TOWARDS THE DEVELOPMENT OF A TOOL TO QUANTIFY AND MONITOR STREAM RESTORATION SUCCESS FOLLOWING REMOVAL OF RIPARIAN ALIEN INVASIVE PLANTS

Report to the

Water Research Commission

by

JP Simaika, S Jacobs, Z Railoun, K Wiener

University of Stellenbosch

WRC Report No. 2460/1/18

ISBN 978-0-6392-0038-5

August 2018

Obtainable from:

Water Research Commission
Private Bag X03
Gezina, 0031

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

Printed in the Republic of South Africa

EXECUTIVE SUMMARY

Rationale

Invasive alien plants pose a major threat to a sustainable future for South Africa's water resources. A recent estimate of efforts to control the major invasive taxa, *Acacia*, *Pinus* and *Hakea*, over the past two decades, puts the cost at R564 million. Already experiencing major drought conditions, South Africa's Cape Floristic Region (CFR) is predicted to become drier overall. Rivers in the area may lose significant stream flow in the near future, causing losses in biodiversity and water quality. Invasive alien plants aggravate the shortfall of available water and pose a serious threat to the functions and processes of the region's ecosystems. Models predicting future consequences of a lack of management of invasive alien plants and water resources in the Western Cape indicate that if the current growth of alien plants goes unchecked from the present state for 100 years, some species might invade up to 62.4% of the catchment area with about 87 million m^3 of water lost due to this invasion. This could severely constrain the economies and quality of life in the CFR' major towns and cities, and lead to major biodiversity losses in a recognised global biodiversity hotspot.

Restored river systems not only provide more fresh water, but also buffer against floods and bank erosion, recycle nutrients, and create habitats for an array of biota. In an effort to control invasive alien plants and eventually restore riparian areas, the government of South Africa initiated the Working for Water (WfW) programme. The major objective of WfW is to restore and maintain natural resources by clearing invasive alien plants while creating jobs and economically empowering unemployed people from historically disadvantaged communities. However, stream restoration mainly focuses on removing invasive alien plants to increase water availability. Riparian zones are left to self-repair without monitoring, which causes them to revert back to alien stands, making future clearing as costly as initial clearing.

Little attention is paid to the high loads of effluent discharge, particularly nitrogen and phosphorus concentrations, that many streams carry. For example, in a comprehensive study of 20 of the largest river catchments in South Africa and their prevailing nutrient status between 1970 and 2005, it was found that nutrient levels generally exceeded water quality guidelines, which threaten both plant and animal biodiversity. Indeed, in the Western Cape, the Berg River system provides favourable conditions for eutrophication for most of the year. The dire findings and future predictions of water scarcity in the CFR highlight the need for more concrete information on the ecology of the effect invasive plants may have before and after clearing on water quality, sedimentation and aquatic biodiversity.

Study aims

The project had three overall study aims:

1. To determine the effects of riparian restoration on stream species functional diversity and abundance of stream species and compare this to natural and invaded conditions.
2. To determine the effect that riparian zone restoration has on nutrient cycling (nitrogen) on streams and compare this to natural and invaded conditions.
3. To quantify the nature and significance of the relationship between functional diversity and abundance of stream organisms and nutrient cycling.

Expected results or products

The expected major contribution of the project was to publish information with the potential to influence policy guidelines on water quality monitoring after removing alien trees and thereafter restoring riparian corridors. In addition, the recommendations from this project were expected to have a direct impact on water quality, and thus enhance indirect benefits derived from streams with high ecological integrity, i.e. functioning of trophic cascades. Finally, by focusing on the dynamics of nutrient inputs by invasive species in the sampled streams, and how the streams are affected by invasion and clearing, this project aimed to contribute to the sustainable management of clean water for delivery to communities. Part of the physical outputs were to include a publication on the targeted management and restoration methods for Cape streams, and a student was to be trained, enhancing and building the capacity in the fields of restoration ecology and freshwater ecology, areas of scientific scarcity in South Africa.

Summary results

Our overall findings indicate that invasive *Acacia* stands had no effect on surface water quality, but on nutrient concentrations (total nitrogen and phosphorus) of infiltrated sediment. Furthermore, where there was a high connectivity between the riparian zone and stream, sedimentation was high and showed no sign of depletion. In contrast, where riparian connectivity was low at the invaded site, sediment loads were low and showed signs of depletion. However, nutrient concentrations of those sediments were also high, and there were elevated nitrogen and phosphorus concentrations in infiltrated sediments and at cleared sites (> 10 years). Annual leaf litterfall of *Acacia mearnsii* in riparian zones was five to seven times greater than that of native vegetation with two peaks in litterfall: one at the end of the dry season in mid-autumn, and the other in mid-summer. Nutrient (nitrogen) concentrations in *Acacia* leaf litter were up to ten times higher than that of native vegetation. While macroinvertebrates responded strongly to differences in invasion status, algae did not. There were only weak relationships between algal biomass and nutrients in streams.

Managing the clearing activities is perhaps most indicative here, as is prioritising areas for clearing. Riparian zones that have dense stands of invasive plants with high connectivity to streams, i.e. low banks, should be prioritised for clearing. This reduces the potential effect of continued sediment and biomass input. Clearing activities need to be geared towards reducing sediment input into the stream channel. These methods may come at increased costs. The water needs of downstream stakeholders need to be considered to choose management methods that reduce sedimentation, for example, simply including immediate replanting with indigenous plants, and active stream stabilisation methods.

This project addresses several needs identified in the National Resource Management (NRM) strategy document, which is deeply rooted also in the overarching vision and mission of the Department of Environmental Affairs regarding safeguarding and enhancing ecological integrity. Particularly relevant is that this project links threats to the functioning of natural systems by invasive alien species. The NRM strategy document also identifies the need for assessing the ecological outcomes of management interventions, such as invasive alien plant control.

The results from the project are not only primarily relevant to the WfW programme, but also to the Working for Ecosystems programme. WfW has identified the needs for ecological and hydrological information such as the need to (a) understand the impact of invasive plants on stream flow resources to prioritise investments in invasive alien plant management; and (b) knowledge on whether activities are improving water quality and flow. Working for Ecosystems seeks to understand the impacts of different invasive plant species on soil stability, erosion and siltation.

While this project did not find that invasion has negative impacts on surface water quality, it needs to be noted that this is entirely subject to catchment characteristics. In other studies outside South Africa, water quality effects have been demonstrated to be significant with related effects on aquatic biota. Thus, the potential negative effects on water quality from nitrogen-fixing invasive woody plants should not be discounted, but catchment characteristics mapped as part of a clearing strategy to target catchments where effects on water quality may be greatest. Nutrient concentrations of infiltrated sediment and the mobilisation of sediment are of concern. In natural streams with natural flow patterns this may be less of a concern, flow-regulated systems are likely to be impacted. The recommendation here is to consider flow management with catchment characteristics and reach consensus on which riparian zones to clear. This should be possible to do using appropriate desktop models.

Study objectives

A major objective of this project was to measure stream faunal and floral recovery based on the biotic measures of aquatic flora (green algae and cyanobacteria), benthic invertebrate species diversity and abundance, and the abiotic indicators of nutrient dynamics as a function of invasion (natural, cleared and invaded). Three research objectives were proposed to:

1. Quantify the effects of riparian restoration on stream species functional diversity and abundance of stream species.

   Are there detectable differences in the functional diversity and abundance of stream species in the control (natural), cleared and invaded sections?

2. Quantify the effect that riparian zone restoration has on nutrient cycling (nitrogen) on streams.

   Does dissolved nitrogen input differ between upstream and downstream sections of the sampling sites? Are there detectable differences in dissolved nitrogen between the control (natural), cleared and invaded sections?

3. Quantify the nature and significance of the relationship between functional diversity and abundance of stream organisms and nutrient cycling.

   Are there correlates between macroinvertebrate species functional diversity and abundance and the amount of available nitrogen in streams? Are there correlates between algal flora functional diversity and abundance and the amount of available nitrogen in streams?

On the advice of the review group, a fourth objective regarding sediment was added. Sediment may be a major driving factor in nutrient cycling, and thus the focus of the project included investigating relative suspended sediment loads and nutrient concentrations.

Study area

Two perennial rivers located in the CFR, namely, Wit River (Bainskloof) and Du Toit's River (Franschhoek Pass) were chosen for this study. Each river had three experimental reaches with different riparian vegetation categories, namely, (a) near-pristine fynbos; (b) cleared of alien vegetation; and (c) invaded. The study sites were located in protected areas that were free of land use that could affect water quality and trophic interactions (e.g. agriculture).

Methodology

Data were assessed during low- and high-energy flow events from January 2016 to February 2017. For the sediment study, nine bedload sediment baskets and one time-integrated suspended sediment sampler were placed at each site to quantify the relative sediment load, and to test for nutrients: total nitrogen, total carbon and total phosphorus. Samples were also used to determine the percentage of clay using particle size analysis to compare nutrient loading between sites. Temporal variation of the water level at the sites was measured every 30 minutes with pressure transmitter probes. In conjunction with the water level data, channel cross sections (five per site) were surveyed. These data were used to calculate discharge by means of a discharge rating curve.

For the litter study, litter traps were placed under both native and alien tree canopies in the near-pristine and invaded sites of the Du Toit's and Wit rivers. Samples were collected monthly from January 2016 to December 2016. Mean monthly values of leaf litterfall, expressed as dry weight in $g \cdot m^{-2}$ were calculated for each trap per site. For each season, leaf litter nitrogen and carbon concentrations (mg/g dry mass) were determined. Foliar samples of *A. mearnsii* and reference fynbos tree species were collected in mid-autumn (early April 2006) for $\delta^{15}N$ analyses. The natural abundance ^{15}N technique was used to determine the nitrogen-fixing ability of *A. mearnsii* growing in the riparian zones of the rivers. The fractional contribution of biologically fixed nitrogen was determined. In order to understand nutrient conservation strategies and determined absorption of nitrogen and phosphorus, the resorption efficiencies of the nutrients were determined from senesced leaves.

In order to test the home field advantage hypothesis that plant leaf litter tends to decompose faster in its native environment and that macroinvertebrates in the same environment favours the plant material above them, fynbos species and *A. mearnsii* leaf were placed litter in fine mesh bags in the native and new reach environments. A total of 384 (192 per river) leaf bags were placed in the river reaches for two experiments – decomposition without herbivory and decomposition with herbivory. Leaf bags were imbedded at both rivers from 4 November 2016 to 6 February 2017. Incubation periods were 14, 28, 48, 64, 80 and 102 days. At the end of each incubation period, 64 bags (32 per river, or eight per experiment per river) were removed.

For the study on benthic organisms, five rocks were collected at each sampling site for macro-invertebrate and algal sampling, and an additional five rocks for chlorophyll analysis. Ten rocks were selected randomly in-stream. Rock depths ranged from 10 cm to 60 cm in riffles and pools. The rocks were sampled starting downstream of the flow direction to avoid disturbing the sampling sites. Each rock was dislodged with as little disturbance as possible and immediately placed in a white plastic meat tray. Excess water was drained carefully without losing macroinvertebrates. The macroinvertebrates were picked off the rock and placed in a 50 ml plastic vial with 70% ethanol. The same rock was scrubbed with a toothbrush for a minimum of five minutes. Up to 45 ml of stream water was used to rinse the rock’s surface at intervals. Once scrubbing was completed, the algal solution was added to a 50 ml vial with 5 ml 95% ethanol. The rock’s area (length × width) was then measured and recorded on prelabelled waterproof self-sticking labels, which were placed on the tubes. Five rocks were collected for chlorophyll a analysis. These were selected from the same biotopes as the previous samples. The same general procedure was followed, except that a total of 50 ml of stream water was used to rinse the rock’s surface at intervals. Once scrubbing was completed, the algal solution was added to a 50 ml vial. The vial was then placed on ice and kept dark and cold in an ice cooler.

Results

Mean suspended sediment yields (SSYs) were significantly higher at the invaded sites than at the cleared and fynbos sites – increasing steadily from up- to downstream at Wit River. The results suggest that SSYs in all sites are supply-limited and subject to depletion, except for the invaded site on the Wit River. During the wet season, up to 80% of the total annual suspended sediment load was transported. Analysis of the hysteresis loops showed that the dominant sources of suspended sediment at the Du Toit’s River were from in-channel sources, which were primarily bank erosion and stored sediment on the riverbed. Additionally, relative specific SSYs were found to be solely related to catchment contributing areas. In contrast, the dominant sources of sediment on the Wit River were from a combination of in-channel and catchment sources. SSYs in the Wit River were found to be inversely related to the catchment contributions, indicating that invaded and cleared ecotones enhanced sediment delivery to the river. The sediment load increase in the invaded site was up to 260% compared with natural fynbos, with only a 60% increase at the cleared site. In contrast, invasion and clearing appeared to have no effect on sediment loads at the Du Toit’s River.

Invasion by *A.mearnsii* and clearing of the invasive plant increased total nitrogen and total phosphorus in infiltrated sediments of the Wit River. Mean total nitrogen concentrations in infiltrated sediments of invaded site were up to 30% and total phosphorous were up to 43% higher than in fynbos site. Furthermore, a long-lasting biogeochemical effect of invasion after clearing was detected at the Wit River regarding total phosphorus concentrations in infiltrated sediment. The mean concentration of total phosphorus was 40% higher in the cleared site than the fynbos site on the Wit River. The phosphorus concentrations in surface water were not significantly different between invasion statuses on both rivers. Similarly, ammonia, nitrate and nitrite concentrations in stream water were similar between invasion statuses.

Annual leaf litterfall of *A.mearnsii* in riparian zones was seven to ten times greater in *A. mearnsii* species (on average 297.49 g/m^2) than native vegetation (on average 34.44 g/m^2), with two peaks in litterfall: one in mid-autumn and the other in mid-summer at both perennial streams. In contrast, the native vegetation only drops leaves once a year in the early summer month of December. *A. mearnsii* also retained higher nitrogen concentrations (with a mean value of 24.82 mg·g^{-1} in leaf litter) than the native species throughout the year, which ranged from 3.23–8.63 mg·g^{-1} between seasons. The $\delta^{15}N/\delta^{14}N$ isotope ratio showed positive $\delta^{15}N$ signatures in *A. mearnsii*, suggesting that the deep rootedness of the species allowed it access to ^{15}N-enriched organic matter in deep soils – an ability that the native plants did not have. The co-occurring native species were more efficient in the resorption of nutrients (nitrogen and phosphorus), which was on par with global averages of 56%. In contrast, *A. mearnsii* was inefficient in the resorption of nitrogen with an average of 21.86% resorbed.

Decay rates differed locally. At all sites, decay rates were initially similar, but diverged to twice as fast at the Wit River for *A. mearnsii*, while at the Du Toit's River, decay rates remained fairly similar. Nearly twice as many genera and up to four times the abundances of herbivores were observed at the Wit River sites. Herbivore preferences for alien vegetation differed locally. Litter bags with alien vegetation had twice the abundances of individuals at the Du Toit's River, whereas at the Wit River, at least for the alien site, there was no clear preference for either fynbos litter or alien litter. Functional feeding groups generally had no clear preference for either alien or native litter in any of the treatments across rivers.

There were no consistent differences between invasion statuses in the rivers, algal or macroinvertebrate densities. There were significant differences in benthic algal density and benthic macroinvertebrate densities and functional feeding groups only during the transition period between spring and summer (month of November). At the Wit River, where differences were found to be significant, the invaded site was dominated by scrapers, deposit feeders and predators. The cleared site had a high density of grazers, and also a very high density of deposit feeders.

Conclusions

- Mountain streams are suspended sediment supply-limited systems. The local hydraulic properties and site characteristics (e.g. channel morphology, lithology, vegetation cover, and topography) are important controls and produce substantial variations in the quantity of suspended sediment.
- The impacts of invasive alien plants on suspended sediment dynamics strongly depend on riparian zone connectivity. Low banks with dense stands of invasive alien plants increase SSYs and maintain sediment supplies in systems normally subject to sediment depletion.
- *A. mearnsii* leaf litter is highly abundant with seven to ten times that of native leaf litterfall. Leaves are dropped twice a year and are high in nutrient content, which is in contrast to native leaf litter, which is nutrient-poor and only shed once a year. This changes the diversity and abundances of macroinvertebrate genera – at least in the short term.
- Indeed, in this study, macroinvertebrates responded strongly to differences in invasion status, while algae did not. Weak relationships between algal biomass and nutrients in stream environments have been attributed to the complex interaction between physical and biological factors in mountain streams.

Recommendations

Management

Here, only two stream systems were investigated. We are cautious, therefore, to make broad management recommendations. However, in principle we suggest that:

- Rivers upstream of reservoirs or wetlands should be prioritised for clearing. This would reduce sedimentation and nutrient loads received.

- Riparian zones slotted for clearing need to be assessed for stream bank zonation and connectivity to the main channel of the stream bank.
- Invasive stands in riparian zones with low banks and high connectivity to main channel need to be prioritised for clearing. Stands with high connectivity to the main channel and vulnerable features (reservoir/wetland) should be prioritised over all other clearing activities in riparian zones.
- Clearing needs to be adapted where connectivity to the stream is high. We suggest that invasive stands could initially be thinned and revegetated with native fynbos plants tolerant of higher nitrogen soil content. While initially more expensive than clearing and leaving woody debris to decay, immediate revegetation may lead to more rapid recovery while at the same time being a cost-effective and rapid method for stabilising stream banks.

Research

This project highlights that although vast amounts of money are spent on clearing invasive alien plants such as *A. mearnsii*, which are touted as among the worst of invaders, knowledge of their basic ecology is lacking. We suggest that the principle of “know your enemy” should applied here. By understanding the ecology and physiology of Australian *Acacias* better, new avenues for the control of these invasive plants, which have not been considered before, become plausible.

- Our study suggests that *A. mearnsii* in established stands do not continually fix nitrogen. The conditions under which the plants fix nitrogen need to be investigated further.
- The long-term effect of *A. mearnsii* leaf litter input on the river food web remains unknown. A study investigating the energy value of *A. mearnsii* leaf litter would increase our understanding whether the leaf litter has any adverse effects on the reproductive potential or survival of aquatic macroinvertebrates. This is important as macroinvertebrates form the basis of the food chain on a relative level, and are important for maintaining fish populations.
- The impacts of riparian zone invasion by *A. mearnsii* depend on the highly complex and dynamic environments they invade, and their severity or benefit to the environment will differ substantially. We recommend that desktop models be established to determine the severity of potential impact in catchments. The same model could be used to help prioritise areas for clearing.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Water Research Commission (WRC) for funding this research project as well as the members of the Steering Committee for their advice, guidance and review of various deliverables and the final report, namely:

Mr B. Madikizela	WRC (Chairperson)
Dr N Allsopp	South African Earth Observation Network
Dr H Dallas	Freshwater Research Centre
Dr E Day	Freshwater Consulting Group
Mr A Khan	Department of Environmental Affairs
Dr P Ntshotsho	Council for Scientific and Industrial Research
Dr K Reinecke	Southern Waters
Dr N Rivers-Moore	Rivers-Moore Aquatics
Dr D Sharp	Department of Environmental Affairs

We would also like to thank Pashni Pillay, Adrian Simmers, Charlene Simaika, Cole Granger, Navan Langeveld and Earlon MCcloen (Project assistant), Kyle (Siva) Gertze, Juandre Ford, Bryan Meyer, Adam Railoun, Nick Marais (extra – project assistance), Imelda Heyns (Department of Water and Sanitation), Celeste Mockey, Riaan Keownn, Nigel Roberston, Annatjie French, Monean Jacobs (University of Stellenbosch Administration), Dr Alyssia Kleinert (Botany), Hanelie Botha (Department of Engineering, University of Stellenbosch), for their participation in the project.

PROJECT TEAM

Project leader	Dr John P Simaika
Researcher	Dr Shayne Jacobs
Student	Mr Zaid Railoun
Student	Mr Kenwinn Wiener
Student	Mr Armand van Wyk
Supervisors	Dr John Simaika[1], Dr Shayne Jacobs[1], Dr Suzanne Grenfell[2]

[1] University of Stellenbosch

[2] University of the Western Cape

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AFDMR%	Ash-free Dry Mass Remaining
ALI	Alien Sampling Site
ANOVA	Analysis of Variance
C	Carbon
CFR	Cape Floristic Region
CLE	Cleared Sampling Site
C:N	Carbon:Nitrogen
CSIR	Council for Scientific and Industrial Research
dbRDA	Distance-based Redundancy Analysis
DistLM	Distance-based Linear Model
EC	Electrical Conductivity
FFG	Functional Feeding Group
HFA	Home Field Advantage
IAP	Invasive Alien Plant
LC/MS	Liquid Chromatography–Mass Spectrometry
LMA	Leaf Mass Area
LSD	Least Significant Difference
MANOVA	Multivariate Analysis of Variance
N	Nitrogen
NAT	Natural Sampling Site
NH_4^+	Ammonium
NO_3^-	Nitrate
N:P	Nitrogen:Phosphorus
NRE	Nitrogen Resorption Efficiency
NRP	Nitrogen Resorption Proficiency
P	Phosphorus
PCA	Principal Component Analysis
pH	Potential of Hydrogen
PRE	Phosphorus Resorption Efficiency
PRP	Phosphorus Resorption Proficiency
PVC	Polyvinyl Chloride
RMANOVA	Repeated Measures Analysis of Variance
SD	Standard Deviation
SE	Standard Error

spp.	Species
SSP	Specific Stream Power
SSY	Suspended Sediment Yield
SSY-A	Area Suspended Sediment Yield
TC	Total Carbon
TN	Total Nitrogen
TP	Total Carbon
USA	United States of America
WfW	Working for Water
WMA	Water Management Area
WRC	Water Research Commission

Page left blank intentionally

CHAPTER 1 – GENERAL INTRODUCTION

Owing to the Cape Floristic Region's (CFR) very high globally distinct diversity in a small area and exceptionally high threats to the area's biodiversity, the CFR is considered a global biodiversity hotspot (Mittermeier et al., 1998). Among the greatest threats to the CFR's biodiversity is invasive alien plants (IAPs) (Raimondo et al., 2009). Indeed, if plant invasions were to reach the full extent of their potential distribution, biodiversity may decline by as much as 40% in the region (Scholes & Biggs, 2005; Van Wilgen et al., 2008). Ongoing invasion, particularly by woody IAPs, threatens to reduce surface water run-off by as much as 36% (Van Wilgen et al., 2008). This in turn can lead to reduced dilution and increased concentrations of nutrients, pollution and suspended solids (Chamier et al., 2012). Coupled with climate change, such reductions will lead to extinctions and enormous economic costs (New, 2002; Richardson & Van Wilgen, 2004).

Several woody IAPs seem to thrive in a Mediterranean climate with nutrient-poor soils, which is characteristic of the Fynbos Biome of the CFR. Alien woody plants, particularly Australian *Acacia* spp. (*Acacia mearnsii*, *A. longifolia* and *A. saligna*) are widespread in the CFR. The most successful of these, measured by biomass and geographic spread, is the black wattle (*A. mearnsii*) (Brown et al., 2004). Black wattles form dense evergreen monoculture stands, effectively shading out any competing vegetation (Werner et al., 2010; Le Maitre et al., 2011). The plants also use more water than indigenous vegetation because of the increased above-ground biomass and higher evapotranspiration rates (Le Maitre et al., 2002), which decrease both surface water run-off and groundwater recharge (Görgens & Van Wilgen, 2004).

Recognising the economic and biodiversity threat, the South African government initiated the Working for Water (WfW) programme in 1995. WfW's mandate is to protect and maximise water resources, and to promote social equity through job creation for marginalised communities (Van Wilgen et al., 1998). Although vast tracts of land are being cleared, with some R564 million spent on clearing in the CFR in the last two decades, estimates are that even under moderate invasion scenarios, much more money would have to be spent to bring invasion under control in the coming two decades (Van Wilgen 2016).

1.1 Sediment Dynamics and Nutrient Loading

Clearly, clearing IAPs holds significant benefits in enhancing run-off from catchments and groundwater recharge. However, clearing also causes the loss of nutrient-rich top soil. Coupled with this is a lack of understanding of how these disturbances in the riparian zones will impact riparian bank stability, streambed morphology and water quality. In South Africa, no studies have quantified the amount of sediment that is released by invasion and subsequent clearing, and the nutrients that nitrogen-fixing invasive plants may be adding to the nutrient-poor aquatic ecosystems of the CFR.

Excessive sediment delivery may change channel morphology, aquatic ecosystems and habitats (Walling, 2008; Vigiak et al., 2016). Estimating the relative suspended sediment loads in rivers that drain areas invaded by woody IAPs and land cleared thereof provides new knowledge regarding sediment delivery to invaded and cleared river catchments. This research is among the first to investigate whether widely colonising invasive *Acacia* spp. do in fact alter the rate and timing of sediment transfer from the catchments to the stream.

Fynbos soils are oligotrophic and particularly poor in nitrogen, which is slow to cycle through the system (Stock et al., 1995). Few fynbos plants fix nitrogen: most are pioneers that colonise after fires and are replaced as other plants move in through succession (Cocks & Stock, 2001). These pioneers are short-lived and only contribute 0.5% of the nitrogen stocks to the ecosystem (Cocks & Stock, 2001). Invasive species such as *Acacia spp.* are by contrast effective nitrogen fixers, altering the nitrogen and carbon cycles, increasing productivity, and soil nitrogen and phosphorus nutrient stocks twofold (Ehrenfeld, 2003) while outcompeting native flora (Maron & Jefferies, 1999). The added nutrient in the riparian zone can strongly influence stream nutrient dynamics, turning the streams from sinks to sources (McClain et al., 2003; Vidon et al., 2010).

Stream ecosystems have a strong capacity to transform and retain nutrients; thus, in-stream biogeochemical processes can further influence nutrient chemistry along the stream (Peterson et al., 2001; Dent et al., 2007). While much attention has been focused on riparian soil biogeochemistry and water quality changes in streams as a function of invasive nitrogen-fixing plants, much less is known about the role of suspended sediment as nutrient carriers.

1.2 Leaf Litter Input and Decomposition

The ecology of *A. mearnsii* nitrogen inputs is not well understood, and require a deeper investigation into the amount and timing of litterfall, leaf nitrogen concentrations, nutrient acquisition and resorption of nutrients. The knowledge gained from this study will give a better insight regarding nitrogen contribution to terrestrial ecosystems and a critical understanding of the nutrient economy of *A. mearnsii* in the nutrient-poor fynbos riparian zones in the CFR in the Western Cape, South Africa.

Leaf litter inputs are a key source of energy for stream organisms (Kuglerova et al., 2017). IAPs dramatically change the quantity and quality of leaf litter inputs available to in-stream macroinvertebrates (Boyero et al., 2012), change consumer densities and growth rates, and lead to substantial changes in ecosystem function and macroinvertebrate communities (Levine et al., 2003). The additional nutrient input from plant invasion may change a river system from autochthon-trophic to allochthon-trophic (Powell et al., 2011). In the CFR, effects of *A. mearnsii* invasion on leaf processing and macroinvertebrate communities in streams remains unstudied.

CHAPTER 2 – GENERAL METHODS

2.1 Climate, Geology and Hydrology

The CFR is characterised by a seasonal Mediterranean climate with cool wet winters and hot dry summers. More than 70% of rain occurs between April and September (Sieben, 2003; Rebelo et al., 2006). The Fynbos Biome is thus characterised by small, narrow streams defined by strongly seasonal patterns of flow, with high flows in winter and spring in response to precipitation, and low flow in summer (Corbacho et al., 2003). Fynbos ecosystems are adapted to periodic fires while soils tend to be old, shallow, sandy and acidic in nature with low nutrient status (Rebelo et al., 2006).

Characteristics of a particular site within the river network in part depend on the geological substrate of the catchment (Reinecke et al., 2007). Site characteristics are in part determined by geology, hydrology and inputs of organic/inorganic material from the surrounding catchment. In the Fynbos Biome, the hard sandstone and granite bedrock has led to constrained river reaches in the headwaters. As a result, erosion is the dominant geomorphological process in mountain stream zones. As elevation is relatively steep, stream flow is high. High-powered headwater stream sections of the river dominate the landscape because of the short distance between the mountains of the Cape Fold Belt and the coast (Prins et al., 2004). The lithology downstream changes to calcareous sand and shale-derived soils (Sieben et al., 2009). As a result of these dominant geological substrates, many river floodplains in the Fynbos Biome are covered with deep sandy alluvium (Rebelo et al., 2006).

2.2 Desktop Site Selection for Surveys

Sample sites were chosen based on the history and intensity of the *Acacia* invasion, the absence of appreciable invasion by IAPs, the management history, and where feasible, the same location where previous studies were carried out, for example, the studies of Reinecke et al. (2007), Vosse (2007), Blanchard and Holmes (2008), Naude et al. (2012), and Jacobs et al. (2013). The sampling design called for nine sites consisting of three natural (reference), three invaded sites and three cleared sites (Figure 1, Table 1). We followed further sampling constraints as in Jacobs et al. (2013): invaded sites had to be predominantly *A.mearnsii* or a mixture of *A.mearnsii* and *A. longifolia*, must have been invaded for at least more than 10 years, and have an aerial cover of at least 50% *A. mearnsii* or a mixture of *Acacia* spp. Cleared riparian sites were prior invaded sites that had been cleared more than seven years ago with regular follow-up treatment, and with *A.mearnsii* as the dominant invader.

Further to these selection criteria, sites were primarily chosen based on their similarity to each other (same or similar geology). However, both upper and lower foothill sections were chosen as not enough sites were found in one longitudinal zone. Upper foothills have a slope of between 0.005 and 0.0019. The main substrate on the riverbed is cobbles or a mixture of bedrock as well as cobbles (Rowntree et al., 2000). Cobbles are characterised as being 64 mm to 256 mm in diameter while gravel is mostly between 2 mm to 4 mm in diameter (Ollis et al., 2013). On occasion, sand and gravel may be present on the riverbed. Upper foothills have pools, riffles and rapids as river flow types. The lengths of the flow types are similar; therefore, the length of the pools will be roughly equal to the length of the riffles or rapids (Rowntree et al., 2000). Generally, the slopes of lower foothills slope range between 0.001 and 0.005. The substrate of the riverbed consists mainly of sand and gravel but occasionally bedrock may also be present. Flow types include pools, riffles and rapids. Sand bars may be present within pools. The extent of pools is often larger than riffles or rapids (Rowntree et al., 2000).

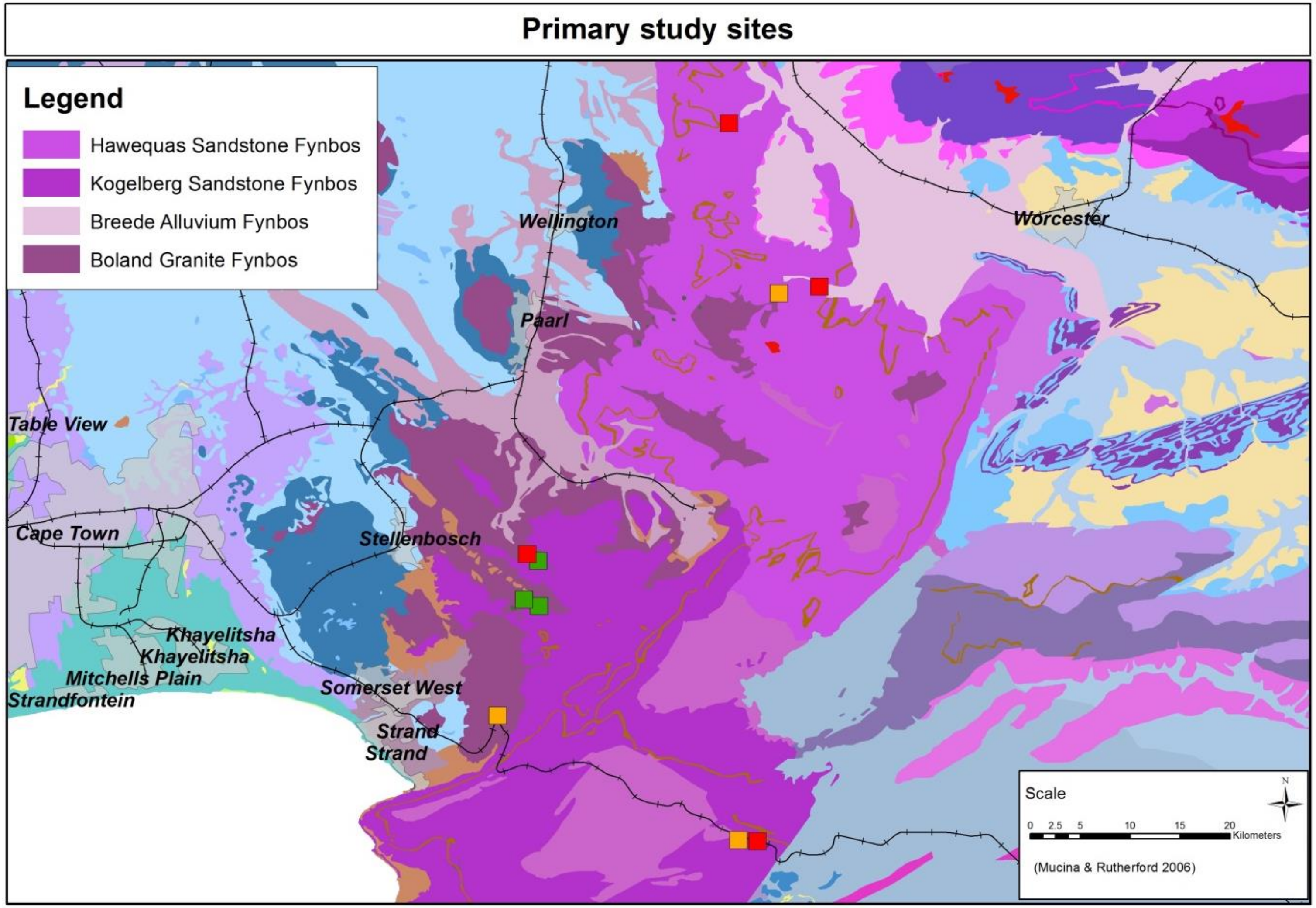


Figure 1: Map showing the location of the sites used in the main part of the study. Natural (reference) sites are indicated with green symbols, invaded sites with red symbols, and cleared sites with orange symbols. The map was created with spatial data provided by the South African National Biodiversity Institute BGIS. The colours denote different vegetation types as described by Mucina and Rutherford (2006). Vegetation types associated with sites are Kogelberg sandstone fynbos (Jakkals; Eerste; Dwars), Hawequas sandstone fynbos (Wit; Molenaars), Boland granite fynbos (Sir Lowry's Pass; Eerste; Dwars), and Breede alluvium fynbos (Molenaars; Wit).

Table 1: Characteristics and site-specific information of the selected sites within the Western Cape region. Information was gathered from local conservation agencies, farm managers and owners (from Jacobs et al., 2013)

Sites	Geology	Invasion status	Coordinates	Landowners	History of invasion	History of clearing	Fire history	Gradient	Longitudinal zone
Upper Eerste River	Sandstone/ granite	Natural	33.9537 S; 18.9788 E	CapeNature	No evidence	None	Burned in March 2009	0.038	Mountain stream transitional
Lower Eerste River	Sandstone/ granite	Natural	33.9895 S; 18.9675 E	CapeNature	No evidence	None	Burned in March 2009 and in 2015	0.058	Mountain stream
Upper Dwars River	Sandstone/ granite	Natural	33.9544 S; 18.9799 E	CapeNature	No evidence	None	Burned in March 2009	0.120	Mountain headwater stream
Wit River	Sandstone/ quartzite	Invaded	33.5384 S; 19.0154 E	Mount Bain Development	Invaded for ≥ 15 years *A. mearnsii*	No evidence	No evidence of fire at the start of the fieldwork, however, burned in March 2012	0.022	Transitional
Lower Molenaars	Sandstone	Invaded	33.7047 S; 19.2332 E	CapeNature	Invaded for > 10 years *A. mearnsii*/ *A. longifolia*	Cleared in summer 2011	Burned in March 2011	0.020	Transitional
Lower Jakkals River	Sandstone	Invaded	33.2088 S; 19.1774 E	Beaumont Wine Farm, Bot River	Invaded for > 15 years *A. mearnsii*/ *A. longifolia*	Yes, some evidence	No evidence of recent fire	0.050	Mountain stream
Lower Dwars River	Sandstone/ granite	Invaded	33.9481 S; 19.9698 E	CapeNature	Invaded for > 10 *years A. mearnsii*/ *A. longifolia*	No evidence	No evidence of recent fire	0.050	Mountain stream
Sir Lowry's Pass River	Sandstone/ granite	Cleared	34.0948 S; 18.9444 E	Wedderville Estate, Sir Lowry's Village	Mixed invasions: *Acacia* spp.+ *Pinus* spp. > 8 years ago	Cleared in 2002 and annual fooled up by landowner: fell and burn	No evidence of recent fire	0.068	Mountain stream

Sites	Geology	Invasion status	Coordinates	Landowners	History of invasion	History of clearing	Fire history	Gradient	Longitudinal zone
Upper Molenaars	Sandstone	Cleared	33.7107 S; 19.1970 E	CapeNature	Invaded for > 7 years mostly *A. mearnsii*	Yes. Initial treatment: 2002–2003. Follow-up treatments clearing treatment: fell and remove	No evidence of fire at the start of the fieldwork; however, burned in March 2011	0.044	Mountain stream
Upper Jakkals	Sandstone	Cleared	33.2169 S; 19.2072 E	CapeNature	*A. mearnsii*/ *A. longifolia* > 12 years	Initial clearance: 1996–1997. Two follow-up treatments. Treatment: fell and burn	Burned in January 2010	0.040	Mountain stream

2.3 Natural Sites

Reference (i.e. control/natural) sites are critical in restoration and rehabilitation efforts to provide compositional and structural information on vegetation communities and on historical disturbance conditions, which provide direction for restoration goals (Blanchard & Holmes, 2008; Sieben & Reinecke, 2008). Natural (control) sites were chosen based on a pristine or near-pristine condition, with no or minimal invasion by woody IAPs, free of, or with a minimal amount of human disturbance (Figure 2, Table 1). Natural sites were chosen to be within or as close as possible to the longitudinal zones (based upon river gradient and other characteristic) (King & Shael, 2001) where the invaded and cleared sites were also located. Currently, selected natural sites are mountain headwater streams with a gradient of 0.04–0.12, within the boundaries of protected areas, and no previous history of invasion.

Figure 2: Example of natural sites (see Table 1 for complete set). Left: Lower Eerste River natural site, with Prionium serratum a prominent component of wet banks. Right: Upper Eerste River natural sites, with dry banks consisting of Brabejum stellatifolium and Metrosideros angustifolia. Photos: S Jacobs

2.4 Cleared Sites

The cleared sites are all mountain streams with gradients ranging from 0.04 to 0.07. Two of the sites are within protected areas and one is on private land (Figure 3, Table 1). These sites have been cleared between 10 and 20 years ago (1996–1997 and 2002–2003). Ideally, cleared sites to be sampled are five years post-clearing and follow-up treatment as sites cleared in the more distant past may have recovered to near-reference condition.

Figure 3: Example of cleared sites (see Table 1 for complete set). Sir Lowry's Pass River site (cleared). Left: Riparian zones supporting high grass cover. Right: Scars present in terrestrial zones caused by slash that was burned in piles. Photos: S Jacobs

2.5 Invaded Sites

Of the invaded sites, two are mountain streams and one is transitional (Figure 4, Table 1). The riparian site consists mainly of *A. mearnsii* or a mixture of *A. mearnsii* and *A. longifolia* invaded for at least more than 10 years, or reinvaded after clearing. The gradients range from 0.02 to 0.05. One site is in a nature reserve, one on a wine farm, and one is home to a development. As some of the previously selected sites have burned or have been cleared since the completion of previous projects, replacement sites were selected based on selection criteria set out above.

Figure 4: Example of invaded sites (see Table 1 for complete set). Left: The Molenaars River invaded site (mostly A. mearnsii). Right: Jakkals River invaded site (mostly A. mearnsii). Photos: S Jacobs

2.6 Description of Site on Rivers

After visiting the selection site, the Wit and Du Toit's Rivers were considered as the most suitable. The main geomorphological features and the invasion status of each of the sampling sites are summarised in Table 2. There are additional factors to consider for each of these rivers.

Wit River:

- The campsite, Tweede Tol, is in close proximity to the cleared site.
- The presence of invasive fish species.
- Interbasin transfer.

The invasive fish species *Micropterus dolomieu* is present downstream of the waterfall upstream of the Tweede Tol camping site (Shelton et al., 2014). The invaded site and the cleared site are both situated below the waterfall. Therefore, fish species composition will be different at the natural sampling point.

Du Toit's River:

- Clearing history of cleared as well as invaded site needs to be confirmed.
- Sewage discharge from camping site upstream of natural site.

The clearing history of the Du Toit's River will be established with the WfW programme or CapeNature prior to initiation of sampling. Owners of the campsite will be contacted regarding the treatment or removal of sewage.

Table 2: Geomorphological characteristics and site-specific information on each of the selected sites within the Western Cape region

Sites	Coordinates (°)	Altitude (m amsl)	Invasion status	History of invasion	Longitudinal zone	Geology	Landowner	Dominant substrate	Comments
Wit River									
Wit River 1	−33.583296 19.134988	330	Natural	None	Mountain stream	Sandstone/ quartzite	CapeNature	Bedrock, large boulders	
Wit River 2	−33.569920 19.129420	294	Cleared	*A. mearnsii*/ *A. longifolia*	Transitional	Sandstone/ quartzite	CapeNature	Bedrock, large boulders	
Wit River 3	−33.551780 19.160930	262	Invaded	Invaded more than 15 years *A. mearnsii*	Transitional	Sandstone/ quartzite	Mount Bain Development	Large Boulders, Stones	
Du Toit's River									
Du Toit's 1	−33.916610 19.162592	601	Natural	None	Mountain stream	Sandstone/ quartzite	CapeNature	Bedrock, large boulders	
Du Toit's 2	−33.946636 19.168288	354	Cleared	*A. mearnsii*	Transitional	Sandstone/ quartzite	CapeNature	Large Boulders, Stones	
Du Toit's 3	−33.954028 19.171662	345	Invaded	*A. mearnsii*	Transitional	Sandstone/ quartzite	CapeNature	Large Boulders, Stones	

CHAPTER 3 – QUANTIFICATION OF RELATIVE SUSPENDED SEDIMENT LOADS IN TWO A. MEARNSII-INFESTED MOUNTAIN STREAMS OF THE CFR, SOUTH AFRICA

3.1 Introduction

Freshwater resources of the CFR are under constant and increasing threat from the establishment and spread of IAPs (Richardson & Van Wilgen, 2004) as well as climate change (New, 2002). IAPs are specialised in establishing in mountain catchments along watercourses of nutrient-poor and disturbed environments, as is characteristic for riparian zones of the Fynbos Biome in the CFR (Versfeld et al., 1998; Cullis et al., 2007). Several Australian *Acacia* spp. (*A. mearnsii*, *A. longifolia* and *A. saligna*) are widespread in the CFR, especially the Berg and Breede River catchments of the Western Cape, with the Breede catchment having a densely invaded area of 84 398 ha (Versveld et al., 1998). The primary invader is *A. mearnsii* (Brown et al., 2004), which tends to form dense evergreen monoculture or near monoculture stands that severely transform and degrade riparian ecosystems and often render the invaded area unsuitable for the survival of indigenous plants (Richardson et al., 1997; Holmes et al., 2005; Werner et al., 2010; Le Maitre et al., 2011).

Comprehensive research has shown that dense IAP stands, especially along watercourses in South Africa, use more water than indigenous vegetation such as plant communities in the fynbos and grassland biomes because of their increased above-ground biomass and evapotranspiration rates (Le Maitre et al., 1996; 2002). This decreases both surface water run-off and groundwater recharge (Görgens & Van Wilgen, 2004) and puts more strain on natural resources and water-dependent ecosystems (i.e. wetlands, estuaries and springs) (Le Maitre et al., 2002; Ehrenfeld, 2003, Van Wilgen et al., 2012). In turn, this can lead to reduced dilution and increased concentrations of nutrients, pollution and suspended solids (Chamier et al., 2012).

Given the increasing spread and threat of IAPs on South Africa's limited water resources, the WfW programme was initiated in 1995 to eradicate and control IAPs. WfW seeks to protect and maximise water resources and enhance sustainability by eliminating IAPs, thus enhancing ecological integrity while promoting social equity through job creation for marginalised communities (Van Wilgen et al., 1998). Currently, extensive areas of land are being cleared under WfW (Jovanovic et al., 2009).

Studies on vegetation recovery following IAP removal in the Western Cape province of South Africa have outlined that natural vegetation recovery is slow, and a trajectory towards restoration does not always occur (Ruwanza et al., 2013a). Despite the gains in terms of water resources through these clearing initiatives, extensive areas are being exposed and have become vulnerable to erosion (Richardson & Van Wilgen, 2004). The dense vegetation overgrowth and increased above-ground biomass associated with IAPs often result in a shift from fynbos (shrubland) to tree-dominated stands, a loss of ground cover, and development of understorey vegetation. This increases soil erodibility, destabilisation and erosion rates; especially during storm events (Rowntree, 1991; Versfeld et al., 1995; Richardson & Van Wilgen, 2004). Thus, the invasion of woody IAPs, such as *A. mearnsii,* and clearing thereof are the main causes of change in vegetation and land cover in the headwater catchments of the Western Cape.

Soil erosion is a major problem confronting land resources in South Africa with 70% of the country's surface area affected by varying types of soil erosion (Le Roux et al., 2007; Le Roux 2011). Scott et al. (1998) estimate that soil erosion rates in undisturbed mountain catchments in the Fynbos Biome are on average very low: less than 1 $t{\cdot}ha{\cdot}yr^{-1}$ compared with the 0.02 $t{\cdot}ha{\cdot}yr^{-1}$ and 0.16 $t{\cdot}ha{\cdot}yr^{-1}$ on afforested slopes. Van der Waal et al. (2012) report increased soil movement on slopes that are invaded and then cleared of *A. mearnsii*, with erosion rates doubling on invaded slopes in the Kouga Mountains, South Africa. Increased soil erosion not only involves reduced soil productivity through loss of fertile topsoil, but also further extends off-site, for example, increased sediment delivery to watercourses and of suspended sediment loads transported by streams and rivers (Imeson, 1974; Wolman, 1977; Walling, 1983; 2005;; De Vente et al., 2007).

The suspended sediment load of a river is sensitive to both climate change and to a wide range of anthropogenic activities (e.g. land clearance, agricultural development and infrastructure development) within its catchment area that could influence sediment yield due to higher rates of soil erosion and sediment mobilisation. Although all streams carry sediments under natural conditions, excessive sediment delivery can: (a) pose a serious threat to water resource development through reservoir sedimentation and siltation of river channels; and (b) influence channel morphology and aquatic ecosystems and habitats (Walling, 2008; Vigiak et al., 2016).

The sediment loads of many of the world's major rivers have changed significantly in recent years due to land use change, reservoir construction and other anthropogenic impacts on their drainage basins (Walling, 2008). However, this aspect has received little attention in South Africa. The amount of sediment transported by rivers is often used as an indicator of catchment disturbance as driven by extrinsic (e.g. climate or land use change) or intrinsic (e.g. vegetation or soil erodibility change) factors (Foster et al., 2012). For example, Grenfell and Ellery (2009) state that suspended sediment concentrations in the Umfolozi River (Kwa-Zulu Natal) are poorly correlated to discharge. Instead, the availability of sediment in the catchment is likely the result of a reduction in vegetation cover through vegetation dieback during dry months, and the capacity of overland flow to transport available sediment into the river channel during the rainy season.

Estimating the relative suspended sediment loads in river-draining areas invaded by woody IAPs and land cleared thereof will provide a good estimate and a more complete understanding of the level of disturbances these changes in vegetation structure have on sediment delivery to these river catchments. This research is among the first to investigate whether widely colonising invasive *Acacia* spp. do in fact alter the rate and timing of sediment transfer from the catchments to the stream. The aim of this study was to:

- Evaluate the spatial and temporal variability and physical properties of relative suspended sediment loads of two mountain streams in the CFR.
- Compare the results to hydrological and geomorphic data.
- Quantify the sediment contributions from near-pristine fynbos, *Acacia*-cleared and *A. mearnsii*-invaded riparian sections.

3.2 Methods

3.2.1 Study site and general setup

The mean annual temperature in the Breede water management area (WMA) is 12°C with a mean annual rainfall of 1800 mm in high lying areas where the rivers are situated (Rebelo et al., 2006). The geology of the two rivers is dominated by Quarzitic Table Mountain Group sandstones (Rebelo et al., 2006). However, the fault system at the Du Toit's River is responsible for a mixture of rock types throughout the course of the river system. The Upper Du Toit's is dominated by sandstone where the lower sections consist of a combination of sandstone, shale, mudstone and siltstone. The hard impermeable quarzitic sandstone produces coarse textured soils with relatively low silt and clay concentrations, which accounts for the clear waters (Le Roux, 2011). The land use in the catchment is dominated by woody IAPs, which are predominantly *A. mearnsii*. The riparian zones of upper reaches of the rivers that occur in protective areas are still mostly in a near-pristine state with fynbos shrubland.

Measurements were set up in two perennial rivers of the CFR, namely, the Wit River (Bainskloof Pass) and Du Toit's River (Franschhoek Pass). Each river had three experimental reaches, namely: (a) near-pristine fynbos, (b) cleared and (c) *Acacia*-invaded (from up- to downstream; Figure 6) at different longitudinal zones and elevations of above sea level (see Table 3 for site characteristics). The reaches were relatively straight to ensure that discharge and sediment transport were distributed uniformly across the channel. Each site was equipped with nine bedload sediment traps that were equally spaced across the channel. The first row of three was placed towards the upstream section of the reach and the next two towards the downstream end. One time-integrated suspended sediment sampler was installed towards the downstream end of each treatment. Data was assessed from February 2016 to January 2017 to cover both high- and low-flow events.

Table 3: Morphometric characteristics of the Du Toit's and Wit rivers. Shear stress and stream power data are given as bankfull flow conditions

River	**Du Toit's**			**Wit**		
Site	**Fynbos**	**Cleared**	**Invaded**	**Fynbos**	**Cleared**	**Invaded**
Catchment elevation (masl)	982	947	924	958	919	901
Drainage area (km^2)	15.6	46.7	49.6	68.5	79.6	86.0
Mean catchment slope (%)[a]	37	44	43	38	37	38
Riverbed slope (%)[b]	5.1	3.4	2	1.5	0.8	2.9
Average channel width (m)	7.6	18.4	17.5	18.7	17.6	21.3
Average channel depth (m)	0.9	0.6	1.0	0.6	0.8	0.9
Cross-sectional area (m^2)	6.8	11.0	17.5	11.2	14.1	18.9
Boundary shear stress (N/m^3)	233	155	165	112	51	291
Specific stream power (W·m^{-2})	1066.1	847.3	1052.3	1407.9	774.7	313.3

a Calculation based on the slope value for each pixel from a digital elevation model of the catchments

b Slope value based on calculation from a total station survey of the channel bed ($\frac{h2-h1}{L}$)

Surveys of channel and riverbed characteristics, and channel and riparian morphological characteristics were assessed through river cross-profile surveys on five predefined transects along each study reach. The channel cross-profiles were surveyed with an electronic total station (Top-Shot GP – I22L) with a standard reflecting prism fixed onto a staff and measuring tape.

The river channel slope was defined by measuring elevations along the channel thalweg using the total station. This was ultimately used to calculate the specific stream power (SSP) of each reach expressed by the following equations:

Total stream power (TSP; W·m^{-1})

Equation 1. $$TSP = \gamma QS$$

SSP (W·m^{-2})

Equation 2. $$SSP = \frac{TSP}{W}$$

Where γ is the specific weight of water (980.7 kg·m^{-3}), *Q* is the discharge (m^3·S^{-1}), *S* is bed slope (m·m^{-1}), *w* is the channel bankfull width (m) (Bagnold, 1966; 1977).

Boundary shear stress (N·m^{-3})

Equation 3. $$\square_0 = p.g.R.S$$

Where $\square_0$ is bed shear stress, *p* is water density (1000 kg·m^{-3}), *g* is acceleration due to gravity (9.80665 m·s$-^2$), *R* is the hydraulic radius at bankfull water level, and *S* is the slope.

3.2.2 Time-integrated samplers

Time-integrated suspended sediment samplers following Phillips et al. (2000) were installed at each reach and emptied at monthly intervals to determine the spatial variation of the suspended sediment load. The suspended sediment samplers were 1 m long and consisted of commercially available polyvinyl chloride (PVC) pipes with an outer diameter of 110 mm and a wall thickness of 4.2 mm. They were sealed with a plastic traffic cone at the inlet and a PVC-threaded end cap at the outlet. A PVC tube with an inner diameter of 4 mm was passed through the funnel and the cap as inlet and outlet. The suspended sediment samplers were mounted parallel to the riverbed attached to two upright pieces of steel rebar driven into the channel bed, with the inlet tube pointing directly into the direction of the flow. The greater cross-sectional area of the main cylinder compared with that of the inlet tube reduces the flow velocity within the samplers. This reduction in flow velocity induces sedimentation of the suspended sediment particles as the water moves through the cylinder towards the outlet tube. The suspended sediment samplers collect a statistically representative sample under field conditions (Phillips et al., 2000).

At the end of each sample period, the contents of each sampler were washed out on-site using distilled water, and collected in a sealable 10 L plastic bucket. The buckets were predisinfected using phosphorus-free cleaning agents. The samplers were returned to their rebar anchorages and the depth was adjusted for monthly stage changes. Material collected by the suspended sediment sampler was allowed to settle (usually over 24 hours) in the buckets and the supernatant was removed by siphoning. Wet samples were transferred to foil buckets and oven-dried at 105°C for 24 hours. The sediment was weighed to determine the suspended sediment load. This was used to calculate the overall suspended sediment discharge for each sample site in $g \cdot d^{-1}$.

3.2.3 Sediment baskets

Fine sediment on the riverbed was determined with sediment basket traps following Bond (2002) and Schindler Wildhaber et al. (2011). Fifty-four traps (27 per river) were buried in the riverbed at three different locations and sampled monthly over one water year. The locations of each river were chosen in a way that the different vegetation conditions (i.e. *Acacia*-invaded, cleared and near-pristine fynbos) were represented. This experimental setup allowed the alien, cleared and natural sections of the rivers to be compared.

The baskets were made of a 50 × 50 piece of squared wire mesh (50 mm mesh) with the following dimensions: length (200 mm), width and depth of 160 mm (200 × 160 × 160). The traps were installed so that the top was flush with the streambed and were filled with clean gravel from the stream reach (grain size > 50 mm) (Figure 5). This setup allowed cobbles to roll over the basket while fine sediment could move through the basket and cobbles. To retrieve the baskets without sediment sample loss, a polyethylene bag with two long handles was placed around the baskets. The bag was pulled to the bottom of the trap before it was buried to enable water to flow through the trap while it was buried. The bag was pulled over the basket during removal to prevent loss of fine sediment.

The traps were buried *in situ* in a hole that was dug in the riverbed. The traps were placed far enough apart and in such a manner that there was no disturbance to the sample when a trap was placed or removed within its vicinity. Each reach was equipped with nine bedload sediment baskets. The first row of three was placed towards the upstream section of the reach and the next two towards the downstream end. The baskets were equally spaced at each of the three predefined traverses. The baskets were emptied at monthly intervals to investigate the monthly bedload sediment. The baskets' sediments were sieved in-field with a 4 mm sieve and refilled with the same gravel during each sampling event. The < 4 mm sediment was taken to the laboratory for drying, sieving and particle size analyses. The monthly infiltrated sediment load (< 2 mm) for the sample sites were weighed and expressed in $kg \cdot m^{-2} \cdot d^{-1}$.

Figure 5: Sediment Infiltration baskets filled with gravel used in capturing fine sediment (left); embedded basket in riverbed (right)

3.2.4 River discharge

Discharge in the Wit River was monitored at high frequencies at the Drosterskloof weir. However, in the Du Toit's River, the flow was not gauged and therefore it was necessary to develop a stage-discharge rating curve. The temporal variation of the water level was measured at 30-minute intervals at each of the six sites using capacitive pressure loggers (HOBO – U20L-02). The pressure was later converted to stage (h) and then transformed into discharge (Q) by means of the corresponding h/Q rating curve. At each site, h/Q rating curves were developed from direct velocity measurements. Velocity measurements were taken at each of established cross sections at each site. Mean velocity and water depth were measured at eight to ten points across each of the cross sections. Velocity was measured using a Marsh–McBirney Electronic Flo-Mate current meter on a top-setting wading rod. Aggregate discharge was calculated using the velocity–area method described by Gordon et al. (1992). The discharge of the river was used to assess temporal variability in suspended sediment concentration, as well as to calculate total sediment loads.

3.2.5 Sample analyses

Particle size distributions of grains with a diameter < 63 µm were measured with a Saturn DigiSizer 5200 (Micrometrics). Grain size fractions were classed according to Wentworth Udden (1922): sand: 63 µm^{-2}·mm; silt: 3.9–63 µm; and clay: < 3.9 µm and used to compare the grain size distributions between sites. The total loads were normalised for the catchment area to compare sites in different longitudinal zones within the catchments.

3.2.6 Statistical analyses

For monthly sediment infiltration rates, a two-way repeated measures analysis of variance (RMANOVA) was applied to test for significant interactive effects between invasion statuses across the sampling period. When the invasion statuses were compared, one-way analyses of variance (ANOVAs) were computed on all data collected irrespective of the months. Least significant differences (LSD) tests were used for post hoc means separations. The Spearman's rank correlation coefficient (rho) was done individually to analyse the relationships between sediment infiltration rates, suspended sediment flux with discharge, and suspended sediment infiltration rates and clay percentage. All statistical analyses in this project were done using the Statistica 13 software package (Statsoft, 2013).

3.3 Results

3.3.1 Channel morphology and hydrology

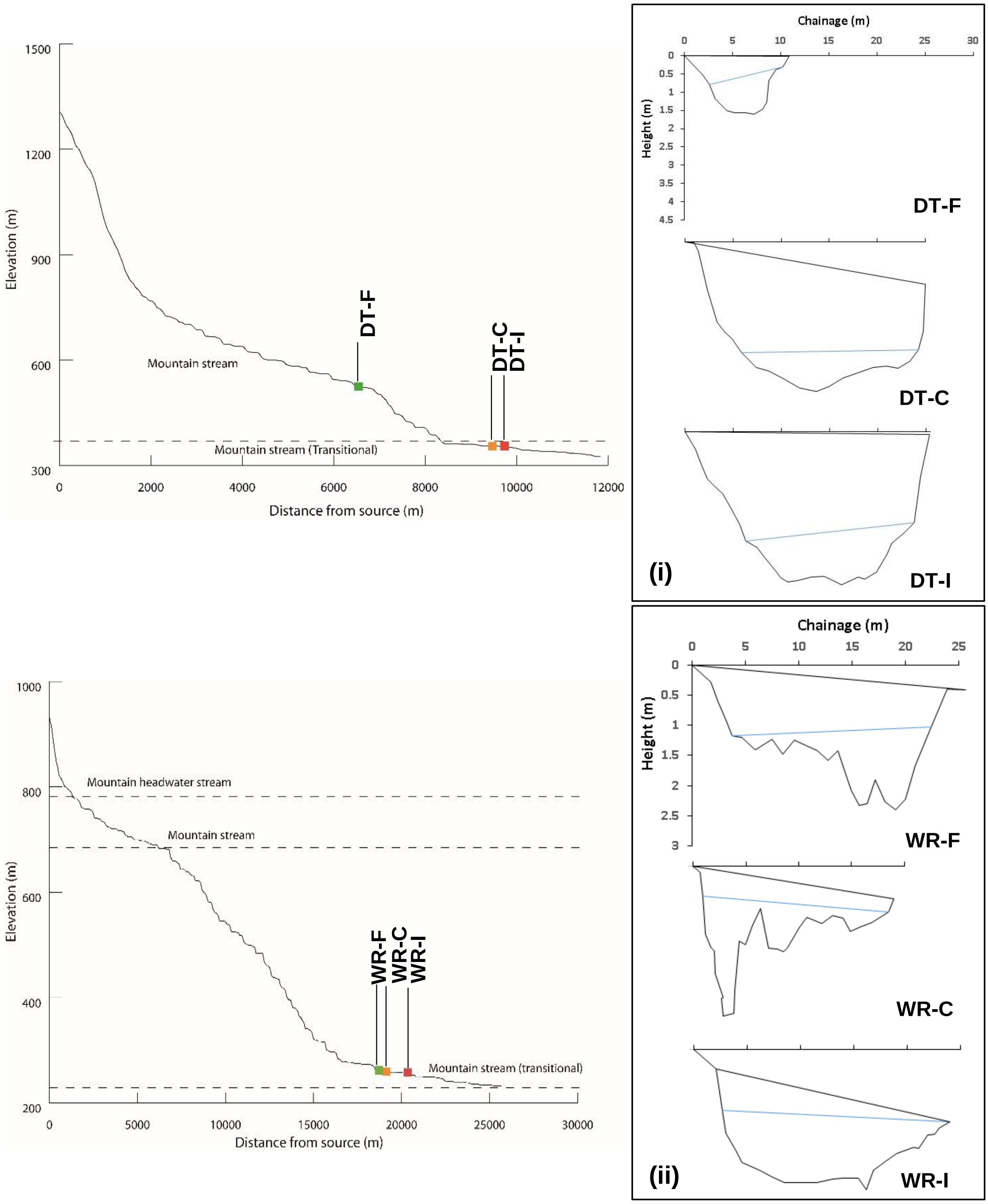


Figure 6: Longitudinal profiles of the (above) Du Toit's and (below) Wit River. Locations and channel cross sections of the three sites in their respective longitudinal zones. Dotted line indicates the transition from one zone to the next based on slope values calculated from 5 m digital elevation models. Inset (i) and (ii) show the channel cross-sectional surveys at each site with the blue line indicating water level at bankfull and black line the macro channel. Abbreviations: DT = Du Toit's River; WR = Wit River; F = Fynbos; C = Cleared; I = invaded

Site abbreviations:

- DT-F = Du Toit's River fynbos site
- DT-C = Du Toit's River cleared site
- DT-I = Du Toit's River invaded site
- WR-F = Wit River fynbos site
- WR-C = Wit River cleared site
- WR-I = Wit River invaded site

In general, the width of the river cross sections at the Du Toit's River increased progressively with distance downstream, ranging from 7.5 m at the upper section (DT-F) to 17–18.38 m in the lower sections (DT-C and DT-I; Table 3). The active channel at the DT-I site was deeply incised into the macro channel with relatively high and steep banks (Figure 6i). Cross sections WR-F and WR-I, located in the Wit River, were similar in terms of channel width riverbank height and depth (Table 3). The macro channel at the WR-C and WR-I sites were more pronounced on the right bank than the left bank (looking downstream) (Figure 6ii). Site DT-F had the highest stream gradient of 5.1% (Table 3). This was followed by sample site DT-C at 3.4% and site DT-I at 2%. Site WR-I had the highest stream gradient of 2.9% at the Wit River followed by WR-F at 1.5% and WR-C at 0.8%.

The SSP between the WR-F and WR-I sites is roughly comparable with 956 $W \cdot m^{-2}$ and 1053 $W \cdot m^{-2}$, respectively (Table 3). The SSP in the WR-C site, 470 W. m^{-2}, is around half that at WR-F and WR-I. The SSPs ($W \cdot m^{-2}$) are different across the three sites at the Du Toit's River, with the SSP in the DT-F site approximately 3.85 times higher than in the DT-I site and twice as high as the DT-C site (Table 3). This indicates that the channel at the DT-F site has a higher sediment transport capacity than DT-C and DT-I.

