properties thought to affect the denitrification potential regardless of invasion status or landscape position for Spring 2012 for the Western Cape. Significant correlations are indicated with an asterisk ($p < 0.05$).

Table 3.2. Spearman's rank correlation coefficient (r^2) for the different treatments of the maximum denitrification potential (DEA) and some of their drivers for Spring 2012 for the Western Cape. Significant correlations are indicated by an asterisk (* $p < 0.05$). Correlations were calculated from all available data irrespective of invasion status or landscape position.

	TREATMENT			
	DEA	DEAC	DEAN	DEA C+N
NH_4^+	0.142	0.170	0.143	0.093
NO_3^-	0.275	0.213	0.119	0.238
pH (water)	-0.246	-0.145	-0.284	-0.087
EC	-0.242	-0.151	-0.430*	-0.270
Organic C	-0.219	-0.154	-0.201	-0.211
Sand	0.096	0.006	0.048	-0.113
Silt	-0.080	0.008	-0.061	0.114
Clay	-0.016	0.022	0.072	0.168

The only observable relationship between the different denitrification treatments and some soil properties during spring in the Western Cape was a negative correlation between the nitrogen amended treatment and the electrical conductivity ($r^2 = -0.430$).

Selected soil physical properties were measured across seasons for the Western Cape and are displayed in Table 2.2.

Table 3.3. Description of a once-off measurement of soil physical properties expressed by invasion status (Western Cape)

Soil property	Natural	Invaded	Cleared
GSWC (%)	11.22 ± 1.66	7.01 ± 1.71	9.86 ± 1.81
VSM (%)	16.20 ± 4.07	11.18 ± 3.11	15.36 ± 2.32
Temp 1 cm (°C)	14.87 ± 3.03	19.23 ± 3.33	20.98 ± 1.49
Temp 6 cm (°C)	14.94 ± 2.36	15.33 ± 2.79	17.84 ± 11.32
pH	4.05 ± 0.46	3.76 ± 0.66	5.14 ± 0.11
EC (µS/m)	24.40 ± 2.63	26.90 ± 4.99	42.09 ± 7.84
Bulk density (g/cm³)	0.85 ± 0.06	1.06 ± 0.05	1.04 ± 0.06
Sand (%)	93.05 ± 0.93	94.10 ± 0.77	92.34 ± 1.13
Silt (%)	3.00 ± 0.59	2.24 ± 0.39	3.66 ± 0.69
Clay (%)	1.60 ± 0.28	1.41 ± 0.51	1.99 ± 0.41
Organic C (%)	3.95 ± 0.32	3.66 ± 0.28	4.00 ± 0.38
Total C (%)	2.38 ± 0.41	2.80 ± 1.13	2.91 ± 0.57
Total N (%)	0.13 ± 0.02	0.25 ± 0.09	0.87 ± 0.50
C:N	18.62 ± 2.76	11.60 ± 2.19	16.64 ± 5.25

The natural, invaded and cleared sites had similar soil moisture contents ($F_{2,28} = 1.640$; $p = 0.212$ and $F_{2,28} = 0.669$; $p = 0.520$), similar temperatures at 1cm and 6cm ($F_{2,28} = 1.251$; $p = 0.302$ and $F_{2,28} = 0.397$; $p = 0.676$) and similar pH's ($F_{2,28} = 1.871$; $p = 0.173$). The measured pH for the fynbos riparian soils was generally below 5, which means that the soils are acidic. The electrical conductivity was higher in cleared sites than natural sites (Fisher LSD = 0.022) and there was no difference in electrical conductivity between natural and invaded sites (Fisher LSD = 0.750). There was significant bulk density differences between the natural, invaded and cleared sites ($F_{2,26} = 4.288$; $p = 0.025$). The soils of natural sites had higher bulk densities than invaded sites (Fisher LSD = 0.017) and cleared sites (Fisher LSD = 0.023), which means natural sites are more compacted and there are less pore spaces in these soils compared to the invaded sites and cleared sites. There was no difference in the soil particle size content of sand in natural invaded and cleared sites ($F_{2,26} = 0.750$; $p = 0.481$). There was also no difference in the soil particle size content of silt in natural, invaded and cleared sites ($F_{2,26} = 1.258$; $p = 0.301$). Once again, there is no difference in the soil particle size content of clay in natural invaded and cleared sites ($F_{2,26} = 0.226$; $p = 0.817$). There is however a significantly higher sand content than silt and clay content in soils under all the invasion statuses (natural: $F_{2,30} = 6155.965$; $p = 0.000$, invaded: $F_{2,21} = 10150.410$; $p = 0.000$ and cleared: $F_{2,27} = 4137.747$; $p = 0.000$). There was no difference for organic carbon ($F_{2,21} = 0.559$; $p = 0.580$) and total carbon ($F_{2,21} = 0.199$; $p = 0.821$) under the different invasion statuses. Total nitrogen and the carbon to nitrogen ratio did not differ significantly when natural, invaded and cleared sites were compared ($F_{2,21} = 1.575$; $p = 0.231$ and $F_{2,21} = 0.712$; $p = 0.502$).

