

# **Determining the biomass and nutrient content of the invasive *Acacia mearnsii* and *Eucalyptus camaldulensis* trees in Fynbos riparian zones**

by

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## **Declaration**

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## Summary

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Alien tree invasion into South African riparian zones remains a significant threat to water resources and biodiversity. While much has been done to limit the spread of invasive alien trees, there are still great strides to be made in successful management of extant invasive alien stands and in reducing the potential for future invasions and re-invasions. This dissertation sought to improve our understanding of stands of *Acacia mearnsii* (Black wattle) and *Eucalyptus camaldulensis* (River red gum) in riparian zones of the Western Cape of South Africa. Information is presented on the extent of invasion in selected catchment areas and its implications for rehabilitation of such sites post-clearing. Additionally, biomass products in the form of wood chips and biochar have been trialled to determine its efficacy as soil amendment agents in agriculture. Sites were selected along the upper to middle Breede River, where stands of either *A. mearnsii* or *E. camaldulensis* were present in high and low densities. Trees were sampled destructively for biomass and nutrient determinations. From different subsets of trees of the same invasive species, wood chips and biochar were produced and used in greenhouse-based experiments on the agricultural crops *Brassica napus* (canola) and *Triticum aestivum* (wheat).

Samples were grouped along density extremes, for which allometric models for biomass and nutrient contents showed divergent patterns throughout. Biomass and nutrient data were used to determine the potential for nutrient export from sites during large-scale clearing operations. It is widely accepted that nutrient accumulation through invasion could impair ecosystem recover. However, it is also estimated here that a high-density site of *A. mearnsii* could store approximately 110 t ha<sup>-1</sup> of AGB, which could contain up to 46 kg of P, 1200 kg of N, and 63 tonnes of C. Similarly, a high-density site of *E. camaldulensis* which has approximately 92 t ha<sup>-1</sup> of aboveground biomass (AGB), could store up to 531 kg of N, 78 kg of P, and 52 tonnes of C. These nutrients can be lost through large-scale biomass removal (including loss of C storage capacity) and most are likely to significantly impact recovery of affected ecosystems. The effect of short-term storage on the allelopathic potential of wood chips was also tested during this study on wheat and canola. For canola, fresh chips of *A. mearnsii* promoted shoot growth more than any of the other treatments. For wheat, however, fresh chips of *E. camaldulensis* were best suited for shoot growth. Allelopathic effects in AGB components of both invasive species were recorded in leachate from photosynthetic components and are minimal in stem material. It is suggested that the bulk stemwood is safest for soil applications and inclusion of other components may be appropriate in smaller quantities. With biochar, it was shown for both crop species that the addition of fertiliser is critical for shoot development. It is suggested that either or both of these biomass products be tested in an ecosystem rehabilitation context.

This study shows the need for a more effective means of invasive alien tree management that considers all the various aspects of invasion in South Africa. Large-scale removal of IAPs in

South Africa presents several opportunities, but could also pose significant future challenges if it is not done with consideration for natural processes and cycles. It is proposed here that the current invasive alien tree management models be re-evaluated to include new information such as soil nutrient enrichment and export, potential use of biomass and biomass products, the place of invasion as both a driver and a consequence of ecological disturbance, and the need for an integrated ecosystem management framework.

## Opsomming

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Indringing van uitheemse bome in Suid-Afrikaanse oewergebiede is steeds 'n bedreiging vir waterbronne en biodiversiteit. Alhoewel baie al gedoen is om die verspreiding van uitheemse indringerplante te beperk, is daar steeds baie stappe wat geneem moet word om hierdie indringerplante suksesvol te bestuur en om potensiele toekomstige indringing te verminder. Hierdie proefskrif het beoog om ons begrip te verbeter rakende indringing deur *Acacia mearnsii* (swart wattel) en *Eucalyptus camaldulensis* (rivier rooigom) in oewergebiede in die Wes Kaap van Suid Afrika. Inligting word aangebied oor die omvang van die indringing in geselekteerde areas in die Breede rivier opvanggebied, en die gevolge daarvan vir rehabilitasie nadat indringer-biomassa verwyder is. Boonop is biomassa-produkte in die vorm van houtskyfies en steenkool aangewend om die doeltreffendheid daarvan as grondwysigingsagente in landbou te bepaal. Persele was gekies lags die hoër-tot-middel- Breërivier waar bosse van *A. mearnsii* of *E. camaldulensis* in hoë en lae digthede aanwesig was. Monsters is geneem deur die gebruik van vernietigende metodes vir die bepaling van biomassa en voedinstowwe in bome. Houtskyfies en steenkool is vervaardig van verskillende onderafdelings van bome van dieselfde indringerspesies, en was gebruik in kweekhuis-eksperimente op die landbougewasse, *Brassica napus* (kanola) en *Triticum aestivum* (koring).

Monsters is volgens digtheidsextreme gegroepeer, waarvoor allometrieëse modelle vir biomassa en voedingsinhoud deurgaans uiteenlopende patrone vertoon het. Data vir biomassa en voedingstowwe is gebruik om die potensiaal vir die uitvoer van voedingstowwe vanaf steekproefpersele tydens grootskaalse skoonmaakoperasies te bepaal. Dit word algemeen aanvaar dat die ophoping van voedingstowwe deur indringing die herstel van die ekosisteem kan beïnvloed. Dit word egter ook hier geskat dat 'n hoëdigheidsgebied van *A. mearnsii* ongeveer 110 ton ha<sup>-1</sup> bo-grondse biomassa (BGB) kan bevat, wat tot 46 kg P, 1200 kg N en 63 ton C kan opberg. Ingselyks kan n *Eucalyptus camaldulensis* hoëdigheidsgebied wat ongeveer 92 t ha<sup>-1</sup> BGB het, kan tot 531 kg N, 78 kg P en 52 ton C opberg. Hierdie voedinstowwe kan verlore gaan deur grootskaalse verwydering van biomassa (inlsuitende n verlies aan koolstofopbergingskapasiteit) en meeste hiervan sal waarskynlik die herstel van geaffekteerde ekosisteme beduidend beïnvloed. Die effek van korttermynopslag op die allelopatiese potensiaal van houtskyfies is ook tydens hierdie studie op koring en kanola getoets. Vir kanola het vars skyfies van *A. mearnsii* die groei van lote meer bevorder as enige van die ander behandelings. Vir koring was die vars skyfies van *E. camaldulensis* egter die beste geskik vir die groei van lote. Allelopatiese effekte in BGB-komponente van albei indringerspesies is opgeteken in logwater van fotosintetiese komponente en is minimaal in stammateriaal. Daar word voorgestel dat die grootmaat stammateriaal die veiligste is vir grondtoediening, en dat ander komponente in kleiner hoeveelhede ingesluit kan word. Met biochar is daar vir albei gewasspesies gewys dat die toevoeging van kunsmis van kritieke belang is vir die ontwikkeling van lote. Daar word voorgestel

dat een of albei van hierdie biomassa-produkte in 'n ekosisteemrehabilitasie-konteks getoets word.

Hierdie studie toon die behoefte aan vir 'n meer effektiewe manier van indringerboombestuur wat alle verskillende aspekte van indringing in Suid-Afrika in ag neem. Grootskaalse verwydering van indringerplante in Suid-Afrika bied verskeie geleenthede, maar dit kan ook belangrike uitdagings vir die toekoms inhou as dit nie gedoen word met inagneming van natuurlike prosesse en siklusse nie. Dit word voorgestel hier dat die huidige modelle rondom die bestuur van indringerplante hervalueer word om nuwe inligting soos verryking en uitvoer van grondvoedingstowwe, potensiële gebruik van biomassa en biomassaprodukte, indringing as 'n drywer en 'n gevolg van ekologiese versteuring, en die behoefte aan 'n geïntegreerde ekosisteembestuursraamwerk in te sluit.

*This dissertation is dedicated to my late mother, Ellen Juba and my late sister, Angelique Joslynne Juba. Rest in peace.*

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## Preface

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This dissertation is presented as a compilation of 8 chapters:

<b>Chapter 1</b>	General Introduction and project aims
<b>Chapter 2</b>	Literature review: Alien plant invasion as an ecological disaster and simultaneous prospect for socio-economic opportunities in South Africa
<b>Chapter 3</b>	The aboveground <i>biomass allometry of the invasive Acacia mearnsii and Eucalyptus camaldulensis</i> <sup>a</sup>
<b>Chapter 4</b>	Nutrient dynamics of invasive <i>Acacia mearnsii</i> and <i>Eucalyptus camaldulensis</i> aboveground biomass components in riparian zones of the western cape, South Africa <sup>a</sup>
<b>Chapter 5</b>	Nutrient export in biomass a potential stressor on riparian zones invaded by <i>Acacia mearnsii</i> and <i>Eucalyptus camaldulensis</i>
<b>Chapter 6</b>	Value added products from invasive biomass: allelopathic potential of various biomass components on growth of wheat and canola <sup>b</sup>
<b>Chapter 7</b>	Value added products: the effect of biochar application on crop health is dependent in the addition of sufficient amounts of fertilizer
<b>Chapter 8</b>	Synthesis: Are we missing critical blind spots in invasive alien tree management?

### Oral presentations

<sup>a</sup> Presented as: Juba, R., Jacobs, S.M., Le Maître, D., (2017). Determining and modelling of biomass and nutrient stocks of *Acacia mearnsii* and *Eucalyptus camaldulensis* in Western Cape riparian zones. South African Association of Botanists, Milnerton.

<sup>a</sup> Presented as: Juba, R., Jacobs, S.M., Jacobs, K., Le Maître, D., Cogill, L., Maubane T., Slabbert, E., & Smart, R. (2017). Assessing ecological impacts of alien clearing methods along Western Cape riparian zones. Fynbos Forum, Swellendam.

<sup>a</sup> Presented as: Juba, R., Jacobs, S.M., Jacobs, K., Le Maître, D., Cogill, L., Maubane T., Slabbert, E., & Smart, R. (2017). Legacy effects of alien clearing methods on riparian functioning; is it sustainable? River Basin Management Conference, Skukuza.

### Poster presentations

<sup>b</sup> Presented as: Juba, R., Jacobs, S.M., & Le Maître, D. (2018). Allelopathic potential of *Acacia mearnsii* and *Eucalyptus camaldulensis* and effects on selected crop species. National Wetlands Indaba, Kimberley.

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## List of Abbreviations and Acronyms

<b>AGB</b>	Aboveground Biomass
<b>ANOVA</b>	Analysis of Variance
<b>ARC</b>	Agricultural Research Council
<b>BD</b>	Basal Diameter
<b>CARA</b>	Conservation of Agricultural Resources Act
<b>CBD</b>	Convention on Biological Diversity
<b>CFK</b>	Cape Floral Kingdom
<b>CFR</b>	Cape Floristic Region
<b>DAFF</b>	Department of Agriculture, Forestry, and Fisheries
<b>DEA&amp;DP</b>	Department of Environmental Affairs and Development Planning
<b>EC</b>	Electrical Conductivity
<b>GHGs</b>	Greenhouse Gases
<b>IAPs</b>	Invasive Alien Plants
<b>ICP-MS</b>	Inductively Coupled Plasma Mass Spectrometry
<b>ICP-OES</b>	Inductively Coupled Plasma- Optical Emission Spectrometry
<b>LSD</b>	Least Significant Difference
<b>MA</b>	Meta-Analysis
<b>NCCRP</b>	National Climate Change Response Policy
<b>NEMA</b>	National Environmental Management Act
<b>NWA</b>	Nation Water Act
<b>OPT</b>	Optimal Partitioning Theory
<b>PCA</b>	Principal Components Analysis
<b>SAPIA</b>	South African Plant Invaders Atlas
<b>TGA</b>	Thermal Gravimetric Analysis
<b>VAIs</b>	Value Added Industries
<b>VAPs</b>	Value Added Products
<b>WfW</b>	Working for Water

## Chapter 1: Background and project aims

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### 1.1 Introduction

Riparian ecotones are hotspots for processes that underlie ecosystems services such as production of clean water, acting as habitat for riparian plant and animal species, and immobilization of toxins (Naiman & Decamps, 1997). An important function of riparian zones is as natural systems for water quality management which provide the same benefits as waste-water treatment works without the construction and maintenance costs. Vegetation in these ecosystems can reduce eutrophication in water caused by high levels of phosphorus and nitrogen (Lowrance et al., 1983; Vymazal, 2014; Wiener et al., 2020), remove heavy metals from soil (Kumar et al., 1995; Raskin et al., 1997) and purify water contaminated by societal effluent (Lowrance et al., 1998; Vymazal, 2006; Vymazal, 2014).

With increasing understanding of riparian and aquatic processes, the roles of various nutrients in supporting riparian ecosystem services are becoming clear. These processes are threatened by invasive alien trees, which are exotic tree species that have become naturalised in a new area and can produce large numbers of reproductive offspring which are able to spread over considerable distances from their parent plants in short amounts of time (Richardson et al., 2000). Increasing knowledge of nutrient cycles, especially in riparian zones, has shed light on the impact of invasive alien *Acacias* on ecosystem functioning, especially in respect of significant shifts in nutrient dynamics (Gaertner *et al.*, 2011; Le Maître *et al.*, 2011; Tye & Drake, 2012; Naudé, 2012; Slabbert et al., 2014; Kambaj Kambol, 2013), leading to local enrichment of nutrients such as nitrogen (N) and phosphorus (P) and in some instances, increases in microbial activity (Slabbert *et al.*, 2014). These studies, however, mainly consider soil nutrient cycles and lack a directed effort at quantifying within-tree stocks, which is important considering the extent of invasions and the prospects of an ever-increasing invasion range (Henderson & Wilson, 2017). Invasive trees in riparian zones can grow in very Closed canopy stands and can reach large sizes, locking up substantial amounts of nutrients in biomass (Hall, 2002; Kering et al., 2012; Madalcho, 2016; Masters et al., 2016). Under natural conditions, nutrients eventually find their way to riparian soils and the aquatic environment where they are considered allochthonous subsidies, important for supporting food webs and ecological function (i.e. interception and sequestration of nutrients from groundwater as it moves through riparian areas into streams). This function is not only disrupted by invasive trees, but also possibly by its management, i.e. slash pile burning and herbicide use (Jacobs et al., 2017). This necessitates an in-depth discussion of invasion, its extent, and its potential as a valuable resource for concerted economic growth which minimizes ecological impacts and enhances efficiency of clearing practices.

Invasion by woody alien species in South Africa is not only an important ecological problem, but also an economic one. This is evident in the Western Cape region, where large-scale invasions in riparian zones by species such as *Acacia mearnsii* de Wild. (Black wattle) and *Eucalyptus camaldulensis* Dehnh (River red gum) have caused significant losses of natural plant diversity and have a greater impact on low flows than native species, requiring the government to allocate funds for their control and eventual eradication. The Working for Water (WfW) program, aimed at eradicating alien plants mainly along river systems, has spent just under R 700 million on alien clearing and education programs in the year 2013-2014. Van Wilgen et al. (2012) later noted that a total of R3.2 Billion was spent between 1995 and 2008 on alien clearing in South Africa; on average R213 million p.a. over 15 years. The expenditure has since increased by up to R2 billion per year and WfW has now spent more than R15 billion on alien plant removal between 1995 and 2017 (Van Wilgen et al., 2020). Van Wilgen et al. (2020) also reported that management of *Acacia* species alone cost up to R3.5 billion between 1995 and 2017.

Two decades since the start of the Working for Water (WfW) project, invasive species are still spreading. According to the South African Plant Invaders Atlas (SAPIA) the extent of invasion has increased considerably between the time of its earlier mapping activities in 2007 (Henderson, 2008) and the latest data made available in 2017 (Henderson and Wilson, 2017). One of the most prolific of these invasive species is *Acacia mearnsii*, an Australian leguminous species which has invaded South African ecosystems after being introduced for its use in the forestry sector. It incurs close to a third of the total resources spent annually on invasions (Wise et al., 2012). Proportional to the annual expenditure on its clearing, Le Maître et al. (2000) reported that *A. mearnsii* uses up to 25% of the total volume of water taken up by invasive trees in the Western Cape. In light of the current drought in the Western Cape, this is an especially important species: according to Morokong et al. (2016), removing aliens in major catchments could be one of the most cost-effective measures to make more water available for household and agricultural use.

In addition to its socio-economic impacts, invasion of riparian zones has important ecological consequences which, in many cases, are well documented. *Acacia mearnsii* is often referred to as a transformer species, as it is known to change soil chemical and biological properties, while also influencing growth of neighbouring native plant species (Naudé, 2012). This leads to eventual enrichment of soil nutrients such as N, and may have far-reaching ecological impacts, including establishment of other nuisance plant species, and possible eutrophication in river systems, while also reducing ecosystem services (Tye & Drake, 2012). This is also true for other invasive acacia species, including *A. saligna*, *A. cyclops*, *A. longifolia*, and *A. dealbata* (Stock et al., 1995; Gaertner et al., 2011; Le Maître et al., 2011). The notorious invasiveness of the species along riparian zones has been ascribed, in part, to its large investments in propagules, which are easily transported downstream by rivers, or stored in the soil to germinate at a later stage (Richardson et al., 2008).

Plants from the *Eucalyptus* and *Pinus* genera are used widely in commercial forestry (Zobel et al., 1987). Through its introduction to several countries, these plants also often become invasive and present a significant threat to local biodiversity (Richardson, 1998). Of the *Eucalyptus* species recorded through a rapid assessment in the Western cape and Mpumalanga provinces (*E. camaldulensis*, *E. cladocalyx*, *E. grandis*, *E. lehmanni*, *E. paniculata*, and *E. sideroxylon*), only *E. camaldulensis* and *E. grandis* were found to be clearly invasive, while the rest of the species were considered naturalized (Forsyth et al., 2004). Henderson (2001) categorised *E. camaldulensis* as a clear transformer species, having potential to significantly alter ecosystems they invade. *Eucalyptus camaldulensis* is also identified as one of the species to create closed canopies, effectively shading out undergrowth and creating a monotypic stand (SAPIA; Henderson, 2007). Despite its apparent effects on ecosystem functioning, plants from this genus are severely understudied in South African ecosystems. Elsewhere, *Eucalyptus* species have been subject of study with specific focus on its volatile compounds and its potential allelopathic properties, primarily in foliage (Kohli & Singh, 1991, Fatunbi et al., 2009). Allelopathy refers to the potential inhibitory effect of a chemical compound on a receiver organism.

However, contrary to their ecological and overall economic cost, invasions can also be used as a tool for job creation and economic development, as the plants have long provided rural communities with a semi-reliable source of products such as firewood, timber, and bark (sold for tannins). Geldenhuys (1999) and Shackleton (2007) also reported the use of fuelwood from invasive alien trees for a large proportion of their household energy needs. On a more industrial scale, invasive biomass is now also being viewed as a viable option for energy production. Recently, Mudavanhu et al. (2016) and Stafford & Blignaut (2017) evaluated the potential benefits of some invasive species along some rivers and invaded plains in South Africa and suggested various avenues for economic gain from such stands. It is thus possible to explore more ways in which woody invasive trees can be utilised for economic and ecological benefit to people and ecosystems affected by them, in order to minimize the amount of water resources lost to woody tree invasion, while also minimizing the net financial cost of clearing. In addition to this, job creation by WfW provides relatively stable income streams to disadvantaged communities where the plants occur.

## 1.2 Problem statement

Literature searches through Google Scholar were not able to find any previous studies of nutrient dynamics and temporal and spatial shifts in nutrient stocks of either *A. mearnsii* or *E. camaldulensis*. In the Western Cape, several studies have focussed on soil nutrient dynamics as affected by invasive alien plants and the legacy after they have been cleared (Witkowski, 1991; Jacobs et al., 2013; Nsikani et al., 2018; Holmes et al., 2020). Although studies of soils associated

with these plants can provide a fairly good idea of the state of nutrients in an area, they do not provide a holistic view of nutrient dynamics and its transfer between ecosystem pools. Studies of soil in isolation also do not provide much insight into temporal changes in nutrient dynamics within plants, as well as its response to differences in trees' spatial arrangement (Closed canopy vs. Open canopy). These studies also do not provide information on the potential nutrient losses following harvesting of the trees, chipping and use of those chips elsewhere, including local and international export.

The development of a holistic view of nutrient dynamics in invaded riparian zones entails include studies which encompass, and give account of, all tree components, across seasons, and in stands of different densities. Also, economic models spurring on the use of invasive biomass for financial benefit are still being developed (Mudavanhu et al., 2016; Vundla et al., 2016; Stafford & Blignaut, 2017; Stafford et al., 2017; Stafford et al., 2018; Gardner et al., 2020; Gaurav et al., 2020), but in many cases lack data on total resource availability (e.g. biomass, nutrients, bark for tannins) and thus lack accurate predictions of sustainability of such ventures. Additionally, advocating for use of invasive biomass in wood chips for agricultural soil augmentation (e.g. Fehmi et al., 2020) does not adequately address the possible negative effects on agricultural crops through, for instance, allelopathic chemicals. Most research on the effects of *Eucalyptus* chemicals has been done on leachates from leaves (Kholi & Singh, 1991; Singh et al., 2005; El-Khawas & Shehata, 2005; Verdeguer et al., 2009; Lisanework & Michelsen, 1993; Zhang et al., 2010; Hegab et al., 2016), and studies describing *Eucalyptus* allelochemical effects on neighbouring plants in an agricultural or in a natural setting are not readily available. It is still unclear whether all parts of the *Eucalyptus* plant produce allelopathic substances, and which parts can actually be used in soil treatment, and whether the theory of hormesis (stimulatory at lower doses and inhibitory at higher doses; Duke et al., 2006) applies to plant allelopathy. The use of invasive biomass in an agricultural context is discussed here with a specific focus on the allelopathic potential of the various aboveground biomass components, as well as wood chips stored over time. Lastly, our understanding of the long-term implications of large-scale removal of invasive biomass from nutrient poor Fynbos and riparian ecosystems disregard the potential impacts of nutrient exports on subsequent ecosystem processes.

This study will discuss the potential effect of alien tree biomass removal on localised nutrient pools and the subsequent implications for ecosystem recovery. I will discuss current extent of four stands of invasive trees of *A. mearnsii* and *E. camaldulensis*, the standing biomass of these trees, nutrient stocks in its various aboveground components, and the effect of season, spacing and life stage on biomass and nutrient allocation.

### 1.3 Research aims and Objectives

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

These can be broken down into 4 objectives, as follows:

1. Document the aboveground biomass ( $\text{kg dw ha}^{-1}$ ) and nutrient stocks ( $\text{kg ha}^{-1}$ ) of *Acacia mearnsii* and *Eucalyptus camaldulensis* in selected Fynbos riparian zones.
2. Determine the possible effect of alien clearing on local nutrient reserves
3. Determine the possible allelopathic effects of harvested plant material of all aboveground components of *A. mearnsii* and *E. camaldulensis* on growth of agricultural crops and report on allelopathic potential of wood chips of different ages.
4. Determine the agricultural potential of wood chips and biochar in soil enhancement for crop growth.

These are shown in Figure 1.1 linked to one another as a conceptual framework of how they contribute towards improving our understanding of the impacts of invasion, clearing and its general management has on subsequent ecosystem recovery.

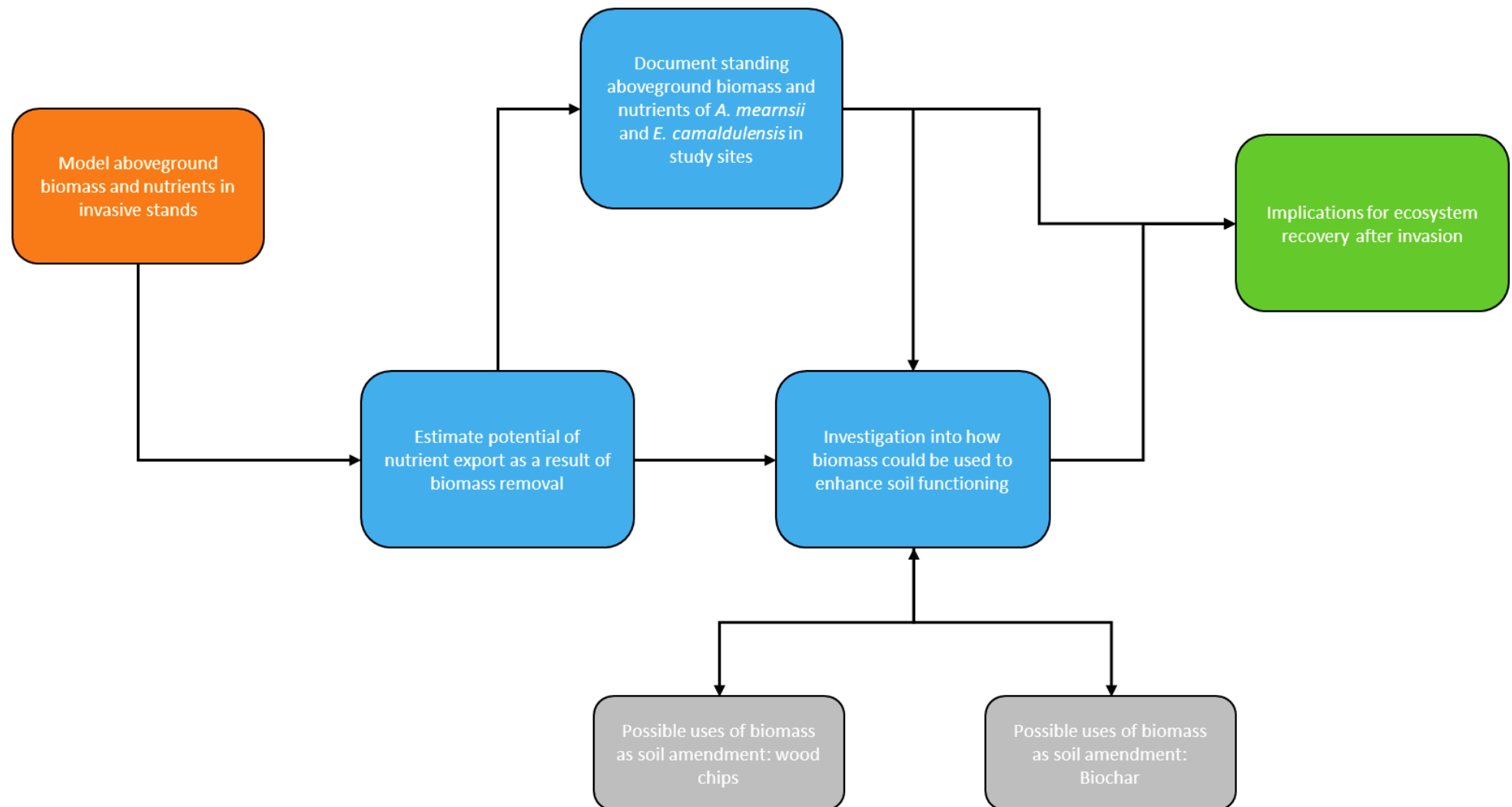


Figure 1.1: Layout of objectives of this study and how they are related and will be used to inform a discussion around the implications of alien clearing and management on ecosystem recovery.



## 1.4 Outcomes

The outcomes of this study are a set of biomass and nutrient allometric models for the invasive *A. mearnsii* and *E. camaldulensis* in selected riparian zones in the Western Cape province of South Africa. These high-resolution models consider environmental, seasonal, and stand age differences, while treating aboveground components (stem, leaves, bark, branches) of trees separately throughout. The models are then used to determine the standing biomass and nutrient stocks in the tested sites, extrapolated from single-tree analyses. The project also reports on possibilities for wood chips and biochar produced from these invasive species to be used as soil amendment on agricultural landscapes and their possible effects on crop development. As a consequence of increasing alien clearing, the study provides an account of the possible implications for nutrient cycle disruptions in sites cleared of invasive alien trees.

## 1.5 Significance

Alien clearing, especially in riparian zones, is necessary to maintain ecological integrity and reduce water wastage, but alien tree removal and rehabilitation are extremely time-consuming and expensive. Clearing methodologies might also significantly affect post-clearing rehabilitation, and it has been shown that felling and removal of invasive biomass is the most effective method of clearing to allow best ecosystem recovery (Blanchard & Holmes, 2008; Holmes et al., 2008). This project seeks to rethink the role of biomass, its constituent nutrient stocks in ecosystem functioning, and the impacts of large-scale tree removal on re-establishment of native species in cleared areas. This project also seeks to provide insight into the potential for using resources from invaded areas as a valuable cost-reduction mechanism to reduce net costs of clearing using income from selling excess biomass.

## 1.6 Scope and limitations

Because of significant site differences strengthened by variations in climate between South African provinces, and also site differences within provinces but between river systems and river reaches, the scope of this research is only applicable to upper to middle reaches of the Breede River catchment. These results and models could possibly be applied elsewhere but need to first be verified locally, and, if necessary, adapted accordingly. Roots of trees were not considered here in discussions of nutrient cycling and movement within trees. Finally, the

allelopathy experiments could not include perennial crops due to time constraints on the project.

## 1.7 Assumptions

This project only considers aboveground biomass. Models and numbers presented here are thus only applicable to stem, bark, branches, and foliar components of tree biomass. While belowground biomass makes up a large proportion of total tree biomass, the tree stumps and roots generally are not removed, so excluding their biomass and nutrient dynamics is not an issue for this study.

This project assumes that the extensive invasions provide a perfect opportunity for the use of biomass in value-added products, while excess biomass can be used for ecosystem rehabilitation. The inferences drawn from this study assume that everyone clearing invaded sites is well enough equipped to remove aliens and use the biomass efficiently. During biomass measurements, stem density counts were made to estimate total stand biomass. These counts were made in Closed canopy stands and for Open canopy trees; the results are extrapolated to a stand level and assume fairly homogenous distribution of stems and stem sizes. Lastly, biomass and nutrient models are created by destructively sampling a small portion of the extant populations and results are again up-scaled to describe stand-level biomass and nutrients, assuming all trees (including resprouts) follow similar trends.

## 1.8 Study area

### General

This study was conducted in the Breede River catchment of the Western Cape of South Africa, within riparian zones invaded by either *Eucalyptus camaldulensis* or *Acacia mearnsii*. The Breede River catchment, about 322 km in length and covering 12 600 km<sup>2</sup> (DEA&DP, 2017), has its source in the Skurweberg mountains between Ceres and Wolseley in the west, and joins the Atlantic Ocean close to Cape Infanta on the Agulhas Plain in the east. The river is joined in the west by the Wit River flowing through Bainskloof, the Molenaars River flowing through Du Toitskloof, the Holsloot River, which connects with the main channel just east of Rawsonville, and the Hex River which joins southeast of Worcester. Further east, the Breede River is joined by the Riviersonderend River and the Buffelsjags River.