3.3.2 Suspended sediment flux

Only a small number of suspended sediment samplers withstood the high-energy flows; consequently, a reduced data set across the study period was available:

Wit River:

- WR-F: n = 9.
- WR-C: n = 10.
- WR-I: n = 12.

Du Toit's River:

- DT-F: n = 11.
- DT-C: n = 11.
- DT-I: n = 12.

This made statistical analyses and comparison between sites difficult. Thus, only general statements concerning the nett suspended sediment loads were possible. Generally, suspended sediment load at the Du Toit's River increased from upstream to downstream, i.e., from site DT-F to site DT-I. The maximum suspended sediment caught by the suspended sediment samplers at the Du Toit's River increased along the river from 4.34 $g \cdot d^{-1}$ at site DT-F and 13.43 $g \cdot d^{-1}$ at the DT-C site to 15.41 $g \cdot d^{-1}$ at the DT-I site (Figure 7c and Figure 7d). The maximum suspended sediment measured at the Wit River was 4.5 $g \cdot d^{-1}$ (Figure 7a).

Spearman rank analyses between suspended sediment samplers and sediment infiltration baskets were only feasible at the most downstream sites (WR-I and DT–I; Figure 7b; Figure 7d) of the rivers. Within and between seasons, strong monthly fluctuations in the suspended sediment loads occurred, which are largely a function of the mean monthly discharge and rainfall. There is an observable pattern in the timing of variations in suspended sediment load (demonstrated in Figure 7). The seasonal suspended sediment loads are also quite varied throughout the monitoring period with individual months contributing to the annual load. The spring and summer months contribute the least to the annual load with 0.16 $g \cdot d^{-1}$ and 0.99 $g \cdot d^{-1}$ respectively (Figure 7). The periods of highest suspended sediment transport are in autumn and winter.

The relationship between monthly rainfall and suspended sediment load reveals periods of the year where there is an abundance of suspended sediment available for transport, followed by depletion. These periods can be visualised and analysed through hysteresis loops (Figure 7). At WR-I, the hysteresis loop is a figure-eight shape. The figure-eight hysteresis loop can be interpreted as a counter-clockwise loop during low rainfall events and a positive clockwise loop during high rainfall events. Figure-eight hysteresis suggests that sediment was derived from remote sources. During summer and spring, suspended sediment transport was relatively low, which yielded only 0.04 g·d⁻¹ and 0.24 g·d⁻¹, respectively. The highest suspended sediment transport was during autumn and winter, which yielded 1.18 g·d⁻¹ and 5.52 g·d⁻¹.

In the Du Toit's River, the relationship between suspended sediment and discharge shows a positive clockwise hysteresis loop (Figure 7c and Figure 7d). Suspended sediment increased rapidly and peaked during the first high intensity rainfall event in June, before peak rainfall in July. After suspended sediment and rainfall reached their peaks, suspended sediment and rainfall decreased considerably. The periods of highest sediment transfer occurred in autumn and winter, which yielded 1.25 g·d⁻¹ and 27.80 g·d⁻¹ of suspended sediment respectively. The dry season (spring and summer) contributed the least suspended sediment with 0.16 g·d⁻¹ and 0.99 g·d⁻¹ respectively. Although there is still relative depletion in January, there is a greater availability of suspended sediment than the levels in August and September.

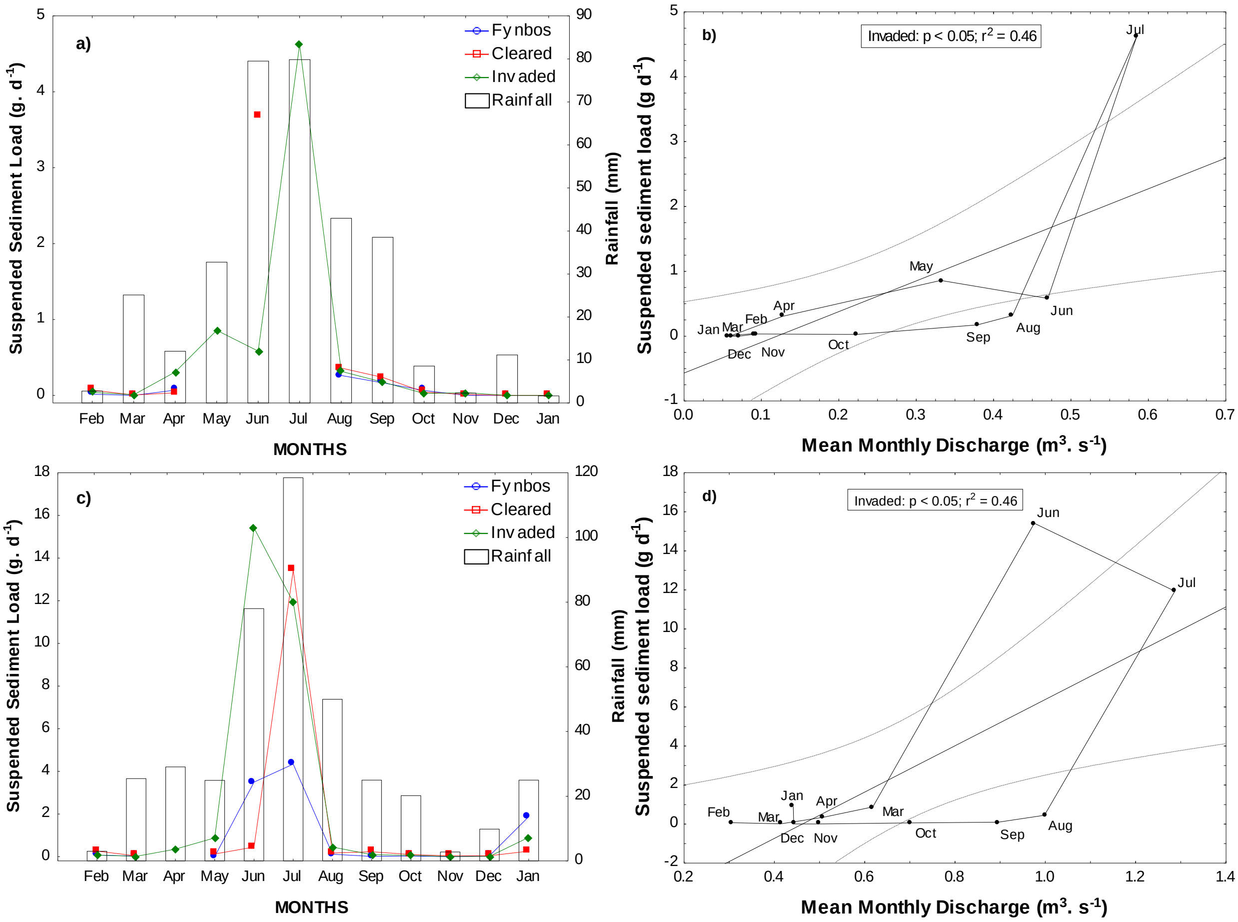


Figure 7: Monthly suspended sediment load caught by suspended sediment samplers compared with rainfall at the three sites for (a) Wit and (c) Du Toit's Rivers and suspended sediment load in relation to discharge at invaded sites for the Wit (b) and (d) Du Toit's Rivers. Note that April at the Du Toit's (fynbos and cleared) and May–July at the Wit River (fynbos and cleared) are not represented in the record. Note the different scales on the suspended sediment load axes

In positive clockwise hysteresis loops, sediments are derived from readily available sources. At the invaded site on the Du Toit's River, sediment transport peaked before rainfall and discharge (Figure 7d). This suggests that sediment has become available for transport throughout the dry season, and as soon as water is available to transport it, it moves. In contrast, in the cleared and fynbos sites, sediment transport peaks at the same time as rainfall. This suggests that less sediment has become available, and the system tends towards being supply-limited at the beginning of the rainfall season. The invaded site is conversely transport-limited at the beginning of the wet season. From this data, it is clear that more sediment is available for erosion and transport at the invaded site. In addition, the cleared site is producing more sediment than the fynbos site.

3.3.3 Temporal and spatial patterns in sediment infiltration

Significant overall temporal variation was observed for the sediment infiltration at the Du Toit's River (ANOVA: $F_{[10,\ 240]} = 64.10$; $p < 0.001$) with values ranging between 0.001 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ during low-flow conditions and 2.10 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ during high flow events (Table 4). Mean sediment infiltration rates during the dry season (October to March) were significantly lower at all sites with a mean of 0.08 ± 0.09 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ compared with the wet season (April to September) with a mean of 0.48 ± 0.26 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ (ANOVA: $F_{[10, 240]} = 64.10$; $p < 0.001$) (see Figure 10a). Similarly, significant temporal variation was observed at the Wit River with sediment infiltration rates ranging of 0.001 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ and 0.90 $kg{\cdot}m^{-2}{\cdot}d^{-1}$. The mean sediment infiltration rate at the Wit River was 0.02 ± 0.04 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ during the dry season (October to April) compared with 0.29 ± 0.16 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ during the wet season (May to September) (ANOVA: $F_{[9, 216]} = 68.16$; $p < 0.001$) (see Figure 10b). At all sites, an exponential increase in fine sediment infiltration rates was observed after the first high intensity rainfall event.

A linear regression model was applied to examine the relationships between sediment infiltration and discharge. The Spearman's correlation coefficients (rho) for the Wit River were all above 0.7 and reached up to 0.94 at the most downstream site (Figure 9a–c). These results suggest a strong relationship between sediment infiltration and water discharge ($R^2 > 0.7$; $p < 0.01$). Sediment infiltration rates show considerable temporal variability in the mean sediment infiltration rates for a given average monthly discharge, resulting in the generation of clockwise hysteresis loops at all sites of the respective rivers.

In February, at the start of the sampling period, discharge and sediment load were both low, at 0.09 $m^3{\cdot}s^{-1}$ and 0.05 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ respectively. In May, the discharge increases substantially to a value comparable to that seen in September (0.33 $m^3{\cdot}s^{-1}$). At the sites, WR-F and WR-C, the increase in sediment load is not of the same magnitude, with total mass of 0.07 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ and 0.13 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ being transported at the respective sites. These values are over 50% smaller than the mass flux during May 2016 (0.17 $kg{\cdot}m^{-2}{\cdot}d^{-1}$ and 0.31 $kg{\cdot}m^{-2}{\cdot}d^{-1}$). These findings suggest that between June and September, there seems to be a depletion of available sediment sources. However, this depletion never occurred at the WR-I site.

Similar patterns were observed at the sites of the Du Toit's River (Figure 9d–f). However, a lower coefficient ($R^2 < 0.5$) was observed at all the sites (Figure 9d–f). In general, relative sediment loads were low at the end of the summer dry season (February and March). The progressive increase in discharge produced a rapid rise in sediment load with high loads on the rising limb acting to produce strong clockwise hysteresis loops. At sites DT-F and DT-C, sediment loads and discharge produced a peak in August, whereas site DT-I produced a peak in May prior to discharge peak. This pattern parallels the data from the suspended sediment samplers (Figure 7c). Throughout the remainder of the monitoring period, sediment loads were reduced dramatically compared with those with comparable discharge. This was an indication of sediment depletion over progressive high flow events at all sites due to the flushing of readily available sediment accumulation in the channel.

Figure 10 shows the relationship between average grain size distribution of the sediment finer than 63 µm and mean sediment infiltration rates (< 2 mm) that infiltrated the samplers. The grain size analyses showed a decrease of silt and clay with increasing fine sediment infiltration in relative values (i.e. fraction of silt and clay of the total fine sediment deposition; Figure 10). Coarser grain sizes (2 mm and < 63 µm) had a tendency to be enriched during periods of higher discharge. Conversely, lowest concentrations of these size classes were found during low-flow periods, particularly over the summer months. The finer grain-size categories (< 63 µm), showed the opposite pattern; tending to be enriched during low-flow periods and depleted during winter peaks flows. During periods with low sediment infiltration rates and low-flow events, up to 0.97% of the sediment consisted of sediment < 63 µm compared with the 0.62% during high flows. Sediments of these size classes are most likely to be transported in suspension (Sear, 1993; Acornley & Sear, 1999).

Figure 8: Photos showing the distinct visual difference between (left) base flow and (right) high-flow conditions at the invaded site on the Wit River

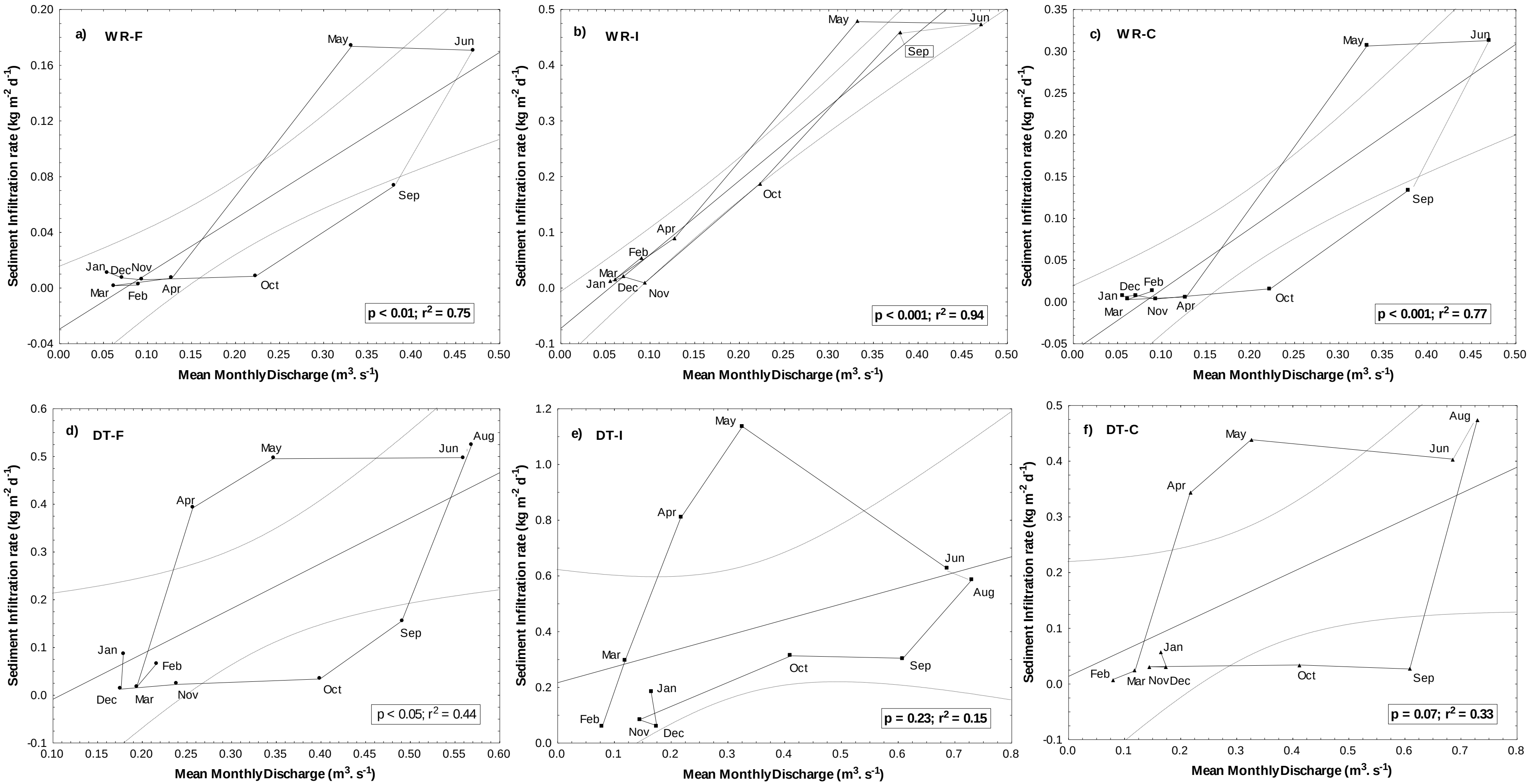


Figure 9: Sediment infiltration rate in relation to mean discharge at sites during the measurement month at (a–c) at Wit River and (d–f) at Du Toit's River. The relationships at the sites are described by clockwise hysteresis loops. Note that the months of July at the Du Toit's and July–August at the Wit River are not represented in the record, which may hide further evidence of monthly hysteresis in the catchment. Dashed lines are the 95% confidence intervals

3.3.4 Spatial pattern in fine sediment infiltration

Significant interaction between sediment infiltration rates and the three sites was noted at the Wit River (two-way ANOVA: $F_{[2, 24]} = 22.92$; $p < 0.001$; Figure 12b). The highest values for sediment infiltration were observed at the most downstream site (WR-I; ANOVA; $p < 0.001$). In general, sediment infiltration rates on the Wit River increased from upstream to downstream (Figure 12b). The averaged sediment infiltration was significantly greater in both WR-C ($0.08\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$; $p < 0.001$) and WR-I ($0.18\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$; $p < 0.001$) than in WR-F ($0.05\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$) (Table 4), especially during a period of high discharge (May to September; Figure 12b). The overall relationships between mean sediment infiltration rates and SSP for sites WR-F, WR-C, and WR-I are shown in Figure 10.

Similarly, significant overall interaction was evident between sediment infiltration rates and sites at the Du Toit's River ($F_{[2, 24]} = 46.74$; $p < 0.001$). The mean sediment infiltration rates observed at the Du Toit's River ranged from $0.21\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$ at DT-F and $0.17\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$ DT-C to $0.41\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$ at DT-I (Table 4). Sediment infiltration rates were significantly greater in DT-I ($0.405\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$) than DT-C ($0.17\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$; $p < 0.001$) and DT-F ($0.21\ \mathrm{kg \cdot m^{-2} \cdot d^{-1}}$; $p < 0.001$), with no differences difference between DT-F and DT-C (ANOVA; $p = 0.054$, Figure 10a).

Table 4: Mean values and ranges of daily sediment (< 2 mm) infiltration rate of the two rivers at the three sites and of the coefficient of variation of the monthly values within the nine samplers per site

Site	Du Toit's (DT)		Wit (WR)	
	Infiltrated sediment ($\mathrm{kg \cdot m^{-2} \cdot d^{-1}}$)	**Coefficient of variation (%)**	**Infiltrated sediment ($\mathrm{kg \cdot m^{-2} \cdot d^{-1}}$)**	**Coefficient of variation (%)**
Fynbos (F)	0.21 (0.001–0.75)**	76 (13.3–187.8)	0.05 (0.001–0.50)*	83 (3–156)
Cleared (C)	0.18 (0.001–0.82)**	103 (25.6–208.8)	0.08 (0.001–0.55)*	83 (14.7–118)
Invaded (I)	0.41 (0.002–2.10)**	73 (15.7–168.2)	0.18 (0.002–0.90)**	102 (22.3–184)

* Differs significantly from the two other sites (one-way ANOVA; $p < 0.05$)

** Differs highly significantly from the two other sites (one-way ANOVA; $p < 0.01$)

Sediment infiltration rates at the Wit River sites appear to be roughly inversely related to SSP. For the traps situated at WR-F, low rates of sediment infiltration were found at SSP greater than $1000\ \mathrm{W \cdot m^{-2}}$, whereas much greater rates were found in the WR-I site for SSP less than $1000\ \mathrm{W \cdot m^{-2}}$ (Figure 10). The higher rates of sediment infiltration in these areas reflect enhanced sediment availability and delivery in the WR-I site. Conversely, on the Du Toit's River, SSP is roughly comparable between the DT-F and DT-I sites; however, DT-I exhibits significantly higher sediment infiltration rates than DT-F (Figure 10).

The cross-sectional variation in sediment infiltration rates among infiltration baskets at each site as expressed by the coefficient of variation was very high with coefficients of variation up to 184% at the Wit River and 208.8% at the Du Toit's River (Table 4). The percentage of silt and clay (< 63 µm) in the mean captured infiltrated sediment was generally highest at DT-C and DT-I and WR-C and WR-I. This is probably attributable to the availability of these particle size classes in the lower parts of the catchments compared with the sites in the upper sections (Figure 9). The fraction of the sediment smaller 63 µm decreased with higher averaged sediment infiltration rates (Spearman rank correlation; $p < 0.05$; Figure 9).

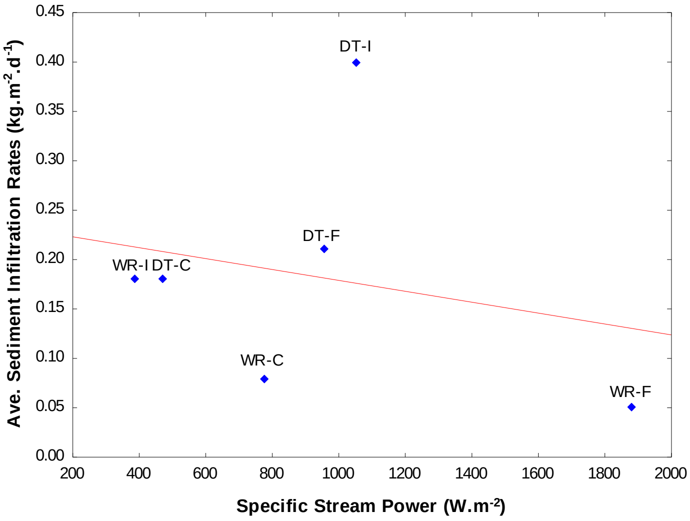


Figure 10: Channel classification, criteria and description of the geomorphic process based on SSP and average sediment infiltration rates. A1 = SSP > 1000 W·m^{-2} and A2 = SSP 300–1000 W·m^{-2}. Floodplain and channel classifications are based on the work of Nanson and Croke (1992)

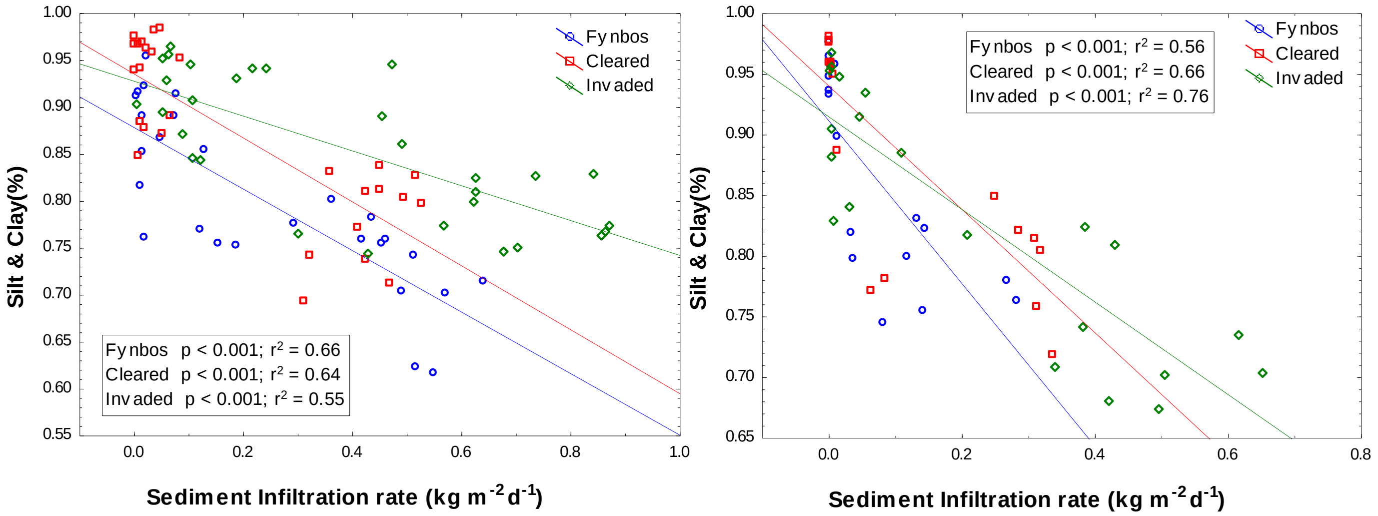


Figure 11: Monthly relative silt and clay infiltration at (left) Du Toit's and (right) Wit River in relation to the mean monthly sediment infiltration rate

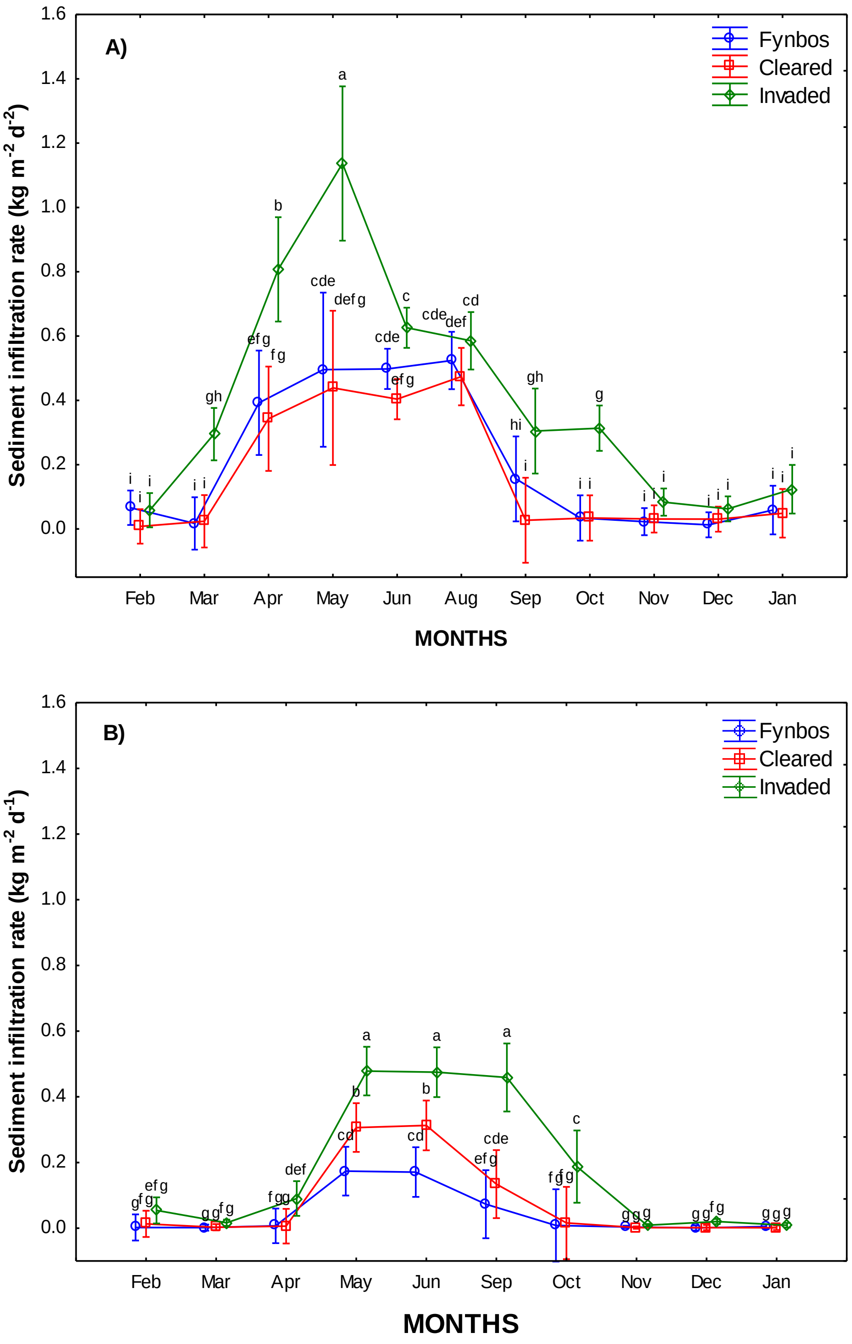


Figure 12: Temporal and spatial variability in fine sediment infiltration rates at (A) Du Toit's River and (B) Wit River across months. Mean site values are indicated by different point symbols and whiskers indicate ±95% confidence interval. LSD tests (p < 0.05) for interaction effects based on two-way RMANOVAs

3.3.5 Catchment suspended sediment yield (SSY)

When the monthly suspended sediment loads are recalculated to represent the area SSY (SSY-A; kg·km^{-2}·yr^{-1}), a different pattern of sediment production and transport is apparent with the smaller subcatchment of the Du Toit's River generating a greater load of suspended sediment per unit area than the larger subcatchments of the Du Toit's River (Figure 13a). Significant interaction was noted between sites and SSY ($F_{[2, 24]} = 59.80$; $p < 0.001$, Figure 14). Mean SSY differed significantly between the upstream site, DT-F, and the most downstream site, DT-I, at the Du Toit's River (one-way ANOVA: $F_{[2, 24]} = 55.65$; $p < 0.001$). The mean SSY decreased significantly from DT-F (0.002 kg·m^{-2}·d^{-1}) to DT-I

(0.001 kg·m^{-2}·d^{-1}; ANOVA; $p < 0.001$) and DT-C (0.0004 kg·m^{-2}·d^{-1}; ANOVA; $p < 0.001$) at the Du Toit's River (Figure 13a).

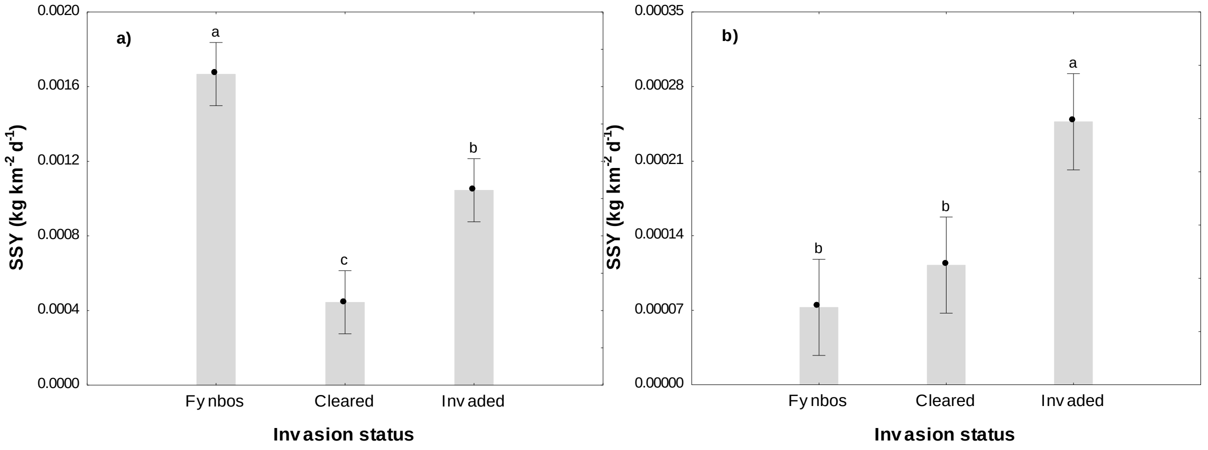


Figure 13: Catchment SSY for invasion statuses at (a) Du Toit's and (b) Wit River. Mean values are indicated by different point symbols and whiskers indicate ±95% confidence interval. Letters indicate significant differences (LSD tests; $p < 0.05$) for interaction effects based on one-way ANOVAs. Note the different scales on the SSY axes

The general decrease in SSY from up- to downstream broadly indicates a general trend that as contributing area increase, SSY decrease, resulting in an inverse relationship (Figure 14). Overall, significant interaction was observed between SSY and sites at the Wit River ($F_{[2, 24]} = 17.43$; $p < 0.001$). SSY differed significantly between WR-F and WR-I (ANOVA; $p < 0.001$). However, mean SSY increases from 0.0001 kg·m^{-2}·d^{-1} at WR-F to 0.0003 kg·m^{-2}·d^{-1} at WR-I (Figure 13b). The increase in SSY from down- to upstream results in a positive relationship between SSY and contributing area (Figure 14).

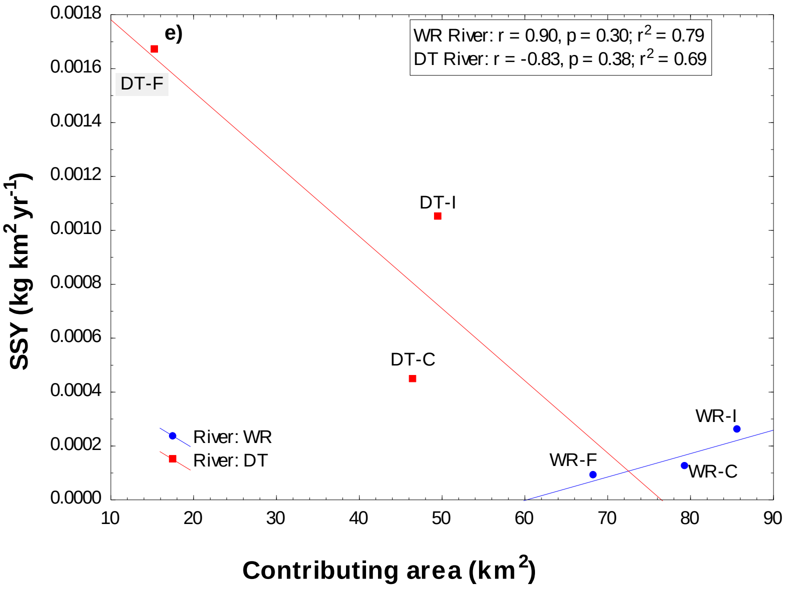


Figure 14: Relationship between catchment contributing area and SSY. Sites on the Du Toit's River are coloured red, and sites on the Wit River are indicated by blue

3.4 Discussion

3.4.1 Temporal variability in sediment infiltration

There was a consistent tendency of higher rates of sediment infiltration to be associated with the higher flow events, particularly during the wet season (autumn and winter). Conversely, lowest infiltration rates were associated with the summer low-flow period. The clear association between sediment infiltration rates and discharge with greater sediment infiltration rates being associated with high winter flows is a feature supported by other investigations (e.g. Acornley & Sear, 1999; Greig et al., 2005; Zimmermann & Lapointe, 2005; Maturana et al., 2014; Milan, 2017).

The association is shown more clearly in Figure 9a–f, where sediment infiltration rate is plotted against discharge. An analysis of the relationship shows that sediment infiltration rates can be characterised by clockwise hysteresis loops at all sites (Figure 9a–f). The initial peak in suspended sediment occurs with marginal increases in discharge until sediment depletion of readily available sources results in a subsequent decline in suspended sediment. The early flushing and exhaustion of readily available sediment often drives clockwise hysteresis loops. This phenomenon is well documented in sediment transfer literature, whereby peak sediment concentrations precede the peak in river discharge and depletion results in a subsequent decline in sediment load before a decrease in discharge (Scott et al., 1998; Salant et al., 2008; Grenfell & Ellery, 2009; Smith & Dragovich, 2009). This also implies that the available sediment is easy to mobilise and quickly entrained and is likely to be within (and proximal) to the river channel (Lefrançois et al., 2007; Bača, 2008; Rodríguez-Blanco et al., 2010). These sediment sources include bed material (Arnborg et al., 1967; Bogen, 1980), bank material (Seeger et al., 2004; Lefrançois et al., 2007; Smith & Dragovich, 2009) and floodplains (Mano et al., 2009).

The assessment of the hysteresis loops on the Du Toit's River separately highlights the control of sediment availability within and proximal to the river channel. The relationship between monthly discharge and sediment infiltration rates also reveals periods of the year where there is a relative abundance and depletion of sediment available for transport. At the invaded site on the Du Toit's River, sediment transport peaks before discharge. This suggests that in this part of the catchment, sediment has become available for transport throughout the dry season, and as soon as water is available to transport it, it moves. The latter feature indicates that the invaded site is transport-limited at the beginning of the wet season.

In contrast, in the cleared and fynbos sites, sediment transport peaks at the same time as discharge. This suggests that less sediment has become available, and the system tends towards being supply-limited to varying degrees. Furthermore, the results indicate that twice as much sediment is available for transport at the invaded site. In addition, the fynbos site is producing more sediment than the cleared site. The actual amount of sediment available for transport is also a major control on rates of sediment transport, as described in Grenfell and Ellery (2009). This is illustrated in Figure 9d–f, where sediment infiltration rates vary considerably during discharge of similar intensity and magnitude. Although discharge is frequently considered the dominant control of temporal variation in suspended sediment (Maturana et al., 2014), variability may also occur due to of sediment availability in the catchment as is evident at the Du Toit's River. The invaded site on the Wit River shows a relatively strong relationship between sediment infiltration rates and discharge compared with the other sites. This finding is often associated with a lack of sediment depletion and constant supply. Sediments tend to accumulate in the catchment during low magnitude flows governed by low transport capacity (transport-limited) whereas during peak transport events, erosional processes and sediment availability become the dominant control (supply-limited) (Grenfell & Ellery, 2009; Perks, 2013). On the contrary, at the cleared and fynbos sites, sediment infiltration peaks with discharge and shows evidence of sediment exhaustion during later stages of the wet season. Again, suggesting that in these sections, less sediment becomes available, and the system tends to be more supply-limited.

Temporal variations in the grain size distribution in the sediment infiltration baskets also reflect changes in flow conditions in the channel in this study, higher winter flows transported greater quantities of varying grain sizes. This result supports the finding that increases in flow facilitate the transport of larger-sized particles (Horowitz, 1991; 2008). However, finer fractions of suspended sediment (< 63 µm) are more prevalent during lower energy flows. Sear (1993), Acornley and Sear (1999), and Schindler Wildhaber et al. (2012) found that during flows, sediment composed of sediment transported in suspension was predominantly < 0.25 mm in size. During high flows, a greater proportion of a diameter between 0.25 mm and 4 mm was transported. During low-flow conditions in summer months, sediments of a size likely to be transported in suspension (< 63 µm) accounted for most infiltrated material.

3.4.2 Spatial variability in sediment infiltration

Sediment infiltration rates were highly variable between sites. Significantly higher sediment infiltration tended to occur in the traps situated in the downstream/invaded reaches of the respective rivers. To develop insight whether this was related to river geomorphological processes or changes in vegetation structure, the SSP was calculated to classify the reaches according to Nanson and Croke's (1992) generic classification of floodplains. SSP has widely been used to assess sediment transport and geomorphic behaviour of river channels (e.g. Bagnold, 1977; Chang, 1979; Nanson & Croke, 1992; Ferguson, 2005; Bizzi & Lerner, 2015).

Based on the commonly used floodplain classification system of Nanson and Croke (1992), the floodplains of all sites were characterised as high-energy non-cohesive floodplains where SSP exceeded 300 W·m^{-2}. The invaded and fynbos sites on the Du Toit's River and fynbos site on the Wit River were classified as A1 orders where SSP is greater than 1000 W·m^{-2}. This indicates that in these sections, the streams have an extremely high capacity to transport sediment. Despite the comparable SSP, the suspended sediment loads at the invaded site on the Du Toit's River were around seven times higher than at the fynbos site on the Wit River and twice as high as the fynbos site on the Du Toit's River. This could be an indication of enhanced sediment availability and transport at the invaded site on the Du Toit's River. On the Wit River, the invaded site was classified as an A2 order, where the stream's capacity to do work is almost four times lower than the fynbos site. Regardless of the lower stream power in WR-I, suspended sediment load in the invaded site was almost 2.6 times higher than the fynbos site. These high sediment infiltration rates in the invaded sites could reflect enhanced input and availability of fine sediment from within the channel and *A. mearnsii*-invaded riparian zones in the downstream sections of these rivers.

The WR-C and DT-C sites did not respond in a linear fashion, but valuable information could be drawn from the results. DT-I compares well with WR-I in terms of both SSP and suspended sediment loads. Suspended sediment load WR-C is two times higher in WR-F. In contrast, at DT-C, suspended sediment load was almost 1.3 times less than in DT-I with no significant difference compared with DT-F. The clearing of IAPs dramatically reduced the protective vegetation cover, which consequently exposed soil surface to increase erosion. Many authors reported that after removing IAPs, native species have the chance to re-establish (Galatowitsch & Richardson, 2005; Blanchard & Holmes, 2008; Reinecke et al., 2008). However, the rate at which this occurs depends on several factors such as invasion intensity and magnitude before clearing. Thus, the variable response in the cleared sites in this study could be related to the rate of native vegetation recovery over the last 10 years and also the time since clearing.

The progressive increase in fine suspended sediment (< 63 µm) infiltration downstream at the Du Toit's River may have been the result of increasing downstream distance causing a longitudinal reduction in slope and shear stress, which created opportunities for the selective deposition of finer particles. Davide et al. (2003) documented a similar phenomenon. They found that with increasing distance downstream, suspended sediments became enriched in the clay fraction (0.4–4 µm) of the Po River, Italy. Therefore, the enrichment of silt and clay-sized particles in the lower reaches of the Du Toit's River could be attributed to the longitudinal reduction of slope and shear stress (shear velocity and stream power) (Sear, 1993).

3.4.3 Catchment-scale SSY

SSYs are often reduced with increasing catchment area (Lane et al., 1997; De Vente et al., 2005). This could be a consequence of a decrease in local slope and the presence of wide floodplains with distance downstream, creating sediment sinks (Walling et al., 1999; Birkinshaw & Bathurst, 2006). When the monthly suspended sediment loads are transformed to represent SSY-A ($kg \cdot km^{-2} \cdot d^{-1}$), a different pattern of sediment production and transport is apparent with the study reach in the smaller subcatchments generating a greater load of suspended sediment per unit area than the reaches in the larger subcatchments of the Du Toit's River (Figure 11a).

Along the Du Toit's River, sediment loads per unit area decrease with increase in contributing area, resulting in an inverse SSY-A relationship, although sediment infiltration rates increase. This could be a consequence of poor vegetation cover and high surface erosion (Dedkov & Mozzherin,1996; Walling & Webb, 1996; Dedkov, 2004; De Vente et al., 2007), which consequently increases the catchment sediment contributions to the river network. This supports the assumption that the change in vegetation structure – natural fynbos shrub to invasive *A. mearnsii* woody trees – and consequent loss of protective ground cover through clearing might contribute significantly to increased suspended sediment loads in lower section of the Du Toit's River.

Conversely, SSY generally increased with an increase in contributing area in the Wit River. The magnitude of increase was proportional to the catchment contributing area resulting in a positive SSY-A relationship. This suggests that suspended sediment originated from and was transported from within channel sources and proximal to the channel (Walling & Webb, 1996). Similarly, Dedkov (2004) reports that a positive relationship between SSY and drainage basin area often results when channel erosion (bank and bed) is the dominant source of suspended sediment. This may occur in headwater areas with good vegetation cover and resistant rocks where erosion rates increase downstream in response to greater entrainment and transport of sediment (Walling & Webb, 1996), or where large volumes of unconsolidated sediment in the channel are available for erosion (Church & Slaymaker, 1989). Therefore, the increase in contributing area and discharge within the channel at the Wit River are clearly important controls that may be expected to produce substantial variation in sediment load downstream.

3.4.4 Impacts of different vegetation conditions on suspended sediment loads

At the natural sites, fynbos shrubland vegetation appeared to make low contributions to the suspended sediment loads of the Wit and Du Toit's Rivers with total contributions of 15% and 27%, respectively. The dense undergrowth in undisturbed natural vegetation provides protection against soil loss through its ability to bind soil, withstand flooding and consequently dampening the speed of overland flow ensuring sediment deposition. The geology and soils are also considered as important controlling variables of soil erosion rate, with quarzitic sandstone having a relatively low erodibility (Scott et al., 1998). Scott et al. (1998) showed that soil erosion rates in an undisturbed fynbos mountain catchment in the Western Cape, with underlying sandstone, are naturally low ($< 1\ t \cdot ha \cdot yr^{-1}$).

The invaded sites on the Wit and Du Toit's Rivers appeared to deliver significantly higher suspended sediment loads with total contributions of 59% and 52% respectively during the monitoring period. The total contribution of the invaded site on the Wit River is almost three times higher than that at the fynbos sites. The contribution of the invaded site on the Du Toit's River is almost twice as high as the fynbos site. The change in vegetation structure and dense overgrowth of these woody invasive trees inhabit vegetation undergrowth and groundcover, which leaves soil unprotected against soil erosion. Scott et al. (1998) found a strong increase in water repellence in soils under *Eucalyptus* and *A. mearnsii* plantations compared with soils under fynbos, which could impede water infiltration and percolation into the soil (Scott et al., 1998; Ruwanza et al., 2013), resulting in increased generation of overland flow and subsequent soil erosion. The doubling of erosion rates found on *Acacia*-invaded slopes in a study by Van der Waal (2009) partially supports this finding. Similarly, Kosmas et al. (1997) recorded increased erosion rates under woody *Eucalyptus* ($23.8\ t \cdot km^{-2} \cdot yr^{-1}$) invasions compared with shrubland ($6.7\ t \cdot km^{-2} \cdot yr^{-1}$) in a Mediterranean region. This was mainly attributed to the loss of native species richness – in particular groundcover and understorey vegetation that no longer bind and protect the soil.

The sites cleared of IAPs in riparian corridors did not respond in a linear fashion, but valuable information can be drawn from the results. The suspended sediment load contribution in the cleared site (26%) of the Wit River was significantly higher than in the fynbos site (15%). In contrast, no significant differences in suspended sediment loads were found between the cleared (22%) and the fynbos site (27%) on the Du Toit's River. The heterogeneity between sites in terms of riparian and stream characteristics, such as stream width, riverbank height and slope of the riparian areas, could explain the unexpected results observed at the Du Toit's River. The fynbos site on the Du Toit's River are characterised by a relatively narrow stream channel and low riverbanks with steep riparian slopes compared with the fynbos site on the Wit River with a wider channel and gentle riparian slopes, similar to the two downstream sites.

The clearing of IAPs dramatically reduce the protective vegetation cover which, in turn, expose soil surface to increase erosion. Many authors reported that after the removal of IAPs, native species have the chance to re-establish (Galatowitsch & Richardson, 2005; Blanchard & Holmes, 2008; Reinecke et al., 2008). In their study on the short-term recovery of native riparian vegetation along the Berg River in the Western Cape (South Africa), Ruwanza et al. (2013) found increased richness, cover and abundance of native vegetation four years after clearing of *Eucalyptus* spp. However, the rate at which this occurs depends on several factors such as invasion intensity and magnitude before clearing. Thus, the variable response in the cleared sites in this study could be related to the rate of native vegetation recovery.

3.5 Conclusions

There is a dearth of information about the temporal, spatial and transfer dynamics of suspended sediment through the hydrological networks that drain mountain catchment areas, with a lack of understanding of sediment dynamics in Western Cape rivers as a whole. The assessment of this variability can provide valuable information on the sediment delivery of these catchments and is a novel approach to assess the provenance of sediment delivery to the rivers.

This study has investigated the spatial and temporal variability of suspended sediment (and its properties) across two mountain catchments in the CFR. The study does not clearly demonstrate the link between IAPs and clearing on suspended sediment loads. Our results showed that the impacts of IAPs were indeed highly context-dependent as patterns of sediment loads did not respond in a linear fashion for the two rivers. This was mainly attributed to the distinctive stream characteristics such as stream width, riverbank height and riparian slope. Although the patterns are inconsistent, valuable trends could still be observed for certain variables. Overall, the invaded sites were found to contribute more than 50% of the total suspended sediment load compared with the relatively low suspended sediment contributions of the sites surrounded by natural fynbos (15–27%).

In general, suspended sediment loads of mountain streams may be characterised as complex and supply-limited systems. The local hydraulic properties and site characteristics such as channel morphology, vegetation cover, and topography are clearly important controls and produce substantial variations in the quantity of suspended sediment. SSY is relatively low, especially when considering the steep terrain and high transport capacity of these streams.

This study provided important benchmark data, furthering the understanding of fluvial geomorphological processes in Western Cape rivers. Additionally, the findings of this study, especially with respect to sediment loads of mountain streams in the CFR, may facilitate future research and highlight problem areas (natural or human-induced changes) at the local catchment scale. Further research needs to be undertaken to enable better understanding of the behaviour of suspended sediments in these river systems and to determine the holistic impacts of IAPs and clearing thereof on these river processes.

CHAPTER 4 – INVESTIGATING THE IMPACTS OF INVASIVE NITROGEN-FIXING *A. MEARNSII* SEDIMENT NUTRIENT DYNAMICS IN MOUNTAIN STREAMS OF THE CFR

4.1 Introduction

Riparian ecosystems in South Africa and particularly the Western Cape are constantly under threat from invasion by woody nitrogen-fixing IAPs (Richardson & Van Wilgen, 2004). Due to these threats, the interaction between invasive plants and indigenous ecosystems has been a major focus in the field of restoration ecology (Ehrenfeld, 2003). The introduction of invasive species into natural ecosystems is the result of human activities (Forsyth et al, 2004; Milton, 2004). One of the key aspects thus far has been the impact of invasive plants on indigenous vegetation and the attributes of natural ecosystems that make them susceptible to invasion (Rejmánek & Richardson, 1996). Many invasive species can transform a landscape and negatively affect ecosystem processes (Richardson & Van Wilgen, 2004). The invasion of non-indigenous species such as Australian *Acacia* in fynbos riparian zones reduced native plant species diversity (Richardson et al., 2007), and reduced stream flows because of increased water use and changes to nutrient cycling (Yelenik et al., 2004).

The fynbos region of the Western Cape is dominated by oligotrophic soils, and characterised by sclerophyllous shrubs (Cowling et al., 2004). In general, fynbos soils are nitrogen-poor and the nitrogen cycle appears to be very slow (Stock et al., 1995). Very few fynbos plants have the ability to fix nitrogen (Cocks & Stock, 2001). Nitrogen-fixing fynbos plants (such as *Encephalartos* spp*.* and *Morella cordifolia*) are short-lived and they only contribute 0.5% of nitrogen stocks to the ecosystem (Cocks & Stock, 2001). In contrast, invasive species such as *Acacia* are effective nitrogen fixers and alter nitrogen and carbon cycles (Richardson & Cowling, 1992; Ehrenfeld, 2003). Invasion by non-indigenous plants often cause an increase in nutrient stocks, as well as an increase in the productivity of many ecosystems regardless of the decrease in plant diversity (Stock et al., 1995; Maron & Connors 1996; Maron & Jefferies 1999; Tye & Drake, 2012).

Le Maitre et al. (1996) modelled the predicted impacts of *Acacia* spp. on water resources. These models suggested that if there is no management plan in place, Western Cape catchments could lose up to 87 million m^3 of water to invasive species. WfW, initiated by the Department of Water Affairs and Forestry in October 1996, aims to control woody IAPs to reduce water use and preserve stream flow in South African catchments. Many alien invasive species that are being targeted by WfW are nitrogen-fixing legumes. For example, *A. saligna*, *A. mearnsii* and *A. cyclops* are commonly found in Western Cape landscapes. While it is generally accepted that clearing IAPs in South Africa will have significant benefits in enhancing run-off from catchments and groundwater recharge, concerns have been raised that some of the nitrogen will be mobilised causing a flux of nitrogen into streams (Jacobs et al., 2013). It is unclear how the clearing of invasive nitrogen-fixing trees in the riparian zones will affect water quality and other aspects of the aquatic ecosystems ecology. Indeed, little attention has been paid to quantify the amount of nutrients that nitrogen-fixing invasive plants may be adding to aquatic systems in South Africa.

Research on the changing levels of nitrogen in riparian soils has largely been documented in central Japan (Akamatsu et al., 2011), New Mexico (DeCant, 2008) and, more recently, in South Africa (Jacobs et al., 2013). Soils under terrestrial Australian *Acacia* species were found to have a twofold increase in soil elemental concentrations compared with adjacent fynbos soils (Musil & Midgley, 1990). This enrichment has been ascribed (Musil & Midgley, 1990) to the higher annual dry litterfall of *Acacia* spp. (Milton 1981b; Mitchell et al., 1986), higher foliar litter nitrogen and phosphorus concentrations (Milton, 1981b), and slow rate of litter decomposition (Milton, 1981b; Mitchell et al., 1986).

Any changes in nutrient cycling within the riparian zone can strongly influence stream nutrient concentrations as these ecosystems are hotspots of biogeochemical processing (become sources rather than sinks) (McClain et al., 2003, Vidon et al., 2010). For instance, the woody nitrogen-fixer

Russian olive (*Elaeagnus angustifolia*) that invades riparian ecosystems in the western United States alters stream nitrogen dynamics (Mineau et al., 2011), while *Falcataria moluccana* can increase stream nitrogen levels up to sixfold (Wiegner et al., 2013). However, it remains unknown how nitrogen-fixing invasive plants increase nitrogen concentrations in streams, although several mechanisms have been proposed (Wiegner et al., 2013). Thus, further research is required to determine which mechanisms, if any, are primarily responsible for the elevated nutrient concentrations in streams. No studies have emphasised the importance of nutrient transport in association with sediments.

Stream ecosystems have a strong capacity to transform and retain nutrients; thus, in-stream biogeochemical processes can further influence nutrient chemistry along the stream (Peterson et al., 2001; Dent et al., 2007). While much attention has been focused on riparian soil biogeochemistry and water quality changes in streams as a function of invasive nitrogen-fixing plants, much less is known about the role of suspended sediment as nutrient carriers. Nutrients, in particular phosphorus, have a relatively higher bonding affinity for clay particles in soils and sediments than the low bonding affinity to water molecules (Paudel et al., 2015). The suspended sediment can have direct and indirect effects on the primary productivity of a stream: directly by affecting photosynthesis by blocking sunlight, and indirectly by affecting water chemistry by releasing and adsorbing nutrients. Permanently suspended particulate matter and resuspension of particulate matter adsorb and regulate release of different forms of nutrients into the water column (Tappin et al., 2010).

The purpose of this study is to determine the impacts of invasive nitrogen-fixing *A. mearnsii* and clearing thereof on nutrient dynamics in suspended sediment of mountain streams in the CFR. Estimating the amount of nutrients attached to sediment in the rivers draining areas invaded by woody IAPs and land cleared thereof will provide good insight and improve understanding of how invasive nitrogen-fixing species change the nutrient contents and delivery to these river systems. This research is among the first to investigate whether widely colonising invasive *Acacia* spp. do in fact alter sediment-associated nutrient concentrations (carbon, nitrogen and phosphorus) in fynbos mountain streams. The aim of this study was to:

- Evaluate the spatial and temporal variability in nutrient concentrations of two mountain streams in the CFR.
- Compare the results to physical and other chemical properties data.
- Quantify the nutrient contributions from near-pristine fynbos, *A. mearnsii*-invaded and *Acacia*-cleared riparian sections.

4.2 Methods

4.2.1 Study sites and design

The rivers selected for this study fall in the Breede WMA in the Western Cape Province, South Africa. The rivers are situated in largely mountainous upper subcatchments and a designated mountain catchment area. Much of it is located within the protected areas. As a result, it is largely undeveloped with no anthropogenic disturbances, with land use primarily limited to recreation and IAPs. The sampling design called for three sites at each river, each consisting of:

- One upper stream natural site (reference) (pristine or near-pristine condition, with no or minimal invasion by woody IAPs, and free of human disturbance).
- A mid-stream cleared riparian site (a previously invaded site that has been cleared more than seven years ago with *A. mearnsii* as the dominant invader).
- An invaded site (predominantly *A. mearnsii* or a mixture of *A. mearnsii* and *A. longifolia* invaded for at least ten or more years).

The dominant lithology in the catchment is Quarzitic Table Mountain Sandstone of the Peninsula formation (Rebelo et al., 2006). The hard impermeable quarzitic sandstone produces shallow, coarse textured soils and are characterised as nutrient-poor, leached and acidic (CapeNature, 2017). The Breede WMA is characterised by a Mediterranean-type climate, with rainfall occurring predominantly in winter (most of which occurs between April and September) while the summer months are notably hot and dry (Deacon et al., 1992; Sieben, 2003). The mean annual precipitation in the catchment varies considerably with values exceeding 1800 mm in the mountainous regions, and values dipping to 300 mm in the lower eastern parts (DEADP 2011). The mean annual temperature for the Breede catchment area is 12°C on higher-lying areas and 19°C on lower-lying areas. The mean annual temperature measured at the study areas for the monitoring period, Wit River (19.3°C) and Du Toit's River (17.8°C) are well within the long-term average for the Breede WMA. The hydrological regime of streams is driven by precipitation. Discharge in the rivers is high in winter with major peaks in May through to August, and lowest between December and March.

Suspended sediment, infiltrated sediment and water samples were collected at three sites from up- to downstream: natural (control), cleared and invaded. For more information regarding the characteristics of the rivers and sites, see Chapter 3 (cf. Table 3).

4.2.2 Sediment collection and analysis

Suspended sediment was sampled with one time-integrated suspended sediment samplers at each site following Phillips et al. (2000). The apertures of the suspended sediment samplers were about 60 mm above the riverbed towards the downstream end of each treatment. Infiltrated sediment samples were collected in nine sediment baskets per site following Bond (2002) and Schindler Wildhaber et al. (2012). The first row of three was placed towards the upstream section of the reach and the next two towards the downstream end. The baskets were spaced equally at each of the three predefined traverses. Suspended sediment samplers and sediment baskets were both emptied monthly. The basket's sediment was sieved with a 4 mm sieve and the basket refilled with the remaining coarse sediment during each sampling event. Sediments < 4 mm were taken to the laboratory for further analyses.

The term 'infiltrated sediment' refers to the total sediment < 2 mm infiltrated during one month in the sediment basket. 'Suspended sediment' refers to the total amount of sediment caught during one month in the suspended sediment samplers. Sediment samples were oven-dried at 105°C for 24 hours and then disaggregated and homogenised using a pestle and mortar. All samples were dry-sieved through a 63 µm mesh to minimise the influence of the grain size as recommended by Horowitz and Elrick (1987). Water was sampled using 250 ml plastic bottles, which were pre-rinsed with stream water. Water was filtered in the field through 0.45 µm Milliex-HP syringe filter units (Whatman, Piscataway, New Jersey, USA). All water samples were immediately placed on ice, transported to the laboratory, and stored frozen until analysis. Water sample collection was done quarterly during periods of low, moderate, intermediate and high flow.

The samples collected by the sediment baskets and suspended sediment sampler were measured for the following parameters: monthly total nitrogen (TN) and total carbon (TC); particle size distribution; and on a seasonal basis, total phosphorus (TP) and iron while aluminium, pH and electrical conductivity (EC) were determined biannually. TC and TN of the infiltrated sediment and suspended sediment were measured by dry combustion followed by a CN elemental analyser (Euro EA Analyser) using standard processing techniques (Sollins et al., 1999) at the Central Analytic Facility, Stellenbosch University (Stellenbosch, Western Cape, South Africa). TP and iron in infiltrated sediment were analysed using a Thermo iCap 6500 Inductively Coupled Plasma Optical Emission Spectrometer, after sample preparation by microwave digestion at the laboratories of the Council for Scientific and Industrial Research (CSIR), Stellenbosch. Water samples were analysed for ammonium, nitrite + nitrate, and orthophosphate by the CSIR laboratories, KZN.

4.2.3 Particle size corrections

Particle size distributions of the previously dried and sieved sediment sample (< 63 µm) from infiltrated sediment were assessed with a high definition digital laser particle size analyser (Micromeritics Saturn DigiSizer 5200). Grain size fractions were classified according to Wentworth Udden; Silt: 63–3.9 µm and clay: < 3.9 µm. The classification was used to determine the percentage of clay in each sediment sample. Previous studies have established that particle size can exert a strong influence on associated geochemical concentrations (Horowitz & Elrick, 1987; Horowitz, 1991; He & Owens, 1995; He & Walling, 1996).

Carbon, nitrogen and phosphorus properties were tested for correlation between sample clay percentage and property concentration within the individual sites. The non-parametric Spearman‘s rho correlation coefficients test was used as both nutrient concentration data and clay fraction data were not normally distributed. Previous studies have commonly based particle size correction factors on specific surface areas ratios; however, due to time and resource constraints, the correction factor of this study was based on clay percentage to further minimise the effect of grain size.

In instances where significant correlation was observed between clay and nutrient concentrations, nutrient concentration values were corrected to account for any particle size dependencies to allow for direct comparison between sites. Where correlation between nutrient concentrations and clay was not significant across all three sites, it was assumed that by disaggregating and sieving samples to < 63 µm during processing, a sufficient account had already been taken of any nominal grain size dependencies. Thus, enabling direct comparison between sediment samples without the requirement for additional correction (Klamer et al., 1990; Wang & Chen, 2000; Whiting et al., 2005; Stutter et al., 2009). However, where the particle size effect correlation test indicated that the nutrient concentrations were significantly correlated with clay percentage across all sites, the particle size correction was considered appropriate. The clay percentage and geochemical concentrations of each sample were then used to make corrections for differences in particle size composition between samples and sites using Equation 4:

Equation 4. $$Cc = C / Cp$$

Where *C* is the measured property concentration, *Cc* the property concentration corrected for particle size to be directly comparable with the value for suspended sediment, *Cp* is the clay percentage of suspended sediment.

4.2.4 Statistical analyses

All statistical analyses for chapter were done using the Statistica 13 software package (Dell Inc., 2015). The null hypotheses that *A. mearnsii* invasion and clearing thereof do not affect TC, TN and TP were tested using ANOVA, and the significance was determined at $p < 0.05$. Fisher’s LSD test was used for post hoc means separations. Non-normal distributions of data for sediment loads and geochemical property concentrations justified the use of non-parametric tests. A combination of Pearson’s (*r*) and Spearman rank correlation (rho) were done individually to test for significant relationships at significance $p < 0.05$ between chemical and physical properties of sediment (< 63 µm) (TC, TN, and TP with clay percentage) and (TC with TN, TC with TP, TN with TP and, lastly, TP with iron).

4.3 Results

4.3.1 Temporal dynamics of carbon and nitrogen in infiltrated and suspended sediments

Wit River

The TN in infiltrated sediment at the Wit River showed significant interaction between seasons (two-way ANOVA: $F_{[9, 45]} = 36.51$; $p < 0.001$; Figure 15c). TN had significantly higher mean concentrations during the autumn months (mean = 1.09 ± 0.34%) than winter months (0.54 ± 0.07%), spring months (0.60 ± 0.12%) and summer months (0.77 ± 0.27%).

The TC concentrations in infiltrated sediment showed a similar temporal pattern to TN in infiltrated sediment with significant overall seasonal trends (two-way ANOVA: $F_{[9, 45]} = 64.35$; $p < 0.001$; Figure 15b), with higher overall mean concentrations in autumn months (21.03 ± 5.07%) and significantly lower concentrations during the winter (11.56 ± 1.42%), spring (13.47 ± 3.77%), and summer (14.19 ± 7.9) months.

The mean TN and TC concentrations were typically lowest during the wet season (with high flows and sediment infiltration rates) and peak concentrations during low flows in the autumn months. The effect of season on C:N ratio showed a significant overall interaction (two-way ANOVA: $F_{[3, 18]} = 31.99$; $p < 0.001$). Overall, lowest C:N ratios were measured during winter (mean = 17.45) and peak ratios during summer (mean = 22.14).

TN and TC concentrations in suspended sediment showed similar temporal patterns as TN and TC concentration in the infiltrated sediment: the smallest concentrations were assessed during winter months with a mean of TN = 0.6% and mean TC = 14.13%. TN and TC concentrations were significantly higher in summer with means of 1.47 and 23.95%, respectively (Figure 15b and Figure 15c).

Du Toit's River

In the Du Toit's River, significant seasonal variability was observed between TN in infiltrated sediment and seasons (two-way ANOVA: $F_{[11, 66]} = 18.21$; $p < 0.001$; Figure 15c). The highest mean TN concentration was observed during summer months (0.68 ± 0.14) relative to autumn (0.43 ± 0.19%), winter (0.40 ± 0.15%) and spring (0.55 ± 0.09%).

The seasonal pattern in TC showed a comparable trend to TN, with significantly higher average TC concentrations during the summer months (13.51 ± 3.12%) and spring (12.78 ± 2.49%) than winter (9.37 ± 3.37%) and autumn (8.72 ± 4.69%) months.

The lowest C:N ratios were measured during summer (19.91) and peak concentrations during winter (23.59). The temporal dynamic of TN and TC concentrations of suspended sediment showed the same characteristics as in infiltrated sediment with low levels at high discharge and high sediment yield during the wet winter months and high levels at low discharge and low sediment yields in the dry summer months.