Selected soil properties were measured once-off to describe and understand the soils better, because these properties are believed not to change in the short-term.

Table 3.4. Description of a once-off measurement of soil physical properties expressed by invasion status during spring for the Eastern-and Southern Cape

	Natural	Invaded	Cleared
GSWC (%)	23.30 ± 3.37	21.66 ± 13.42	8.13 ± 6.08
pH	4.56 ± 0.24	5.92 ± 0.03	5.68 ± 0.03
EC (µS/m)	44.46 ± 8.65	76.96 ± 10.69	30.60 ± 5.56
Sand (%)	80.83 ± 2.62	90.00 ± 3.00	91.72 ± 3.18
Silt (%)	12.83 ± 1.92	4.75 ± 1.75	3.89 ± 1.79
Clay (%)	6.34 ± 0.73	5.25 ± 1.25	4.39 ± 1.26
Organic C (%)	4.30 ± 0.62	2.18 ± 0.53	0.73 ± 0.49

Natural, invaded and cleared sites had similar soil moisture contents ($F_{2,5} = 1.529$; $p = 0.303$). The pH was affected by invasion status ($F_{2,5} = 19.978$; $p = 0.004$) and was significantly lower in natural sites than invaded sites (Fisher LSD = 0.003) and cleared sites (Fisher LSD = 0.004). Electrical conductivity was affected by invasion status ($F_{2,5} = 6.982$; $p = 0.036$) and was higher in invaded sites than natural sites (Fisher LSD = 0.048). There was significantly less sand in natural sites than cleared sites (Fisher LSD = 0.040), but no difference in natural and invaded sites (Fisher LSD = 0.096). There was significantly more silt in natural sites than invaded (Fisher LSD = 0.035) and cleared sites (Fisher LSD = 0.017). Clay content did not differ between the natural, invaded and cleared sites ($F_{2,5} = 0.805$; $p = 0.498$). Cleared sites had significantly less organic carbon than natural sites (Fisher LSD = 0.005).

4. Discussion

The maximum denitrification potential that was measured for the fynbos riparian ecotones of the study in the Western Cape and the Southern- and Eastern Cape during spring of 2012 was in the same order of magnitude compared to the denitrification potential of other studies that were conducted in Mediterranean riparian ecotones by Pinay et al. (2000) and Bernal et al. (2007). The maximum denitrification potential for the Western Cape and the Southern- and Eastern Cape during spring of 2012 was an order of magnitude lower than the denitrification potential of the same study sites in the Eastern-and Southern Cape summer 2012. The difference in the maximum denitrification potential for the same study sites in the Eastern- and Southern Cape indicates that there is temporal variability between spring and summer. Bernal et al. (2007) found higher N₂O emissions in the laboratory compared to *in situ* soil core samples in the summer, but no difference in the field N₂O emissions and laboratory N₂O emissions in winter. Temperature was a limiting factor in the denitrification process during summer in the Eastern- and Southern Cape as a result of similar field N₂O emissions between laboratory- and field conditions. This finding proves that there is no spatial variability in the maximum denitrification potential of soil micro-organisms compared to laboratory conditions during winter. The finding by Bernal et al. (2007) also proves that there is spatial variability between laboratory- and field analyses due to different results even though field conditions were mimicked in the laboratory. Another possibility for the lower denitrification potential of the Eastern- and Southern Cape in summer is due to NO₃⁻ that leached to deeper soil layers during this rainy season when the denitrification

potential measurements were taken. The higher denitrification potential in spring for the same study sites implies that NO_3^- became more available in spring for denitrification to take place, provided there are favourable conditions.