The flows in the river are heavily influenced by agricultural water use, mainly in the form of irrigation (DEA&DP, 2017). Besides significant annual fluctuations in available water in the river as a result of strongly seasonal rainfall, water quality is increasingly being affected by land-use along the river (DEA&DP, 2017). The impacts of these land-uses include increased in salinity and pulses of agrochemicals or its overall concentrations as a result of agricultural intensification. These could be exacerbated by nutrient enrichment and decreases in microbial quality of the water due to dysfunctional wastewater treatment works, while the prominent dairy industry also significantly affects water quality and, as a result, impacts aquatic biodiversity. These activities have been highlighted as important sources of declines in water quality, leading to compromised aquatic ecosystems, and negatively affecting human health and downstream production systems (UNEP, 2016).

## Study sites

This study was done in 4 sites along the Breede River (Figure 1.2) that have been chosen to represent sites containing either of the two invasive species, with distinct areas of high and low density, and with multiple growth stages evident.

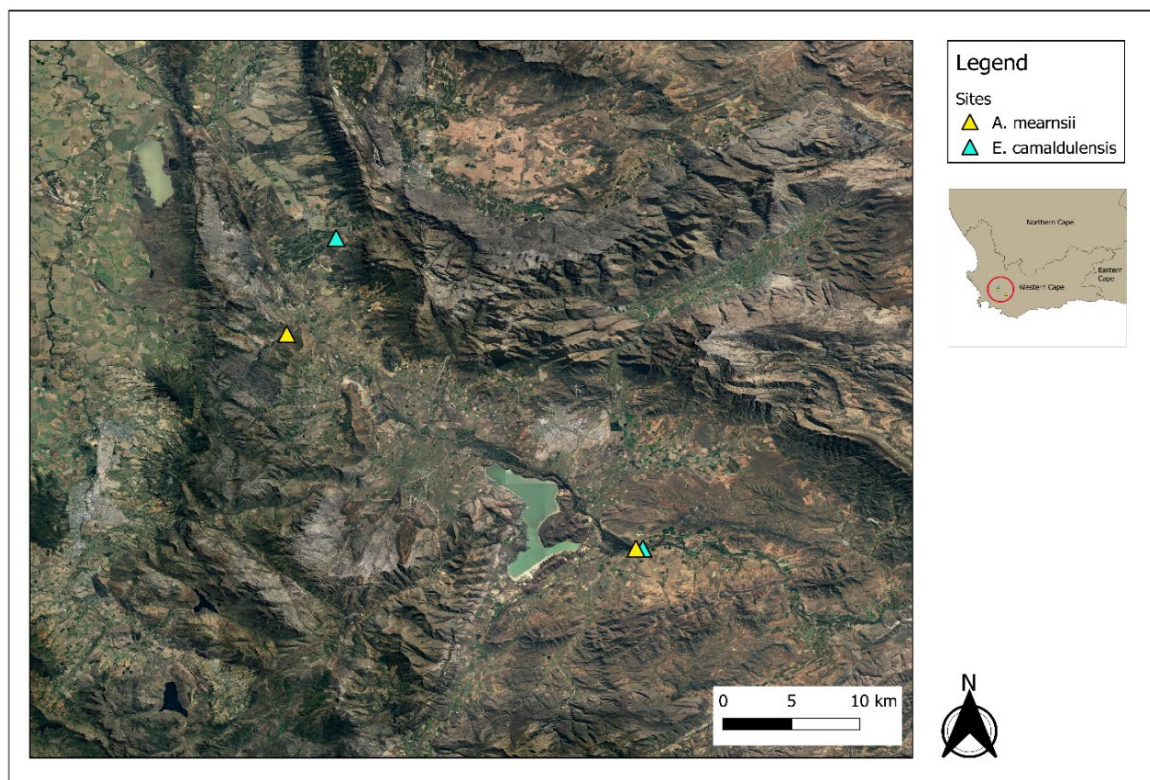


Figure 1.2: Map showing the location of the study sites in the Western Cape.



The first site at Wolseley is invaded by *Eucalyptus camaldulensis* and is situated on a mixed fruit farm (Figure 1.3). The site neighbours the Romansrivier, which is a tributary of the Breede. According to the landowner, the site has been invaded by *E. camaldulensis* for at least 50 years. The area between Wolseley and Tulbagh receives a mean of 370 mm of rainfall annually, which fluctuates between 210 mm and 610 mm, and mean daily temperatures that range between 4.8 °C in July and 29.3 °C in February (Rebello et al., 2006). The second site is located at the bottom of the Bainskloof/Bastaainskloof valley, next to the Wit River. This site is highly invaded by *Acacia mearnsii*. Clearing has been done in the area before, but wattles have since grown back vigorously. The mountainous area surrounding the river receives up to 1200 mm of rainfall annually, but which could range between 530 mm and 2140 mm, and a mean daily temperature of 4.4 °C during July and 25.4 °C in during February (Rebello et al., 2006). Lastly, two adjacent sites are located at Alfiesdrift, between Worcester and Robertson, next to the farm Alfalfa. These sites contain both *A. mearnsii* and *E. camaldulensis* in discrete stands of varying densities, and is located on the Breede River main channel. This site is the only site below the Brandvlei dam. The site is known to receive an average of 345 mm of rainfall annually, and experience mean daily temperatures of between 5 °C during July and 29.6 °C during February (Rebello et al., 2006).

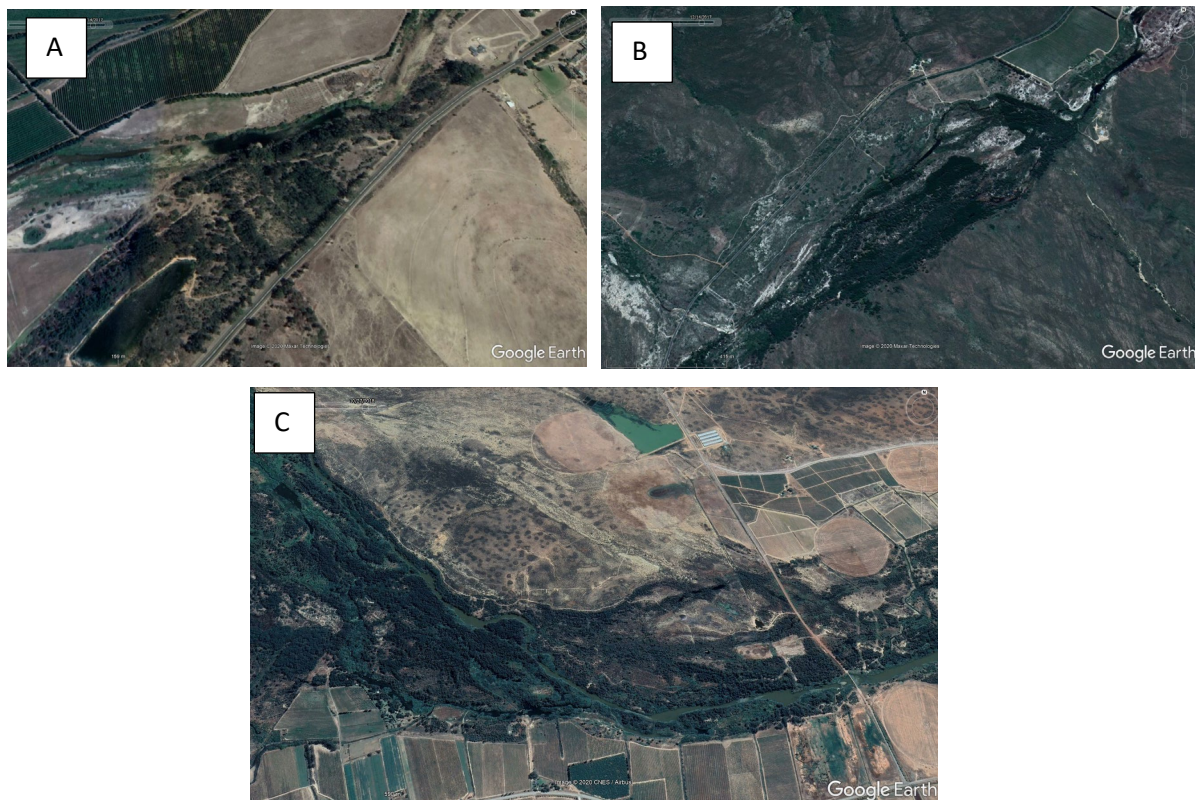


Figure 1.3: Sampling sites used during this study showing an *E. camaldulensis* site at Wolseley (A), an *A. mearnsii* site at Bainskloof (B) and a site invaded by both species in (C; *A. mearnsii* centre to left and *E. camaldulensis* towards the right)

## Stand densities

Throughout this study, sites are referred to as either Open or Closed canopy. This is based on a Normalised Difference Vegetation Index (NDVI) generated for each of the sites, which is a standardized, dimensionless index that quantifies vegetation vigour through the formula:

$$NDVI = (NIR - Red) / (NIR + Red) \quad (1.1)$$

Where NIR= near-infrared reflectance in the NIR spectral band and Red is the reflectance in the visible red band (Yengoh et al., 2015). The concept is based on the reflection of green light by chlorophyll and the absorption of red and blue light (Yengoh et al., 2015). In this instance, NDVI is used to identify sites of high-density invasion in predominantly Fynbos landscapes, and was paired with visual inspections to confirm species and to estimate canopy cover. Data were derived from the Fruitlook platform.

As a rule, Open canopy sites are described as sites with a vegetation index of less than 0.4 and canopy cover of invasion of less than 25%, while Closed canopy sites were those with a vegetation index of greater than 0.65 and canopy cover of more than 75% (Figure 1.4).

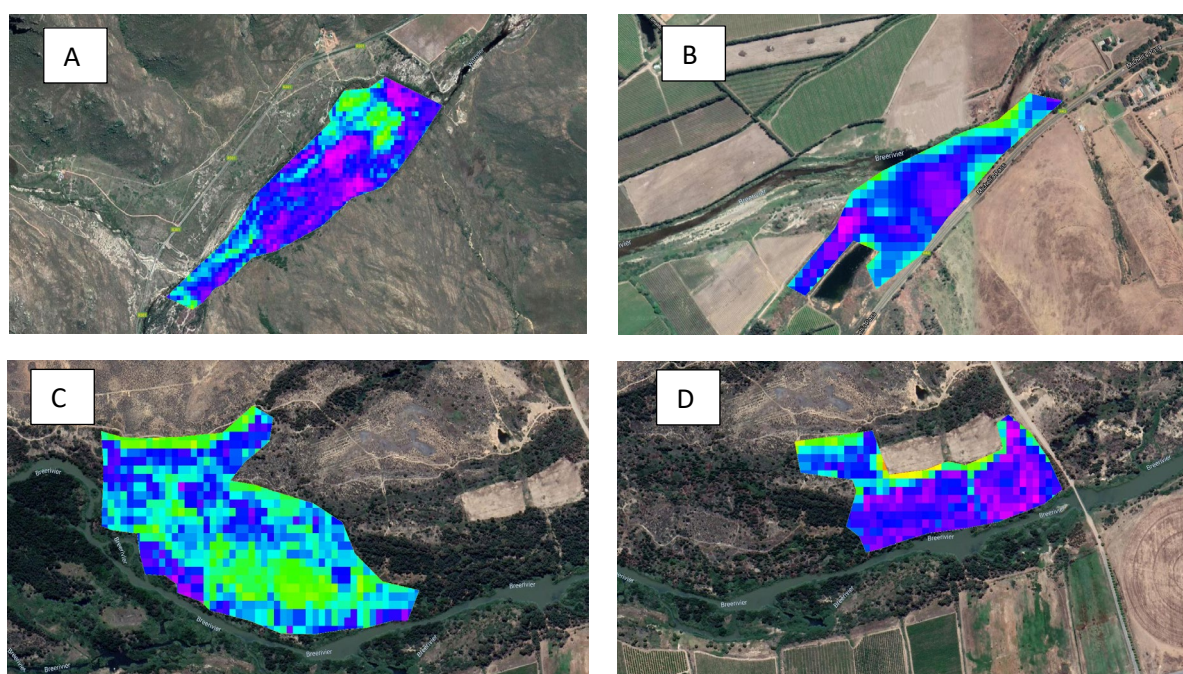


Figure 1.4: Maps produced from vegetation indices of sites investigated during this study. Darker shades show a higher vegetation index and thus an indication of greater biomass and plant vigour. All images are dated to April-August 2016. The sites shown here are Bainskloof (A; *Acacia mearnsii*), Wolseley (B; *Eucalyptus camaldulensis*), and Alfalfa (C; *Acacia mearnsii* and D; *Eucalyptus camaldulensis*). Pixel sizes are 20 m x 20 m (Source: Fruitlook)

## Zonation of sites

Table 1.1: Geomorphological zonation of sites use during this study, along with its longitudinal zones along the river, following Rowntree et al. (2000).

Site name	Invasive species	GPS coordinates	Longitudinal zone
Wolseley	<i>E. camaldulensis</i>	33°26'18.35"S; 19°13'36.26"E	Foothill; Cobble bed
Bainskloof	<i>A. mearnsii</i>	33°32'24.98"S; 19°10'36.26"E	Foothill; Cobble bed
Alfalfa	<i>E. camaldulensis</i>	33°46'2.79"S; 19°32'13.11"E	Lowland; Sand-bed
Alfalfa	<i>A. mearnsii</i>	33°46'3.34"S; 19°31'48.05"E	Lowland; Sand-bed

## Geology and flora

This study is conducted in the upper and middle reaches of the Breede River Catchment. The Upper Breede Catchment is characterized by Quartzitic Table Mountain sandstone in the mountains, with Bokkeveld and Malmesbury shale, and Enon conglomerate formations in the lower parts. Towards the middle reaches, on the low-lying plains surrounding Worcester, the geology loses its Enon conglomerate feature, and is mostly made up of Quartzitic Table Mountain Sandstone in the mountains and Bokkeveld and Malmesbury shale deposits in the valley bottom (Rebelo et al., 2006). The dominant vegetation type in the surrounding area is Breede Quartzitic Fynbos, while the floodplain of this area is classified as Breede Alluvium Fynbos (Rebelo et al., 2006), characterised by an average of 480 mm of annual rainfall (mostly between May and August) which supports relatively tall-growing shrubs growing on sandy soils. The geology of the floodplain is derived from Malmesbury and Bokkeveld group shale deposits (Rebelo et al., 2006).

## 1.9 Chapters overview

This dissertation is written as a literature review, four full-length scientific articles, one short note, and a synthesis of findings, all woven together with a common theme of aboveground biomass and nutrients in two invasive alien tree species.

**Chapter 2** discusses the available literature on plant invasion globally and locally. The chapter outlines some of the economic possibilities from invasive biomass in South Africa, including its potential for agricultural use as a soil amendment. This chapter also contains the theoretical framework for this study.



**Chapter 3** presents allometric models of biomass of *A. mearnsii* and *E. camaldulensis*, along with an in-depth look at the dynamics of the various aboveground biomass components as affected by tree growth stage and stem densities. Models generated in this chapter are based on stem basal diameter and are used to determine the biomass of the various aboveground tree components in the study sites investigated here.

**Chapter 4** builds on chapter 3 by using biomass data and laboratory analyses to determine nutrient dynamics in the aboveground biomass components of the two invasive species as a factor of plant age group, stem densities, and season. Models are also presented here to determine nutrient stocks in the field through basal diameter measurements.

**Chapter 5** considers the potential effects of alien clearing and export of nutrients in biomass on ecosystem nutrient balances by providing an account of the total nutrient exports that can be expected in typical sites invaded by *A. mearnsii* and *E. camaldulensis*.

**Chapter 6** Looks at the agricultural potential of invasive biomass in the form of wood chips. This chapter is inspired by the current perception amongst farmers of allelopathy of wood chips and aimed to address these. All the various aboveground biomass components are tested in terms of their allelopathic effects on wheat and canola through a petri dish germination experiment and a tunnel experiment.

**Chapter 7** is an extended discussion of biomass use in agriculture. In this chapter, biochar made from *A. mearnsii* and *E. camaldulensis* is used in ex-situ experiments with varying amounts of fertilizer to test its effectiveness on selected agricultural crops as affected by fertilizer addition. This chapter also provides a nutrient analysis of the biochar samples.

**Chapter 8** consolidates all findings during this study and makes suggestions for future studies. Conclusions are presented in a systemic manner through a short summary.

## 1.10 References

- Blanchard, R., & Holmes, P. M. (2008). Riparian vegetation recovery after invasive alien tree clearance in the Fynbos Biome. *South African Journal of Botany*, 74(3), 421-431.
- De Wit, M. P., Crookes, D. J., & Van Wilgen, B. W. (2001). Conflicts of interest in environmental management: estimating the costs and benefits of a tree invasion. *Biological invasions*, 3(2), 167-178.
- DEA&DP (2017). Environmental Resources Protection Plan for the Breede River Catchment in the Western Cape. Western Cape Government; Department of Environmental Affairs and Development Planning.
- Duke, S. O., Cedergreen, N., Velini, E. D., & Belz, R. G. (2006). Hormesis: is it an important factor in herbicide use and allelopathy?. *Outlooks on Pest Management*, 17(1), 29-33

- El-Khawas, S. A., & Shehata, M. M. (2005). The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on monocot (*Zea mays* L.) and dicot (*Phaseolus vulgaris* L.) plants. *Biotechnology*, 4(1), 23-34.
- Fatunbi, A. O., Dube, S., Yakubu, M. T., & Tshabalala, T. (2009). Allelopathic potential of *Acacia mearnsii* De wild. *World Applied Sciences Journal*, 7(12), 1488-1493.
- Fehmi, J. S., Rasmussen, C., & Gallery, R. E. (2020). Biochar and woodchip amendments alter restoration outcomes, microbial processes, and soil moisture in a simulated semi-arid ecosystem. *Restoration Ecology*.
- Forsyth, G. G., Richardson, D. M., Brown, P. J., & 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(1-2), 75-77.
- Gaertner, M., Richardson, D. M., & Privett, S. D. (2011). Effects of alien plants on ecosystem structure and functioning and implications for restoration: insights from three degraded sites in South African fynbos. *Environmental management*, 48(1), 57-69.
- Gardner, D. (2020). The benefits of clearing invasive alien plants: good governance. *FarmBiz*, 6(3), 36-37.
- Gaurav, G. K., Mehmood, T., Cheng, L., Klemeš, J. J., & Shrivastava, D. K. (2020). Water hyacinth as a biomass: A review. *Journal of Cleaner Production*, 122214.
- Hegab, M. M., Gabr, M. A., Al-Wakeel, S. A. M., & Hamed, B. A. (2016). Allelopathic potential of *Eucalyptus rostrata* leaf residue on some metabolic activities of *Zea mays* L. *Universal Journal of Plant Science*, 4(2), 11-21.
- Henderson L. (2001). *Alien Weeds and Invasive Plants. A complete guide to declared weeds and invaders in South Africa*. Agricultural Research Council, Pretoria
- Henderson, L. (2007). Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). *Bothalia*, 37(2), 215-248.
- Henderson, L., & Wilson, J. R. (2017). Changes in the composition and distribution of alien plants in South Africa: An update from the Southern African Plant Invaders Atlas. *Bothalia-African Biodiversity & Conservation*, 47(2), 1-26.
- Holmes, P. M., Esler, K. J., Gaertner, M., Geerts, S., Hall, S. A., Nsikani, M. M., Richardson, D. M., & Ruwanza, S. (2020). Biological invasions and ecological restoration in South Africa. In *Biological Invasions in South Africa* (pp. 665-700). Springer, Cham.
- Holmes, P. M., Esler, K. J., Richardson, D. M., & Witkowski, E. T. F. (2008). Guidelines for improved management of riparian zones invaded by alien plants in South Africa. *South African Journal of Botany*, 74(3), 538-552.
- Jacobs, S., Cogill, L., Jacobs, K., Juba, R., Maubane, T., Slabbert, E., & Smart, R. (2017). Assessing the impact of selected methods of removal of invasive alien *Acacia mearnsii* and *Eucalyptus camaldulensis* on fynbos riparian ecosystem function. *Report to the Water Research Commission. WRC Report No. 2343/1/16*
- Jacobs, S., Naude, M., Slabbert, E., Kambaj, O., Fourie, M., Esler, K., & Cowan, D. (2013). Identifying relationships between soil processes and biodiversity to improve restoration of riparian ecotones invaded by invasive acacias. *Report to the Water Research Commission. Report No. 1927/1*
- Kambaj Kambol, O. (2013). In situ and ex situ soil respiration in natural, *Acacia*-invaded and cleared riparian ecotones in the Fynbos Biome (MSc thesis, Stellenbosch: Stellenbosch University).
- Kohli, R. K., & Singh, D. (1991). Allelopathic impact of volatile components from *Eucalyptus* on crop plants. *Biologia plantarum*, 33(6), 475.
- Kohli, R. K., & Singh, D. (1991). Allelopathic impact of volatile components from *Eucalyptus* on crop plants. *Biologia plantarum*, 33(6), 475.



- Kumar, P. N., Dushenkov, V., Motto, H., & Raskin, I. (1995). Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental science & technology*, 29(5), 1232-1238.
- Le Maître, D. C., Gaertner, M., Marchante, E., Ens, E. J., Holmes, P. M., Pauchard, A., & Richardson, D. M. (2011). Impacts of invasive Australian acacias: implications for management and restoration. *Diversity and Distributions*, 17(5), 1015-1029.
- Lisanework, N., & Michelsen, A. (1993). Allelopathy in agroforestry systems: the effects of leaf extracts of *Cupressus lusitanica* and three *Eucalyptus* spp. on four Ethiopian crops. *Agroforestry systems*, 21(1), 63-74.
- Lowrance, R. (1998). Riparian forest ecosystems as filters for nonpoint-source pollution. In *Successes, limitations, and frontiers in ecosystem science* (pp. 113-141). Springer, New York, NY.
- Lowrance, R. R., Todd, R. L., Fail, J., Hendrickson, O., Leonard, R., & Asmussen, L. E. (1983). Waterborne nutrient budgets for the riparian zone of an agricultural watershed. *Agriculture, ecosystems & environment*, 10(4), 371-384.
- Morokong, T., Blignaut, J., Nkambule, N. P., Mudavanhu, S., & Vundla, T. (2016). Clearing invasive alien plants as a cost-effective strategy for water catchment management: The case of the Olifants River catchment, South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 774-787.
- Mudavanhu, S., Blignaut, J., Nkambule, N., Morokong, T., & Vundla, T. (2016). A cost-benefit analysis of using Rooikrans as biomass feedstock for electricity generation: A case study of the De Hoop nature reserve, South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 788-813.
- Naudé, M. (2012). Fynbos riparian biogeochemistry and invasive Australian *Acacias* (MSc thesis dissertation, Stellenbosch: Stellenbosch University).
- Nsikani, M. M., van Wilgen, B. W., & Gaertner, M. (2018). Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N<sub>2</sub>-fixing woody species: implications for ecological restoration. *Restoration ecology*, 26(2), 235-244.
- Raskin, I., Smith, R. D., & Salt, D. E. (1997). Phytoremediation of metals: using plants to remove pollutants from the environment. *Current opinion in biotechnology*, 8(2), 221-226.
- Rebelo, A. G., Boucher, C., Helme, N., Mucina, L., Rutherford, M. C., Smit, W. J., Powrie, L. W., Ellis, F., Lambrechts, J. J. N., Scott, L., Radloff, F. G. T., Johnson, S.D., Richardson, D. M., Ward, R. A., Procheş, S. M., Oliver, E. G. H., Manning, J. C., Jürgens, N., McDonald, D. J., Janssen, J. A. M., Walton, B. A., le Roux, A., Skowno, A. L., Todd, S. W., & Hoare, D. B. (2006). Fynbos biome. In *The vegetation of South Africa, Lesotho, and Swaziland* (No. 19, pp. 52-219). SANBI.
- Richardson, D. M. (1998). Forestry trees as invasive aliens. *Conservation biology*, 12(1), 18-26.
- Richardson, D. M., & Kluge, R. L. (2008). Seed banks of invasive Australian *Acacia* species in South Africa: role in invasiveness and options for management. *Perspectives in Plant Ecology, Evolution and Systematics*, 10(3), 161-177.
- Rowntree, K. M., Wadeson, R. A., & O'Keefe, J. (2000). The development of a geomorphological classification system for the longitudinal zonation of South African rivers. *South African Geographical Journal*, 82(3), 163-172.
- Shackleton, C. M., McGarry, D., Fourie, S., Gambiza, J., Shackleton, S. E., & Fabricius, C. (2007). Assessing the effects of invasive alien species on rural livelihoods: case examples and a framework from South Africa. *Human Ecology*, 35(1), 113-127.
- Singh, H. P., Batish, D. R., Setia, N., & Kohli, R. K. (2005). Herbicidal activity of volatile oils from *Eucalyptus citriodora* against *Parthenium hysterophorus*. *Annals of applied biology*, 146(1), 89-94.
- Slabbert, E., Jacobs, S. M., & Jacobs, K. (2014). The soil bacterial communities of South African Fynbos riparian ecosystems invaded by Australian *Acacia* species. *PloS one*, 9(1).

- Stafford, W. H., Von Maltitz, G. P., & Watson, H. K. (2018). Reducing the costs of landscape restoration by using invasive alien plant biomass for bioenergy. *Wiley Interdisciplinary Reviews: Energy and Environment*, 7(1), e272.
- Stafford, W., & Blignaut, J. (2017). Reducing landscape restoration costs: Feasibility of generating electricity from invasive alien plant biomass on the Agulhas Plain, South Africa. *Ecosystem Services*, 27, 224-231.
- Stafford, W., Le Maitre, D., Forsyth, G., Blanchard, R., von Maltitz, G., & Oboyade, A. (2017). Bioenergy potential from invasive alien plant biomass in South Africa. *In 2017 International Conference on the Industrial and Commercial Use of Energy (ICUE)* (pp. 1-7). IEEE.
- Stock, W. D., Wienand, K. T., & 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 15 N natural abundance values. *Oecologia*, 101(3), 375-382.
- Tye, D. R., & 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.
- UNEP (2016). A snapshot of the world's water quality: towards a global assessment. Nairobi, United Nations Environment Programme (UNEP)
- Van Wilgen, B. W., Forsyth, G. G., Le Maître, D. C., Wannenburgh, A., Kotzé, J. D., van den Berg, E., & Henderson, L. (2012). An assessment of the effectiveness of a large, national-scale invasive alien plant control strategy in South Africa. *Biological Conservation*, 148(1), 28-38.
- Van Wilgen, B. W., Wilson, J. R., Wannenburgh, A., & Foxcroft, L. C. (2020). The extent and effectiveness of alien plant control projects in South Africa. *In Biological Invasions in South Africa* (pp. 597-628). Springer, Cham.
- Verdeguer, M., Blázquez, M. A., & Boira, H. (2009). Phytotoxic effects of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. *Biochemical Systematics and Ecology*, 37(4), 362-369.
- Vundla, T., Blignaut, J., Nkambule, N., Morokong, T., & Mudavanhu, S. (2016). The opportunity cost of not utilising the woody invasive alien plant species in the Kouga, Krom and Baviaans catchments in South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 814-830.
- Vymazal, J., & Březinová, T. (2014). Long term treatment performance of constructed wetlands for wastewater treatment in mountain areas: four case studies from the Czech Republic. *Ecological engineering*, 71, 578-583.
- Vymazal, J., Greenway, M., Tonderski, K., Brix, H., & Mander, Ü. (2006). Constructed wetlands for wastewater treatment. *In Wetlands and natural resource management* (pp. 69-96). Springer, Berlin, Heidelberg.
- Wiener, K. D., Simaika, J. P., Grenfell, S. E., & Jacobs, S. M. (2020). Effects of invasive N2-fixing *Acacia mearnsii* on sediment nutrient concentrations in mountain streams: Implications of sediment geochemistry for ecosystem recovery. *CATENA*, 195, 104786.
- Wise, R. M., Van Wilgen, B. W., & Le Maître, D. C. (2012). Costs, benefits, and management options for an invasive alien tree species: The case of mesquite in the Northern Cape, South Africa. *Journal of Arid Environments*, 84, 80-90.
- 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*, 1-15
- Yengoh, G. T., Dent, D., Olsson, L., Tengberg, A. E., & Tucker III, C. J. (2015). Use of the Normalized Difference Vegetation Index (NDVI) to Assess Land Degradation at Multiple Scales: Current Status, Future Trends, and Practical Considerations. Springer.
- Zhang, C., & Fu, S. (2010). Allelopathic effects of leaf litter and live roots exudates of *Eucalyptus* species on crops. *Allelopathy Journal*, 26(1), 91-100.
- Zobel, B. J., Wyk, G. V., & Stahl, P. (1987). *Growing exotic forests*. John Wiley & Son

## Chapter 2: Literature review: Alien plant invasion as an ecological disaster and simultaneous prospect for socio-economic opportunities in South Africa

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### 2.1 Introduction

Invasions by alien species are, next to human-induced climate change, regarded as one of the greatest threats to South Africa's biodiversity due to their serious impacts on ecological processes (D'Antonio & Vitousek, 1992), and as a driver of ecological change (Sala, 2000; Van Wilgen et al., 2020). In South Africa, it is estimated that 180 alien plant species have invaded about 18 million hectares of the country (Le Maître et al., 2016). Henderson (2007) noted that 31 of the most important alien invaders are Australian species, most of which are trees. The South African Plant Invaders Atlas (SAPIA, 2007) illustrated the then state of plant invasion in the country and highlighted plants from the genera *Acacia* and *Eucalyptus* as the most important threat to Fynbos ecosystems (13 species of the former and seven of the latter; Henderson, 2007). Since the implementation of the SAPIA mapping program, continuous monitoring of invasives has shown that in some cases, such as for *Acacia* species, the plants have increased their distribution across the country, despite the best efforts of removal programs (Henderson & Wilson, 2017). According to Henderson & Wilson (2017), South Africa has a total of 773 alien plant species, most of which showed a range expansion in the study period between 2006 and 2016, as shown through data gathered from the South African Plant Invaders Atlas (SAPIA). Of these, the families *Fabaceae* (containing the *Acacia* genus) and *Myrtaceae* (containing the *Eucalyptus* genus) are ranked first and third on the list respectively of alien plant taxa in the country (Henderson & Wilson, 2017). The spread of these, and other invasive alien species needs to be curbed through a well-orchestrated and effective program.