Overall, TN concentrations showed significant inverse relationships with the total amount of suspended sediment and averaged infiltrated sediment (Figure 16a; Figure 16b). Infiltrated sediment and suspended sediment generally increased with increasing discharge (cf. Chapter 3). However, lowest TN concentrations in infiltrated sediment and suspended sediment were measured during high flows and sediment yields in winter and peak concentrations during base flows and low sediment loads in summer (Figure 16a; Figure 16b). This finding is also supported by the significant positive relationships between the clay fraction and TN in infiltrated sediment (Figure 16d). The clay fraction in infiltrated sediment is generally higher during the base flow than in high flows (cf. Chapter 3). A significant positive correlation exists between TN and TC concentrations in captured sediments (Figure 16c). The linear regressions between TC and TN gives an R^2 for infiltrated sediment of 0.81 and of 0.74 for suspended sediment (Figure 16c).

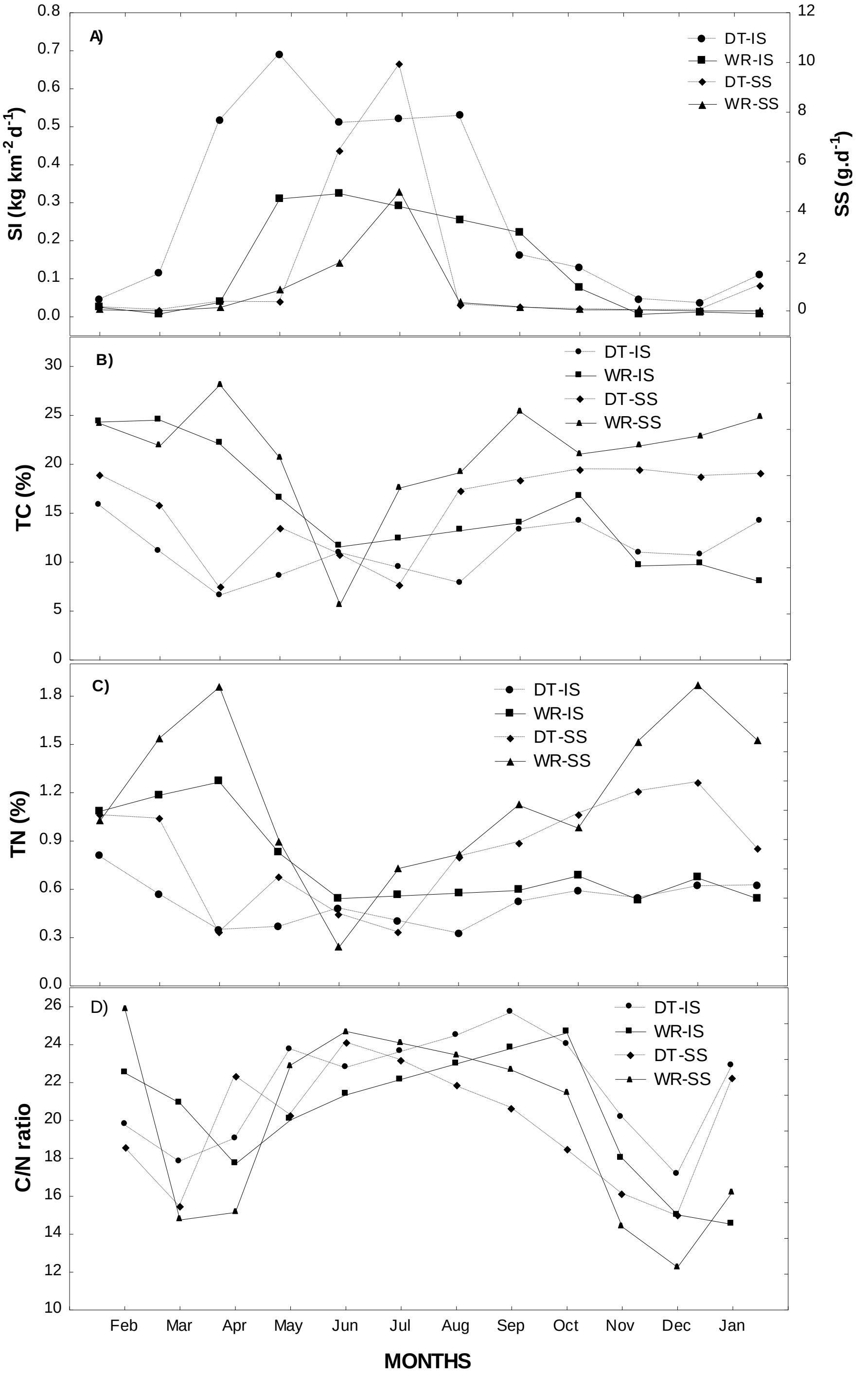


Figure 15: Sediment and nutrient dynamics during the field period. (A) monthly sediment infiltration rates (infiltrated sediment; < 2 mm) and the monthly suspended sediment; (B) TN of infiltrated sediment and suspended sediment; (C) TC of the monthly infiltrated sediment and suspended sediment; (D) C:N ratios of infiltrated sediment and suspended sediment. It should be noted that the patterns of infiltrated sediment suspended sediment, TN, TC and C:N ratios depicted were consistent for the sites on both the Wit (WR) and Du Toit's (DT) River

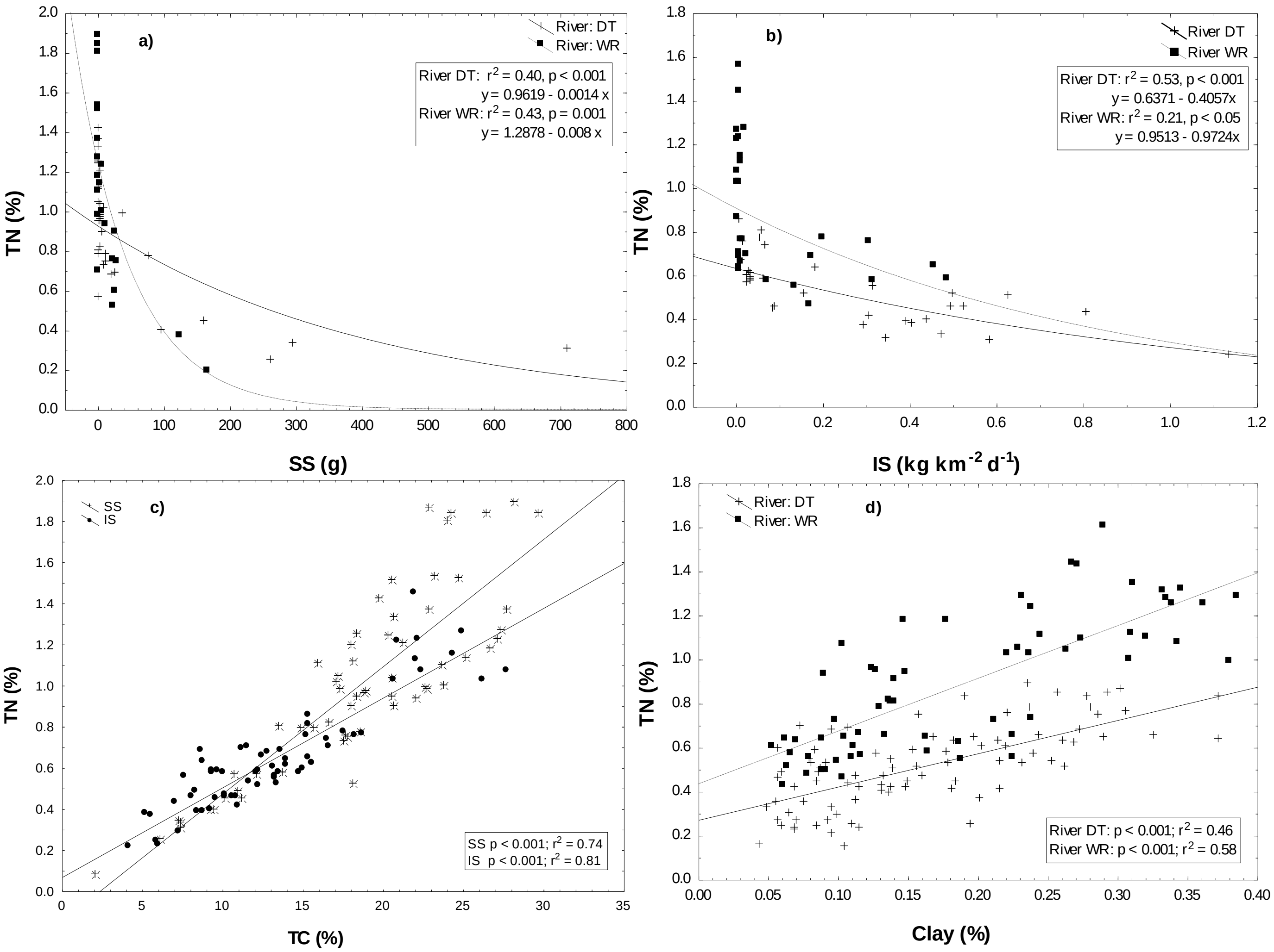


Figure 16: Relationship between TN and (a) average infiltrated sediment (< 2 mm); (b) total suspended sediment; (c) clay and silt fraction of infiltrated sediment and (d) TC. Solid squares and dotted lines: Wit River (WR), crosses and solid lines: Du Toit's River (DT)

4.3.2 Spatial dynamics of carbon and nitrogen in infiltrated sediment and suspended sediment

The interaction between invasion statuses were not significant for TN in infiltrated sediment at the Wit River (two-way ANOVA: F $_{[2,\ 6]}$ = 4.796; p = 0.001). Nevertheless, when TN was averaged over the course of the sampling period, there was a general trend towards enhanced TN concentrations in the invaded site. The highest mean was found at the invaded site (mean = 0.83 ± 0.23%) compared with the cleared (0.78 ± 0.30%) and fynbos sites (0.77 ± 0.37%).

Significant interaction was observed between TC and invasion statuses (two-way ANOVA: F $_{[2,\ 6]}$ = 32.96; p < 0.001). TC increased in a way similar to TN from fynbos (mean = 14.31 ± 6.19) to the cleared (15.18 ± 6.44%) and invaded sites (17.81 ± 6.31%). The invaded site had high C:N ratios (mean: 21.28) and differed significantly from cleared (p < 0.05) and natural (p = 0.01) sites with means of 19.46 and 18.95, respectively (Table 5).

Table 5: Comparison of average values (± standard deviation) at the three sites sampled of TC, TN and TP in infiltrated sediment (IS) and suspended sediment (SS)

Rivers	**Wit**			**Du Toit's**		
Sites	**Fynbos**	**Cleared**	**Invaded**	**Fynbos**	**Cleared**	**Invaded**
TC IS (%)	14.3 ± 6.2	15.2 ± 6.4	17.8 ± 6.3	12.9 ± 3.5	10.9 ± 4.5	10 ± 3.6
TN IS (%)	0.77 ± 0.37	0.78 ± 0.30	0.83 ± 0.23	0.56 ± 0.15	0.53 ± 0.21	0.48 ± 0.17
TC SS (%)	24.66 ± 4.09	23 ± 3.78	23.36 ± 3.68	16.67 ± 4.75	16.98 ± 3.78	15.15 ± 5.77
TN SS (%)	1.33 ± 0.43	1.11 ± 0.52	1.14 ± 0.48	0.85 ± 0.31	0.93 ± 0.29	0.80 ± 0.42
TP IS ($mg \cdot g^{-1}$)	0.47 ± 0.33	0.88 ± 0.69	0.78 ± 0.51	0.41 ± 0.22	0.59 ± 0.27	0.59 ± 0.31
C:N in IS	18.95 ± 0.64	19.5 ± 0.39	21.3 ± 0.58	22.98 ± 0.82	21.08 ± 0.41	21.11 ± 0.83
C:N in SS	19.49 ± 3.94	21.58 ± 7.30	19.51 ± 4.29	20.14 ± 3.37	18.78 ± 2.75	19.73 ± 3.88

In the Du Toit's River, TN concentration in infiltrated sediment differed significantly between invasion statuses (two-way ANOVA: $F_{[2, 6]} = 14.68$; $p < 0.01$). TN content was significantly lower in the invaded site (mean = 0.48 ± 0.17%) than in the cleared (0.53 ± 0.21%; $p < 0.05$) and fynbos sites (0.56 ± 0.15%; $p < 0.01$), with no differences between fynbos and cleared ($p = 0.07$).

Similarly, the highest mean TC content was found at the fynbos site (12.87 ± 3.53%) and was significantly higher than cleared (10.87 ± 4.52%; $p < 0.05$) and invaded sites (10.02 ± 3.64; $p < 0.01$).

The C:N ratios differed significantly between sites (two-way ANOVA: $F_{[2, 6]} = 6.70$; $p < 0.05$). Fynbos had the highest C:N ratios (mean = 22.98) and differed significantly from invaded (mean = 21.11; $p < 0.05$) and cleared sites (mean = 21.08; $p < 0.05$). No significant differences were found between the cleared and invaded sites ($p = 0.95$) (Table 5).

The TN concentration of the suspended sediment showed a different spatial pattern as TN concentration of the infiltrated sediment (Table 5): the highest concentrations were found at the cleared site with a mean of 0.94%; fynbos and invaded sites had lower concentrations with means of 0.85% and 0.80%, respectively (Table 5).

The TC in suspended sediment increased similar to the TNs from the cleared site (16.98 ± 3.78) to the fynbos (16.67 ± 4.75) and invaded (15.15 ± 5.77) sites. The increase of TC in the suspended sediment from the invaded site with a mean of 14.50% to the fynbos and cleared sites with means around 16% was significant. Overall, mean TN and TC concentrations in suspended sediment was 0.34% and 4.8%, respectively, higher than in infiltrated sediment.

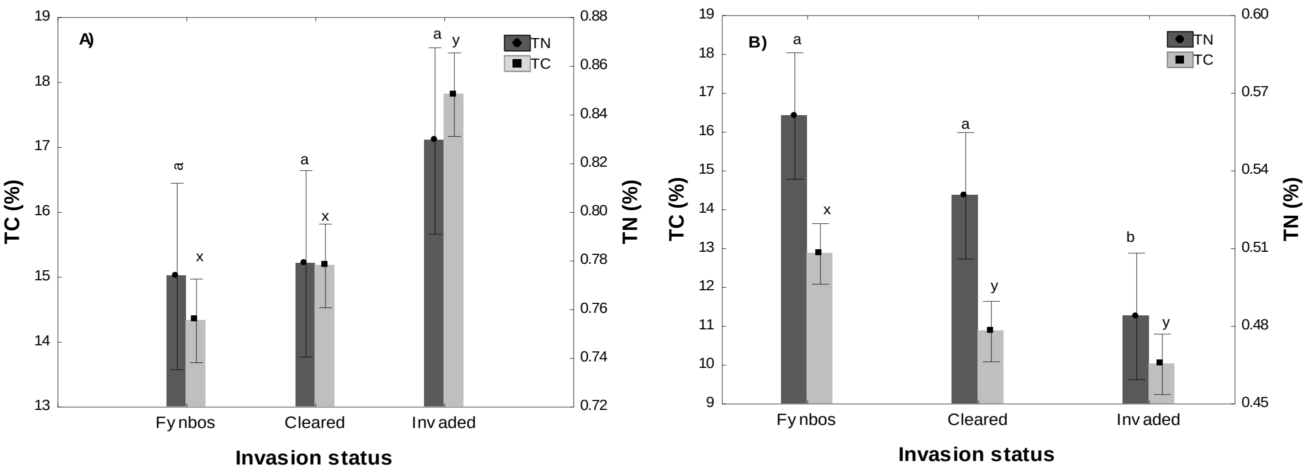


Figure 17: TN and TC in infiltrated sediment (< 63 µm) for invasion statuses at (A) Wit and (B) Du Toit's River. Mean values are indicated by different point symbols and whiskers indicate ± 95% confidence interval. Letters indicate significant differences (LSD tests; p < 0.05) for interaction effects based on one-way ANOVAs

TC and TN in infiltrated sediment are highly correlated with one another at all sites (r = 0.90; Table 6). Clay fraction of infiltrated sediment was significantly correlated with TN. The linear regressions between the clay fraction and TN gives R^2 values for the natural and cleared sites greater than 0.60 (Table 7). The invaded sites on the Du Toit's and Wit River had relatively lower R^2 values of 0.26 and 0.47, respectively (Table 7). Overall, C:N ratios showed a significant and inverse correlation with iron concentrations (r = −0.84; p < 0.001) and clay percentage (r = −0.54; p < 0.001) (Table 6).

Table 6: Pearson's correlation coefficient (r) for selected soil physical and chemical properties of infiltrated sediment. Correlations were calculated from all available data irrespective of invasion status; however, different variables differed in the regularity of sampling: monthly (TC, TN, clay percentage, stream temperature: °C), seasonally (TP and iron) and biannually (aluminium and pH). Correlation values represent only instances where comparisons could be made

	TP ($mg \cdot g^{-1}$)	**TN (%)**	**TC (%)**	**C:N**	**Iron ($mg \cdot g^{-1}$)**	**Aluminium ($mg \cdot g^{-1}$)**	**pH (H_2O)**	**Clay (%)**
Temperature in water (°C)	0.84**	0.50**	0.29*	−0.60**	0.96**	N/A	−0.80**	0.81**
TP ($mg \cdot g^{-1}$)		0.32**	−0.07	−0.67**	0.90**	0.57*	−0.27	0.89**
TN (%)		–	0.90**	N/A	0.39**	N/A	N/A	0.65**
TC (%)			–	N/A	−0.45**	N/A	N/A	0.25**
C:N ratio				–	−0.84**	0.03	N/A	−0.54**
Iron ($mg \cdot g^{-1}$)					–	0.54**	−0.17	0.95**
Aluminium ($mg \cdot g^{-1}$)						–	−0.05	0.33*
pH (H_2O)							–	−0.34
Clay (%)								–

* Significant relationship between the two properties at p < 0.05

** Highly significant relationship between the two properties at p < 0.01

4.3.3 Spatial and temporal dynamics of TP in riverbed sediments

Significant seasonal trends were noted for TP in infiltrated sediment on the Wit River ($F_{[3,\,18]} = 290.38$; $p < 0.001$) with significantly higher concentrations in summer (1.63 ± 0.39 mg g^{-1}) relative to autumn (0.50 ± 0.11 mg g^{-1}), winter (0.32 ± 0.11 mg g^{-1}) and spring (0.52 ± 0.18 mg g^{-1}). TP differed significantly between invasion statuses (two-way ANOVA: $F_{[2,\,6]} = 18.07$; $p < 0.01$). On average over the sampling period, invaded sites had significantly higher TP concentrations (mean = 0.78 ± 0.51 mg·g^{-1}) than natural sites (0.47 ± 0.33 mg·g^{-1}; $p < 0.05$), but no significant difference was apparent between invaded and cleared sites (0.88 ± 0.69 mg·g^{-1}; $p = 0.3729$).

Similar spatial trends were observed at the Du Toit’s River, with significant differences between invasion statuses ($F_{[2,\,6]} = 6.04$; $p < 0.05$). Natural sites had significant lower mean TP concentration than invaded ($p < 0.01$) and cleared sites ($p < 0.01$) with no significant differences between cleared and invaded sites ($p = 0.9819$). TP concentrations differed significantly between invasion statuses with lowest concentration in the fynbos sites (0.41 ± 0.22 mg·g^{-1}), followed by higher concentrations in the cleared (0.59 ± 0.27 mg·g^{-1}) and invaded sites (0.59 ± 0.31 mg·g^{-1}). Significant seasonal trends were observed for TP in infiltrated sediment seasons ($F_{[3,\,18]} = 46.10$; $p < 0.001$) with significant higher mean concentration in summer (0.98 ± 0.25 mg·g^{-1}) compared with spring (0.46 ± 0.1 mg·g^{-1}), autumn (0.40 ± 0.16 mg·g^{-1}) and winter (0.41 ± 0.08 mg·g^{-1}).

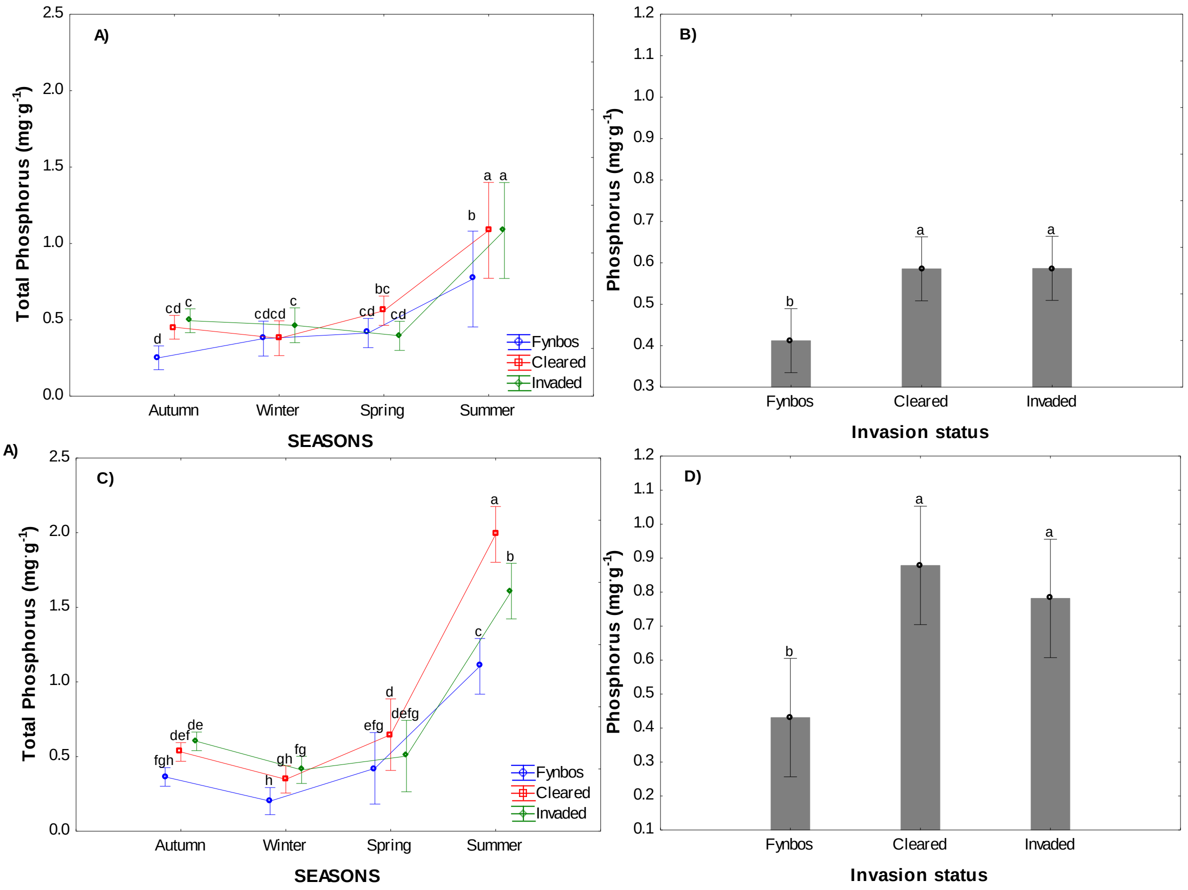


Figure 18: TP in infiltrated sediment (< 63 µm) for invasion statuses across seasons. Mean values are indicated by different point symbols and whiskers indicate ±95% confidence interval. Letters indicate significant differences (LSD tests; p <0.05) for interaction effects based on RMANOVAs: invasion status and seasons. Mean seasonal measurements for TP are depicted in (A) invasion statuses at the Du Toit’s River and (C) at the Wit River. Mean seasonal measurements for TP are depicted in the bar graphs for Du Toit’s River (B) and (D) Wit River for invasion statuses. In (B) and (D), letters represent significant differences (LSD tests; p < 0.05) based on one-way ANOVAs

TP concentrations in infiltrated sediment were significantly correlated with clay percentage, iron and aluminium concentrations, regardless of invasion status. TP showed a significant inverse relationship with C:N ratios (Table 6). TP concentrations had significant relationship with TN. pH (H_2O) had no effect on TP ($r = -0.27$). C:N ratios showed a significant and inverse correlation with iron concentrations and clay percentage.

4.3.4 Spatial variation in nutrient content between invasion statuses

In this section, the nutrient concentration measured in Section 4.3.2 and 4.3.3 has been corrected for differences in particle size composition. The method accounts for variations in particle size composition via the clay percentage of the samples. The general trend is that an increase in nutrient concentration is associated with a decrease in particle size. This equates to an increase in specific surface area and the number of potential sorption sites with decreasing particle size. It was found that grain size composition differed both spatially and temporally between the invasion statuses on the two rivers (see Section 3.2.1 for explanation). Therefore, to minimise the influence of grain size variations on nutrient concentrations, a particle size correction factor was applied to allow the direct comparisons between invasion statuses.

Particle size effect correction test

The correlation analysis of the association between clay percentage and nitrogen, phosphorus and iron concentrations values for the individual sites from the respective rivers within this study indicated that particle size influence was highly significant across the geochemical properties and sites concerned. Therefore, particle size correction was considered appropriate. The correlations between TC and clay percentage for the individual sites from the rivers was variable across sites resulting in a non-linear relationship. Thus, it was considered inappropriate to apply particle size correction to the TC concentration values.

Table 7: Spearman's correlation coefficients ρ (rho) and significance for clay percentage versus selected nutrient properties in infiltrated sediment (< 63 µm) from fynbos, cleared and invaded sites on the rivers

Rivers	**Du Toit's**			**Wit**		
	Fynbos	**Cleared**	**Invaded**	**Fynbos**	**Cleared**	**Invaded**
TP ($mg \cdot g^{-1}$)	0.63**	0.90**	0.47**	0.53*	0.97**	0.98**
TN (%)	0.62**	0.64**	0.26**	0.64**	0.79**	0.47**
TC (%)	0.36**	0.30**	0.08	0.17*	0.00	0.08
Iron ($mg \cdot g^{-1}$)	1.00**	0.92**	0.90**	0.73*	0.99**	1.00**

* Correlation is significant at $p < 0.05$
** Correlation is significant at $p < 0.01$

Between site variations in TN content

Significant interaction between invasion statuses and rivers was observed (two-way ANOVA: $F_{[2, 12]} = 29.11$; $p < 0.01$; Figure 19). The invaded site on the Wit River had significantly higher TN content (mean = 5.75%) than the fynbos sites on the Wit (4.44; $p < 0.001$) and Du Toit's River (4.58%; $p < 0.001$), with no differences between the fynbos sites ($p = 0.56$).

Conversely, the invaded site on the Du Toit's River had significantly lower TN content (3.52%) than the fynbos sites on both the Du Toit's ($p < 0.001$) and Wit River ($p < 0.01$; Figure 19). Mean TN content for the cleared sites on the Wit and Du Toit's Rivers, 3.25% and 3.76% respectively, was significantly lower than both fynbos sites (p < 0.001) and the invaded site on the Wit River (p < 0.001). The differences between both cleared sites and the invaded site on Du Toit's River were not significant for TN (p > 0.05) whereas significant differences were noted between the cleared sites (p > 0.05; Figure 19).

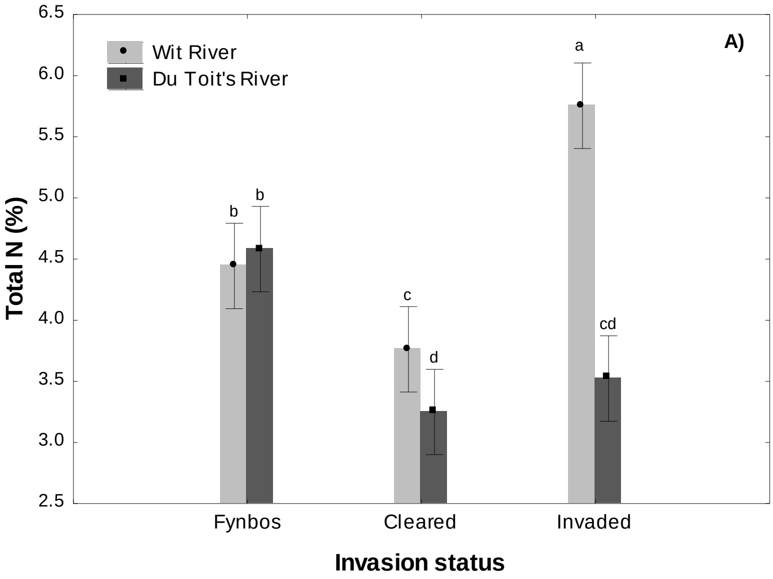


Figure 19: Mean TN in infiltrated sediment for invasion statuses across rivers. Bars represent means and whiskers represent ± 95% confidence intervals for TN. Significance levels (LSD post hoc test; p < 0.05) are indicated by different letters for two-way ANOVAs. TN concentrations are particle size corrected

Between site variations in TP content

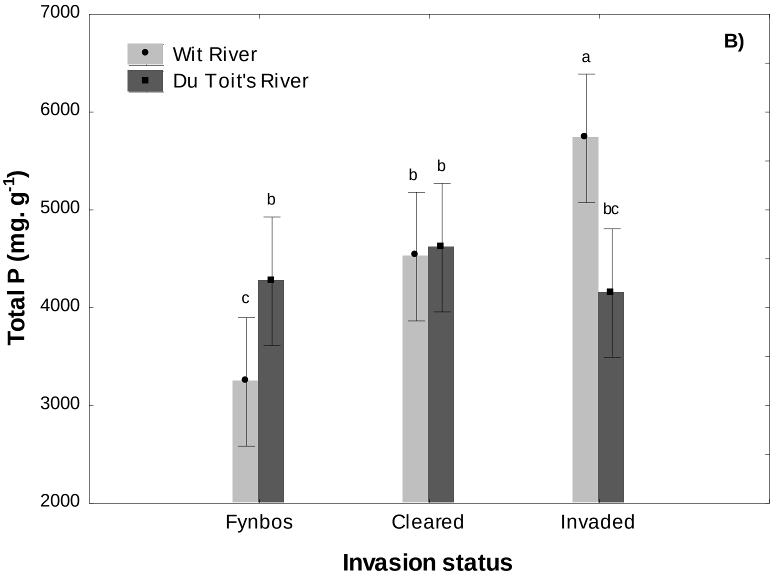


Figure 20: Mean TP in infiltrated sediment for invasion statuses across rivers. Bars represent means and whiskers represent ± 95% confidence intervals for TP. Significance levels (LSD post hoc test; p < 0.05) are indicated by different letters for two-way ANOVAs. TP concentrations are particle size corrected

Interaction between invasion statuses and rivers for TP in sediment infiltration was significant (two-way ANOVA: $F_{[2, 12]} = 9.60$; $p < 0.01$; Figure 20). The invaded site on the Wit River had a significantly higher TP content (mean = 5731.73 $mg \cdot g^{-1}$) than the fynbos site on the Wit River ($p < 0.001$) and the Du Toit's River ($p < 0.01$) with mean values of 3242.73 $mg \cdot g^{-1}$ and 4271.05 $mg \cdot g^{-1}$, respectively. The invaded site on the Du Toit's River had significantly lower TP content (4150.01) than the invaded site on the Wit River ($p < 0.01$; Figure 20).

The mean TN content did not differ between the invaded site on the Du Toit's and the cleared sites on both the Du Toit's and Wit River ($p > 0.05$). Similarly, no differences were noted between the invaded and fynbos site on the Du Toit's River ($p = 0.78$; Figure 20).

4.3.5 Spatial and temporal variability in stream water nutrient concentrations

Ammonium (NH_4^+) concentrations were below the detection limit of 10 $\mu g \cdot \ell^{-1}$ in May 2016 at all sites. The highest mean NH_4^+ concentrations on the Wit River and Du Toit's River were observed in November with maximum values of 86 $\mu g \cdot \ell^{-1}$ and 80.3 $\mu g \cdot \ell^{-1}$, respectively. Averaged over the course of the sampling period, fynbos sites had the highest NH_4^+ concentrations (mean = 56.9 $\mu g \cdot \ell^{-1}$) relative to the cleared (45.9 $\mu g \cdot \ell^{-1}$) and invaded sites (44 $\mu g \cdot \ell^{-1}$) on the Du Toit's River; however, this was not significant (one-way ANOVA: $F_{[2, 24]} = 0.39$; $p = 0.68$; Table 8).

The highest mean NH_4^+ concentrations on the Wit River were observed at the cleared site with a mean of 34.3 $\mu g \cdot \ell^{-1}$ compared with the fynbos and invaded sites with means of 31.2 $\mu g \cdot \ell^{-1}$ and 26.8 $\mu g \cdot \ell^{-1}$, respectively (Table 8). Again, no significance was found between invasion statuses (one-way ANOVA: $F_{[2, 30]} = 0.14$; $p = 0.87$). This is supported by the substantial variation in NH_4^+ concentrations among the sites with different invasion statuses.

Nitrate + nitrite ($NO_3^- + NO_2^-$) concentrations were below the detection limits of 5 $\mu g \cdot \ell^{-1}$ in May 2016, June 2016 and February 2017 at all sites. $NO_3^- + NO_2^-$ concentrations peaked in September at 9 $\mu g \cdot \ell^{-1}$ at the Du Toit's River and 25 $\mu g \cdot \ell^{-1}$ at the Wit River. Averaged over the course of the sampling period, $NO_3^- + NO_2^-$ concentrations on the Du Toit's River increased from the fynbos site (mean = 6.7 $\mu g \cdot \ell^{-1}$) to the cleared and invaded sites with means of 7.7 $\mu g \cdot \ell^{-1}$ and 8.3 $\mu g \cdot \ell^{-1}$, respectively (Table 8). $NO_3^- + NO_2^-$ concentrations on the Wit River were highest at the cleared site (13.7 $\mu g \cdot \ell^{-1}$) compared with the fynbos and invaded sites with means around 7.7 $\mu g \cdot \ell^{-1}$ (Table 8). However, $NO_3^- + NO_2^-$ data were not sufficient for a valid statistical analysis.

Orthophosphate (PO_4^{3-}) concentrations ranged from below the detection limit (4 $\mu g \cdot \ell^{-1}$) to 126.29 $\mu g \cdot \ell^{-1}$ in the Wit River and below the detection limit to 67.65 $\mu g \cdot \ell^{-1}$ in the Du Toit's River. The invaded site in the Wit River showed relatively higher PO_4^{3-} concentration with a mean of 26.6 $\mu g \cdot \ell^{-1}$ compared with the cleared and fynbos sites with means of 10 $\mu g \cdot \ell^{-1}$ and 4.8 $\mu g \cdot \ell^{-1}$, respectively (Table 8).

Despite the apparent spatial differences across invasion statuses, PO_4^{3-} concentrations were not significantly different (one-way ANOVA: $F_{[2, 21]} = 1.59$; $p = 0.23$). Similarly, the interaction between invasion status and PO_4^{3-} concentrations was not significant (one-way ANOVA: $F_{[2, 12]} = 1.87$; $p = 0.20$). This was mainly due to the high standard deviations in PO_4^{3-} concentrations that indicates the high variability among samples in the respective sites (Table 8). Nevertheless, mean PO_4^{3-} concentrations in the Du Toit's River were highest at the cleared site with a mean concentration of 32.5 $\mu g \cdot \ell^{-1}$. Mean PO_4^{3-} concentrations in the fynbos and invaded sites were 10.7 $\mu g \cdot \ell^{-1}$ and 22.4 $\mu g \cdot \ell^{-1}$, respectively (Table 8). PO_4^{3-} concentrations in the Du Toit's River ranged from below the detection limit (4 $\mu g \cdot \ell^{-1}$) to 26.67 $\mu g \cdot \ell^{-1}$ in the fynbos site, 67.65 $\mu g \cdot \ell^{-1}$ in the cleared site, and 33.33 $\mu g \cdot \ell^{-1}$ in the invaded site.

Table 8: Comparison of mean values (± standard deviation) of nutrient concentrations for ammonium (NH_4^+), nitrate + nitrite (NO_3^- + NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-}) between invasion statuses of the respective rivers across the study period. All concentrations are reported in $\mu g \cdot \ell^{-1}$

Rivers	**Wit**			**Du Toit's**		
	Fynbos	**Cleared**	**Invaded**	**Fynbos**	**Cleared**	**Invaded**
NH_4^+	31.2 ± 34.7	34.3 ± 35.1	26.8 ± 28.5	56.9 ± 39.7	45.9 ± 34.4	44 ± 31.8
NO_3^- + NO_2^-	7.7 ± 3.5	13.7 ± 7.5	7.7 ± 3.5	6.7 ± 3.6	7.7 ± 3.5	8.3 ± 4.1
NO_3^-	1.9 ± 3.5	3.4 ± 7.5	1.9 ± 3.5	1.3 ± 1.2	0.7 ± 1.5	1.5 ± 2.3
PO_4^{3-}	4.8 ± 5.6	10.0 ± 16.2	26.6 ± 40.8	10.7 ± 14.6	32.5 ± 25.2	22.4 ± 10.7

4.4 Discussion

4.4.1 Temporal variability in sediment-bound nutrient concentrations

Significant temporal variations for nutrient (TC, TN and TP) concentrations in infiltrated sediment and suspended sediment were noted in the two streams assessed. The minimum TN and TC concentrations in infiltrated sediment concurred with high discharge and high suspended sediment loads in winter. This is supported by a study by Acornley and Sear (1999) that reported approximately 21% organic carbon in the suspended sediment during low flows in summer and 11% during high flows in winter in two rivers in Hampshire, England. Schindler Wildhaber et al. (2012) also reported significant temporal variation in TC and TN in infiltrated sediment and suspended sediment with low concentrations at high discharge and high sediment yield, and high concentrations at low discharge and low sediment yields. The pattern can be partially explained by the dilution of TC and TN during high sediment loads with mineral matter derived from sources within or proximal to the channel (Zhang et al., 2009). This pattern was attributed by Sear et al. (2008) and Schindler Wildhaber et al. (2012) to the low specific gravity of TC, which holds it in suspension longer than inorganic material. Thus, the TC proportion increases during periods of low flows compared with coarse inorganic material, which may also hold for this study. The significant positive relationship between the concentration of TN in infiltrated sediment and the clay fraction (sediment < 63 µm) and the significant inverse relationship between TN and infiltrated sediment and suspended sediment supports this assumption. Like TC, fine clay particles are also held in suspension more easily than coarser grained sediment and settle out during low energy flows (Schindler Wildhaber et al., 2012).

Furthermore, the supply of materials to the streams varies significantly with the season due to the timing of leaf litterfall and soils nutrient cycling processes in the riparian zones. For example, peak litterfall rates reported for *A. mearnsii*-invaded riparian zones in the Western Cape, South Africa, concurred with the first seasonal rains in autumn while indigenous fynbos litterfall rates peak in summer (see Chapter 5 of this report). Further, Naude (2012) found that nitrate (NO_3^-) concentrations were highest in autumn with maximum ammonium (NH_4^+) concentrations observed in summer for riparian soils in the south of the Western Cape, South Africa. During heavy rainfall events in the wet season (autumn to spring), higher water levels increase the connectivity between the riparian zone and stream channel, enabling overbank flow to access, leaf litter and nutrient-rich soils on the riverbanks and in riparian zones (Burt, 1997). Similarly, increased run-off in the catchment connects and delivers distant sources of sediment to the river channel (Walling, 1983). These terrigenous materials are transferred to the river channel where it is stored (deposited) and mineralised during low-flow periods in summer. The explanation holds particularly for the Wit River where riverbanks are low. In addition, high stream temperatures during the summer months (average: 21.12°C) may also be an important factor stimulating/promoting in-stream mineralisation of leaf litter and nitrification processes as reported by Naude (2012) for soils in

Mediterranean riparian ecosystems because elevated nitrogen in streams concur with peak NH_4^+ concentrations in riparian soils.

TP concentrations in infiltrated sediment have been found to remain relatively constant during the high-flow period (autumn to spring). The phosphorus concentrations during the period of low flow in summer were significantly higher than that during high flows. The difference in phosphorus concentrations during the high flows compared with the low-flow period might suggest that phosphorus sources in the rivers were not depleted or that they were replenished during the wet season and accumulated in the channel during summer low-flow conditions. Phosphorus ions, particularly phosphates (PO_4^{3-}), are highly reactive ions and are readily absorbed onto negatively charged clay particles in soils and sediments by bonding chemically to positively charged particles such as iron and aluminium hydroxides (Addiscott & Whitmore, 1991) or substitution of phosphates for silicates in the clay structure (Harper et al., 2008). Therefore, freshwater systems receive most of their phosphorus through soil erosion (Correl, 1998). Soil erosion rates increase during consecutive storm events due to high rainfall and overland flow, which may result in increased sediment delivery to streams (Hicks et al*.,* 2000). Thus, the elevated phosphorus retained in riparian soils during summer and autumn as reported by Naude (2012) can readily be flushed to the stream through surface pathways during winter storm events (Van der Perk, 2006). However, these fine clay particles will remain in suspension and mix with inorganic mineral matter remobilised from the riverbed until conditions are favourable for it to settle out of the water column.

This pattern also can be attributed to the supply of sediment-bound phosphorus derived from terrigenous soil erosion, which involves nutrient-rich sediment and leaf litter from riparian areas. The supply of materials to the streams varies significantly with season due to the timing of leaf litter input, mineralisation and soil nutrient cycling processes. For example, peak litterfall rates in *A. mearnsii*-invaded riparian zones concurred with the first seasonal rains in autumn (see Chapter 5 in this report). Besides the seasonal supply of nutrient-rich material from riparian zones, the seasonal variation in the intrinsic stream characteristics (i.e. temperature, flow and sediment loads) could also be important driving factors. The significant inverse relationship between stream temperature and C:N ratios in infiltrated sediment provide evidence that the seasonal variation of the stream temperature has a pronounced effect on rate of carbon mineralisation. Thus, the increase of nutrients during summer period could also be as result of a combination of warmer stream temperatures that stimulate microbial activity and the mineralisation of large stocks of fynbos and *A. mearnsii* leaf litter on the streambed.

C:N ratios have been largely used as a proxy to discriminate between autochthonous and allochthonous sources of TC and TN in sediment (Gordon & Goni, 2003; Wu et al., 2007; Zhang et al., 2007; Ramaswamy et al., 2008). The mean C:N ratios of infiltrated sediment ranged between 18.72 and 21.27 for the sites on the Wit River, and between 20.80 and 22.98 for the three sites on the Du Toit's River (Table 5). The C:N ratios of the suspended sediment were comparable to the C:N ratio of the infiltrated sediment. Mean C:N ratios were between 18.72 and 22.98 for infiltrated sediment and suspended sediment at all three sites on the respective rivers. The C:N ratios of infiltrated sediment and suspended sediment were generally higher than the typical C:N ratio of autochthonous material (> 10; Meyers, 1994; Meyers & Teranes, 2001), thus input from allochthonous sources (e.g. soil or litter) were evident at both rivers. C:N ratios in infiltrated sediment showed a significant and strong inverse correlation with iron concentrations. This inverse trend is expected because the electrons transferred to iron electron acceptors could be a major pathway for organic carbon mineralisation (Lovely & Phillips, 1986).

The most important drivers of temporal variations of the nutrient concentrations in this study are likely to be a function of two factors: (a) variation in environmental conditions such as river discharge, rainfall, temperature and sediment loads; and (b) variation of source input and delivery such as litterfall and soil erosion. Therefore, the presence of invasive nitrogen-fixing *A. mearnsii* at the downstream sites of these rivers could increase stream nutrient concentrations, which are derived from large stocks of nutrient-rich leaf litter and soils. Thus, during heavy rains, the riparian zone and stream channel are connected and run-off may deliver nutrient-rich sediments to the channel, which accumulate during low flows.

4.4.2 Spatial variability in sediment-bound nutrient concentration

The two rivers in this study are predominantly underlain by Peninsula Formation quarzitic sandstone. Soils in the Fynbos Biome derived from sandstone are generally characterised as acidic and deficient in essential nutrients such as nitrogen and phosphorus (Rebelo et al., 2006; Soderberg & Compton, 2007; Cramer, 2010). Therefore, the soils contribute very little nutrients, specifically phosphorus, to the ecosystem. The mean phosphorus content of the soils in the fynbos range between 0.003 $mg \cdot P \cdot g^{-1}$ and 0.02 $mg \cdot P \cdot g^{-1}$ (Hawkins et al., 2005) with mean TN concentrations of 0.09% (Cowling & Holmes, 1992). The TP concentration increased from the upstream fynbos sites to the two downstream cleared and invaded sites on the rivers. The increase of TP in infiltrated sediment from up- to downstream can be explained by the degree of nitrogen-fixing *A. mearnsii* infestations in the riparian zone at the downstream sites.

The fynbos sites are mostly surrounded by pristine fynbos vegetation, while the cleared sites were cleared from nitrogen-fixing invasive woody trees. Nitrogen-fixing *A. mearnsii* trees heavily infest the invaded sites. The TC and TN concentrations in infiltrated sediment increased similarly to TP from up- to downstream on the Wit River. Previous studies have shown that leaf litter from riparian trees, especially nitrogen-fixing species, can enhance in-stream nutrient cycling because of its high nutrient content and degradability (Compton et al., 2003; Volk et al., 2003; Mineau et al., 2011). The conflicting results obtained from the Du Toit's River, where TN and TC decrease from up- to downstream, could be explained by heterogeneity between sites in terms of channel and riparian zone characteristics (Schlosser & Karr, 1981). The high terraces with wide, gentle sloping riparian areas observed at the invaded and cleared sites on the Du Toit's River could reduce the connectivity between riparian sources and the stream channel; consequently trapping leaf litter and nutrient-rich sediment in the riparian zone. Thus, the riparian zone at the Du Toit's River may have played a significant role as a buffer to nutrients entering the stream.

In general, the TN and TC concentrations of the infiltrated sediment and suspended sediment are relatively high. Schindler Wildhaber et al. (2012) reported mean TN concentrations of 0.2% and 0.47%, respectively, with a mean of 0.33% in the Enziwigger River in Switzerland, which is impacted by different land uses (including forest, pasture and arable farmland). The TC concentrations ranged from 1.7% to 6.6% with a mean of 4.2%. The TC concentrations of infiltrated sediment in this study were similar to those of Sear et al. (2008).

The mean nitrogen concentrations reported for mountain fynbos soil were more than five times lower than the nitrogen concentrations observed in this study (Cowling & Holmes, 1992). Similarly, mean phosphorus values for the respective invasion statuses exceed that of the background soil concentrations provided by Hawkins et al. (2005) and Stock and Verboom (2012). Likewise, the maximum phosphorus concentrations in sediments along the highly impacted Berg River in the Western Cape, South Africa, reported by Struyf et al. (2012) were found to be almost ten times lower than that found in the invaded sites of this study. Concordantly, TN and TP concentrations reported in this study were considerably higher, suggesting that invasive nitrogen-fixing *A. mearnsii* and clearing thereof may have played a significant role in suspended sediment nutrient concentrations in these rivers.

The phosphorus in fresh waters is mostly available at a slightly acidic pH between 6 and 7 (Naiman et al. 2002). At lower pH, phosphorus combines readily with metals (aluminium, iron and manganese) (Tate et al., 1995) while at a higher pH, it associates with calcium. The correlation analysis used to examine the relationships between TP concentrations and related metals (iron and aluminium) and clay fraction (< 3.9 µm) of infiltrated sediment showed that there were significant and strong relationships between TP and iron, and clay. The strong correlation between iron and TP concentrations in infiltrated sediment and the low pH values (< 6) in these systems suggests that phosphorus forms a less soluble complex with iron ions (hydroxides) and adheres to clay particles. Therefore, the strong absorptive reaction between phosphorus and iron hydroxides on clay particles represents a substantial accumulating sink of phosphorus (Lambers et al., 2006). These sorption interactions potentially reduce the phosphorus concentration in stream water and its bioavailability to plants and aquatic biota. Iron-bonded phosphorus is released as inorganic phosphate when (a) redox reaction is satisfied; (b) under high flows and/or anoxic conditions (Webster et al., 2001; Dallas & Day, 2004; Sinkko et al., 2011); or (c) in a high pH (i.e. close to 8) environment (Gomez et al., 1999; Hou et al., 2013).

4.4.3 Impacts of nitrogen-fixing *A. mearnsii* on nutrient contents in fynbos streams

4.4.4 Effects sediment-bound nutrient content

The TN and TP contents were significantly higher in the Wit River site invaded with *A. mearnsii* compared with sections with native fynbos vegetation. This was expected because of the greater input of nitrogen-rich leaf litter to the *A. mearnsii*-invaded sites compared with the fynbos sites (see cf. Naude, 2012). The annual litterfall of alien *A. mearnsii* was about 297.29 $g \cdot m^{-2}$, being greater than that of indigenous mountain fynbos riparian vegetation (68.88 $g \cdot m^{-2}$) in the riparian zones of the Western Cape, South Africa (see Chapter 5 of this report). The phosphorus and nitrogen content of the *Acacia* litter exceeded that of litter from the indigenous vegetation. The close proximity of invasive trees and the low riverbanks results in a high degree of lateral connectivity between riparian area and stream at the invaded site on the Wit River. This could facilitate direct leaf litter input and lateral transport through surface run-off into streams, where it is stored and mineralised in the streambed and consequently releases nutrients (especially nitrogen) under favourable conditions (Strauss & Lamberti, 2000, Bernhardt & Likens, 2002, Starry et al., 2005)

Sediment nutrient concentrations (TN and TP) were strongly correlated with clay particles found in this study. Therefore, it is possible that nitrogen- and phosphorus-rich sediments from *A. mearnsii*-invaded and cleared riparian zones, as discussed in Chapter 3, are mobilised during winter high flows through soil erosion, and thus contribute to the enhanced nitrogen and phosphorus concentrations noted in the Wit River. The impacts of invasive nitrogen-fixing on soil nitrogen stocks in riparian zones have been well documented in central Japan (Akamatsu et al., 2011), in semi-arid New Mexico (DeCant, 2008) and South Africa (Jacobs et al., 2013).

In their study, Jacobs et al. (2013) found that soil nitrogen and phosphorus stocks in *Acacia*-invaded riparian zones were significantly enhanced compared with natural fynbos riparian soils in the south of the Western Cape, South Africa. The increase has been attributed to the large stocks of riparian nitrogen-rich leaf litter and increased mineralisation rates that were stimulating (promoting) these enhanced nutrient concentration in surface soils of *Acacia*-invaded riparian zones (Naude, 2012). A similar result was found in riparian soils invaded by Russian olive along the Rio Grande River in New Mexico, USA. In this study, DeCant (2008) showed a 55% increase in TN and around four times the amount of available nitrogen compared with soils under cottonwood; however, there was little focus on phosphorus. However, the impacts of nitrogen-fixing invasive plants on phosphorus cycling still remain poorly understood (Chamier et al., 2012). The soil phosphorus status was altered by invasion of alien legumes, such as *A. cyclops* and *A. saligna*, which were essentially due to large litter accumulations and rapid turnover rates (Witkowski & Mitchell, 1987). Due to their higher growth rates and ability to fix atmospheric nitrogen, Australian *Acacias* may require reasonably high sources of soil phosphorus, consequently increasing the rate at which inorganic phosphorus enters soil pools (Juba 2012).

Conversely, TN and TP content were significantly lower in the invaded section on the Du Toit’s River than the invaded section on the Wit River. The different pattern observed at the Du Toit’s River might be a result of the combination of both between site variation in stream characteristics (such as stream width, size of streambed, stream temperature and riverbank height and riparian topography; cf. Chapter 3). Moreover, the proximity of the invasive trees to the stream channel, abundance and density could also be considered as important controlling variables in the transfer of nitrogen-rich leaf litter and nutrients between riparian soil and streams.

In contrast to the Wit River, a relatively wide stream channel with wide, gentle sloping riparian zones and high terraces (riverbanks) characterise the cleared and invaded site on the Du Toit's River. These stream characteristics could reduce the connectivity (supply-delivery) between the riparian zone and stream channel in these sections, which consequently lead to efficient trapping of *A. mearnsii* litter and nutrient-rich sediment in these areas (riparian zone and floodplains). This most likely leads to immobilisation of nutrients by elevated soil processes (nitrification, denitrification and soil respiration) to occur in the floodplain and riparian zones rather than in the stream, thus preventing nutrients entering the stream (Naiman et al., 2005; Jacobs et al., 2006). Therefore, channel characteristics and the proximity of the invasive *A. mearnsii* trees to the stream channel could potentially regulate stream nutrient fluxes. Therefore, each particular stream should be investigated and managed independently for site-specific characteristics and effects.

4.4.5 Effects on stream nutrient concentrations

The increase in $NO_3^- + NO_2^-$ concentrations in streams is the most commonly documented effect of nitrogen-fixing invasive plants on water quality (Compton et al., 2003; Hurd & Raynal, 2004; Mineau et al., 2011; Wiegner et al., 2013). In our study, there was a marginal increase in $NO_3^- + NO_2^-$ concentrations in the cleared sites of both rivers. The greatest concentration increase was in the cleared site on the Wit River where $NO_3^- + NO_2^-$ increased by ~5 $\mu g \cdot \ell^{-1}$. The effects of nitrogen-fixing trees on stream NH_4^+ concentrations were similar to that reported for $NO_3^- + NO_2^-$. In this study, small increases in NH_4^+ concentrations were observed in the fynbos sites compared with the invaded sites. This pattern was consistent across streams; however, it was not significant. Similar to this study's findings, several studies have reported no effect of nitrogen-fixing trees on stream NH_4^+ concentrations (Compton et al., 2003; Volk et al., 2003; Wiegner et al., 2013). Invasion statuses had less effect on stream water concentrations of NH_4^+ and $NO_3^- + NO_2^-$ than on suspended sediment TN concentrations in this study. The high average stream temperature and the loss of nitrogen through denitrification and biological uptake likely explain the low available nitrogen concentrations between indigenous fynbos and invaded areas.

The effects of nitrogen-fixing trees on stream phosphorus concentrations have not been well documented and reported effects are equivocal. In this study, *A. mearnsii*-invaded sections showed much higher concentrations of PO_4^{3-} than natural fynbos sections, but the difference was not significant due to the high variability among samples. The greatest concentration increase was in the *A. mearnsii*-invaded site on the Wit River, where PO_4^{3-} increased by ~450%, and at the cleared site on the Du Toit's River (~200%). Similar to this study's findings, Wiegner et al. (2013) reported no significant change in total dissolved phosphorus concentrations in Hawaiian streams below sites invaded by *F. moluccana* stands. A similar result was found in two western United States streams draining *E. angustifolia*-invaded riparian zones, where PO_4^{3-} concentrations are unaffected. In contrast, Volk et al. (2003) report significantly higher TP concentration in streams draining nitrogen-fixing *Alnus rubra*-infested riparian areas compared with streams surrounded by old growth coniferous forests in the Pacific Northwest. Similar to this study's findings, Wiegner et al. (2013) reported no significant change in total dissolved phosphorus concentrations in Hawaiian streams below sites invaded by *F. moluccana* stands. A similar result was found in two western United States streams draining *E. angustifolia*-invaded riparian zones where PO_4^{3-} concentrations were unaffected.

In general, it is worth noting that stream nutrient concentrations showed no significant longitudinal trends. Thus, this finding evidenced that stream water chemistry may not be affected to the same degree as suspended sediment in this study by an invasion of *A. mearnsii*. The potential of intrinsic stream characteristics such as stream temperature, stream size and in-stream processes to counterbalance additional nutrient inputs is considered to be a key controlling variable. The disproportionally high TN and TP concentrations in suspended sediment could be a result of the input of nutrient-rich sediments from invaded riparian areas and mineralisation of large stocks of leaf litter stored in the riverbed that increase the release of nutrients in sediments. Several studies have also recognised that nutrients are mainly bound to clay particles in sediment (Steegen et al., 2001; Verstraeten & Poesen, 2002). Thus, clay particles can become a substantial sink for nutrients, regulating its release to pore water and overlying stream water.

4.5 Conclusion

Stream nutrient content was quantified for sections draining three different invasion statuses in two fynbos mountain streams. This study offered several insights into the nutrient dynamics of fynbos mountain streams and the extent to which these nutrients are altered by *A. mearnsii* infestations and clearing. The data supports the hypothesis that invasion by nitrogen-fixing *A. mearnsii* changes the suspended sediment nutrient content. Pronounced temporal variation in sediment nutrient concentrations was observed in this study. The analysis of the temporal variation showed that nutrient concentrations in sediment infiltration was predominantly influenced by timing of litterfall and variation in transport conditions such as discharge, precipitation and sediment loads. The mean phosphorus concentrations exceeded the upper limit ($0.02\ mg \cdot P \cdot g^{-1}$) of the background phosphorus concentrations suggested for soils in the Fynbos Biome (suggesting an additional source of phosphorus in these rivers). Rainfall frequency and intensity are constantly declining in the Western Cape, which could result in reduced streamflow and a lower dilution capacity of streams. Thus, we expect the residence time and nutrient concentrations in these rivers to increase further.

The nutrient content (nitrogen and phosphorus) from the invaded site on the Wit River differed significantly from the two natural fynbos sites. The large amount of litter with its high nutrient content from *A. mearnsii* might explain the increase of nitrogen and phosphorus in sediments. Conversely, due to the difference in stream characteristics (i.e. riverbank height, stream width) at the invaded site on the Du Toit's River, invasive species had a smaller impact on the nutrient content. The nutrient content in the cleared sites showed a trajectory towards pre-invasion levels, although phosphorus content in the cleared site on the Wit River was significantly higher than the fynbos site. Therefore, after IAP clearing, the time required for nutrient contents to reach levels similar to pre-invasion will depend on the rate at which fynbos riparian vegetation species recolonise/re-establish. This could also suggest that different types of channels route their nutrients differently through the catchment. Thus, due to site-specific stream characteristics, each stream should be considered, investigated and managed independently.

The results suggest that invasive nitrogen-fixing *A. mearnsii* and clearing thereof have a greater influence on sediment-associated nutrients content in fynbos streams than on surface water quality. Furthermore, this study adds to the growing knowledge of in-stream nutrient biogeochemistry by showing that and that Mediterranean streams are hotspots for nutrient processing (Peterson et al., 2001; Dent et al., 2007; Bernal et al., 2015).

CHAPTER 5 – NITROGEN INPUTS FROM INVASIVE *A. MEARNSII* INTO RIPARIAN ZONES IN THE CFR

5.1 Introduction

The maintenance of biodiversity is a major challenge for ecosystem management. After habitat loss, IAP species is the second-biggest threat to global biodiversity (D'Antonio & Meyerson, 2002; Richardson & Van Wilgen, 2004). Riverine systems, including riparian habitats, are extremely vulnerable to invasion and the spread of non-native species (Hood & Naiman 2000; Kuglerova et al., 2017). The ability of IAPs to establish themselves in non-native environments depends on their ability to suppress or compete with indigenous riparian vegetation for essential resources such as light, water and nutrients (Vitousek et al., 1997b; Morris et al., 2011). The successful invasion of exotic species in riparian vegetation communities can be harmful to riparian biodiversity and ecosystem process such as nutrient cycling, soil microbial community and soil nitrogen levels (Richardson et al., 2007; Kuglerova et al., 2017). Australian *Acacias*, which consist of 1012 species in the subgenus *Phyllodineae* native to Australia (Richardson et al., 2011), are some of most pervasive alien plants groups in the world (Richardson & Van Wilgen, 2004; Lowe et al., 2008). These Australian *Acacias* compete successfully in disturbed environments such as riparian corridors and post-fire environments (Richardson et al., 2007; Morris et al., 2011) and particularly in nutrient-limited Mediterranean-type ecosystems such as the Portuguese dunes and the CFR in the Fynbos Biome, South Africa (Witkowski, 1991; Brown et al., 2004; Marchante et al., 2008).

In the invasive flora of South Africa, the most common Australian leguminous *Acacia* spp. (*A. mearnsii, A. longifolia, A. saligna*) that invade the CFR in the fynbos plant communities are able to fix nitrogen from the atmosphere, which form root nodules where symbiotic nitrogen-fixing bacteria make nitrogen available for the plant to absorb (Drake, 2011). These woody invaders alter nitrogen, carbon, phosphorus and water cycles (Le Maitre et al., 1996; Yelenik et al., 2004; Morris et al., 2011). Previous studies (Milton, 1981b; Witkowski, 1991; Yelenik et al., 2004, 2007; Naude, 2012) have shown how Australian woody perennial shrubs increase available nitrogen in the soil through increased inputs of nitrogen-rich litter. Nitrogen cycling in the natural vegetation of the Fynbos Biome is a slow process due to the vegetation consisting largely of sclerophyllous shrubs – a structural type characteristic of nutrient-poor substrates in Mediterranean climate regions (Beadle, 1966). Fynbos plants grow slowly and have high nutrient cycling prior to leaf abscission (Stock & Allsopp, 1992). Many studies found that natural vegetation has litter with low levels of nitrogen concentrations, high C:N ratios, and slower rates of decomposition than the nitrogen-fixing plants they are normally compared with (Witkowski, 1991; Liao et al., 2008). Native nitrogen-fixing plants exist in the fynbos, but they are short-lived and distributed erratically in the post-fire plant species community. However, these early colonising species do not deposit enough of their nitrogen-rich leaf litter to have an impact on soil nitrogen dynamic status, thus making them a lesser threat to ecosystem processing (Yelenik et al., 2004). *Acacia* spp. stands trees are in most instances three times taller than fynbos species in the surrounding area and contains a larger quantity of biomass than their native counterparts (Miton, 1981a; Yelenik et al., 2004; 2007).

Many studies indicate that *A. mearnsii* influences ecosystem functioning and native diversity (Caldeira et al., 2001; Forrester et al., 2007; Moyo & Fatunbi, 2010; Drake, 2011; Tye & Drake, 2012; Van der Colff et al., 2017) and increased water uses that moderate stream flow (Le Maitre et al., 2002). *A. mearnsii* is a nitrogen-fixer that can nodulate under the most extreme water- and nutrient-limited conditions (Rodríguez-Echevarria et al., 2009; Sprent, 2009; Crous, 2010) due to specialised mechanisms such as extensive root systems (shallow or deep), symbiotic nitrogen fixation and nutrient conservation strategies (Morris et al., 2011; Potgieter, 2012). The ability of *A. mearnsii* to maintain a high growth rate permits it to compete successfully with native species and eventually overgrow them (Milton, 1981a; Witkowski, 1991; Werner et al., 2010). *A. mearnsii* alters native plant communities more profoundly than it could through direct competition. The dense stands of *A. mearnsii*, which are mostly large dense thick stands of evergreen shrubs between 1 m and 20 m tall, differ tremendously from most native species (riparian community plants), thus allowing *A. mearnsii* to occupy previously unexploited natural environments (Rascher et al., 2012). The dense stands and rapid growth rates of *A. mearnsii* consequently lead to higher above-ground biomass than that of native species (Moyo & Fatunbi, 2010; Rascher et al., 2012). This ultimately results in a substantial increase of nitrogen, which is known to contribute to the long-term maintenance of increased nitrogen stocks in terrestrial and aquatic ecosystems (Vitousek et al., 1997b; Witkowski, 1991; Chapin et al., 2002; Yelenik et al., 2007). These unusual traits of *A. mearnsii* include: greater size, higher growth rates, greater live tissue and litter nutrient concentrations, nitrogen-fixing capabilities and nutrient allocation patterns (Witkowski, 1991; Ehrenfeld, 2003; Liao et al., 2008; Morris et al., 2011; Tye, 2013). Therefore, invasive *Acacia*, especially *A. mearnsii*, is considered a successful and persistent nitrogen-fixing species in any terrestrial ecosystem (Lawrie, 1981; Vitousek et al., 1997a; Yelenik et al., 2004; Rodríguez–Echeverría et al., 2011).