The Eastern- and Southern Cape displayed interesting trends in the maximum denitrification potential during summer where there was lower denitrification activity in the nitrogen amended treatment than the control as well as a higher denitrification potential in wet banks and dry banks compared to the upper terrestrial sites, which proves that there is spatial variability between the riparian ecotones and the upper terrestrial sites. These differences in the denitrification potential can be explained by the presence of indigenous legumes (*Virgilia* spp.) and nitrogen-fixing bacteria that is associated with the root nodules of *Virgilia* spp. that is mostly found in the dry banks and wet banks of the fynbos riparian ecotones in the Eastern- and Southern Cape. This tree is ubiquitous in riparian zones of the Southern Cape and parts of the Eastern Cape (Magadlela, 2013) and was dominant in two of three study sites. This indigenous nitrogen-fixing tree species is contributing towards NO_3^- availability in the wet banks and dry banks and there are more favourable conditions for denitrification to take place, which are increased soil moisture (see Table 3.4.) and NO_3^- availability in this case. The soils in Eastern- and Southern Cape have higher silt and clay content (see Table 3.4.) and are thus providing a larger surface area around the soil particle allows a larger denitrifying soil microbial community to establish, which will also make NO_3^- more abundant and therefore increase the denitrification ability of the soils in the Eastern- and Southern Cape. Ecological theory suggests that soil microbial populations around *Virgilia* trees might include denitrifiers that that have been established in wet banks and dry banks for a long period. Further, higher silt and clay contents have been found to cause an increase in denitrification activity by Pinay et al. (2000), which is linked to the higher nutrient retention ability compared to sand dominated soils (see Table 3.4.).

This might also explain the lower denitrification activity in the cleared sites compared to the natural and invaded sites. *Virgilia* trees are a natural part of the landscape, thus denitrification potential might be high due to constant high N inputs from N-rich litter. Similar results were reported in a study by Vermes and Myrold (1992) who found that indigenous nitrogen fixing trees that were established much longer than stands of trees that were recently cut down had significantly higher denitrification potentials (three orders of magnitude higher) due to a better established denitrifying soil microbial community. Thus invasion might not significantly alter the denitrification potential, as *Acacia* trees might simply replace indigenous legumes. However, this situation changes when invasive trees are cleared – now N-rich litter is not added to the soil in large amounts, thus the denitrifier population may reduce (abundance and community structure; see Slabbert 2012), which is reflected in lower denitrification potential in invaded sites. Similarly, root associated microbes may also be altered when clearing takes place as roots quickly die, and root-associated microbes become inactive, which may explain why sites that were cleared of invasive alien *Acacia* spp. are not reducing NO_3^- from the system in the form of N_2O gas through the process of denitrification.

There were no clear trends in the interactions between the different maximum denitrification potential treatments and the environment for the Western Cape, except for one random correlation where electrical conductivity had a negative impact on denitrification potential when amended with nitrate (see Table 3.2.), because nitrates were most likely transported away from the microsites where the denitrifying microbial populations occurred and nitrates not utilised by denitrifying micro-organisms in the denitrification process. The sand content in the Eastern- and Southern Cape had a negative relationship with the control maximum denitrification potential treatment (see Table 3.1.), which can be explained by the lack of nutrient retention and water storage ability, but there was a positive relationship between organic carbon and the DEA control as well as a positive correlation between the DEA control and the organic carbon and the silt content (see Table 3.1.), which means that organic carbon plays a role in providing carbon under natural conditions and that the silt content may have increased areas with larger microsites that have the capacity for denitrifying micro-organisms to develop larger communities in the silt fraction of the soil compared to sand. A positive relationship was also found when the DEA treatment was amended with nitrogen in the clay particle fraction of the soil where denitrifying micro-organisms tended to be more active when nitrates were not limiting anymore.