A major concern regarding invasion by alien plants in South Africa is water use. Le Maître et al. (2000) estimated that the top ten most invasive species in the Fynbos Biome account for 88% of the total water uptake among invasive plants, the greatest of which is *Acacia mearnsii* (up to 1503 mm/ year in winter rainfall zones receiving between 906 mm and 1050 mm of rainfall per year ; Dye & Jarman, 2004). It is now estimated that invasion in South Africa could lead to runoff reduction of up to 1.44 billion m<sup>3</sup> year (Le Maître et al., 2016) and is projected to reach up to 3.15 billion m<sup>3</sup> year within 25 years (assuming a spread rate of 10% per year; Le Maître et al., 2020). Invasion in South Africa is, however, not confined to Fynbos riparian zones and may occur elsewhere, even flourishing in arid areas such as the Northern Cape (e.g. *Prosopis* spp.; Versveld et al., 2000 ; Shackleton et al., 2017). Recent destructive fires in the Southern Cape region illustrate the potential of invasion in higher-lying areas, as fires were greatly exacerbated by presence of IAPs (Kraaij et al., 2018 ; Forsythe et

al., 2019). However, the methods employed to remove aliens could have significant impacts on future prospects of restoration. For instance, when invasive stands are cleared, and the biomass removed or burned, the nutrients stored within the biomass are potentially also removed from that system, depending on its volatility in cases where biomass is burned. Nutrient export in this way may have an impact on soil nutrient stocks and significantly disrupt the system's nutrient cycles, and its effects can further be exacerbated through burning of biomass (Jacobs et al., 2017). It should, however, also be noted that leguminous species such as *A. mearnsii* could increase soil nitrogen through nitrogen fixing and may impair unassisted ecosystem recovery (Geartner et al., 2011). In such cases, complete removal of biomass (including litter) could decrease nitrogen deposition in such sites, and would assist in creating an appropriate environment for the establishment of native species (Nsikani et al., 2018).

## **2.2 Biomass allometry and nutrient dynamics of invasive alien trees**

The South African Environmental Observation Network (SAEON) has developed a carbon sinks atlas, which makes use of remote sensing, biomass modelling, and historic land cover data to monitor country-wide carbon stocks and flows (Feig et al., 2017; DEFF; 2020). Remote sensing applications such as LiDAR (light detection and ranging) have also been used to estimate fuel loads in Savanna areas to determine the impact of communal land use on tree conservation (Wessels et al., 2010). However, tree biomass modelling using destructive methodologies in South Africa has largely been confined to the forestry sector (Dovey, 2005; Dovey, 2012; Muyambo, 2017), where models are aimed at determining biomass of plantations of various *Eucalyptus* and *Pinus* species. These studies provide a good reference for the measurement of tree biomass in managed plantations, and the partitioning of nutrients and biomass to various tree components. There are, however, major differences between the dynamics of plantations and that of natural forests, including growth form of trees (allometry), environmental stressors, species interactions, tree life stages, and internal factors, such as phenology. There are also management activities in plantations directed at increasing volume and quality of merchantable wood per unit area used, in contrast to natural forests in South Africa. For instance, there has been a growing awareness of the value of growing forestry trees in deliberate mixtures with predetermined species that would enhance their productivity (Forrester et al., 2005; Kelty, 2006; Nicholls et al., 2006). Some trees have been found to increase stand-level productivity through positive interactions with forestry species such as complementary resource use and increase in nitrogen availability (in the case of nitrogen-fixing species) (Forrester et al., 2006; Kelty, 2006). Other physical management practices such as pruning (Pinkard, 2002; Montagu et al., 2003), residue management, spacing, weed

control, and fertilizer additions during early life stages (de Moraes Goncalves et al., 2004; Munoz et al., 2008) also affect wood quality and overall tree allometry in plantations. Management of plantations thus typically result in significant differences in productivity, and data generated from such systems cannot easily be compared to wild forest systems.

Another difference between managed plants and those growing in wild forests is natural resource allocation patterns are not modified by management practices to maximise yield. Fife et al. (2008), for instance, suggested that evergreen trees may move nutrients between different tree components during different stages of the plants' lifetime to accommodate its changing needs as it enters various life stages. This was also found by Lodige et al. (2014), who showed in a greenhouse experiment that differences in tree size of *Fagys sylvatica* (European beech) and *Picea abies* (Norway spruce) correlated with differences in partitioning of aboveground biomass. They also noted that environmental conditions such as drought and lack of sufficient light could (to a lesser extent than tree size) skew aboveground biomass partitioning. Poorter & Nagel (2000) term this change in allocation as a result of tree growth "ontogenic drift", suggesting that larger plants typically have to invest relatively more resources in support structures and leaf area. Importantly, Poorter & Nagel (2000) also note that, even though ontogeny plays a big role in allocation of biomass, specific environmental conditions can also be instrumental in determining the form and functioning of individual plants in their specific environments. This theory is supported by Wu et al. (2008), who showed that drought conditions around seedlings of *Sophora davidii* (David's mountain laurel) resulted in greater root growth, while minimizing height, leaf mass and leaf area, and total biomass. Canham et al. (1996) tested four woody species: *Acer rubrum*, *Pinus strobus*, and the light and water tolerant species *Acer saccharum* and *Quercus rubra*. Contrary to the later findings by Wu et al. (2008), they found that the latter two species did not change their root allocation patterns in response to a decrease in available soil resources, while the former two species showed a significant reduction in relative belowground growth as a response to increased resources. Vascular plant growth could be generalized through a set of mathematical equations that describe biomass allocation and account for the constant relationship between length and diameter of stems and branches as a means to avoid breaking (West et al., 1999).

Ultimately, resource allocation to biomass components is described by the Optimal Partition Theory (OPT), which suggests that plants allocate resources to where it can be applied most effectively towards satisfying the needs of the system (Thornley, 1972). However, there are several other mechanisms that also drive plant growth form like, for instance, within-species competition as a result of resource use overlap (Enquist et al., 1998), or physical constraints such as tree height as a result of stem diameter and strength (Niklas,

1994). It has also been suggested that population density of plants is not only inversely proportional to average mass of individuals, but that population densities are a function of average individual mass and not the other way around (Enquist et al., 1998). This refers to changes in access to resources for individuals as a result of changes to the average individual mass of a population. Resource availability to growth is thus in many instances responsible for the allometric growth of plants. In the Fynbos soils of South Africa, nutrient availability is often a limiting factor for plant growth, resulting in characteristic slow-growing, hardy shrubs dominating the landscape (Witkowski, 1988). Successful invasion by alien trees thus requires traits enabling efficient use of resources to allow fast growth in order to compete with native vegetation and other invasive plant species, which could possibly be explained by phenotypic expressions of resource allocation. Additionally, traits such as an ability to fix nitrogen through associations with root microbes (Witkowski, 1991) could negate the requirement for efficient resource use and allow species to escape certain resource limitations.

Another important factor in resource use and availability is seasonal changes, as trees can significantly alter their resource allocation as a response to external seasonal factors such as changes in ambient temperatures and amounts and types of precipitation (Pugliese, 1988). Moreover, the continuous-time model presented by Pugliese (1988) suggests a few important facets of resource allocation in plants, namely: i) the allocation of resources to vegetative biomass components is that which allows maximum potential for both reproduction and building up of reserves (carbohydrates) at the end of each growing season; ii) a trade-off exists between growth rates and reproductive function, whereas no reproduction takes place until an optimum size is reached, and that reproductive function does not express itself at the expense of maintaining size. These temporal changes in resource allocation could also be affected by internal phenological cycles such as seeding, which could require mobilization and re-allocation of large amounts of nutrients. It is thus necessary to account for seasonal differences in allocation of nutrients to various tree components of *A. mearnsii* and *E. camaldulensis* to improve the accuracy of allometric models throughout the year. Seasonal variations in nutrient concentrations have been documented for litter of the leguminous *A. saligna* and *A. cyclops* (Witkowski, 1991b) but none for *A. mearnsii* or *E. camaldulensis*.

Givnish & Vermeij (1976) presented leaf size and shape as an evolutionary trait to optimize harvesting of light energy, and to optimize leaf temperature for photosynthesis when the leaf is active, while preventing mortality when the leaf is not active. Rozendaal et al. (2006) noted physical differences in leaves from sunny and shaded sides of trees and attributed that to temperature regulation, and they reported higher nutrient contents in leaves more exposed to sunlight. Differences might even sometimes be seen in the colour of leaves harvested from sunny and shaded sides of a tree, leaves on the sunny side being slightly darker (Bergen,



1904; Moore et al., 1998). Plasticity in leaf physical and chemical characteristics within species as a response to sunlight intensity could thus be an important variable when discussing stand-level nutrient dynamics, especially in forests where shading plays a major role in tree growth (Popma et al., 1992; Rozendaal et al., 2006). These differences in biomass allometry and allocation of nutrients are important considerations for the use of biomass in agriculture or even in determining restoration potential of cleared sites. Overall, documentation of these and other processes assist greatly in our understanding of the physiological, phenological, and ecological aspects of plants.

Likewise, plasticity in leaf physical and chemical characteristics within species as a response to sunlight intensity could also be an important variable when discussing stand-level nutrient dynamics, especially in forests where shading plays a major role in tree growth (Popma et al., 1992; Rozendaal et al., 2006). An experiment on Western tent caterpillars' (*Malacosoma californicum*) inclination for leaves from the sunny side of northern hemisphere trees showed that caterpillars fed from leaves from the sunny side had significantly more body mass than those from the shaded side (Moore et al., 1998). The study concluded that leaves on the sunny side typically had higher nitrogen contents than others, and also had lower moisture contents. These differences in biomass allometry and allocation of nutrients are important considerations for the use of biomass in agriculture or even in determining restoration potential of cleared sites. More importantly in terms of economic potential of invasive plants, accurate modelling of biomass and nutrients could present an opportunity for more informed development of biomass products. Where not explicitly and statistically tested during sampling, including an appreciation for these variations in the sampling design could significantly increase the applicability of data generated from such studies.

### **2.3 Uses of invasive alien biomass**

While alien plant invasions in South Africa present a major threat to local biodiversity and ecosystem services, and have great potential to reduce productivity of agricultural land, they are potentially a valuable resource. In the Kromme, Baviaans and Kouga rivers, Vundla et al. (2016) reported potential for value added products from invasive species such as *A. mearnsii* and *A. saligna* and *Pinus* spp. and highlighted their potential for economic growth for the region. They suggested a partnering of clearing contractors with value added industries to co-finance projects, which was shown to reduce costs of alien removal and improve prospects of restoration. The National Climate Change Response Policy (NCCRP) requires a combined approach of methods to reduce greenhouse gas (GHG) emissions in South Africa (DEA,

2011). The Agriculture sector, including Forestry is identified by the NCCRP as having an important role to play in reducing current emissions of GHGs, while contributing to local economic growth. Value-added Industries (VAIs) using invasive biomass are regarded as an approach that would reduce environmental impacts of alien plant invasions and current clearing methods (such as slash-and-burn), provide financial relief for individuals involved in their processes, and provide the agricultural sector with valuable products such as biochar, wood chips, firewood, building material and wood pellets. Invasive species are widely used within rural areas as building material and for firewood (Shackleton et al., 2007). Pulp made from *A. mearnsii* chips, in particular, is in great demand in Asian markets, which was estimated in 2011 to support a US\$ 4.3b industry, based on the exportation of chips from countries like South Africa, Brazil, and Indonesia (see Griffin et al., 2011). Wood chips are also currently used locally as mulch for agricultural land, providing soil with enhanced water retention capabilities, while serving as a substrate for microbial activity.

Le Maître et al. (2000) estimated that about 10 million hectares of South African land is under invasion by exotic trees and shrubs. About 3.7 million hectares or 28% of the Western Cape was invaded to some degree, comprising 37% of the total invaded area in the country. The Breede River catchment was estimated to be 48% invaded (Le Maître et al., 2000). The Western Cape, in particular, thus has great potential to spearhead the use of alien biomass for economic benefit of rural communities. Electricity could also potentially be produced from invasive biomass as a substitute for fossil fuels. Stafford & Blignaut (2017) modelled biomass on the Agulhas Plain in the South-western part of the Western Cape and reported that up to 15 years of 2.6 MW electricity can be created from current stands of invasive alien trees in the region. These estimates only accounted for biomass within reach of nearby roads (200 m) and on slopes of less than 20°, and thus only consider about 36% of the total available biomass (Stafford & Blignaut, 2017). These findings echo and build on earlier estimations by Mudavanhu et al. (2016), who suggested the possibility of bio-electricity production from *Acacia cyclops* as a replacement to diesel. Economically, this is viable and potentially fairly sustainable in the medium to long term.

Another such product, biochar, (charcoal used as a mechanism for soil carbon storage) also has great potential for agricultural application as soil amendment. Little research has, however, been published in the South African context on the use of invasive plant biomass as feedlot in biochar production. One of the major points of contention in biochar production appears to be its nutrient filtering capacity, with some suggesting that it effectively reduces nutrient availability to crops (Sika & Hardie 2014). However, it has been reported widely that the presence of biochar has a positive effect on crop development (Chan et al., 2008; Steiner et al., 2008) and that this could be enhanced by treatment with an appropriate fertilizer addition



(Olivier, 2011). The effectiveness of biochar is also largely dependent on other factors during its production, including feedstock type (Chan et al., 2008; Gaskin et al., 2010; Aghoghovwia, 2018) and pyrolysis regime (Van Zwieten et al., 2010; Carrier et al., 2012). This has not been sufficiently reported on and represents a significant gap in our understanding of the product and its variable features. It is likely that invasive species such as *A. mearnsii* and *E. camaldulensis* could service a biochar economy in South Africa's nutrient-poor soils where it could assist with slow release of nutrients and housing of beneficial soil microbial communities. Wood chips, for instance, also require relatively little input costs as the process mainly involves cutting down stands of invasive trees, chipping, and transport. Cohen et al. (2015) deemed wood chips as a high value product that had great potential in the Western Cape. Of great interest in the context of the Western Cape is the use of chips on soil to assist in soil water retention and to improve microbial activity in areas where rainfall is becoming less frequent and less reliable. However, allelopathic properties have been associated with invasive trees in South Africa (Fatunbi et al., 2009; Ruwanza et al., 2015) and little is known about the direct impacts of *Acacia* and *Eucalyptus* biomass on locally produced crops.

## 2.4 Invasive biomass for agricultural use: issues of allelopathy

The need to utilize biomass for agriculture is often hampered by the occurrence of allelopathic compounds found within plants. Studies on allelochemistry have provided some insights into the different allelochemicals produced by plants as defensive mechanisms (de Moral & Muller, 1970, Gross, 1999). These chemicals have been grouped according to their molecular structures and, hence, their possible effects on receptor plants. Seigler (2006) summarizes some of these allelochemicals as follows: acetylenic compounds, which are generally important for plant-algal interactions, waxes which protect plants from attacks by fungi and bacteria, polyketides produced by fungi, which are mycotoxic (toxic to mammals), and polyketides, which are known to break down cell membranes. Tannins, known to occur widely in *Acacia* species, have also been known to be algicidal (Gross, 1999). Some allelochemicals identified in *E. camaldulensis* include *p*-cymene,  $\alpha$ -phellandrene, cuminal, phellandral, cineole,  $\alpha$ -pinene,  $\beta$ -pinene, and geranial (del Moral & Muller, 1970). These have been shown to inhibit the growth of some species, while not having any negative effect on the growth of others (Ahmed et al., 2008). Most published research on the effects of *Eucalyptus* chemicals has, however been done on leachates from fresh and dried leaves (e.g. Kholi & Singh, 1991; Lisanewok & Michelsen, 1993; Singh et al., 2005; El-Khawas & Shehata, 2005; Verdeguer et al., 2009; Zhang et al., 2010; Hegab et al., 2016; Table 2.2), and studies describing *Eucalyptus* allelochemical effects on neighbouring plants in an agricultural or in a natural setting are not

readily available. It is thus still unclear whether all parts of the *Eucalyptus* plant produce allelopathic substances, and which parts can be used in soil mulching for the purpose of ecosystem restoration and/or growth of agricultural crops.

Of all the studies listed below for *Acacia* species (Table 2.1), Swaminatham *et al.* (1989) was the first to suggest that bark extract may have stronger allelopathic potential than leaves. Interestingly, however, most studies since have still focussed their attention on leaf extracts of *Acacia* species. Fatunbi *et al.* (2009) again reported significant allelopathic effects of bark leachates of *A. mearnsii* on dry weight of cabbage (*Brassica oleracea*) and Africa love-grass (*E. curvula*). Bark, however, did not have a negative effect on germination of maize and also did not appear to negatively affect growth of any of the tested receiver plants. Conversely, Mehmood *et al.* (2011) reported that bark extract suppressed root and shoot growth of the weed *P. hysterophorus*. It is thus possible that the defining factor in allelopathy is more related to the physiological tolerance (or naivety) of the receiver plant and that allelopathy is relative rather than absolute.

In all but one study on *Eucalyptus* allelopathy (Zhang *et al.*, 2010; Table 2.2), only leaf allelopathy is considered. All of the studies in Tables 2.1 and 2.2 reported moderate to severe cases of allelopathic influence on growth of receiver plant species when treated with extracts from *Eucalyptus* leaf material. There is thus currently a need to explore other possibilities for handling excess biomass that could be economically and ecologically justifiable. Because of the great financial burden to remove aliens, Acosta *et al.* (2016) stressed the importance of new methods to control aliens and their spread. As can be seen by new initiatives surrounding use of invasive biomass, it is an opportune moment to consider invasions as a potentially valuable resource, as opposed to a persistent condition that threatens livelihoods and biodiversity. Clearing is not done fast enough to offset the rate of spread of IAPs, leading to a net increase in IAP range (Henderson & Wilson, 2017). Given the overwhelming presence of invasive alien trees in South Africa, the current annual expenditure on its control, and the differential impacts of various clearing methodologies on nutrient cycling and ecosystem rehabilitation, it is important that we consider options for control of aliens that are economically viable and offset the cost of clearing, and ecologically sound in that they do not jeopardise recovery of previously invaded sites.

Table 2.1: List of most relevant publications reporting possible allelopathic effects of various biomass components of various *Acacia* species on receiver crop and/or weed species. (Continues on next page).

<b>Acacia species</b>	<b>Biomass component</b>	<b>Receiver</b>	<b>Outcomes</b>	<b>Reference</b>
<b><i>A. auriculiformis</i></b>	Leaves	<i>Brassica juncea</i> , <i>Phaseolus mungo</i> , <i>Raphanus sativus</i> <i>Vigna unguiculata</i> <i>Cicer arietinum</i>	Inhibits germination and root development. Dosage dependent.	Hoque et al. (2003)
<b><i>A. auriculiformis</i></b>	Leaves	<i>Zea mays</i>	Inhibits germination root development and shoot growth. Dosage dependent.	Oyun (2006)
<b><i>A. confusa</i> <i>A. aulacocarpa</i> <i>A. auriculiformis</i> <i>A. cincinnata</i> <i>A. crassicarpa</i> <i>A. leptocarpa</i> <i>A. margium</i> <i>A. polystachya</i> <i>A. torfilis</i></b>	Leaves (dry)	<i>Lactuca sativa</i> <i>Medicago sativa</i> <i>Brassica rapa</i>	Overall growth inhibition at low doses.	Chou et al. (1998)
<b><i>A. mearnsii</i></b>	Leaves, stem, bark, root	<i>Brassica oleracea</i> <i>Eragrostis curvula</i> <i>Zea Mays</i>	Inhibited germination. Inhibition dependent on the biomass component used for extract.	Fatunbi et al. (2009)
<b><i>A. mearnsii</i></b>	Leaves, branches	<i>Conyza sumatrensis</i> <i>Trifolium spp.</i> <i>Echinochloa utilis</i> <i>Lactuca sativa</i>	Inhibited germination. The study also reports a stronger inhibitory effect on dicots than monocots.	Schumann et al. (1995)

<b>Acacia species</b>	<b>Biomass component</b>	<b>Receiver</b>	<b>Outcomes</b>	<b>Reference</b>
<b><i>A. leucopholea</i></b>	Leaves	<i>Sorghum vulgare</i> <i>Arachis hypogaea</i>	Inhibited germination, growth of roots and shoots with increases in dose concentrations.	Jayakumar & Manikandan (2005)
<b><i>A. saligna</i></b>	Leaves, stems	<i>Triticum aestivum</i> <i>Brassica napus</i>	Inhibited growth and germination at low doses of leaf extract. Leaves show greater inhibitory potential than stems and canola is affected more than wheat.	Kamel & Hammad (2015)

Table 2.2: List of most relevant publications reporting possible allelopathic effects of various biomass components of various *Eucalyptus* species on receiver crop and/or weed species (Continues on next page).

<b><i>Eucalyptus</i> species</b>	<b>Biomass component</b>	<b>Receiver</b>	<b>Outcomes</b>	<b>Reference</b>
<b><i>E. globulus</i> <i>E. citriodora</i></b>	Leaves	<i>Phaseolus aureus</i> <i>Lens esculentum</i> <i>Hordeum vulgare</i> <i>Avena sativa</i>	Dose-related growth inhibition through impairment of photosynthetic and respiratory activity.	Kholi & Singh (1991)
<b><i>E. citriodora</i></b>	Leaves	<i>Parthenium hysterophorus</i> (weed)	Dose-related effects; complete inhibition of germination at high doses. The study also reports lowered chlorophyll content and lowered respiratory activity due to treatment with Eucalyptus oils.	Singh et al. (2005)
<b><i>E. rostrata</i></b>	Leaves	<i>Zea Mays</i> <i>Phaseolus vulgaris</i>	Inhibited germination. The study also found changes in internal biochemical processes, consistent with increases in dose concentrations.	El-Khawas & Shehata (2005)
<b><i>E. camaldulensis</i></b>	Leaves	<i>Amaranthus hybridus</i> <i>Portulaca oleracea</i>	Complete inhibition of germination of both test species.	Verdeguer et al. (2009)
<b><i>E. globulus</i> <i>E. camaldulensis</i> <i>E. saligna</i></b>	Leaves	<i>Cicer arietinum</i> <i>Zea mays</i> <i>Pisum sativum</i> <i>Eragrostis tef</i>	Dose-dependent growth reduction and germination.	Lisanework & Michelsen (1993)
<b><i>E. grandis</i> <i>E. urophylla</i></b>	Roots Leaves	<i>Raphanus raphanistrum</i> <i>Cucumis sativus</i>	Extracts from leaf litter of all <i>Eucalyptus</i> species inhibited root	Zhang et al. (2010)

<b><i>E. citriodora</i></b>		<i>Brassica rapa</i>	and shoot growth increasingly at increasing doses for <i>R. raphanistrum</i> and <i>B. rapa</i> but not for <i>C. sativus</i> . Root results were variable.	
<b><i>E. camaldulensis</i></b>	Leaves, root, bark, fruit	<i>Lycopersicon esculentum</i>	Inhibitory effect is dose-dependent. Most pronounced in leaf extract treatment.	Fikreyesus et al. (2011)
<b><i>E. rostrata</i></b>	Leaves	<i>Zea mays</i>	The study reports a stimulatory effect in low doses and an inhibitory effect in higher doses.	Hegab et al. (2016)

## 2.5 Impacts of invasive alien tree management on nutrient cycling; returns and export

Nutrient cycling through invasive forest biomass in Fynbos riparian zones is an important facet of stand productivity, but still most research in the area is focussed mainly on soil conditions as affected by these stands. Invasive alien plant impacts on soil physicochemical properties in riparian zones of the Western Cape have been the subject of several studies discussing the effects of invasive alien plants on surrounding ecosystem properties, including soil physicochemical properties (Naudé, 2012; Kambaj Kambol, 2013), soil microbial communities (Slabbert et al., 2014), and surrounding vegetation (Naudé, 2012). Typically, these studies compare sites that have been invaded to sites that have been cleared and sites in pristine condition. Naudé (2012) for instance worked in stands of *Acacia mearnsii* and found significant increases in available N in soils where these trees grow and attributed that to their ability to fix nitrogen through symbioses with soil bacteria and through litterfall. The study (Naudé, 2012) reported retarded recovery of indigenous woody cover after clearing, as invasive grasses were most prevalent in these cleared sites. According to Nsikani et al. (2018), the establishment of native species could be negatively affected by increases in soil nutrient levels, especially N and P, as a result of the invasion, which could lead to the establishment of secondary invaders. Similar studies by Kambaj Kambol (2013) and Fourie (2014) discussed impacts of invasive *Acacia* species on different soil physicochemical properties and reported significant influences on soil nutrient dynamics. Nutrient deposition as a result of invasions could thus have detrimental impacts on ecosystem recovery. In the naturally nutrient-poor Fynbos, issues such as nitrogen fixation by invasive legumes have been linked to lowered prospects of recovery after alien clearing (Nsikani et al., 2018). In these instances, removal of excess nutrients through removal of litter may be necessary (Nsikani et al., 2018).

An aspect of invasion and clearing techniques much less discussed in South African literature is the export of nutrients from cleared sites. As mentioned above, some species may be able to enrich their environments with elements such as N while alive, and if left to decompose on site, nutrients removed from the soil can be expected to be returned. This is, however, not the case in some instances, as practices such as fell and burn, or complete biomass removal from riparian zones are fairly common. Studies describing this are mostly done in tropical forests (Kauffman et al., 1993; Kauffman et al., 1994; Markewitz et al., 2004). All these studies reported high losses in components such as C and N after biomass was cleared and burned; Kauffman et al. (1994) reported losses of up to 56% of total stocks of carbon and 68% of nitrogen stocks due to this practice in Brazil. Markewitz et al. (2004) showed that a change of land use from tropical forest to a secondary forest (forest recovering

after mature forest is removed for pastures) leads to significant long-term decreases in total nutrient stocks.

Thus, while accumulation of some nutrients has been identified as a threat to ecosystem recovery, potential export of others through removal of invasive biomass has not received the same attention. It is thus still unclear whether nutrient export from cleared sites has an impact on ecosystem recovery. Sankaran *et al.* (2008), however, recorded potential rates of nutrient export in *E. grandis* and *E. tereticornis* in plantations in India and suggest that these exports would not be likely to be replaced by atmospheric sources. The study also concluded that the removal method is important in site nutrient dynamics. The bulky stems generally have much lower concentrations of nutrients (in this case N, P, K, Ca, and Mg), which, when removed, only accounts for small quantities of the total available pools of the nutrients tested. These quantities increase significantly when leaves, bark and branches are removed along with the stem. Although this study refers to plants grown in plantations, the implications are potentially the same for natural ecosystems where IAPs are removed.

It is thus important to conserve local nutrient cycles and this needs to consider the entire suite of nutrients taken up by IAPs, the source of each of these nutrients, and how this compares to impacts from native plant species. Attiwill *et al.* (1993) described nutrient cycling in an ecosystem, where nutrients can be seen as distributed between live wood (standing tree), dead wood (litter and dead stems and branches), atmospheric, and soil nutrients (Figure 2.1). Elements like N, P and cations would possibly be diminished in availability as organic matter inputs are reduced. This is especially relevant for stands of evergreen trees which can store nutrients in their biomass for several years before senescence. It is thus probable that clearing, and removal methods greatly influence future nutrient cycling and hence prospects of rehabilitation.



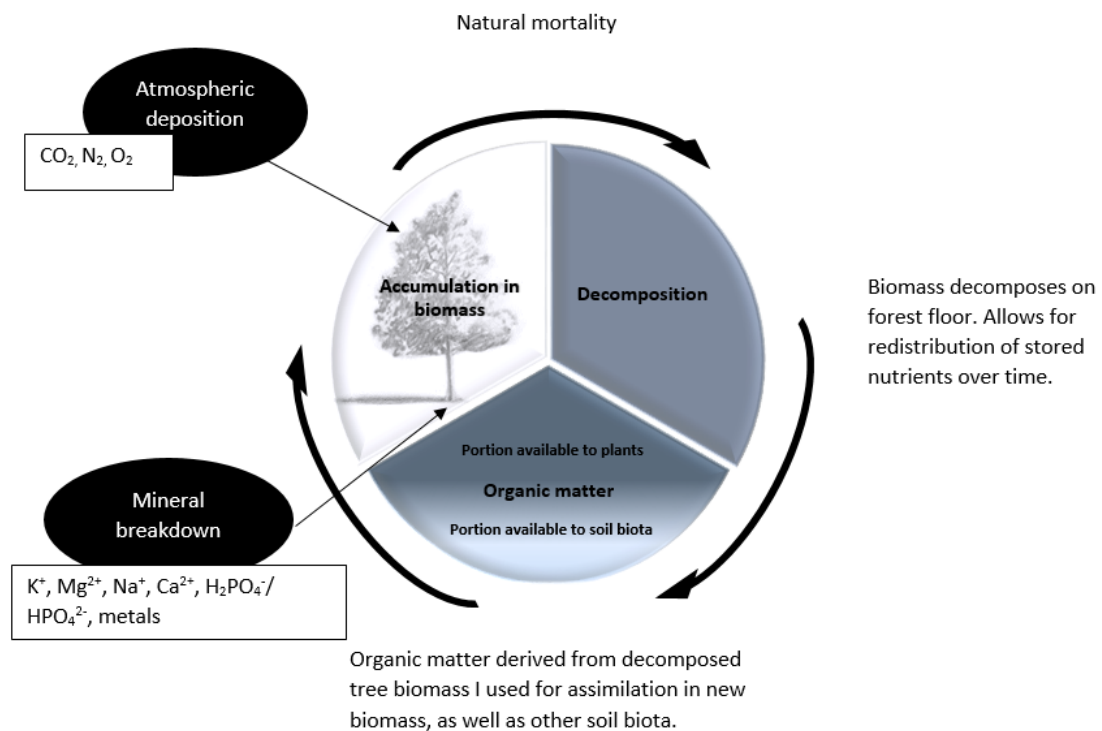


Figure 2.1: Cycling of nutrients in a generic ecosystem showing some of the major phases through which nutrients go between plants and soil.