However, the extent of *A. mearnsii* nitrogen inputs (litterfall, nitrogen concentrations, nutrient acquisition, resorption of nutrients) is not well understood in the Fynbos Biome of the nutrient-impoverished ecosystem of the CFR. To examine this, we quantified the difference in litter quantity and quality between the woody *A. mearnsii* compared with the predominantly riparian native species *B. stellatifolium* (*Proteaceae*) and *M. angustifolia* (*Myrtaceae*). The two indigenous species are seen as important keystone species of the south of the Western Cape Mediterranean-type riparian systems of the Fynbos Biome in the CFR. Moreover, the knowledge gained around this study will give a better insight of nitrogen contribution to terrestrial ecosystems and a critical understanding of the nutrient economy of *A. mearnsii* in the nutrient-poor fynbos riparian zones in the CFR in the Western Cape, South Africa.

5.2 Methods

5.2.1 Study area in the Fynbos Biome, CFR

This study was conducted in the mountain streams and mountain transitional zones of the Fynbos Biome in the CFR (Reinecke et al., 2013) in the Breede WMA in the Western Cape. Two perennial rivers were chosen for the study. The Wit River (Figure 21A) is a small perennial tributary of the Breede River. The Du Toit's River (Figure 21B) forms part of the Breede system but is essentially a tributary of the Riviersonderend River, which is shortened by the Theewaterskloof Reservoir (Tharme & Brown, 2004). Along each river, two vegetation states or treatments were identified: (a) near-pristine site, and (b) invaded site (Figure 22). Near-pristine sites had to be characterised with typical mountain fynbos, including *B. stellatifolium,* and *M. angustifolia*, and short indigenous trees, bushes and sedges with no alien invasive trees present (Figure 22A). Invaded sites had to include dominant *A. mearnsii*, while some *A. longifolia* may be present, with IAP cover of over 50% at the sites. Sites were arranged from up- to downstream with near-pristine sites being upstream and invaded sites being downstream. In addition, no commercial or agricultural areas had to be in close vicinity of the study sites.

The climate is of Mediterranean type, which is characterised by dry summers and wet winters. After the first heavy rains, mountain streams and rivers in the CFR have surface flow in winter. In summer, streams are reduced to perennial pools and section of riffles resulting in periodic water shortages (Goldblatt & Manning, 2000; Tharme, 2010). These climate conditions only occur in the western part of the Fynbos Biome, with approximately 60% of the rain falling being between 1000 mm and 2000 mm per year, with the exception of some areas exceeding 3000 mm (Sieben, 2003). In the Fynbos Biome, vegetation distribution in upper catchments regions is typically characterised by sclerophyllous fynbos and sandstone soils, which are very acidic and nutrient-poor (Samways et al., 2011; Day & King, 1995). Sampling started in January 2016 and endured until December 2016.

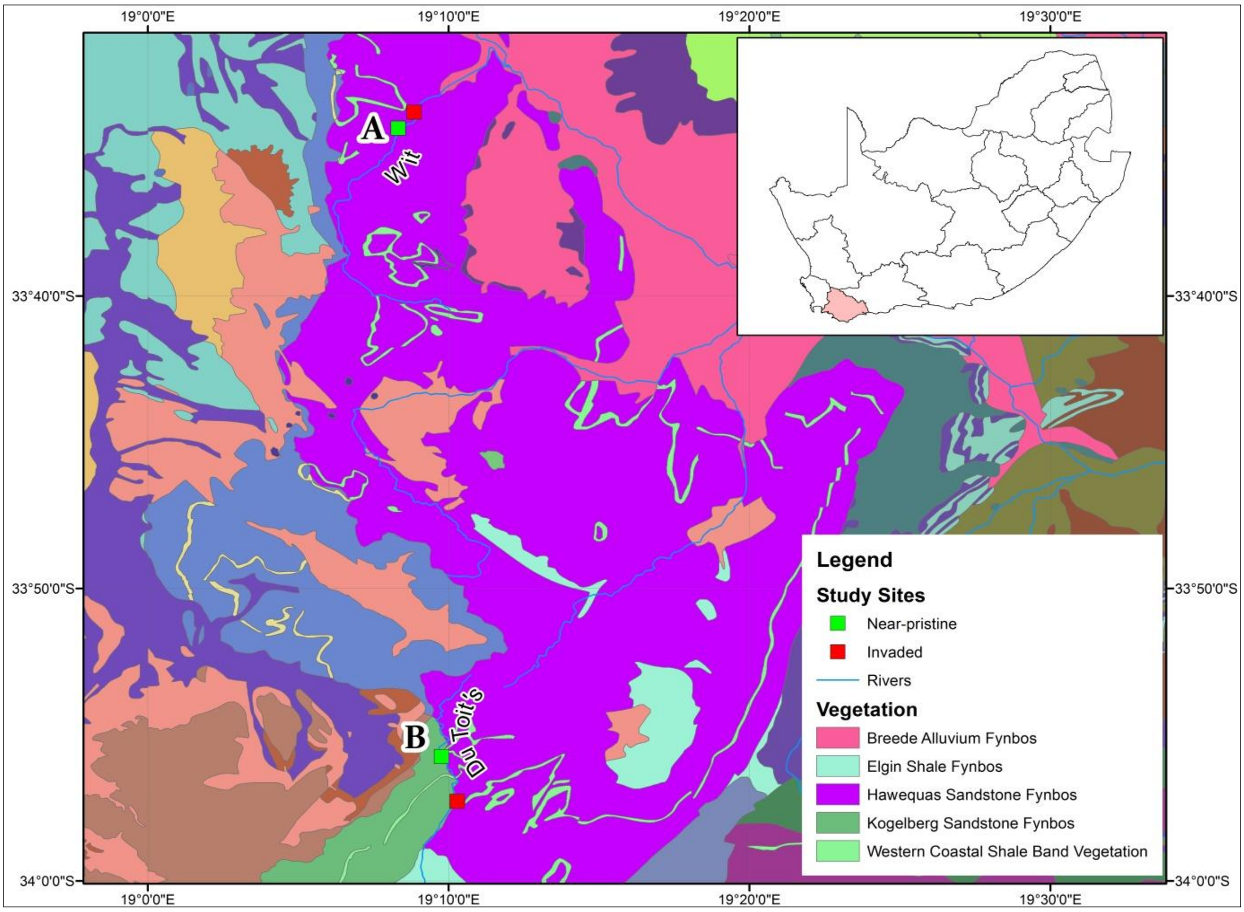


Figure 21: Location of the two perennial rivers in the Western Cape, Breede WMA: (A) Wit and (B) Du Toit's River and the four treatment sites: green = near-pristine; red = invaded site

Figure 22: Photographs of the different invasion treatments: (a) near-pristine and (b) invaded site

5.2.2 Leaf litterfall traps and seasonal carbon and nitrogen concentrations

Litter traps were placed under both native and alien tree canopies in the near-pristine and invaded sites of the Du Toit's and Wit rivers. At the invaded sites, ten traps were placed in a random order under a closed canopy of *A. mearnsii* trees. Here, a closed canopy was to have at least 50% cover or more. Five traps were placed for each of the two native tree species, namely, *B. stellatifolium* and *M. angustifolia*. Traps could not be placed randomly as the vegetation cover of the native species was not as dense as that of the invaded species. In some cases, especially in near-pristine sites, trap fixtures such as rope and tie straps were used to fix the traps sturdily on the ground as the study is in mountainous plots (Staelens et al., 2003). Litterfall and other inputs were collected in a fine mesh (0.5 mm) net that was suspended at a top height between 1.0 m and 1.3 m with a known catching area of 1.149 m^2. Each trap was attached to three steel rebar rods that were equally spaced to ensure adequate water drainage and a depth of 0.5 m prevent litter from blowing out of the traps. The method of Staelens et al. (2003) was followed to construct litterfall traps.

In the laboratory, a 250 µm sieve was used to exclude other plant components and retain only leaf litter material (active tissue or some cases senesced leaves). The leaf litter was oven-dried at 60°C for 24 hours on a top-loading analytical balance accurate to 0.1 g until a constant weight was achieved. Samples were collected monthly from January 2016 to December 2016. Mean monthly values expressed in g·m^{-2} were calculated for each trap per site. Leaf samples for each month (12 months) were weighed out to approximately 5 g for each species, grounded in a rotary mill and sieved through a 150 µm sieve to a homogeneous powder. This was for green leaves as they receive the most attention due to elevated nitrogen concentrations, which are generally thought to be due to high turnover rates, which are significant in plant productivity and nitrogen-fixation strategies (Witkowski, 1991; Yelenik et al., 2004; Craine et al., 2009; Drake, 2011) in *A. mearnsii*. The same procedure was done for the native species. The homogeneous powder of the different species was weighed between 0.5–0.8 mg in tin foil cups on an ultra-microbalance (Mettler Toledo XP6) as prescribed by laboratory standards by the Geology Department at the University of Stellenbosch to determine seasonal nitrogen and carbon concentrations (reported in milligram per gram dry mass) expressed as elemental composition using an Elementar Vario EL Cube hosted within an ICP–MS and XRF Unit of Central Analytical Facilities. The carbon elemental composition was measured concurrently and the C:N ratio calculated.

5.2.3 Isotope analysis (nitrogen acquisition strategies using ^{15}N natural abundance)

The natural abundance ^{15}N technique was used determine the nitrogen-fixing ability of *A. mearnsii* growing in the riparian zones of the two perennial rivers in the present study [Shearer & Kohl, 1986; Jacobs et al., 2006; see review of Boddey et al. (2000) for a detailed description of the method]. We sampled foliar material from *A. mearnsii* trees and nearby reference tree species. Foliar samples were collected in mid-autumn (early April 2006) for δ^{15}N analyses. Forty trees from each species were selected for sampling. Samples of the leaves were selected specifically from the upper canopy of the tree, and only leaves without noticeable signs of disease were used. Reference plant samples were collected from three non-fixing reference plants (*B. stellatifolium*, *M. angustifolia* and *S. angustifolia*) growing within a 5 m radius of *A. mearnsii*-invaded riparian plots as all species in the vicinity had to access nitrogen from the same soil pool (Jacobs et al., 2006).

Foliar samples were dried for three days at 70°C and then ground in a rotary mill homogenous powder, weighed to 0.5–0.8 mg using an ultra-microbalance (Mettler Toledo XP6) as prescribed by laboratory standards for leaf material. Samples were prepared for carbon and nitrogen elemental compositions and isotope analysis using a Flash HT Plus elemental analyser coupled to a Delta V Advantage isotope ratio mass spectrometer by a ConFloIV interface. The carbon and nitrogen isotope values were corrected against an in-house standard (Merck Gel) and a Urea Working Standard (IVA Analysentechnik e.K., Meerbusch, Germany). Laboratory standards and blanks were run after every 24 unknown samples. Sample analyses were carried out at iThemba Laboratories, Johannesburg. Stable isotopic data are reported in standard delta notation (δ^{15}N, relative to atmospheric nitrogen reference standard). The fractional contribution of biologically fixed nitrogen (% Ndfa) in nitrogen-fixing species was estimated using Equation 5.

Equation 5. $$\% Ndfa = 100\ \frac{(\delta^{15}\mathrm{N}\ Ref - \delta^{15}\mathrm{N} Fixing\ plant)}{\delta^{15}\mathrm{N} Ref - \beta}$$

In the equation, $\delta^{15}N_{Ref}$ is the δ^{15}N from external sources other than atmospheric nitrogen (in a non-fixing reference plant in the same area), $\delta^{15}N_{Fixing\ plant}$ is the δ^{15}N of the potential nitrogen-fixing *Acacia*, and β is the δ^{15}N value of the *Acacia* grown without access to soil nitrogen. Previous studies in southern Africa (Stock et al., 1995; Woghiren, 2002; Pule-Meulenberg & Dakora, 2009) used by β values of 0–1.3, and −2% and species-specific values (Tye & Drake, 2012) for *Acacia* species in southern Africa. In the present study, the best estimated value was 0 as it gave the most best yielded conservative estimates of the percentage nitrogen fixed.

5.2.4 Resorption efficiencies (retranslocation of nutrients in *A. mearnsii* and native plants)

The resorption efficiency of nutrients was determined from senesced leaves in the study. This gives us the ability to understand absorption of nutrients, mainly nitrogen and phosphorus (Zhang et al., 2014) in the riparian zones of the two perennial rivers, which can be a key component of adaptive mechanisms to conserve nutrients. Samples of mature green leaves and senesced leaves were collected from the native species in the pristine sites and *A. mearnsii* in the invaded sites at each river to estimate nutrient cycling. Five trees were selected for sampling from each species in December (early summer) 2016. Senesced leaves are those in which an abscission layer has formed with the major pulse of resorption occurring in this stage (Norby et al., 2000; Wright & Westoby, 2003). These leaves are generally a different colour (often red or yellow) and can be removed from the twig by light flick of the leaf. Senesced leaves were collected directly from the plants rather than collecting from the leaf litter traps. This was mainly done to avoid decomposition of litter and leaching of nutrients from leaves.

Leaf material was collected randomly at the collection time from different points at the tree canopy position and pooled by species for nutrient analysis. In the laboratory they were oven-dried at 60°C for 72 hours to determine dry mass. Samples were finely grounded to a homogenous powder using a Hammer Mill with a sieve size of 250 µm and stored in plastic micro-centrifuge tubes. Finally, samples were transported to a commercial laboratory, namely, Bemlab in Somerset West, South Africa, for nitrogen and phosphorus analysis using a Leco instrument and combustion method for nitrogen, and ICP instrument for phosphorus. The mean and standard errors of nitrogen and phosphorus concentrations were calculated for green and senesced leaves component for each species. The nitrogen and phosphorus contents were calculated per unit mass (reported in milligram per gram dry mass). Resorption efficiency and proficiency were calculated per individual, and then pooled by species for the purpose of comparison to provide insight into both the intraspecific and interspecific variation in resorption constraints. The percentage nitrogen and phosphorus reduction between green and senesced leaves was calculated using Equation 6:

Equation 6. $$\text{Resorption efficiency (RE)} = \frac{[\text{nutrient}]green - [\text{nutrient}]sen)}{[\text{nutrient}]green} \times 100$$

The mean nitrogen or phosphorus concentration in mature green leaves are $[nutrient]_{green}$ and $[nutrient]_{sen}$, which refer to the senesced leaf nitrogen or phosphorus concentrations (Aerts, 1996). Leaf senescence was sometimes difficult to judge, especially for the species of *B. stellatifolium*. Some of the senesced leaves collected have not completely undergone the process at time of collection. Therefore, we chose to use the 0.10 quartile values for $[nutrient]_{sen}$ under the presumption that they were leaves that had undergone the process of complete senescence or near-complete senescence. The resorption proficiency was determined using $[nutrient]_{sen}$ as defined in Equation 6.

5.2.5 Statistical analysis

The data were analysed using the Statistica version 13 software package (Dell Inc., 2015). Litterfall production, carbon and nitrogen concentrations were analysed using a two-way RMANOVA to test significant interactive effects between litterfall, carbon and nitrogen concentrations across sampling period followed by the LSD post hoc test for means comparison when significant differences were found. To test the difference between foliar $\delta^{15}N$ and nitrogen percentage values in *A. mearnsii* and associated reference plants, a one-way ANOVA was used, which was followed by a Mann–Whitney U test as data were not distributed normally. These were followed by the Games–Howell post hoc test for means comparison when significant differences were found.

5.3 Results

5.3.1 Annual litterfall, nitrogen and carbon concentrations, and nitrogen return to soil estimation of *A. mearnsii* and co-occurring native species in CFR riparian zones

At the Wit River, *A. mearnsii* produced more mean leaf litterfall than the two co-occurring native species in each month ($F_{[22,187]} = 2.9704$; $p < 0.001$) and different seasons ($F_{[6.51]} = 2.7615$; $p < 0.001$) (Figure 23). This was especially evident in summer (January) and autumn (April). The highest mean litterfall for *A. mearnsii* at the Wit River was in April (mean = 51.23 g/m^2) with a similar litterfall rate in January with a mean of 43.47 g/m^2. Conversely, the litterfall rate for the two co-occurring native species (*B. stellatifolium* and *M. angustifolia*) remained relatively constant throughout the year with peaks of mean = 16.06 g/m^2 and mean = 28.41 g/m^2, respectively, in December. Through different seasons, the highest litterfall for *A. mearnsii* occurred in autumn (mean = 33.89 g/m^2) rather than winter (mean = 15.58 g/m^2; $p < 0.001$) and spring (mean = 20.99 g/m^2; $p < 0.001$), with no significant difference between autumn and summer (mean = 33.85 g/m^2; $p = 0.99$). Seasonal litterfall rates for *B. stellatifolium* were relatively constant through the year (mean = 36.26 g/m^2). The litterfall rate of *M. angustifolia* was significantly higher in summer (mean = 15.46 g/m^2) than autumn (3.25 g/m^2; $p < 0.001$), winter (1.99 g/m^2; $p < 0.001$) and spring (4.57 g/m^2; $p < 0.001$). Overall, the litterfall rate of *A. mearnsii* was significantly higher than the co-occurring native species ($p < 0.001$).

A similar temporal trend was observed at the Du Toit's River under the dense *A. mearnsii* stands with autumn (April) exhibiting one peak, and only one peak in summer (December) for the native species under near-pristine plots. The highest mean litterfall for *A. mearnsii* at Du Toit's River was in April (mean = 77.99 g/m^2). On the other hand, the litterfall rate for the two co-occurring native species (*B. stellatifolium* and *M. angustifolia*) remained relatively constant throughout the year; however, there were significantly higher litterfall rates for *M. angustifolia* (mean = 30.32 g/m^2; $p < 0.05$) in December. Through different seasons, there were significantly higher litterfall rates for *A. mearnsii* in autumn (mean = 49.84 g/m^2) relative to winter (mean = 21.88 g/m^2; $p < 0.001$), spring (mean = 19.83 g/m^2; $p < 0.001$) and summer (mean = 36.97 g/m^2; $p < 0.001$). The highest litterfall peak in *B. stellatifolium* was in the summer month, which was significantly different from autumn (mean = 36.97 g/m^2; $p < 0.01$), winter (mean = 1.96 g/m^2; $p < 0.01$) and spring (mean = 2.35 g/m^2; $p < 0.01$). Similar results are found in the *M. angustifolia* with the highest litterfall peak in summer.

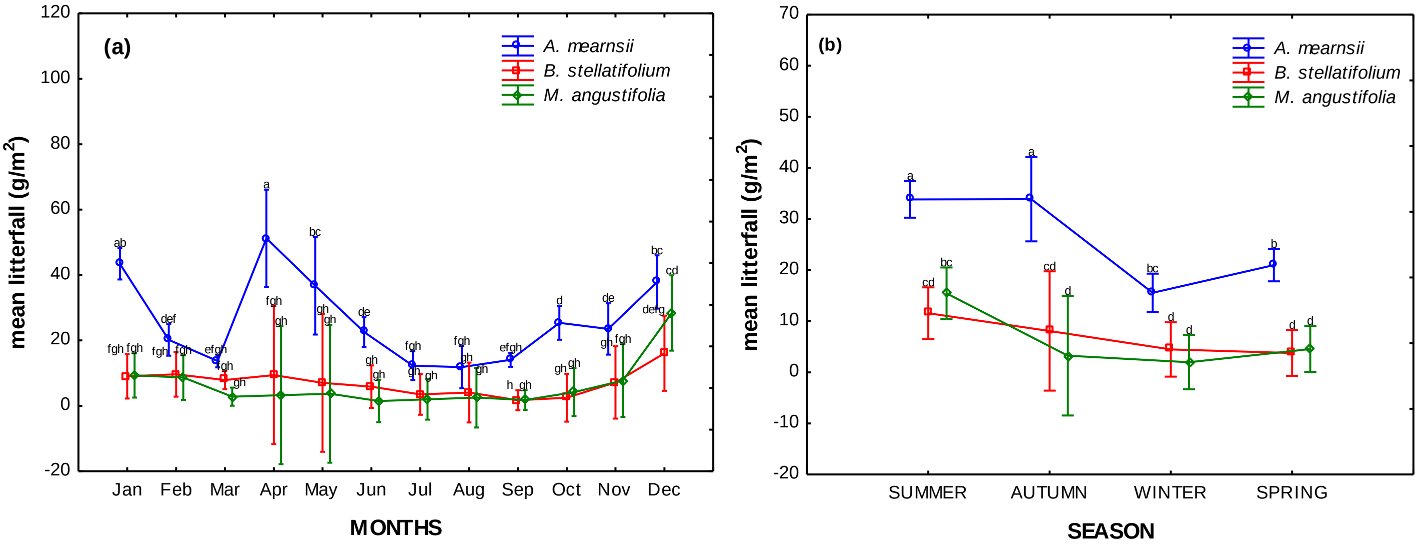


Figure 23: Patterns of (a) mean and (b) seasonal (g/m^2) for A. mearnsii and co-occurring native species growing in near-pristine and invaded areas in riparian zones at the Wit River. Letters denote significant differences (LSD test; $p < 0.05$) based on two-way RMANOVA: (a) ($F_{[22,187]} = 2.97$; $p < 0.001$; (b) $F_{[6.51]} = 2.7615$; $p < 0.01$) using all the data collected over the year and different seasons

Little variation (< 5 $mg\cdot g^{-1}$) was observed through seasons in nitrogen concentrations for *A. mearnsii* at the Wit River (Figure 25a). The highest mean nitrogen concentrations found in *A. mearnsii* was in autumn (31.05 $mg\cdot g^{-1}$) with no significant differences between summer (25.58 $mg\cdot g^{-1}$) and winter (25.38 $mg\cdot g^{-1}$; $p = 0.93$). The mean nitrogen percentage in *B. stellatifolium* was highest in summer (8.36 $mg\cdot g^{-1}$) with no significant difference fond between autumn (5.45 $mg\cdot g^{-1}$; $p = 0.08$) and spring (5.28 $mg\cdot g^{-1}$; $p = 0.09$). However significant differences are found between summer and winter (3.23 $mg\cdot g^{-1}$; $p < 0.001$). A similar trend in nitrogen concentrations is noted in *M. angustifolia* with the highest mean nitrogen concentrations being in summer (7.55 $mg\cdot g^{-1}$) with a similar nitrogen percentage in autumn with a mean of 7.34 $mg\cdot g^{-1}$.

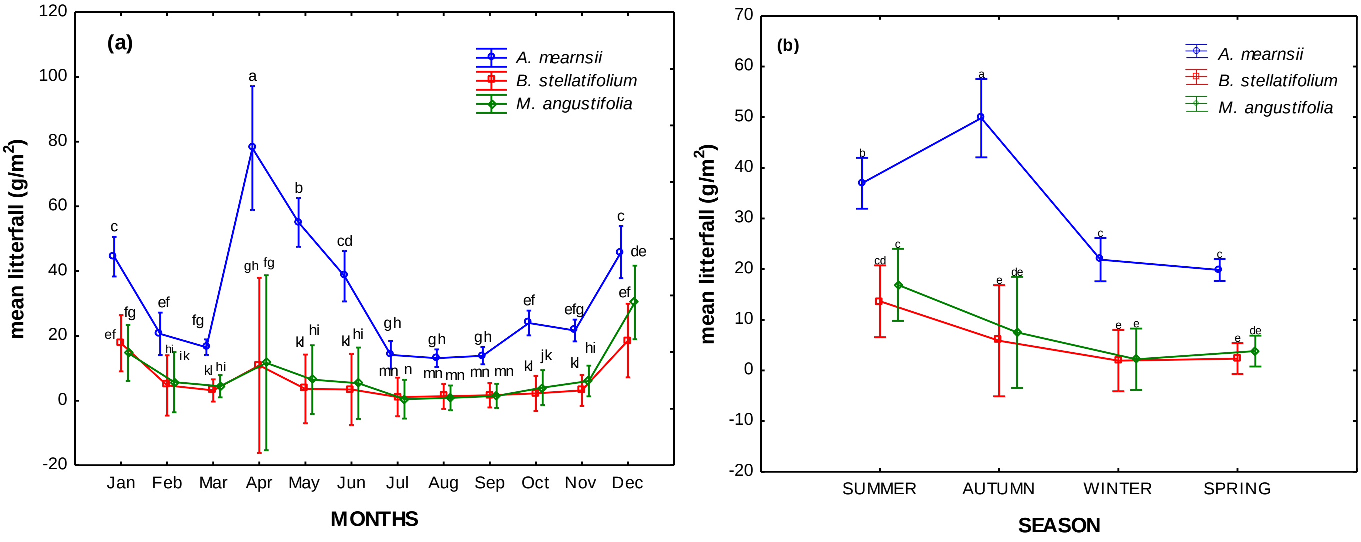


Figure 24: Patterns of (a) mean and (b) seasonal litterfall (g/m^2) for A. mearnsii and co-occurring native species growing in near-pristine and invaded areas in riparian zones at the Du Toit's River. Letters denote significant differences (LSD test; $p < 0.05$) based on two-way RMANOVA: (a) (F [$_{22,187}$] = 7.24; $p < 0.001$; (b) F [$_{6.51}$] = 10.11; $p < 0.001$) using all the data collected over the year and different seasons

Similar temporal variation (< 5 $mg\cdot g^{-1}$) through seasons was observed at the Du Toit's River for *A. mearnsii* (Figure 25b). The nitrogen concentrations appear to be the highest in autumn (27.97 $mg\cdot g^{-1}$) with no significant difference found between summer (25.44 $mg\cdot g^{-1}$) and winter (24.30 $mg\cdot g^{-1}$; $p = 0.27$). The co-occurring native species of *B. stellatifolium* and *M. angustifolia* remain relatively constant throughout the year but with difference found between seasons. *B. stellatifolium* had the highest mean nitrogen concentrations in summer (5.86 $mg\cdot g^{-1}$) with no significant difference found between autumn (5.71 $mg\cdot g^{-1}$; $p = 0.89$) and spring (4.88 $mg\cdot g^{-1}$; $p = 0.35$), but significant differences were found in winter (3.52 $mg\cdot g^{-1}$; $p < 0.05$). The highest mean nitrogen concentration was in autumn (7.99 $mg\cdot g^{-1}$) for *M. angustifolia*. There was significant difference between autumn and summer (5.94 $mg\cdot g^{-1}$; $p < 0.01$), but no significant difference in between winter (4.69 $mg\cdot g^{-1}$; $p < 0.001$) and spring (5.25 $mg\cdot g^{-1}$; $p < 0.05$). Overall at both Wit and Du Toit's River, *A. mearnsii* was significantly higher in mean nitrogen percentage through seasons ($p > 0.001$).

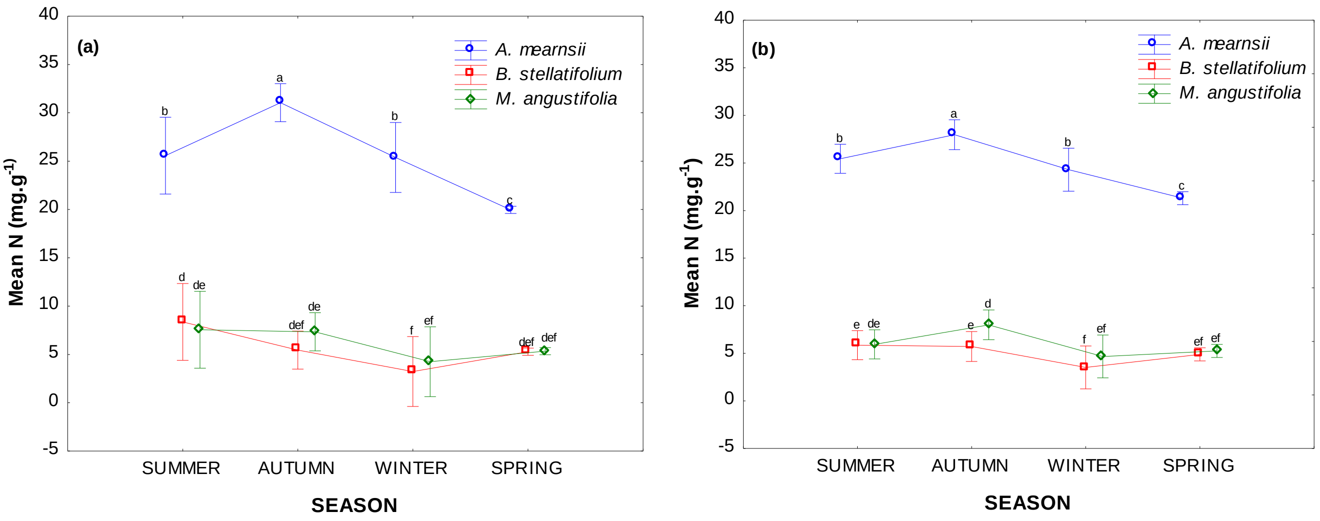


Figure 25: Mean seasonal patterns of nitrogen concentrations (%) for A. mearnsii and co-occurring native species growing in near-pristine and invaded areas in riparian zones at (a) Wit River and (b) Du Toit's River. Letters denote significant differences (LSD test; $p < 0.05$) based on two-way RMANOVAs: (a) ($F_{[6.18]}$ =5.4708; $p < 0.001$; (b) $F_{[6.18]}$ = 3.4645; $p < 0.01$) using all the data collected over different seasons

The C:N ratios in *A. mearnsii* were relatively constant from summer until spring at Wit River. In the co-occurring native species, *B. stellatifolium*, the highest C:N ratios were found in winter (mean = 69.27%) with a similar percentage found in spring (mean = 68.90%). *M. angustifolia* had the highest C:N ratios in spring (mean = 68.75%) with comparable values found in summer (mean = 67.98%; p = 0.90). Overall, co-occurring native species were significantly higher than *A. mearnsii* through seasons (p < 0.001, Figure 26a). Similar trends are observed at Du Toit's River with C:N ratios remaining relatively constant through different seasons in *A. mearnsii* with no significant differences. The highest C:N ratio in *B. stellatifolium* was evident in winter (mean = 62.49%) but in comparison to autumn (mean = 50.32%; p < 0.001), a significant difference was found between seasons but no significant difference between summer (mean = 60.68%; p = 0.65) and spring (mean = 58.04%; p = 0.27), respectively. Summer (mean = 59.87%) and spring (mean = 62.51%) were relatively similar in C:N ratios between the two seasons in *M. angustifolia* with autumn being significantly different from spring, winter and summer (p < 0.001). Generally at the Du Toit's River, co-occurring native species were significantly higher in C:N ratios than *A. mearnsii* through seasons (p < 0.001; Figure 26b). The results in C:N ratios are noted to be the inverse of mean nitrogen concentrations.

On an annual basis, *A. mearnsii* had significantly more litterfall (273.66 mg·g^{-1}·y^{-2} ± 37.76) than co-occurring native species [*B. stellatifolium* (36.26 mg·g^{-1}·y^{-2} ± 5.79); *M. angustifolia* (33.56 mg·g^{-1}·y^{-2} ± 11.14) at riparian plots at Bainskloof Pass (Table 9, site a)]. The same observations were made at Franschhoek Pass for *A. mearnsii* (321.32 mg·g^{-1}·y^{-2} ± 58.93), which had considerably higher litterfall than co-occurring native species [*B. stellatifolium* (29.80 mg·g^{-1}·y^{-2} ± 9.00); *M. angustifolia* (38.14 mg·g^{-1}·y^{-2} ± 11.95) at the respective riparian plots (Table 9; Site b)]. These were based on annual mean nitrogen concentrations, which showed *A. mearnsii* being six times greater (25.28 ± 2.27) than co-occurring native species *B. stellatifolium (*4.03 ± 1.11); *M. angustifolia* (4.84 ± 0.52) at Bainskloof Pass. These estimates were similar for annual mean nitrogen concentrations at the Franschhoek Pass between *A. mearnsii* and co-occurring native species.

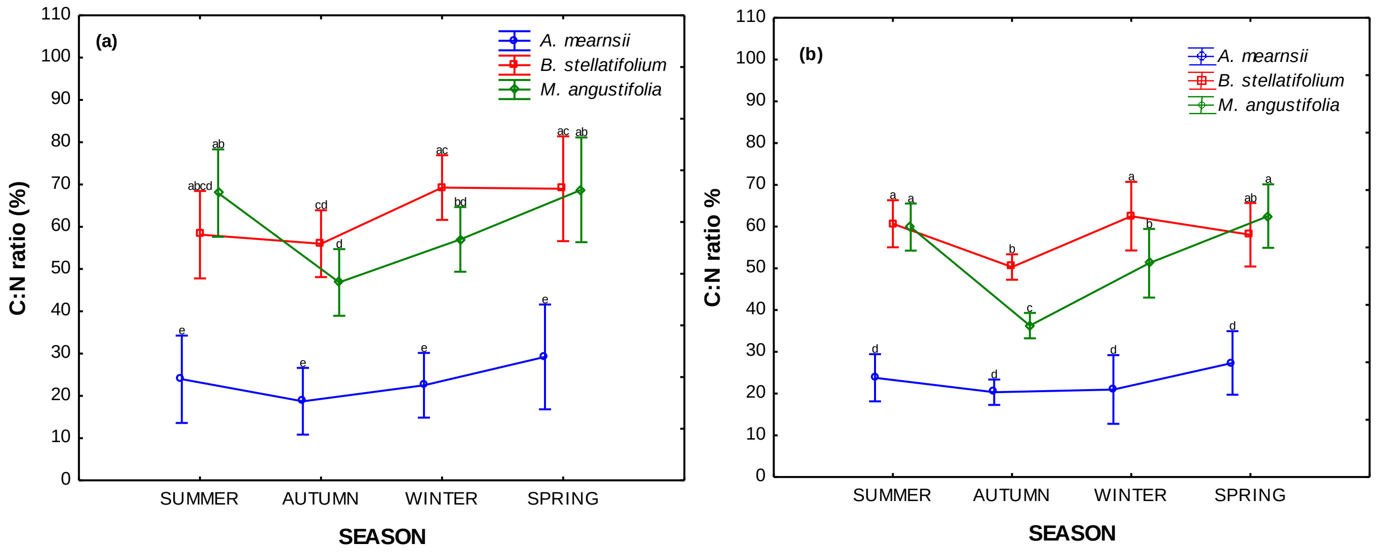


Figure 26: Seasonal patterns of C:N ratio concentrations (%) for A. mearnsii and co-occurring native species growing in near-pristine and invaded areas in riparian zones at (a) Wit River and (b) Du Toit's River. Letters denote significant differences (LSD test; $p < 0.05$) in two-way RMANOVAs (a) (F [$_{6.18}$] =1.4412; p = 0.253555; and (b) F [$_{6.18}$] = 4.2496; $p < 0.01$) using all the data collected over different seasons

However, results based on annual mean C:N ratios showed that the co-occurring native species had significantly higher ratios than *A. mearnsii* at both sites (Table 9: Site a, b), but based on results on annual mean nitrogen returned to soil, the observation was made that *A. mearnsii* had significantly more litter nitrogen concentrations return to soil than the co-occurring species between riparian plots of near-pristine and invaded sites (Table 9: Site a, b).

Table 9: Mean annual litterfall, nitrogen percentage, C:N ratio % and seasonal nitrogen percentage returned to soil. The data reflect means ± [standard error (SE)]

	Nitrogen	**Annual litterfall (g·m^{2}·y^{-1}) [SE]**	**Annual mean nitrogen % (mg·g^{-1}) [SE]**	**Annual mean C:N ratio % (mg·g^{-1}) [SE]**	**Annual mean nitrogen % return (mg nitrogen m^{2}·y^{-1}) [SE]**
Site (a): Bainskloof Pass					
A. mearnsii	10	273.66 ± 37.76	25.28 ± 2.27	23.59 ± 2.17	6896.27 ± 1554.43
B. stellatifolium	5	36.26 ± 5.79	4.03 ± 1.11	63.09 ± 3.51	163.83 ± 86.51
M. angustifolia	5	33.56 ± 11.14	4.84 ± 0.52	60.14 ± 5.18	165.73 ± 93.51
Site (b): Franschhoek Pass					
A. mearnsii	10	321.32 ± 58.93	24.35 ± 1.37	23.11 ± 1.60	8072.98 ± 2186.06
B. stellatifolium	5	29.80 ± 9.00	3.10 ± 0.28	57.88 ± 2.68	142.60 ± 96.33
M. angustifolia	5	38.14 ± 11.95	3.61 ± 0.42	52.47 ± 5.97	182.28 ± 102.31

5.3.2 Nitrogen-fixation status of *A. mearnsii* in riparian zones in the CFR

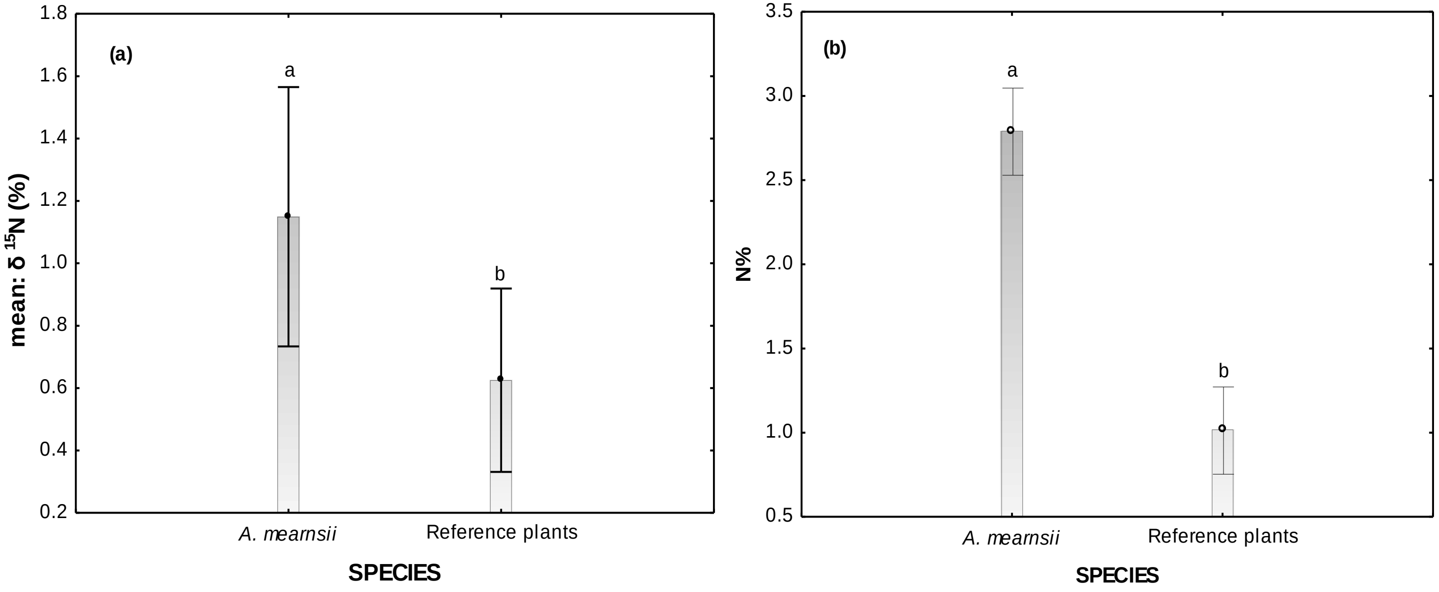


Figure 27: Mean foliar δ^{15}N (a) and nitrogen concentration (b) in A. mearnsii and associated reference plants. Fully expanded mature leaves collected during April 2016 from the Wit River study site. Values are represented as means; error bars indicate standard error

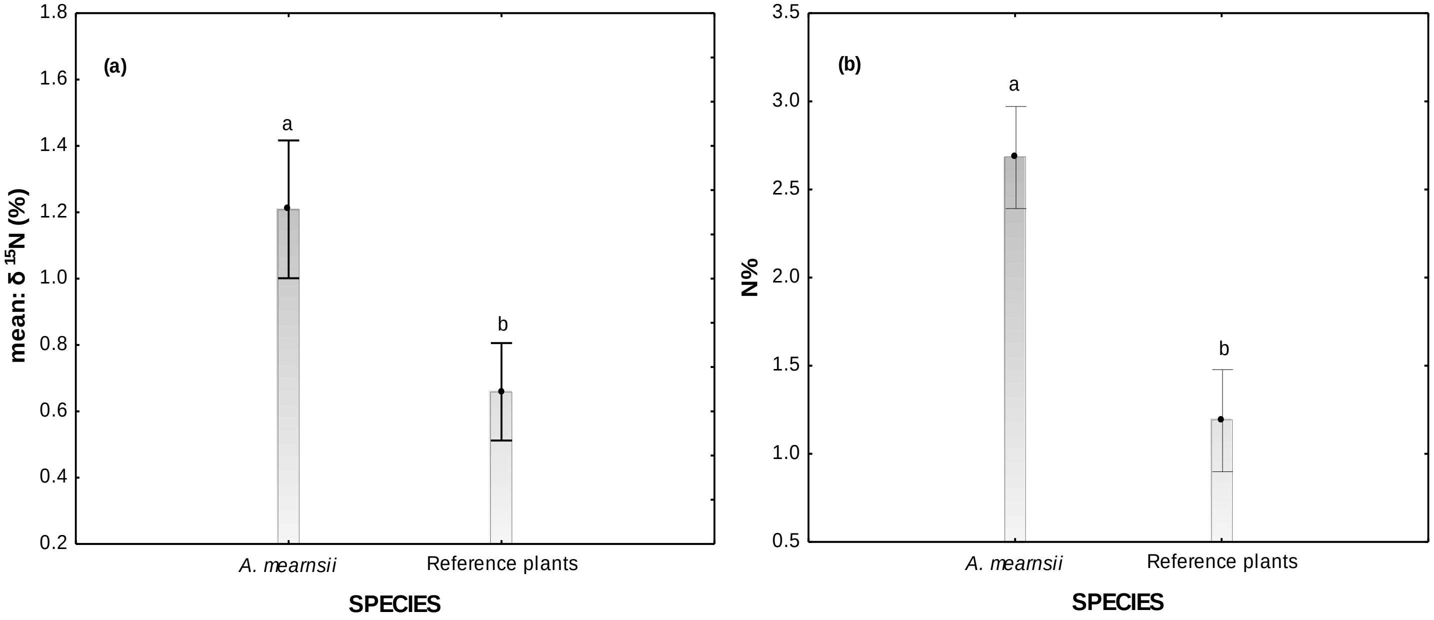


Figure 28: Mean foliar δ^{15}N (a) and nitrogen concentration (b) in A. mearnsii and associated reference plants. Fully expanded mature leaves collected during April 2016 from the Du Toit's River study site. Values are represented as means, error bars indicate standard error

Table 10: δ^{15}N (‰) and nitrogen (%) in leaves of A. mearnsii and reference species plants growing in the Bainskloof and Franschhoek Pass riparian zones. Values represent independent replicates of leaf samples from different trees. The data reflect means ± [SE]

	Site (a): Bainskloof Pass		**Site (b): Franschhoek Pass**	
Nitrogen	*A. mearnsii*	Reference trees	*A. mearnsii*	Reference trees
	10	10	10	10
Range of δ^{15}N (‰)	0.69 to 1.69	0.32 to 1.09	0.78 to 1.68	1.38 to 1.79
Mean δ^{15}N (‰) [SE]	1.15 [0.11]	0.64 [0.10]	1.21 [0.11]	1.61 [0.05]
Range of percent (%) leaf nitrogen	1.90 to 3.42	0.81 to 1.27	1.97 to 4.11	1.06 to 1.42
Mean nitrogen percentage [SE]	2.79 [0.17]	1.02 [0.04]	2.69 [0.19]	1.19 [0.03]
Mean C:N [SE]	22.18 [1.25]	32.92 [0.06]	26.81 [1.00]	30.27 [0.51]

Leaf δ^{15}N (‰) values of *A. mearnsii* ranged from 0.69 to 1.69 ‰, and for the reference plants species from 0.32 to 1.09 (‰) at the Wit River invaded site (Figure 27). There was significant difference found (Mann–Whitney U test; $p < 0.05$) between *A. mearnsii* and reference trees at the Wit River site in mean leaf δ^{15}N (‰) values. Leaf nitrogen concentrations in *A. mearnsii* ranged from 1.90% to 3.42% and reference plant species from 0.81% to 1.27% at the study site with significant differences apparent between species ($p < 0.05$).

At the Du Toit's River, similar results were found with significant differences in mean leaf δ^{15}N (‰) between *A. mearnsii* and reference plant species (Figure 28) with significant mean differences found ($p < 0.001$). Similarly mean nitrogen concentrations also had significant differences between species at the Du Toit's River with *A. mearnsii* being the highest.

5.3.3 Nutrient allocation and conservation strategies in *A. mearnsii* and competing co-occurring native species

5.3.3.1 Foliar nutrient content (nitrogen and phosphorus) and N:P ratios concentrations

The mean nutrient concentrations in the mature green leaves sampled during early autumn (early April) varied significantly between species at both Bainskloof and Franschhoek Pass sites. Green leaf nitrogen concentrations varied from mean = 27.08 mg·g^{-1} for *A. mearnsii*, mean = 8.02 mg·g^{-1} for *B. stellatifolium* and mean = 8.76 mg·g^{-1} for *M. angustifolia* at the Bainskloof Pass. A similar trend was observed at the Franschhoek Pass site where mature green leaves varied significantly between species. Green nitrogen concentrations varied from mean = 27.48 mg·g^{-1} for *A. mearnsii*, mean = 8.34 mg·g^{-1} for *B. stellatifolium*, and mean = 8.72 mg·g^{-1} for *M. angustifolia.*

Green mature leaf nitrogen concentrations varied from senesced leaf nitrogen concentrations in both sites (

Table 11,

Table 12). A similar pattern was observed for phosphorus concentrations, where green leaf nitrogen concentrations varied from senesced leaf nitrogen concentrations. The N:P mass ratios in mature leaves ranged from 14.56 mg·g^{-1} to 38.56 mg·g^{-1} in the three species at Bainskloof Pass and from 10.56 mg·g^{-1} to 32.75 mg·g^{-1} between species at the Franschhoek Pass site. In senesced leaves, variation in data ranged from 24.70 mg·g^{-1} to 43.00 mg·g^{-1} between species. In Franschhoek, variation in senesced leaves was from 21.50 mg·g^{-1} to 45.67 mg·g^{-1} between species. The results show variation in N:P ratio in senesced leaves when compared with mature green leaf N:P ratios, with notably only *A. mearnsii* in Bainskloof Pass having little variation between senesced and mature green leaf N:P ratios. The expectation was for mature green leaves in the *A. mearnsii* to differentiate from the other two species, with higher N:P ratios found in *A. mearnsii* at both sites. Senesced leaves of both *B. stellatifolium* and *M. angustifolia* had higher N:P ratios than *A. mearnsii* at both sites. Senesced leaves of both *B. stellatifolium* and *M. angustifolia* were also in higher N:P ratios than *A. mearnsii* mature green leaves N:P ratios, while N:P ratios in *A. mearnsii* senesced leaves and green leaves did not differ in N:P ratios.

5.3.3.2 Nutrient resorption efficiencies in above-ground components

In both study sites, *A. mearnsii* was remarkably more efficient at phosphorus resorption than nitrogen resorption. However, phosphorus resorption of both co-occurring native species at both sites were significantly more efficient at phosphorus resorption than nitrogen resorption. Calculated on a mass basis, nitrogen resorption efficiency (NRE) from senesced leaves for *A. mearnsii* was 25%, *B. stellatifolium* 56% and *M. angustifolia* 51%. On average, *A. mearnsii* translocates a quarter of nitrogen contained in the leaves for reuse after senescence, and *B. stellatifolium* and *M. angustifolia* translocate half of their nitrogen in leaves after senescence at Bainskloof Pass. A similar reuse of nitrogen after senescence was observed at Franschhoek for *A. mearnsii* (37%), *B. stellatifolium* (56%) and (53%) for *M. angustifolia*. However, there was variation in NREs between *A. mearnsii* and co-occurring native species at both sites (see

Table 11 and

Table 12). Between resorption efficiencies, phosphorus resorption was much higher than nitrogen resorption. Phosphorus resorption values ranged in *A. mearnsii* (mean 42.64%), *B. stellatifolium* (mean 76.67%) and *M. angustifolia* (mean 69.00%) at Bainskloof Pass. Similar range of values were observed for the Franschhoek Pass site, with the following mean ranges: *A. mearnsii* (53.43%), *B. stellatifolium* (78.11%) and *M. angustifolia* (79.33%). *A. mearnsii* phosphorus resorption efficiency was double the efficiency of nitrogen resorption at both sites (

Table 11, resorption efficiencies), suggesting that nutrient resorption within *A. mearnsii* operates independently.

Resorption proficiencies determine the amount nutrient concentrations a plant is able to use in the senescence stage (colour often red or yellow) and conserve nutrient resources. Senesced leaves at the Bainskloof Pass were different between *A. mearnsii* (~22 $mg{\cdot}g^{-1}$) and the co-occurring native species, namely, *B. stellatifolium* (~3 $mg{\cdot}g^{-1}$) and *M. angustifolia* (~6 $mg{\cdot}g^{-1}$). A similar observation was made at the Franschhoek site (see

Table 12). Moreover, the low leaf nitrogen concentrations seen in the two co-occurring native species were largely due to their high leaf mass area (LMA) (

Table 11).

Table 11: Nitrogen and phosphorus mean concentrations, N:P ratios of green and senesced leaves' nitrogen and phosphorus resorption proficiencies/efficiencies for the Bainskloof and Franschhoek Pass sites. Values are shown as (mean ± SE)

	A. mearnsii	***B. stellatifolium***	***M. angustifolia***
Bainskloof Pass	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Green mature leaf concentrations (mg·g^{-1})			
Nitrogen	27.08 ± 0.41	8.02 ± 0.24	8.76 ± 0.79
Phosphorus	1.12 ± 0.19	0.44 ± 0.04	0.46 ± 0.02
N:P ratio	27.05 ± 4.31	18.57 ± 0.97	19.02 ± 1.20
Senesced leaf concentration, mg·g^{-1} (resorption proficiency)			
Nitrogen (NRP)	22.04 ± 1.46	3.54 ± 0.16	5.98 ± 0.94
Phosphorus (PRP)	0.8 ± 0.09	0.1 ± 0.00	0.14 ± 0.02
N:P ratio	28.25 ± 1.57	35.40 ± 1.57	36.80 ± 2.03
Resorption efficiencies (%)			
Nitrogen (NRE)	18.60 ± 5.27	55.79 ± 1.90	50.56 ± 3.40
Phosphorous (PRE)	42.64 ± 2.67	76.67 ± 1.67	69.00 ± 6.00

Table 12: Nitrogen and phosphorus mean concentrations, N:P ratios of green and senesced leaves nitrogen and phosphorus resorption proficiencies/efficiencies for the Bainskloof and Franschhoek Pass sites. Values are shown as (mean ± SE)

	A. mearnsii	***B. stellatifolium***	***M. angustifolia***
Franschhoek Pass	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Green mature leaf concentrations (mg·g^{-1})			
Nitrogen	27.48 ± 1.10	8.34 ± 0.37	8.72 ± 0.34
Phosphorus	1.00 ± 0.11	0.52 ± 0.10	0.58 ± 0.02
N:P ratio	28.34 ± 1.94	17.71 ± 2.20	15.11 ± 0.80
Senesced leaf concentration, mg·g^{-1} (resorption proficiency)			
Nitrogen (NRP)	17.24 ± 1.41	3.60 ± 0.18	4.06 ± 0.19
Phosphorus (PRP)	0.46 ± 0.07	0.1 ± 0.00	0.12 ± 0.02
N:P ratio	38.89 ± 2.56	36.00 ± 1.84	36.30 ± 4.12
Resorption efficiencies (%)			
Nitrogen (NRE)	25.11 ± 4.02	56.15 ± 4.19	53.47 ± 1.19
Phosphorous (PRE)	53.43 ± 4.94	78.11 ± 3.63	79.33 ± 3.23

5.4 Discussion

5.4.1 Litterfall estimates of *A. mearnsii* and co-occurring native species

The study was aimed to estimate leaf litterfall of *A. mearnsii* in the riparian zones of the Wit River and Du Toit's River in the Breede WMA in the CFR. The results agree with previous findings (Milton, 1981b; Witkowski, 1991; Yelenik et al., 2004; 2007; Inagaki & Ishizuka, 2011; Naude, 2012) that found greater litterfall in *Acacia* species than native vegetation. It is evident that the growth strategy of *A. mearnsii* is distinctively different from co-occurring species. Thus, the results presented on average for leaf litterfall at the Wit River (273.66 ± 37.76 g/m^2) and Du Toit's River (321.32 ± 58.93 g/m^2) are in range with other *Acacia* species [*A. cyclops* (377.52), *A. longifolia* (335.03), *A. melanoxylon* (318.80) and *A. saligna* (488.83)] found in the Milton (1981b) study in the southern Western Cape. In a later study, Witkowski (1991) worked on the coastal lowlands of the southern Western Cape and compared bimonthly litterfall data of *Acacia* species (*A. saligna* and *A. cyclops*) to native vegetation. Witkowski (1991) found that these *Acacia* species' litterfall production was greater than the native vegetation's. However, Witkowski's (1991) study is not comparable with Milton (1981b) and the present study as it was done on a bimonthly basis, but similar figures are seen for annual production of leaf litter with an estimate of 40% of leaf fall for *A. saligna* (178.35 g/m^2) and *A. cyclops* (201.72 g/m^2) when compared with the native vegetation *Leucospermum parole* (155.39 g/m^2) and *Pterocelastrus tricuspidatus* (140.22 g/m^2). A later study done by Yelenik et al. (2007) in Riverlands Nature Reserve north of Cape Town, South Africa, found that *A. saligna* had a higher amount of litterfall (404.16 ± 35.1 g·m^2·y^{-1}) than fynbos vegetation (102.26 ± 16.8 g·m^2·y^{-1}). The results in the Yelenik et al. (2007) published paper are in the same range as the results found for *A. mearnsii* and the co-occurring native species in the present study for *A. mearnsii*. Likewise, a study by Naude (2012) in riparian zones within the south Western Cape region found that invaded sites of *A. mearnsii* and *A. longifolia* were twice as much as the fynbos vegetation in the study that largely consisted of leaves. Nevertheless, these estimates of litterfall were only shown annually (Milton, 1981b; Naude, 2012; Yelenik et al., 2004) or bimonthly (Witkowski, 1991). Thus, it was difficult to compare results through a temporal spectrum.

The mean annual litterfall estimates seen in *A. mearnsii* are seen in other parts of the world. A study done by (Saharjo & Watanbe, 2009) in Sumatra, Indonesia, found the leaf litterfall to be in similar range (446 g/m^{-2}) for *A. dealbata*. Furthermore, the values of co-occurring native species (*B. stellatifolium* and *M. angustifolia*) in near-pristine sites are considerably lower than values found in the other studies for native species (Milton, 1981b; Witkowski, 1991; Yelenik et al., 2007). In addition, the results found in *A. mearnsii* are not based on all production parts (i.e., flowers, seeds, twigs) of the plant but only the leaf material. The leaves of *A. mearnsii* contain elevated nitrogen concentrations that can change ecosystem processing due to high turnover rates, significance in plant productivity and nitrogen-fixation strategies (Yelenik et al., 2004; Craine et al., 2009; Drake, 2011). Moreover, the data presented might be biased as native species traps were placed under single trees and *A. mearnsii* under dense stands and might be an over- or underestimation of leaf litterfall for the respective species.

The temporal trends in litterfall of *A. mearnsii* demonstrate two peaks: one being at the end of the dry season, April (mid-autumn), and one being January (mid-summer). In a study near Bungendore, New South Wales in Australia, Grant et al. (1994) studied the pollination and breeding system of an *A. mearnsii* population over three flowering seasons (1987–1990). The study found that the species had two heavy and one light flowering season. Many authors (Moncur et al., 1989; Campbell, 2000; Nyoka, 2003; Dell'Porto et al., 2006) state that the flowering season of *A. mearnsii* are in the months of September to November, and occurs generally for 8 to 10 weeks but it can be irregular as described by Bonney (2003). The flowering stage for the *A. mearnsii* in the present study was in the same months as pale yellow flowers were apparent, but flowers were assumed to have only fallen only in December, which could be seen as an irregular period (Figure 29).

The flowering stage of *A. mearnsii* was accompanied with leaf litterfall and observed in litterfall for December, which potentially supports the pattern by Moncur et al. (1989) and Bonney (2003). The leaf litterfall peak in autumn, in particular the month of April, is postulated to be the senescence stage of *A. mearnsii* before the winter season, which is the species' growing season. The theory is that *A. mearnsii* recycles nutrients in autumn, which coincides with new leaf growth as the wet season approaches. Gallinat et al. (2015) suggests that IAPs extend their growing season in autumn, which allows them to require more carbon, which alters ecosystems and potentially the local climate.

In addition, Richardson et al. (2009) indicated that warm temperatures could increase nitrogen mineralisation rates, which could lead to increased plant uptake and elevated levels of foliar nitrogen, which could stretch over the entire growing season. In the study, the same findings can be postulated for *A. mearnsii* as high rates of litterfall were apparent in April, which led to elevated nitrogen concentrations. *A. mearnsii* stands in the present study have formed under climate conditions, which have variability in seasonal temperatures with flowering occurring during warm summer months, leaf drops in autumn and dormancy in cold winters. The variability referred to is also described for temperate deciduous forest in Europe, America and the southern slopes of the Himalayas (Röhrig & Ulrich, 1991). However, the early leaf emergence and longer maintaining of green leaves in *A. mearnsii* compared with native species give *Acacia* an advantage in transporting nutrients to the roots for storage to ensure better winter survival and possibly spring growth.

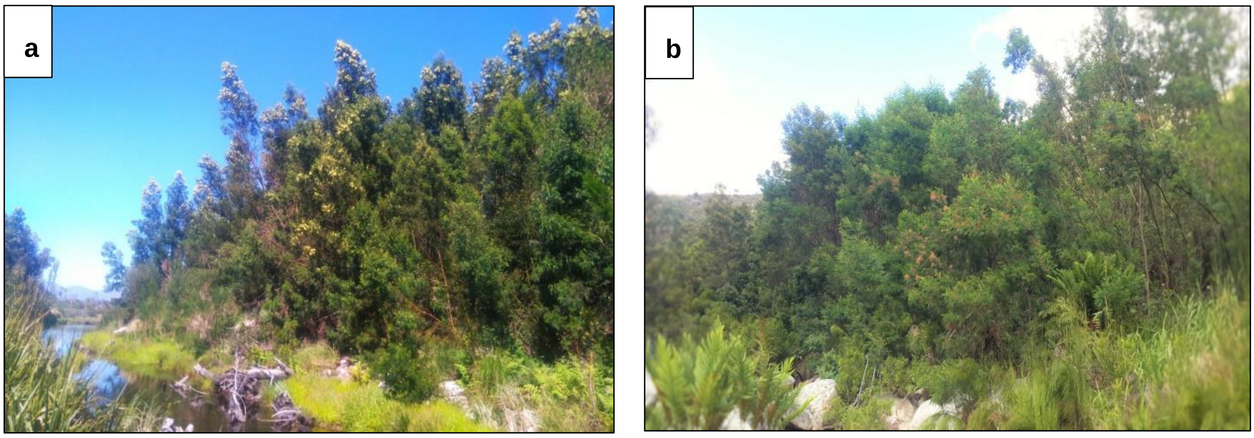


Figure 29: Representation of the flowering season (early) at the (a) Du Toit's River invaded site on 20 October 2016 showing pale yellow flowers and late flowering season at the (b) Wit River invaded site on 11 November 2016 showing orange flowers

5.4.2 Seasonal carbon and nitrogen concentrations, and nitrogen return to soil

The annual mean nitrogen concentrations of *A. mearnsii* and co-occurring native species were relatively distinctive, which was expected as there are major differences between species' lifespan history traits such as phenology, growth form and nitrogen-fixation capacity (Drake, 2011; Tye, 2013). The high concentration of nitrogen in leaves of *A. mearnsii* is due to the ability of the species to fix nitrogen (Yelenik et al., 2004; 2007; Van der Colff et al., 2017). The results found agree with Tye (2013) who found similar ranges of mean annual nitrogen concentrations between seasons for *A. dealbata* in Mpumalanga, South Africa. Results found in other studies (Yelenik et al., 2004; 2007; Juba, 2012, unpublished data; Tye & Drake, 2012; Maoela; 2015) for co-occurring native species agree with the low nitrogen concentration found in the present study. *A. mearnsii* and co-occurring native species exhibit different seasonal allocations of nutrient, which could both be for storage or for uptake early in the flowering and leaf litterfall season. Leaves senescence typically coincides with new leaf growth (Chapin & Shaver, 1989).

The co-occurring species (*B. stellatifolium* and *M. angustifolia)* allocated a higher proportion of nutrient for storage or uptake at the end of the year (December, early summer), whereas the invasive *A. mearnsii* had two senescence stages (December, early summer; April, early autumn). It is therefore sensible to presume that *A. mearnsii* is less reliant on long distance seasonal translocation of nitrogen than the co-occurring native species (Tye, 2013). Non-legumes species as the co-occurring native species tend to produce leaf material with lower year round nutrient concentrations and longer lifespans as observed in nutrient resorption efficiencies (see

Table 11 and

Table 12, resorption efficiencies). This may be reflected in structural components such as lignin and cellulose that have greater C:N content ratios in leaves and the specific plant's leaf longevity as well as the exchange in nutrient conservation (Killingbeck, 1993; 1996; Aerts, 1995; Tye, 2013). A good example of the nutrient conservation strategy of the co-occurring native species is the high nitrogen concentrations of both species in summer at both sites and particularly the month of December as observed in

Table 11 and

Table 12 in the high green leaf concentrations. Moreover, seasonal allocation of nitrogen is likely dependent on either nutrient availability in the environment or interspecific requirement of nitrogen in different seasons as components can differ both spatially and temporarily (Tolsma et al., 1987; Fife et al., 2008). Between species, co-occurring species' low leaf nitrogen concentration tend to show that they occur in phosphorus-limited landscapes and are largely due to their high LMA, which can essentially dilute all nutrients (Lambers et al., 2010). We did not measure LMA in the present study. Tye & Drake (2012) and Witkowski (1991) found difference in foliar nutrients between invasive and non-legumes species from the same habitat as the present study.

Based on the return of nitrogen to soil (mg nitrogen $m^{-2} \cdot y^{-1}$) as quantified by Yelenik et al. (2004), the results agree with previous findings (Witkowski, 1991; Stock et al., 1995; Yelenik et al., 2004, 2007; Naude, 2012) in the south Western Cape of the impacts of *Acacia* spp. on terrestrial soil nitrogen stocks. In the present study, the results show that that *A. mearnsii* had greater mean annual nitrogen return to soil than native vegetation. Therefore, it can be surmised that the high litterfall rate and constant nitrogen concentrations found in *A. mearnsii* throughout the year enhance nutrient concentrations especially nitrogen in the soils under *A. mearnsii* canopies at invaded sites. The enhancing factors are mainly due to attribute difference in leaf chemistry of the alien, namely, *A. mearnsii*. The increase in soil nitrogen is expected as *A. mearnsii* was not limited by nitrogen supply as the species is a nitrogen-fixer (Forrester et al., 2007; Naude; 2012; Boudiaf et al., 2013). The high nitrogen return from *A. mearnsii* in the invaded riparian plots could have down-regulated or decreased NREs as the sites are high in soil nitrogen availability (Naude, 2012) as observed in the study. Even though no soil nitrogen data is presented for *A. mearnsii*-invaded sites, there is a considerable amount of literature to support the arguments that nitrogen fixers (notably *Acacia* spp.) have an impact on soil nitrogen dynamics (Witkowski, 1991; Yelenik et al., 2004; Naude, 2012). In addition, Ehrenfeld (2003), and Liao et al. (2008) in meta-analyses studies on nitrogen-fixing plants reported high levels of TN in soil and available nitrogen. Similar results are found in the Portugal sand dune ecosystems by Marchante et al. (2008) and Hellmann et al. (2011) who reported increased available inorganic nitrogen for *A. longifolia* in uninvaded environments.