The difference in soil processes in different parts of the river reach was also not included in the scope of the study, but similarities in the parent material (sandstone) of the mountain catchments resulted in the formation of sandy soils of the fynbos riparian ecotones in the past, caused present similarities in some soil processes. Denitrification was one of the measured soil process that was similar under different invasion statuses and landscape positions. There may however be a different effect of denitrification on removing excess nitrogen from lower parts of the river (foothill streams for example) where differences in particle sizes may be expected. There may be an increase in denitrification in these lower parts of the river, because higher levels of sand and silt are expected as you move down the river. This increase in silt and clay content further down a river is supported by the river continuum concept of Vannote et al. (1980).

The maximum denitrification potential for the Western Cape was also similar across different landscape positions and under different invasions statuses, which implies that soil moisture was not a limitation in the denitrification process. The N_2O emissions from the field and laboratory incubations were in the same order of magnitude and it can be implied that N_2O emissions from the field are not only the product of anaerobic denitrification, but also a product of aerobic soil processes such as nitrogen mineralization. This is also supported by work done by Kambol (2013) on soil respiration processes that were the highest in summer for the same study sites, which imply that the upper parts of these fynbos riparian ecotone soils are driven by aerobic respiration processes. Bernal et al. (2007) also found a similar denitrification potential for the different treatments, which means that denitrification in their Mediterranean-type ecosystem is not limited by the availability of carbon and nitrogen, but that there are other factors that limit the process of denitrification across Mediterranean-type ecosystems. Soil nutrients can be a limiting factor for denitrification to take place, for instance

carbon is used as an energy source and nitrate as an electron acceptor in the absence of oxygen. This study did not find carbon or nitrogen to be a limiting factor for denitrification to take place in the Western Cape riparian ecotones (see Table 3.3.), because there was a lack of difference with various DEA treatments where sufficient carbon and nitrogen was supplied and anaerobic conditions induced for soil micro-organisms to perform denitrification in the incubated soils (see Table 3.2.).

5. Conclusion

The maximum denitrification potential for the Western Cape is similar across different invasion statuses and landscape positions and in the same order of magnitude compared to measured rates in other MTEs, and nitrogen and carbon does not appear to be limited. The maximum denitrification potential for the Eastern- and Southern Cape during summer is one order of magnitude lower compared to spring, which implies that conditions for denitrification are not conducive during summer, but more favourable during spring. Leaching of nitrate is a limiting factor during summer in the Eastern- and Southern Cape when the maximum denitrification potential was measured, which implies that soil moisture is also a limiting factor for denitrification to take place. An indigenous legume, *Virgilia* spp. is driving the denitrification potential in the wet and dry banks of the Eastern- and Southern Cape due to constant high N inputs from N-rich litter. Invasion by *Acacia* spp. does not seem to have an effect on the denitrification potential, because the *Acacia* trees are replacing an indigenous nitrogen-fixing tree species that is more effective in reducing NO_3^- from the system than when invasive alien *Acacias* are cleared. The sand, silt and clay content and the organic carbon content is also affecting the denitrification potential of the Eastern and Southern Cape. It is clear from these findings that the Western Cape reacts very differently to the impacts of invasive alien *Acacia* spp. and clearing on the denitrification potential of the fynbos riparian ecotones compared to the Eastern and Southern Cape.

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CHAPTER 4: CONCLUDING REMARKS, MANAGEMENT RECOMMENDATIONS AND FUTURE RESEARCH

1. Main findings

1.1. Trends in nitrogen cycling, availability and reduction

The nitrogen mineralization rates across seasons in the current study (Table 4.1.) are comparable to other MTEs, although the IAPs and different positions in the landscape did not have a significant effect on nitrogen mineralization. There are similarities in nitrogen mineralization activity between natural, invaded and cleared sites as well as a similarity in riparian ecotones (wet and dry banks) and upper terrestrial sites. These similarities in nitrogen mineralization activity is a result of the main factor of high sand content in the soil, which limits N mineralization under natural conditions in the field due to a short supply of suboptimal factors (e.g. water, temperature, low nutrient retention or the microbial population was less than ideal). There is however a tendency for N mineralization activity to be higher in the riparian zones than the upper terrestrial areas (Table 4.1.) due to one of the factors that is in short supply, especially in the invaded and cleared sites, that reduces nitrogen in the system through possible leaching.