Rapid clearing of alien trees, however, requires effective removal of biomass to prevent structural damage to infrastructure (e.g. waterways) and to minimize the risk of severe fires. Biomass is thus often burned under controlled conditions. However, burning of inappropriately large amounts of biomass could significantly impair an ecosystem's capacity for recovery (Jacobs et al., 2017). The presence of greater fuel loads associated with invasive alien trees could facilitate fires of greater intensity and have a significant effect on a catchment's hydrology (Le Maître et al., 2014), by temporarily increasing soil hydrophobicity (Fox et al., 2007; Mirbabaei et al., 2013) and encouraging soil erosion (Neary et al., 1999; Doerr et al., 2009). By altering soil properties, fires may even prevent re-establishment of vegetation, disturbance to microbial communities, (Neary et al., 1999; Korb et al., 2004) and altering soil physicochemical properties (Jacobs et al., 2017). In their review of alien clearing methods, Blanchard & Holmes (2008) concluded that the "fell-and-remove" option was better than "fell-and-burn" and "fell-only", as the method allowed for restored sites that most resembled reference conditions. The fell and burn practice was ranked the least favourable as it significantly reduced the ability of indigenous vegetation to re-establish as a result of mortality

to indigenous resprouters and reduction of viable seed banks (Blanchard & Holmes, 2008). Pretorius et al. (2008) supported these findings, noting that in post-clearing and post-burn sites, the overwhelming majority of re-establishing plants are woody aliens, including the resprouting individuals that survived burning. However, Vosse et al. (2008) and Fourie (2008) found that riparian seedbanks may still largely be intact after heavy invasion and that an invaded riparian zone could potentially recover by itself if clearing takes seedbank viability into consideration. Disturbances such as fires and complete removal of biomass lead to nutrient losses in the form of wind-blown ash, soil erosion, and percolation of solutes (Foster & Bhatti, 2006). It is possible to quantify nutrient export and its implications for future biological growth. To draw reliable estimates of nutrient exports from activities such as fell-and-burn compared to regular harvesting requires consideration of plant and stand-level variations in nutrient and biomass allocation, the environmental conditions that dictate such variation, and the possible levels of plasticity that can be found at these levels.

## **2.6 Theoretical framework**

Woody invasive biomass has been used successfully in various value-added products that include wood chips, biochar, wood pellets, furniture, and bioelectricity. Products used in agriculture, specifically wood chips and biochar need to be tested to determine efficacy towards soil and crop improvement. Given the possibilities for successful incorporation of invasive biomass in value added products partnered with an appropriate financing mechanism, it can be envisaged that clearing of invasive trees would be more successful. This would, however (due to increased area being cleared) require greater inputs into rehabilitation efforts, which are likely to be affected by nuanced effects of invasive trees on previously invaded sites including, but not limited to, deposition of nutrients in originally nutrient-poor sites, and export of others. It is important to determine within-tree nutrient status, which could be paired with soil data to provide a holistic view of nutrient flows in invaded systems, and promote better management of sites after clearing. Until now, most nutrient-based research has focused on soil (Witkowski, 1991; Jacobs et al., 2013) and litter (Nsikani et al., 2018; Railoun, 2018) as affected by IAPs in the Fynbos. However, there seems to be little literature on nutrients within AGB components of standing invasive biomass and is mostly focused on N and P (Tye, 2014; Van der Colff et al., 2018). Figure 2.2 shows the flow of resources through a plant and attempts to describe its niche as an open system within a greater ecological system. For invasive alien trees, the flow of resources between the tree and its immediate environment would be similar to other plant species, with differences in how its acquired (e.g. legumes v non-legumes).

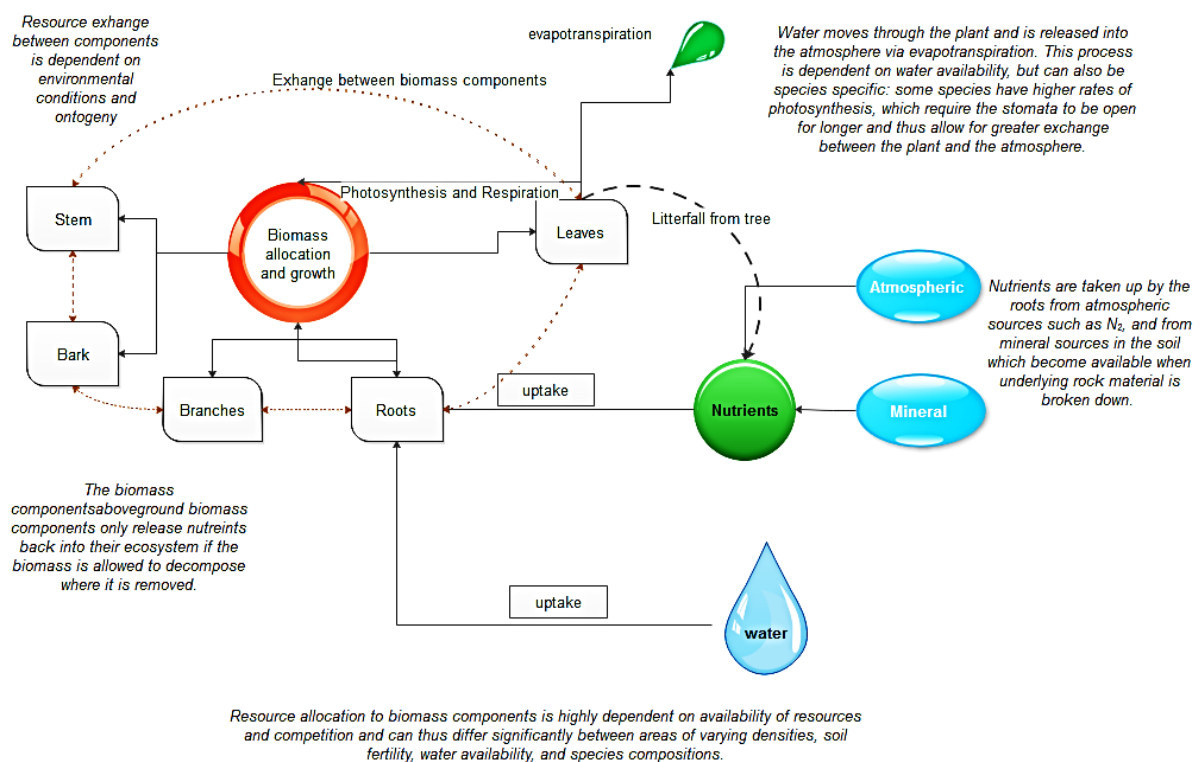


Figure 2.2: Major flow of resources from the ecosystem through the plant and the internal exchange of resources as a result of changing environmental conditions.

While it is widely accepted that invasive alien plant species pose significant threats to local biodiversity, ecosystem functioning, and hydrological functioning of catchments, the immediate ecological environment of these species are not the only systems affecting, or being affected by, their presence. Some IAPs in South Africa form part of wider social, economic, and agricultural systems, which make its management more complex than a one-dimensional approach to clearing and rehabilitation. Understanding the wider systems surrounding invasion could assist in identifying new, more effective ways of managing plant invasion in the country. Considering the more than 20 years of activity of Working for Water, plant invasion could, for instance, be portrayed as an important source of employment for marginalized communities. Working for Water positioned itself as an important employment program through alien clearing, especially in rural South Africa where employment opportunities are rare. In itself, this approach is counterintuitive and often at odds with itself, as effective removal of invasive alien trees inevitably leads to job losses. Additionally, invasive trees have become important socially as building material, firewood, fencing materials, and medicines, and provide recreational value such as shade and aesthetics in some instances. This was shown by Shackleton et al. (2007), who suggested that some communities even

prefer greater densities of invasive alien plants to service their dependence on the plants. Similarly, the *Eucalyptus* genus is one of the more agriculturally important genera due to their interaction with bees (Johannsmeyer, 2016), and it has even been suggested that *Eucalyptus* species only be removed in high-risk ecosystems such as riparian zones, to sustain honeybee populations (Hutton-squire, 2014). It is more than likely that the negative impacts of IAPs far outweigh the potential positives shown in Figure 2.3, but these aspects of invasion need to be considered nonetheless in order to further inform its management. Purely by its utilitarian value, it may even be necessary to propose zones for woodlots outside of riparian zones, where the rate of spread is contained but the species can still be used as firewood, building materials, sources of nectar, etc.

Figure 2.3 shows the connectivity of invasive alien trees in South Africa with various systems. Despite this apparent interconnectedness observed from Fig. 2.3, the presence of IAPs is still largely discussed only in terms of its ecological, hydrological, and economic impacts in areas where they occur. In these cases, there are still major gaps in our understanding of the presence of IAPs, and our management strategies. To broaden the discussion of invasion by *A. mearnsii* and *E. camaldulensis* in the Western cape, this dissertation will discuss the presence of these species in selected riparian zones and its management as a potential agricultural resource through the use of wood chips as biochar for soil amendment. While it is acknowledged that not all the aspects presented in Fig. 2.3 are addressed fully here, the aim is to stimulate discussions further to recognise the importance of other aspects of invasion in informing its management.

Figure 2.4 shows a layout of how the chapters' in this study are related and build on to one another. The study starts with creating allometric models for biomass and nutrients in aboveground biomass (AGB) components of *A. mearnsii* and *E. camaldulensis*, distinguishing between trees that grow in Closed canopy stands and Open canopy trees, trees from different age groups, and distinguishing between the contributions of each of the biomass components. This dataset is then used to estimate the potential for nutrient export in the event of complete removal of the AGB from invaded riparian zones, where a comparison is made between the leguminous *Acacia* and the non-leguminous *Eucalyptus*, followed by a discussion of the potential impact on the ecosystem post-removal. Lastly, a discussion is formulated around the use of biomass products as a soil amendment tool in agriculture. Here, I look at the use of wood chips and biochar and their potential for agricultural use and discuss these in the context of their perceived flaws (allelopathy in wood chips and low nutritional value in biochar). Although this is done in an agricultural context, the findings from these could be equally pertinent in management of exposed soils post-clearing.

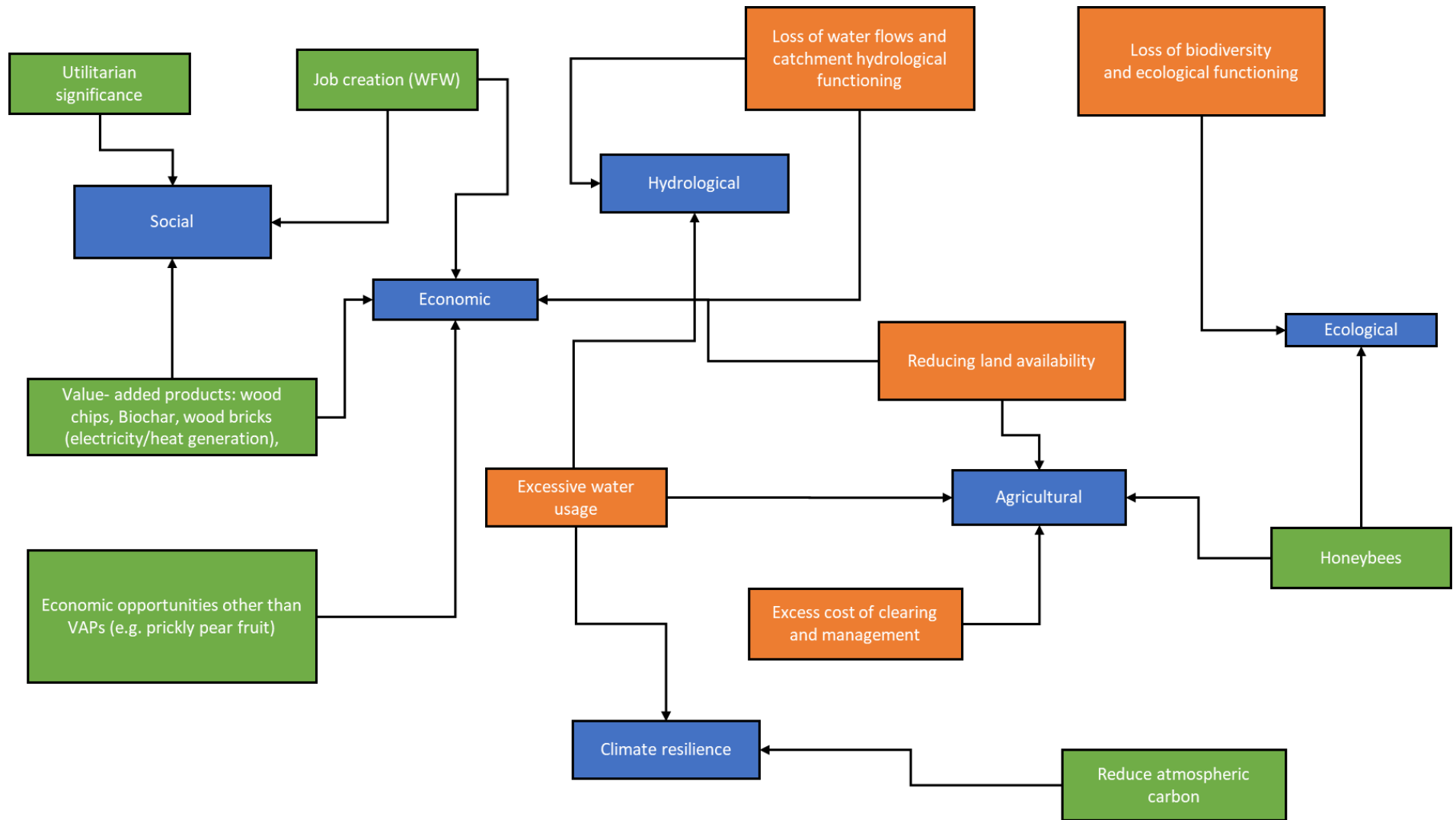


Figure 2.3: Different systems likely affected by alien tree invasion in South Africa and their interconnectivity. Orange depicts net negative effects, green depicts net positive effects, and the blue boxes show some of the different systems affected by IAPs.

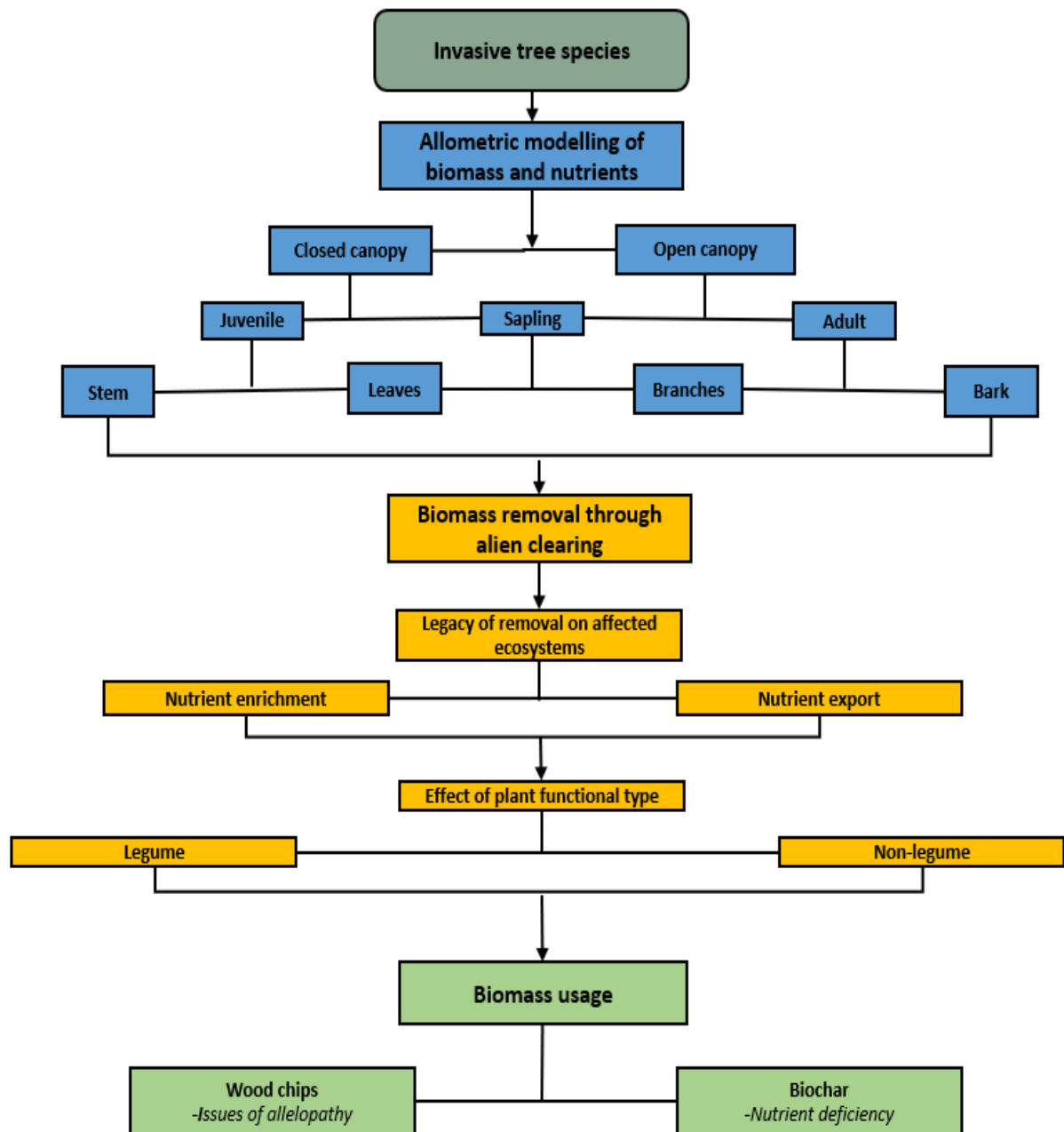


Figure 2.4: Study framework showing the progression from modelling stands of invasive biomass and its nutrients to alien clearing and its impacts on affected ecosystems.

## 2.7 References

- Abbas, T., Zahir, Z. A., & Naveed, M. (2017). Bioherbicidal activity of allelopathic bacteria against weeds associated with wheat and their effects on growth of wheat under axenic conditions. *BioControl*, 62(5), 719-730.
- Acosta, A. L., Giannini, T. C., Imperatriz-Fonseca, V. L., & Saraiva, A. M. (2016). Worldwide alien invasion: A methodological approach to forecast the potential spread of a highly invasive pollinator. *PLoS one*, 11(2).
- Aghoghovwia, M. P. (2018). *Effect of different biochars on inorganic nitrogen availability* (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Ahmed, R., Hoque, A. R., & Hossain, M. K. (2008). Allelopathic effects of *Leucaena leucocephala* leaf litter on some forest and agricultural crops grown in nursery. *Journal of Forestry Research*, 19(4), 298.
- Attiwill, P. M., & Adams, M. A. (1993). Nutrient cycling in forests. *New phytologist*, 124(4), 561-582.
- Bergen, J. Y. (1904). Transpiration of sun leaves and shade leaves of *Olea europaea* and other broad-leaved evergreens. *Botanical Gazette*, 38(4), 285-296.
- Blanchard, R., & Holmes, P. M. (2008). Riparian vegetation recovery after invasive alien tree clearance in the Fynbos Biome. *South African Journal of Botany*, 74(3), 421-431.
- Canham, C. D., Berkowitz, A. R., Kelly, V. R., Lovett, G. M., Ollinger, S. V., & Schnurr, J. (1996). Biomass allocation and multiple resource limitation in tree seedlings. *Canadian Journal of Forest Research*, 26(9), 1521-1530.
- Carrier, M., Hardie, A. G., Uras, Ü., Görgens, J., & Knoetze, J. H. (2012). Production of char from vacuum pyrolysis of South-African sugar cane bagasse and its characterization as activated carbon and biochar. *Journal of Analytical and Applied Pyrolysis*, 96, 24-32.
- Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., & Joseph, S. (2008). Agronomic values of greenwaste biochar as a soil amendment. *Soil Research*, 45(8), 629-634.
- Chou, C. H., Fu, C. Y., Li, S. Y., & Wang, Y. F. (1998). Allelopathic potential of *Acacia confusa* and related species in Taiwan. *Journal of Chemical Ecology*, 24(12), 2131-2150.
- Cohen, B., Logan, A., Pieterse, R., & Swanepoel, E. (2015). Casidra: Economic study on the use of cleared alien biomass for commercial exploitation. *The Green House*.
- D'Antonio, C. M., & Vitousek, P. M. (1992). Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual review of ecology and systematics*, 23(1), 63-87.
- De Moraes Gonçalves, J. L., Stape, J. L., Laclau, J.-P., Smethurst, P., & Gava, J. L. (2004). Silvicultural effects on the productivity and wood quality of eucalypt plantations. *Forest Ecology and Management*, 193(1-2), 45-61. <https://doi.org/10.1016/j.foreco.2004.01.022>
- Del Moral, R., & Muller, C. H. (1970). The allelopathic effects of *Eucalyptus camaldulensis*. *American Midland Naturalist*, 254-282.
- Department of Environmental Affairs (DEA). (2011). National climate change response white paper.
- Department of Environmental Affairs, Forestry, and Fisheries (DEFF). (2020). South African Carbon Sinks Atlas. <https://ccis.environment.gov.za/carbon-sinks/#/explore-data>. Date accessed: 11 August 2020.



- Doerr, S. H., Shakesby, R. A., & MacDonald, L. H. (2009). Soil water repellency: a key factor in post-fire erosion. In *Fire effects on soils and restoration strategies* (pp. 213-240). CRC Press.
- Dovey, S. B. (2012). *Effects of clear felling and residue management on nutrient pools, productivity and sustainability in a clonal eucalypt stand in South Africa* (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Du Toit, B. & Dovey, S.B. (2004). Effects of harvesting on nutrient pools and stand growth in *Eucalyptus* plantation.pdf. (n.d.).
- Du Toit, B. (2003). Effects of site management operations on the nutrient capital of a eucalypt plantation system in South Africa, (199), 15–25.
- Duke, S. O., Cedergreen, N., Velini, E. D., & Belz, R. G. (2006). Hormesis: is it an important factor in herbicide use and allelopathy?. *Outlooks on Pest Management*, 17(1), 29-33.
- El-Khawas, S. A., & Shehata, M. M. (2005). The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on monocot (*Zea mays* L.) and dicot (*Phaseolus vulgaris* L.) plants. *Biotechnology*, 4(1), 23-34.
- Ericsson, T., Rytter, L., & Vapaavuori, E. (1996). Physiology of carbon allocation in trees. *Biomass and Bioenergy*, 11(2–3), 115–127. [https://doi.org/10.1016/0961-9534\(96\)00032-3](https://doi.org/10.1016/0961-9534(96)00032-3)
- Fatunbi, A. O., Dube, S., Yakubu, M. T., & Tshabalala, T. (2009). Allelopathic potential of *Acacia mearnsii* De Wild. *World Applied Sciences Journal*, 7(12), 1488-1493.
- Feig, G. T., Joubert, W. R., Mudau, A. E., & Monteiro, P. (2017). South African carbon observations: CO<sub>2</sub> measurements for land, atmosphere, and ocean. *South African Journal of Science*, 113(11-12), 1-4.
- Fife, D. N., Nambiar, E. K. S., & Saur, E. (2008). Retranslocation of foliar nutrients in evergreen tree species planted in a Mediterranean environment. *Tree physiology*, 28(2), 187-196.
- Forests, S., Of, J., & Science, F. (2015). Estimating biomass and macronutrient content of some commercially important plantation species in South Africa, (SEPTEMBER 2009). <https://doi.org/10.2989/SF.2009.71.3.9.921>
- Forrester, D. I., Bauhus, J., & Cowie, A. L. (2005). On the success and failure of mixed-species tree plantations: lessons learned from a model system of *Eucalyptus globulus* and *Acacia mearnsii*. *Forest Ecology and Management*, 209(1–2), 147–155. <https://doi.org/10.1016/j.foreco.2005.01.012>
- Forrester, D. I., Bauhus, J., & Cowie, A. L. (2006). Carbon allocation in a mixed-species plantation of *Eucalyptus globulus* and *Acacia mearnsii*. *Forest Ecology and Management*, 233(2–3), 275–284. <https://doi.org/10.1016/j.foreco.2006.05.018>
- Forsythe, G., Le Maître, D., Van den Dool, R., Walls, R., Pharaoh, R., and Fortune, G. (2019). *The Knysna Fires of 2017: Learning from this disaster*. Stellenbosch University and Santam.
- Foster, N. W., & Bhatti, J. S. (2006). Forest ecosystems: nutrient cycling. *Encyclopedia of soil science*, 1, 718-721.
- Fourie, M. (2014). Investigating soil nitrogen dynamics in natural, invaded and cleared fynbos riparian ecotones and implications for riparian functioning (*MSc thesis*, Stellenbosch: Stellenbosch University).

- Fox, D. M., Darboux, F., & Carrega, P. (2007). Effects of fire-induced water repellency on soil aggregate stability, splash erosion, and saturated hydraulic conductivity for different size fractions. *Hydrological Processes: An International Journal*, 21(17), 2377-2384.
- Gaskin, J. W., Speir, R. A., Harris, K., Das, K. C., Lee, R. D., Morris, L. A., & Fisher, D. S. (2010). Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. *Agronomy Journal*, 102(2), 623-633.
- Givnish, T. J., & Vermeij, G. J. (1976). Sizes and shapes of liane leaves. *The American Naturalist*, 110(975), 743-778.
- Griffin, A. R., Midgley, S. J., Bush, D., Cunningham, P. J., & Rinaudo, A. T. (2011). Global uses of Australian *Acacias* – recent trends and future prospects. *Diversity and Distributions*, 837–847. <https://doi.org/10.1111/j.1472-4642.2011.00814.x>
- Gross, E. (1999). Allelopathy in benthic and littoral areas : case studies on allelochemicals from benthic cyanobacteria and submersed macrophytes. In: INDERJIT, , ed. and others. Principles and Practices in Plant Ecology. Boca Raton, Fla.:CRC Press, pp. 179-199
- Hall, J.P., 2002. Sustainable production of forest biomass for energy. *For. Chron.* 78, 391–396. doi:10.5558/tfc78391-3
- Hegab, M. M., Gabr, M. A., Al-Wakeel, S. A. M., & Hamed, B. A. (2016). Allelopathic potential of *Eucalyptus rostrata* leaf residue on some metabolic activities of *Zea mays* L. *Universal Journal of Plant Science*, 4(2), 11-21.
- Henderson, L. (2007). Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). *Bothalia*, 37(2), 215-248.
- Henderson, L., & Wilson, J. R. (2017). Changes in the composition and distribution of alien plants in South Africa: An update from the Southern African Plant Invaders Atlas. *Bothalia-African Biodiversity & Conservation*, 47(2), 1-26.
- Holmes, P. M., Esler, K. J., Richardson, D. M., & Witkowski, E. T. F. (2008). Guidelines for improved management of riparian zones invaded by alien plants in South Africa. *South African Journal of Botany*, 74(3), 538-552.
- Hoque, A. R., Ahmed, R., Uddin, M. B., & Hossain, M. K. (2003). Allelopathic effect of different concentration of water extracts of *Acacia auriculiformis* leaf on some initial growth parameters of five common agricultural crops. *Journal of Agronomy*.
- Hutton-Squire, J. P. (2014). Historical relationship of the honeybee (*Apis mellifera*) and its forage; and the current state of beekeeping within South Africa (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Jacobs, S., Cogill, L., Jacobs, K., Juba, R., Maubane, T., Slabbert, E., & Smart, R. (2017). Assessing the impact of selected methods of removal of invasive alien *Acacia mearnsii* and *Eucalyptus camaldulensis* on fynbos riparian ecosystem function. *Report to the Water Research Commission*. WRC Report No. 2343/1/16
- Jayakumar, M., & Manikandan, M. (2005). Allelopathic potential of *Acacia leucopholea* on groundnut and sorghum. Research Department of Botany, VHNSH College, Virudhunagar, 626(001).
- Kambaj Kambol, O. (2013). *In situ and ex situ soil respiration in natural, Acacia-invaded and cleared riparian ecotones in the Fynbos Biome* (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Kamel, M., & Hammad, S. A. (2015). The allelopathic effect of the exotic tree *Acacia saligna* on the germination of wheat and canola. *Ecologia Balkanica*, 7(1).

- Kauffman, J. B., Cummings, D. L., & Ward, D. E. (1994). Relationships of fire, biomass and nutrient dynamics along a vegetation gradient in the Brazilian cerrado. *Journal of Ecology*, 519-531.
- Kauffman, J. B., Sanford Jr, R. L., Cummings, D. L., Salcedo, I. H., & Sampaio, E. V. S. B. (1993). Biomass and nutrient dynamics associated with slash fires in neotropical dry forests. *Ecology*, 74(1), 140-151.
- Kelty, M. J. (2006). The role of species mixtures in plantation forestry. *Forest Ecology and Management*, 233(2–3), 195–204. <https://doi.org/10.1016/j.foreco.2006.05.011>
- Kering, M.K., Butler, T.J., Biermacher, J.T., Guretzky, J.A. (2012). Biomass Yield and Nutrient Removal Rates of Perennial Grasses under Nitrogen Fertilization. *Bioenergy Res.* 5, 61–70. doi:10.1007/s12155-011-9167-x
- Kohli, R. K., & Singh, D. (1991). Allelopathic impact of volatile components from *Eucalyptus* on crop plants. *Biologia plantarum*, 33(6), 475.
- Kollman, J., Dietz, H., & Edwards, P. J. (2004). Allocation, plasticity and allometry, 6(2002), 205–206.
- Korb, J. E., Johnson, N. C., & Covington, W. W. (2004). Slash pile burning effects on soil biotic and chemical properties and plant establishment: recommendations for amelioration. *Restoration Ecology*, 12(1), 52-62.
- Kraaij, T., Baard, J. A., Arndt, J., Vhengani, L., & Van Wilgen, B. W. (2018). An assessment of climate, weather, and fuel factors influencing a large, destructive wildfire in the Knysna region, South Africa. *Fire Ecology*, 14(2), 4.
- Le Maître, D. C., Blignaut, J. N., Clulow, A., Dziki, S., Everson, C. S., Görgens, A. H., & Gush, M. B. (2020). Impacts of plant invasions on terrestrial water flows in South Africa. In *Biological Invasions in South Africa* (pp. 431-457). Springer, Cham.
- Le Maître, D. C., Forsyth, G. G., Dziki, S., & Gush, M. B. (2016). Estimates of the impacts of invasive alien plants on water flows in South Africa. *Water Sa*, 42(4), 659-672.
- Le Maître, D. C., Versfeld, D. B., & Chapman, R. A. (2000). Impact of invading alien plants on surface water resources in South Africa: A preliminary assessment. *WaterSA*, 26 (3)
- Lisanework, N., & Michelsen, A. (1993). Allelopathy in agroforestry systems: the effects of leaf extracts of *Cupressus lusitanica* and three *Eucalyptus* spp. on four Ethiopian crops. *Agroforestry systems*, 21(1), 63-74.
- Lodge, C. (2014). How do Size and Resource Availability Control Aboveground Biomass Allocation of Tree Seedlings? *Forest Research: Open Access*, 3(3). <https://doi.org/10.4172/2168-9776.1000123>
- Markewitz, D., Davidson, E., Moutinho, P., & Nepstad, D. (2004). Nutrient loss and redistribution after forest clearing on a highly weathered soil in Amazonia. *Ecological Applications*, 14(sp4), 177-199.
- Masters, M.D., Black, C.K., Kantola, I.B., Woli, K.P., Voigt, T., David, M.B., DeLucia, E.H., 2016. Soil nutrient removal by four potential bioenergy crops: *Zea mays*, *Panicum virgatum*, *Miscanthus x giganteus*, and prairie. *Agric. Ecosyst. Environ.* 216, 51–60. doi:10.1016/j.agee.2015.09.016
- Mehmood, K., Asif, H. M., Bajwa, R., Shafique, S., & Shafique, S. (2011). Phytotoxic potential of bark extracts of *Acacia nilotica* and *Syzygium cumini* against *Parthenium hysterophorus*. *Pak. J. Bot.* 43(6), 3007-3012.
- Mirbabaei, S. M., Shahrestani, M. S., Zolfaghari, A., & Abkenar, K. T. (2013). Relationship between soil water repellency and some of soil properties in northern Iran. *Catena*, 108, 26-34.