The substantial addition of nitrogen-rich litterfall in autumn could contribute to the soil nitrogen being enriched in the present study and therefore lead to the decomposition of the substantial nitrogen-rich litter, which replete the invaded environment and is eventually taken up by the plant. The change in ecosystem nitrogen is mostly associated with the physiological characteristic of the invasive, namely, *A. mearnsii* (Ehrenfeld, 2003; Naude, 2012). Biological traits such as larger size, high growth rate and higher inputs of nitrogen-enriched litter with rapid turnover rates are seen as the most plausible explanations in more nitrogen return to the soil for *A. mearnsii* at the invaded site at the two perennial rivers.

5.4.3 *A. mearnsii* and reference plants leaf $\delta^{15}N$ signatures

The results showed that all *A. mearnsii* (n = 10) at each invaded riparian plot had positive $\delta^{15}N$ signatures. This was not expected, as it indicated the *Acacia* spp. access to soil nitrogen. The key determination of leaf nitrogen isotope composition is the isotope ratio of the external nitrogen source and plant mechanisms (Evans, 2001; Tye, 2013). To address these, possible disputes should be suggested. Many *Acacia* spp. in southern Africa are deep-rooted, which is a physiological function to overcome water and nutrient limitation in arid and semi-arid environments (Morris et al., 2011; Tye, 2013). The deep-rooted *Acacia* spp. can access more diverse nitrogen pools than species in the adjacent environment. They can also take up the same form of nitrogen (i.e., NH^4) than surrounding reference plant species. The concept is maintained by Shearer and Kohl (1986) and Högberg (1997) who stated that with increasing depth in the soil profile, $\delta^{15}N$ consequently increases. The pattern is the combined effects of ^{15}N enriched of organic matter due to microbial decomposition in deep soils, the remoteness of atmospheric inputs and, lastly, the production of fresh litterfall that is depleted in ^{15}N in relation to the soil nitrogen (Högberg, 1997; Tye, 2013). The effect of these processes is important as it suggests that deep-rooted species may be taking up nitrogen with a δ^{15}N that is enriched in relation to a shallow-rooted *Acacia* spp. For example, in a field experiment in Hluhluwe-iMfolozi Park at Kruger National Park, Cramer et al. (2007) reported that deep-rooted *Acacia* spp. had more positive δ^{15}N values than shallow-rooted species.

To further support the positive δ^{15}N signatures in *A. mearnsii*, the geographical location should be considered. The invaded sites in the study are located in CFR in the Fynbos Biome, which is situated in the mountain transitional zones (Reinecke et al., 2013). It is characterised by a seasonal Mediterranean climate with cool wet winters and hot dry summers (Rebelo et al., 2006). The study of Cramer et al. (2007) at the Hluhluwe-iMfolozi Park reported higher rainfall (780 mm per annum) than at Kruger (550 mm per annum). Aranibar et al. (2003) explained that with decreasing rainfall, δ^{15}N increases.

A. mearnsii leaves were sampled in mid-autumn (April), which was at the end of the dry season. Therefore, results found for *A. mearnsii* agree with the general pattern observed by many authors that on a regional and global scale, foliar δ^{15}N increases as precipitation decreases (Austin & Vitousek, 1998; Aranibar et al., 2004; Craine et al., 2009). Nonetheless, other authors (Bai et al., 2012; Houlton & Bai, 2009) also mentioned that the controls of atmospheric deposition on nitrogen cycling are in many instances ignored in nitrogen isotopic studies as nitrogen isotopes from atmospheric deposition and biological nitrogen fixation are presumed to be constant over large regional scales. Moreover, results found in the present study indicate that the use of physiological function (rooting depth) to overcome water limitation and the microclimate conditions of the mountainous regions in the Fynbos Biome of the CFR are contributing factors in the δ^{15}N enrichment of the invasive but not the only factors.

Secondly, the resorption of nutrient particularly nitrogen from leaves can lead to δ^{15}N positive enrichment signatures of leaf nitrogen due to preferred resorption of the light isotope, which happens through metabolic fractionation. In New England, Drake et al. (2008) found a positive correlation between nutrient resorption efficiency and differences between δ^{15}N in leaves (green and senesced) in *Spartina* spp. (salt marsh grasses) in natural enriched salt marshes. Nutrient resorption data collection took place in December as the native species' senescence stage was estimated to be in that month. However, the data calculated for NRE for *A. mearnsii* might be an underestimation of the resorption of nutrients of the species. In the study, Ndfa% in *A. mearnsii* was also calculated; however, results were not significant. Nitrogen fixation is not a contribution in δ^{15}N positive enrichment signatures. Several studies (Tye & Drake, 2012; Van Der Colff et al., 2017) have found relatively negative δ^{15}N signatures in nitrogen-fixing acacias leaf litter; however, to our knowledge, no studies indicate δ^{15}N positive enrichment signatures resulting from nutrient resorption processes. Instead we posit that nutrient resorption is apparent in the month of April for *A. mearnsii* and that is related to positive leaf δ^{15}N signatures.

The technique of the ^{15}N natural abundance has many limitations. Firstly, the ^{15}N abundance of plant-available soil nitrogen can be a value close to zero or in some cases too variable for the methodology to be applied in an ecological background (Högberg 1997; Gehring & Vlek, 2004). Regarding reference plants, it is assumed that the ^{15}N enrichment of these plants reflect the ^{15}N enrichment of the soil nitrogen taken up by the invasive, which sets a significant limitation that should be considered when using this approach (Andrews et al., 2011). It is therefore anticipated that the reference plants should be similar to the invasive plant species in life form, period of growth rate, root characteristics and form of nitrogen taken up from the soil. However, this is usually not the case in many field settings. Thus, it is encouraged to use more than one reference species in a field study (Jacobs et al., 2006; Andrews et al., 2011). Therefore, in the results found it is plausible to understand that it might be likely that we could have overestimated the contribution of nitrogen fixation. Even though the ^{15}N natural abundance method has inadequate quantifiable estimations, it is regarded as a suitable qualitative measure of natural setting, which is accepted by many authors (Boddey et al., 2000; Jacobs et al., 2006; Tye, 2013).

Therefore, a general assumption can be drawn that that the reference species in the study was a fair baseline δ^{15}N signature of plant-available nitrogen in the soil as they grew in close proximity of the *A. mearnsii* infestation.

Another factor to plausibly consider is that *A. mearnsii* acquired more inorganic nitrogen from the soil. In a study by Goi et al. (1993), *A. auriculiformis* acquired a high uptake from soil N, which resulted in increased leaf δ^{15}N due to discrimination against δ^{14}N as preferential efflux of δ^{14}N from the roots (Evans, 2001; Tye, 2013). Therefore, positive values found in δ^{15}N and nitrogen concentrations in the present study were reported in their study (Martinelli et al., 1999; Cramer et al., 2007). The positive values found in (Martinelli et al., 1999) were related to greater fractionation (isotopic) in a more stocked nitrogen-replete system. As such, the suggestion can be made that in the month of April, high nitrogen return to soils in *A. mearnsii*-invaded riparian plots could have contributed to the stocked nitrogen-replete system. Sites with high nitrogen availability are more likely to have with plants with higher nitrogen concentrations, which tend to correlate positively with positive plant δ^{15}N signatures (Martinelli et al., 1999; Cramer et al., 2007).

Isotopic fractionation can take place at different stages (uptake, transport or assimilation) of a plant's nitrogen cycling and is different between plants species using different strategies at each step (Craine et al., 2015). A good account of this, plants associated with different types of mycorrhizal fungi facilitate nitrogen acquisition from the soil will have different δ^{15}N values (see review by Boddey et al., 2000). Craine et al. (2009) showed on a global scale that different types of mycorrhizal fungi associated with plants can show different variation in leaf δ^{15}N values for the meta-analysis of 900 plants sampled. Additionally, the study showed that the type of mycorrhizal association can considerably influence leaf δ^{15}N values, with ericoid mycorrhizal (EM, 3.2 ‰) and ectomycorrhizal (ECM, 5.9 ‰) plants being generally more depleted in leaf δ^{15}N than non-mycorrhizal plants. Arbuscular mycorrhizal plants were shown to be more intermediate in isotopic values and on average depleted by 2‰ in comparison to non-mycorrhizal plants. These fungi facilitators can influence the nitrogen isotopic configurations in plants and other ecosystems pools (Hobbie & Högberg, 2012; Craine et al., 2015).

However, these differences found in (Craine et al., 2009) might be extreme cases as they depend on the plant's reliance on fungi for nitrogen and the supply and demand of nitrogen from the plant. Under natural conditions, mycorrhizal associations are found in 82% of higher plants (Chapin et al., 2002), which can explain the pattern of leaf δ^{15}N values in *A. mearnsii*. Therefore, the nitrogen demand and mycorrhizal associations of *A. mearnsii* have to be considered. In general, African *Acacias* have the ability to form arbuscular mycorrhizas that assist in the uptake of nitrogen and phosphorus from soil pools (Rodríguez–Echeverría et al., 2009; Tye, 2013). *Acacia* spp. from the subgenus *Phyllodineae* can form both arbuscular and ectomycorrhizal mycorrhizal features, which give the authorisation to become invasive and good in land repossession as nodulation is a species-specific characteristic of the subgenus *Phyllodineae*. As such, the positive leaf δ^{15}N signature in *A. mearnsii* could be through mycorrhizal association as well. The results found numerous or conflicting interpretations that can be put forward for the positive leaf ^{15}N indicators found. Certain authors mentioned (Handley & Raven, 1992; Tye, 2013) that the natural abundance δ^{15}N plant tissues are functions of all the foundation and metabolic effects to which their nitrogen pools have been subjected; as such, all the above mentioned topics could be a combined contributing factors.

Resorption efficiencies in A. mearnsii and competing co-occurring native species

Several studies (Aerts, 1996; Ratnam et al., 2008, Tang et al., 2013; Van der Colff et al., 2017) indicate that in any part of the world, most woody plant species conserve phosphorus more tightly than nitrogen. The pattern was shown in the present study at both the Wit River and Du Toit's River with phosphorus resorption being more efficient than nitrogen resorption. NREs were lowest in nitrogen withdrawn from leaves during senescence in *A. mearnsii*. At both sites, higher nitrogen was withdrawn from leaves during senescence in both *B. stellatifolium* and *M. angustifolia*. The NREs values reported for

A. mearnsii were similar to values reported by Tye (2013) for *A. dealbata* (~27%) in the Komati Wildlife Reserve in Mpumalanga, South Africa, but lower than most values reported for associated species growing in the Savanna of South Africa [i.e. Kruger National Park, 58% reported by Ratnam et al. (2008) for fine the Garden Route National Park – leaf trees]. Van der Colff et al. (2017) also recently reported NREs of ±50% in a study at the Garden Route National Park. The values reported for *A. mearnsii* were lower than the global average (56%) for woody plants, which were corrected and reported by Aerts (1996) and Van Heerwaarden (2003).

The low nitrogen resorption values suggest that nitrogen resorption is inefficient in *A. mearnsii* at both sites. On the other hand, NREs reported for *B. stellatifolium* and *M. angustifolia* were on par with the global mean average for nitrogen (56%) and phosphorus (52%) for evergreen shrubs and trees (Aerts, 1996). Moreover, the results suggest that nitrogen resorption is more efficient for co-occurring native species at both study sites. PREs reported in the study for *A. mearnsii* were lower than values reported by He et al. (2011) (up to 80% for arid *Acacia* species in the north-western Australia). The inefficient resorption values found in *A. mearnsii* were not caused by the delay of senescence and can be clarified by the high phosphorus resorption values of *A. mearnsii*. In addition, the resorption of phosphorus over nitrogen seemed to be favoured in *A. mearnsii*, which is shown in the high N:P ratios. The low nitrogen resorption values for *A. mearnsii* found in the present study are observed in other IAP studies (Killingbeck, 1993; Tang et al., 2013). *A. mearnsii* cycles a sufficient amount of phosphorus before leaf abscission, but nitrogen cycling is observed not be as proficient in cycling nutrients as phosphorus. The suggestion is that the invasive species could acquire more inorganic mineral nitrogen from the soil due to high nitrogen-rich litterfall in December and the use of nitrogen fixation in *A. mearnsii* at invaded plots. Van der Colff et al. (2017) stated that *A. mearnsii* reserved its use of symbiotic nitrogen fixation even under changing environmental conditions as it retains its nutrient economy.

To explain these patterns, some factors can be put forward. Firstly, species differentiate in leaf longevity (lifespan based on timing of leaf litterfall). *A. mearnsii* and the co-occurring native species require different but large proportions of leaf nitrogen to be stored in accessible nutrient pools (i.e. structural compounds), which leads to different kinetics of retranslocation (Tye, 2013). In an ecological setting, plants with low leaf mass per unit area have leaves that tend to have short lifespans and high nitrogen concentrations (Wright & Westoby, 2003) such as *A. mearnsii*. Confounding these explanations are nitrogen-fixation capabilities, a characteristic of nitrogen-fixing plants, which are known to influence nitrogen resorption (Killingbeck, 1993; 1996). This can be reasoned with the high nitrogen-fixation capacity of *A. mearnsii* at both sites as indicated by the ^{15}N natural abundance data. *A. mearnsii* could be increasing nitrogen supply from the roots (nitrogen availability) and therefore shift between a cross-over point between the cost point of resorption and nutrient acquisition, which lead to a lower NRE. The interpretations are supported by other studies (Evans et al., 1989; Killingbeck, 1996; Lima et al., 2006) that reported that with the abundant increase in soil nitrogen availability (nitrogen return to soil) and the occurrence of symbiotic nitrogen fixation, a decrease in NRE is expected even though nitrogen fixation is an energy costly process and the ability to fix nitrogen does not mean an inability to resorb nutrients (Houlton et al., 2008). The other postulation is that the cost-related association of nitrogen-fixing rhizobia is less than the cost of resorption at molecular level even though the energy cost of resorption remains anonymous (Houlton et al., 2008). Another contradicting fact is that the root systems of Australian *Acacia* spp. lack cluster roots, which make them incapable of accessing soil phosphorus. However, *Acacia* spp. have specialised extensive root systems and greater mycorrhizal symbionts for enhanced phosphorus acquisition through symbiotic nitrogen fixation, which has a high demand for phosphorus (Allsopp & Stock, 1993). A total of 20% of plant phosphorus are allocated to nodules during nitrogen fixation with the processes taking a large amount of energy as they access a greater pool of phosphorus (Potgieter, 2012). To consider everything in context of *A. mearnsii* (higher litterfall, quantities of nitrogen-rich litter and nitrogen-fixation process), it is therefore plausible to assume that they have a weaker capacity to conserve nutrients (Witkowski, 1991). There is a paradoxical mystery of how *A. mearnsii* satisfy their phosphorus demands and are successful in the study sites.

To satisfy their phosphorus requirements, some *Acacia* spp. may occupy sites with high phosphorus availability as seen with *A. cyclops* in the Fynbos Biome or sites with low phosphorus availability such as *A. saligna* in the Cape lowlands (Witkowski & Mitchell, 1987; Witkowski, 1994). The extensive root system of *Acacia* spp. can increase nutrient uptake by enlarging the soil volume and substantially increasing the number of places for mycorrhizal establishment (Hoffman & Mitchell, 1986; Power, 2010). Moreover, Witkowski (1994) found in the south Western Cape in the CFR that *A. saligna* root penetration was faster than *A. cyclops* over a month period, which potentially could have assisted them in tapping into the water table. Cramer et al. (2007) stated that greater water availability can contribute to phosphorus acquisition via mass flow. Furthermore, these occupations to satisfy their phosphorus demands and the use of deep extensive root system and symbiotic association with mycorrhizal fungi most likely contribute to their success in the Fynbos Biome and particularly *A. mearnsii* phosphorus-acquisition strategies in the present study.

The co-occurring native species effectively recycles nutrients before leaf abscission as high NREs and PREs are found in both species, which were higher than the global average (52%) reported by Aerts (1996). Mean PREs ranged from 50.56 ± 3.40 to 79.33 ± 3.23 between *B. stellatifolium* and *M. angustifolia*, which were values higher than 34 evergreen species (mean = 34%) reported by Tang et al. (2013) in China. However, the riparian native species *B. stellatifolium* (*Proteaceae*) and *M. angustifolia* (*Myrtaceae*) are the only indigenous species of their genus in South Africa and are seen as key species in the south Western Cape Mediterranean-type riparian systems in the CFR (Galatowitsch & Richardson, 2005; Crous et al., 2010). The old, climatically buffered, infertile landscape as defined by Lambers et al. (2010) is ancient inhabit oligotrophic (Power, 2010). The native plants are better adapted to the soil of the CFR and are richly represented on the impoverished soils of the CFR and therefore developed long-term adaption strategies to phosphorus limitations on CFR soils (Killingbeck et al, 1990).

The two native species employ root adaptions such as root clusters to assist in phosphorus acquisition in severely impoverished soils, which is effectively a mining strategy (Lambers et al., 2013). Clusters roots can more easily access soluble phosphorus from the highly weathered and leached ancient soils of the CFR than species with mycorrhizal symbioses (Lambers et al., 2008a; Power, 2010; Potgieter, 2012). In the native species, phosphorus diffusion restricts phosphorus supply to terrestrial plants in natural ecosystems. This happens when phosphorus in the soils are commonly low and dry. Therefore, phosphorus rarely reaches the root system to meet the plant's needs (Prenzel, 1979). However, the mining strategy of these indigenous plants (due to cluster roots) effectively acquires phosphorus through their own species' specific adaption strategy (Lambers et al., 2010; Power, 2010) and, coincidently, the plants remobilise nutrients more efficiently (Aerts, 1995; Wright et al., 2004).

In the native species (*B. stellatifolium* and *M. angustifolia*), low nitrogen concentrations and high PREs are found, which ultimately make them efficient at nutrient conservation (Aerts & Chapin, 2000) due to their mining strategies. The native species both have sclerophyllous leaves, which have longer lifespans than non-sclerophyllous leaves (invader species). These plant species can enhance nutrient conservation as they can increase their nutrient preservation times (Wright & Westoby, 2003; Tye, 2013). Furthermore, the high PRE values in the native species exhibit the true maximum resorption potential of the species because of the long-term adaptation to phosphorus improvised soils as in the CFR. Places such as the Fynbos Biome in the CFR are generally inclined to have a richer degree of sclerophyll plants than plant species in eutrophic environments (Power, 2010; Potgieter, 2012).

However, it is debatable whether Mediterranean ecosystems such as the CFR in the Fynbos Biome are nitrogen- or phosphorus-limited (Sardans et al., 2004; Diaz et al., 2012; Potgieter, 2012). Zhang et al. (2014) stated that plants under phosphorus limitations should have higher PREs. To assess the nutrient limitation in the CFR at present, the use of the N:P ratios in an ecology system are well supported in literature by Craine et al. (2008) and Zhang et al. (2014). Green leaf N:P ratios have been noted as a good forecaster of nutrient limitation. Based on a mass concentration, nitrogen limitation is indicated by < 14, 14 < N:P, nitrogen and phosphorus co-limitation (< 16) and, lastly, N:P > 16 indicates phosphorus limitation (Zhang et al., 2014).

Native and IAP species exceeded the threshold for a phosphorus-limited system, which indicate that the study sites are situated on phosphorus-limited mountainous regions of Breede WMA. The prediction of plants adapted to nutrient-poor systems are easily identified in the low nutrient concentrations in senesced leaves and higher NRE as they grow in infertile habitats, which have low soil nutrient availability (Richardson et al., 2005; Tang et al., 2013, Zhang et al., 2014) as *B. stellatifolium* and *M. angustifolia*. However, nitrogen-fixing plants growing in phosphorus-limited regions tend to have low NREs with high NRPs (Tang et al., 2013) and employ root adaptations; mycorrhizae symbionts assist in phosphorus-acquisition. The results show two different nutrient conservation strategies between the notorious invasive, *A. mearnsii*, and the indigenous native keystone species. These adaption strategies are species-specific as *A. mearnsii* displays a scavenging strategy with nitrogen fixation and the native species a mining strategy to acquire phosphorus (Lambers et al., 2008b; 2010) on the rich plant diversity and severely phosphorus-impoverished soils of the CFR. These strategies are used by other plant species in landscapes such as south-western Australia.

When comparing the present results (nutrient resorption efficiencies) to other studies, it is important to acknowledge that the current estimates for resorption efficiency are articulated on a mass basis and not LMA as done by other authors (Killingbeck, 1996; He et al., 2011). Calculating leaves on a mass basis instead of leaf area basis has its advantages. A good example is the reduced effect by the loss of the LMA during the senescence stage whereas expressing it on an area basis can accurately represent nett nutrient movement from senescing leaves. *A. mearnsii* has a relative small LMA, which could be become problematic and subject to considerable error as it could be complicated to measure. Therefore, we chose to limit our expression of resorption efficiencies to mass basis (concentrations on mass basis) only and acknowledge that our mass-based estimates are likely to overestimate resorption efficiencies (He et al., 2011; Tye, 2013).

5.5 Conclusion

To our knowledge, this work demonstrates that the invasion of *A. mearnsii* in South African fynbos is supplemented by an alteration in the nutrient cycling, most notably nitrogen. These findings suggest that high bioactive nitrogen inputs from *A. mearnsii* can be expected through continuous accumulated nitrogen-rich litterfall, nitrogen-fixing capacity, and acquisition and conservation strategies of the species, which ultimately lead to high nitrogen return to the soil. The government properties of *A. mearnsii* that differ from the native species they normally are compared with, can make the species a successful and competitive invasion species on the phosphorus-deprived environment of the CFR. *A. mearnsii* can maintain its nutrient status regardless of the available nutrients and, in addition, has other mechanisms to compensate for low nutrient availability. Nonetheless, the results suggest that invasive *A. mearnsii* and the co-occurring native species (*B. stellatifolium* and *M. angustifolia*) function differently in the riparian zones of the Breede WMA in the Western Cape, South Africa. The work presented here is a pilot study to better understand the nutrient economy of *A. mearnsii* in the nutrient-poor fynbos riparian zones in the CFR in the Western Cape, South Africa.

CHAPTER 6 – TESTING HOME FIELD ADVANTAGE EFFECTS ON DECOMPOSITION RATES AND MACROINVERTEBRATE COMMUNITIES OF NATIVE AND INVASIVE LITTER SPECIES IN FYNBOS MOUNTAIN STREAMS IN THE CFR

6.1 Introduction

The decomposition of plant material (litter) is among the most studied in terrestrial ecosystem ecology and has been well documented for decades; particularly at site level (Parton et al., 2007; Austin et al., 2014). Leaf litter breakdown is regulated by the physiochemical environment, litter quality and the composition and abundance of macroinvertebrate communities (Reinhart & VandeVoort, 2006). These factors are seen as the main drivers of decomposition in terrestrial systems (Hättenschwiler et al., 2005). In aquatic environments, stream characteristics (temperature, flow regime, nutrients and substrate) can also affect decomposition rates and are suggested to be different in up- and downstream reaches and different microhabitats (pools, runs and riffles) within stream ecosystems (Maamri et al., 2001; Sponseller & Benfield, 2001).

Firstly, leaf litter inputs are seen as a key source of energy for aquatic ecosystems and freshwater biota among streams (fungi, bacteria, and macroinvertebrates) (Negrete-Yankelevich et al., 2008; Ayres et al., 2009; Kuglerova et al., 2017). Secondly, leaf litter inputs from different plant species are different in structure. Chemical properties such as leaf shape, species-specific leaf area, different nitrogen concentrations, C:N ratios and lignin concentrations (Aerts, 1997; Gholz et al., 2000; Ayres et al., 2009) are seen as factors that explain 70% of disparity in leaf litter decomposition (Gholz et al., 2000; Parton et al., 2007). Understanding these interchanging regulating factors can illuminate the influences that change the processes of decomposition rates and macroinvertebrate communities on leaf litter processing within aquatic environments (Leroy et al., 2006).

The feedback loop between litter inputs and the native soil community could create likely competition among macroinvertebrates communities for the litter they receive. Leaf litter between species has been shown to have a large variation in chemical and physical traits (Jewell et al., 2015). For that reason, litter has been recognised as an important source of nutrients and energy for macroinvertebrate communities. Recently, it was thought that the breakdown and mining of plant resources were mostly used by generalist consumers. However, there is significant growing evidence that plant species have species-specific or affinity effects to certain macroinvertebrate communities (Veen et al., 2015). As a result, decomposer communities may become adapted and form a specialised affinity effect to the litter they encounter over an extended period. They become efficient at breaking down their own litter matrix (absorbing nutrients) from the riparian plant community above them (Ayres et al., 2009). Consequently, this at-home benefit has been referred to as the home field advantage (HFA) hypothesis where litter decomposes faster in its home environment than from its origin (Gholz et al., 2000; Ayres et al., 2006). Hence, it creates a specialised affinity effect from in-stream macroinvertebrate communities to the above-ground component, which is generated through litter input. This specialised litter affinity effect from macroinvertebrate communities is driven by three key interacting drivers: the plant community on the adjacent riparian zone, leaf litter quality, and incubation conditions (Veen et al., 2015). The effect can be measured in an ecological time over weeks or months (Jewell et al., 2015).

Nonetheless, there is significant evidence in literature (Freschet et al., 2012; Veen et al., 2015) that tested for, but did not show the occurrence of HFA, hence the conditions under which it exists are uncertain. Litter diversity in a home environment is not always associated with faster decomposition rates and macroinvertebrate litter affinity effects (Austin et al., 2014; Veen et al., 2015). There is evidence of HFA effects where invertebrate decomposer communities become adjusted to feeding on their home turf litter but are less efficient at breaking down the foreign litter regardless of plant diversity or C:N ratios (Veen et al., 2015). However, the variation observed could only account for 8% of litter decomposition processes and are undetected in macroinvertebrate litter affinity effects (Veen et al., 2015; Baroudi, 2016). The difference in effects is vastly variable depending on both biotic and abiotic factors of the ecosystem, which influence the litter decomposition rates (Veen et al., 2015). It is not yet known if disturbing events such as the introduction of IAPs can affect macroinvertebrate litter affinity effects. For example, the invasion of nitrogen-fixing plants could change aquatic environments due to the chemical and physical traits, and differences in leaves from native vegetation (Morris et al., 2011; Jacobs et al., 2013). To better understand how and when decomposition rates and macroinvertebrate communities interrelate with litter to influence HFA effects, it is crucial to explore the significant drivers of the interaction between the plant community, litter type and environmental conditions (Freschet et al., 2012). Yet, it is unclear at the present time if litter of a different type would affect macroinvertebrate communities in an HFA microsite as vegetation peaks (litterfall period) generally follow invertebrate species richness (Buddle et al., 2006); therefore, these mechanistic links need added examination (Van der Wal et al., 2013).

The invasion of IAP species in riparian zones is associated with modifications in quantity and quality of leaf litter inputs available to in-stream macroinvertebrates (Braatne et al., 2007; Boyero et al., 2012). These modifications often affect consumer densities and growth and can lead to substantial changes in ecosystem function and macroinvertebrate communities (Boyero et al., 2012; Levine et al., 2003). Additionally, some taxa can increase decomposition rates and favour certain litter inputs, which mainly rely on the particular style in which they feed (Meyer et al., 2011; Baroudi, 2016). As an example, some macroinvertebrates may feed on nitrogen-rich litter and consequently are able to break down leaf litter more effectively than other invertebrates feeding on leaf litter of an inferior quality (Garcia-Palacios et al., 2013; Jia et al., 2015). The change in litter type due to riparian invasion by IAPs may slow down or accelerate litter decomposition and to extend change macroinvertebrate communities in undisturbed areas as they are associated with changes in community loss and species composition (Boyero et al., 2012). Nevertheless, some global studies also show no effects (Ehrenfeld, 2003; Lecerf et al., 2007).

Lecerf et al. (2007) found no significant changes in macroinvertebrate communities after introducing exotic Japanese knotweed into streams in the United Kingdom and France. The study also found that the decomposition rates between exotic and native species were comparable, suggesting that the species shared similar chemical traits. Similarly, in a field experiment in three streams located in the Chehalis River Basin, Washington, Claeson et al. (2014) found that introducing invasive knotweed leaf litter was related to higher macroinvertebrate abundance and taxa richness than the native alder and cottonwood in one stream but not in the others. Furthermore, Reinhart and VandeVoort (2006) in their study in Rattlesnake Creek in Missoula, Montana, found that leaf decomposition rates were no different between exotic and native species. They concluded that aquatic organisms did not prefer one litter species over the other. The variation in results observed across the world in different stream ecosystems could likely be site-specific, and plants species of these sites might have a unique impact in its localised environment (Leroy et al., 2006; Kuglerova et al., 2017).

In addition, faster decomposition of leaf litter from IAPs compared with native species litter has been reported when the invasive plants were nitrogen-fixing plants and the native species were not (Witkowski, 1991). The physiological characteristics of IAPs of high nutrient concentrations, species-specific leaf area and nitrogen-fixing capabilities are key functions in faster decomposition rates when compared with native species (Allison & Vitousek, 2004; Morris et al., 2011). On the contrary, slower decomposition rates of IAPs were found in numerous studies (Witkowski, 1991; Drenovsky & Batten, 2007). These conflicting findings suggest that more effort needs to go in improving our capabilities to predict the impact of IAPs on real environmental ecosystems in field situations (Davis et al., 2011; Furey et al., 2003). Many studies testing the HFA hypothesis across the globe in terrestrial ecosystems (Vivanco & Austin, 2008; Ayres et al., 2006; Jacob, 2010; Austin et al., 2014) have found inconsistent results; for example, some have shown decomposition rates accelerated in their native as opposed to foreign environments. However, a study by Veen et al. (2015) suggested that the current understanding of the HFA phenomenon is not adequate to generalise across diverse systems as environmental characteristics of HFA remain unexplored. The study also revealed that dissimilarity between litter characteristics could be a stronger predictor of HFA effects of decomposition and possibly macroinvertebrate affinity effects.

However, in the CFR of the Fynbos Biome in South Africa, an understanding of the HFA in litter decomposition rates and macroinvertebrate community structure is incomplete, which limits our understanding of the highly diverse hydrological and biological components of the Mediterranean streams in the CFR. Witkowski (1991) found that litter decomposition rates of the invasive nitrogen-fixing shrub (*A. cyclops*) were slower than the native species (*P. tricuspidatus*) in coastal lowlands of Cape fynbos in the Western Cape. In the same study in a different field setting, Witkowski (1991) found that litter decomposition rates of the nitrogen-fixing *A. saligna* were faster than the co-occurring native species (*L. parole*). The differences in rates are subject to litter quality characteristics between species and nutrient conservation, such as retranslocation of leaf nutrients during senescence (Ehrenfeld, 2003; Allison & Vitousek, 2004). However, none of these studies have been done in riparian environments.

The region has been recognised as a global diversity hotspot owing to its susceptibility to numerous processes that threaten the exceptional biodiversity (Mittermeier et al., 1998). Riparian zones in the CFR are extremely vulnerable to natural disturbances such as flood rushes and fire, which are known to influence riparian systems intensely (Naude, 2012; Maoela, 2015). The flora in riparian areas is 66% naturally spread through the CFR with 33% being woody plants (Galatowitsch & Richardson, 2005; Naude, 2012). However, the biggest threat to the biodiversity of the CFR is the persistent manifestation of IAP species. It is estimated that IAPs have invaded 10 million ha countrywide, with the Fynbos Biome being by far the most affected and the most ecologically harmful (Le Maitre et al., 2002).

The success of IAPs is mainly their good settling capabilities in disturbed areas (Witkowski, 1991; Marchante et al., 2008). Especially the region in the Berg and Breede River catchments of the Western Cape is particularly invaded with the Breede catchment having a heavily invaded region of 84 398 ha (Versfeld, 1995). Alien trees in the CFR, especially Australian leguminous *Acacia* spp. (*A. mearnsii, A. longifolia, A. saligna*), have dense canopies that produce increased nitrogen-rich leaf litter input that may change the river system from autochthotrophic to allochthotrophic (Sakai et al., 2001; Powell et al., 2011). The timing of leaf litter inputs (Le Maitre et al., 1996) and the chemical properties (nitrogen-rich litter and low C:N ratios) may affect decomposition rates, abundance and diversity of invertebrate taxa due to the difference in leaf litterfall quantity and quality between invasive *Acacia* and native species in the Fynbos Biome (King et al., 1987; Rutt et al., 1989). Replacing native riparian tree species with IAPs is likely to affect and modify aquatic habitats of macroinvertebrate communities in adjacent streams (Richardson & Van Wilgen 2004; Reinhart & VandeVoort, 2006; Samways et al., 2011).

Despite the information on the impact of invasion of *A. mearnsii* on terrestrial communities, the possible effects of *A. mearnsii* invasion on leaf processing and macroinvertebrates communities in-stream remain unstudied. Therefore, a trait-based approach to the HFA hypothesis could prove useful between the invasive *A. mearnsii* and native species in the Mediterranean stream of the CFR. The study investigates the impact of *A. mearnsii* invasion and the impact they contribute to ecology in fynbos mountain streams in the CFR. The following is done:

- Compare the decomposition rates (used as a broad inclusive term of both bacterial/fungal decomposition and macroinvertebrate herbivory) between the invasive *A. mearnsii* and a co-occurring native species (here after “fynbos species”).
- Determine if HFA plays a role between *A. mearnsii* and fynbos species in bacterial/fungal decomposition when placed far from origin.
- Determine if HFA plays a role in freshwater macroinvertebrate herbivory of *A. mearnsii* and fynbos species in their native reaches and away from origin.

6.2 Methods

6.2.1 Study area

The study was conducted in mountain and foothill stream sections of the Mediterranean-type CFR vegetation in the Breede WMA, Western Cape. The vegetation distribution in upper catchments regions are typically characterised by sclerophyllous fynbos and sandstone soils, which are acidic and nutrient-poor (Day & King, 1995; Samways et al., 2011). The climate is Mediterranean type characterised by dry summers and wet winters. After the first heavy rains, mountain streams and rivers in the CFR have surface flow in winter. In summer, streams are reduced to perennial pools and a section of riffles resulting in periodic water shortages (Goldblatt & Manning, 2000; Tharme, 2010).

Two perennial rivers were chosen for the study. The Wit River is a small perennial tributary of the Breede River. The Du Toit's River forms part of the Breede system, but is essentially a tributary of the Riviersonderend River, which is shortened by the Theewaterskloof Reservoir (Tharme & Brown, 2004) (Figure 30). Along each river, two vegetation treatments were identified, namely, near-pristine and invaded sites. Near-pristine sites were predominant with typical natural fynbos plant species (consisting mostly of *B. stellatifolium* and *M. angustifolia*) and some scattered *Searsia angustifolia* in the Du Toit's River and *Erica caffra* and *Elegia capensis* at the Wit River with no alien invasive trees present at both sites. Invaded sites had to include alien trees such as *A. mearnsii*, which had to account for 50% cover at sites. Secondly, no commercially afforested or agricultural areas had to be at study sites. Table 13 and Table 14 summarise the river characteristics and treatments of each study site. Sampling started on 4 November 2016 in mid-spring and endured until 6 February 2017 in mid-summer.

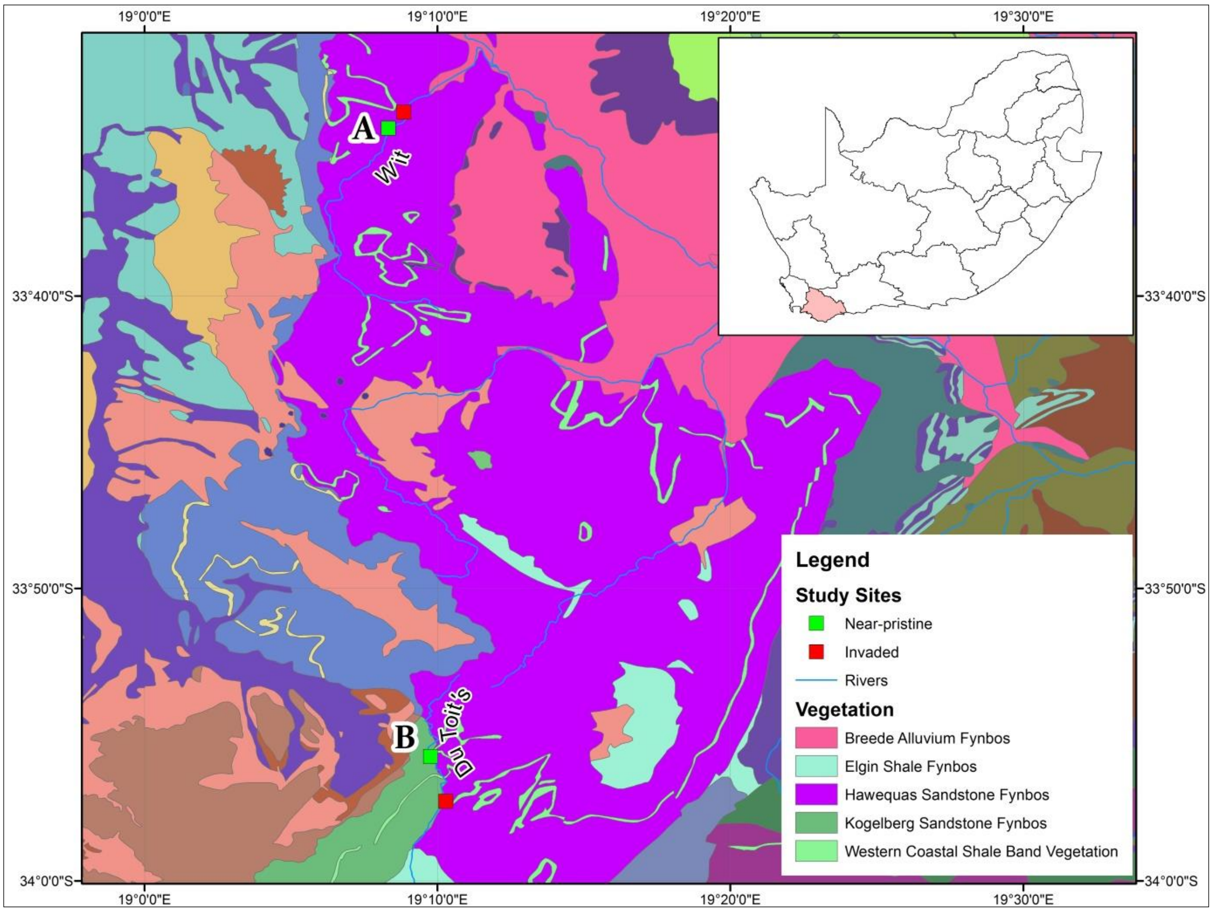


Figure 30: Location of the two perennial rivers in the Western Cape, Breede WMA: Wit and Du Toit's River, and the four treatment sites (green: near-pristine; red: invaded site)

Table 13: Summary of site characteristics of the Wit River and associated invasion statuses (near-pristine and invaded) in the Breede WMA. Values are means [SE] and were recorded from the start to the end of the field experiment

Site: Wit River	Near-pristine	Invaded
Catchment elevation (m amsl)	958.40	901.40
Reach length (m)	140	210
Wetted channel width (m)%	9.46	15.30
Range of average water temperature (˚C)	14.52 to 29.15	14.80 to 29.15
Mean water temperature (˚C) [SE]	22.15 [1.92]	22.07 [1.93]
Range of discharge ($m^3 \cdot s^{-1}$)	0.76 to 0.81	0.66 to 0.77
Mean discharge ($m^3 \cdot s^{-1}$)	0.78 [0.01]	0.69 [0.02]
Range of pH	4.42 to 4.74	4.41 to 4.80
Mean pH [SE]	4.61 [0.05]	4.60 [0.10]
Range of EC ($\mu S \cdot cm^{-1}$)	74.8 to 75.2	74.40 to 75.00
Mean EC ($\mu S \cdot cm^{-1}$)	75.03 [0.06]	74.64 [0.08]
Microhabitats	Pool	Pool
Substratum characteristics	Vertical gradient, physical features including wide single channels dominated by large boulders, medium-sized cobbles with few riffles and patches of sand in certain segments of the reach, alongside the wetted channel, banks comprise medium-sized cobbles and bedrock.	Substratum is dominated by small cobbles and gravel through the reach with large boulders in some section of the reach, major biotopes are riffles with occasional backwaters and no cascades, alongside the wetted channel banks comprises sand, woody shrubs and riparian vegetation.

Table 14: Summary of site characteristics of the Du Toit's River and associated invasion statuses (near-pristine and invaded) in the Breede WMA. Values are means [SE] and were recorded from the start to the end of the field experiment

Site: Du Toit's River	Near-pristine	Invaded
Catchment elevation (m amsl)	981.50	924.20
Reach length (m)	110	130
Wetted channel width (m)%	3.38	8.58
Range of average water temperature (˚C)	12.69 to 19.09	12.40 to 22.24
Mean water temperature (˚C) [SE]	18.85 [2.42]	20.43 [1.94]
Range of discharge ($m^3 \cdot s^{-1}$)	0.16 to 0.40	0.43 to 0.71
Mean discharge ($m^3 \cdot s^{-1}$)	0.23 [0.03]	0.51 [0.04]
Range of pH	4.76 to 5.12	4.50 to 5.10
Mean pH [SE]	4.94 [0.05]	4.70 [0.08]
Range of EC ($\mu S \cdot cm^{-1}$)	69.30 to 74.20	70.20 to 74.40
Mean EC ($\mu S \cdot cm^{-1}$)	72.61 [1.10]	73.56 [0.56]
Microhabitats	Pool	Pool
Substratum characteristics	Vertiginous gradient, physical features displaying features of south Western Cape rivers, including single, narrow channels dominated by riffles that is short and shallow and sections of cascades with deep bedrock-bottomed pools.	Substratum is dominated by small rounded cobbles, large cobbles, gravel and small boulders, riverbanks are dominated with woody shrubs and sand that is relatively high in certain segments and throughout the reach major biotopes are pools and occasional backwater with no cascades.

6.2.2 Experimental set up for decomposition and herbivory

Leaves of *Acacia* species (*A. mearnsii*) and two native species (*B. stellatifolium* and *M. angustifolia*) were collected from single trees in December 2015 and November 2016 just before abscission or shortly after they had fallen (Norby et al., 2000). All leaf material was picked for both decomposition and macroinvertebrate experiments and was oven-dried at 50°C for 24 hours. To test for differences in decomposition and herbivory between leaf types, treatment (non-pristine or invaded) leaf bags were imbedded at both rivers (Wit River and Du Toit's River at the different treatment sites) from 4 November 2016 to 6 February 2017 (incubation periods of 14, 28, 48, 64, 80 and 102 days). Leaf bags were made from fine mesh (0.5 mm) for the decomposition experiment to exclude macroinvertebrates (Webster & Benfield, 1986). Herbivory bags were made from fine coarse mesh (0.5 mm with a 2 mm screening window) to allow access to macroinvertebrate herbivores. The surface area of leaf bags was 0.04 m^2. A total of 5.0 g of *A. mearnsii* leaves was placed into litter bags. For the native species, litter was weighed out to 2.5 g for each, mixed and placed into litter bags. A total of 384 (192 per river) leaf bags were fabricated for both experiments and placed in the field. For the different experiments, four leaf bags of each species were grouped together to a steel rod with nails at each treatment. The steel rod was used an anchor against any objects that might flow downstream due to strong flow variability (Webster & Waide, 1982).

In order to test the HFA hypothesis that plant leaf litter tends to decompose faster in its native environment and that macroinvertebrates in the same environment favour the plant material above them (Austin et al., 2014), fynbos species litter (HFA) and *A. mearnsii* (HFA) decomposition bags were placed in the native reach environment [i.e. near-pristine = fynbos species (HFA); foreign litter which was the *A. mearnsii* litter bags] and in the invaded site. Similarly, the same procedure was done for the herbivory experiment to test the HFA hypothesis. At every incubation date, eight bags per treatment (near-pristine or invaded) were retrieved, which consisted of the HFA and foreign leaf bags. The same procedure was followed for the macroinvertebrate experiment. In total, 32 bags were retrieved per river system at every incubation period.

The Hierarchical Framework for Stream Habitat Classification by Frissel et al. (1986) was followed to place both experiments leaf bags. Microhabitat subsystems are systems defined by Frissel et al. (1986) as patches within pool/riffle systems that have homogeneous environmental conditions (temperature, discharge and substrate characterisation). Decomposition and herbivore leaf bags were placed in sections where microhabitat subsystems were detected within pool systems (Frissell et al., 1986). Mediterranean climate streams are categorised by successive floods and droughts that are variable through seasons due to high annual and inter-annual discharge (Gasith & Resh, 1999). Pools are seen as habitats that hold inputs of leaf litter and other debris, which accumulate and create new microhabitats through different seasons (Gasith & Resh, 1999; Bonada et al., 2007). The same applied for the placement of decomposition bags, which were placed in pool habitats as these sections of a stream are generally undisturbed from flow variability and major changes in synergistic factors (water temperature, pH and conductivity) (Grab, 2014).

Leaf bags for decomposition and macroinvertebrate experiments were collected in ziplock bags and transported to the laboratory. The bags were disassembled, where the remaining leaf material in each bag was carefully washed off and sieved (250 µm) to remove debris and invertebrates. Decomposition leaves were placed in paper bags with labels and dried at 50°C for 24 hours to attain a constant dry mass. The invertebrates retained on the sieve were preserved in 90% ethanol in 50 ml vials for later identification. The dry mass of each bag was placed into a muffle furnace at 550°C to determine the ash-free dry mass. The ash-free dry mass represents the decomposition rate over time and is expressed as the ash-free dry mass remaining (AFDMR%) per treatment and litter type. Macro-invertebrates were identified to genus level using an Olympus SZ compound microscope (Model SZ2-ILST) and the Water Research Commission freshwater invertebrate guides (Day et al., 2002; De Moor et al., 2003a, 2003b; Stals & De Moor, 2007).

The identification of genera belonging to the functional feeding groups (FFGs) of scrapers, deposit feeders and predators were computed according to their FFGs. The genera of these FFGs were then summed within families and the families were summed into FFGs. FFGs were assigned using a modified version by Merrit and Cummins (1984). Finally, FFG abundance was standardised to 1 m^2 according to the available surface area of the mesh bag before calculating abundances of the FFGs.
Statistical analysis

Statistica version 13 software package (Dell Inc., 2015) was used to analyse difference in leaf types in different treatments using repeated measurements (ANOVA). If significant differences were found, the repeated ANOVA was followed by an LSD post hoc test for means comparison. The PRIMER v6 PERMANOVA+ software package (Anderson et al., 2008; Clarke & Warwick, 2001; Clarke & Gorley, 2006) was used for all ordination and multivariate analyses, which required constructing the Euclidean distance and Bray–Curtis similarity matrices. The factors tested in PERMANOVA were "week, site and treatment" (a fixed factor with 12 levels). Differences in macroinvertebrate abundances (measured indirectly as FFGs and directly as proportions of genera) were assessed using a multi-dimensional scaling based on a Bray–Curtis similarity matrix of the square-root transformed abundances. Multi-dimensional scaling was used to test differences between macroinvertebrate assemblages between the two sites (rivers), two different treatments (near-pristine and invaded) and two litter groups across 12 weeks. Environmental factors were investigated using the following procedure: Water temperature (°C) at each treatment of each river was recorded every 30 minutes throughout the experiment (November 2106–February 2017) with a HOBO U20L Water Level Logger (Onset Computer Corporation Inc., Pocasset, MA, USA).

Instantaneous discharge measurements were made at every sampling event with a handheld Marsh–McBirney Model 2000 flow meter from Southern Waters Consultancy. Similarly, stream water pH and EC were recorded in the field throughout the experiment using a Multiparameter pH/ORP/EC/TDS/ Salinity/DO/Pressure/ Temperature Waterproof Meter from the University of the Western Cape, Earth Sciences Department. Discharge ($m^3 \cdot s^{-1}$), temperature (°C), conductivity ($\mu S \cdot cm^{-1}$) and pH were entered into a Draftsman's Plot (PRIMER) to assess multivariate normality and autocorrelation. Noticeable variables with autocorrelation ($-0.75 < R > 0.75$) were removed and the remaining variables that showed skewness were square-root, fourth-rooted or log-transformed. The remaining variables were only transformed based on the degree of skewness and normalised. Nutrient concentrations in-stream were not measured during the specific study period as many authors (Lambers et al., 2008b; Lambers et al., 2010; Potgieter, 2012) described Mediterranean streams in CFR as nutrient-limited (nitrogen and phosphorus) streams.

6.3 Results

6.3.1 Decomposition rates in near-pristine and alien-invaded reaches

The decomposition rates are far greater in *A. mearnsii* than fynbos species across incubation weekdays at the treatments at both study areas of the Wit River and Du Toit's River. In near-pristine and invaded reaches, the decay rates of *A. mearnsii* were faster than the fynbos species, indicating that the decomposability of the species are not site-dependent (Table 15). Furthermore, the results indicate that HFA only plays a role in alien-invaded reaches for *A. mearnsii* (HFA) but not fynbos species (HFA) in near-pristine reaches.

Table 15: The decay rates over 102 days (grams/per day %) in near-pristine and invaded reaches at the Wit River and Du Toit's River sites. Decay rates were calculated at each retrieval period (day) of four leaf bags per leaf litter type. Values reflect means ± [STDEV]

	Decay rates across sampling days (g/d)						
	N	14	28	48	64	80	102
Wit River							
Near-pristine							

		Decay rates across sampling days (g/d)					
	N	**14**	**28**	**48**	**64**	**80**	**102**
Fynbos species (HFA)	4	4.51	2.16	1.21	0.73	0.55	0.42
		[0.11]	[0.06]	[0.04]	[0.07]	[0.06]	[0.15]
A. mearnsii	4	4.23	1.94	0.72	0.54	0.18	0.15
		[0.41]	[0.04]	[0.16]	[0.02]	[0.07]	[0.09]
Alien-invaded							
***A. mearnsii* (HFA)**	4	4.22	2.08	0.94	0.32	0.26	0.19
		[0.32]	[0.18]	[0.16]	[0.10]	[0.07]	[0.01]
Fynbos species	4	5.17	2.40	1.32	0.99	0.56	0.38
		[0.14]	[0.02]	[0.15]	[0.06]	[0.08]	[0.07]
Du Toit’s River							
Near-pristine							
Fynbos species (HFA)	4	4.70	2.06	1.12	0.79	0.56	0.34
		[0.11]	[0.09]	[0.02]	[0.03]	[0.08]	[0.06]
A. mearnsii	4	4.35	1.85	0.91	0.68	0.29	0.20
		[0.32]	[0.81]	[0.12]	[0.20]	[0.00]	[0.02]
Alien-invaded							
***A. mearnsii* (HFA)**	4	3.29	1.55	0.82	0.34	0.26	0.20
		[0.47]	[0.19]	[0.24]	[0.11]	[0.04]	[0.04]
Fynbos species	4	4.28	1.83	1.07	0.81	0.42	0.29
		[0.71]	[0.59]	[0.05]	[0.09]	[0.03]	[0.03]

The decomposition rates of *A. mearnsii* are significantly faster than the fynbos species at both the near-pristine and invaded reaches at the Du Toit’s River (see Figure 31a, Figure 31b). Decomposition rates for *A. mearnsii* were faster than fynbos species from 48 days with a mean = 34.77% compared with 58.20% ($p > 0.001$). From day 64 until day 102, *A. mearnsii* decomposed much faster than the fynbos species ($p > 0.001$). Mean values ranged from 20% to 15.28% for *A. mearnsii* and from 46.78% to 43.18% for fynbos species. The HFA effect for fynbos species is seen at day 0 to day 14, which supports the theory of faster decomposition rates in its home environment.

Similarly at the Wit River invaded reach, decomposition rates of *A. mearnsii* were significantly faster than the fynbos species ($p < 0.001$). *A. mearnsii* decomposed faster than fynbos species from day 14 until day 102 (F $_{[6.36]}$ = 6.52; $p < 0.001$). Mean values for *A. mearnsii* ranged from 59.77% to 19.75% between day 14 and day 102. Fynbos species generally decreased decomposition rates between days with a relative constant rate between day 46 and day 102. HFA for *A. mearnsii* is apparent as it decomposes faster through all incubation days.

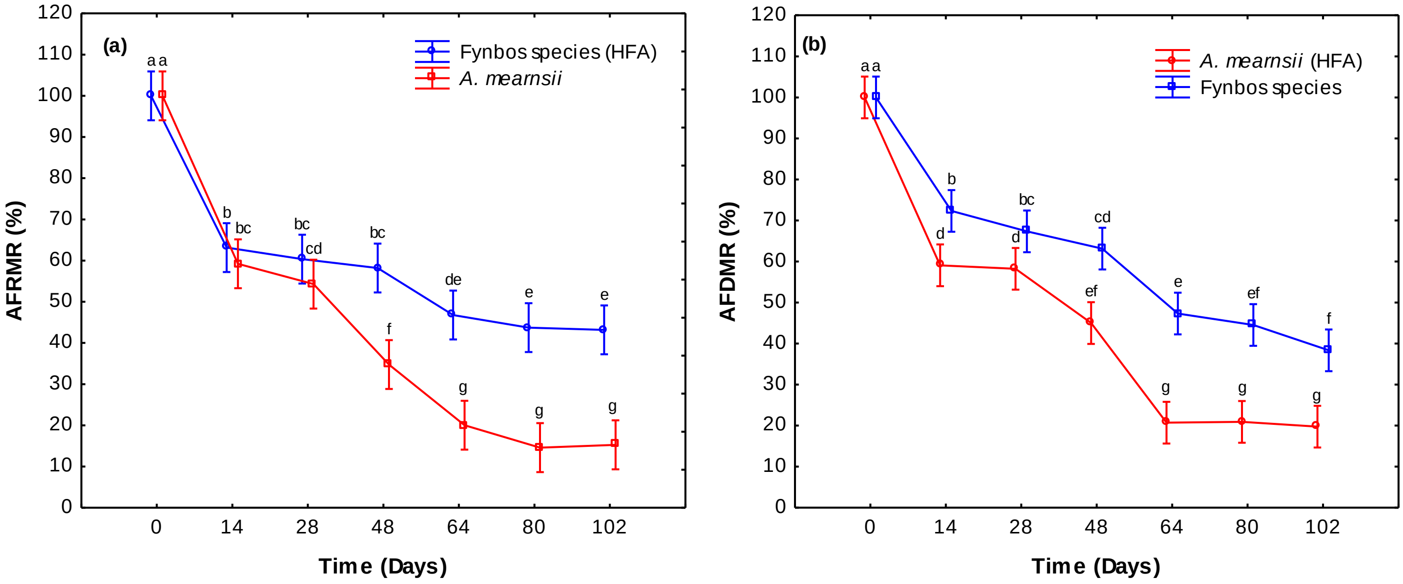


Figure 31: AFDMR% over 102 days incubation period of (a) fynbos species (HFA) and A. mearnsii in near-pristine reaches; and (b) A. mearnsii (HFA) and fynbos species in invaded reaches at Wit River. Letters represent significant differences (LSD test; $p < 0.05$) based on a two-way RMANOVA: (a) F $_{[6,\ 36]}$ = 9.80; $p < 0.001$; (b) F $_{[6.36]}$ = 6.52; $p < 0.001$)

At the Du Toit's River in the near-pristine reach from day 0 to day 48, decomposition rates between fynbos species (HFA) and *A. mearnsii* were the same (F $_{[6.36]}$ = 2.24; $p = 0.62$). However, from day 64 there were significant differences between species with fynbos species (HFA) (mean = 50.67%) decomposing much slower than *A. mearnsii* (mean = 30.35%, $p < 0.001$). The same trend followed up to day 102, with *A. mearnsii* decomposing much faster from day 80 (mean = 22.84%; $p < 0.001$) and at day 102 with a mean value of 20.87%; $p < 0.01$ than fynbos species (HFA) in near-pristine reaches.

At the invaded reach at Du Toit's River, *A. mearnsii* (HFA) decomposed faster on day 14 (mean = 46.02%) than fynbos species (mean = 59.02%; $p < 0.001$). However, no significant differences were found between species on day 28 (F $_{[6.36]}$ = 2.31; $p = 0.12$). In addition, from day 48 to day 102, significant differences were found between species with mean differences of 39.55% on day 48 for *A. mearnsii* (HFA) and 51.27% for fynbos species. This pattern held until day 80 with F $_{[6.36]}$; $p < 0.001$ differences found between species and on day 102, a mean mass loss difference of 9.26%; $p < 0.005$ between species was apparent.

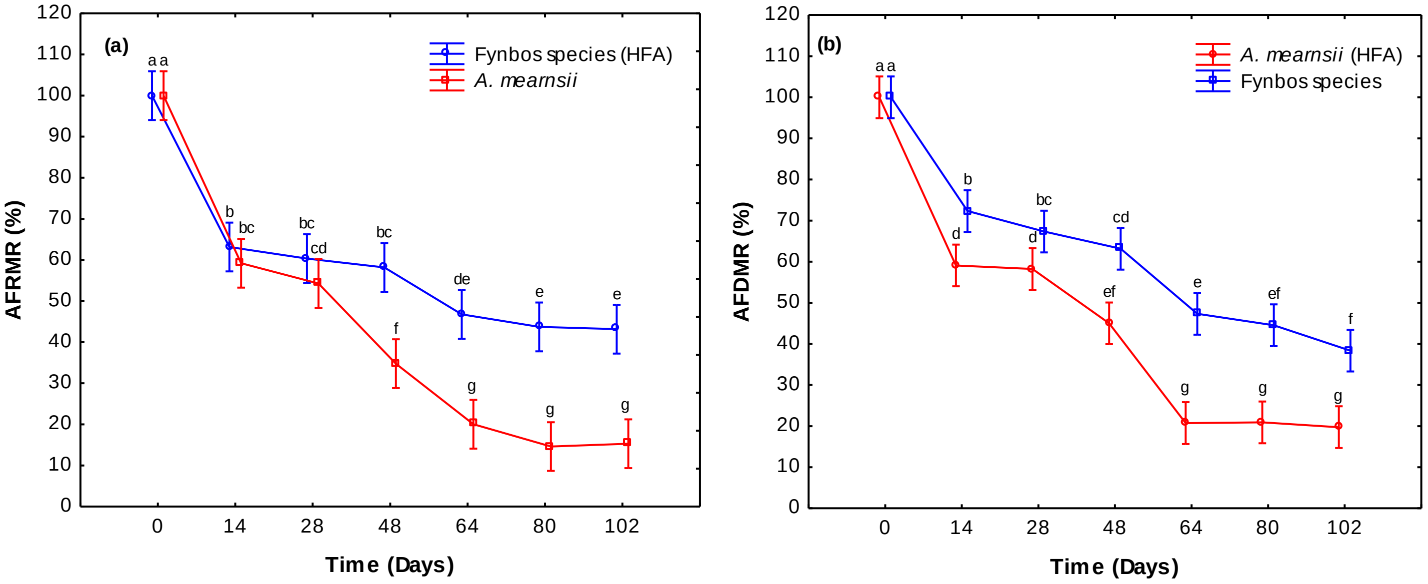


Figure 32: AFDMR% over 102 days incubation period of (a) fynbos species (HFA) and A. mearnsii in near-pristine reaches; and (b) A. mearnsii (HFA) and fynbos species in invaded reaches at Franschhoek Pass. Letters represent significant differences (LSD test; $p < 0.05$) based on a two-way repeated measure (a) ANOVA ($F_{[6,\ 36]} = 2.24$; $p = 0.06$; (b) $F_{[6.36]} = 2.31$; $p = 0.055$)

The scatter plot of mean nitrogen concentrations against AFDMR% shows that faster decomposition rates are related to greater nitrogen concentrations in *A. mearnsii* in all sites. Lower nitrogen concentrations are related to slower decomposition rates in fynbos species (Figure 33). In addition, higher C:N ratios are related to slower decomposition rates as shown by fynbos species and faster decomposition rates are related to *A. mearnsii*.

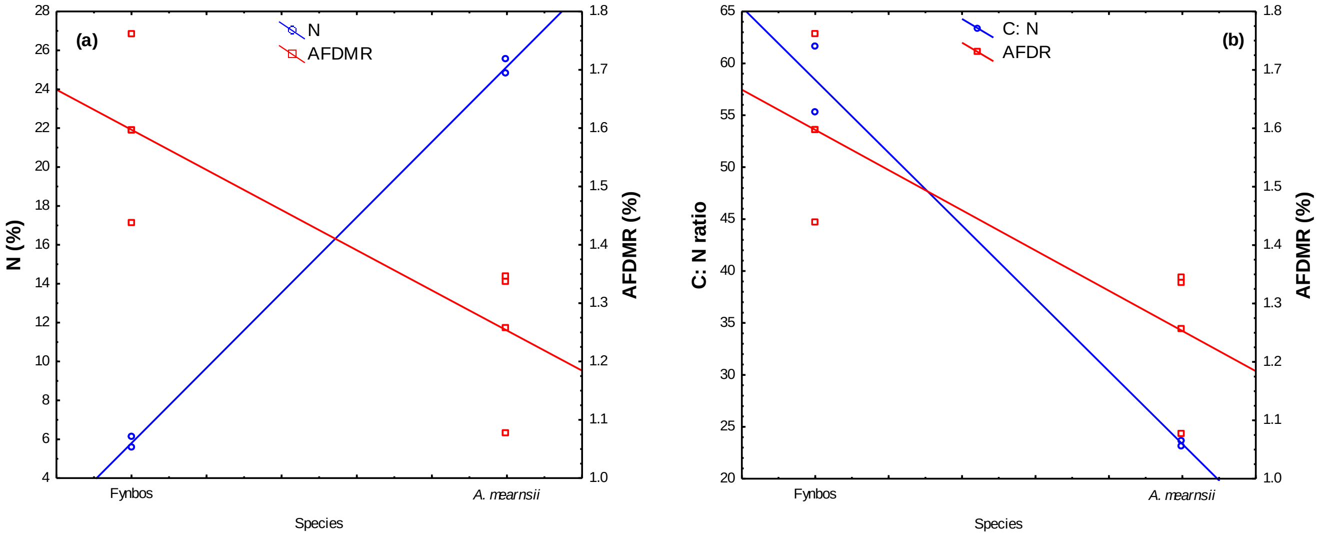


Figure 33: Scatter plots of mean (a) nitrogen concentrations (b) C:N ratios against AFDMR% of fynbos species and A. mearnsii in near-pristine and invaded reaches

6.3.2 Macroinvertebrate abundances between near-pristine and invaded reaches and general observations

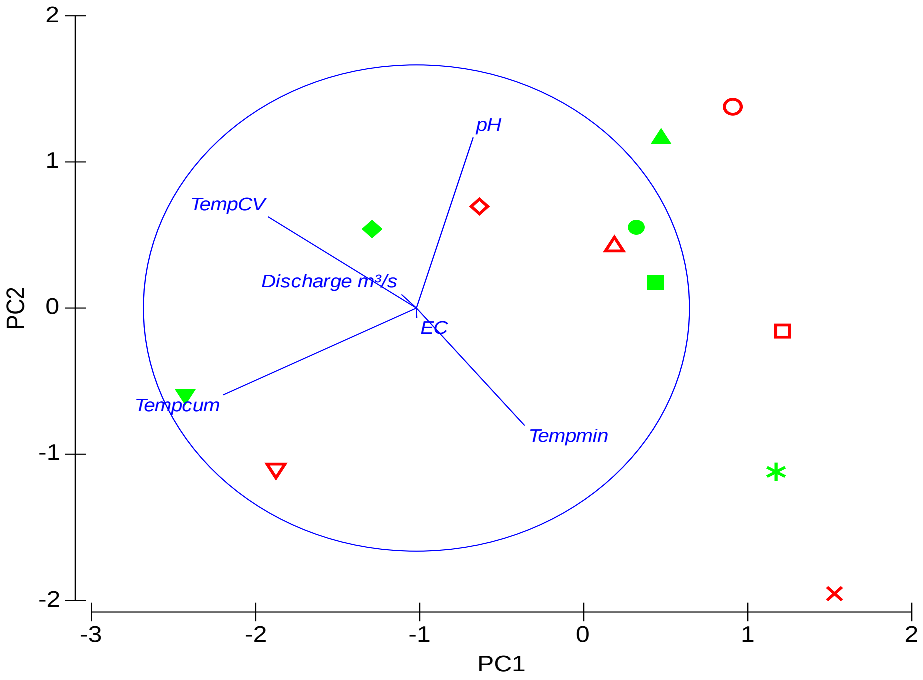
A total of 56 taxa were represented by the 1207 macroinvertebrates sampled across different treatments at both study areas. At the Wit River, a total of 977 macroinvertebrates were sampled at both treatments, but only 247 macroinvertebrates were sampled at both treatments at the Du Toit's River.