Ammonium becomes more available over time in the riparian zones (Table 4.1.) due to higher litterfall rates and higher nitrogen content of the litter. Invaded sites produce significantly higher levels of available NO_3^- compared to cleared areas as a result of higher levels of NH_4^+ in the invaded sites and leaching of NO_3^- in the cleared sites (Table 4.1.). The driver for available nitrogen in fynbos riparian ecotones is NO_3^- , even though NH_4^+ becomes more dominant over time. The denitrification process is not influenced significantly by invasion status or landscape position (Table 4.1.) due to the sandy nature of the soil that does not support ideal conditions for denitrification to take place under field conditions (low NO_3^- retention and too short time periods of water saturated soils). Nitrification contributed to N_2O emissions that were measured under field conditions (Table 4.1.) more so than anaerobic biological denitrification. The importance of denitrification as a pathway to reduce NO_3^- in fynbos riparian ecotones is overestimated.

These different dynamics of nitrogen cycling under different invasion statuses and landscape positions (Table 4.1.) can be used to evaluate the impacts of invasion and clearing on riparian functioning and make recommendations from the results obtained in this study. The impact of invasive alien *Acacia* spp. is not significant on the N mineralization and denitrification process across seasons, although there was more NH_4^+ and NO_3^- available in the invaded dry banks (Table 4.1.). The lack of difference in N mineralization activity may prove that soil micro-organisms are not influenced by invasive alien Acacias, but that there is still the potential for groundwater or surface water to be polluted by higher levels of NO_3^- compared to reference sites. It appears that soil recovery processes in the form of N_2O release to the atmosphere has not yet taken place and that

riparian zones do not produce more N₂O than upper terrestrial areas (Table 4.1.), which means riparian zones are not contributing more towards climate change.

1.2. *Maximum denitrification potential*

There is no difference in the maximum denitrification potential for the various invasion statuses and landscape positions of the Western Cape (see Table 4.1.) and the maximum denitrification potential is in the same order of magnitude compared to other MTEs. Nitrogen and carbon does not appear to be limited in the Western Cape. The Eastern- and Southern Cape has a maximum denitrification potential that is one order of magnitude lower in summer than spring, which implies that conditions for denitrification are more favourable during spring than summer. One possible limiting factor for the maximum denitrification potential of the Eastern- and Southern Cape in summer is leaching of NO₃⁻ during the high rainfall summer season, which implies that the source of NO₃⁻ is a limiting factor for denitrification to take place. One of the drivers of the maximum denitrification potential in the wet and dry banks of the Eastern- and Southern Cape is an indigenous legume, *Virgilia* spp. that constantly adds high levels of nitrogen to the system in the form of nitrogen-rich litter.

Maximum denitrification potential does not seem to be influenced by the invasion of *Acacia* spp., because they are replaced by an indigenous nitrogen-fixing tree species that is more effective in reducing NO₃⁻ from the system than when invasive alien Acacias are cleared (Table 4.1.). It is clear from these findings that the Western Cape reacts very differently to the impacts of invasive alien *Acacia* spp. and clearing on the denitrification potential of the fynbos riparian ecotones compared to the Eastern and Southern Cape.

1.3. *Relationships between N processes and the environment*

Nitrogen mineralization was not significantly affected by the environment, but that the underlying factor may be due to similarities in soil microbial activity across all the study sites. Nitrogen availability in the fynbos riparian ecotones of the Western Cape is driven by NH₄⁺ and NO₃⁻, but is more strongly influenced by NO₃⁻, which has higher concentrations than NH₄⁺ (Table 4.1.). Nitrification occurs more regularly than ammonification and plays a greater role in making nitrogen available for plant and/or microbial uptake. The actual field N₂O emissions are positively influenced by the availability of NO₃⁻ and bulk density. Denitrification is responsible for only a part of the actual field N₂O emissions due to a lower potential for the fynbos riparian ecotones to produce N₂O under laboratory conditions that simulate an ideal environment for denitrification to take place. Nitrogen mineralization through the process of nitrification makes up the other part of the field N₂O emissions that were measured during the study under field conditions, which was higher than the potential for denitrification to occur (Table 4.1.). Fortunately riparian zones do not only rely on denitrification as a pathway to reduce nitrogen from the environment. Unfortunately the alternative ways to get rid of nitrogen such as the mobilization of sediment during floods and vertical leaching of nitrogen in the form of nitrate during wet periods may lead to contamination of water resources.