- Mohler, C. L., Marks, P. L., & Sprugel, D. G. (1978). Stand structure and allometry of trees during self-thinning of pure stands. *The Journal of Ecology*, 599-614.
- Montagu, K. D., Kearney, D. E., & Smith, R. G. B. (2003). The biology and silviculture of pruning planted eucalypts for clear wood production - A review. *Forest Ecology and Management*, 179(1-3), 1-13. [https://doi.org/10.1016/S0378-1127\(02\)00579-0](https://doi.org/10.1016/S0378-1127(02)00579-0)
- Moore, L. V., Myers, J. H., & Eng, R. (1988). Western tent caterpillars prefer the sunny side of the tree, but why?. *Oikos*, 321-326.
- Mudavanhu, S., Blignaut, J., Nkambule, N., Morokong, T., & Vundla, T. (2016). A cost-benefit analysis of using Rooikrans as biomass feedstock for electricity generation: a case study of the De Hoop nature reserve, South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 788-813.
- Müller, I., Schmid, B., & Weiner, J. (2000). The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants, 3, 115-127.
- Müller, I., Schmid, B., & Weiner, J. (2000). The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspectives in plant ecology, evolution and systematics*, 3(2), 115-127.
- Muñoz, F., Rubilar, R., Espinosa, M., Cancino, J., Toro, J., & Herrera, M. (2008). The effect of pruning and thinning on above ground aerial biomass of *Eucalyptus nitens* (Deane & Maiden) Maiden. *Forest Ecology and Management*, 255, 365-373. <https://doi.org/10.1016/j.foreco.2007.09.063>
- Muyambo, P. (2017). *Modelling aboveground biomass and nutrient export in South African Pinus elliotii* (Doctoral dissertation).
- Naiman, R. J., & Decamps, H. (1997). The ecology of interfaces: riparian zones. *Annual review of Ecology and Systematics*, 28(1), 621-658.
- National Renewable Energy Laboratory. (2012). International Trade of Wood Pellets.
- Naudé, M. (2012). Fynbos riparian biogeochemistry and invasive Australian *Acacias* (MSc thesis, Stellenbosch: Stellenbosch University).
- Neary, D. G., Klopatek, C. C., DeBano, L. F., & Ffolliott, P. F. (1999). Fire effects on belowground sustainability: a review and synthesis. *Forest ecology and management*, 122(1-2), 51-71.
- Nichols, J. D., Bristow, M., & Vanclay, J. K. (2006). Mixed-species plantations: Prospects and challenges. *Forest Ecology and Management*, 233(2-3), 383-390. <https://doi.org/10.1016/j.foreco.2006.07.018>
- Nsikani, M. M., van Wilgen, B. W., & Gaertner, M. (2018). Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N<sub>2</sub>-fixing woody species: implications for ecological restoration. *Restoration ecology*, 26(2), 235-244.
- Olivier, C. F. (2011). An investigation into the degradation of biochar and its interactions with plants and soil microbial community (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Oyun, M. B. (2006). Allelopathic potentialities of *Gliricidia sepium* and *Acacia auriculiformis* on the germination and seedling vigour of maize (*Zea mays* L.). *American Journal of Agricultural and Biological Science*, 1(3), 44-47.

- Picard, N., S., L., & Henry, M. (2012). Manual for building tree volume and biomass allometric equations: from field measurement to prediction. *Manual for building tree volume and biomass allometric equations: from field measurement to prediction*, FAO; Food and Agricultural Organization of the United Nations.
- Pinkard, E. a. (2002). Effects of pattern and severity of pruning on growth and branch development of pre-canopy closure *Eucalyptus nitens*. *Forest Ecology and Management*, 157(1–3), 217–230. [https://doi.org/10.1016/S0378-1127\(00\)00647-2](https://doi.org/10.1016/S0378-1127(00)00647-2)
- Poorter, H. A., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO<sub>2</sub>, nutrients and water: A quantitative review. *IMF Occasional Papers*, 27(189), 595–607. [https://doi.org/10.1071/PP99173\\_CO](https://doi.org/10.1071/PP99173_CO)
- Popma, J., Bongers, F., & Werger, M. J. A. (1992). Gap-dependence and leaf characteristics of trees in a tropical lowland rain forest in Mexico. *Oikos*, 207-214.
- Pretorius, M. R., Esler, K. J., Holmes, P. M., & Prins, N. (2008). The effectiveness of active restoration following alien clearance in fynbos riparian zones and resilience of treatments to fire. *South African Journal of Botany*, 74(3), 517-525.
- Pretzsch, H., Grote, R., Reineking, B., Rötzer, T. H., & Seifert, S. T. (2008). Models for forest ecosystem management: a European perspective. *Annals of botany*, 101(8), 1065-1087.
- Pugliese, A. (1988). Optimal resource allocation in perennial plants: a continuous-time model. *Theoretical Population Biology*, 34(3), 215-247.
- Railoun, M. Z. (2018). *Impacts of the invasive tree Acacia mearnsii on riparian and instream aquatic environments in the Cape Floristic Region, South Africa* (MSc thesis, Stellenbosch: Stellenbosch University).
- RHP. (2011). River Health Programme state of rivers report: rivers of the Breede Water Management Area, 71.
- Richardson, D. M., Pyšek, P., Rejmánek, M., Barbour, M. G., Panetta, F. D., & West, C. J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and distributions*, 6(2), 93-107.
- Rozendaal, D. M. A., Hurtado, V. H., & Poorter, L. (2006). Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Functional Ecology*, 20(2), 207-216.
- Ruwanza, S., Gaertner, M., Esler, K. J., & Richardson, D. M. (2015). Allelopathic effects of invasive *Eucalyptus camaldulensis* on germination and early growth of four native species in the Western Cape, South Africa. *Southern Forests: A Journal of Forest Science*, 77(2), 91-105.
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R. Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D. M., Mooney, H. A., Oesterheld, M., Poff, N. L., Sykes, M. T., Walker, B. H., Walker, M., & Wall, D. H. (2000). Global biodiversity scenarios for the year 2100. *Science*, 287(5459), 1770-1774.
- Sankaran, K. V., Mendham, D. S., Chacko, K. C., Pandalai, R. C., Pillai, P. K. C., Grove, T. S., & O'Connell, A. M. (2008). Impact of site management practices on growth of *Eucalyptus* plantations in the monsoonal tropics in Kerala, India. *Site Management and Productivity in Tropical Plantation Forests*, 23.



- Schumann, A. W., Little, K. M., & Eccles, N. S. (1995). Suppression of seed germination and early seedling growth by plantation harvest residues. *South African Journal of Plant and Soil*, 12(4), 170-172.
- Seigler, D. S. (2006). Basic pathways for the origin of allelopathic compounds. In *Allelopathy* (pp. 11-61). Springer, Dordrecht.
- Shackleton, C. M., McGarry, D., Fourie, S., Gambiza, J., Shackleton, S. E., & Fabricius, C. (2007). Assessing the effects of invasive alien species on rural livelihoods: case examples and a framework from South Africa. *Human Ecology*, 35(1), 113-127.
- Shackleton, R. T., Le Maître, D. C., van Wilgen, B. W., & Richardson, D. M. (2017). Towards a national strategy to optimise the management of a widespread invasive tree (*Prosopis* species; mesquite) in South Africa. *Ecosystem Services*, 27, 242-252.
- Sika, M. P., & Hardie, A. G. (2014). Effect of pine wood biochar on ammonium nitrate leaching and availability in a South African sandy soil. *European journal of soil science*, 65(1), 113-119.
- Singh, H. P., Batish, D. R., Setia, N., & Kohli, R. K. (2005). Herbicidal activity of volatile oils from *Eucalyptus citriodora* against *Parthenium hysterophorus*. *Annals of applied biology*, 146(1), 89-94.
- Slabbert, E., Jacobs, S. M., & Jacobs, K. (2014). The soil bacterial communities of South African Fynbos riparian ecosystems invaded by Australian *Acacia* species. *PloS one*, 9(1).
- Stafford, W. H., Von Maltitz, G. P., & Watson, H. K. (2018). Reducing the costs of landscape restoration by using invasive alien plant biomass for bioenergy. *Wiley Interdisciplinary Reviews: Energy and Environment*, 7(1), e272.
- Stafford, W., & Blignaut, J. (2017). Reducing landscape restoration costs: Feasibility of generating electricity from invasive alien plant biomass on the Agulhas Plain, South Africa. *Ecosystem Services*.
- Steiner, C. (2008). Biochar carbon sequestration. *University of Georgia, Biorefining and Carbon Cycling Program, Athens, GA*, 30602.
- Sterner, R. W., & Elser, J. (2008). Ecological stoichiometry: overview. In *Encyclopedia of Ecology, Five-Volume Set* (pp. 1101-1116). Elsevier.
- Swaminathan, C., Rai, R. V., & Suresh, K. K. (1989). Allelopathic proclivities of *Acacia nilotica* (L.) Willd. ex Del. *Journal of Tropical Forest Science*, 56-60.
- Terera, F., Gaertner, M., Jacobs, S. M., & Richardson, D. M. (2015). *Eucalyptus camaldulensis* invasion in riparian zones reveals few significant effects on soil physico-chemical properties. *River Research and Applications*, 31(5), 590-601.
- Thornley, J. H. M. (1972). A model to describe the partitioning of photosynthate during vegetative plant growth. *Annals of Botany*, 36(2), 419-430.
- Tye, D. R. (2014). *Nutrient and biomass allocation strategies in an invasive Australian Acacia and a co-occurring native Acacia in South Africa* (Doctoral dissertation, University of Witwatersrand).
- Van der Colff, D., Dreyer, L. L., Valentine, A., & 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, 358-364.
- Van Wilgen, B. W., Richardson, D. M., Le Maître, D. C., Marais, C., & Magadlela, D. (2001). The economic consequences of alien plant invasions: examples of impacts and approaches to

- sustainable management in South Africa. *Environment, development, and sustainability*, 3(2), 145-168.
- Van Wilgen, N. J., van Wilgen, B. W., & Midgley, G. F. (2020). Biological invasions as a component of South Africa's global change research effort. In *Biological Invasions in South Africa* (pp. 855-878). Springer, Cham.
- Van Zwieten, L., Kimber, S., Morris, S., Chan, K. Y., Downie, A., Rust, J., Joseph, S., & Cowie, A. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and soil*, 327(1-2), 235-246.
- Verdeguer, M., Blázquez, M. A., & Boira, H. (2009). Phytotoxic effects of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. *Biochemical Systematics and Ecology*, 37(4), 362-369.
- Versfeld, D. B., Le Maître, D. C., & Chapman, R. A. (1998). Alien invading plants and water resources in South Africa: a preliminary assessment. Report to the Water Research Commission. Report number TT99/98.
- Vosse, S., Esler, K. J., Richardson, D. M., & Holmes, P. M. (2008). Can riparian seed banks initiate restoration after alien plant invasion? Evidence from the Western Cape, South Africa. *South African Journal of Botany*, 74(3), 432-444.
- Vundla, T., Blignaut, J., Nkambule, N., Morokong, T., & Mudavanhu, S. (2016). The opportunity cost of not utilising the woody invasive alien plant species in the Kouga, Krom and Baviaans catchments in South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 814-830.
- Waring, R. H. (1987). Characteristics of trees predisposed to die. *Bioscience*, 37(8), 569-574.
- Weiner, J. (2004). Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 6(4), 207-215.
- Wessels, K. J., Mathieu, R., Erasmus, B. F. N., Asner, G. P., Smit, I. P. J., Van Aardt, J. A. N., Main, R., Fisher, J., Marais, W., Kennedy-Bowdoin, T., Knapp, D.E., Emerson, R., & Jacobson, J. (2011). Impact of communal land use and conservation on woody vegetation structure in the Lowveld savannas of South Africa. *Forest Ecology and Management*, 261(1), 19-29. doi:10.1016/j.foreco.2010.09.012
- West, G. B., Brown, J. H., & Enquist, B. J. (1999). A general model for the structure and allometry of plant vascular systems. *Nature*, 400(6745), 664-667.
- Witkowski, E. T. F. (1988). Response to nutrient additions by the plant growth forms of sand-plain lowland fynbos, South Africa. *Vegetatio*, 79(1-2), 89-97.
- 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*, 1-15.
- Wu, F., Bao, W., Li, F., & Wu, N. (2008). Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. *Environmental and Experimental Botany*, 63(1-3), 248-255. <https://doi.org/10.1016/j.envexpbot.2007.11.00>
- Zhang, C., & Fu, S. (2010). Allelopathic effects of leaf litter and live roots exudates of *Eucalyptus* species on crops. *Allelopathy Journal*, 26(1), 91-100.



## Chapter 3: The aboveground biomass allometry of the invasive *Eucalyptus camaldulensis* and *Acacia mearnsii*

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### 3.1 Abstract

Modelling of invasive biomass is a useful tool to make informed management decisions regarding large stands of invasive trees in South African riparian zones. Trees in plantations have been modelled extensively, where management activities are directed towards a specific growth form, or wood quality objective. This is not the case in natural forests, where variable species compositions and densities translate into variable growth forms and allometry of the plant. Through destructive sampling of *Acacia mearnsii* and *Eucalyptus camaldulensis* in the Breede River Catchment, allometric models were generated for estimation of aboveground biomass (AGB) from stem basal diameter measurements. Models were generated by sampling of 72 individuals per species; six individuals from three life stages each (juvenile, sapling, adult) were harvested from two stem density extremes (Closed canopy vs. Open canopy), at two different sites each (Bainskloof and Alfalfa for *A. mearnsii* and Wolseley and Alfalfa for *E. camaldulensis*). After trees were destructively sampled, all samples were categorized depending on their position on the plant, i.e. leaves, stem, branches, and bark, and were treated this way throughout the study. This study confirms that individuals' growth form differs enough between stands of different densities to warrant separate modelling. Tree allometry is related to life stage and, to a certain extent, the age of the stand as a whole. Allometric growth form is shown by the changes in allocation of biomass proportion to different biomass components through the different life stages and suggests that models for adult trees could differ significantly from those for younger trees. Large-scale sampling could minimise the effect of this variation on allometric models. For increased accuracy in remote sensing measurements, it is suggested that these variables are incorporated into biomass estimation models that make allowance for the variations in aboveground biomass distributions.

### 3.2 Introduction

Optimal Partition Theory (OPT) states that resources are allocated to organs/components that require it the most to best serve the needs of the system (Thornley, 1972; Geng et al., 2007). This theory seeks to explain the size ratios between various components of that organism as a physical expression of internal and external stimuli (Niklas, 2006; Chave et al., 2014). Growth form of a tree, for instance, is significantly affected by environmental factors

such as resource availability (light, water, and nutrients), competition from other species, and even competition from conspecifics due to an overlap in resource use as a result of crowding (Enquist et al., 1998). In forestry, these factors are highly controlled to maximize stem growth (Pretzsch et al., 2008) whereas in wild forests environmental factors are ever-changing and uncontrolled. Additionally, tree growth and resource allocation are also affected by other internal factors such as maximum height as a function of stem diameter (Niklas, 1994).

Niklas & Enquist (2001; 2002) have shown that growth of plant components is related to their photosynthetic function in the plant, resulting in either allometric (biomass growth of different parts resulting in differential proportions over time) or isometric (proportions of biomass components remain relatively stable) growth, influencing storage dynamics, and can also be heavily influenced by resource availability. These proportions can differ by several orders of magnitude between sites; in areas of low nutrient availability trees could preferentially invest in increased root growth (Chapin, 1990), allowing for the fine root system to come into contact with more nutrients to assist aboveground growth. Likewise, competition for sunlight greatly influences variables like tree height in relationship to stem diameter and leaf mass (Poorter & Nagel, 2000; Rijkers et al., 2000). Acquisition of sufficient sunlight requires rapid growth toward the canopy in Closed canopy stands, whereas Open canopy trees could grow wider for maximum interception (King, 1990), increasing investment in branches and foliage. Plants thus respond to environmental factors which influence their growth form throughout its development, so that for the same species, different allometric models can be generated to describe the growth of the different components and at different life stages (Niklas, 2006). Modelling of biomass of whole systems through upscaling of rigorous single-tree analyses could thus provide useful and reliable insights into the flow of resources in forests and the effect of internal and external factors on resource partitioning in individual trees.

Modelling of tree growth and biomass allocation in plantations helps managers make informed decisions to maximize yield of merchantable wood (Dovey & Du Toit, 2004; Ounban et al., 2016; Muyambo, 2017). Modelling of tree biomass in natural forests is, however, complicated due to the high variability in growth form and the within-plant variability in resource allocation as a result of local environmental conditions. It is less often reported on for natural forests in modern times, and in the few cases where it has been done, research appears mainly directed towards the role of forests in carbon sequestration (Masera et al., 2003; Latte et al., 2013; Sist et al., 2013; Chave 2014; Diédhiou et al., 2016). Beets et al. (2012), used a combination of stem diameter at breast height (DBH; 1.3 m) and tree height (H) measurements to model various indigenous trees and tree fern species in New Zealand using the equation

$$Y = aX^b \quad (4.1)$$

where  $a$  and  $b$  are parameters,  $X$  is DBH x H and  $Y$  refers to stem + branch (>10cm diameter) volume.

However, to simplify in-field measurements to model AGB, methodologies need to be simplified and ideally only have one independent variable. To test this, Afromontane forests in Ethiopia were modelled and showed that the use of Diameter at Breast Height (DBH; 1.3 m) or Diameter at stump height (DBH; 0.3 m) could be used effectively to describe more than 96% of aboveground biomass variability (Tetemke et al., 2019) while using equation 4.1, where  $a$  and  $b$  are parameters,  $X$  is DBH or DBS, and  $Y$  is component biomass. Mugasha et al. (2013) also showed that DBH and tree height (H) together could be used to explain up to 97% of biomass variation but that the addition of H only marginally improved model accuracy. The use of DBH or Basal Diameter (BD) has also been shown to effectively model biomass of *Prosopis* spp. as an invasive tree in Kenya (Muturi et al., 2011). The use of DBH or BD/DBS should thus be sufficient to model biomass of *A. mearnsii* and *E. camaldulensis* in invasive stands in South Africa.

Although modelling of biomass is a useful tool that can be used to advocate for the importance of forests in, for instance, carbon sequestration (e.g. Mandal et al., 2013; Phiri et al., 2015), it is just as useful in advising economic models aimed at clearing and selling biomass as value added products (Mugido et al., 2014). Biomass modelling is a potentially useful management planning tool that could allow for the estimation of standing biomass in invasive stands and inform possible uses such as amounts available for wood chip or charcoal production. Adding value to biomass products could be especially relevant in the Western Cape of South Africa, where invasion threatens local water supply and biodiversity in the Cape Floral Kingdom (CFK). The province is one of the most highly invaded provinces in the country (Le Maître et al., 2000; Henderson 2007; Henderson & Wilson, 2017) and one of its major river systems, the Breede River, which is a catchment critical for agriculture, household use, and industry in the Boland region, is also highly invaded, largely by *Acacia mearnsii* and *Eucalyptus camaldulensis*.

Allometric models have not yet been developed to determine biomass of stands of invasive trees in South Africa and this exposes a significant gap in our understanding of the extent of biomass distribution in invasive stands. In this chapter, models are presented for determination of biomass of the aboveground tree components (stem, leaves, branches, and bark) of *A. mearnsii* and *E. camaldulensis* at four locations along the Breede River. Models were created from various sizes of trees, ranging from juveniles to adult trees and at two density extremes.

This was done to include the potential variation caused by changes in growth form due to these factors. Here I test the hypothesis that tree growth form and allometry of *Acacia mearnsii* and *Eucalyptus camaldulensis* are affected significantly by stem densities and life stage, with significant implications for accuracy of allometric modelling.

### 3.3 Materials and methods

#### Sampling

Destructive sampling was done on six (6) individuals each from different age groups (juveniles, saplings, and adults) and at two density extremes (Closed canopy vs. Open canopy) and at two sites each, for a total of 72 individuals per species. Stem density extremes used here are contextual rather than literal, as approximate canopy cover was used as a proxy for this (Closed canopy: >75% canopy cover; Open canopy: <25% canopy cover). This is because the effects of stem densities at different life stages are not comparable and absolute stem densities would only yield accurate comparisons in an even-aged stand. The study sites used for this chapter include two sites for *E. camaldulensis* (Alfalfa and Wolseley) and two sites for *A. mearnsii* (Alfalfa and Bainskloof). Sampling was done with a chainsaw and in-field weighing was done with a digital 30 kg scale, accurate to two decimals. For biomass measurements, methods applied by Brown (1997) were used, where aboveground components (leaves, stem, bark, and branches) were separated from each other in the field for further separate analyses (Figure 3.1).

Leaves were removed by hand through stripping and separating them from twigs. The stem was cut into smaller 1 - 1.5 m pieces, after which the bark could be removed by hitting it with the blunt side of an axe until it freely separates from the stem. For modelling purposes, stem basal diameter was recorded using a pair of callipers and tree height was recorded after the tree was felled. Stem diameter at breast height (DBH; 1.3 m) could not be used for modelling as some of the samples had a maximum height of less than 1.3 m. Stem basal diameter was thus chosen as a metric for modelling that could be used for all size ranges.



Figure 3.1: Biomass sampling, separation, and measurements in the field and preparation for further analyses.

All aboveground components were weighed, after subdividing large pieces using a chainsaw, using a 4-decimal digital scale, after which a subsample was taken and dried in an oven at 45°C until a stable dry mass was achieved. Biomass of subsamples was then determined in the lab by weighing the dried samples and applying with the following equation:

$$\text{Dry mass (\%)} = (dm/wm) * 100 \quad (4.2)$$

Where: dm= average dry mass of subsample (g), and wm= average wet mass of subsample (g). The percentage dry mass is then upscaled to the rest of the tree using the equation:

$$\text{Total biomass} = ((dm/wm) * 100) * x \quad (4.3)$$

Where x = mass of the field wet sample (g)



## Statistical analyses

To determine the differences in mass allocation to various aboveground biomass components, a Factorial ANOVA was used after a normal distribution was confirmed with a Kruskal Wallis test. Where significant differences were found, a post-hoc Fisher's LSD test was used to determine the source of variation.

A Pearson correlation matrix was produced, which reveals the relationship of different biomass components to each other, and also the relationships of various nutrients to one another. The PCA is also used to determine the effect of stem densities on plant component growth and biomass allocation. To test the extent to which stand density in a wild forest has an effect on tree growth form, a simple regression was produced for both invasive species showing tree height in relation to basal diameter. Modelling of biomass and nutrients involved best-fit regressions of the biomass component over the measured basal diameter of the stem (30 cm). Most models presented here are power models, following the format of equation 1, as this format has been used widely. However, because a variety of regressions exist that would help make allometric biomass and nutrient models as accurate as possible, multiple regressions are tested throughout this study to ensure best-fit models are used through  $R^2$  values. Statistical analyses were done using Statistica® 13.

## 3.4 Results

The differences between Closed canopy trees and open- canopy trees with regards to their height-to-BD (Basal Diameter) relationship is shown in Fig. 3.2 (A) for *Acacia mearnsii* and Fig 3.2 (B) for *Eucalyptus camaldulensis*. Figures 3.2 A and B suggest that *A. mearnsii* is more affected by intraspecific competition for light than *E. camaldulensis*, as trees in Closed canopy *A. mearnsii* sites showed more height gain than Open canopy ones. Given the power models for all of the categories, the slowest-growing trees were Open canopy *A. mearnsii* (~5.25 m high at 5 cm BD and ~8.76 m high at 10 cm diameter). Both Closed canopy and Open canopy *Eucalyptus camaldulensis* trees had faster growth rates than *A. mearnsii*. For instance, *E. camaldulensis* trees were on average 5.54 m and 5.83 m high for Closed canopy and Open canopy trees, respectively at 5 cm BD and 9.11 m and 9.57 m at 10 cm BD for Closed canopy and Open canopy trees, respectively.



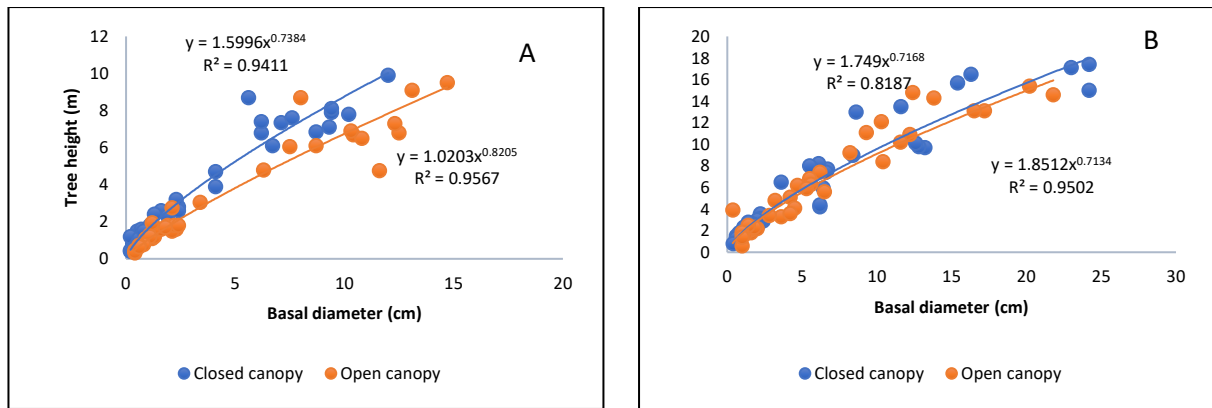


Figure 3.2 Differences in growth rates of trees growing in Closed canopy stands and Open canopy stands for *A. mearnsii* (A) and *E. camaldulensis* (B).

### ***Acacia mearnsii***

To determine the change in the percentage of the total biomass occupied by the various biomass components as a factor of stem densities (Closed canopy vs Open canopy) and plant life stage (juvenile, sapling, and adult), a factorial ANOVA and a post-hoc LSD test were used.

Figure 3.3 shows Open canopy trees (*A. mearnsii*) having a significantly greater leaf component than Closed canopy growing trees (LSD;  $P < 0.05$ ) but in both cases decline significantly from the juvenile to adult stage. This is the inverse of the trend taken by the stem components, (Fig 3.3 B) which increases significantly from the juvenile to adult stage as would be expected for trees. The stem component of Open canopy trees is the greatest at the adult stage; the lowest percentage of stem component is found in juveniles for both density extremes which, in both cases, is significantly less than the adult and sapling stages. Adult Closed canopy trees had a significantly greater stem biomass component than that of juveniles and saplings from both densities (LSD;  $P < 0.05$ ), while Open canopy adults do not increase their stem biomass component after the sapling stage.

The bark component of *A. mearnsii* also decreases significantly as trees grow bigger, with adult plants having significantly less biomass allocated to the bark component than juvenile trees (Fig 3.3 C). Bark component of Open canopy trees remained unchanged throughout the various life stages (LSD;  $P \geq 0.05$ ). The branch component of Closed canopy trees increases constantly but not significantly (LSD;  $P \geq 0.05$ ) from juvenile to the adult stage (Fig 3.3 D), while it decreases significantly in the Closed canopy stands (LSD;  $P < 0.05$ ).