During the sampling events at the Du Toit's River, leaf packs were found outside the stream in various weeks, indicating some human interference that may have displaced the leaf bags. Leaf bags in the invaded reach at the Du Toit's River also had bags floating on the water, which suggests some flow disturbance; thus, some bags had no macroinvertebrates present in leaf packs at retrieval time.

6.3.2.1 Habitat conditions in two perennial rivers and different treatments

The principal component analysis (PCA) for the Wit River explained 76.7% of the variation between sampling points on the first two axes based on environmental variables comprising pH, EC, Tempmin, Tempcum and TempCV. The first axis (PCA1) explained 47.3% of this variation and was driven by Tempcum and TempCV that had the strongest correlations (−0.708 and −0.544 respectively). Tempcum was highest at week 4 irrespective of the nature of the site. The second axis (PCA2) explained 29.4% of the variation and was driven by pH and Tempmin that had the strongest correlations (0.702 and −0.483 respectively). Tempmin separated the sampling points at week 4 from the others irrespective of the nature of the site. Discharge and EC contributed very little to variation among sampling points. However, none of the environmental variables listed separated sampling points according to week of sampling or the nature of the site.

The PCA for Du Toit's River explained 62.7% of the variation between sampling points on the first two axes based on environmental variables comprising pH, EC, Tempmin, Tempcum and TempCV. PCA1 explains 42.7% of the variation that was driven by EC (conductivity), which had the highest eigenvalues (0.920). The pH and discharge had the smallest contribution in eigenvalues on PCA 1. On PCA2, Tempcum (−0.786) and TempCV (−0.524) had the eigenvalues with Tempmin having minimal contribution.



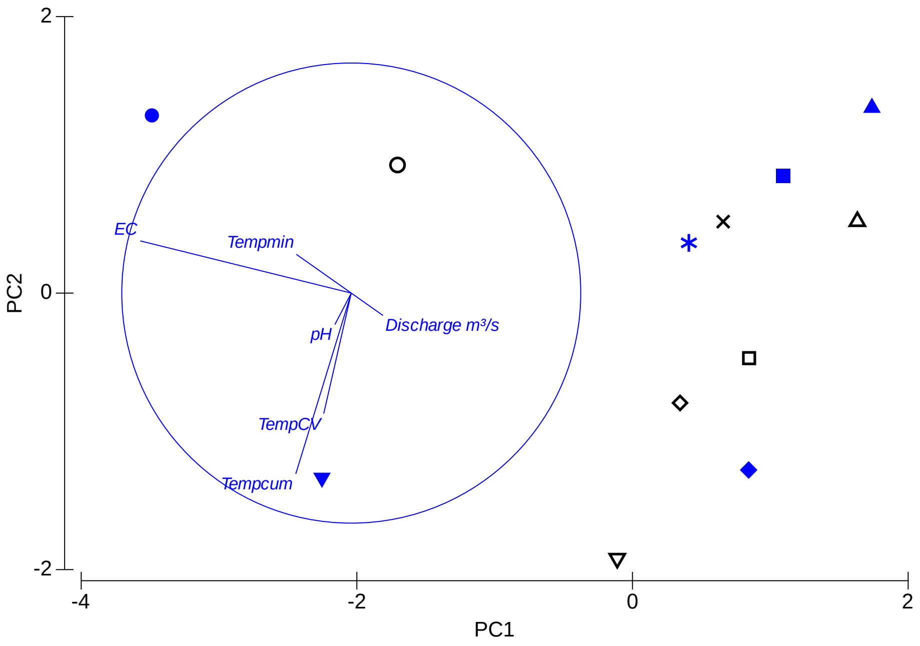


Figure 34: PCA of the environmental conditions in-stream at the study site of the Wit River (above) and Du Toit's River (below). At Wit River, treatments are presented as follows: green = near-pristine reaches; red = invaded reaches. At Du Toit's River: blue = near-pristine reaches; black = invaded reaches. Different shapes represent different weeks. Triangles (2 weeks); upside-down triangle (4 weeks); squares (6 weeks); diamond (8 weeks); circle (10 weeks) and asterisk or × (12 weeks)

6.3.3 FFGs in between fynbos species and *A. mearnsii* litter types in different treatments

6.3.3.1 Wit River macroinvertebrate FFG richness

Macroinvertebrate FFGs had no preferences for either fynbos species litter or *A. mearnsii* litter in the near-pristine site (Table 16). In the near-pristine site, no distinct clusters based on litter type [fynbos species (HFA) or *A. mearnsii*] were found. However, FFG abundances increased from week 2 until week 10 irrespective whether the litter type was from its home environment (HFA) or not (Figure 35). In week 10, mean scraper abundance [mean = 548.50 (135.96) m^{-2}] appeared to be greatest in *A. mearnsii* litter type (Table 16). In addition, predator abundances seem to be greatest in week 12 for fynbos species (HFA) litter with a mean value of 280.25 m^{-2}.

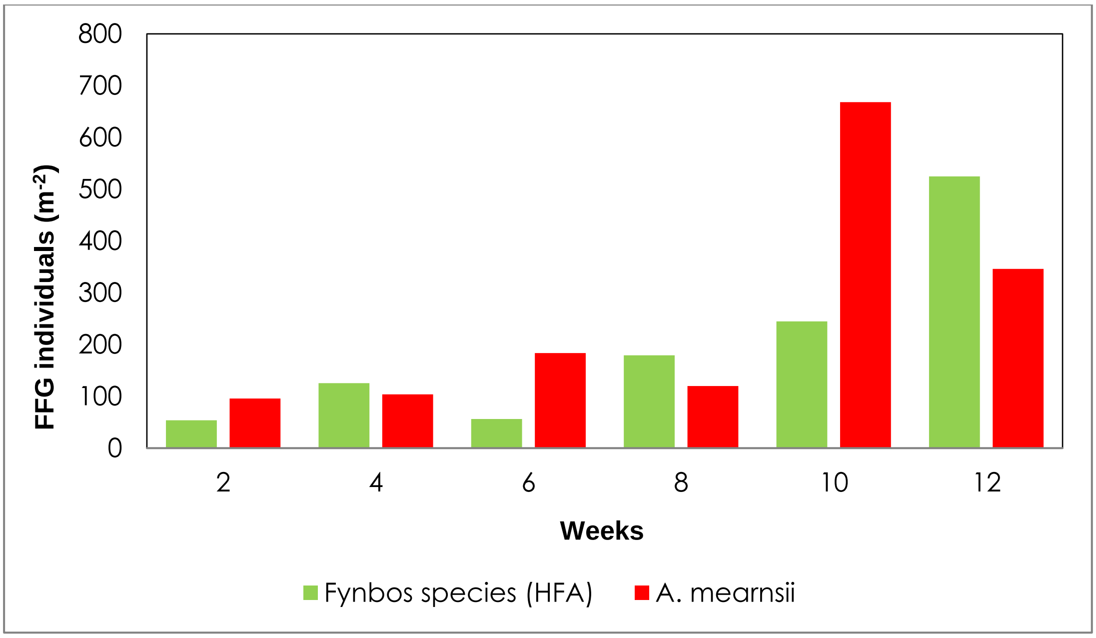


Figure 35: Mean abundances of FFGs (m^{-2}, n = 3) of deposit feeders, scrapers and predators at the Wit River near-pristine site between two litter types [green = fynbos species (HFA); red = A. mearnsii] over a 12-week sampling period

At the invaded site, FFG abundance increased from week 2 to week 12, irrespective of whether the litter type was from its home environment (Figure 36; Table 17). In addition, scraper and predator abundance are greatest in week 10 with means of 471.25 m^{-2} and 119.25 m^{-2}, respectively. These means contribute to the major pulse increase in FFG individuals in week 10 for both litter types.

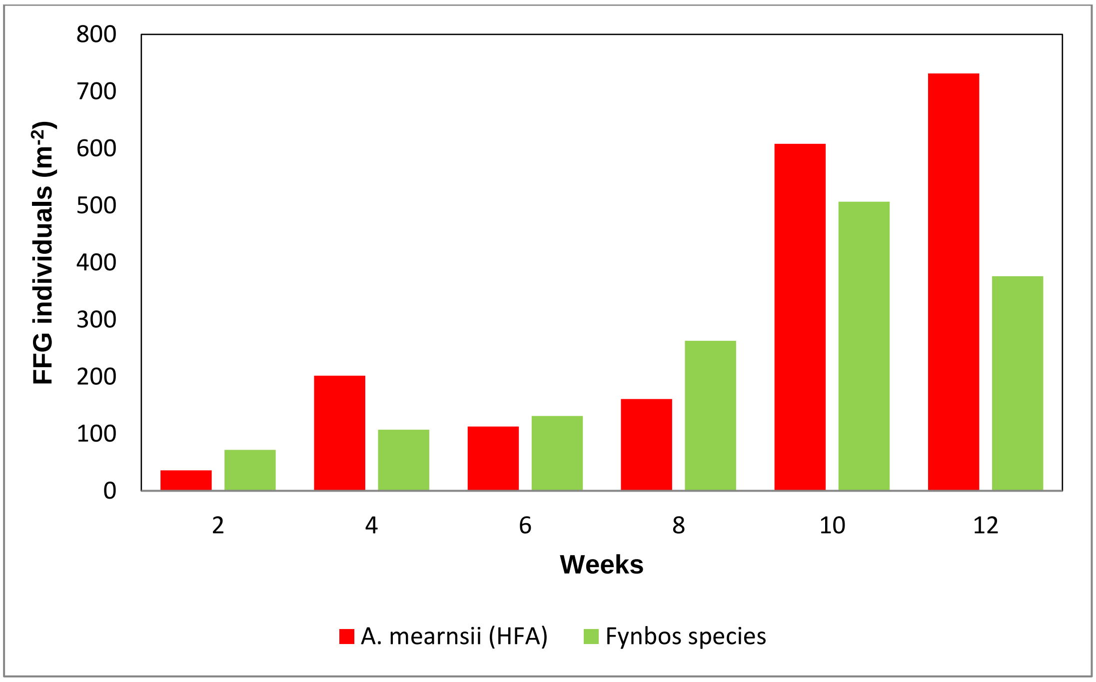


Figure 36: Mean abundances of FFGs (m^{-2}, n = 3) of deposit feeders, scrapers and predators at the Wit River, invaded site between two litter types [red = A. mearnsii (HFA); green = fynbos species] over a 12-week sampling period

Table 16: Mean abundances (individuals m^{-2}, ± SD, n = 4) of FFGs (deposit feeders, scrapers and predators) recorded at the Wit River near-pristine site over incubation weeks/intervals and different litter types (FS = fynbos species; AM = A. mearnsii). HFA = indicates home field advantage litter type; (*) indicates less than four sampling size; and (X) no data sampled

Wit river: Near-pristine (a)

Weeks/interval:	2		4		6		8		10		12	
Litter type:	FS (HFA)	AM	FS (HFA)	AM	FS (HFA)	AM	FS (HFA)	AM	FS (HFA)	AM	FS (HFA)	AM
FFGs (m^2):												
Deposit feeder [SE]:	12.00 [6.93]	24.00*	124.50 [26.57]	32.00 [6.93]*	32.00 [18.33]	56.00 [6.93]*	X	18.00 [18.00]	48.00 [21.91]	101.50 [26.35]	196.75 [79.81]	155.25 [46.02]
Scraper [SE]:	24.00 [9.80]	X	59.75 [12.6.93]	55.67 [18.06]	24.00 [12.00]	103.67 [6.64]	173.00 [22.72]	89.80 [50.07]	197.00 [60.30]	548.50 [135.96]	48.00 [9.80]	184.75 [64.65]
Predator [SE]:	18 [18.00]*	72.00*	18 [18.00]*	16.00 [13.86]*	X	24.00 [12.00]	6.00 [6.00]	X	X	72.00*	280.25 [107.21]	6.00*
Total FFGs per litter type	54.00	72.00	125.50	103.70	56.00	183.67	179.00	119.80	245.00	668.00	525.00	346.00

Table 17: Mean abundances (individuals m^{-2}, ± SD, n =4) of FFGs (deposit feeders, scrapers and predators) recorded at the Wit River invaded site over incubation weeks/ intervals and different litter types (FS = Fynbos species; AM = A. mearnsii). HFA = indicates home field advantage litter type; (*) indicates less than four sampling size; and (X) no data sampled

Wit River: Invaded site												
Weeks/Interval:	2		4		6		8		10		12	
Litter type:	AM (HFA)	FS	AM (HFA)	FS	AM (HFA)	FS	AM (HFA)	FS	AM (HFA)	FS	AM (HFA)	FS
FFGs (m^2):												
Deposit feeder [SE]:	12.00 [6.93]	48.00 [16.97]	53.75 [14.87]	41.50 [14.77]	59.00 [6.35]	77.50 [26.35]	35.50 [11.50]	18.00 [18.00]	119.25 [68.12]	256.25 [22.83]	361.75 [91.25]	232.75 [39.54]
Scraper [SE]:	X	12.00 [8.49]*	65.50 [40.57]	35.75 [22.76]	41.75 [17.75]	35.50 [20.50]	125.25 [32.85]	89.80 [50.07]	471.25 [226.86]	226.75 [96.18]	351.75 [177.04]	131.25 [58.74]
Predator [SE]:	24.00 [16.97]	12.00 [8.49]*	12.00 [6.93]*	30.00 [18.00]*	12.00*	18.00 [6.00]	X	X	215.00	24.00 [16.97]	18.00 [6.93]	12.00*
Total FFGs per litter type	36.00	72.00	202.00	107.25	112.75	131.00	160.75	119.80	608.50	507.00	731.50	376.00

6.3.3.2 Du Toit's River macroinvertebrate FFG richness

FFG abundance showed no preference for either fynbos species (HFA) or *A. mearnsii* litter in the near-pristine reach (Figure 37). The abundances of FFGs did not increase from week 2 to week 12 irrespective of litter type (Table 18). Deposit feeders are the highest in week 12 with a mean 381.5 [119.50] m^{-2}.

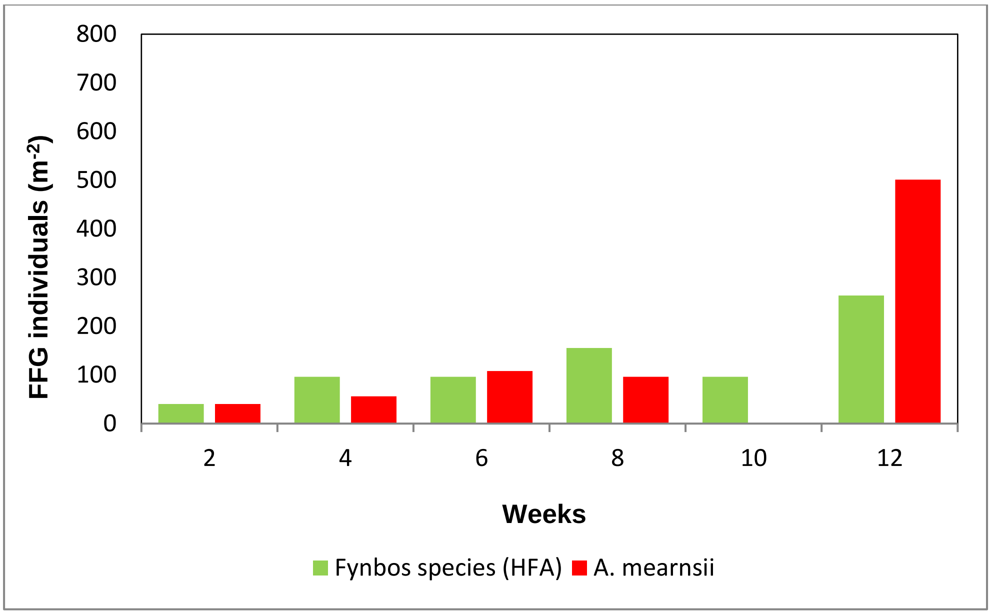


Figure 37: Mean abundances of FFGs (m^{-2}, n = 3) of deposit feeders, scrapers and predators at the Du Toit's River, near-pristine reach between two litter types (green = fynbos species (HFA); red = A. mearnsii) over a 12-week sampling period

In the invaded site, FFG abundance showed no preference for either *A. mearnsii* litter (HFA) or fynbos species. FFG abundance did not increase from week 2 to week 12 irrespective of litter type. Scraper abundances were highest in week 6, irrespective of litter type. Deposit feeders are greatest in week 2 and predator abundances are highest in week 4.

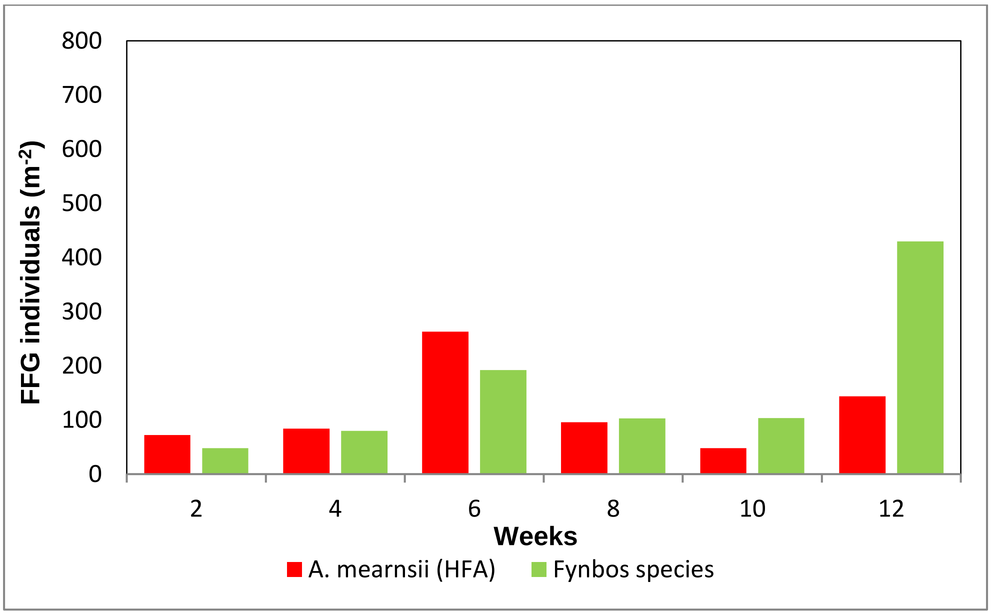


Figure 38: Mean abundances of FFGs (m^{-2}, n = 3) of deposit feeders, scrapers and predators at the Du Toit's River, invaded reach between two litter types [green = fynbos species; red = A. mearnsii (HFA)] over a 12-week sampling period

Table 18: Mean abundances (individuals m^{-2}, ± SD, n = 4) of FFGs (deposit feeders, scrapers and predators) recorded at the Du Toit's River, near-pristine site over incubation weeks/intervals and different litter types (FS = fynbos species; AM = A. mearnsii). HFA = indicates home field advantage litter type; (*) indicates less than four sampling size; and (X) no data sampled

Du Toit's River: Near-pristine												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**
FFGs (m^2):												
Deposit feeder [SE]:	24.00 [0.00]	24.00*	48.00*	X	48.00*	X	X	X	24.00*	X	215.00*	381.5 [119.50]*
Scraper [SE]:	X	X	24.00*	32.00 [6.93]	X	36.00 [12.00]	107.50 [35.50]	48.00*	24.00*	X	48.00*	95.50 [23.50]*
Predator [SE]:	16.00 [9.80]	24.00	24.00*	24.00*	48.00 [24.00]	72.00*	48.00*	48.00*	48.00*	X	X	24.00*

Du Toit's River: Near-pristine												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**	**FS(HFA)**	**AM**
Total FFG per litter type	40.00	40.00	96.00*	56.00	96.00	108.00	155.50	96.00	96.00	X	263.00	501.00

Table 19: Mean abundances (individuals m^{-2}, ± SD, n = 4) of FFGs (deposit feeders, scrapers and predators) recorded at the Du Toit's River, invaded site over incubation weeks/ intervals and different litter types (FS = fynbos species; AM = A. mearnsii). HFA = indicates home field advantage litter type; (*) indicates less than four sampling size; and (X) no data sampled

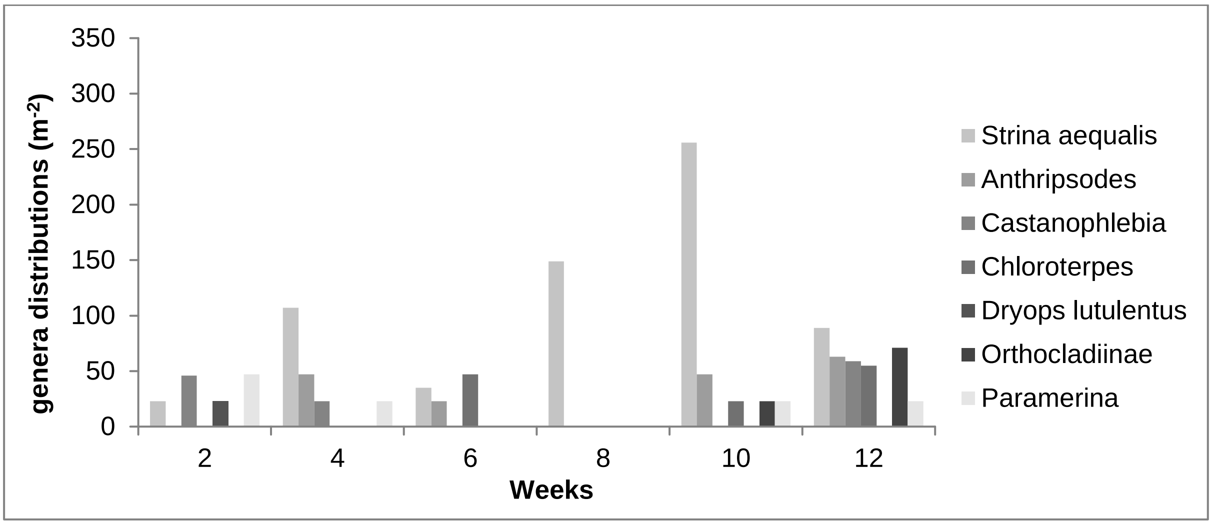
Du Toit's River: Invaded site												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	AM(HFA)	FS	AM(HFA)	FS	AM(HFA)	FS	AM(HFA)	FS	AM(HFA)	FS	AM(HFA)	FS
FFGs (m^2):												
Deposit feeder [SE]:	48.00*	24.00*	36.00 [12.00]*	24.00*	X	X	24.00 *	32.00 [6.93]	24.00*	32.00 [8.00]	48.00*	310.25 [76.05]

Du Toit’s River: Invaded site

Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Scraper [SE]:	X	X	24.00*	32.00 [6.93]	191.00 [39.67]	144.00	48.00*	47.75 [16.74]	24.00*	47.75 [16.74]	36.00 [12.00]	95.67 [56.04]
Predator [SE]:	24.00*	24.00*	24.00*	24.00*	72.00 [8.00]	48.00	24.00*	24.00*	X	24.00*	59.50 [35.50]	24.00*
Total FFG per litter type	72.00	48.00	84.00	80.00	263.00	192.00	96.00	103.00	48.00	103.75	143.50	429.92

6.3.3.3 *Wit river macroinvertebrate genera distributions*

In the near-pristine reach at the Wit River, genera abundances showed no preference for either fynbos species (HFA) or *A. mearnsii* litter type (Figure 39). However, genera abundances increased from week 2 until week 10 irrespective of litter type with mean values gradually increasing per week (Table 20). The genus accounting for most increase over weeks was *Strina aequalis* with week 10 fynbos species (HFA) litter being the highest in mean (256.00 m^{-2}) value (Figure 39). In the *A. mearnsii* litter type, *S. aequalis* was also the highest throughout the weeks: in week 10 it showed a mean value of 299.00 m^{-2}.



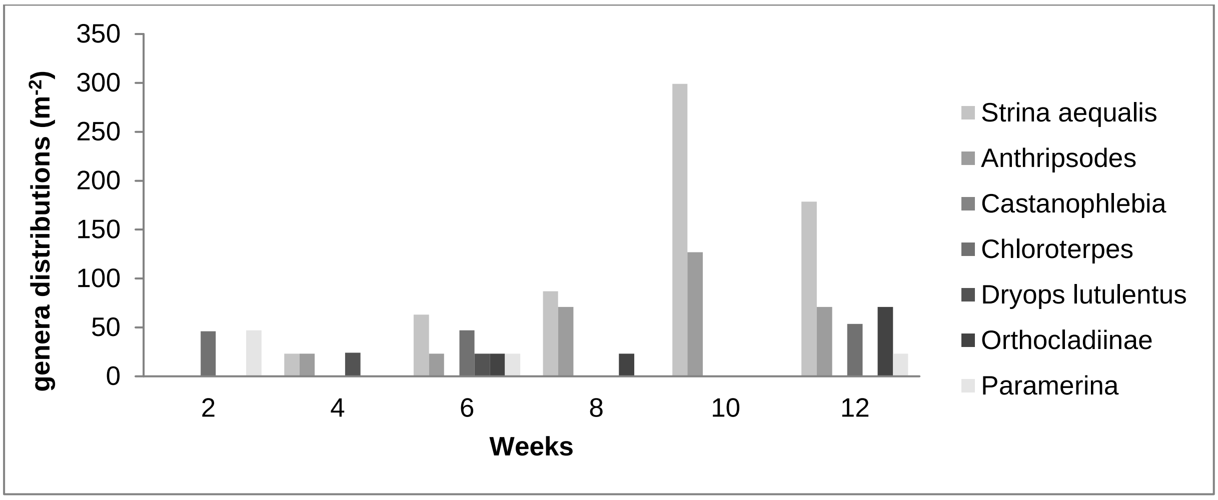


Figure 39: Mean genera (m^{-2}) abundances (above) of fynbos species (HFA) and (below) A. mearnsii litter type in near-pristine reach at the Wit River. Missing gaps represent no data sampled in each week

Genera abundance in the invaded site showed a slight preference for either *A. mearnsii* (HFA) or fynbos species litter type over the weeks (Table 21, Figure 40). In the *A. mearnsii* (HFA) litter type, *S. aequalis* was the highest in week 10 with a mean value of 325.67 m^{-2} and *Athripsodes* the second-highest in week 12 respectively in *A. mearnsii* (HFA) litter type. In the fynbos species litter in the invaded site, the genera distribution of *S. aequalis* and *Athripsodes* accounted for the most abundance on the fynbos species litter type.

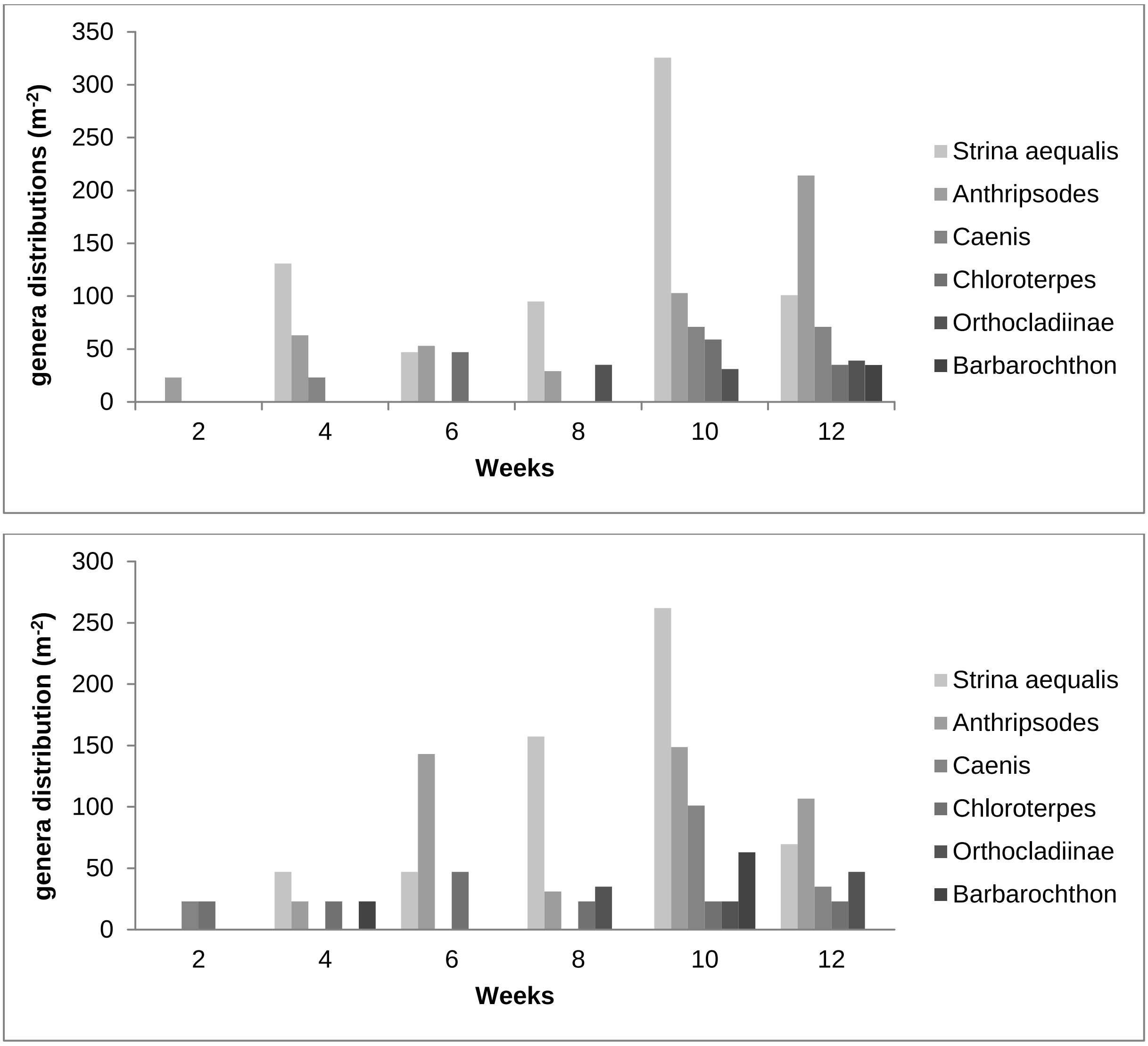


Figure 40: Mean genera abundances (above) of A. mearnsii (HFA) litter and (below) fynbos species litter type in the invaded reach at the Wit River

Table 20: Mean (individuals m^{-2}, ± SE, n = 4) genera distributions represented the most at the Wit River near-pristine reach over incubation weeks/intervals and different litter types (FS = fynbos species; AM = A. mearnsii). HFA = indicates home field advantage litter type; (*) indicates less than four sampling size; and (X) no data sampled

Wit River: near-pristine site												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**
Genera distributions (m^{-2}):												
Strina aequalis	23.00*	X	107.00 [36.00]	23.00*	35.00*	63.00 [21.17]	148.75 [26.40]	87.00 [55.66]	256.00 [83.52]	299.00 [91.93]	89.00 [24.74]	178.50 [57.27]
Athripsodes	X	X	118.00 [29.39]	23.00*	23.00*	23.00*	X		47.00*	127.00 [21.17]	63.00 [8.00]	71.00 [31.75]
Castanophlebia	46.00*	X	23.00*	X	X	X	X	X	X	X	59.00 [12.00]	X

Wit River: near-pristine site												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**
Chloroterpes	X	46.00*	X	X	47.00*	47.00*	X	X	23.00*	X	55.00 [8.00]	53.67 [17.33]
Dryops lutulentus	23.00*	X	X	24.00*	X	23.00*	X	X	X	X	X	X
Orthocladiinae	X	X	X	X	X	23.00*	X	23.00*	23.00*	X	71.00*	71.00*
Paramerina	47.00*	47.00*	23.00*	X	X	23.00*	X	X	23.00*	X	23.00*	23.00*
Total genera distribution per litter type	129.00	103.00	271.00	70.00	102.00	202.00	148.75	101.00	372.00	426.00	360.00	397.00

Table 21: Mean (individuals m^{-2}, ± SE, n = 4) genera distributions represented the most at the Wit River invaded reach over incubation weeks/intervals and different litter types (FS = fynbos species; AM = A. mearnsii). HFA = indicates home field advantage litter type; (*) indicates less than four sampling size; and (X) no data sampled

Wit River: invaded site												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**
Genera distributions (m^{-2}):												
Strina aequalis	X	X	131.00 [29.39]	47.00*	47.00 [24.00]	47.00 [24.00]	95.00 [18.79]	157.33 [92.93]	325.67 [45.15]	262.00 [112.42]	101.00 [24.74]	69.67 [25.33]
Athripsodes	23.00*	X	63.00 [40.00]	23.00*	53.00 [15.01]	143.00*	29.00 [6.00]	31.00 [8.00]	103.00 [34.87]	148.75 [47.13]	214.25 [74.84]	106.75 [37.07]
Caenis	X	23.00*	23.00*	X	X	X	X	X	71.00 [21.91]	101.00 [28.35]	71.00 [41.57]	35.00 [12.00]

Wit River: invaded site												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**
Chloroterpes	X	23.00*	X	23.00*	47.00*	47.00 [24.00]	X	23.00*	59.00 [15.49]	23.00*	35.00 [11.49]	23.00*
Orthocladiinae	X	X	X	X	X	X	35.00*	35.00 [12.00]	31.00 [8.00]	23.00*	39.00 [19.50]	47.00 [13.86]
Barbarochthon	X	X	X	23.00*	X	X	X	X	X	63.00 [21.17]	35.00*	X
Total genera distribution per litter type	23.00	46.00	217.00	70.00	147.00	237.00	159.00	246.33	589.57	620.75	495.25	281.42

6.3.3.4 Du Toit's River macroinvertebrate genera distributions

Genera abundance showed no preference for either fynbos species *or A. mearnsii* litter in the near-pristine reach (Figure 41). There was no increase of genera distributions found across weeks and many weeks showed no data available. *Aprionyx* abundances are the greatest in fynbos species (HFA) litter type across weeks with week 4 accounting for the highest abundances found with a mean value of 47.00 m^{-2}. *Paramerina* also showed abundances across weeks but with no increase. In the *A. mearnsii* litter type in the near-pristine site, there was no increase in genera abundance numbers with *Caenis* being the highest in week 12 with a mean value of 345.50 m^{-2}.

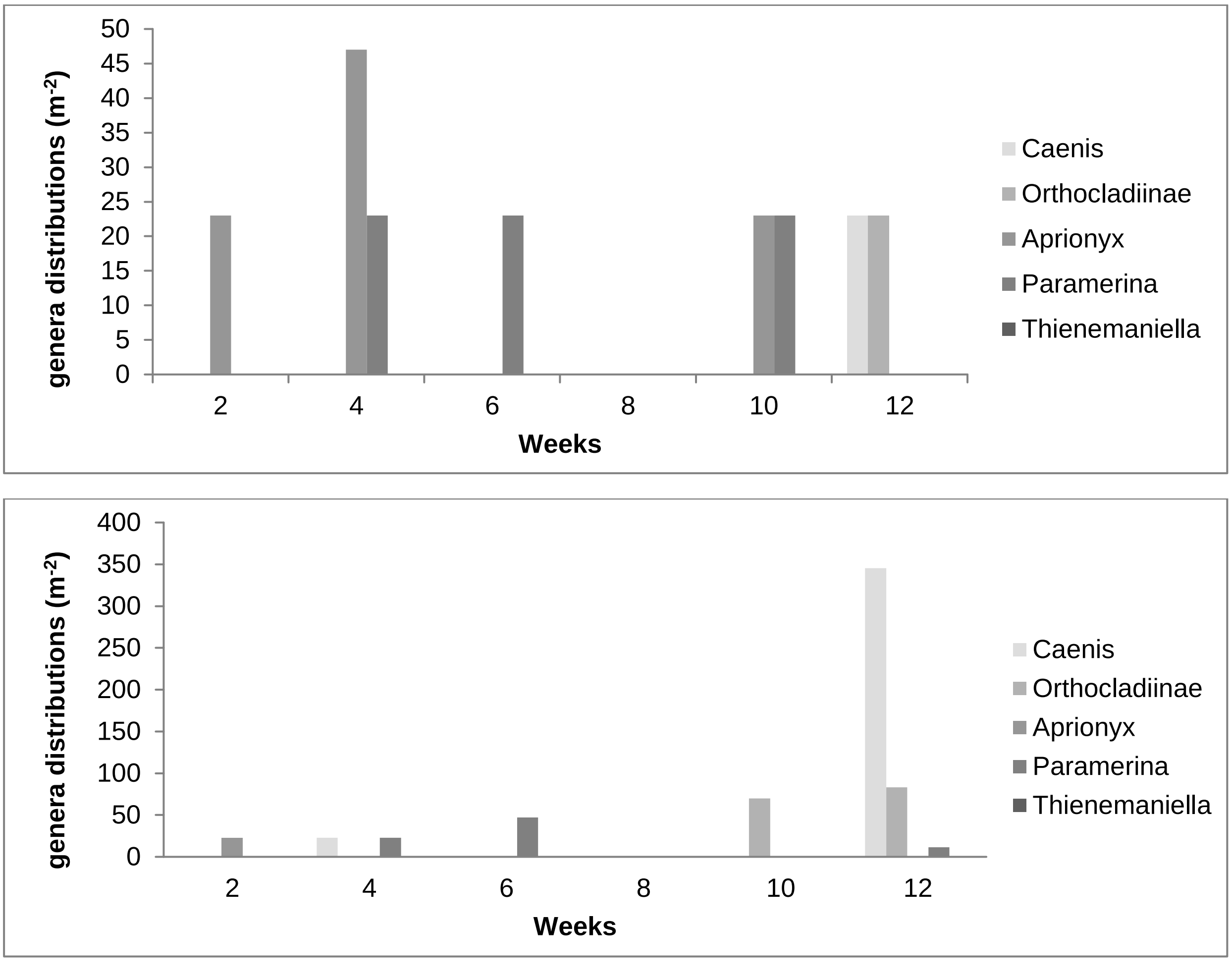


Figure 41: Mean genera abundances (above) of fynbos species (HFA) litter and (below) A. mearnsii litter type in the near-pristine reach at the Du Toit's River. Note the different scales of the y-axes

Similarly at the invaded reach, genera abundances showed no preference for either *A. mearnsii* (HFA) or fynbos species litter type (Figure 42). In addition, no increase in genera abundances were found across weeks and earlier weeks showed no data available. However, *both A. mearnsii* (HFA) and fynbos species litter type showed high mean values for *Caenis* in week 12.

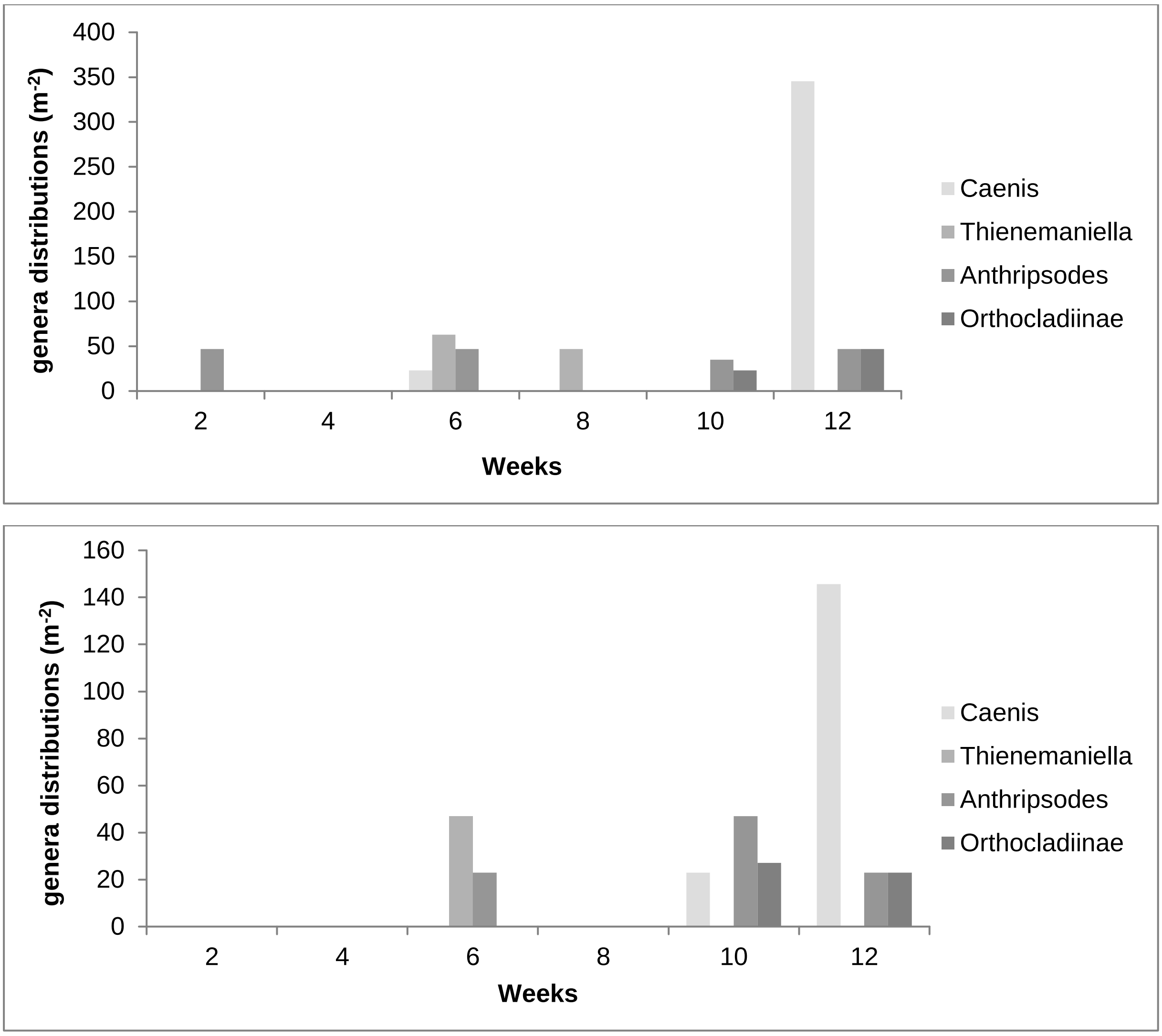


Figure 42: Mean genera abundances (above) of A. mearnsii (HFA) litter and (below) fynbos species litter type in the invaded reach at the Du Toit's River. Note the different scales of the y-axes

Table 22: Mean (individuals m^{-2}, ± SE, n = 4) genera distributions represented the most at the Du Toit’s River near-pristine site over incubation weeks/intervals and different litter types (FS = Fynbos species; AM = A. mearnsii). HFA = indicates home field advantage litter type; (*) indicates less than four sampling size; and (X) no data sampled/available

Du Toit’s River: near-pristine site

Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**
Genera distributions (m^2):												
Caenis	X	X	X	23.00*	X	X	X	X	X	X	23.00*	345.50 [131.50]
Orthocladiinae	X	X	X	X	X	X	X	X	X	70.00 [12.00]*	23.00*	83.00 [36.00]
Aprionyx	23.00*	23.00*	47.00*	X	X	X	X	X	23.00*	X	X	X
Paramerina	X	X	23.00*	23.00*	23.00*	47.00*	X	X	23.00*	X	X	11.50*

Du Toit’s River: near-pristine site												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**	**FS (HFA)**	**AM**
Thienemaniella	X	X	X	X	X	X	119.00*	X	X	X	X	X
Total genera distribution per litter type	23.00	23.00	70.00	46.00	23.00	47.00	119.00	X	46.00	70.00	46.00	440.00

Table 23: Mean (individuals m^{-2}, ± SD, n = 4) genera distributions represented the most at the Du Toit's River invaded site over incubation weeks/intervals and different litter types (FS = fynbos species; AM = A. mearnsii). HFA = indicates home field advantage litter type; (*) indicates less than four sampling size; and (X) no data sampled

Du Toit's River: invaded site

Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**
Genera distributions (m^2):												
Caenis	X	X	X	X	23.00*	X	X	X	X	23.00*	345.50 [107.05]	145.67 [61.47]
Thienemaniella	X	X	X	X	63.00 [31.02]	47.00*	47.00 [13.86]	X	X	X	X	X
Athripsodes	47.00*	X	X	X	47.00*	23.00*	X	X	35.00 [9.70]	47.00	47.00 [24.00]	23.00*
Orthocladiinae	x	X	X	X	X	X	X	X	23.00*	27.15 [13.86]	47.00 [24.00]	23.00*

Du Toit’s River: invaded site												
Weeks/Interval:	**2**		**4**		**6**		**8**		**10**		**12**	
Litter type:	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**	**AM (HFA)**	**FS**
Total genera distribution per litter type	47.00	X	X	X	133.00	70.00	47.00	X	58.00	74.14	439.5	191.67

6.4 Discussion

6.4.1 Decomposition rates between Fynbos species and *A. mearnsii*

The results found in the present study supported previous findings that invasive plants decompose at a faster rate than native species (Witkowski, 1991; Allison & Vitousek, 2004; Claeson et al., 2014). The results indicate that *A. mearnsii* litter decompose more rapidly than native species litter regardless of the local environment or the treatment. Allison and Vitousek (2004) found that invasive species decomposed faster than native species in the nutrient-poor ecosystems of the Hawaiian Islands. In addition in the CFR of the Western Cape, Witkowski (1991) found that the decomposition rate of *Acacia* species (*A. saligna*) was faster than the native sclerophyllous shrub (*L. parole*). Witkowski (1991), Allison and Vitousek (2004), and Hickman et al. (2013) suggest that the difference in decomposition rates were likely due to the litter quality characteristics between the species.

The outcome from the present study supports this conclusion as we found faster decomposition rates in *A. mearnsii* in both near-pristine and invaded reaches of both study streams in the Breede WMA. Jo et al. (2016) found that decomposition rates over time were related to high nitrogen concentrations in litter. In the present study, the litter from the invasive nitrogen-fixing *A. mearnsii* is considerably different in litter quality (nitrogen and C:N ratios concentrations) than the fynbos species. The differences in litter quality between native and invasive species have been shown in other studies (Santiago, 2007; Gießelmann et al., 2010; Prescott et al., 2000) as the main factor in faster decomposition rates for invasive species over time. In the present study, climate can be excluded as a factor as treatments per site and river catchments are reasonably close to one another, which is supported by other authors (Meentemeyer, 1978; Gholz et al., 2000). Therefore, the major contributing factor of decay rates of *A. mearnsii* in both near-pristine and invaded reaches at both rivers are likely related to intrinsic variables of leaf litter biochemistry (Perez-Harguindeguy et al., 2000; Ehrenfeld, 2003; Liao et al., 2008; Morris et al., 2011). However, this may not be the only reason for faster decomposition rates.

Generally, decomposition experiments show that within a stream hierarchy (up- and downstream), breakdown of leaf litter is different on a site level (Sponseller & Benfield, 2001; Leroy et al., 2006). This is mainly due to these sections having unique in-stream characteristics as water temperature, flow regime, pH, conductivity and microhabitats (Frissel et al., 1986; Leroy et al., 2006). In the study stream, characteristics between near-pristine and invaded reaches are fairly similar at the Wit River. Stream environmental factors of average temperature, discharge, pH and EC show little variation in change through the study period at the Wit River reaches. At the Du Toit's River, a similar pattern in stream characteristics between pristine and invaded reaches in pH and conductivity (EC) is observed. However, range of discharge rates ($m^3 \cdot s^{-1}$) between near-pristine (0.16 to 0.40) and invaded reaches (0.43 to 0.71) are different and could account for slower decay rates in near-pristine sites during incubation days. Claeson et al. (2014) found that in the Chehalis River Basin, Washington, USA, decay rates were affected by physical abrasion of leaf litter at intermediate stage of decay as stream flow ranged from winter low flows to irregular bankfull flow events. Therefore, physical abrasion due to the discharge regime between sites could account for the slower and faster decay rates.

Mediterranean climate streams are characterised by high inter-annual and intra-annual variability in discharge, which can play a role in decay rates (Gasith & Resh, 1999). In addition, Graça et al. (2001) stated that the discharge regime across different environmental gradients needs to be considered when predicting litter decay rates. Moreover, temperature seems to play a role in retarding decay rates in near-pristine sites. Between sites, mean water temperature differentiate with 1.58˚C with the near-pristine site being colder over incubation days. Braatne et al. (2007) studied Japanese knotweed along riparian corridors in the Clearwater River Basin, USA, and found that lower temperatures decreased decomposition rates in the intermediate stage for all leaf types measured. The reduced decay rates in the near-pristine sites are well supported by the study in the Clearwater River Basin. Another factor to consider in the experiment is litter bag size. In the study, fine mesh (0.5 mm) litter bags were used to exclude meso- and macroinvertebrate fauna (Webster & Benfield, 1986). Thus, the assumption drawn from the decomposition study is that there was minimal interaction between macroinvertebrates and leaf litter. Therefore, results found in the decomposition rates in the study are not influenced by macroinvertebrate communities, but rather bacterial/fungal communities (Graça et al., 2001).

Leaf litter from different plant species decomposes at different rates within a stream due to difference in leaf traits, which accounts for 70% of the variation explained in decomposition processes (Hickman et al., 2013; Kuglerova et al., 2017). The other 30% is explained by factors such as stream characteristics (Leroy et al., 2006) and HFA effects (Gholz et al., 2000). In the study at both rivers reaches (near-pristine and invaded), trait differences can be seen as the main reason for decay rates between *A. mearnsii* and the fynbos species. The study reaches in the study are relatively similar (except the near-pristine site in Du Toit's River) and treatments are largely close to each other, which show less heterogeneous in-stream characteristics. Hence, the results confirm that the litter decay rates of *A. mearnsii* in its native environment or home field are the same as in unexploited areas (e.g. near-pristine sites). As a consequence, ecosystem functions in undisturbed streams may be affected by *A. mearnsii* as the invasion of the species can increase the amount of leaf material entering the streams and the litter thereof decomposing at significant rate when a certain threshold is met.

6.4.2 Macroinvertebrate herbivore litter affinity effects in home and away environments

There was no general preference of FFGs or of genera diversity and abundance for either fynbos species or *A. mearnsii* litter regardless of the local environment or the treatment in the study. In the earlier weeks at the Wit River in the near-pristine reach, there was no increase in FFGs in both litter types. However, a general increase in FFGs was apparent from week 8 to week 12. The taxa richness and diversity of macroinvertebrates coincided with changes in the quantities of litter inputs and the seasonal timing of inputs particularly in summer the month of December. The sclerophyllous and evergreen vegetation in the Mediterranean streams of the CFR are typically characterised by a more prolonged period of litterfall that extends from summer (sclerophyllous trees) to autumn (Maamri et al., 1994).

At the Wit River Bainskloof Pass in Chapter 3, a substantial amount of litter input is dropped on the adjacent riparian zone from both *B. stellatifolium* and *M. angustifolia*. The input of litter is distinguished as the leaf abscission stage for these native species. It is therefore likely that the macroinvertebrate communities are seasonally dependent on the pulse of leaves (senesced) entering the stream in summer. Similar results are found in riparian corridors in the USA by Claeson et al. (2014) and Reinhart and VandeVoort (2006) where sites received a diverse input of litter types, which resulted in an upturn in invertebrate communities. In genera distributions, *S. aequalis* accounted for most of the abundances across weeks that showed a preference either for fynbos species or *A. mearnsii* litter type. This suggests that the quality of different litter (invasive vs. native) regulates the abundance of these organisms across weeks.

Similar findings were made by Januasauskaite and Straige (2011) in a laboratory experiment that resembled natural conditions. They found that litter inputs regulated the abundances of the organism in their study. The finding also suggests that the introduction of the *A. mearnsii* litter type may effectively be used by *S. aequalis* as a potential energy source. In addition, the substrate characteristic of the near-pristine site, which is constituted of large substratum material, facilitate foraging of macroinvertebrate communities. The rough surface topography creates microhabitats, which are accompanied with season leaf litter inputs that accelerate macroinvertebrate community diversity in a stream. This is observed in other studies such as Rempel et al. (2000) and Holomuzki and Messier (1993).

In the invaded reach at the Wit River, a similar make-up was apparent in earlier weeks with FFGs generally increasing from week 8. However, a much greater number of FFGs was recorded, which coincides with the timing of leaf litter input and the resources that *A. mearnsii*-infested sites may hold. Deposit feeders, scrapers and predators are substantially high in these sites, which gives an indication that they may feed on nutrient-rich litter that the *A. mearnsii*-infested sites may hold. The substantial amount of litter input in summer and December are apparent in Chapter 3, which enters the streams readily as the adjacent invaded riparian zones are in close proximity.

Reinhart and VandeVoort (2006) indicated that a significant macroinvertebrate community response and change are inevitable when 50% of the knotweed species invade the riparian areas in their study. Therefore, the hypothesis can be made that with an increased invasion of riparian zones by *A. mearnsii*, an increase in macroinvertebrate communities is witnessed in the present study. However, the invaded site in the study also holds many native species, particularly *B. stellatifolium* and *M. angustifolia*, which are known to be important keystone species in the mountainous riparian regions of the Fynbos Biome in the CFR (Crous, 2010). Consequently, in-stream ecosystem functions may not be significantly affected in terms of HFA affinity effects for *A. mearnsii* litter type as these species are naturally confined in the infested vicinities.

In the near-pristine reach at the Du Toit’s River, no general preference was found between litter types in FFGs and genera distributions. Deposit feeders were found to be the greatest in *A. mearnsii* litter type week 12, preferably *Caenis* with fynbos species litter also favoured by deposit feeders. *Aprionyx*, which are also deposit feeders, were greatest on fynbos species litter type in week 4. However, FFGs and genera were most likely regulated by different litter quality types in week 12 and not by adjacent riparian litter even though the inputs from native riparian species sampled in Chapter 3 were substantial.

The inconsistent results found in macroinvertebrate communities found are related to stream characteristics and human interference. The site is situated in the mountain stream section of the Du Toit’s River 2 km downstream from the Mont Rochelle Nature Reserve, which has a vertiginous gradient and narrow channels – a physical feature that is obvious in the upper stream sections of Franschhoek Pass. Stream characteristics of cool water temperatures and steep river slope can lead to overall low macro- and meso-invertebrate diversity and abundances as explained by Wall et al. (2008) and Tharme (2010). Due to human interference, many bags were lost during the sampling weeks. Bags were placed outside the stream or displaced from the original sampling point, thus common trends could not be observed.

In the invaded site, no preferences were found between litter types in FFG abundances, but a preference and an increase were found in genera distributions across weeks. FFGs show no increase between weeks but only a slight peak in FFG in week 12 for the fynbos species litter type. A substantial quantity of litter inputs from *A. mearnsii* invasion on the riparian zone proceeded to fall in the adjacent stream directly or laterally as banks on the site were relatively high and steep. However, the distribution of FFGs per se were not as significant as other sites, which could be due to substratum characteristics. The substrate is dominated with small cobbles and gravel with sand being the dominant characteristic of most of the river segment. The invaded site at the Du Toit’s River appeared to deliver approximately 52% of its suspended sediment load across the year (see Chapter 4 in this report). The study also suggested low sedimentation infiltration rates were associated with low flows in summer, resulting in sedimentation fluxes in-stream to be settled at substratum level. In addition, this is well supported by the study of Rempel et al. (2000) in the Frazer River in British Columbia that stated that the diversity of taxa and benthic habitats in a stream section would be less suitable to sedimentary conditions. The high sedimentary conditions are allied with high silt levels as stated by other authors (Dallas & Day 1993; Samways et al., 2011) and also associated with low reduction in benthic invertebrates but not all species, as some favour these sites. As such, high numbers of *Caenis* (family – *Canidae*) are present in week 12 irrespective of the litter type, which could imply that the species could favour alien-infested sites. Samways et al. (2011) found a good account of this in the upper catchments of the CFR streams, Western Cape, where *Caenis* were most present in alien-disturbed environment, particularly *A. mearnsii.* The abundant numbers in *Caenis* could be due to the attractive food sources and nutrient-rich leaf litter these invaded sites may hold.

Macroinvertebrate communities in Mediterranean streams in the CFR are highly diverse and may exceed the diversity of plant communities in the adjacent riparian zone (Taylor et al., 2014). The understanding is that the functional mechanisms and litter affinity effects of these macroinvertebrates are in their primitive stages. There is proof of HFA effects in study, where macroinvertebrate communities feed on their home turf litter, but there are also effects that show that macroinvertebrates take advantage of the available resources introduced over space and time, which shifts their feeding preferences. Moreover, the hydrological regimes in the Mediterranean streams of the CFR are unpredictable and characterised by discharge that is season-dependent, which leads to periods of winter floods and droughts in other seasons.

The unpredictable seasonal discharge miseries establish a selective pressure on communities of macroinvertebrates to favour certain seasons as these changes in the hydrologic regime may be favoured by short life-cycled and small body-sized macroinvertebrate communities (as in the present study). In addition, leaf packs from week 6 to week 12 of the species used in the study experienced their leaf litterfall peaks in summer, particularly December, which made the choice of season more relevant under an ecological perspective. However, leaves mostly accumulated in pool microhabitats where macroinvertebrates are mostly present in certain seasons (summer in the present study) (Frissel et al., 1986), which could have led to an overestimation of macroinvertebrate communities on leaf bags. The ecological data obtained from one river cannot necessarily be extrapolated to another, with each river having to be assessed individually to seek common trends.

6.5 Conclusion

This study demonstrates that the invasion of *A. mearnsii* in undisturbed environments may have an effect on aquatic systems. The effect of *A. mearnsii* leaf litter due to key chemical traits may extend beyond the local area of invasion and to other undisturbed environments (Reinhart & VandeVoort, 2006). In the present study, the decay rates of the IAP appear to be same in pristine and infested environments. This demonstrates the potential that *A. mearnsii* has to change macroinvertebrate communities, at least in the short term. This context is well described by Callaway and Ridenour (2004) who noted that the chemical complexes produced by IAP affect macroinvertebrates in the area they invade as these organisms are not well adapted to these exotic compounds. Furthermore, the adaptation of macroinvertebrate communities to *A. mearnsii* litter could potentially modify aquatic ecosystems and lead to substantial change to macroinvertebrate communities through season/s if they invade. The occurrence is well observed in the increase in FFGs and taxon numbers in the present study, which limits the HFA of the organisms to their homegrown litter; however, diversity of macro-invertebrate appears to remain the same (Allison, 2012). The invasive plant material transports nutrient-rich leaf litter (an estimate of 48 600 to 70 200 seeds per kilogram) (Moyo & Fatunbi, 2010), which is therefore a key factor in changing trophic dynamics and energy chains in-stream and undisturbed areas downstream (Le Maitre, 2002; Chamier et al., 2012; Naude, 2012). Future research should focus on assessing the impacts of *A. mearnsii* leaf litter inputs and seed dispersal on streams, which must be considered during the formulation of riparian restoration strategies in the mountain streams of the CFR.

CHAPTER 7 – EFFECT OF RIPARIAN ZONE INVASION BY ALIEN TREES ON AQUATIC ALGAE AND INVERTEBRATE DIVERSITY ON MEDITERRANEAN MOUNTAIN STREAM ECOSYSTEMS

7.1 Introduction

Invasion by alien species is a major threat that follows biodiversity after habitat loss due to destruction (D'Antonio & Meyerson, 2002; Richardson & Van Wilgen, 2004). Alien invasive woody plants, specifically from the genera *Acacia*, *Pinus* and *Eucalyptus*, take up more water than indigenous fynbos species in the CFR, which places an increasing strain on this natural fynbos ecosystem, as well as aquatic ecosystems due to reductions in stream flow (Le Maitre et al., 2002; Ehrenfeld, 2003; Van Wilgen et al., 2012) and shading-in of stream habitat (Samways et al., 2011).

The CFR in South Africa is one of only six floral kingdoms of the world, which are exceptionally high in plant species richness (Mucina & Rutherford, 2006). The CFR is a centre of endemism for many plant taxa (Van Wyk & Smith, 2001; Born et al., 2007). The CFR also has the highest degree of *A. mearnsii* invasion in South Africa. Two attributes in particular have contributed to its success: *A. mearnsii* is specialised in invading nutrient-poor environments, as are characteristic to the CFR (Stock et al., 1995); and in disturbance events, the species establishes itself faster than indigenous vegetation (Morris et al., 2011). In addition to suppressing indigenous fynbos growth, IAPs alter nitrogen and carbon cycles (Ehrenfeld, 2003). Indeed, coupled with its negative impact on water resources, its ability to fix nitrogen has earned *Acacia* the rank as the most damaging invasive species in the Fynbos Biome (Brown et al., 2004).

In response to the clear need for intervention, the WfW programme was established in 1996 by the Department of Water and Sanitation, formerly known as the Department of Water Affairs and Forestry (Van Wilgen et al., 1998). This programme includes both ecological and socio-economic objectives. Ecological objectives include clearing IAPs to increase the amount of run-off from catchments while ensuring the conservation of biodiversity. Socio-economic objectives include uplifting previously disadvantaged communities, doing training and building capacity (Enright, 2000). The WfW programme is the only government-funded stream restoration programme in South Africa. This programme has focused mainly on removing IAPs to restore crucial ecosystem services such as producing clean water. It is unclear what impacts the changes in water quality have on the biotic components of the aquatic ecosystems of concern with the invasion and clearing (Chamier et al., 2012). Indeed, little attention has been paid in South Africa to quantify the amount of nitrogen that nitrogen-fixing IAPs may be adding to streams.

Riparian zones play a major role in ecosystem processes and food webs. Allochthonous input from riparian plants into the food web of the aquatic ecosystem includes leaf litter, wood and nutrients (Goldman, 1961; Wallace & Eggert, 1997; Baxter et al., 2005). Research on leaf litter input into streams as well as the breakdown and changes in nutrient levels in streams has been done mainly in the USA. Increased nitrogen inputs have been reported from streams that drain stands of native nitrogen-fixing Russian olive (*E. angustifolia*) (Mineau et al., 2011), and exotic nitrogen-fixing *Albizia* trees (*F. moluccana*). *E. angustifolia* was shown to alter dissolved organic nitrogen levels in-stream. Leaf litter breakdown of *F. moluccana* was higher than indigenous *Metrosideros polymorpha* trees. *F. moluccana* had higher C:N ratios and, due to the higher decomposition rates, nitrogen levels were higher (MacKenzie et al., 2013).

Nutrient levels in-stream have been investigated in terms of both successes of the application of different restoration methods as well as a comparison of natural as well as invasive species present in the USA (for example, Filoso & Palmer, 2011; Mineau et al., 2011). By contrast, in South Africa, water quality after clearing remains largely undetermined. The impacts of clearing on soil nutrient cycling within the riparian zone has recently been investigated (Naude, 2012; Jacobs et al., 2013). Soil nutrient cycling is altered by the introduction of *Acacia* spp. In areas where invasion has occurred, *A. mearnsii* increases nitrate concentration. Even at cleared sites, riparian soils (dry banks) may have high nitrogen content for up to seven years as denitrification rates remain low (Jacobs et al., 2013).