Organic carbon provides energy to the soil microbial biomass and the silt content allows more micro-organisms to occur within the soil. The soil nitrogen cycling processes are influenced by organic carbon content and include ammonification, nitrification, and the production of N_2O gas from denitrification (under anaerobic conditions) or nitrification (under aerobic conditions). An increase in organic carbon cause an increase in nitrogen cycling, especially in areas that are prone to receive organic matter such as the invaded sites and dry banks, which have enhanced inputs of nitrogen in the form of NH_4^+ and NO_3^- (Table 4.1.). A negative relationship between NO_3^- and EC implies that a low electrical conductivity reduces the ability of the soil to transport other nutrients. The sand, silt and clay content and the organic carbon content is also affecting the denitrification potential of the Eastern and Southern Cape.

The trends in nitrogen stocks, mineralization, available nitrogen, actual immobilization and potential immobilization are indicated for the natural, invaded and cleared sites in comparison to the natural sites for one season, spring in the Western Cape in Table 4.1.

Table 4.1. Summary of the nitrogen stocks and associated process, the reduction thereof and the maximum denitrification potential for one season, spring, in the Western Cape. The up arrows indicate if invasion has a negative impact or heading towards recovery (down arrow) compared to the reference sites. Up arrows indicate an increase in the form of nitrogen or activity and the horizontal arrow indicates a transition from natural sites to being invaded and then the transition from invaded sites that was cleared; red indicates a negative disturbance and green indicates a trend towards recovery.

		Natural		Invaded		Cleared	
							
N stocks (kg/ha)	Total N	2 525.37	→	6 747.69	↑	8246.93	↑
	Organic N	2513.38	→	6 717.31	↑	8229.30	↑
	Inorganic N	11.99	→	30.38	↑	17.63	↑
Mineralization (kg/ha/day)	Organic N turnover	0.88	→	-0.313	↓	0.40	↑
Available N (kg/ha)	NH ₄ ⁺	0.27	→	2.73	↑	2.15	↓
	NO ₃ ⁻	11.72	→	27.65	↑	15.48	↓
Actual immobilisation (kg/ha/day)	Actual field N ₂ O	4.59	→	20.67	↑	9.27	↓
Potential immobilisation (kg/ha/day)	DEA	8.32	→	10.54	↑	6.31	↓
	DEAC	7.81	→	9.94	↑	6.53	↓
	DEAN	7.18	→	10.42	↑	7.02	↓
	DEA C+N	7.95		9.72	↑	5.97	↓

There was a general trend for invasion to increase the nitrogen content and activity, except for the organic nitrogen conversion (nitrogen mineralization), which showed a decline and a tendency for nitrogen stocks and mineralization to increase following clearing, but a decrease in available nitrogen and N₂O emissions that may lead towards recovery of riparian functioning in terms of nitrogen cycling (Table 4.1.).

2. Recommendations

This study investigated some of the impacts that invasive alien *Acacia* spp. have on fynbos riparian ecotones and what the impact of clearing is. The main focus of the study was to investigate nitrogen mineralization, nitrogen availability, field N₂O emissions and the maximum denitrification potential on the changes that invasive alien plants may have on the system and whether clearing efforts leads to the restoration of these zones through the conversion of NO₃⁻ to N₂O that is rapidly converted in the atmosphere to N₂. It is important to know that there is no one simple solution or quick fix for the restoration of the fynbos riparian ecotones due to the dynamic nature of the functioning of nutrient

cycling (Naude, 2012) and the potential for denitrification to take place under favourable conditions. It is clear that invasive alien *Acacia* spp. adds nitrogen to the system.

There are some soil properties that cannot be influenced by human intervention when attempting to restore these zones through alien vegetation clearing. They include soil moisture, soil temperature, pH, electrical conductivity, soil particle size content, organic carbon content and the presence of soil micro-organisms. When attempting to restore these systems with clearing of the invasive alien vegetation the whole system and all the factors that affect nitrogen cycling must be taken into account. It is not beneficial to remove the invasive alien vegetation during a season or conditions where nitrates can be leached and have a negative impact on the groundwater or even the surface water bodies in these areas.