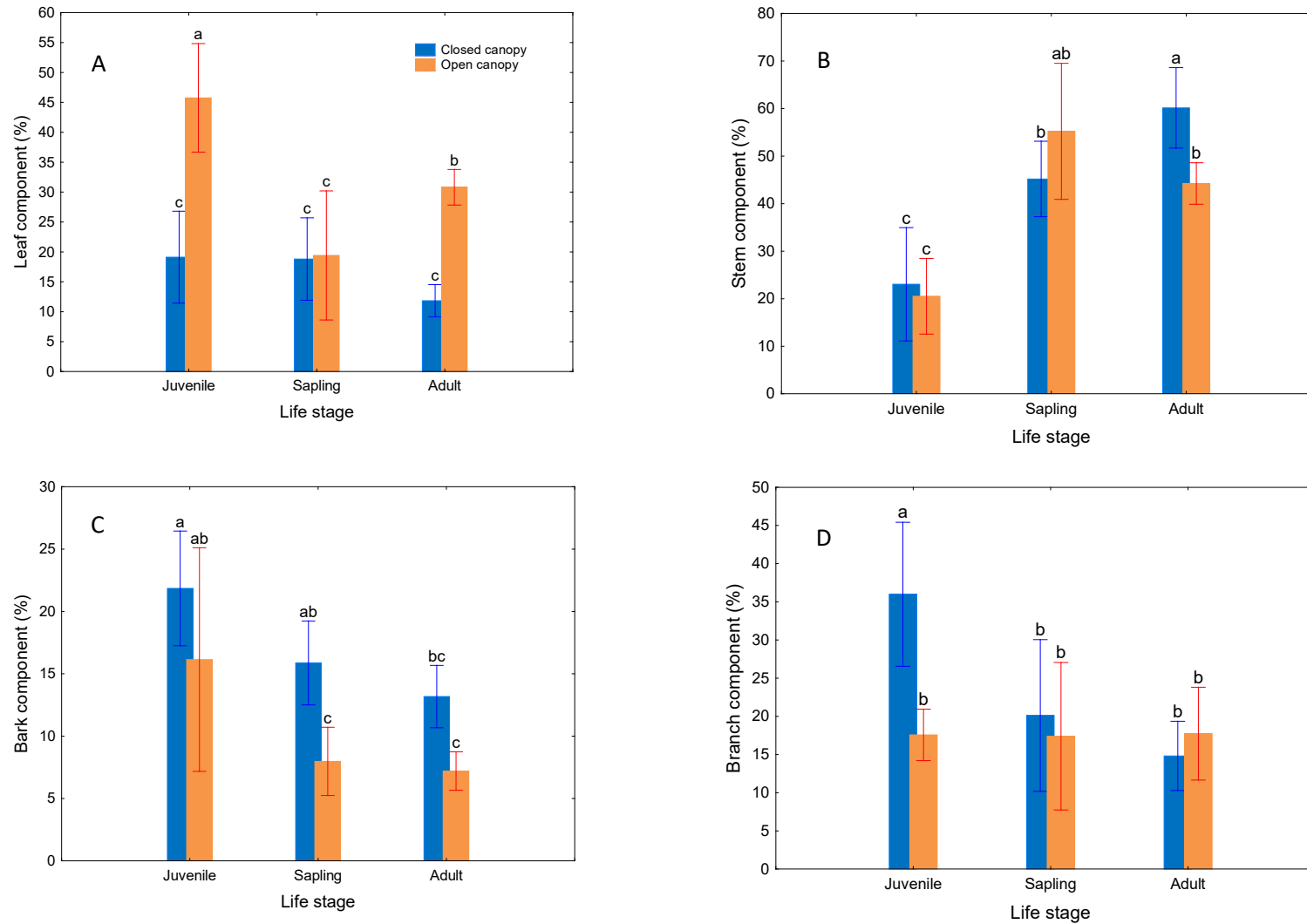


Figure 3.3: Biomass allocation changes of *Acacia mearnsii* between three life stages; juvenile, sapling, and adult. Components are shown in each of the various figures: Leaves (A), Stem (B), Bark (C), and Branches (D). Letters above error bars show where significant differences were found (Fisher's LSD test;  $P < 0.05$ ); error bars denote 0.95 confidence intervals.

Results from a Principal Components Analysis are shown in Figure 3.4 and a correlation matrix in Table 3.1. This analysis was done to determine the relationships of six variables (tree height, stem basal diameter, stem biomass, leaf biomass, bark biomass, and branches biomass) to one another and the influence of stem density extremes on those variables. Density is depicted as 1 or 2 (1= Closed canopy stands; 2= Open canopy stands). Figure 3.4 shows how adult trees are clustered around the third (Closed canopy) and fourth (Open canopy) quadrants. Tree height is strongly associated with trees in Closed canopy stands, while Open canopy stands favoured increased growth of leaves and branches. All variables are weighted heavily towards adult trees, showing increase in biomass of the various components and increments in diameter and height with tree age. This can also be seen in Figure 3.4 (B), where saplings and juveniles are clustered together, and adults are clustered on the opposite side of the plane. This plot shows that the distinction between growth forms of saplings and juveniles and between different stem densities are similar relative to adult trees.

Table 3.1: Pearson correlation matrix of the various Closed canopy *A. mearnsii* aboveground tree components to one another and to stem basal diameter (BD) and tree height.

	BD	Height	Stem	Leaf	Bark	Branch	Total
BD							
Height	0.962						
Stem	0.887	0.794					
Leaf	0.974	0.934	0.928				
Bark	0.935	0.877	0.978	0.962			
Branch	0.878	0.821	0.71	0.868	0.757		
Total	0.93	0.848	0.992	0.964	0.987	0.789	

Table 3.2: Pearson correlation matrix of the various Open canopy *A. mearnsii* aboveground tree components to one another and to stem basal diameter (BD) and tree height.

	BD	Height	Stem	Leaf	Bark	Branch	Total
BD							
Height	0.949						
Stem	0.988	0.954					
Leaf	0.981	0.942	0.989				
Bark	0.954	0.87	0.937	0.942			
Branch	0.87	0.778	0.848	0.825	0.9		
Total	0.988	0.933	0.989	0.982	0.967	0.912	

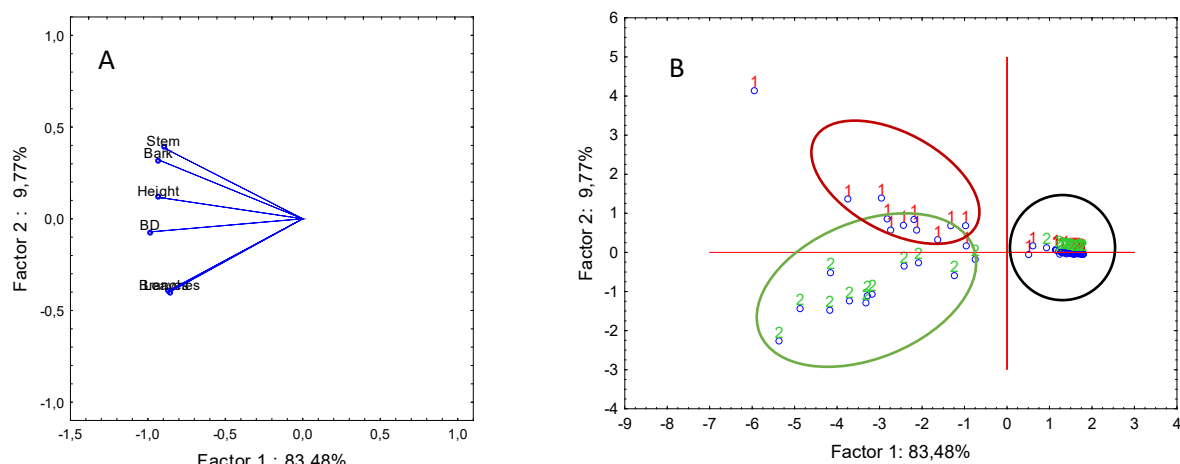


Figure 3.4: Ordination of six variables used to describe *A. mearnsii* biomass allocation (A) as a factor of stem densities (B). Circles represent Closed canopy stands (red; 1) and Open canopy stands (green; 2). The black circle represents juveniles and saplings from both density extremes. Factors considered describe a total of 93.25% of the variation in the dataset used.

Tree height and basal diameter (BD) are tested against aboveground biomass components of black wattles (Table 3.3). As a descriptive variable, BD explains more variation in the datasets of the biomass components than tree height. However, all variables tested showed high correlations with each other ( $R^2 > 0.5$ ). Models were developed from the above data for the biomass allocation of each of the aboveground biomass components of *A. mearnsii* (Table 3.2), using stem basal diameter (cm) as independent variable.

Table 3.3: Predictive models generated from power regressions of biomass data over all size ranges of *A. mearnsii*, distinguishing between Closed canopy stands and Open canopy stands. Stem diameter (cm; x) can be used to determine biomass (kg dw).

Component	Closed canopy		Open canopy	
	Regression	$R^2$	Regression	$R^2$
<b>Stem</b>	$y = 0.047x^{2.42}$	0.967	$y = 0.02x^{2.645}$	0.964
<b>Leaves</b>	$y = 0.02x^{1.918}$	0.911	$y = 0.025x^{2.416}$	0.918
<b>Bark</b>	$y = 0.027x^{1.91}$	0.955	$y = 0.008x^{2.147}$	0.914
<b>Branches</b>	$Y = 0.034x^{1.767}$	0.883	$y = 0.012x^{2.407}$	0.928
<b>Total</b>	$y = 0.156x^{2.052}$	0.9475	$Y = 0.079x^{2.326}$	0.973

### ***Eucalyptus camaldulensis***

Figure 3.5 (A) shows a distinct difference in the relative contribution of the leaf component between Closed canopy stands and Open canopy stands of *E. camaldulensis*. This is consistent for all three age groups: foliage occupying the greatest biomass proportion in the sapling stage, as expected. In all stages, trees in Closed canopy stands had significantly less leaves (Fig 3.5 A) at similar basal diameters than those growing freely (LSD;  $P < 0.05$ ). This trend is inverted for the stem component, with the Closed canopy trees investing significantly more energy into stem development than Open canopy trees (Fig 3.5 B). Bark and branches showed no clear trend; however, saplings from Closed canopy trees had a significantly greater bark and branch proportion than any of the other categories (Figure 3.5 C and D).

Juveniles that grow in Closed canopy stands also have significantly less of their mass in? bark and branch components than other categories (LSD;  $P < 0.05$ ). The percentage change of the branch component in *E. camaldulensis* showed a significant difference between trees from different life stages in Closed canopy stands (LSD;  $P < 0.05$ ). The branch component peaks for Closed canopy stands at the sapling stage and then decreases towards the adult stage. For Open canopy trees, there is a constant but non-significant decrease in branch component from juvenile to adult, as expected.

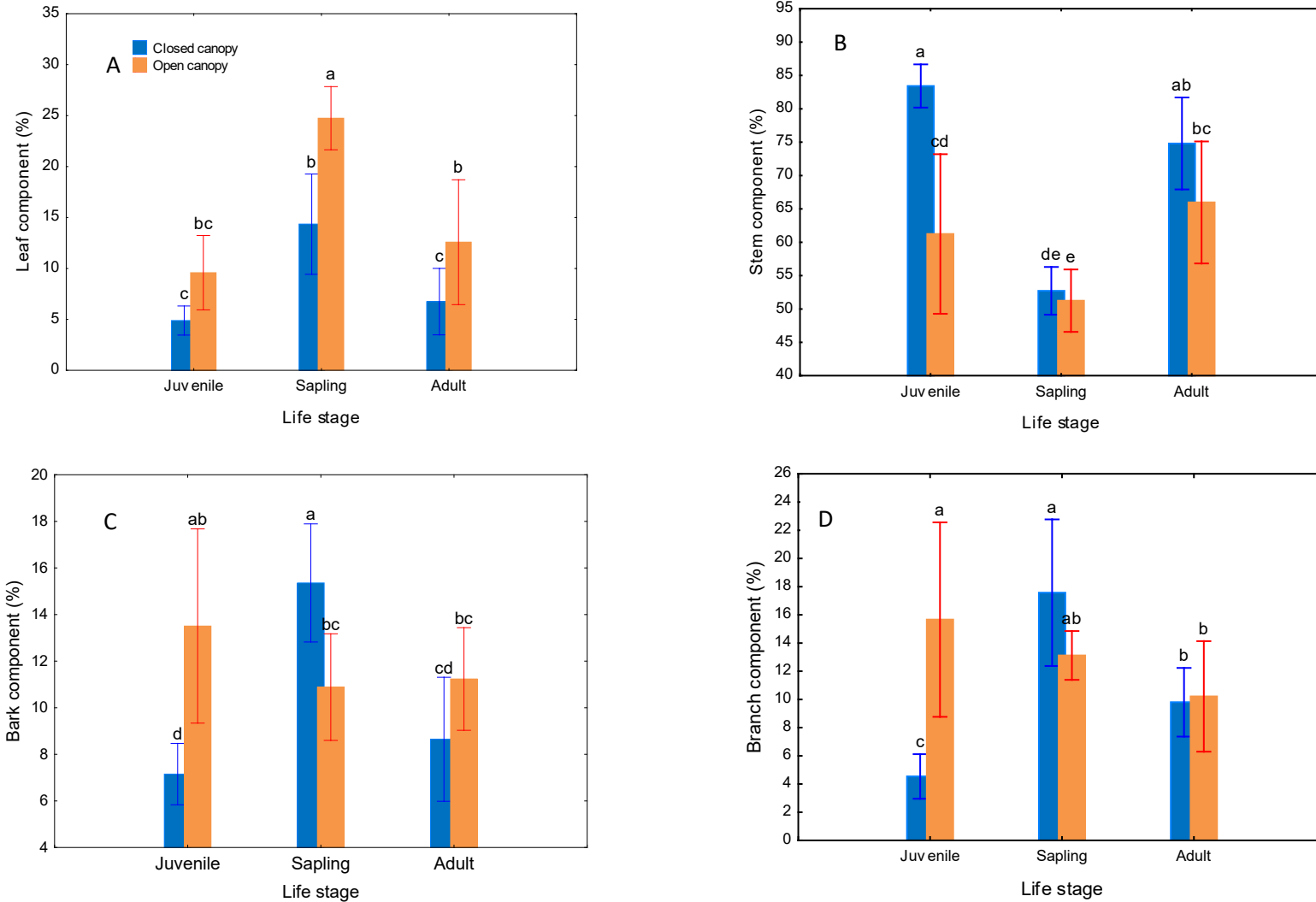


Figure 3.5: Biomass component changes of *Eucalyptus camaldulensis* between three life stages; juvenile, sapling, and adult. Components are shown in each of the various figures: Leaves (A), Stem (B), Bark (C), and Branches (D). Letters above error bars show where significant differences were found (Fisher's LSD test;  $P < 0.05$ ); error bars denote 0.95 confidence intervals.

Table 3.4: Pearson correlation matrix of the various Closed canopy *E. camaldulensis* aboveground components to one another and to stem basal diameter (BD) and tree height.

	BD	Height	Stem	Leaf	Bark	Branch	Total
BD							
Height	0.94						
Stem	0.937	0.852					
Leaf	0.67	0.751	0.523				
Bark	0.905	0.793	0.92	0.504			
Branch	0.864	0.742	0.898	0.506	0.988		
Total	0.946	0.857	0.996	0.553	0.947	0.929	

Table 3.5: Pearson correlation matrix of the various Open canopy *E. camaldulensis* aboveground components to one another and to stem basal diameter (BD) and tree height.

	BD	Height	Stem	Leaf	Bark	Branch	Total
BD							
Height	0.953						
Stem	0.826	0.749					
Leaf	0.917	0.889	0.729				
Bark	0.866	0.79	0.97	0.74			
Branch	0.929	0.684	0.836	0.923	0.841		
Total	0.867	0.787	0.996	0.788	0.978	0.852	

Similar to *A. mearnsii*, leaf biomass of *E. camaldulensis* was highly correlated with plants from Open canopy sites and there is a clear distinction between adults and younger plants (Figure 3.6). Branch, stem, and bark material, however, show greater correlation with trees in Closed canopy stands.

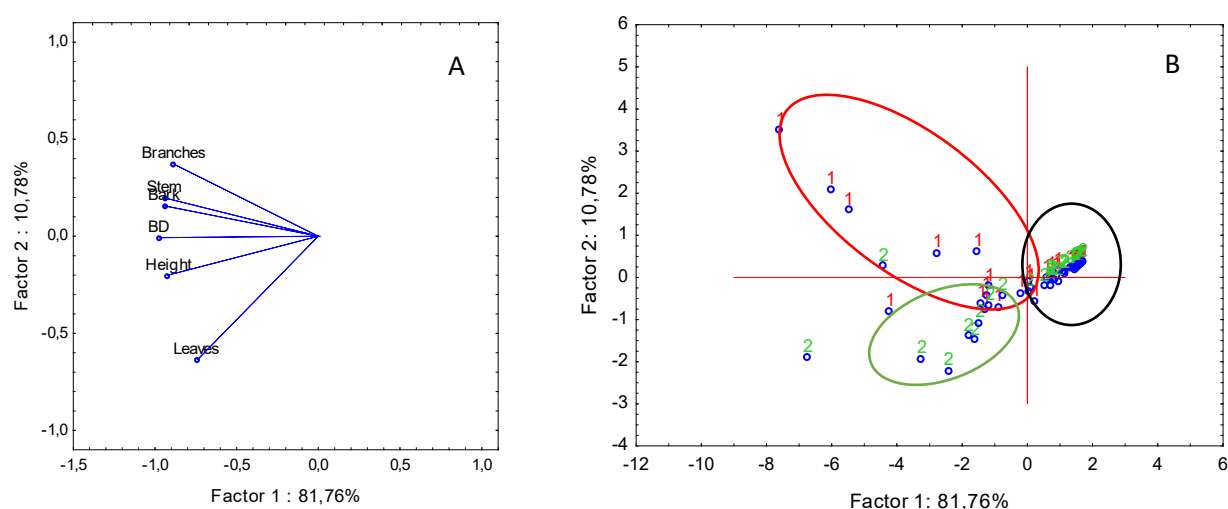


Figure 3.6: Ordination of six variables used to describe *E. camaldulensis* biomass allocation (A) as a factor of stem densities (B). Circles represent Closed canopy stands (red; 1) and Open canopy stands (green; 2). The black circle represents juveniles and saplings from both density extremes. Factors used describe a total of 89% of the variation in the dataset.



Models for *E. camaldulensis* were also developed using basal diameter (cm; x) as independent variable (Table 3.4).

Table 3.6: Predictive models generated from power regressions of biomass data over all size ranges of *E. camaldulensis*, distinguishing between Closed canopy stands and Open canopy stands. Stem diameter (cm; x) can be used to determine biomass.

Component	Closed canopy	R <sup>2</sup>	Open canopy	R <sup>2</sup>
	Regression		Regression	
<b>Stem</b>	$y = 0.052x^{2.34}$	0.903	$y = 0.035x^{2.484}$	0.881
<b>Leaves</b>	$y = 0.004x^{2.389}$	0.866	$y = 0.007x^{2.387}$	0.828
<b>Bark</b>	$y = 0.007x^{2.397}$	0.934	$y = 0.008x^{2.389}$	0.94
<b>Branches</b>	$y = 0.005x^{2.574}$	0.897	$y = 0.009x^{2.27}$	0.81
<b>Total</b>	$y = 0.073x^{2.37}$	0.922	$y = 0.065x^{2.439}$	0.88

### 3.5 Discussion

Allometric modelling of biomass and nutrients is a useful tool in forestry to estimate stand productivity as a factor of carbon sequestration and nutrient use efficiency, and to determine the effect of large-scale harvesting on soil nutrient reserves. It is also useful for economic models aimed at value added biomass products. This chapter presents various best-fit models for aboveground biomass components of *A. mearnsii* and *E. camaldulensis* that could be used for either purpose. Models presented here support Niklas's (2006) notion of allometric rather than isometric growth in trees, and show that proportions of biomass allocated to different AGB components is variable between life stages. All models show similar trends in biomass response to an increase in stem diameter, measured at stem Basal Diameter (BD) as most juveniles measured did not yet reach sufficient height for measurements at the Diameter of Breast Height (1.3 m). The high predictability of variables (R<sup>2</sup>) suggests that it is possible to produce these models for any stand of sufficient size of invasive trees and could be used effectively in stand management.

Some of the principles of the Optimal Partition Theory are evident in the findings from this study. Relatively conspicuous differences such as branch and leaf biomass can be ascribed to a response to availability of sunlight. The differences in access to resources such as sunlight and the constant competition with conspecifics in Closed canopy stands result in visible growth form differences compared to those growing freely. Lödige et al. (2014), however, also found that soil moisture and nutrient content have a significant influence on aboveground biomass allocation in some trees. Moreover, this holds true for temporal changes in requirements within

plants, which can result in translocation of resources between components. Poorter and Nagel (2000) point out that larger plants typically have to invest relatively more resources in support structures and leaf area. It is also known that plant growth is affected by external factor such as crowding/ intraspecific competition (Enquist et al., 1998) but may also be limited by resource allocation to height growth as a function of stem diameter/ strength (Niklas, 1994). Research shows that some *Eucalyptus* species may have high light-use efficiency (Binkley et al., 2009), enabling steady and improved growth rates when soil conditions are optimal. It has also been reported that this increased light-use efficiency of *Eucalyptus* species allows it to dominate *Acacia* species in mixed-species plantations. This is shown by Lemaire et al. (2013), who reported the results for *E. grandis* and *A. mangium*, significantly affecting how close these could be planted to each other. In the current study, it is shown that *E. camaldulensis* grows much faster than *A. mearnsii* trees, suggesting that it would outcompete *A. mearnsii* in an even-aged stand where the only limiting factor is light availability. Interestingly, the differences in growth rates between Closed canopy and Open canopy *E. camaldulensis* trees was not clear during the current study. It is thus likely that light is not the most limiting factor, as can be expected in nutrient-poor Fynbos soils. Trends for *A. mearnsii*, however, show that light-limited plants in Closed canopy stands grow tall more rapidly than Open canopy trees; however, not at the same rates as *E. camaldulensis* trees. As shown in the Principal Components Analyses (PCAs) in this study, *Acacia mearnsii* trees from Closed canopy stands were greatly influenced by intraspecific competition for sunlight, resulting in tall thin trees in Closed canopy stands, whereas Open canopy trees were more likely to exhibit a proportionately greater biomass of leaves and branches, as can be seen in the steeper slopes of models (Table 3.3).

Being a strong nitrogen fixer, *A. mearnsii* is able to manipulate its soil environment to favour its growth in nutrient-poor Fynbos riparian soils (Naude, 2012). It has previously been documented that *Eucalyptus* trees deplete soil nutrient reserves fast and have a significant effect on sites with known low levels of nutrients (Turner & Lambert, 1986); nutrients may thus become so limited in an unmanaged forest that tree height growth is limited by the ability to efficiently distribute available resources. While it is shown here that tree growth form differs as a result of life stage, the effect of competition for light is more evident in *A. mearnsii* stands than *E. camaldulensis* stands. However, there are statistical differences in biomass allocation of both species between life stages and between stand densities. The study hypothesis (tree growth form of both *Acacia mearnsii* and *Eucalyptus camaldulensis* is affected significantly by stem densities and life stage and affects its modelling) could thus not be rejected for either species.

### 3.6 Conclusions

This study has shown that increases in stem density increase the proportion of biomass allocated to the stem and proportionately reduce that allocated to other components (especially leaves and branches). Tree form has been shown here to be largely a result of stem densities but could also be related to stand age and access to resources which are not density-dependent. Biomass determinations of both *A. mearnsii* and *E. camaldulensis* can be done with relative accuracy within sites tested here. This can be done with simple basal diameter measurements and using allometric models produced during this study. It is uncertain whether these models are applicable to sites outside of the study area or for plants outside of the size range tested here. It is thus recommended that small trials precede any management activity based on data generated from these models. With a greater sample size, these models will also become more and more accurate for the sites used during this study and more broadly applicable to others. As proposed by Vundla et al. (2016) and Stafford & Blignaut (2017), value adding to invasive biomass, specifically stem biomass, has great economic potential for rural communities and for South Africa's economy. Economic models for Value Added Products (VAPs) from various components invasive biomass such as those put forward by Stafford & Blignaut (2017) could benefit greatly from models such as these to drive more effective and financially sustainable clearing of stands of invasive alien trees in riparian zones.

### 3.7 References

- Beets, P. N., Kimberley, M. O., Oliver, G. R., Pearce, S. H., Graham, J. D., & Brandon, A. (2012). Allometric equations for estimating carbon stocks in natural forest in New Zealand. *Forests*, 3(3), 818-839.
- Binkley, D., Stape, J. L., Bauerle, W. L., & Ryan, M. G. (2010). Explaining growth of individual trees: light interception and efficiency of light use by *Eucalyptus* at four sites in Brazil. *Forest Ecology and Management*, 259(9), 1704-1713.
- Brown, S. (1997). *Estimating biomass and biomass change of tropical forests: a primer* (Vol. 134). Food & Agriculture Org.
- Chapin, F. S., Schulze, E., & Mooney, H. A. (1990). The Ecology and Economics of Storage in Plants. *Annual Review of Ecology and Systematics*, 21(1), 423-447. <https://doi.org/10.1146/annurev.es.21.110190.002231>
- Chave, J., Réjou-Méchain, M., Búrquez, A., Chidumayo, E., Colgan, M. S., Delitti, W. B., Duque, A., Eid, T., Fearnside, P. M., Goodman, R. C., Henry, M., Martinez-Yrizar, A., Mugasha, W. A., Muller-Landau, H. C., Mencuccini, M., Nelsonm B. W., Ngomanda, A., Nogueria, E. M., Ortiz-Malavassi, E., Pelissier, R., Ploton, P., Ryan, C. M., Saldarriaga, J. G., & Vieilledent, G. (2014). Improved allometric models to estimate the aboveground biomass of tropical trees. *Global change biology*, 20(10), 3177-3190.

- Diédhiou, I., Diallo, D., Mbengue, A. A., Hernandez, R. R., Bayala, R., Diémé, R., Diédhiou, P.M., & Sène, A. (2017). Allometric equations and carbon stocks in tree biomass of *Jatropha curcas* L. in Senegal's Peanut Basin. *Global Ecology and Conservation*, 9, 61-69.
- Du Toit, B., Dovey, S. B., Fuller, G. M., & Job, R. A. (2004). Effects of harvesting and site management on nutrient pools and stand growth in a South African eucalypt plantation. *Site Management and Productivity in Tropical Plantation Forests*, 31.
- Geng, Y. P., Pan, X. Y., Xu, C. Y., Zhang, W. J., Li, B., & Chen, J. K. (2007). Plasticity and ontogenetic drift of biomass allocation in response to above- and below-ground resource availabilities in perennial herbs: A case study of *Alternanthera philoxeroides*. *Ecological Research*, 22(2), 255–260. <https://doi.org/10.1007/s11284-006-0017-9>
- Henderson, L. (2007). Invasive, naturalized, and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). *Bothalia*, 37(2), 215-248.
- Henderson, L., & Wilson, J. R. (2017). Changes in the composition and distribution of alien plants in South Africa: An update from the Southern African Plant Invaders Atlas. *Bothalia-African Biodiversity & Conservation*, 47(2), 1-26.
- King, D. A. (1990). Allometry of saplings and understorey trees of a Panamanian forest. *Functional Ecology*, 4(1), 27–32. <https://doi.org/10.2307/2389648>
- Latte, N., Colinet, G., Fayolle, A., Lejeune, P., Hébert, J., Claessens, H., & Bauwens, S. (2013). Description of a new procedure to estimate the carbon stocks of all forest pools and impact assessment of methodological choices on the estimates. *European journal of forest research*, 132(4), 565-577.
- Le Maire, G., Nouvellon, Y., Christina, M., Ponzoni, F. J., Gonçalves, J. L. D. M., Bouillet, J. P., & Laclau, J. P. (2013). Tree and stand light use efficiencies over a full rotation of single-and mixed-species *Eucalyptus grandis* and *Acacia mangium* plantations. *Forest Ecology and Management*, 288, 31-42.
- Le Maître, D. C., Versfeld, D. B., & Chapman, R. A. (2000). Impact of invading alien plants on surface water resources in South Africa: A preliminary assessment. *WaterSA*, 26 (3)
- Lödige, C., Schall, P., & Ammer, C. (2014). How do size and resource availability control aboveground biomass allocation of tree seedlings? *Forest Research: Open Access*, 3(3).
- Mandal, R. A., Dutta, I. C., Jha, P. K., & Karmacharya, S. B. (2013). Evaluating sustainability in community and collaborative forests for carbon stocks. *Proceedings of the International Academy of Ecology and Environmental Sciences*, 3(2), 76.
- Masera, O. R., Garza-Caligaris, J. F., Kanninen, M., Karjalainen, T., Liski, J., Nabuurs, G. J., Pussinen, A., De Jong, B. H. J., & Mohren, G. M. J. (2003). Modeling carbon sequestration in afforestation, agroforestry and forest management projects: the CO2FIX V. 2 approach. *Ecological modelling*, 164(2-3), 177-199.
- Mugasha, W. A., Eid, T., Bollandssås, O. M., Malimbwi, R. E., Chamshama, S. A. O., Zahabu, E., & Katani, J. Z. (2013). Allometric models for prediction of above-and belowground biomass of trees in the miombo woodlands of Tanzania. *Forest Ecology and Management*, 310, 87-101.
- Mugido, W., Blignaut, J., Joubert, M., De Wet, J., Knipe, A., Joubert, S., Cobbing, B., Jansen, J., Le Maître, D., & Van der Vyfer, M. (2014). Determining the feasibility of harvesting invasive alien plant species for energy. *South African Journal of Science*, 110(11-12), 01-06.
- Muturi, G. M., Kariuki, J. G., Poorter, L., & Mohren, G. M. (2012). Allometric equations for estimating biomass in naturally established *Prosopis* stands in Kenya. *Journal of Horticulture and Forestry*, 4(4), 68-77.
- Muyambo, P. (2017). *Modelling aboveground biomass and nutrient export in South African Pinus elliottii* (Doctoral dissertation).

- Naudé, M. (2012). *Fynbos riparian biogeochemistry and invasive Australian acacias* (MSc thesis, Stellenbosch: Stellenbosch University).
- Niklas, K. J. (2006). A phyletic perspective on the allometry of plant biomass-partitioning patterns and functionally equivalent organ-categories. *New Phytologist*, 171(1), 27-40.
- Niklas, K. J. (2006). Plant allometry, leaf nitrogen and phosphorus stoichiometry, and interspecific trends in annual growth rates. *Annals of Botany*, 97(2), 155-163.
- Niklas, K. J., & Enquist, B. J. (2001). Invariant scaling relationships for interspecific plant biomass production rates and body size. *Proceedings of the National Academy of Sciences*, 98(5), 2922-2927.
- Niklas, K. J., & Enquist, B. J. (2002). On the vegetative biomass partitioning of seed plant leaves, stems, and roots. *The American Naturalist*, 159(5), 482-497.
- Ounban, W., Puangchit, L., & Diloksumpun, S. (2016). Development of general biomass allometric equations for *Tectona grandis* Linn. f. and *Eucalyptus camaldulensis* Dehnh. plantations in Thailand. *Agriculture and Natural Resources*, 50(1), 48-53.
- Phiri, D., Ackerman, P., Wessels, B., du Toit, B., Johansson, M., Säll, H., Lundqvist, S., & Seifert, T. (2015). Biomass equations for selected drought-tolerant eucalypts in South Africa. *Southern Forests: A Journal of Forest Science*, 77(4), 255-262.
- Poorter, H. A., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO<sub>2</sub>, nutrients and water: A quantitative review. *IMF Occasional Papers*, 27(189), 595-607. [https://doi.org/10.1071/PP99173\\_CO](https://doi.org/10.1071/PP99173_CO)
- Pretzsch, H., Grote, R., Reineking, B., Rötzer, T. H., & Seifert, S. T. (2008). Models for forest ecosystem management: a European perspective. *Annals of botany*, 101(8), 1065-1087.
- Rijkers, T., Pons, T. L., & Bongers, F. (2000). The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. *Functional Ecology*, 14(1), 77-86.
- Sist, P., Mazzei, L., Blanc, L., & Rutishauser, E. (2014). Large trees as key elements of carbon storage and dynamics after selective logging in the Eastern Amazon. *Forest Ecology and Management*, 318, 103-109.
- Stafford, W., & Blignaut, J. (2017). Reducing landscape restoration costs: Feasibility of generating electricity from invasive alien plant biomass on the Agulhas Plain, South Africa. *Ecosystem Services*, 27, 224-231.
- Tetemke, B. A., Birhane, E., Rannestad, M. M., & Eid, T. (2019). Allometric Models for Predicting Aboveground Biomass of Trees in the Dry Afromontane Forests of Northern Ethiopia. *Forests*, 10(12), 1114.
- Thornley, J. H. M. (1972). A model to describe the partitioning of photosynthate during vegetative plant growth. *Annals of Botany*, 36(2), 419-430.
- Turner, J., & Lambert, M. J. (1986). Effects of forest harvesting nutrient removals on soil nutrient reserves. *Oecologia*, 70(1), 140-148.
- Vundla, T., Blignaut, J., Nkambule, N., Morokong, T., & Mudavanhu, S. (2016). The opportunity cost of not utilising the woody invasive alien plant species in the Kouga, Krom and Baviaans catchments in South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 814-830.