Research by Jovanovic et al. (2009) compared the nitrogen levels in groundwater underlying fynbos (natural), cleared and invaded riparian areas. The species composition of the invaded area contained mainly *A. saligna* and other invasive *Acacia* species. Groundwater resources underlying the invaded riparian area had higher levels of nitrogen than cleared and fynbos sites (Jovanovic et al., 2009). After clearing, nitrate was released into the groundwater due to high residual nitrogen reserves in the rooting zones of nitrogen-fixing *Acacia* species, increased recharge rates as well as a decrease in evapotranspiration (Jovanovic et al., 2009). However, the link between increased nitrate and a possible nitrogen flux in surrounding aquatic ecosystems has not as yet been determined.

IAPs contribute to increased biomass and litter input into streams. The rate of litter breakdown by periphyton and macroinvertebrates differs between invasive species and natural vegetation (Chamier et al., 2012). If the differences in litter input from invasive treatments (*A. mearnsii* and/or *A. longifolia*) differ dramatically from indigenous fynbos vegetation, this has the potential to alter the nutrient cycle of the aquatic ecosystem. Eutrophication may occur at these sites due to the increase in metabolisation of nutrients. In turn, eutrophication may lead to changes in species functional diversity and abundance (Chamier et al., 2012).

In this study, aquatic algae, macroinvertebrates and their FFGs will be used to investigate the effects of riparian zone invasion on two fynbos mountain streams located in the CFR. Algae in open-canopied rivers are reportedly more reliable indicators of trophic status in rivers than is the measurement of chemical parameters such as nitrogen and phosphorus (Ewart-Smith & King, 2012). Since algae are important primary producers in rivers, they are important sources of energy for the rest of the aquatic ecosystem. Macroinvertebrates feed on algae and detritus including leaf litter, aquatic plants or other invertebrates. Some macroinvertebrates are directly reliant on algae as their main food source (MacKenzie et al., 2013). Due to higher nitrogen contents, some invasive species' decomposition rates are higher than indigenous species (MacKenzie et al., 2013; Wiegner et al., 2013). The ease of decomposition of leaf litter as a food source for macroinvertebrates is dependent on the nitrogen content, tannin content and leaf toughness. Macroinvertebrates also feed on algal biomass formed on the leaf litter surface. Decomposition of leaf litter and subsequent release of nitrogen therefore depend on both periphyton and macroinvertebrate communities (MacKenzie et al., 2013). Increased nitrogen levels can in turn affect algal community structure and macroinvertebrate assemblages.

The objectives of this study are to investigate the effects of invasion status: firstly, on algal density (both in terms of biomass measured as density and chlorophyll a concentration) and diversity; secondly, on macroinvertebrate density and FFG; thirdly, on the importance of environmental parameters (flow, water temperature, nitrogen concentration, sediment load) on structuring algal and macroinvertebrate assemblages.

7.2 Methods

Site selection

The CFR of South Africa has a Mediterranean-type climate with low summer rainfall and high winter rainfall. With its exceptionally high biodiversity coupled with high anthropogenic pressure on the biodiversity, the CFR is considered a global biodiversity hotspot. For this study, we selected two perennial fynbos mountain streams in the CFR. These rivers were the Wit River (Bainskloof Pass, Wellington, Western Cape) and the Du Toit's River (Franschhoek Pass, Franschhoek, Western Cape). At each river, experimental sections were chosen according to invasion status. The statuses were near-pristine fynbos (here also referred to as natural), cleared and invaded. See Figure 1 for the relative locations of the sites, and Table 3 for the site characteristics.

7.2.1 Sample collection and identification

At each sampling site, rocks were collected for macroinvertebrate and algal sampling. At each site, a total of ten medium-sized rocks (mean area 189 cm^2) were selected randomly to preserve the samples. Rock depths ranged from 10 cm to 60 cm in riffles and pools. The rocks were sampled starting down-stream of the flow direction to avoid disturbing the sampling sites. Each rock was dislodged with as little disturbance as possible, and immediately placed in a white plastic meat tray. Excess water was carefully drained without losing macroinvertebrates. The macroinvertebrates were removed from the rock, and placed in a 50 ml plastic vial with 70% ethanol. The same rock was then scrubbed with a toothbrush for a minimum of five minutes. Up to 45 ml of stream water was used to rinse the rock's surface at intervals. Once scrubbing was complete, the algal solution was added to a 50 ml vial with 5 ml 95% ethanol. The rock's dimensions (length × width × height) were then measured and recorded on prelabelled water-proof self-sticking labels, which were placed on the tubes.

An additional five rocks were collected for chlorophyll a analysis. These were selected from the same biotopes as previous samples. The same general procedure was followed, except that stream water was used to rinse the rock's surface (to a total of 50 ml). Once scrubbing was complete, the algal solution was added to a 50 ml vial. The vial was then placed on ice and kept dark and cold in an ice cooler. All samples were submitted to the CSIR for chlorophyll a analyses on the same day of sample collection.

The 50 ml sample for algal identification was shaken vigorously and transferred to a beaker. A standard kitchen blender was used to blend the sample for 10 seconds to ensure that all clumps and colonies were broken up, and that a homogenised and representative sample was achieved. The 5 ml sample was removed and centrifuged for 10 minutes at 3000 rpm. The supernatant of 4.5 ml was removed, and the pellet of 0.5 ml retained for algal identification and enumeration.

An aliquot of the sample was placed on a haemocytometer using a Pasteur pipette. The sample was first observed under low magnification of 160× to note algal density and distribution. The 400× objective was then used for identification and enumeration. Starting at the top of the first square of the grid, all algae cells were counted. Normally, if a count of 400 cells is reached, the squares counted are noted, if not, the entire grid is observed to ensure precision of cell densities. In the samples of this study, abundances were low and so all grids were counted (total area of 9 mm^2).

Cell density of the samples was calculated as described in Biggs and Kilroy (2000) and subsequently corrected to rock area (mm^2) using the following expression:

$= xy + xz + yz\ (r^2 = 0.93) \times 100$ [modified from Ewart-Smith and King (2012)]

Since algal cell counts are not representative of actual biomass present in the sample, the data were size-adjusted. Databases such as AlgaeBase (www.algaebase.org) and literature (Taylor et al., 2007) were consulted to obtain length and breadth measurements. It was assumed that a cylindrical shape would approximate most growth forms reasonably well and thus volume was calculated as $\pi \times r^2 \times h$. In many cases, it was not possible to get clear information on either or both dimensions from the literature. If a sample came from a well-represented group such as diatoms, the median was chosen as the size distribution was highly skewed, whereas groups represented by fewer taxa were assigned average sizes; where only one representative was found, the size of that taxon was used.

Invertebrates were identified to genus using Day and De Moor (2002); Day et al. (2003); De Moor et al., (2003a; 2003b); and Stals and De Moor, 2007), and placed into FFGs using Ewart-Smith and King (2012). Macroinvertebrate density was expressed as frequency of individuals/mm^2.

7.2.2 Environmental variables

Three replicate surface water samples were taken at each of the sampling sites in May, July and November 2016, and analysed for ammonia, nitrate + nitrite, nitrite and orthophosphate. During most periods and at most sites, nutrients were low or below detectable limits, which supports the general finding that Mediterranean streams in the CFR are nutrient-limited (Lambers et al., 2008b; 2010; Potgieter, 2012).

Capacitive pressure loggers (HOBO – U20L-02, Onset Computer Corporation Inc., Pocasset, MA, USA) were placed at both rivers and set to measure the water level and temperature at 30-minute intervals at each of the six sites. Discharge was calculated as described in Chapter 3. In addition to the above variables, stream water pH and EC were recorded *in situ* using a handheld multiprobe. Sediment and nutrient (percentage nitrogen and percentage carbon) load data were also used here. For a description of how data were collected, see Chapter 4.

7.2.3 Statistical analyses

The software package PRIMER v6+ PERMANOVA was used for all statistical analyses (Clarke & Warwick, 2001; Clarke & Gorley, 2006; Anderson et al., 2008). Algal and macroinvertebrate densities were square-root transformed prior to analysis of the data based on Bray–Curtis distance. Environmental variables and stream characteristics were checked for correlation using a Draftsman's plot. Variables that were highly correlated ($-0.75 < r > 0.75$) were removed from further analysis. Prior to normalisation on all variables, transformations were applied to individual variables to reduce skew. Pairwise comparisons at each river of algal and macroinvertebrate densities and functional groups were made between the term 'RixTr' for pairs of levels of factor 'Treatment' using the PERMANOVA function, set to do 999 Monte Carlo permutations. Distance-based linear model (DistLM) and distance-based redundancy analysis (dbRDA) routines were used on algal and macroinvertebrate resemblance data. DistLM is a distance-based regression approach and dbRDA is an ordination that visualises fitted models from DistLM (Anderson et al., 2008). DistLM routines were set to choose the BEST variables, and selected based on the adjusted R^2 value. BEST is a procedure that examines the value of the selection criterion for all possible combinations of predictor variables.

7.3 Results

7.3.1 Algal densities

Algae were resented by 21 families comprising 36 genera and morphogenera and 22 471 cells. The Wit River was more diverse (19 families, 26 morphogenera) than the Du Toit's River (16 families, 22 morphogenera). However, samples from the Wit River had lower densities (0.11 density/mm^2) than those from the Du Toit's River (0.13 density/mm^2). Permutational multivariate analysis of variance (MANOVA) revealed significant differences between rivers, invasion statuses and months and their combined factors (Table 24).

Considering the invasion statuses in each month at the Du Toit's River, algal densities differed only between the cleared and near-natural (CLE:NAT) sites in November and December. The pattern at Bainskloof was similar with significantly higher densities at the cleared site than the natural site in November, and the cleared and alien site in January.

Table 24: Permutational MANOVA on algal densities based on 999 permutations of residuals under a reduced model. Factors were river (ri), treatment (tr) and month (mo), with factor combinations (x) of these factors. Abbreviations: df = degrees of freedom; SS = sum of squares; MS = means of squares; Pseudo-F = F value; p = p-value based on 999 Monte Carlo permutations

Source	df	SS	MS	Pseudo-F	p
Ri	1	13 941	13 941	15.08	0.00
Tr	2	6 657	3 328	3.60	0.00
Mo	4	28 554	7 138	7.72	0.00
rixtr	2	3 914	1957	2.12	0.02

rixmo	4	15 856	3 964	4.29	0.00
trxmo	8	12 852	1 607	1.74	0.01
rixtrxmo	8	11 620	1 453	1.57	0.02

Table 25: Pairwise comparisons of algal densities between the term 'RixTr' for pairs of levels of factor 'Treatment' using permutational MANOVA with Monte Carlo permutation of 126 repeats at the Du Toit's River (Franschhoek Pass) and Wit River (Bainskloof Pass). Significant p values are in bold font. Abbreviations: t = t-value; p = p-value; ALI = alien site; CLE = cleared site; NAT = near-natural/reference site

River	Pairs	October		November		December		January		February	
Du Toit's River	CLE:NAT	1.03	0.39	**2.45**	**0.03**	1.85	0.06	1.30	0.21	0.77	0.60
	CLE:ALI	0.99	0.42	1.53	0.14	0.97	0.44	1.22	0.26	0.84	0.51
	NAT:ALI	0.80	0.52	1.46	0.15	1.62	0.08	1.81	0.05	1.31	0.19
Wit River	CLE:NAT	0.41	0.87	**2.17**	**0.03**	1.42	0.18	0.80	0.54	0.65	0.61
	CLE:ALI	1.59	0.14	1.07	0.38	0.76	0.57	1.57	0.13	1.86	0.06
	NAT:ALI	1.15	0.29	1.75	0.08	1.27	0.25	**2.30**	**0.04**	1.12	0.34

7.3.2 Chlorophyll a

Chlorophyll a concentrations differed between months and treatments (Table 26). At the Du Toit’s River, the pair CLE:NAT differed in both November and December, and the pair CLE:ALI differed significantly in November, with mean chlorophyll a concentrations being highest at the cleared sites in both months and intermediate at the alien site. Only in December at the Wit River, chlorophyll a concentrations were significantly higher at the alien site than those at the natural site.

Table 26: Pairwise comparisons of chlorophyll concentrations between the term 'RixTr' for pairs of levels of factor 'Treatment' using permutational MANOVA with Monte Carlo permutation of 126 repeats at the Du Toit's River (Franschhoek Pass) and Wit River (Bainskloof Pass). Significant p values are in bold font. Abbreviations: t = t-value; p = p-value; ALI = alien site; CLE = cleared site; NAT = near-natural/reference site

River	Pairs	October		November		December		January		February	
Du Toit's River	CLE:NAT	0.43	0.78	**2.88**	**0.01**	**2.56**	**0.03**	0.79	0.53	1.13	0.31
	CLE:ALI	0.87	0.44	**3.03**	**0.01**	0.65	0.60	0.92	0.43	0.34	0.83
	NAT:ALI	1.28	0.24	0.33	0.82	1.34	0.20	1.44	0.15	0.98	0.36
Wit River	CLE:NAT	0.99	0.39	n/a	n/a	2.09	0.06	1.63	0.13	1.27	0.24
	CLE:ALI	1.37	0.18	1.10	0.30	0.81	0.44	0.43	0.67	1.30	0.22
	NAT:ALI	0.32	0.88	n/a	n/a	**2.81**	**0.02**	1.69	0.14	0.07	0.97

7.3.3 Macroinvertebrate densities

From the Du Toit’s River, 18 families were recorded, representing 33 genera. The most dominant families (and feeding groups) were *Chironomidae* (scrapers), *Baetidae* (deposit feeders), and *Leptophlebiidae* (deposit feeders). At the Wit River, 19 families were recorded, representing 34 genera. The most dominant families (and feeding groups) were *Chironomidae* (scrapers), *Simuliidae* (filter feeders) and *Leptophlebiidae* (deposit feeders).

At family and genus level, macroinvertebrate densities did not differ between months or treatments at the Du Toit’s River (Table 27). FFGs also had similar distributions among treatments and between months, the only exception being the NAT:ALI pair in January (Table 27). At the family level, the Wit River repeated this pattern except for the month of November where all invasion status comparison pairs differed significantly. At the Wit River, genus level densities also differed between invasion statuses (CLE:ALI and NAT:ALI) in October. In November, dominant FFGs and families at the near-natural site were the deposit feeders (*Baetidae*), whereas at the cleared site deposit feeders (*Leptophlebiidae*) and grazers dominated (*Petrothrincidae*). The alien site was dominated by scrapers (*Chironomidae*) (Figure 43 and Figure 44).

Table 27: Pairwise comparisons of macroinvertebrate densities at the family and genus level and of functional groups between the term 'RixTr' for pairs of levels of factor 'Treatment' using permutational MANOVA with Monte Carlo permutation of 126 repeats at the Du Toit's River (Franschhoek Pass) and Wit River (Bainskloof Pass). Significant p values are in bold font. Abbreviations: t = t-value; p = p-value; ALI = alien site; CLE = cleared site; NAT = near-natural/reference site

Comparison at family level

River	**Pairs**	**October**		**November**		**December**		**January**		**February**	
		t	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**
Du Toit's River	CLE:NAT	1.42	0.12	1.50	0.09	1.63	0.10	1.32	0.20	1.08	0.33
	CLE:ALI	1.04	0.38	0.91	0.49	1.08	0.37	0.90	0.48	0.84	0.58
	NAT:ALI	1.16	0.28	1.42	0.11	1.70	0.07	1.50	0.11	1.84	0.09
Wit river	CLE:NAT	1.16	0.27	1.75	**0.03**	1.35	0.18	0.66	0.72	0.81	0.57
	CLE:ALI	1.32	0.14	3.83	**0.00**	1.21	0.25	1.22	0.24	0.76	0.66
	NAT:ALI	1.15	0.27	1.83	**0.03**	0.91	0.49	1.06	0.35	1.04	0.39

Comparison at genus level

River	**Pairs**	**October**		**November**		**December**		**January**		**February**	
		t	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**
Du Toit's River	CLE:NAT	1.69	0.05	1.20	0.23	1.12	0.30	1.28	0.16	1.09	0.35
	CLE:ALI	1.17	0.22	0.73	0.65	0.66	0.73	0.93	0.49	0.84	0.57
	NAT:ALI	1.43	0.08	1.20	0.23	1.25	0.19	1.36	0.13	1.37	0.22
Wit River	CLE:NAT	1.24	0.23	1.75	0.04	1.31	0.18	0.75	0.64	0.98	0.45
	CLE:ALI	1.66	0.04	3.51	0.00	1.12	0.30	1.18	0.24	0.73	0.73
	NAT:ALI	1.94	0.02	1.72	0.02	0.76	0.69	1.03	0.36	0.93	0.49

Comparison of FFGs

River	**Pairs**	**October**		**November**		**December**		**January**		**February**	
		t	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**
Du Toit's River	CLE:NAT	0.66	0.73	0.47	0.83	0.74	0.59	2.00	0.05	0.61	0.63
	CLE:ALI	0.69	0.68	0.70	0.64	0.80	0.51	1.80	0.07	0.78	0.55
	NAT:ALI	0.56	0.87	n/a	n/a	1.28	0.24	3.97	0.00	1.40	0.23
Wit River	CLE:NAT	1.12	0.29	1.20	0.26	0.47	0.83	0.79	0.62	1.15	0.29
	CLE:ALI	1.05	0.35	4.00	0.00	0.66	0.67	1.85	0.07	0.69	0.67
	NAT:ALI	1.20	0.24	1.81	0.03	0.60	0.76	1.44	0.13	1.31	0.18

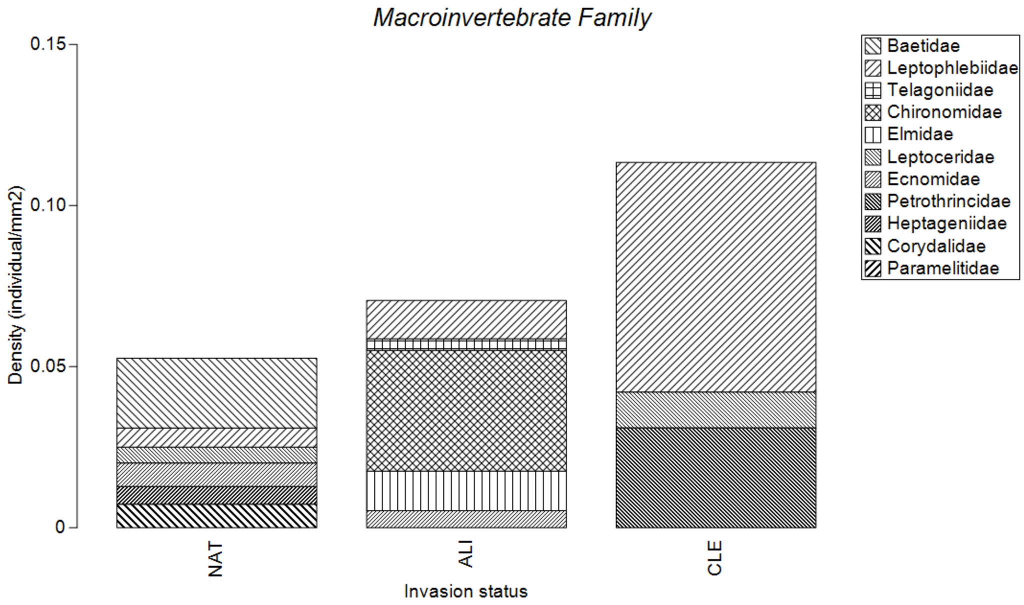


Figure 43: Box plot of macroinvertebrate family density (individual/mm^2) at near-natural (NAT), cleared (CLE) and alien (ALI) sampling sites at the Wit River during November

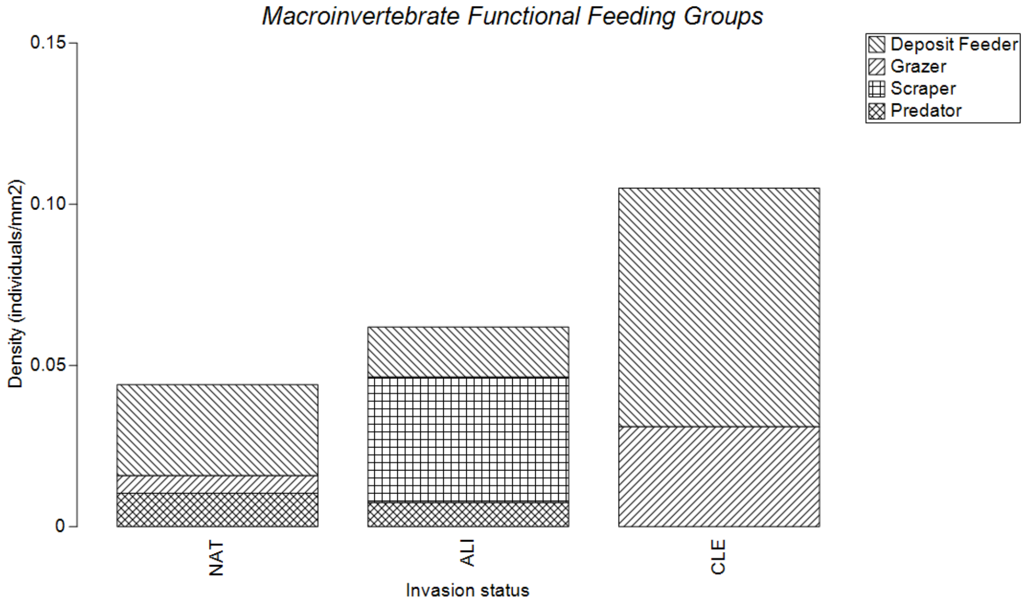


Figure 44: Box plot of macroinvertebrate FFG density (individual/mm^2) at near-natural (NAT), cleared (CLE) and alien (ALI) sampling sites at the Wit River during November

7.3.4 Environmental variables

The first axis of the dbRDA for the Du Toit's River described 63.5% of the fitted variation and 12.3% of the total variation (Figure 45). The second axis described 19.8% of the fitted and only 3.8% of the total variation. Water temperature had a strong influence on community structure as evidenced by the long parallel line to the first axis. Marginal tests (Pseudo-F = 4.067; p = 0.005) and BEST variable (adjusted R^2 = 0.081) confirmed the relative importance of temperature. Flow appeared to have an influence but marginal test results were not significant. Addition of the variable would result in a best solution of only 8.9%. The overall weak model and low explanatory power of the variables suggested that other physical or non-local variables were more important in structuring in-stream communities at the Du Toit's River.

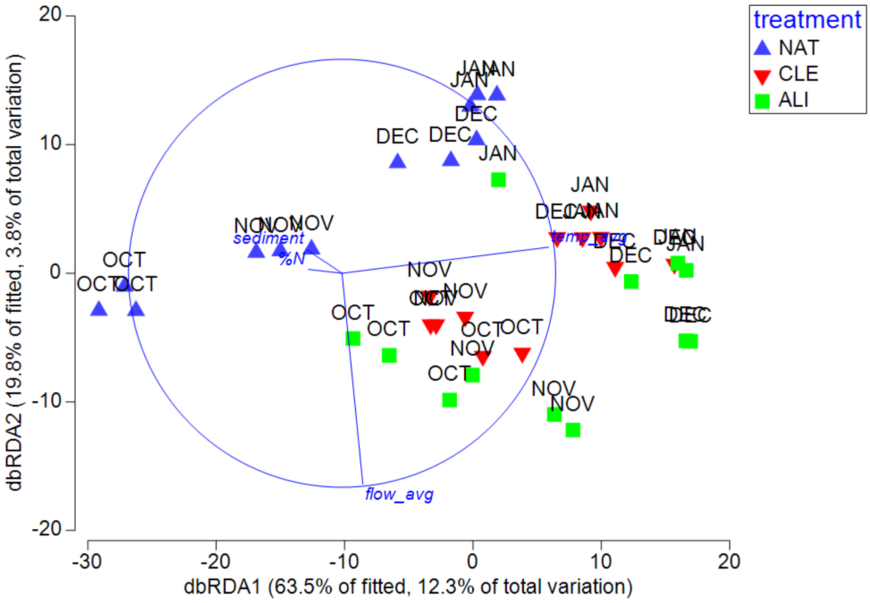


Figure 45: dbRDA of DistLM analysis at the Du Toit's River on the effect of environmental variables on structuring algal communities. Based on a square-root transformed Bray–Curtis similarity matrix of algal density. Fitted variation is the variance within the linear model; total variation is the variance within the original data

The first axis of the dbRDA for the Wit River described 80.9% of the fitted variation and 27.5% of the total variation (Figure 46). The second axis described 9.3% of the fitted and only 3.2% of the total variation. At the Wit River, water temperature also had a strong influence on community structure as evidenced by the long parallel line to the first axis. Marginal tests (Pseudo-F = 10.113; p = 0.001) and BEST analysis (adjusted R^2 = 0.211) confirmed the relative importance of temperature. The addition of flow would only marginally improve the best model (adjusted R^2 = 0.232). The percentage nitrogen content of sediment appeared to be important to the overall model, where inclusion of the variable brought the best solution to the adjusted R^2 = 0.251. The marginal tests, however, were not significant. In this DistLM also, the overall weak explanatory power of the model and the variables suggested that there were other important drivers of algal community structure.

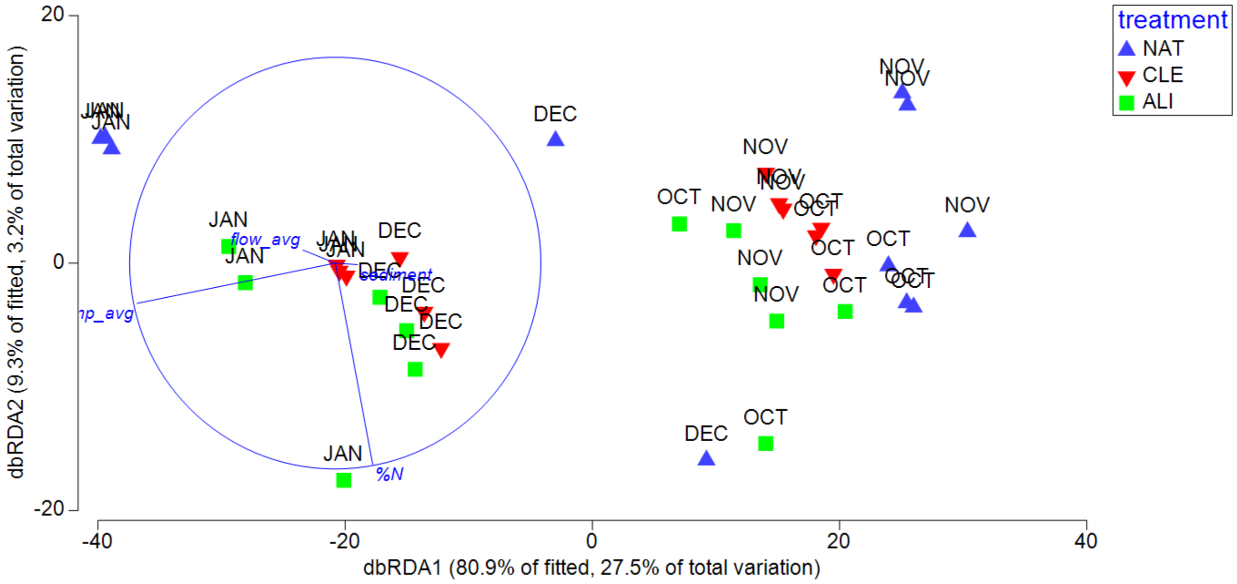


Figure 46: dbRDA of DistLM analysis on Wit River on the effect of environmental variables on structuring algal communities. Based on a square-root transformed Bray–Curtis similarity matrix of algal density. Fitted variation is the variance within the linear model; total variation is the variance within the original data

The first axis of the family level dbRDA for the Du Toit's River described 71.5% of the fitted variation and 10.3% of the total variation (Figure 47). The dbRDA plot suggested strong influence from flow and temperature, but marginal tests were significant only for flow (Pseudo-F = 3.051; p = 0.015). The best solution using a single variable was flow (adjusted $R^2 = 0.062$) and the best overall solution selected both flow and temperature (adjusted $R^2 = 0.078$). The partitioning between invasion statuses are very clear in the plot.

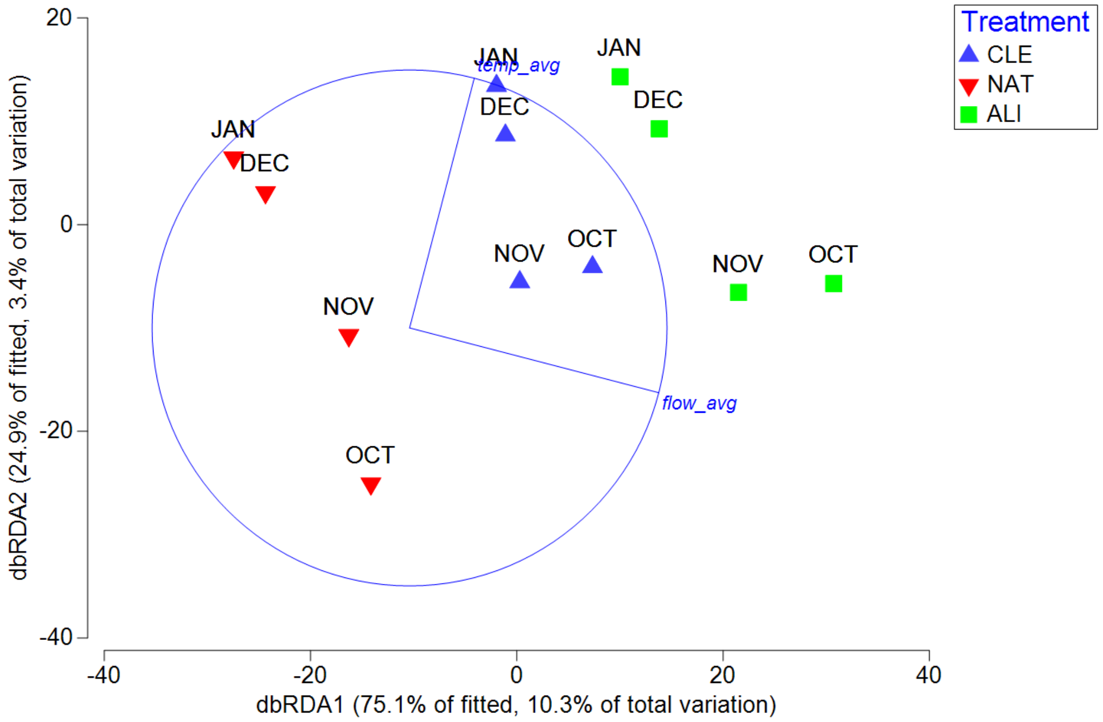


Figure 47: dbRDA of DistLM analysis on the Du Toit's River on the effect of environmental variables on structuring family level macroinvertebrate communities. Based on a square-root transformed Bray–Curtis similarity matrix of macroinvertebrate density. Fitted variation is the variance within the linear model; total variation is the variance within the original data

At the family level, partitioning of the sites along a gradient of invasion status was less clear at the Wit River than the Du Toit's River (Figure 48). Alien sites were more spread and similar to cleared sites, while there was a general difference between cleared and alien sites. The first axis of the dbRDA plot explained 45.3% of the variation but only 5.9% of the total variation. The second axis explained 38.4% of the fitted variation and 5% of the total variation. As at the Du Toit's River, marginal tests were also not significant here. However, in contrast to the Du Toit's, sediment was the most important variable (adjusted $R^2 = 0.054$) and the best model was solution was based on sediment and flow (adjusted $R^2 = 0.087$).

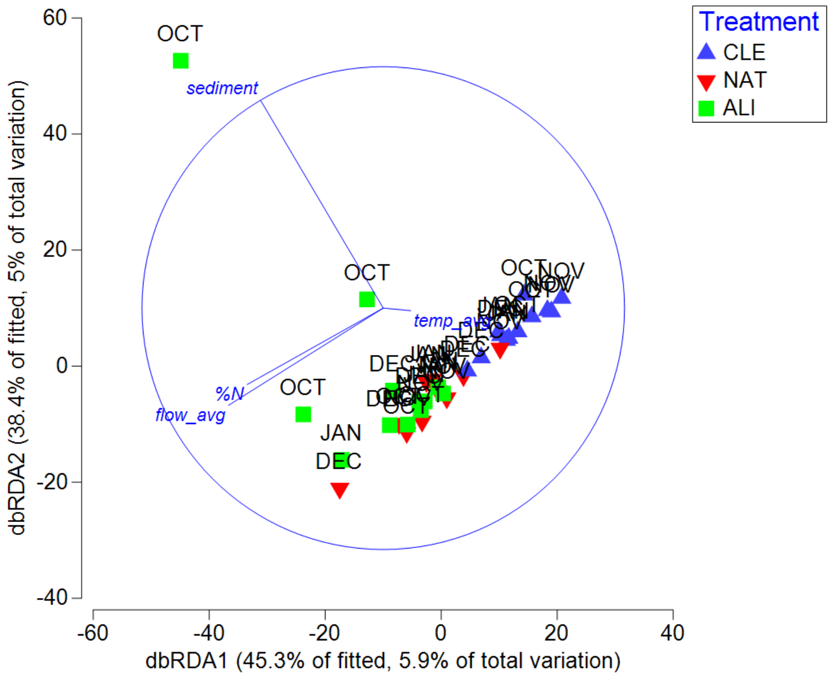


Figure 48: dbRDA of DistLM analysis on the Wit River on the effect of environmental variables on structuring family level macroinvertebrate communities. Based on a square-root transformed Bray–Curtis similarity matrix of macroinvertebrate density. Fitted variation is the variance within the linear model; total variation is the variance within the original data

At the genus level, DistLM results and dbRDA plots were similar to those reported to at the family level, and the plots are therefore not shown here. At the Du Toit's River, the first dbRDA axis explained 71.3% of the fitted variance and 7.1% of the total variance. The second axis explained 28.7% of the fitted variance and 2.8% of the total variance. Marginal tests for variables were not significant. The best solution using a single variable was flow (adjusted $R^2 = 0.028$) and the best overall solution selected both flow and temperature (adjusted $R^2 = 0.037$).

The first axis of the dbRDA plot for the Wit River explained 40.6% of the variation, but only 5.3% of the total variation, while the second axis explained 26.7% of the fitted variation and 3.5% of the total variation. Marginal tests on the variables were not significant. Sediment was the most important variable (adjusted $R^2 = 0.046$) and the best model was solution was based on sediment and temperature (adjusted $R^2 = 0.079$) rather than flow, which was important at the family level.

The first axis of the dbRDA plot on FFGs of the Du Toit's River described 100.9% of the fitted variation, and 15.7% of the total variation (Figure 49). The second axis described −0.9% of the fitted and −0.1% of the total variation. Water flow had a strong influence on feeding community structures, particularly in December and January in the natural sites. Marginal tests (Pseudo-F = 4.123; p = 0.024) and BEST analysis (adjusted $R^2 = 0.092$) confirmed the relative importance of flow. Percentage nitrogen content of sediment also appeared to have an influence, but marginal test results were not significant, and the variable would improve a global model only marginally to 9.6%.

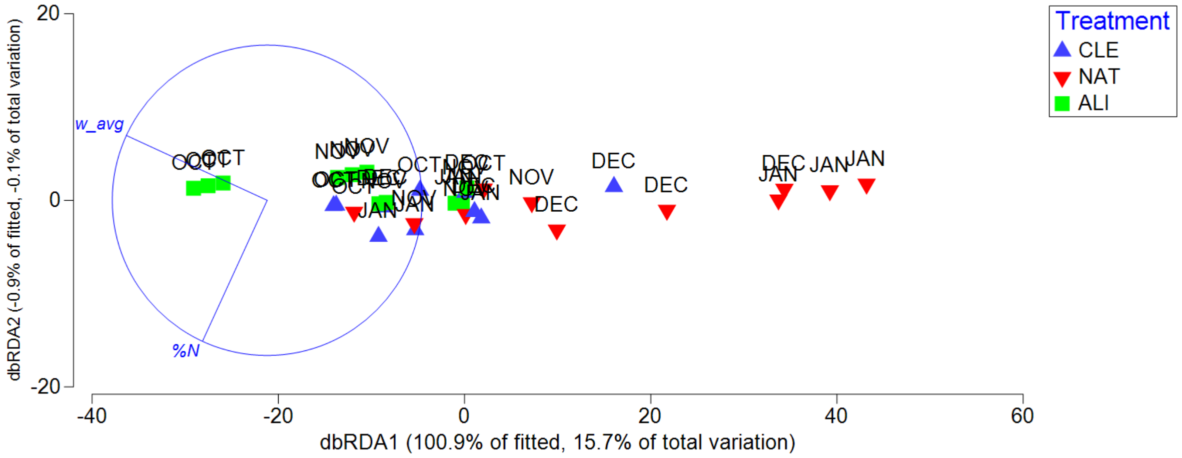


Figure 49: dbRDA of DistLM analysis on the Du Toit's River on the effect of environmental variables on structuring macroinvertebrate FFGs. Based on a square-root transformed Bray–Curtis similarity matrix of macroinvertebrate density. Fitted variation is the variance within the linear model; total variation is the variance within the original data

The first axis of the dbRDA plot on FFGs for the Wit River explained 57% of the variation, but only 9.3% of the total variation, while the second axis explained 39.9% of the fitted variation and 6.5% of the total variation. Marginal tests on the variables were significant only for sediment (Pseudo-F = 2.86; p = 0.043). Sediment was the most important variable (adjusted R^2 = 0.053) and the best model was solution was based on sediment and flow (adjusted R^2 = 0.079) as it also was the family level (see Figure 48).

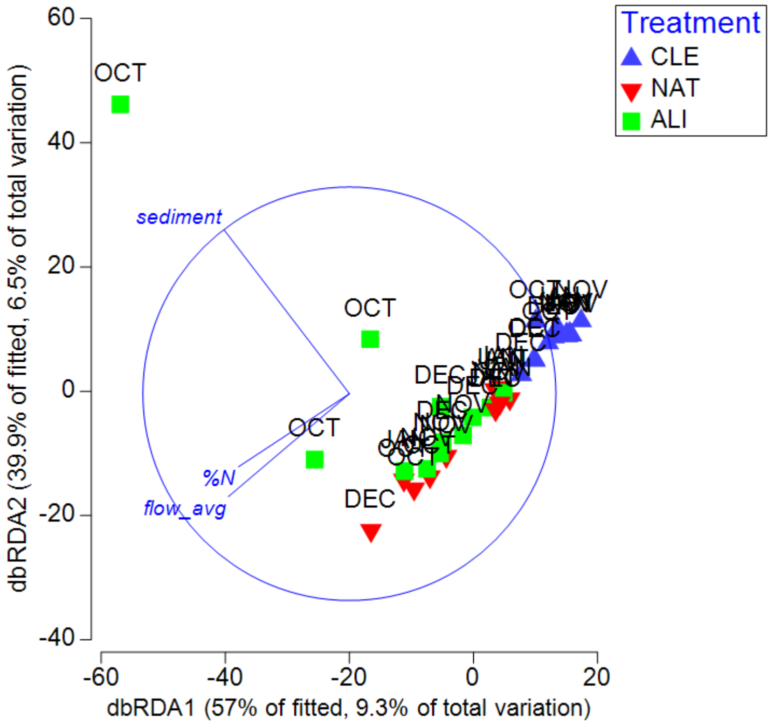


Figure 50: dbRDA of DistLM analysis on the Wit River on the effect of environmental variables on structuring macroinvertebrate FFGs. Based on a square-root transformed Bray–Curtis similarity matrix of macroinvertebrate density. Fitted variation is the variance within the linear model; total variation is the variance within the original data

7.4 Discussion

Nitrogen-fixing alien plants are known to increase stream nitrogen levels equivalent to nitrogen from small-scale agricultural plots (Jaynes et al., 2001). Here, we investigated the effect of riparian zone invasion by, and clearing of, invasive alien Australian black wattle (*A. mearnsii*) of aquatic flora and fauna in two mountain streams in the CFR, South Africa.

Surface water samples at alien sites were not higher in nutrients (ammonia, nitrite, nitrate, orthophosphate) than cleared or natural sites. However, nutrients including nitrogen and phosphorus may be adsorbed to the bottom sediment and can accumulate over time in the river sediment (Oberholster et al., 2013). We therefore hypothesized that benthic algal biomass at invaded sites would be greater than at natural sites due to higher nutrient content in sediment and surface water. This increase in algal biomass was in turn thought to increase benthic macroinvertebrate biomass, particularly the grazer functional group. Higher algal biomass would decrease species diversity.

Over the five-month sampling period, there were no consistent differences between invasion statuses in the rivers. There were only significant differences in benthic algal density and benthic macroinvertebrate densities and FFGs during the transition period between spring and summer. Indeed, macroinvertebrates responded strongly to differences in invasion status, while algae did not. Weak relationships between algal biomass and nutrients in stream environments in New Zealand have been attributed to the complex interaction between physical and biological factors in those streams (Clausen & Biggs, 1998).

At the Wit River, where significant differences between invasion statuses were detected, cleared sites had higher algal biomass than alien sites (intermediate) and natural sites (lowest). A reason for this may be that cleared sites are, like natural sites open, but more disturbed as vegetation cover is less established, which contributes to greater sediment input to the stream. While alien sites were open-canopied, shade across sections of the stream could have had an impact on algal growth. Yet, alien sites were dominated by scrapers, deposit feeders and predators. By contrast it was the cleared site that had a high density of grazers, and also a very high density of deposit feeders. The consistent differences in the biotic responses between invasion statutes may have been due to the change in sedimentation and transport of sediment between October and November (see Chapter 4).

Temperature or flow were in nearly all instances the most important factors in structuring benthic algal and macroinvertebrate communities. The role of flow on community structures at the Du Toit's River was especially evident, with particularly the cleared sites spread parallel to the first axis. At the Wit River, sediment played a key role, and the differences between invasion statuses were not clear from the dbRDA plots. Another possibility is the high leaf litter input during the period, including November (see Chapter 6). However, this does not explain why the differences do not persist as might be expected in December and January. A possibility may be that the biota adapts to the change in flow reduction and sediment transport and spread across sites as there are fewer environmental constraints of differences. The very low explanatory powers of the variables and the models for both rivers suggest that there are other local physical or non-local variables that are important drivers in structuring benthic communities in-stream.

In summary, nitrogen-fixing *A. mearnsii* did not increase in-stream nitrogen as has been reported for other species, despite the fact that the species has far higher leaf litter input into streams, and at that twice a year (see Chapter 6). This could be due to high banks, reducing the effects of the riparian zone invasion, high flow rates and a geology that does not allow for significant nutrient deposition. Nevertheless, given the physiological similarity of *A. mearnsii* and other nitrogen-fixing invaders, its effect on water quality in streams of the CFR should not be underestimated. Instead the findings from this study should potentially be used to estimate the vulnerability of streams to nitrogen pollution by *A. mearnsii*.

CHAPTER 8 – GENERAL SUMMARY AND CONCLUSIONS

There is a significant gap in the knowledge in mountain catchments in the CFR of biophysical processes such as sediment dynamics, as well as knowledge on some biological processes such as decomposition, which prevents a more complete understanding of how these rivers function. This is especially pertinent with the changes in structure of riparian plant communities with the introduction of IAPs in many of these rivers, as well as with clearing by government agencies and private landowners. To address this, we chose two rivers in the Western Cape where several biophysical processes were studied at reaches that had natural fynbos riparian vegetation, or had been cleared by WfW or landowners, or had stands of alien invasive *A. mearnsii*. The reaches were chosen in the sequence natural, cleared and invaded from upstream to downstream. Equipment was installed to gather data on steam flow and other biophysical parameters, while equipment to track sediment movement patterns was constructed and installed. Over the course of more than one year, patterns emerged that shed some new light on fynbos river functioning, the impact of alien invasive *Acacia* and the effect of clearing on fynbos river functioning.

We found high spatial and temporal variability in sediment characteristics and flux, with the Du Toit's River showing higher suspended sediment flux and higher sediment infiltration in sediment baskets. There were also differences between the sites with little consistency between sites of like invasion status (natural, cleared, invaded). In general, sediment dynamics are the result of the distinctive stream attributes such as stream width, riverbank height and riparian slope. However, alien invasive species do have some impact on sediment flux and yield, where the bank is low, and the river connected. Indeed, at a connected invaded site, sediment is supplied, whereas in sites with low connectivity or other invasions statuses (cleared, natural), sediment yield is depleted. From literature (Hupp & Osterkamp, 1996; Tabacchi et al., 2000; Corenblit et al., 2009) there is a likelihood that alien invasive species such as *A. mearnsii* growing in large and dense stands will lead to higher deposition, and when clearing takes place, this sediment is again released to the flowing waters. The results show that the impacts of IAPs were however highly context-dependent, as patterns of sediment loads vary both between and among rivers and reaches with different invasion statuses. However, as sediment makes a significant contribution to stream nutrient stocks and fluxes, the high sediment flux associated with invaded and cleared areas, especially, may have a significant impact of nutrient cycling in rivers. This aspect was further addressed looking at the impact of *A. mearnsii* on leaf litter production, litter decomposition and sediment nutrient stocks.

In terms of sediment nutrient dynamics, the variable characteristics of the two rivers again played a significant role. Naude (2012) showed higher nitrogen stocks on riparian soils of *A. mearnsii*-invaded riparian zones and a return to pre-invasion levels in cleared riparian zones. However, Fourie (2014) showed no evidence of significant denitrification in these same soils, leading to the conclusion that most nitrogen must be lost to the stream, and an expectation that nitrogen in sediment will be higher. This was the case for the sediment in the invaded reaches in the Wit River, which showed the same trends in TN as was found for Naude (2012) in riparian soils. However, it was not evident in the Du Toit's River, where the natural and cleared reaches showed higher sediment nitrogen than the invaded site. While river characteristics cannot be excluded, the role of historical land use may likewise not be excluded in the Du Toit's catchment: Naude (2012) clearly showed consistent elevated TN in riparian soils only in invaded reaches, with low TN levels in natural riparian soils. However, she also showed high available nitrogen in cleared soils – a legacy effect of clearing – as was the case in the current study, which appeared to be lightly invaded before clearing and follow-up some years before the current monitoring period.

The invaded and cleared sites also showed higher phosphorus values, which are consistent with Naude's (2012) findings that phosphorus cycles appear to be altered in invaded riparian soils, though this was not evident in available phosphorus in her study. It is possible that TP stocks are higher in invaded reaches, and that this phosphorus is released to the river in the form of litter when invaded, and in both litter and sediment subsequent to clearing, as was found here. At the Du Toit's River sites, the role of riparian geomorphology cannot be excluded as the sides of the river consisted of high terraces made out of sediment.

In summary, it is clear that invasion by *A. mearnsii* can change sediment nutrient concentrations; however, this may be a context-dependent response, which depends on geomorphology and other factors. The role of litter in invaded reaches and the role of nitrogen in decomposition were also investigated in this study.

A. mearnsii produced more litter than the two native trees, and the litter of *Acacia* trees were also enriched in nitrogen. This supports the results that were obtained from the Wit River sites, where high sediment nitrogen values were observed in the invaded reach, and the work of Naude (2012), who found high nitrogen in riparian soils underneath stands of *A. mearnsii* in the Wit River catchment. Another missing part of the nitrogen cycle in invaded riparian ecotones in the Fynbos Biome is the breakdown of nitrogen in-stream to produce sediment nitrogen dynamics. Invaded riparian ecotones showed higher litterfall, and had litter with higher nitrogen content, which also decomposed faster than native trees. *A. mearnsii* leaf litter had consistently lower dry mass in litter bags installed in the stream after the full duration of the experiment (102 days) than native species, though it appeared that native species, in the initial phase of decomposition, held a slight advantage. There was also not strong evidence of the HFA tested, as was observed in other ecosystems (Gholz et al., 2000; Ayres et al., 2009). In summary, this research closed an important gap in the understanding of nitrogen dynamics in riparian and aquatic ecosystems, especially regarding production of nitrogen-rich litter, decomposition and sediment nitrogen dynamics.

The research provides a baseline for further studies in sediment and nutrient dynamics of invaded and cleared rivers, in comparison with natural stream reaches in the Fynbos Biome. The overall aim of development of a tool to quantify and monitor stream restoration success following removal of riparian IAPs was partially achieved, in that the first step was taken, namely closing many of the gaps that existed in our knowledge on the impact of invasion by nitrogen-fixing alien invasive species, and assessing the changes brought on by clearing these alien invasive species.

In general, we recommend that rivers upstream of reservoirs or wetlands be prioritised for clearing, as this would reduce sedimentation and nutrient loads received. Riparian zones slotted for clearing should be assessed for stream bank zonation and connectivity to the main channel of the stream bank. Invasive stands in riparian zones with low banks and high connectivity to the main channel should be prioritised for clearing. Stands with high connectivity to the main channel and vulnerable features (reservoir or wetland) should be prioritised over all other clearing activities in riparian zones. Finally, clearing needs to be adapted where connectivity to the stream is high. We suggest that invasive stands could initially be thinned and revegetated with native fynbos plants tolerant of higher nitrogen soil content. While initially more expensive than clearing and leaving woody debris to decay, immediate revegetation may lead to more rapid recovery, and at the same time be a cost-effective and rapid method for stabilising stream banks.

REFERENCES

Acornley, R.M. and Sear, D.A. (1999) Sediment transport and siltation of brown trout (Salmo trutta l.) Spawning gravels in chalk streams. Hydrological Processes (13), 447–458.

Addiscott, T.M. and Whitmore, A.P. (1991) Simulation of salute leaching in soils of deferring permeabilities. Soil Use and Management (7), 94–102.

Aerts, R. (1995) The advantages of being evergreen. Trends in Ecology & Evolution 10(10), 402–407.

Aerts, R. (1996) Nutrient resorption from senescing leaves of perennials: Are there general patterns? Journal of Ecology 84(4), 597–608.

Aerts, R. (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. Oikos (79), 439–449.

Aerts, R. and Chapin F.S. (2000) The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. Advances in Ecological Research (30), 1–67.

Akamatsu, F., Ide, K., Shimano, K. and Toda, H. (2010) Nitrogen stocks in a riparian area invaded by N-fixing black locust (Robinia pseudoacacia L.). Landscape and Ecology Engineering (7), 109–115.

Allison, S.D. (2012) A trait-based approach for modelling microbial litter decomposition. Ecology Letters (15), 1058–1070.

Allison, S.D. and Vitousek, P.M. (2004) Rapid nutrient cycling in leaf litter from invasive plants in Hawaii. Oecologia (141), 612–619.

Allsopp, N. and Stock, W.D. (1993) Mycorrhizal status of plants growing in the Cape Floristic Region, South Africa. Bothalia (23), 91–104.

Anderson, M.J., Gorley, R.N. and Clarke, K.R. (2008) PERMANOVA+ for Primer: Guide to software and statistical methods. PRIMER-E, Plymouth, UK. 214pp.

Andrews, M., James, E.K., Sprent, J.I., Boddey, R.M., Gross, E. and Do Reis Jr, F.B. (2011) Nitrogen fixation in legumes and actinorhizal plant in natural ecosystems: Values obtained using 15N natural abundance. Plant Ecology and Diversity 4 (2–3), 130–140.

Aranibar, J.N., Macko, S.A., Anderson, I.C., Potgieter, A.L.F., Sowry, R. and Shugart, H.H. (2003) Nutrient cycling responses to fire frequency in the Kruger National Park (South Africa) as indicated by stable isotope analysis. Isotopes in Environmental and Health Studies 39 (2),141–158.

Aranibar, J. N. et al. (2004) Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands. Global Change Biology(10), 359–373.

Arnborg, L., Walker, H. J., Peippo, J. (1967) Suspended load in the Colville River, Alaska, 1962. Geografiska Annaler, Series A (49), 131 - 144.

Austin, A.T. and Vitousek, P. (1998) Nutrient dynamics on a precipitation gradient in Hawaii. Oecologia (113), 519–529.

Austin, A.T. and Vitousek, P. (2004) Nutrient dynamics on a precipitation gradient in Hawaii: Biogeochemical cycles in arid and semiarid ecosystems. Oecologia (141), 221–235.

Austin, A.T., Vivanco, L., Gonzalez-Arzac, A. and Perez, L.I. (2014) There's no place like home? An exploration of the mechanisms behind plant litter-decomposer affinity in terrestrial ecosystems. New Phytologist (204), 307–314, [doi: 10.1111/nph.12959].

Ayres, E., Dromph, K.M. and Bardgett, R.D. (2006) Do plant species encourage soil biota that specialise in the rapid decomposition of their litter? Soil Biology & Biochemistry (38), 183–186.

Ayres, E. et al. (2009) Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology & Biochemistry, 1–5.

Bača, P. (2008) Hysteresis effect in suspended sediment concentration in the Rybárik Basin, Slovakia. Hydrological Sciences, 53: 224–235.

Bagnold, R.A. (1977) Bed load transport by natural rivers. Water Resources Research 13: 303–312.

Bai, E., Houlton, B. and Wang, Y. (2012) Isotopic identification of nitrogen hotspots across natural terrestrial ecosystems. Biogeosciences, 9: 3287–3304.

Baroudi, R.H. (2016) Interactive effects of litter quality and invertebrates on litter decomposition rates across a successional gradient. MSc thesis. College of Bowling Green State University.

Baxter, C.V., Fausch, K.D. and Saunders, W.C. (2005) Tangled webs: Reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology, 50: 201–220. doi:10.1111/j.1365-2427.2004. 01328.x

Beadle, N.C.W. (1966) Soil phosphate and its role in molding segments of the Australian flora and vegetation, with special reference to xeromorphy and sclerophylly. Ecology, (47) 992-1007.

Bernal, S., Lupon, A., Ribot, M., Sabater, F. and Martí, E. (2015) Riparian and in-stream controls on nutrient concentrations and fluxes in a headwater forested stream. Biogeosciences (12), 1941-1954.

Bernhardt, E.S. and Likens, G.E. (2002) Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. Ecology (83), 1689-1700.

Biggs, B.J.F. and C. Kilroy. (2000). Stream Periphyton Monitoring Manual. NIWA, Christchurch. 215pp.

Bizzi, S. and Lerner, D.N. (2015) The use of stream power as an indicator of channel sensitivity to erosion and deposition processes. River Research and Applications, 31: 16–27.

Blanchard, R. and Holmes P.M. (2008) Riparian vegetation recovery after invasive alien tree clearance in the Fynbos Biome. South African Journal of Botany, 74: 421–431.

Boddey, R.M., Peoples, M.B., Palmer, B. and Dart, P.J. (2000) Use of 15N natural abundance technique to quantify biological nitrogen fixation by woody perennials. Nutrient Cycling in Agroecosystems (57), 235–270.

Bogen, J. (1980) The hysteresis effect of sediment transport system. Norwegian Journal of Geography (34), 45 - 54.

Bonada, N., Doledec, S. and Statzner, B. (2007) Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. Global Change Biology (13), 1658-1671.

Bond, N.R. (2002) A simple device for estimating rates of fine sediment transport along the bed of shallow streams. Hydrobiology (468), 155–161.

Bonney, N. (2003) (2nd ed.). What seed is that? Neville Bonney, Tantanoola, SA.

Born, J., Linder, H.P. and Desmet, P. (2007) The Greater Cape Floristic Region. Journal of Biogeography, 34: 147–162. doi:10.1111/j.1365-2699.2006.01595.x

Boudiaf, I., Baudoin, E., Sanguin, H., Beddiar, A., Thioulouse, J., Galiana, A., Prin, Y., Le Roux, C., Lebrun, M. and Duponnois, R. (2013) The exotic legume tree species, Acacia mearnsii, alters microbial soil functionalities and the early development of a native tree species, Quercus suber, in North Africa. Soil Biology and Biochemistry (65) 172-179.

Boyero, L., Barmuta, L.A., Ratnarajah, L., Schmidt, K. and Pearson. R.G. (2012) Effects of exotic riparian vegetation on leaf breakdown by shredders: A tropical–temperate comparison. Freshwater Science (31), 296–303.

Braatne, J.E., Mazeika, S., Sullivan, P. and Chamberlain, E. (2007) Leaf decomposition and stream macroinvertebrates colonisation of Japanese knotweed on Invasive Plant Species. International Review of Hydrobiologia, 92(6), 656–665.

Brown, C.A., Boucher, C., Pienaar, E. and Pemberton, E. (2004) Project report: Effects of alien invasives on the Breede River. Department of Water Affairs and Forestry: 1–69.

Buddle, C.M., Langor, D.W., Pohl, G.R. and Spence, J.R. (2006) Arthropod responses to harvesting and wildfire: Implications for emulation of natural disturbance in forest management. Biological Conservation, 128(3), 346–357. doi: 10.1016/j.biocon.2005.10.002

Burt TP. 1997. The hydrological role of buffer zones within the drainage basin system. In Buffer Zones: Their Processes and Potential in Water Protection, Haycock NE, Burt TP, Goulding K, Pinay G (eds). Quest Environmental: UK, pp. 21 – 32.

Caldeira, M.V.W., Schumacher, M.V. and Spathelf, P. (2002) Quantification of nutrient content in above-ground biomass of young Acacia mearnsii De Wild., provenance Bodalla. Annals of Forest Science, 59(8), 833–838.

Callaway, R.M. and Ridenour, W.M., 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment (2), 436-443.

Campbell, P. (2000) Wattle control. Plant Protection Research Institute, Handbook no. 3, Pretoria.

CapeNature. (2017) Hottentots-Holland Nature Reserve Complex: Protected areas management plan 2017–2021. Internal Report, CapeNature, Cape Town. 121pp.

Chamier, J., Schachtschneider, K., Le Maitre, D.C., Ashton, P.J. and Van Wilgen, B.W. (2012) Impacts of invasive alien plants on water quality, with particular emphasis on South Africa. Water SA, 38(2), 345–356.

Chang, H.H. (1979) Minimum stream power and river channel patterns. Journal of Hydrology, 41: 303–327.

Chapin, F.S., Matson, P.A. and Mooney, H.A. (2002) Principles of terrestrial ecosystem ecology. Springer, NY.

Chapin, F.S. and Shaver, G.R. (1989) Differences in growth and nutrient use among arctic plant growth forms. Functional Ecology (3), 73–80.

Church, M., and Slaymaker, O., 1989, Disequilibrium of Holocene sediment yield in glaciated British Columbia: Nature, v. 337, p. 452–454, doi: 10.1038 /337452a0.

Claeson, S.M., LeRoy, C.J., Barry, J.R. and Kuehn, K.A. (2014) Impacts of invasive riparian knotweed on litter decomposition, aquatic fungi, and macroinvertebrates. Biological invasions, (16), 1531-1544.

Clarke, K.R. and Gorley, R.N. (2006) PRIMER v6: User Manual/Tutorial. PRIMER-E: Plymouth Marine Laboratory, Plymouth, UK. 190pp.

Clarke, K.R. and Warwick, R.M. (2001) Change in marine communities: An approach to statistical analysis and interpretation, 2nd ed. PRIMER-E: Plymouth Marine Laboratory, Plymouth, UK.

Clausen B. and Biggs B.J.F. (1998) Stream flow variability indices for riverine environmental studies. Hydrology in a changing environment. In: Proceedings of the British Hydrological Society International Conference, pp. 357–364.

Cocks, M. and Stock, W.D. (2001) Field patterns of nodulation in fifteen Aspalathus species and their ecological role in the fynbos vegetation of southern Africa. Basic and Applied Ecology (2),115–125.

Compton, J.E., Church, M.R., Larned, S.T. and Hogsett, W.E. (2003) Nitrogen export from forested watersheds in the Oregon Coast Range: The role of N_2-fixing red alder. Ecosystems (6), 773–785.

Corbacho, C., Sánchez, J.M. and Costillo, E. (2003). Patterns of structural complexity and human disturbance of riparian vegetation in agricultural landscapes of a Mediterranean area. Agriculture, Ecosystems & Environment (95), 495-507.

Corenblit, D., Steiger, J., Gurnell, A.M., Tabacchi, E. and Roques, L. (2009) Control of sediment dynamics by vegetation as a key function driving biogeomorphic succession within fluvial corridors. Earth Surface Processes and Landforms, 34(13), pp.1790–1810.

Correl, D.L. (1998) The role of phosphorus in the eutrophication of receiving waters: A review. Journal of Environmental Quality (27), 261–266.

Cowling, R.M. and Holmes, P.M. (1992) Flora and vegetation. In: Cowling, R.M. (ed.) The Ecology of Fynbos. Nutrients, Fire and Diversity. Cape Town: Oxford University Press. 23–61.

Cowling, R.M., Richardson, D.M. and Mustart, P.J. (2004) Fynbos. In: Cowling, R.M., Richardson, D.M. and Pierce, S.M. (eds) Vegetation of Southern Africa. Cambridge: Cambridge University Press, 99–167.

Craine, J. M. et al. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytologist, 183(4), 980–992.

Craine, J.M. et al. (2015) Ecological interpretations of nitrogen isotopes ratios of terrestrial plant and soils. Journal of Plant and Soil, 396 (1–2), 1–26.

Craine, J.M., Morrow, C. and Stock, W. (2008) Nutrient concentration ratios and co-limitation in South African grasslands. New Phytologist (179), 829–836.

Cramer, M.D. (2010) Phosphate as a limiting resource: Introduction. Plant Soil (334), 1–10.

Cramer, M.D., Chimphango, S.BM., Van Cauter, A., Waldram, M.S and Bond, W.J. (2007) Grass competition induces N_2 fixation in some species of African Acacia. Journal of Ecology, 95(5), 1123–1133.

Crous, C.J. (2010) Vulnerability of selected native and invasive woody species to streamflow variability in Western Cape fynbos riparian ecotones. MSc thesis. Stellenbosch University, South Africa.

Cullis, J., Görgens, A. and Marais, C. (2007) A strategic study of the impact of invasive alien vegetation in the mountain catchment areas and riparian zones of South Africa on total surface water yield. Water SA (33), 35-42.

D'Antonio, C. and Meyerson, L.A. (2002) Exotic plant species as problems and solutions in ecological restoration: A synthesis. Restoration Ecology (10), 703–713.

Dallas, H.F. and Day, J.A. (1993) The effect of water quality variables on riverine ecosystems: a review. Freshwater Research Unit, University of Cape Town.

Dallas, H.F. and Day, J.A. (2004) The effect of water quality variables on aquatic ecosystems: A review. WRC Report No. TT 224/04. Water Research Commission, Pretoria, 224 pp.

Davide, V., Pardos, M., Diserens, J., Ugazio, G., Thomas, R. and Dominik, J. (2003) Characterisation of bed sediments and suspension of the river Po (Italy) during normal and high flow conditions. Water Research, 37, 2847–2864.

Davis, M.A. et al. (2011) Don't judge species on their origins. Nature (474), 153–154.

Day, J.A. and De Moor I.J. (eds). (2002) Guides to the freshwater invertebrates of southern Africa. Volume 6: Arachnida and Mollusca (Araneae, water mites and Mollusca). WRC No. TT 182/02. Water Research Commission, Pretoria. 141 pp.

Day, J.A., Harrison A.D., De Moor I.J. (eds). (2003) Guides to the freshwater invertebrates of southern Africa. Volume 9: Diptera. WRC Report No. TT 201/02. Water Research Commission, Pretoria. 200 pp.

Day, J.A. and King, J.M. (1995) Geographical patterns, and their origins, in the dominance of major ions in South African Rivers. South African Journal of Science, 91(6), 299–306.

Deacon, H.J., Jury, M.R. and Ellis, F. (1992) Selective time and regime. In: Cowling R.M. (Ed.), Fynbos: Nutrients, Fire and Diversity. London: Oxford University Press, 6–23.