The invaded sites have the most available NH_4^+ and NO_3^- in winter and is the very last season that can be considered for clearing efforts to take place in future. There is the most soil moisture available of all the seasons due to high rainfall. This can cause leaching of NO_3^- to take place or conversely if leaching of NO_3^- does not take place there is the possibility for NO_3^- to be converted to N_2O through denitrifying soil micro-organisms if anaerobic soil conditions occur. Lower soil temperatures during winter may limit the activity of denitrification and recovery of soils from excess nitrogen. It is recommended that clearing efforts take place in spring when there are sufficient moisture levels in the soil after the winter season in order for denitrification to reduce nitrogen from the system, even if denitrification is not the most effective process.

The soil micro-organisms can perform their normal functions and still make nitrogen available due to the higher temperatures and they may also take up the excess nitrogen that is released as a result of the symbiosis with alien bacteria that is broken when the invasive alien vegetation is cleared. The lower levels of soil moisture during spring will reduce the potential leaching of NO_3^- in the fynbos riparian ecotones that is dominated by sandy soils with very little silt and clay, which makes these ecosystems even more prone to nutrient loss due to a low nutrient retention capacity. It is also very important to prevent environmental degradation in these systems as these riparian ecotones are part of catchment areas that assist in securing clean water fit for human consumption when we consider the fact that we are actually a water scarce country and need to start looking after our water resources from now on and must continue to do so in future.

Clearing of IAPs can also not take place with the expectation that spontaneous recovery of the structure and function of the riparian ecotones will take place such as planting resilient indigenous riparian trees, because it may not happen naturally and it may be necessary to put active restoration efforts in place following clearing. It has been observed in this study that there are still legacy effects of enhanced nitrogen in the form of mainly NO_3^- , but also NH_4^+ (Table 4.1.). These legacy effects remain for up to seven years after alien vegetation clearing have taken place and has not yet recovered from added nitrogen inputs to date in the example of this study. The invasive alien Acacias

add more nitrogen to the system than the natural vegetation. When clearing has taken place it is recommended to remove the leaves that are high in nitrogen content and leave the woody debris on the ground to prevent excessive runoff and removal of the topsoil layer through erosion. The woody debris will also assist in preventing extreme loss of soil moisture by creating a cooler micro-habitat where they cover the soil surface. It also serves as refuge areas for seeds that are transported down the river and may facilitate the establishment of indigenous plant species.

3. Future research

This study laid the foundation for the investigation of nitrogen mineralization, available nitrogen, field N_2O emissions and the maximum denitrification potential in fynbos riparian ecotones as well as some of the soil physical and chemical properties that play a role in maintaining ecosystem functioning in terms of nutrient cycling and the effect of clearing on these systems compared to natural vegetation. It is necessary that the leaching potential be determined before any management decisions regarding clearing is considered in fynbos riparian ecotones. This study made assumptions on the leaching of NO_3^- by taking into account the soil moisture levels and the availability of NO_3^- in different seasons, but especially during winter when it was expected that leaching may be higher due to higher rainfall in this season. The availability of nitrogen was not determined after incubations took place and this might have shed some valuable light on the availability of the substrate for denitrification to take place in the future. The nitrogen content after incubation could have been a good indication of whether nutrients were depleted by denitrifying soil micro-organisms. The nitrogen content after incubation could also be an indication of whether the N_2O emissions from the field were actually from denitrifying soil micro-organisms.

It will also be valuable to determine the optimum temperature and soil moisture under which the various nitrogen cycling processes take place through laboratory incubations, but preferably out in the field as these studies are very limited in fynbos riparian ecotones specifically in the Western Cape. It will also add value to do a study on the nitrogen-fixation ability of indigenous nitrogen-fixing species that occur in the fynbos riparian ecotones of the Western Cape and to compare them to the Eastern- and Southern Cape species to determine possible differences in the amount of nitrogen in the system that originated from the atmosphere, the only part of the nitrogen cycle that was not measured in the current study. This may give an indication of whether the invasive alien vegetation have the same nitrogen utilisation strategies in areas where nitrogen is already limited and to show whether the invasive alien vegetation have a competitive advantage over indigenous species for nitrogen usage. This may assist further understanding of the nutrient cycling in alien nitrogen-fixing plant species and how to best manage them in future by clearing efforts that are costly and sometimes ineffective.

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