## Chapter 4: Nutrient contents of aboveground biomass components of the invasive *Eucalyptus camaldulensis* and *Acacia mearnsii* in riparian zones of the Western Cape, South Africa

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### 4.1 Abstract

Invasive trees in South African riparian zones have been shown to significantly alter nutrient cycles, which may remain disrupted for extended periods after their removal. In the nutrient-poor but botanically diverse Fynbos ecosystems, disruption or alteration of nutrient cycles could amplify the known effects of invasion by alien trees on these ecosystems. Previous research has focused largely on the effects of invasive alien trees on soil and hydrological function of invaded riparian zones and wetlands. Nutrient dynamics in the aboveground biomass components of invasive trees are not widely reported on but could provide crucial insight into the total effect of IAPs on ecosystem nutrient cycles. This study reports on the nutrient dynamics of aboveground components of the invasive *Acacia mearnsii* and *Eucalyptus camaldulensis* in South Africa's Breede River catchment in the Western Cape as a result of plant age group (juvenile, sapling, or adult), stem densities (Closed canopy or Open canopy), and biomass components (stem, leaves, branches, and bark). Nutrient contents, except carbon, were generally lowest in the bulky stem material and most concentrated in leaves for both tree species. Stem material had significantly greater C:N ratios but significantly lower N:P ratios than any of the other components for both species. Trends were similar between stands of different densities. Foliar C:N ratios have been extensively reported on before and their ecological implications are well understood; low foliar C:N ratios could suggest fast decomposition potential of material and thus rapid release of nutrients into soil and nearby water systems. Through exploring N:P ratios of the biomass of the two invasive species, and a discussion of their potential relationship with soil nutrient availability, this study provides an opportunity for further investigation into the role of soil conditions in the establishment of invasive alien species in nutrient-poor fynbos ecosystems. It shows that establishment and removal of invasive alien plants from Fynbos riparian zones could significantly impact local nutrient cycles through both nutrient addition, and abstraction. By reporting on spatial and temporal influences on within-tree nutrient dynamics, this study makes it possible to accurately model the actual effect of these invasive trees on riparian zone nutrient cycles and, more importantly, the potential effects of abrupt clearing and removal of these plants from invaded areas. To enable accurate and useful determinations of nutrient stocks



within invasive stands of *A. mearnsii* and *E. camaldulensis*, allometric models are presented here that use stem basal diameter (BD) as the independent variable.

## 4.2 Introduction

Plasticity in resource allocation in plants can be attributed to the plant's response to internal and external factors which largely determine its physiological requirements (Attiwill & Adams 1993). In natural forests, allocation of any resource to a plant organ is species-specific and can be influenced by the age of the plant, competition for resources as a result of stand density, and could also respond to changes in climatic or seasonal weather conditions (Weiner, 2004). This is likely to differ from resource allocation in plantation forestry where stands are evenly spaced and of a similar age, minimizing the effects of competition and age-related differences in resource allocation. These management activities make forest nutrient fluxes more predictable and enables reliable tracking and (where necessary) replenishment, whereas nutrient distributions in natural or invasive stands are much more complex due to inherent heterogeneity in stand age structure, spacing, species composition, and environmental influences.

Plants require a wide range of nutrients to sustain their metabolism, growth, and reproduction, most of which are available from the soil (Schulze, 2004). Chapin et al. (1986) noted that the supply of a nutrient in the soil, as well as the actual uptake of a nutrient by the plant determines the rate of absorption of that nutrient. Plants growing in infertile soils thus often appear prone to storing excess nutrients at the expense of a fast growth rate (Chapin, 1980).

There is extensive research done on stoichiometry and allometry in ecology, including how these are affected by, for instance, nutrient availability and other environmental variables (Muller et al., 2000; Kollman et al., 2004; Weiner, 2004; Sterner & Elser, 2008), and self-regulating mechanisms as a response to stem densities (Mohler et al., 1978; Pretzsch et al., 2008). It has also been proposed that the parts of the plant most limited in terms of available resources will enjoy relatively greater growth to increase acquisition of that resource (Ericsson et al., 1996). Drought-induced stress (e.g. as would be occurring in summer in Mediterranean regions) would, for instance, reduce shoot growth and cause a change in nutrient allocation to aboveground components, while increasing root growth (Ericsson *et al.*, 1996). While these responses can easily be observed through biomass measurements, the effects of internal and external changes on nutrient allocation within trees is not often reported on for natural stands of trees.



Data are available on the indirect effects of stands of *A. mearnsii* on nutrient cycling in Fynbos soils (i.e. associations with root bacteria and increases in soil enzymes; Jacobs et al., 2013; Slabbert *et al.*, 2014), but nutrients released into soil directly from plant material have not yet been quantified. Thus there is a gap in knowledge of the total inputs of nutrients to the affected ecosystem. There is also biomass and nutrient data available for *A. mearnsii*, grown in plantations (Dovey, 2005), which provides a solid baseline for the measurement of tree biomass in managed plantations, and the partitioning of nutrients and biomass to various tree components. It is still unclear whether these data translate into stands where *A. mearnsii* has become invasive. This chapter investigates nutrient allocation to different aboveground biomass components of *Acacia mearnsii* and *Eucalyptus camaldulensis* to determine whether temporal events such as life stage, and season, and density extremes (Closed canopy vs. Open canopy) have a significant effect on this allocation of nutrients.

Here, I hypothesised that:

- Aboveground nutrient allocation for both invasive species is affected by plant life stage and stem density.
- The leguminous *A. mearnsii* contains greater concentrations of N and P in biomass than the non-leguminous *E. camaldulensis*

## 4.3 Methods

### Study sites

This study was conducted in four riparian sites of the Breede River Catchment in the Western Cape, two of which each had stands of *Eucalyptus camaldulensis*, and two of *Acacia mearnsii* (Figure 1). The first *E. camaldulensis* site was on a private farm near the town of Wolseley (33°26'18.35"S; 19°13'36.26"E) and the second one on a dairy farm between Worcester and Robertson, below the Brandvlei dam (33°46'2.79"S; 19°32'13.11"E). The second *E. camaldulensis* site is adjacent to the first *A. mearnsii* site, which is situated on the same farm but forms a distinct stand to the south of the *E. camaldulensis* stand (33°46'3.34"S; 19°31'48.05"E). The other *A. mearnsii* site was at Bainskloof near Wellington, along the Wit River, which is a tributary of the Breede River (33°32'24.98"S; 19°10'36.26"E) (Figure 4.1).

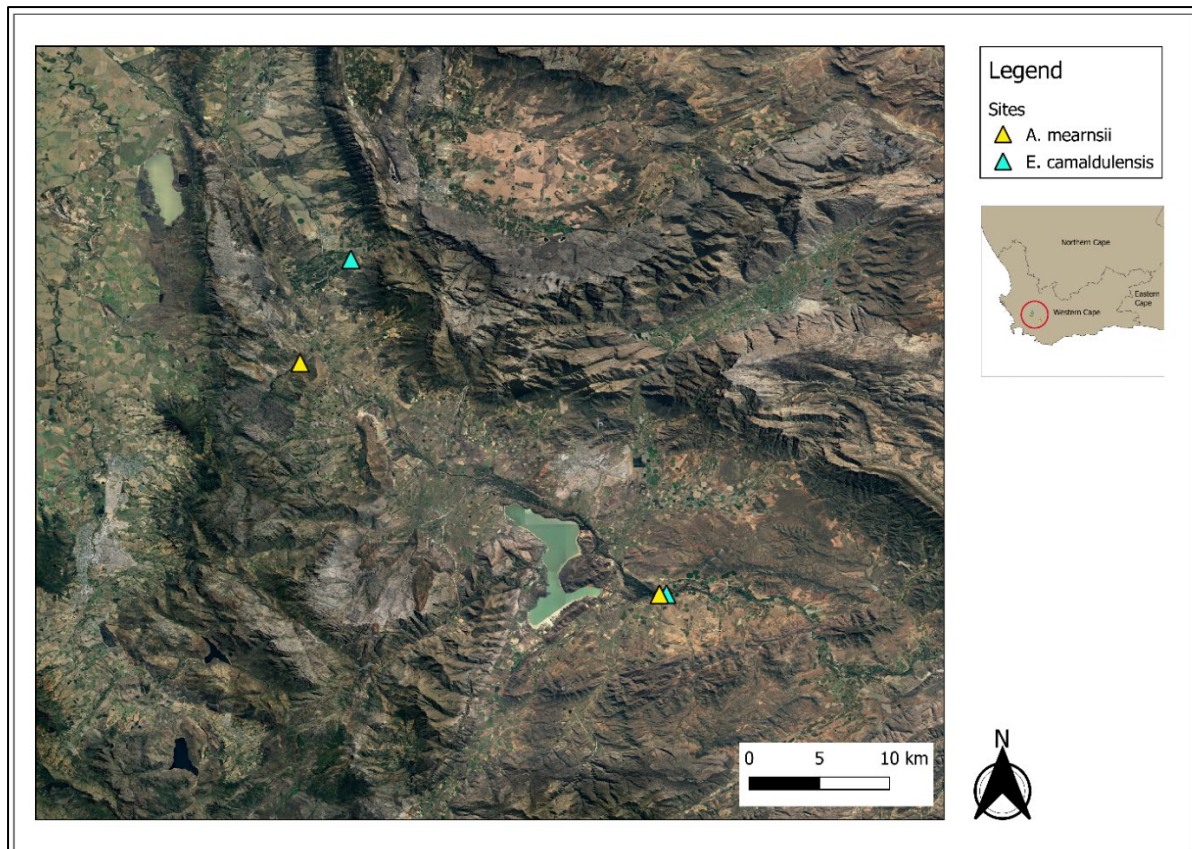


Figure 4.1: Location of study sites within the Western Cape of South Africa

Trees of *E. camaldulensis* and *A. mearnsii* were harvested and all aboveground components separated into: stem, leaves, bark, and branches. Three adult samples of each species were harvested in sites of high density (Closed canopy) and three each at sites of low density (Open canopy). Closed canopy sites were sites that had more than 75% canopy cover of either *E. camaldulensis* or *A. mearnsii*, while Open canopy sites had less than 25% canopy cover of either species. Sampling was also done on juvenile plants and saplings at the same densities as above for adult trees. Sampling was done during the summer. Adult trees were then also harvested during the following winter to account for internal movement of nutrients between aboveground biomass components as a response to seasonal weather patterns.

Nutrients were quantified by a commercial laboratory, Bemlab, located in Somerset West, South Africa. Dry samples were cut into smaller pieces using a band saw, after which they were crushed with a Netzsch Condux LV15M toothed disk mill to allow for further processing. An Ultra Centrifugal Retsch ZM200 mill was used to mill the crushed samples into a powder using a screen of 0.5 mm aperture size. Samples were stored in 15 ml plastic vials for laboratory analyses. Nutrient analyses using the following methods: Phosphorus (P), Potassium (K), Magnesium (Mg), Calcium (Ca) and Sodium (Na) contents were determined

using the Ash method (Lambert, 1976), which includes treating the ashed sample with a 50% solution of hydrochloric acid (HCl) and measuring the contents using a Perkin Elmer Optima 7300 DV ICP-OES (Inductively Coupled Plasma- Optical Emission Spectrometry) spectrometer. Carbon (C) and Nitrogen (N) contents were determined through Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Snook, 1992) using a LECO-CNS 2000 analyser.

## Statistics

Normal distribution within the datasets was confirmed with a Shapiro-Wilks test for normality. A 3-way factorial ANOVA was conducted for tests with three independent variables with the configuration of species-biomass component-life stage, and species-biomass component-density. Where significant differences or interactions between variables were found ( $P < 0.05$ ), a post-hoc Fisher's LSD test was used throughout. Where no significant differences were found, no further tests were conducted. Statistical analyses were done using Statistica® 13. Nutrient ratios were developed from percentage values derived from lab analyses and were mass-based. Allometric models were generated from best-fit models generated in Microsoft Excel. Modelling of nutrients involved best-fit regressions of the biomass component over the measured basal diameter of the stem. In this study, each model is developed on a per case basis; most models follow the standard power model structure, as previously used by Beets et al. (2012) and Tetemke et al. (2019) which follows the format:

$$Y = aX^b \quad (4.1)$$

Where  $a$  and  $b$  are parameters,  $X$  is Basal Diameter (BD) and  $Y$  is the nutrient mass (kg; tonnes for C).

## 4.4 Results

### Influence of stem density and life stage on tissue nutrient contents in *Acacia mearnsii*

Carbon percentage between biomass components and life stages of *A. mearnsii* showed significant differences (ANOVA:  $F_{[6, 176]} = 2.178$ ;  $P = 0.046$ ; Table 4.1). Carbon percentage differed significantly between the various biomass components for both densities and in both cases %C in leaves was significantly less than in the other biomass components (LSD;  $P < 0.05$ ), as expected. Carbon percentage was constant throughout life stages in most

instances; however, %C in branches of Juveniles of Closed canopy trees had significantly lower %C than saplings ( $P=0.015$ ) and adults ( $P=0.028$ ) and the bark component of this sample had significantly more %C at the juvenile stage than at sapling stage ( $P=0.039$ ). Carbon (C) was the greatest in the stem material but did not differ significantly from branch and bark materials. Leaves, overall, had the lowest %C (between  $55.14\% \pm 0.17$  in adult Open canopy trees, and  $55.49\% \pm 0.37$  in juvenile Open canopy trees), and Closed canopy and Open canopy trees showed similar %C in the leaves of *A. mearnsii*. There were significant differences in Nitrogen percentages between different tree components and densities of *A. mearnsii* (ANOVA:  $F_{[6, 174]}=4.362$ ;  $P<0.001$ ; Figure 4.3). Nitrogen percentage was highest in the leaves of all samples of *A. mearnsii* tested. This was significantly higher than the other components except for the bark and branches samples of the Closed canopy-growing juveniles (LSD;  $P>0.05$ ). Nitrogen in foliage of juvenile Open canopy trees ( $3.89 \pm 0.13\%$ ) was significantly higher than all other foliar samples ( $P<0.001$ ). The lowest levels of foliar %N were found in Closed canopy juveniles ( $2.75 \pm 0.29\%$ ) and Open canopy adults ( $2.66 \pm 0.29\%$ ). There was no significant difference in foliar %N in the sapling stage between the two densities ( $P=0.439$ ). Stem material had the lowest %N content over all life stages (between  $0.25 \pm 0.10\%$  in Open canopy saplings and  $0.48 \pm 0.24\%$  in Closed canopy saplings) and there were no significant differences between these samples ( $P>0.05$ ).

Density also had a significant impact on %P content ( $F_{[1, 174]}=0.904$ ;  $P<0.001$ ), as did biomass component ( $F_{[3, 174]}= P<0.001$ ). There was significant interaction between different biomass components between life stages ( $F_{[6, 174]}=0.848$ ;  $P<0.001$ ; Figure 4.4). Phosphorus content of aboveground biomass was highest in bark material of both Open canopy and Closed canopy juveniles. In foliage of Open canopy juveniles, %P content was significantly higher than in Open canopy saplings (LSD;  $P<0.001$ ). The highest foliar %P was found in adults of Closed canopy *A. mearnsii* trees ( $0.104 \pm 0.05\%$ ) and was significantly higher than in leaves of Closed canopy saplings and juveniles ( $P<0.001$ ). Phosphorus percentage was also lowest in stem samples throughout (as low as  $0.03 \pm 0.02\%$  in Closed canopy juvenile stems). Potassium (%K) was also greatest in leaf material of *A. mearnsii* and was significantly higher than the other components ( $P<0.05$ ). Open canopy trees had significantly higher stem and bark %K than trees in Closed canopy stands, and there were no significant differences for the other biomass components. Bark and branches in Open canopy trees had significantly higher %Ca than Closed canopy stands. There were no significant differences for leaves and stems. Mg was also the highest in leaves and the lowest in the stems but showed no significant differences between densities. Sodium (Na) content was the highest in branches of *A. mearnsii* but only the Closed canopy growing trees were significantly higher than all the other components (including branches of Open canopy trees).

**Table 4.1:** *Acacia mearnsii* life stage, density, and biomass component (mean  $\pm$  SD). Letters in superscript show where significant differences were found (Fisher's LSD: Significance level at  $P < 0.05$ .)

	Density	Life stage	C (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)
Bark	Closed canopy	Juvenile	57.35 $\pm$ 0.51 <sup>abc</sup>	2.35 $\pm$ 0.69 <sup>ce</sup>	0.10 $\pm$ 0.07 <sup>ac</sup>	0.21 $\pm$ 0.10 <sup>af</sup>	0.39 $\pm$ 0.15 <sup>ce</sup>	0.16 $\pm$ 0.10 <sup>ab</sup>	857.13 $\pm$ 489.6 <sup>efghi</sup>
		Sapling	56.69 $\pm$ 0.05 <sup>d</sup>	2.40 $\pm$ 0.91 <sup>cde</sup>	0.06 $\pm$ 0.02 <sup>abd</sup>	0.22 $\pm$ 0.10 <sup>af</sup>	0.27 $\pm$ 0.10 <sup>cefg</sup>	0.15 $\pm$ 0.07 <sup>ab</sup>	666.43 $\pm$ 284.67 <sup>fghi</sup>
		Adult	57.19 $\pm$ 0.64 <sup>abd</sup>	1.19 $\pm$ 0.27 <sup>fh</sup>	0.05 $\pm$ 0.02 <sup>bdef</sup>	0.12 $\pm$ 0.20 <sup>bc</sup>	0.71 $\pm$ 0.27 <sup>d</sup>	0.20 $\pm$ 0.12 <sup>ac</sup>	933.36 $\pm$ 450.1 <sup>efg</sup>
	Open canopy	Juvenile	57.12 $\pm$ 0.61 <sup>ad</sup>	1.84 $\pm$ 0.71 <sup>g</sup>	0.12 $\pm$ 0.07 <sup>c</sup>	0.23 $\pm$ 0.07 <sup>af</sup>	0.50 $\pm$ 0.17 <sup>cd</sup>	0.16 $\pm$ 0.10 <sup>ab</sup>	843.00 $\pm$ 141.7 <sup>efgh</sup>
		Sapling	57.06 $\pm$ 0.39 <sup>ad</sup>	2.14 $\pm$ 0.69 <sup>cg</sup>	0.08 $\pm$ 0.02 <sup>ab</sup>	0.22 $\pm$ 0.07 <sup>af</sup>	0.62 $\pm$ 0.12 <sup>d</sup>	0.17 $\pm$ 0.05 <sup>ab</sup>	897.83 $\pm$ 318.7 <sup>efg</sup>
		Adult	57.32 $\pm$ 0.29 <sup>abc</sup>	1.48 $\pm$ 0.29 <sup>f</sup>	0.06 $\pm$ 0.02 <sup>bd</sup>	0.17 $\pm$ 0.05 <sup>ab</sup>	0.63 $\pm$ 0.24 <sup>d</sup>	0.18 $\pm$ 0.12 <sup>ab</sup>	781.60 $\pm$ 160.8 <sup>efghi</sup>
Branches	Closed canopy	Juvenile	56.92 $\pm$ 0.32 <sup>ad</sup>	1.26 $\pm$ 0.24 <sup>fh</sup>	0.06 $\pm$ 0.00 <sup>bdef</sup>	0.05 $\pm$ 0.05 <sup>cd</sup>	0.23 $\pm$ 0.05 <sup>efg</sup>	0.15 $\pm$ 0.05 <sup>ab</sup>	2775.50 $\pm$ 601.3 <sup>a</sup>
		Sapling	57.68 $\pm$ 0.54 <sup>bc</sup>	1.18 $\pm$ 0.29 <sup>fh</sup>	0.05 $\pm$ 0.02 <sup>bdef</sup>	0.05 $\pm$ 0.07 <sup>d</sup>	0.30 $\pm$ 0.10 <sup>cefg</sup>	0.15 $\pm$ 0.02 <sup>ab</sup>	2227.09 $\pm$ 394 <sup>b</sup>
		Adult	57.60 $\pm$ 0.34 <sup>bc</sup>	1.19 $\pm$ 0.17 <sup>fh</sup>	0.05 $\pm$ 0.02 <sup>bdef</sup>	0.06 $\pm$ 0.05 <sup>cd</sup>	0.38 $\pm$ 0.17 <sup>ce</sup>	0.16 $\pm$ 0.05 <sup>ab</sup>	1651.83 $\pm$ 389.7 <sup>c</sup>
	Open canopy	Juvenile	57.38 $\pm$ 0.93 <sup>abc</sup>	1.07 $\pm$ 0.20 <sup>fh</sup>	0.07 $\pm$ 0.02 <sup>abd</sup>	0.03 $\pm$ 0.05 <sup>d</sup>	1.25 $\pm$ 0.49 <sup>a</sup>	0.18 $\pm$ 0.02 <sup>ab</sup>	1534.50 $\pm$ 425.5 <sup>cd</sup>
		Sapling	56.98 $\pm$ 0.51 <sup>d</sup>	1.05 $\pm$ 0.07 <sup>h</sup>	0.06 $\pm$ 0.02 <sup>bd</sup>	0.07 $\pm$ 0.05 <sup>cd</sup>	1.61 $\pm$ 0.59 <sup>b</sup>	0.14 $\pm$ 0.02 <sup>ab</sup>	1481.08 $\pm$ 266.4 <sup>cd</sup>
		Adult	56.91 $\pm$ 0.27 <sup>d</sup>	1.20 $\pm$ 0.20 <sup>fh</sup>	0.04 $\pm$ 0.02 <sup>def</sup>	0.05 $\pm$ 0.05 <sup>d</sup>	0.36 $\pm$ 0.17 <sup>ce</sup>	0.13 $\pm$ 0.02 <sup>b</sup>	1701.42 $\pm$ 382.3 <sup>c</sup>
Leaves	Closed canopy	Juvenile	55.29 $\pm$ 0.34 <sup>e</sup>	2.75 $\pm$ 0.29 <sup>de</sup>	0.04 $\pm$ 0.02 <sup>def</sup>	0.17 $\pm$ 0.10 <sup>ab</sup>	0.19 $\pm$ 0.12 <sup>efg</sup>	0.12 $\pm$ 0.05 <sup>b</sup>	1083.22 $\pm$ 361.7 <sup>def</sup>
		Sapling	55.14 $\pm$ 0.17 <sup>e</sup>	3.20 $\pm$ 0.42 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>bdef</sup>	0.23 $\pm$ 0.05 <sup>af</sup>	0.24 $\pm$ 0.20 <sup>efg</sup>	0.16 $\pm$ 0.02 <sup>ab</sup>	1196.58 $\pm$ 317.5 <sup>de</sup>
		Adult	55.29 $\pm$ 0.17 <sup>e</sup>	2.81 $\pm$ 0.24 <sup>e</sup>	0.10 $\pm$ 0.02 <sup>c</sup>	0.35 $\pm$ 0.7 <sup>e</sup>	0.70 $\pm$ 0.15 <sup>d</sup>	0.24 $\pm$ 0.02 <sup>c</sup>	2194.25 $\pm$ 640.2 <sup>b</sup>
	Open canopy	Juvenile	55.49 $\pm$ 0.37 <sup>e</sup>	3.89 $\pm$ 0.34 <sup>b</sup>	0.08 $\pm$ 0.02 <sup>ab</sup>	0.26 $\pm$ 0.03 <sup>f</sup>	0.33 $\pm$ 0.15 <sup>cef</sup>	0.18 $\pm$ 0.02 <sup>abc</sup>	1197.45 $\pm$ 236 <sup>de</sup>
		Sapling	55.37 $\pm$ 0.22 <sup>e</sup>	3.36 $\pm$ 0.47 <sup>a</sup>	0.10 $\pm$ 0.02 <sup>c</sup>	0.30 $\pm$ 0.07 <sup>e</sup>	0.37 $\pm$ 0.24 <sup>ce</sup>	0.18 $\pm$ 0.05 <sup>abc</sup>	1474.83 $\pm$ 807.2 <sup>cd</sup>
		Adult	55.11 $\pm$ 0.24 <sup>e</sup>	2.66 $\pm$ 0.29 <sup>de</sup>	0.10 $\pm$ 0.02 <sup>c</sup>	0.30 $\pm$ 0.08 <sup>e</sup>	0.54 $\pm$ 0.17 <sup>d</sup>	0.17 $\pm$ 0.05 <sup>ab</sup>	2376.58 $\pm$ 450.5 <sup>ab</sup>
Stem	Closed canopy	Juvenile	57.63 $\pm$ 0.44 <sup>bc</sup>	0.47 $\pm$ 0.15 <sup>j</sup>	0.03 $\pm$ 0.02 <sup>ef</sup>	0.05 $\pm$ 0.01 <sup>d</sup>	0.13 $\pm$ 0.05 <sup>fg</sup>	0.03 $\pm$ 0.00 <sup>d</sup>	475.50 $\pm$ 132.1 <sup>hi</sup>
		Sapling	57.30 $\pm$ 0.61 <sup>abc</sup>	0.48 $\pm$ 0.24 <sup>j</sup>	0.03 $\pm$ 0.02 <sup>ef</sup>	0.06 $\pm$ 0.01 <sup>cd</sup>	0.10 $\pm$ 0.02 <sup>fg</sup>	0.03 $\pm$ 0.02 <sup>d</sup>	408.33 $\pm$ 163.4 <sup>i</sup>
		Adult	57.78 $\pm$ 0.47 <sup>c</sup>	0.47 $\pm$ 0.22 <sup>j</sup>	0.03 $\pm$ 0.02 <sup>f</sup>	0.04 $\pm$ 0.01 <sup>d</sup>	0.09 $\pm$ 0.05 <sup>g</sup>	0.03 $\pm$ 0.02 <sup>d</sup>	520.42 $\pm$ 270.1 <sup>ghi</sup>
	Open canopy	Juvenile	57.48 $\pm$ 0.42 <sup>abc</sup>	0.27 $\pm$ 0.24 <sup>j</sup>	0.03 $\pm$ 0.02 <sup>ef</sup>	0.14 $\pm$ 0.02 <sup>b</sup>	0.08 $\pm$ 0.07 <sup>g</sup>	0.04 $\pm$ 0.02 <sup>d</sup>	479.33 $\pm$ 236.4 <sup>hi</sup>
		Sapling	57.65 $\pm$ 0.24 <sup>bc</sup>	0.25 $\pm$ 0.10 <sup>j</sup>	0.03 $\pm$ 0.02 <sup>ef</sup>	0.21 $\pm$ 0.02 <sup>af</sup>	0.09 $\pm$ 0.07 <sup>fg</sup>	0.04 $\pm$ 0.00 <sup>d</sup>	430.90 $\pm$ 166 <sup>hi</sup>
		Adult	57.28 $\pm$ 0.54 <sup>ab</sup>	0.45 $\pm$ 0.10 <sup>j</sup>	0.03 $\pm$ 0.02 <sup>ef</sup>	0.09 $\pm$ 0.01 <sup>cd</sup>	0.09 $\pm$ 0.02 <sup>g</sup>	0.03 $\pm$ 0.02 <sup>d</sup>	423.33 $\pm$ 139.5 <sup>hi</sup>



## **Influence of stem density and life stage on tissue nutrient content of *Eucalyptus camaldulensis***

Carbon percentage (%C) of *E. camaldulensis* differed significantly between biomass components (ANOVA;  $F_{[3; 172]}=131$ ;  $P<0.001$ ; Table 4.1). Neither stem density nor life stage affected C content of aboveground biomass of *E. camaldulensis* ( $P\geq 0.05$ ). The biomass component with the lowest concentration of C was foliage (between  $55.12 \pm 0.22\%$  for Open canopy adult trees, and  $55.58 \pm 0.22\%$  in foliage of Closed canopy saplings).

Nitrogen percentages of *E. camaldulensis* differed significantly between biomass components and life stages (ANOVA:  $F_{[6; 172]}=6.992$ ;  $P<0.001$ ), and between the two densities ( $F_{[1; 172]}=5.55$ ;  $P=0.19$ ). Nitrogen percentages were the highest in leaves of *E. camaldulensis* and were significantly higher than any other biomass component for both density extremes (LSD;  $P<0.05$ ). Juvenile samples of Closed canopy stands had significantly higher %N than Open canopy trees for all other foliage samples ( $P<0.05$ ), the lowest of which was found in Open canopy saplings ( $1.56 \pm 0.32\%$ ). In Open canopy and Closed canopy trees, the sapling stage showed the lowest percentages of leaf N, while this life stage showed an increase in %N allocation to bark material. In both densities stem material had the lowest percentages of N but there were no significant differences between stem material from different life stages of densities ( $P\geq 0.05$ ). The most significant differences in %P percentages have been found between biomass components ( $F_{[3; 172]}=112.018$ ;  $P=0.001$ ).