DEADP, 2011. Western Cape Integrated Water Resources Management Action Plan: Draft Status Quo Report, Western Cape Department of Environmental Affairs and Development Planning.

DeCant, J.P. (2008) Russian olive, Elaeagnus angustifolia, alters patterns in soil nitrogen pools along the Rio Grande River, New Mexico, USA. Wetlands (28), 896–904.

Dedkov, A. (2004) The relationship between sediment yield and drainage basin area. In: Sediment Transfer through the Fluvial System, International Association of Hydrological Sciences, Wallingford, Oxon, UK, Publication 288: 197–204.

Dedkov, A.P. and Moszherin, V.I. (1996) Erosion and sediment yield: Global and regional perspectives. In: Proceedings of the Exeter Symposium, July 1996. International Association of Hydrological Sciences Publication, 236, 29–33.

Dell'Porto, D.M., Costa, S.C, Araújo, A.P., Minho. and Abdalla., A.L. (2006) Effects of condensed tannin from Acacia mearnsii on sheep infected naturally with gastrointestinal Helminthes. Veterinary Parasitology (15), 132–137.

De Moor, I.J., Day, J.A. and De Moor, F.C. (eds). (2003a) Guides to the freshwater invertebrates of southern Africa. Volume 7: Insecta I (Ephemeroptera, Odonata and Plecoptera). WRC Report No. TT 207/03, Water Research Commission, Pretoria. 288 pp.

De Moor, I.J., Day, J.A. and De Moor, F.C. (eds). (2003b) Guides to the freshwater invertebrates of southern Africa. Volume 8: Insecta II (Hemiptera, Megaloptera, Neuroptera, Trichoptera and Lepidoptera). WRC Report No. TT 214/03, Water Research Commission, Pretoria. 209 pp.

Dent, C.L., Grimm, N.B., Martí, E. Edmonds, J.W. Henry, J.C. and Welter, J.R. (2007) Variability in surface-subsurface hydrologic interactions and implications for nutrient retention in an arid-land stream. Journal of Geophysical Research (112), G04004, 1–13.

De Vente, J., Poesen, J., Arabkhedri, M. and Verstraeten, G. (2007) The sediment delivery problem revisited. Progress in Physical Geography, 31, 155–178.

Diaz, T., Martins-Louc, M.A., Sheppard., L. and Cruz., C. (2012) The strength of the biotic compartment in retaining nitrogen additions prevents nitrogen losses from a Mediterranean maquis. Biogeosciences (9), 193–201.

Drake, D.C. (2011) Invasive legumes fix N_2 at high rates in riparian areas of an N-saturated, agricultural catchment. Journal of Ecology, 99(2), 515–523.

Drake, D.C., Peterson, B.J., Deegan, L.A., Harris, L.A., Miller, E.E. and Warren, R.S. (2008) Plant nitrogen dynamics in fertilized and natural New England salt marshes: a paired 15N tracer study. Marine Ecology Progress Series (354), 35-46.

Drenovsky, R.E. and Batten, K.M. (2007) Invasion by Aegilops triuncialis (barb goatgrass) slows carbon and nutrient cycling in a serpentine grassland. Biological Invasions (9), 107–116.

Ehrenfeld, J.G. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems (6), 503–523.

Enright, W.D. (2000) The effect of terrestrial invasive alien plants on water scarcity in South Africa. Physics and Chemistry of the Earth (B) 25, 237–242.

Evans, R.D. (2001) Physiological mechanisms influencing plant nitrogen isotope composition. Trends in Plant Science, 6(3), 121–126.

Evans, J. et al. (1989) N_2 fixation and its value to soil N increase in lupin, field pea and other legumes in southeastern Australia. Australian Journal of Agricultural Research (40), 791–805.

Ewart-Smith, J.I. and King, J. (2012) The relationship between periphyton, flow and nutrients in foothill rivers of the south-western Cape, South Africa. WRC Report No. 1676/1/12, Water Research Commission, Pretoria. 340 pp.

Ferguson, R.I. (2005) Estimating critical stream power for bedload transport calculations in gravel-bed rivers. Geomorphology, 70, 33–41.

Fife, D.N., Nambiar, E.K.S. and Saur, E. (2008) Retranslocation of foliar nutrients in evergreen tree species planted in a Mediterranean environment. Tree Physiology, 28(2), 187–196.

Filoso, S. and Palmer, M.A. (2011) Assessing stream restoration effectiveness at reducing nitrogen export to downstream waters. Ecological Applications, 21, 1989–2006.

Forrester, D.I., Schortemeyer, M., Stock, W.D., Bauhus, J., Khanna, P.K. and Cowie, A.L. (2007) Assessing nitrogen fixation in mixed- and single-species plantations of Eucalyptus globulus and Acacia mearnsii. Tree Physiology, 27(9), 1319–1328.

Forsyth, G.G., Richardson, D.M., Brown, P.J. and Van Wilgen, B.W. (2004) A rapid assessment of the invasive status of Eucalyptus species in two South African provinces: working for water. South African Journal of Science, (100), pp.75-77.

Foster, I.D.L., Rowntree, K.M., Boardman, J. and Mighall, T.M. (2012) Changing sediment yield and sediment dynamics in the Karoo uplands, South Africa: Post-European impacts. Land Degradation and Development, 23, 508–522. doi:10.1002/ldr.2180.

Fourie, M.J., 2014. Investigating soil nitrogen dynamics in natural, invaded and cleared fynbos riparaian ecotones and implications for riparian functioning (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Freschet, G.T., Aerts, R. and Cornelissen, J.H.C. (2012) Multiple mechanisms for trait effects on litter decomposition: Moving beyond home-field advantage with a new hypothesis. Journal of Ecology (100), 619–630.

Frissell, C.A., Liss, W.J., Warren, C.E. and Hurley, M.D. (1986) A hierarchical framework for stream habitat classification: Viewing streams in a watershed context. Environmental Management, 10(2), 199–214.

Furey, C., Tecco, P.A., Harquindequy, N.P., Giorgis, M.A. and Grossi, M. (2003) The importance of native and exotic plant identity and dominance on decomposition patterns in mountain woodlands of central Argentina. Octa Oecologica, 1–8.

Galatowitsch, S. and Richardson, D.M. (2005) Riparian scrub recovery after clearing of invasive alien trees in headwater streams of the Western Cape, South Africa. Biological Conservation (12), 509–521.

Gallinat, A.S., Primack, R.B. and Wagner, D.L. (2015) Autumn, the neglected season in climate change research. Trends in Ecology & Evolution (30) 169-176.

Garcia-Palacios, P., Maestre, F.T., Kattge, J. and Wall, D.H. (2013) Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. Ecology Letters, 16(8), 1045–1053.

Garland, G., Hoffman, M.T. and Todd, S. (1999) Soil degradation. In: Hoffman. M.T., Todd, S., Ntshona, Z and Turner, S. (eds) Land Degradation in South Africa. Department of Environmental Affairs and Tourism, Pretoria.

Gasith, A. and Resh, V.H. (1999). Streams in Mediterranean climate region: Abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology, Evolution, and Systematics (30), 51–81.

Gehring, C. and Vlek, P.L.G. (2004) Limitations of the N natural abundance method for estimating biological nitrogen fixation in Amazonian forest legumes. Basic Applied Ecology (5), 567–580.

Gholz, H.L., Wedin, D.A., Smitherma, S.M., Harmon, M.E. and Parton, W.J. (2000) Longterm dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Global Change Biology (6), 751–765.

Gießelmann, U.C., Martins, K.G., Brändle, M., Schädler, M., Marques, R. and Brandl, R. (2010) Diversity and ecosystem functioning: Litter decomposition dynamics in the Atlantic rainforest. Applied Soil Ecology (46), 283–290.

Goi, S.R., Sprent, J.I., James, E.K. and Jacob-Neto, J. (1993) Influence of nitrogen form and concentration on the nitrogen fixation of Acacia auriculiformis. Symbiosis (14), 115–112.

Goldblatt, P. and Manning, J. (2000) Cape plants: A conspectus of the Cape flora of South Africa. Strelitzia 9. National Botanical Institute, Pretoria, and Missouri Botanical Garden, Missouri.

Goldman, C.R. (1961) The contribution of alder trees (Alnus tenuifolia) to the primary productivity of Castle Lake, California. Ecology, 42, 283–288.

Gomez, E. Durrillon, C., Rofes, G. and Picot, B. (1999) Phosphate adsorption and release from sediments of brackish lagoons: pH, O_2 and loading influence. Water Research (33), 2437–2447.

Gordon, S.E. and Ghoni, M.A. (2003) Sources and distribution of terrigenous organic matter delivered by the Atchafalaya River to sediment in the northern Gulf of Mexico. Geochimica et Cosmochimica (67), 2359–2375.

Görgens, A.H.M. and Van Wilgen, B.W. (2004) Invasive alien plants and water resources in South Africa: Current understanding, predictive ability and research challenges. South African Journal of Science, 100, 27–33.

Grab, S. (2014) Spatio-temporal attributes of water temperature and macroinvertebrate assemblages in the headwaters of the Bushmans River, southern Drakensberg. Water SA (40),19–26.

Graça, M.A.S., Ferreira R.C.F. and Coimbra, C.N. (2001) Litter processing along a stream gradient: The role of invertebrates and decomposers. Journal of North American Benthological Society (20), 408–419.

Grant, J.E., Moran G.F. and Moncur, M.W. (1994) Pollination studies and breeding system in Acacia mearnsii. In: Australian Tree Species Research in China (A. Brow, ed.). ACIAR Proceedings (48), 165–170.

Greig, S.M., Sear, D.A., and Carling, P.A. (2005) The impact of fine sediment accumulation on the survival of incubating salmon progeny: Implications for sediment management. Science of the Total Environment, 344, 241–258.

Grenfell, S.E. and Ellery, W.N. (2009) Hydrology, sediment transport dynamics and geomorphology of a variable flow river: The Mfolozi River, South Africa. Water SA, 35: 271–282.

Gordon, N.D., McMahon, T.A., Finlayson, B.L. (1992) Stream hydrology: an introduction for ecologists. John Wiley and Sons, West Sussex, England.

Handley, L.L. and Raven, J.A. (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant, Cell & Environment, 15(9), 965–985.

Harper, D., Zalewski, M. and Pacini, N. (2008) Ecohydrology: Processes, Models and Case Studies: An Approach to the Sustainable Management of Water Resources. Trowbridge, UK: Cromwell Press, 391pp.

Hättenschwiler, S., Tiunov, A.V. and Scheu, S. (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics (36), 191–218.

Hawkins, H., Wolf, G. and Stock, W.D. (2005) Cluster roots Leucadendron laureolum (Proteaceae) and Lupinus albus (Fabaceae) take up glycine intact: An adaptive strategy to low mineral nitrogen in soils? Annals of Botany (96), 1275–1282.

He, Q.I. and Owens, P.H. (1995) Determination of suspended sediment provenance using caesium-137, unsupported lead-210 and radium-226: a numerical mixing model approach. I.D.L. Foster, A.M. Gurnell, B.W. Webb (Eds.), Sediment and water quality in river catchments, Wiley, Chichester, pp.207-227.

He, Q. and Walling, D.E. (1996) Interpreting particle size effects in the adsorption of 137Cs and unsupported 210Pb by mineral soils and sediments. Journal of Environmental Radioactivity, (30), pp.117-137.

He, H., Bleby, T.M., Veneklaas, E.J and Lambers, H. (2011) Dinitrogen-fixing Acacia species from phosphorous-impoverished soils resorb leave phosphorous efficiency. Plant, Cell and Environment (34), 2060–2070.

Hellmann, C., Sutter, R., Rascher, K.G., Màguas, C., Correia, O. and Werner, C. (2011) Impact of an exotic N_2-fixing Acacia on composition and N status of a native Mediterranean community. Acta Oecologica (37), 43–50.

Hickman, J.E., Ashton, I.W., Howe, K.M. and Lerdau, M.T, (2013) The native–invasive balance: implications for nutrient cycling in ecosystems. Oecologia (173), 319-328.

Hicks, D.M., Gomez, B. and Trustrum, N.A. (2000) Erosion thresholds and suspended sediment yields, Waipaoa River basin, New Zealand. Water Resources Research (36), 1129-1142.

Hobbie, E.A. and Högberg, P. (2012) Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. New Phytologist, 196(3), 67–382.

Hoffman, M.T. and Mitchell, D.T. (1986) Root morphology of legume spp. in the south western Cape and the relationship of vesicular-arbuscular mycorrhizas with dry mass and phosphorus content of Acacia saligna seedlings. South African Journal of Botany (52), 316–320.

Högberg, P. (1997) ^{15}N natural abundance in soil-plant systems. New Phytologist 137(2), 179–204.

Holmes, P.M., Richardson, D.M, Esler, K.J., Witkowski, E.T.F. and Fourie, S. (2005) A decision-making framework for restoring riparian zones degraded by invasive alien plants in South Africa. South African Journal of Science, 101, 553–564.

Holomuzki, J.R. and Messier, S.H. (1993) Habitat selection by the stream mayfly Paraleptophlebia guttata. Journal of the North American Benthological Society (12), 126-135.

Hood, G.W. and Naiman, R.J. (2000) Vulnerability of riparian zones to invasion by exotic vascular plants. Plant Ecology (148), 105–114.

Horowitz, A.J. (1991) A primer on sediment trace element chemistry. Chelsea, MI: Lewis Publishers. 1–136.

Horowitz, A.J. (2008). Determining annual suspended sediment and sediment-associated trace element and nutrient fluxes. Science of the Total Environment, 400: 315–343.

Horowitz, A.J. and Elrick, K.A. (1987) The relation of stream sediment surface area, grain size and composition to trace element chemistry. Applied Geochemistry (2), 437–451.

Hou, D., He, J., Lu, C., Sun, Y., Zhang, F. and Otgonbayer, K. (2013) Effects of environmental factors on nutrient release at sediment-water interface and assessment of trophic status for a typical shallow lake, northwest China. The Scientific World Journal (2013), 1–16.

Houlton, B.Z. and Bai, E. (2009) Imprint of denitrifying bacteria on the global terrestrial biosphere. In: Proceedings of the National Academy of Sciences (106), 21713–21716.

Houlton, B.Z., Wang Y.P., Vitousek P.M. and Field, C.B. (2008) A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature (454), 327–334.

Hupp, C.R. and Osterkamp, W.R. (1996) Riparian vegetation and fluvial geomorphic processes. Geomorphology, 14(4), pp.277–295.

Hurd, T.M., and Raynal, D.J. (2004) Comparison of nitrogen solute concentrations within alder (Alnus incana ssp. rugosa) and non-alder dominated wetlands. Hydrological Processes (18), 2681–2697.

Imeson, A.C. (1974) The origin of sediment in a moorland catchment with special reference to the role of vegetation. Fluvial Processes in Instrumented Watersheds, Transactions of the Institute of British Geographers, Special Publication No. 6, 69–72.

Inagaki, M. and Ishizuka, S. (2011) Ecological impact on nitrogen and phosphorous cycling of a wide spread fast growing leguminous tropical forest plantation tree species, Acacia mangium. Diversity (3), 712–720.

Jacob, M. (2010) Leaf litter decomposition in temperate deciduous forest stands with a decreasing fraction of beech (Fagus sylvatica). Oecologia (164), 1083‑1094.

Jacobs, S. et al. (2013) Identifying relationships between soil processes and biodiversity to improve restoration of riparian ecotones invaded by exotic acacias. WRC Report No. 1927/1/13, Water Research Commission, Pretoria.

Jacobs, S.M., Petit, N.E. and Naiman, R.J. (2006) Nitrogen fixation by the savanna tree Philenoptera violacea (Klotzsch) Schrire (Apple leave) of different ages in a semi-arid riparian landscape. South African Journal of Botany (73), 163–167.

Januasauskaite, D. and Straige, L. (2011) Leaf litter decomposition: Differences between alien and native maple species. Baltic Forestry, 17(2), 189–196.

Jaynes, D.B., Colvin, T.S., Karlen, D.L., Cambarella, C.A. and Meek, D.W. (2001) Nitrate loss in subsurface drainage as affected by nitrogen fertilizer rate. Journal of Environmental Quality, 30, 1305–1314.

Jewell, M.D., Shipley, B., Paquette, A., Messier, C. and Reich, B.P. (2015) A trait-based test of home-field advantage in mixed-species tree litter decomposition. Annals of Botany (105), 1–8.

Jia, Y.Y. et al. (2015) Insight into the indirect function of isopods in litter decomposition in mixed subtropical forests in China. Applied Soil Ecology (86), 174–181.

Jo, I, Fridley, J.D. and Frank, D.A. (2016) More of the same? In situ leaf and root decomposition rates do not vary between 80 native and nonnative deciduous forest species. New Phytologist (209), 115–122.

Jovanovic, N.Z. et al. (2009) Nitrogen dynamics in land cleared of alien vegetation (Acacia saligna) and impacts on groundwater at Riverlands Nature Reserve (Western Cape, South Africa). Water SA (35), 37–44.

Juba, R. (2012) Determining leave nutrient stocks in one invasive and two native fynbos riparian species. Honours thesis (Unpublished). University of Stellenbosch.

Killingbeck, K.T. (1993) Inefficient nitrogen resorption in genets of the actinorhizal nitrogen-fixing shrub Comptonia peregrina: Physiological ineptitude or evolutionary tradeoff. Oecologia (94), 542–549.

Killingbeck, K.T. (1996) Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency. Ecology, 77(6), 1716–1727.

Killingbeck, K.T., May, J.D. and Nyman, S., 1990. Foliar senescence in an aspen (Populus tremuloides) clone: the response of element resorption to interramet variation and timing of abscission. Canadian Journal of Forest Research (20), 1156-1164.

King, J.M., Day, J.A., Henshall-Howard M.P. and Davies B.R. (1987) Leaf-pack dynamics in a south-western Cape mountain stream. Freshwater Biology (18), 325–340.

King, J.M. and Schael, D.M. (2001) Assessing the ecological relevance of a spatially-nested geomorphological hierarchy for river management. WRC Report No. 754/1/01. Water Research Commission, Pretoria.

Klamer, J.C., Hegemain, W.J.M. and Smedes, F. (1990) Comparison of grain size correction procedures for organic micropollutants and heavy metals in marine sediments. Hydrobiologia (208), 213–220.

Kosmas, C. et al. (1997) The effect of land use on runoff and soil erosion rates under Mediterranean conditions. Catena, 29, 45–59.

Kuglerova, L., Garcia, L., Pardo, I., Mottiar, Y. and Richardson J.S. (2017) Does leave litter from invasive plants contribute the same support of a stream ecosystem function as native vegetation? Ecosphere 8(4), 1–18.

Lambers, H., Ahmedi, I., Berkowitz, O., Dunne, C., Finnegan, P.M., Hardy, G.E.S.J., Jost, R., Laliberté, E., Pearse, S.J. and Teste, F.P. (2013) Phosphorus nutrition of phosphorus-sensitive Australian native plants: threats to plant communities in a global biodiversity hotspot. Conservation Physiology, 1(1).

Lambers, H., Brundrett, M.C., Raven, J.A. and Hopper, S.D. (2010) Plant mineral nutrition in ancient landscapes: High plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant and Soil, 334(1–2), 11–31.

Lambers, H., Pons, T.L. and Chapin, F.S. (2008a) Plant physiological ecology, 2nd ed. New York, NY: Springer-Verlag.

Lambers, H., Raven, J.A., Shaver, G.R. and Smith, S.E. (2008b) Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution (23), 95–103.

Lambers, H., Shane, M.W., Cramer, M.W., Pearse, M.D., Veneklaas, S.J. and Erik, J. (2006) Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. Annals of Botany (98), 693–713.Lane, L.J., Hernandez, M. and Nichols, M. (1997). Processes controlling sediment yield from watersheds as functions of spatial scale. Environmental Modelling, 12, 355–369.

Lecerf, A., Patfield, D., Boiche, A., Riipinen, M.P., Chauvet, E. and Dobson, M. (2007) Stream ecosystems respond to riparian invasion by Japanese knotweed (Fallopia japonica). Canadian Journal of Fisheries and Aquatic Sciences (64), 1273–1283.

Lefrançois, J., Grimaldi, C., Gascuel-Odoux, C. and Gillet, N. (2007) Suspended sediment and discharge relationships to identify bank degradation as a main sediment source on small agricultural catchments. Hydrological Processes, 21, 2923–2933.

Le Maitre, D.C., Van Wilgen, B.W., Chapman, R.A. and McKelly, D.H. (1996) Invasive plants and water resources in the Western Cape Province, South Africa: Modelling the consequences of a lack of management. Journal of Applied Ecology (33), 161–172.

Le Maitre, D.C., Van Wilgen, B.W., Gelderblom, C.M., Bailey, C., Chapman, R.A. and Nel, J.A. (2002) Invasive alien trees and water resources in South Africa: Case studies of the costs and benefits of management. Forest Ecology and Management (160), 143–159.

Le Maitre, D.C., Gaertner, M., Marchante, E., Ens, E.J., Holmes, P.M., Pauchard, A., O'Farrell, P.J., Rogers, A.M., Blanchard, R., Blignaut, J. and Richardson, D.M., 2011. Impacts of invasive Australian acacias: implications for management and restoration. Diversity and Distributions (17), 1015-1029.

Leroy, C.J. and Marks, J.C. (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. Freshwater biology, 51(4), pp.605-617.

Le Roux, J.J., Newby, T.S. and Sumner, P.D. (2007) Monitoring soil erosion in South Africa at a regional scale: Review and recommendations. South African Journal of Science, 103, 329–335.

Le Roux, J.J., 2011. Monitoring soil erosion in South Africa at a regional scale. ARC report no. GW/A/2011/23. Argricultural Reseach Council, Pretoria, pp. 34.

Levine, J.M., Vila, M., D'Antonio, C.M., Sukes, J.S., Grigulis, K. and Lavorel, S. (2003) Mechanisms underlying the impacts of exotic plant invasions. In: Proceedings of the Royal Society of London B (270), 775–781.

Liao, C. et al. (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: A meta-analysis. New Phytologist, 177(3), 706–714.

Lima, A.L.D.S., Zanella, F., Schiavianto, M.A. and Haddad, C.R.B. (2006) N availability and mechanisms of N conservation in deciduous and semideciduous tropical forest legume trees. Acta Botanica Brasilica (20), 625–632.

Lovely D.R., Phillips E.J.P (1986) Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. Applied and Environmental Microbiology (52), 751–757.

Lowe, S.R., Woodford, D.J., Impson, N.D and Day, J.A. (2008) The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. African Journal of Aquatic Science (33), 51–62.

Maamri, A., Chergui, H. and Pattee, E. (1994) Allochthonous input of coarse particulate organic matter to a Moroccan mountain stream. Acta Oecologica (France).

Maamri, A., Bärlocher, F., Pattee, E. and Chergui, H. (2001) Fungal and bacterial colonization of Salix pedicellata leaves decaying in permanent and intermittent streams in eastern Morocco. International Review of Hydrobiology (86), 337–348.

MacKenzie, R.A., Wiegner, T.N., Kinslow, F., Cormier, N. and Strauch, A.M. (2013) Leaf-litter inputs from an invasive nitrogen-fixing tree influence organic-matter dynamics and nitrogen inputs in a Hawaiian river. Freshwater Science 32, 1036–1052.

Mano, V., Nemery, J., Belleudy, P. and Poirel, A. (2009) Assessment of suspended sediment transport in four alpine watersheds (France): Influence of the climatic regime. Hydrological Processes, 23, 777–792.

Maoela, M.A. (2015) Tree health in near pristine, heavily invaded and restored riparian zones: The role of pests and fungal pathogens. PhD Thesis. University of Stellenbosch, Stellenbosch.

Marchante, E., Kjoller, A., Struwe, S. and Freitas, H. (2008) Short and long-term impacts of Acacia longifolia invasion on the belowground processes of a Mediterranean coastal dune ecosystem. Applied Soil Ecology (40), 210–217.

Maron, J.L. and Connors, P.G. (1996) A native nitrogen-fixing shrub facilitates weed invasion. Oecologia, (105), 302-312.

Maron, J.L. and Jefferies, R.L. (1999) Bush lupine mortality, altered resource availability, and alternative vegetation states. Ecology (80), 443–454

Martinelli, L.A. et al. (1999) Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. Biogeochemistry (46), 45–65.

Maturana, O., Tonina, D., McKean, J.A., Buffington, J.M., Luce, C.H., and Caamano, D. (2014) Modelling the effects of pulsed versus chronic inputs on salmonid spawning habitat in a low-gradient gravel-bed river. Earth Surface Processes and Landforms, 39, 877–889.

McClain, M.E. et al. (2003) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems (6), 301–312.

Meentemeyer, V. (1978) Macroclimate and lignin control of litter decomposition rates. Ecology (59), 465–72.

Merrit, R. and Cummins, K.W., (1983) An Introduction to he Acuatic Insects of North America. Kendall.

Meyer, W.M., Ostertag, R. and Cowie, R.H. (2011) Macro-invertebrates accelerate litter decomposition and nutrient release in a Hawaiian rainforest. Soil Biology and Biochemistry, 43(1), 206–211.

Meyers, P.A. and Teranes, J.L. (2001) Tracking Environmental Change Using Lake Sediments: Physical and Geochemical Methods. 239-269. In: Last, W.M. (Ed.), Tracking Environmental Change Using Lake Sediments. In: Smol, J.P. (Ed.), Physical and Geochemical Methods, vol 2. Kluwer Academic Publishers, Dortrecht, p. 504

Meyers, P.A. (1994) Preservation of elemental and isotopic source identification of sedimentary organic matter. Chemical Geology (114), 289–302.

Milan, D. (2017) Controls on spatial and temporal variations in sand delivery to salmonid spawning riffles. Hydrological Processes, 31, 2179–2195.

Milton, S.J. (1981a) Above-ground biomass of Australian acacias in the southern Cape, South Africa. South African Journal of Botany (47), 701–716.

Milton, S.J. (1981b) Litterfall of the exotic Acacias in the south-western Cape. South African Journal of Botany (47), 147–155.

Milton, S.J. (2004) Grasses as invasive alien plants in South Africa. South African Journal of Science (100), 69–75.

Mineau, M.M., Baxter, C.V. and Marcarelli, A.M. (2011) A non-native riparian tree (Elaeagnus angustifolia) changes nutrient dynamics in streams. Ecosystems (14), 353–365.

Mitchell, D.T., Coley, P.G.F., Webb, S. and Allsopp, N. (1986) Litterfall and decomposition processes in the coastal fynbos vegetation, south Western Cape, South Africa. Journal of Ecology *(*74), 977–993.

Mittermeier, R.A. et al. (2005) Hotspots revisited: Earth’s biologically richest and most endangered terrestrial ecoregions. Conservation International and Agrupacion Sierra Madre, Monterrey, Cemex.

Mittermeier, R. A., Myers, N., Thomsen, J. B., Da Fonseca, G. A. and Olivieri, S. (1998). Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. Conservation Biology (12), 516-520.

Moncur, M.W., Moran, G.F., Boland, D.J. and Turner, J. (1989) Floral morphology and breeding systems of Acacia mearnsii De Wild. Paper to workshop, Use of Australian Trees in China, Guangzhou, 266–276.

Morris, T.L., Esler, K.J., Barger, N.N., Jacobs, S.M. and Cramer, M.D. (2011) Ecophysiological traits associated with the competitive ability of invasive Australian acacias. Diversity and Distributions, 17(5), 898–910.

Moyo, H.P.M. and Fatunbi, O. (2010) Utilitarian perspective of the invasion of some South African biomes by Acacia mearnsii. Global Journal of Environmental Research, 4(1), 6–17.

Mucina, L. and Rutherford, M. (2006) The Vegetation of South Africa, Lesotho and Swaziland. Pretoria: South African National Biodiversity Institute, 749–790.

Musil, C.F. and Midgley, G.F. (1990) The relative impact of invasive Australian Acacias, fire and season on the soil chemical status of a sand plain lowland fynbos community. South African Journal of Botany (56), 419–427.

Naiman, R.J., Bilby, R.E., Schindler, D.E. and Helfield, J.M. (2002) Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems (5), 399-417.

Naiman, R.J., Bechtold, J.S., Drake, D.C., Latterell, J.J., O'keefe, T.C. and Balian, E.V., 2005. Origins, patterns, and importance of heterogeneity in riparian systems. In Ecosystem function in heterogeneous landscapes (pp. 279-309). Springer, New York, NY.

Nanson, G.C. and Croke, J.C. (1992) A genetic classification of floodplains. Geomorphology, 4, 459–486.

Naudé, M. (2012) Fynbos riparian biogeochemistry and invasive Australian Acacias. MSc Thesis. University of Stellenbosch, Stellenbosch. 192pp.

Negrete-Yankelevich, S., Fragoso, C., Newton, A.C., Russell, G. and Heal, O.W. (2008) Species-specific characteristics of trees can determine the litter macroinvertebrate community and decomposition process below their canopies. Plant and Soil (307), 83–97.

New, M. (2002) Climate change and water resources in the southwestern Cape, South Africa. South African Journal of Science, 98, 1–8.

Norby, R.J., Long, T.M., Hartz-Rubin., J.S. and O’Neill, E.G. (2000) Nitrogen resorption in senescing tree leaves in a warmer, $C0_2$-enriched atmosphere. Journal of Plant and Soil (224), 1.

Nyoka, B.I. (2003) Biosecurity in forestry: A case study on the status of invasive forest trees species in Southern Africa. Forest Biosecurity Working Paper FBS/1E. Forestry Department. FAO, Rome.

Oberholster, P., Dabrowski, J. and Botha, AM. (2013) Using modified multiple phosphorus sensitivity indices for mitigation and management of phosphorus loads on catchment level. Fundamental and Applied Limnology, 182, 1–16.

Ollis, D., Snaddon, K., Job, N. and Mbona, N., 2013. Classification system for wetlands and other aquatic ecosystems in South Africa: User manual: Inland systems. South African National Biodiversity Institute.

Parton, W.J. et al. (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. Science (315), 361–364.

Paudel, B., Montagna, P.A. and Adams, L. (2015) Variations in the release of silicate and orthophosphate along a salinity gradient: Do sediment composition and physical forcing have roles? Estuarine, Coastal and Shelf Science (157), 42–50.

Pérez-Harguindeguy, N., Díaz, S., Cornelissen, J.H., Vendramini, F., Cabido, M. and Castellanos, A. (2000) Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant and Soil (218), 21-30.

Perks, M.T. (2013) Spatial and temporal dynamics of fine fluvial sediment transfer: Implications for monitoring and management of upland river systems, Durham theses, Durham University. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/7307/.

Peterson, B.J. et al. (2001) Control of nitrogen export from watersheds by headwater streams. Science (292), 86–90.

Phillips, J.M., Russell, M.A. and Walling, D.E. (2000) Time-integrated sampling of fluvial suspended sediment: A simple methodology for small catchments. Hydrological Processes (14), 2589–2602.

Potgieter, G. (2012) N and P limitation of fynbos plants and the nutritional status of legume habitats in the Cape Floristic Region. MSc Thesis. Department of Botany, University of Cape Town, Cape Town.

Powell, K.I., Chase, J.M. and Knight, T.M. (2011) A synthesis of plant invasion effects on biodiversity across spatial scales. American Journal of Botany (98), 539–548.

Power, S. (2010) Soil P availability limits legume persistence and distribution in the fynbos of the Cape Floristic Region. MSc Thesis. Department of Botany, University of Cape Town, Cape Town.

Prenzel, J. (1979) Mass flow to the root system and mineral uptake of a beech stand calculated from 3-year field data. Plant and Soil (51), 39–49.

Prescott, C.E., Zabe, K.L.M., Staley, C.L. and Kabzems, R. (2000) Decomposition of broadleaf and needle litter in forests of British Columbia: Influences of litter type, forest type, and litter mixtures. Canadian Journal of Forest Research (30), 1742–1750.

Prins, N., Holmes, P.M. and Richardson, D.M. (2004) A reference framework for the restoration of riparian vegetation in the Western Cape, South Africa, degraded by invasive Australian Acacias. South African Journal of Botany (70), 767–776.

Pule-Meulenberg, F and Dakora, F.D. (2009) Assessing the symbiotic dependency of grain and tree legumes on N_2 fixation for their N nutrition in five agro-ecological zones of Botswana. Symbiosis, 48(1), 68–77.

Raimondo, D., Von Staden, L., Foden, W., Victor, J.E., Helme, N.A., Turner, R.C., Kamundi, D., Manyama, P. (Eds.), (2009). Red List of South African Plants, Strelitzia 25, South African National Biodiversity Institute, Pretoria.

Ramaswamy, V. et al. (2008) Distribution and sources of organic carbon, nitrogen and their isotopic signatures in sediments from ayeyarwady (irrawaddy) continental shelf, Northern Andaman Sea. Marine Chemistry (111), 137–150.

Rascher, K.G., Hellman, C., Maguas, C. and Werner, C. (2012) Community scale ^{15}N isoscapes: Tracing the spatial impact of an exotic N_2-fixing invader. Ecology Letters (15), 484–491.

Ratnam, J., Sankaran, M., Hanan, N., Grant, R. and Zambatis, N. (2008) Nutrient resorption patterns of plant functional groups in a tropical savanna: Variation and functional significance. Oecologia, 157(1), 141–151.

Rebelo, A.G., Boucher, C., Helme, N., Mucina, L. and Rutherford, M.C. (2006). Fynbos biome. In: Mucina, L., Rutherford, M.C. (Eds.), The Vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria, South Africa. Strelitzia (19), 53–219.Reinecke, M.K., Esler, K., Brown, C. and King, J. (2013) Link between lateral riparian vegetation zones and flow. MSc Thesis. University of Stellenbosch, Stellenbosch.

Reinecke, M.K., Pigot, A.L., and King, J.M. (2008) Spontaneous succession of riparian fynbos: Is unassisted recovery a viable restoration strategy? South African Journal of Botany, 74, 412–420.

Reinecke, M.K., King, J.M., Holmes, P.M., Blanchard, R., Malan, H.L. (2007). The nature and invasion of riparian vegetation zones in the South Western Cape. Report to the WRC on Project K5/1407.

Reinhart, K.O. and VandeVoort, R. (2006) Effect of native and exotic leaf litter on macroinvertebrate communities and decomposition in a western Montana stream. Diversity and Distributions (12), 776–781.

Rejmánek, M. and Richardson, D.M. (1996) What attributes make some plant species more invasive? Ecology (77), 1655–1661.

Rempel, L.L., Richardson, J.S. and Healey, M.C. (2000) Macroinvertebrate community structure along gradients of hydraulic and sedimentary conditions in a large gravel-bed river. Freshwater Biology (45), 57-73.

Richardson, D.M. and Cowling, R.M. (1992) Why is mountain fynbos invasible and which species invade? In: Fire in South African Mountain Fynbos (B.W. Van Wilgen, D.M. Richardson, F.J. Kruger and H.J. Van Hensbergen, eds.). Berlin: Springer-Verlag, 161–181.

Richardson, D.M., Macdonald, I.A.W., Hoffmann, J.H. and Henderson, L. (1997) Alien plant invasion. In: Vegetation of Southern Africa (R.M. Cowling, D.M. Richardson, S.M. Pierce, eds.). Cambridge, UK: Cambridge University Press, 535–570.

Richardson, D.M. et al. (2007) Riparian vegetation: Degradation, alien plant invasions and restoration prospects. Diversity and Distributions (13), 126–139.

Richardson, D.M. et al. (2011) Human-mediated introductions of Australian acacias: A global experiment in biogeography. Diversity and Distributions (17), 771–787.

Richardson, S.J., Peltzer, D.A., Allen, R.B. and McGlone, M.S. (2005) Resorption proficiency along a chronosequence: responses among communities and within species. Ecology (86) 20-25.

Richardson, D.M and Van Wilgen, B.W. (2004) Invasive alien plants in South Africa: How well do we understand the ecological impacts? South African Journal of Science (100), 45–52.

Rodríguez-Blanco, M.L., Taboada-Castro, M.M. and Taboada-Castro, M.T. (2010) Linking the field to the stream: Soil erosion and sediment yield in a rural catchment, NW Spain. Catena, In Press, Corrected Proof.

Richardson, A.E., Barea, J.M., McNeill, A.M. and Prigent-Combaret, C. (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil (321), 305-339.

Röhrig E. & Ulrich B. (1991) Ecosystems of the world: 7. Temperate Deciduous Forests. Elsevier, Amsterdam.

Rodríguez-Echeverría, S., Crisóstomo, J.A., Nabais, C. and Freitas, H. (2009) Belowground mutualists and the invasive ability of Acacia longifolia in coastal dunes of Portugal. Biological Invasions (11), 651–661.

Rodríguez-Echeverría, S., Le Roux, J., Crisóstomo, J. and Ndlovu, J. (2011) Jack-of-all-trades and master of many? How does associated rhizobial diversity influence the colonization success of Australian Acacia species? Diversity and Distributions (17), 946–957.

Rowntree, K.M. (1991) An assessment of the potential impact of alien invasive vegetation on the geomorphology of the river channels in South Africa. Southern African Journal of Aquatic Science, 17, 28–43.

Rowntree, K.M., Wadeson, R.A. and O'Keeffe, J. (2000) The development of a geomorphological classification system for the longi- tudinal zonation of South African rivers. South African Geo- graphical Journal (82), 163–172.

Rutt, G.P., Weatherley N.S. and Ormerod S.J. (1989) Microhabitat availability in Welsh moorland and forest streams as a determinant of macroinvertebrate distribution. Freshwater Biology (22), 247–261.

Ruwanza, S., Gaertner, M., Esler, K. J. and Richardson, D.M. (2013) Both complete clearing and thinning of invasive trees lead to short-term recovery of native riparian vegetation in the Western Cape, South Africa. Applied Vegetation Science, 16,193–204.

Saharjo, B.H. and Watanbe, H. (2009) Estimation of litterfall and seed production of Acacia mangium in a forest plantation in South Sumatra, Indonesia. Forest Ecology and Management (130), 265–268.

Sakai, A. et al. (2001) The population biology of invasive species. Annual Reviews of Ecology and Systematics (32), 305–332.

Salant, N.L., Hassan, M.A. and Alonso, C.V. (2008) Suspended sediment dynamics at high and low storm flows in two small watersheds. Hydrological Processes, 22, 1573–1587.

Samways, M.J., Sharratt, N.J. and Simaika, J.P. (2011) Effect of alien riparian vegetation and its removal on a highly endemic river macroinvertebrate community. Biological Invasions, 13(6), 1305–1324.

Santiago, L.S. (2007) Extending the leaf economics spectrum to decomposition: Evidence from a tropical forest. Ecology (88), 1126–1131.

Sardans, J., Rodà, F. and Peñuelas, J. (2004) Phosphorus limitation and competitive capacities of Pinus halepensis and Quercus ilex subsp. rotundifolia on different soils. Plant Ecology, (174) 307.

Schindler Wildhaber, Y., Michel, C., Burkhardt–Holm, P., Bänninger, D. and Alewell, C. (2012) Measurement of spatial and temporal fine sediment dynamics in a small river. Hydrology and Earth System Sciences (16), 1501–1515.

Schlosser, I.J. and Karr, J.R. (1981) Riparian vegetation and channel morphology impact on spatial patterns of water quality in agricultural watersheds. Environmental Management (5), 233-243.

Scholes, R.J. and Biggs, R. (2005) A biodiversity intactness index. Nature (434), 45.

Schulze, E.D., Gebauer, G., Ziegler, H. and Lange, O.L. (1991) Estimates of nitrogen fixation by trees on an aridity gradient in Namibia. Oecologia 88(3), 451–455.

Scott, D.F., Versfeld, D.B. and Lesch, W. (1998) Erosion and sediment yield in relation to afforestation and fire in the mountains of the Western Cape Province, South Africa. South African Geographical Journal, 80, 52–59, DOI: 10.1080/03736245.1998.9713644.

Sear, D.A. (1993). Fine sediment infiltration into gravel spawning beds within a regulated river experiencing floods: Ecological implications for salmonids. Regulated Rivers Research and Management, 8, 373–390.

Sear, D.A., Frostick, L.B., Rollinson, G. and Lisle, T.E. (2008) The significance and mechanics of fine-sediment infiltration and accumulation in gravel spawning beds. In, Sear, David A. and DeVries, Paul (eds.) Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. Bethesda, USA. American Fisheries Society, pp. 149-174.

Seeger, M., Errea, M.P., Beguería, S., Arnáez, J., Martí, C. and García-Ruiz, J.M. (2004) Catchment soil moisture and rainfall characteristics as determinant factors for discharge/suspended sediment hysteretic loops in a small headwater catchment in the Spanish Pyrenees. Journal of Hydrology, 288, 299–311.

Shearer, G. and Kohl, D.H. (1986) N_2-fixation in field settings: Estimations based on natural ^{15}N abundance. Australian Journal of Plant Physiology (13), 699–756.

Shelton, J.M., Samways, M.J. and Day, J.A. (2015) Predatory impact of non-native rainbow trout on endemic fish populations in headwater streams in the Cape Floristic Region of South Africa. Biological Invasions (17), 365-379.

Sieben, E.J.J. (2003) The riparian vegetation of the Hottentots Holland Mountains, Western Cape, South Africa. Unpublished PhD Thesis, University of Stellenbosch, Stellenbosch.

Sieben, E.J.J., Mucina, L., Boucher, C. (2009) Scaling hierarchy of factors controlling riparian vegetation patterns of the Fynbos Biome at the Western Cape. Journal of Vegetation Science (20), 17-26.

Sieben, E.J.J., Reinecke, M.K. (2008) Description of reference conditions for restoration projects of riparian vegetation from the species-rich fynbos biome. South African Journal of Botany (74), 401-411.

Sinkko, H. et al. (2011) Phosphorus chemistry and bacterial community composition interact in brackish sediments receiving agricultural discharges. Public Library of Science One (6), e21555, 1–13.

Smith, H.G. and Dragovich, D. (2009) Interpreting sediment delivery processes using suspended sediment-discharge hysteresis patterns from nested upland catchments, south-eastern Australia. Hydrological Processes, 23, 2415–2426.

Soderberg, K. and Compton, J.S. (2007) Dust as a nutrient source for fynbos ecosystems, South Africa. Ecosystems (10), 550–561.

Sponseller, R.A. and Benfield, E.F. (2001) Influences of land use on leaf breakdown in southern Appalachian headwater streams: A multiple-scale analysis. Journal of the Northern American Benthological Society (20), 44–59.

Sprent, J.I. (2009) Legume Nodulation: A Global Perspective. Chichester, UK: Wiley-Blackwell.

Staelens, J., Nachtergale, L., Luyssaert, S. and Lust, N. (2003) A model of wind-influenced leave litterfall in a mixed hardwood forest. Journal of Forest Research, 33(2), 201–209.

Stals, R. and De Moor, I.J. (eds). 2007. Guides to the freshwater invertebrates of southern Africa. Volume 10: Coleoptera. WRC Report No. TT 320/07, Water Research Commission, Pretoria. 263pp.

Starry, O.S., Valett, H.M. and Schreiber, M.E., (2005) Nitrification rates in a headwater stream: influences of seasonal variation in C and N supply. Journal of the North American Benthological Society (24), 753-768.

Statsoft., 2013. Inc. Tulsa, OK74104, USA.Steegen, A., Govers, G., Takken, I., Nachtergaele, J., Poesen, J. and Merckx, R. (2001) Factors controlling sediment and phosphorus export from two Belgian agricultural catchments. Journal of Environmental Quality (30), 1249-1258.

Stock, W.D. and Allsopp, N. (1992) Functional perspective of ecosystems. In: The Ecology of Fynbos: Nutrients, Fire and Diversity (R.M. Cowling, ed.). Cape Town: Oxford University Press, 206–225.

Stock, W.D. and Verboom, G.A. (2012) Phylogenetic ecology of foliar N and P concentration and N:P ratios across Mediterranean-type ecosystems. Global Ecology and Biogeography (21), 1147–1156.

Stock, W.D., Wienand, K.T. and Baker, A.C. (1995) Impacts of invading N_2-fixing Acacia species on patterns of nutrient cycling in two Cape ecosystems: Evidence from soil incubation studies and 15N natural abundance values. Oecologia, 101(3), 375–382.

Strauss, E.A. and Lamberti, G.A. (2000) Regulation of nitrification in aquatic sediments by organic carbon. Limnology and Oceanography (45),1854-1859.

Struyf, E. et al. (2012) Nitrogen, phosphorus and silicon in riparian ecosystems along the Berg River (South Africa): The effect of increasing human land use. Water SA (38), 597–606.

Stutter, M.I., Langan, S.J., Lumsdon, D.G. and Clark, L.M. (2009) Multi-element signatures of stream sediments and sources under moderate to low flow conditions. Applied Geochemistry (24), 800–809.

Tabacchi, E., Lambs, L., Guilloy, H., Planty-Tabacchi, A.M., Muller, E. and Decamps, H. (2000) Impacts of riparian vegetation on hydrological processes. Hydrological Processes, 14(16-17), 2959–2976.

Tang, L.Y., Han, W.X. and Chen, Y.H. (2013) Resorption proficiency and efficiency of leave nutrients in woody plants in eastern China. Journal of Plant Ecology (6), 408–17.

Tappin, A.D., Millward, G.E. and Fitzsimons, M.F. (2010) Particle-water interactions of organic nitrogen in turbid estuaries. Marine Chemistry (122), 28–38.

Tate, C.M., Broshears, R.E. and McKnight, D.M. (1995) Phosphate dynamics in an acidic mountain stream: interactions involving algal uptake, sorption by iron oxide, and photoreduction. Limnology and Oceanography (40), 938-946.

Taylor, J.C., Harding, W.R. and Archibald, C.G.M. (2007) An illustrated guide to some common diatom species from South Africa. WRC Report No. TT 282/07, Water Research Commission, Pretoria. 225pp.

Taylor, D.L., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C. and Ruess, R.W. (2014) A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecological Monographs (84), 3–20.

Tharme, R.E. (2010) Ecologically relevant flow for riverine benthic macroinvertebrates: Characterization and application. PhD Thesis. University of Cape Town, Cape Town.

Tharme, R.E. and Brown, C.A. (2004) Preliminary assessment of the ecological flow requirements of the Du Toit's River, Franschhoek. Final report to Cape Nature Conservation. Freshwater Research Unit, University of Cape Town, 42pp.

Tolsma, D.J., Ernst, W.H.O., Verweij, R.A. and Vooijs, R. (1987) Seasonal variation of nutrient concentrations in a semi-arid savanna ecosystem in Botswana. Journal of Ecology 75(3), 755–770.

Tye, D. (2013) Nutrient and biomass allocation strategies in an invasive Australian Acacia and a co-occurring native Acacia in South Africa. MSc Thesis. School of Animal Plant and Environmental Science, University of Witwatersrand, Johannesburg. Tye, D.R.C. and Drake, D.C. (2012) An exotic Australian Acacia fixes more N than a coexisting indigenous Acacia in a South African riparian zone. Plant Ecology, 213(2), 251–257.

U.S. EPA. 2007. "Method 3535A (SW-846): Solid-Phase Extraction (SPE)," Revision 1. Washington, DC.

Van der Colff, D., Dreyer, L.L., Valetine, A. and Roets, F. (2017) Comparison of nutrient cycling abilities between the invasive Acacia mearnsii and the native Virgilia divaricata trees growing sympatrically in forest margins in South Africa. South African Journal of Botany (111), 358–264.

Van der Perk, M. (2006) Soil and Water Contamination. Leiden, The Netherlands: Taylor and Francis/ Balkeman, 389pp.

Van der Waal B.W. (2009) The influence of Acacia mearnsii invasion on soil properties in the Kouga Mountains, Eastern Cape, South Africa. MSc thesis, Rhodes University: Grahamstown

Van der Waal, B.W., Rowntree, K.M. and Radloff, S.E. (2012) The effects of Acacia mearnsii invasions and clearing on soil loss in the Kouga Mountains, Eastern Cape. Land Degradation and Development, 23, 577–585.

Van der Wal, A., Geydan, T.D., Kuyper, T.W. and De Boer, W. (2013) A thready affair: Linking fungal diversity and community dynamics to terrestrial decomposition processes. FEMS Microbiology Reviews (37), 477–494.

Van Heerwaarden, L.M., Toet, S. and Aerts, R. (2003) Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. Oikos (101), 664-669.

Van Wilgen, B.W., Le Maitre, D.C. and Cowling, R.M. (1998) Ecosystem services, efficiency, sustainability and equity: South Africa's Working for Water programme. Trends in Ecology and Evolution, (13), 378–378.

van Wilgen, B.W., Reyers, B., Le Maitre, D.C., Richardson, D.M. and Schonegevel, L. (2008) A biome-scale assessment of the impact of invasive alien plants on ecosystem services in South Africa. Journal of Environmental Management, (89), 336-349.

Van Wilgen, B.W. et al. (2012) An assessment of the effectiveness of a large, national-scale invasive alien plant control strategy in South Africa. Biological Conservation, 148, 28–38. doi:10.1016/j.biocon.2011.12.035

van Wilgen, B.W., Fill, J.M., Baard, J., Cheney, C., Forsyth, A.T. and Kraaij, T., 2016. Historical costs and projected future scenarios for the management of invasive alien plants in protected areas in the Cape Floristic Region. Biological Conservation (200) 168-177.

Van Wyk, A.E. and Smith, G.F. (2001) Regions of floristic endemism in southern Africa: A review with emphasis on succulents. Pretoria: Umdaus Press, 207pp.

Veen, G.F., Freschet, G.T., Ordonez, A. and Wardle, D.A. (2015) Litter quality and environmental controls of home-field advantage effects on litter decomposition. Oikos (124), 187–195.

Versfeld, D. (1995) Catchment management and soil resources. In: Managing Fynbos Catchments for Water (C. Boucher and C Marais, eds.). FRD Programme Report Series 24, Pretoria. 64–74.

Versfeld, D.B., Le Maitre, D.C. and Chapman, R.A., 1998. Alien invading plants and water resources in South Africa. Report No. TT 99/98. Water Research Commission, Pretoria, p.276.

Verstraeten, G. and Poesen, J. (2002) Regional scale variability in sediment and nutrient delivery from small agricultural watersheds. Journal of Environmental Quality (31), 870-879.

Vidon, P. et al. (2010) Hot spots and hot moments in riparian zones: Potential for improved water quality management. Journal of the American Water Resources Association (46), 278–298.

Vigiak, O., Malagó, A., Bouraoui, F., Grizzetti, B., Weissteiner, C.J. and Pastori, M., 2016. Impact of current riparian land on sediment retention in the Danube River Basin. Sustainability of Water Quality and Ecology (8), 30-49.

Vitousek, P.M. et al. (1997a) Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications, 7(3), 737–750.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmánek, M. and Westbrooks, R. (1997b) Introduced species: A significant component of human-caused global change. New Zealand Journal of Ecology, 21(1), 1–16.

Vivanco, L. and Austin, A.T. (2008) Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. Journal of Ecology, 96(4), 727–736.

Volk, C.J., Kiffney, P.M. and Edmonds, R.L. (2003) Role of riparian red alder in the nutrient dynamics of coastal streams of the Olympic Peninsula, Washington, USA. American Fisheries Society Symposium (34), 213–225.

Vosse, S. (2007) The restoration potential of fynbos riparian seed banks following alien clearing. Unpublished M.Sc. Thesis, Stellenbosch University

Wall, D.H. et al. (2008) Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. Global Change Biology (14), 1–17.

Wallace, J.B., Eggert, S.L. (1997) Multiple trophic links of a forest stream linked to terrestrial litter inputs. Science (80), 277, 102.

Walling, D.E. (1983) The sediment delivery problem. Journal of Hydrology, 65, 209–237.

Walling, D.E. (2005) Tracing suspended sediment sources in catchment and river systems. Science of the Total Environment, 344 (1–3), 159–184.

Walling, D.E. (2008) The changing sediment loads of the world's rivers. In: Proceedings of the International Symposium of Sediment Dynamics in Changing Environments, Christchurch, New Zeeland, Wallingford UK, IAHS Press, IAHS Publication 325, 325–38.

Walling, D.E. and Webb, B.W. (1996). Erosion and sediment yield: A global overview. In: Erosion and Sediment Yield: Global and Regional Perspectives (D.E. Walling and B.W. Webb (eds.). International Association of Hydrological Sciences publication no. 236. Wallingford: IAHS Press.

Walling, D. E., Q. He, Q., Blake, W.H., (1999) Use of 7 Be and 137Cs measurements to document short- and medium-term rates of water induced soil erosion on agricultural land, Water Resource Research (35), 3865 - 3874.

Wang, F. and Chen, J. (2000) Relation of sediment characteristics to trace metal concentrations: A statistical study. Water Research (34), 694–698.

Webster, I.A., Ford, P.W. and Hancock, G. (2001) Phosphorus dynamics in Australian lowland rivers. Marine and Freshwater Research (52), 127–137.

Webster, J.R. and Benfield, E.F. (1986) Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics (17), 567–594.

Webster, J.R. and Waide, J.B. (1982) Effects of forest clearcutting on leaf breakdown in a southern Appalachian stream. Freshwater Biology (12), 331-344.

Wentworth, C.K. (1922). A scale of grade and class terms for clastic sediments. Journal of Geology, 30, 377–392.

Werner, C., Zumkier, U., Beyschlag, W. and Màguas, C. (2010) High competitiveness of a resource demanding invasive acacia under low resource supply. Plant Ecology (206), 83–96.

Whiting, P.J., Matisoff, G., Fornes, W. and Soster, F.M. (2005) Suspended sediment sources and transport distances in the Yellowstone River basin. Geological Society of America Bulletin (117), 515–529.

Wiegner, T.N., Hughes, F., Shizuma, L.M., Bishaw, D.K. and Manuel, M.E. (2013) Impacts of an invasive N_2-fixing tree on Hawaiian stream water quality. Biotropica (45), 409–418.

Witkowski, E.T.F. (1991) Effects of invasive alien Acacias on nutrient cycling in the coastal lowlands of the Cape Fynbos. Journal of Applied Ecology (28), 1–15.

Witkowski, E.T.F. (1994) Growth of seedlings of the invasive, Acacia saligna and Acacia cyclops in relation to soil phosphorus. Austral Ecology (19), 290–296.

Witkowski, E.T.F. and Mitchell, D.T. (1987) Variations in soil phosphorus in the fynbos biome, South Africa. The Journal of Ecology, 1159-1171.

Witkowski, E.T.F. and Mitchell, D.T (1987) Variations in soil phosphorus in the fynbos biome, South Africa. Journal of Ecology (75), 1159–1171.

Wolman, M.G. (1977) Changing needs and opportunities in the sediment field. Water Resources Research, 13, 50–54.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z. and Bongers, F. (2004) The worldwide leave economics spectrum. Nature (428), 821–827

Wright. I.J. and Westoby, M. (2003) Nutrient concentration, resorption and lifespan: Leaf traits of Australian sclerophyll species. Functional Ecology (17),10–9.

Wu, Y. et al. (2007) Sources and distribution of carbon within the Yangtze river system. Estuary, Coastal Shelf Science (71), 13–25.

Yelenik, S.G., Stock, W.D. and Richardson, D.M. (2004) Ecosystem level impacts of invasive Acacia saligna in the South African fynbos. Restoration Ecology (12), 44–51.

Yelenik, S.G., Stock, W.D. and Richardson, D.M. (2007) Functional group identity does not predict invader impacts: Differential effects of nitrogen-fixing exotic plants on ecosystem function. Biological Invasions (9), 117–125.

Zhang, J., Wu, Y., Jennerjahn, T.C., Ittekot, V. and He, Q. (2007) Distribution of organic matter in changjiang (Yangtze river) estuary and stable carbon and nitrogen isotopic ratios: Implications for source discrimination and sedimentary dynamics. Marine Chemistry (106), 111–126.

Zhang, L., Wang, H., Zou, J.W., Rogers, W.E. and Siemann, E. (2014) Non-native plant litter enhances soil carbon dioxide emissions in an invaded annual grassland. PLoS ONE (9), 1–8.

Zhang, S., Lu, X.X., Sun, H.Q., Han, J.T. and Higgitt, D.L. (2009) Geochemical characteristics and fluxes of organic carbon in a human-disturbed mountainous river (the Luodingjiang River) of the Zhujiang (Pearl River), China. Science of the Total Environment (407), 815–825.

Zimmermann, A.E. and Lapointe, M. (2005). Sediments infiltration traps: Their use to monitor salmonid spawning habitat in headwater tributaries of the Cascapedia River, Quebec. Hydrological Processes, 19, 4161–4177.

Page left blank intentionally

APPENDICES

Herbicide analyses

River water sampling

Sampling took place at Bainskloof and Franschhoek in the Western Cape, South Africa. River water samples were collected in the Wit River that flow through Bainskloof Pass. River water samples were also collected in the Du Toit's River that flow through Franschhoek Pass. Schott bottles (500 ml) were used to sample the surface water and sediment water from three different sites. Site A was a site invaded by invasive species, Site B was a cleared site and Site C was a natural site. The samples were kept on ice and transported to the laboratory where the solid phase extractions followed.

Solid phase extraction

The Max solid phase extraction column worked best to extract glyphosate, triclopyr, picloram and fluroxypyr. A United States Environmental Protection Agency guideline (U.S. EPA, 2007) was followed to condition the Max column. A 6 cc/150 mg cartridge was used to load 500 ml of river water onto the cartridge. The column was conditioned by loading 2 ml methanol, 4 ml 0.5 M NaOH and 2 ml ultrapure water first. The column was then rinsed using 4 ml 0.5 M NaOH and 2 ml ultrapure water after which 500 ml of the sample was loaded using auto-sampler tubes. The sample was diluted using 4 ml of a 2% formic acid in methanol mixture. The elution deviated from the EPA method due to the polarity of picloram, fluroxypyr and triclopyr. After the elution step, the sample was evaporated using an analytical nitrogen evaporator whereafter the sample was reconstituted in ultrapure water and sent for Liquid chromatography–mass spectrometry (LC/MS) analysis.

LC/MS conditions

Analytical grade glyphosate, triclopyr, fluroxypyr and picloram were obtained from Sigma-Aldrich (Germany). The standards were made up using ultrapure water in amber LC/MS vials. The standards were made using concentrations ranging from 10 ppm–0,001 ppm. 2,4-Dichlorophenyl acetic acid was used as an internal standard. The samples and standard curve samples were spiked using a known concentration of 1 ppm. A Waters Xevo triple quadrupole mass spectrometry system (Central Analytical Facility, Stellenbosch) was used for the analysis and was operated using a 0.3 ml/min flow rate. For glyphosate, a Thermo Hypercarb (5 µm, 2.1 × 100 mm) column was used with two solvents, 1% acetic acid and methanol in 1% acetic acid respectively. For picloram, triclopyr and fluroxyoyr a Waters BEH C18 (1.7 µm, 2.1 × 100 mm) column was used with two solvents, 0.1% formic acid and acetonitrile respectively.

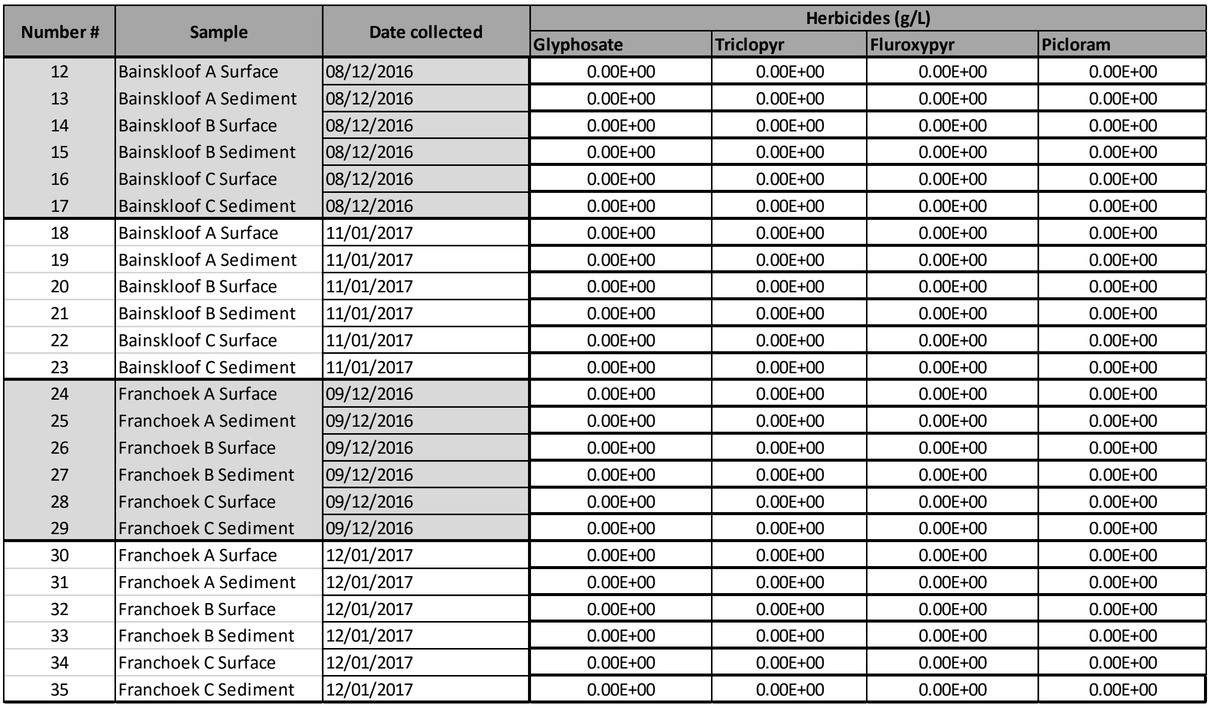
Table 28: Parent ions and daughter ions with their respective cone and collision energy for each active ingredient and DCPAA (internal standard)

Analyte	Parent ion m/z	Daughter ion m/z	Dwell (s)	Cone (V)	Collision energy(V)
DCPAA (infiltrated sediment)	203	159	0.016	5	10
	205	161	0.016	5	10

Analyte	Parent ion m/z	Daughter ion m/z	Dwell (s)	Cone (V)	Collision energy(V)
Glyphosate	170	60	0.161	15	20
	170	88	0.161	15	20
Triclopyr	254	196	0.016	25	10
	256	198	0.016	25	10
Fluroxypyr	255	181	0.016	30	25
	255	237	0.016	30	10
Picloram	241	195	0.016	25	25
	241	223	0.016	25	15

Results

Table 29: Results obtained from LC/MS analysis. Concentrations in ppm



Number #	Sample	Date collected	Herbicides (g/L)			
			Glyphosate	Triclopyr	Fluroxypyr	Picloram
12	Bainskloof A Surface	08/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
13	Bainskloof A Sediment	08/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
14	Bainskloof B Surface	08/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
15	Bainskloof B Sediment	08/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
16	Bainskloof C Surface	08/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
17	Bainskloof C Sediment	08/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
18	Bainskloof A Surface	11/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
19	Bainskloof A Sediment	11/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
20	Bainskloof B Surface	11/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
21	Bainskloof B Sediment	11/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
22	Bainskloof C Surface	11/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
23	Bainskloof C Sediment	11/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
24	Franchoek A Surface	09/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
25	Franchoek A Sediment	09/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
26	Franchoek B Surface	09/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
27	Franchoek B Sediment	09/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
28	Franchoek C Surface	09/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
29	Franchoek C Sediment	09/12/2016	0.00E+00	0.00E+00	0.00E+00	0.00E+00
30	Franchoek A Surface	12/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
31	Franchoek A Sediment	12/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
32	Franchoek B Surface	12/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
33	Franchoek B Sediment	12/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
34	Franchoek C Surface	12/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00
35	Franchoek C Sediment	12/01/2017	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Conclusion

No active ingredients were found from the Bainskloof and Franschhoek samples that were taken. This could be that the active ingredients were below the limit of detection for the LC/MS machine or that there was no active ingredient present in the river water sampled.