Phosphorus showed similar trends to nitrogen, being significantly greater in the leaves than in any other biomass component for both densities. Sapling samples from Closed canopy stands had significantly higher %P than Closed canopy adults (LSD;  $P=0.027$ ), while all other leaf samples were not significantly different ( $P\geq 0.05$ ). Potassium (K) percentage was highest in the leaves of *E. camaldulensis* and was in all cases significantly higher than the other biomass components. The lowest %K percentages were found in stem material throughout. Juveniles of both density extremes had significantly higher foliar %K than saplings and adults. Calcium percentages were also lowest in stem material of *E. camaldulensis* and did not differ between life stages. In Closed canopy stands, juveniles had significantly higher percentages of Ca in leaves and branches than in the adult stage; this is not the case in Open canopy trees. There were no significant seasonal differences in %Ca between various aboveground biomass components for either density extreme. Sodium (Na) content was highest in foliar material of both Closed canopy growing and Open canopy trees and was significantly higher than other components. Life stage had no significant impact on Na contents of any of the biomass components.

**Table 4.2: *Eucalyptus camaldulensis* life stage, density, and biomass component (mean  $\pm$  SD). Letters in superscript show where significant differences were found (Fisher's LSD: Significance level at  $P < 0.05$ ).**

	Density	Life stage	C (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)
Bark	Closed canopy	Juvenile	57.50 $\pm$ 0.44 <sup>abc</sup>	0.34 $\pm$ 0.05 <sup>eh</sup>	0.05 $\pm$ 0.00 <sup>ab</sup>	0.30 $\pm$ 0.05 <sup>af</sup>	0.90 $\pm$ 0.56 <sup>bcd</sup>	0.16 $\pm$ 0.10 <sup>ab</sup>	532.13 $\pm$ 167.69 <sup>ghij</sup>
		Sapling	55.92 $\pm$ 0.69 <sup>d</sup>	0.74 $\pm$ 0.42 <sup>fg</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	0.33 $\pm$ 0.10 <sup>af</sup>	0.64 $\pm$ 0.17 <sup>a</sup>	0.15 $\pm$ 0.07 <sup>ab</sup>	893.45 $\pm$ 263.57 <sup>efg</sup>
		Adult	57.25 $\pm$ 0.59 <sup>abc</sup>	0.33 $\pm$ 0.07 <sup>eh</sup>	0.05 $\pm$ 0.02 <sup>ab</sup>	0.27 $\pm$ 0.05 <sup>af</sup>	0.66 $\pm$ 0.29 <sup>cd</sup>	0.20 $\pm$ 0.12 <sup>ac</sup>	615.67 $\pm$ 84.19 <sup>fghi</sup>
	Open canopy	Juvenile	57.21 $\pm$ 1.10 <sup>abc</sup>	0.42 $\pm$ 0.17 <sup>eh</sup>	0.05 $\pm$ 0.02 <sup>ab</sup>	0.30 $\pm$ 0.07 <sup>af</sup>	0.76 $\pm$ 0.24 <sup>bcd</sup>	0.16 $\pm$ 0.10 <sup>ab</sup>	809.89 $\pm$ 219.96 <sup>efg</sup>
		Sapling	55.47 $\pm$ 0.32 <sup>de</sup>	0.54 $\pm$ 0.17 <sup>ef</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	0.29 $\pm$ 0.05 <sup>af</sup>	0.57 $\pm$ 0.15 <sup>bc</sup>	0.17 $\pm$ 0.05 <sup>ab</sup>	924.17 $\pm$ 922.17 <sup>ef</sup>
		Adult	57.18 $\pm$ 0.71 <sup>abc</sup>	0.43 $\pm$ 0.07 <sup>eh</sup>	0.03 $\pm$ 0.00 <sup>a</sup>	0.30 $\pm$ 0.05 <sup>af</sup>	0.71 $\pm$ 0.51 <sup>d</sup>	0.18 $\pm$ 0.12 <sup>ab</sup>	762.50 $\pm$ 358.68 <sup>efg</sup>
Branches	Closed canopy	Juvenile	57.25 $\pm$ 0.51 <sup>abc</sup>	0.81 $\pm$ 0.15 <sup>fg</sup>	0.07 $\pm$ 0.24 <sup>ab</sup>	0.35 $\pm$ 0.02 <sup>af</sup>	0.51 $\pm$ 0.12 <sup>ab</sup>	0.15 $\pm$ 0.05 <sup>ab</sup>	1095.50 $\pm$ 556.01 <sup>de</sup>
		Sapling	57.63 $\pm$ 0.56 <sup>ac</sup>	0.64 $\pm$ 0.15 <sup>fg</sup>	0.09 $\pm$ 0.02 <sup>b</sup>	0.32 $\pm$ 0.05 <sup>af</sup>	0.56 $\pm$ 0.20 <sup>cd</sup>	0.15 $\pm$ 0.02 <sup>ab</sup>	716.91 $\pm$ 152.06 <sup>fgh</sup>
		Adult	57.06 $\pm$ 0.64 <sup>b</sup>	0.79 $\pm$ 0.17 <sup>g</sup>	0.08 $\pm$ 0.02 <sup>b</sup>	0.34 $\pm$ 0.07 <sup>af</sup>	0.86 $\pm$ 0.27 <sup>cd</sup>	0.16 $\pm$ 0.05 <sup>ab</sup>	747.67 $\pm$ 349.35 <sup>efg</sup>
	Open canopy	Juvenile	57.18 $\pm$ 0.61 <sup>abc</sup>	0.72 $\pm$ 0.12 <sup>fg</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	0.38 $\pm$ 0.05 <sup>a</sup>	1.06 $\pm$ 0.42 <sup>cd</sup>	0.18 $\pm$ 0.02 <sup>ab</sup>	623.20 $\pm$ 179.55 <sup>ghi</sup>
		Sapling	57.13 $\pm$ 0.64 <sup>ab</sup>	0.65 $\pm$ 0.12 <sup>fg</sup>	0.06 $\pm$ 0.02 <sup>ab</sup>	0.34 $\pm$ 0.07 <sup>af</sup>	0.44 $\pm$ 0.12 <sup>d</sup>	0.14 $\pm$ 0.02 <sup>ab</sup>	795.33 $\pm$ 148.19 <sup>efg</sup>
		Adult	57.23 $\pm$ 0.61 <sup>abc</sup>	0.61 $\pm$ 0.12 <sup>fg</sup>	0.05 $\pm$ 0.02 <sup>ab</sup>	0.37 $\pm$ 0.12 <sup>a</sup>	0.52 $\pm$ 0.34 <sup>d</sup>	0.13 $\pm$ 0.02 <sup>b</sup>	835.67 $\pm$ 263.52 <sup>efg</sup>
Leaves	Closed canopy	Juvenile	55.26 $\pm$ 0.56 <sup>de</sup>	2.46 $\pm$ 0.34 <sup>a</sup>	0.20 $\pm$ 0.05 <sup>cd</sup>	0.88 $\pm$ 0.20 <sup>b</sup>	0.57 $\pm$ 0.22 <sup>ab</sup>	0.12 $\pm$ 0.05 <sup>b</sup>	1696.40 $\pm$ 898.03 <sup>abc</sup>
		Sapling	55.58 $\pm$ 0.22 <sup>de</sup>	1.77 $\pm$ 0.37 <sup>cd</sup>	0.25 $\pm$ 0.01 <sup>c</sup>	0.68 $\pm$ 0.20 <sup>c</sup>	0.78 $\pm$ 0.15 <sup>bc</sup>	0.16 $\pm$ 0.02 <sup>ab</sup>	1898.67 $\pm$ 569.78 <sup>a</sup>
		Adult	55.30 $\pm$ 0.27 <sup>e</sup>	2.09 $\pm$ 0.49 <sup>b</sup>	0.19 $\pm$ 0.01 <sup>d</sup>	0.67 $\pm$ 0.17 <sup>c</sup>	0.43 $\pm$ 0.20 <sup>bc</sup>	0.24 $\pm$ 0.02 <sup>c</sup>	1797.08 $\pm$ 605.20 <sup>ab</sup>
	Open canopy	Juvenile	55.11 $\pm$ 0.32 <sup>e</sup>	1.90 $\pm$ 0.49 <sup>bc</sup>	0.21 $\pm$ 0.05 <sup>cd</sup>	0.76 $\pm$ 0.05 <sup>bc</sup>	0.68 $\pm$ 0.37 <sup>cd</sup>	0.18 $\pm$ 0.02 <sup>abc</sup>	1362.00 $\pm$ 668.98 <sup>cd</sup>
		Sapling	55.39 $\pm$ 0.24 <sup>de</sup>	1.56 $\pm$ 0.32 <sup>d</sup>	0.22 $\pm$ 0.05 <sup>cd</sup>	0.49 $\pm$ 0.07 <sup>d</sup>	0.67 $\pm$ 0.17 <sup>ab</sup>	0.18 $\pm$ 0.05 <sup>abc</sup>	1383.00 $\pm$ 431.48 <sup>cd</sup>
		Adult	55.12 $\pm$ 0.22 <sup>e</sup>	2.10 $\pm$ 0.32 <sup>b</sup>	0.20 $\pm$ 0.07 <sup>cd</sup>	0.57 $\pm$ 0.17 <sup>d</sup>	0.13 $\pm$ 0.02 <sup>bcd</sup>	0.17 $\pm$ 0.05 <sup>ab</sup>	1476.42 $\pm$ 435.42 <sup>bcd</sup>
Stem	Closed canopy	Juvenile	57.74 $\pm$ 0.17 <sup>c</sup>	0.26 $\pm$ 0.02 <sup>h</sup>	0.03 $\pm$ 0.00 <sup>a</sup>	0.16 $\pm$ 0.02 <sup>e</sup>	0.10 $\pm$ 0.02 <sup>e</sup>	0.03 $\pm$ 0.00 <sup>d</sup>	305.33 $\pm$ 104.25 <sup>ij</sup>
		Sapling	57.61 $\pm$ 0.37 <sup>ac</sup>	0.24 $\pm$ 0.10 <sup>h</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	0.19 $\pm$ 0.05 <sup>e</sup>	0.09 $\pm$ 0.02 <sup>e</sup>	0.03 $\pm$ 0.02 <sup>d</sup>	285.25 $\pm$ 66.5 <sup>ij</sup>
		Adult	57.53 $\pm$ 0.22 <sup>abc</sup>	0.28 $\pm$ 0.05 <sup>h</sup>	0.03 $\pm$ 0.02 <sup>a</sup>	0.14 $\pm$ 0.05 <sup>e</sup>	0.11 $\pm$ 0.05 <sup>e</sup>	0.03 $\pm$ 0.02 <sup>d</sup>	200.50 $\pm$ 62.32 <sup>j</sup>
	Open canopy	Juvenile	57.73 $\pm$ 0.20 <sup>c</sup>	0.20 $\pm$ 0.07 <sup>h</sup>	0.05 $\pm$ 0.02 <sup>ab</sup>	0.19 $\pm$ 0.05 <sup>e</sup>	0.09 $\pm$ 0.02 <sup>e</sup>	0.04 $\pm$ 0.02 <sup>d</sup>	270.58 $\pm$ 74.37 <sup>ij</sup>
		Sapling	57.68 $\pm$ 0.42 <sup>c</sup>	0.23 $\pm$ 0.07 <sup>h</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	0.25 $\pm$ 0.10 <sup>fe</sup>	0.09 $\pm$ 0.02 <sup>e</sup>	0.04 $\pm$ 0.00 <sup>d</sup>	380.08 $\pm$ 149.98 <sup>hij</sup>
		Adult	57.58 $\pm$ 0.34 <sup>abc</sup>	0.30 $\pm$ 0.07 <sup>eh</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	0.19 $\pm$ 0.05 <sup>e</sup>	0.57 $\pm$ 0.24 <sup>e</sup>	0.03 $\pm$ 0.02 <sup>d</sup>	286.50 $\pm$ 60.62 <sup>ij</sup>



## Nutrient stoichiometry and relationships with tree age group

Stem material had the highest C:N ratios for all life stages (between 235 and 276 for *E. camaldulensis* and 175 and 292 for *A. mearnsii*; Figure 4.2). For *A. mearnsii*, C:N ratio in stem material was, in all cases, significantly higher than any of the other components ( $P < 0.05$ ). The overall lowest stem C:N ratios were reported in both species at the adult stage. For leaf material, the lowest C:N ratios are reported for leaf material of both species at both species and through all life stages. The C:N ratio of bark material of all life stages of *E. camaldulensis* was significantly higher than that of *A. mearnsii* at corresponding life stages ( $P < 0.05$ ).

There was significant interaction for C:N ratio between biomass component and species ( $F_{[3;346]} = 9.02$ ;  $P < 0.001$ ; figure 4.3) but none between density and biomass component ( $F_{[3;346]} = 2.44$ ;  $P = 0.06$ ). The highest C:N ratio was found in stem material of Open canopy stems of *E. camaldulensis* ( $295.81 \pm 96$ ), which was significantly greater than all other non-stem samples (LSD;  $P < 0.05$ ), but not different to any other stem sample. For both species, leaves had the lowest C:N ratios but there were no statistical differences between *A. mearnsii* and *E. camaldulensis* samples or between Closed canopy and Open canopy trees.

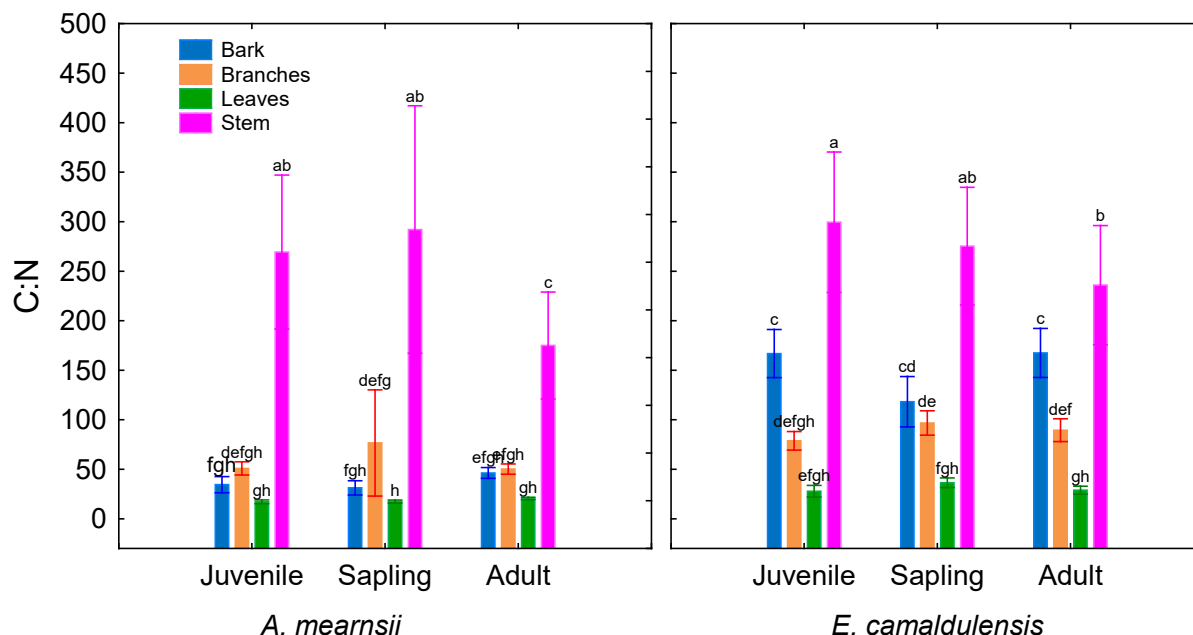


Figure 4.2: C:N ratios of *E. camaldulensis* and *A. mearnsii* across various life stages (juvenile, sapling and adult) and between aboveground biomass components (bark, branches, leaves, and stem). Letters above error bars show where significant differences were found (Fisher's LSD test;  $P < 0.05$ ); error bars denote 0.95 confidence intervals.

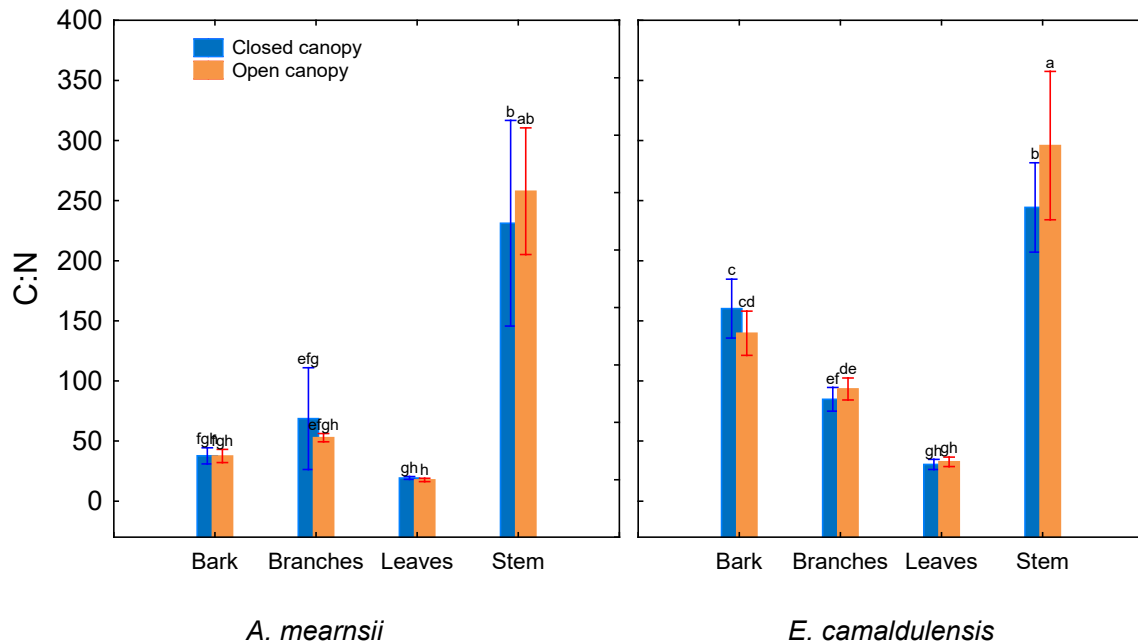


Figure 4.3: C:N ratios of *E. camaldulensis* and *A. mearnsii* between aboveground biomass components (bark, branches, leaves, and stem) and across the two density extremes (Closed canopy and Open canopy). The graph shows a combination of all 3 age classes (Juvenile, sapling, and adult). Letters above error bars show where significant differences were found (Fisher's LSD test;  $P < 0.05$ ); error bars denote 0.95 confidence intervals.

For N:P ratios, there was significant interaction between species, biomass component, and life stage (ANOVA;  $F_{[6; 346]} = 8.30$ ;  $P < 0.001$ ; Figure 4.4). The N:P ratio of leaves of *A. mearnsii* juveniles ( $64.36 \pm 18.38$ ) was significantly higher than any of the other samples (LSD:  $P < 0.05$ ), except that of sapling leaves from Closed canopy sites ( $P = 0.1$ ). Leaves of *A. mearnsii* saplings also had significantly higher C:N ratios than all other sample types, except juveniles of the same species. There were no significant differences between any of the stem samples ( $P \geq 0.05$ ).

For N:P ratios there was also significant interaction between species and biomass component (ANOVA;  $F_{[3; 346]} = 9.92$ ;  $P < 0.001$ ; Figure 4.5) and between species and stem density ( $F_{[1; 346]} = 7.84$ ;  $P = 0.005$ ), but none between biomass component and density ( $F_{[3; 346]} = 0.09$ ;  $P = 0.97$ ). Leaves of Closed canopy *A. mearnsii* had the highest N:P ratios ( $57.08 \pm 20.78$ ), which was significantly higher than any of the other samples. Except for branch material, all biomass components of Closed canopy samples of *A. mearnsii* showed significantly higher N:P ratios than the same biomass components in Open canopy trees. The N:P ratios of *A. mearnsii* samples were also consistently higher than *E. camaldulensis* samples.

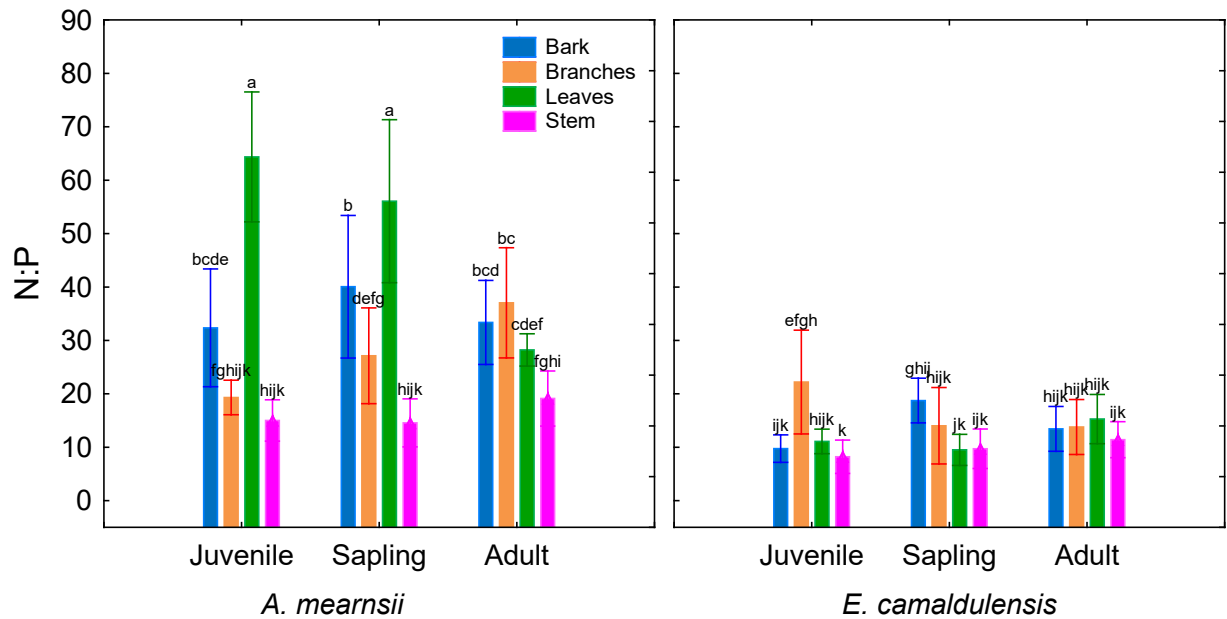


Figure 4.4: N:P ratios of *E. camaldulensis* and *A. mearnsii* across various life stages (juvenile, sapling and adult) and between aboveground biomass components. Letters above error bars show where significant differences were found (Fisher's LSD test;  $P < 0.05$ ); error bars denote 0.95 confidence intervals.

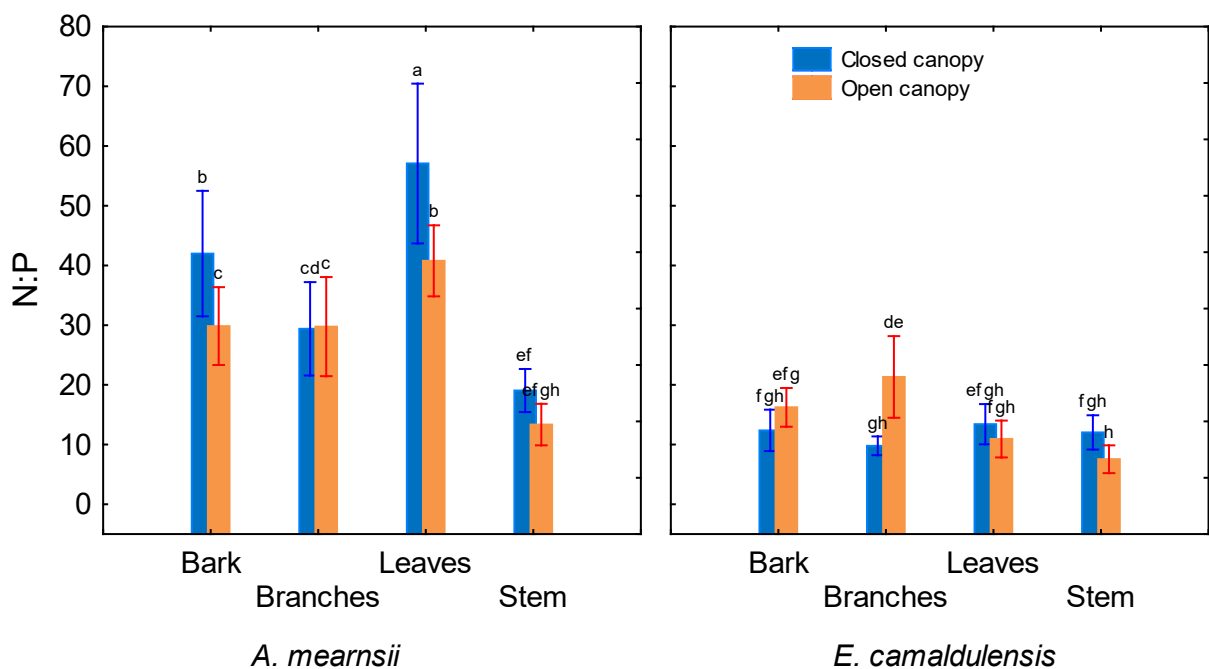


Figure 4.5: N:P ratios of *E. camaldulensis* and *A. mearnsii* between aboveground biomass components (bark, branches, leaves, and stem) and across the two density extremes (Closed canopy and Open canopy). The graph shows a combination of all 3 age classes (Juvenile, sapling, and adult). Letters above error bars show where significant differences were found (Fisher's LSD test;  $P < 0.05$ ) and error bars denote 0.95 confidence intervals.

**Nutrient allometry of *Acacia mearnsii* and *Eucalyptus camaldulensis***

Models based on stem diameter (cm) could be developed to determine nutrient stocks of various aboveground tree components and are shown in Table 4.5 for *A. mearnsii* and *E. camaldulensis* in Table 4.6. These models include the entire size range of individuals found in the Bainskloof and Alfalfa study sites and distinguish between Open canopy and Closed canopy plots.

Table 4.3: Models and goodness of fit values ( $R^2$ ) of selected nutrients of *A. mearnsii* with stem basal (BD) as independent variable (x; cm) for Closed canopy and Open canopy trees, calculated as grams.

Nutrient	Density	Biomass component							
		Leaves	$R^2$	Stem	$R^2$	Bark	$R^2$	Branches	$R^2$
N	Closed canopy	$y = 0.663x^{1.932}$	0.996	$y = 0.16x^{2.517}$	0.922	$y = 0.509x^{1.683}$	0.972	$y = 0.598x^{1.678}$	0.908
	Open canopy	$y = 0.931x^{2.081}$	0.995	$y = 0.043x^{2.726}$	0.907	$y = 0.214x^{2.246}$	0.971	$y = 0.106x^{2.667}$	0.929
P	Closed canopy	$y = 0.012x^{2.199}$	0.979	$y = 0.01x^{2.479}$	0.935	$y = 0.017x^{1.635}$	0.911	$y = 0.025x^{1.574}$	0.851
	Open canopy	$y = 0.02x^{2.303}$	0.986	$y = 0.006x^{2.612}$	0.949	$y = 0.009x^{2.242}$	0.820	$y = 0.007x^{2.284}$	0.859
K	Closed canopy	$y = 0.045x^{2.157}$	0.982	$y = 0.021x^{2.367}$	0.955	$y = 0.044x^{1.727}$	0.950	$y = 0.006x^{1.807}$	0.89
	Open canopy	$y = 0.069x^{2.248}$	0.987	$y = 0.044x^{2.253}$	0.93	$y = 0.029x^{1.918}$	0.774	$y = 0.003x^{2.544}$	0.959
C	Closed canopy	$y = 12.764x^{1.918}$	0.985	$y = 24.683x^{2.51}$	0.966	$y = 15.097x^{1.888}$	0.974	$y = 27.143x^{1.715}$	0.911
	Open canopy	$y = 13.925x^{2.225}$	0.963	$y = 14.858x^{2.508}$	0.954	$y = 6.735x^{2.363}$	0.955	$y = 6.458x^{2.567}$	0.924
Ca	Closed canopy	$y = 0.050x^{2.315}$	0.862	$y = 0.0367x^{2.44}$	0.938	$y = 0.093x^{1.924}$	0.808	$y = 0.141x^{1.757}$	0.905
	Open canopy	$y = 0.067x^{2.430}$	0.913	$y = 0.018x^{2.574}$	0.924	$y = 0.0631x^{2.342}$	0.922	$y = 0.057x^{2.356}$	0.8
Mg	Closed canopy	$y = 0.033x^{2.123}$	0.981	$y = 0.0104x^{2.385}$	0.941	$y = 0.035x^{1.995}$	0.941	$y = 0.063x^{1.703}$	0.883
	Open canopy	$y = 0.044x^{2.179}$	0.98	$y = 0.0089x^{2.477}$	0.93	$y = 0.019x^{2.265}$	0.949	$y = 0.016x^{2.517}$	0.915
Na	Closed canopy	$y = 0.027x^{2.145}$	0.98	$y = 0.0168x^{2.55}$	0.959	$y = 0.007x^{2.317}$	0.765	$y = 0.105x^{1.562}$	0.884
	Open canopy	$y = 0.029x^{2.49}$	0.98	$y = 0.0111x^{2.466}$	0.944	$y = 0.003x^{2.733}$	0.749	$y = 0.015x^{2.651}$	0.907

Table 4.4: Models and goodness of fit values ( $R^2$ ) of selected nutrients of *E. camaldulensis* with stem basal diameter as independent variable (x; cm) for Closed canopy stands and Open canopy individuals, calculated as grams.

Nutrient	Density	Biomass component							
		Leaves	$R^2$	Stem	$R^2$	Bark	$R^2$	Branches	$R^2$
N	Closed canopy	$y = 0.105x^{2.246}$	0.891	$y = 0.058x^{2.752}$	0.875	$y = 0.024x^{2.420}$	0.893	$y = 0.014x^{2.951}$	0.92
	Open canopy	$y = 0.159x^{2.511}$	0.79	$y = 0.058x^{2.739}$	0.874	$y = 0.033x^{2.445}$	0.886	$y = 0.0861x^{2.223}$	0.863
P	Closed canopy	$y = 0.011x^{2.242}$	0.75	$y = 0.01x^{2.524}$	0.883	$y = 0.002x^{2.442}$	0.901	$y = 0.003x^{2.387}$	0.877
	Open canopy	$y = 0.02x^{2.383}$	0.789	$y = 0.01x^{2.825}$	0.928	$y = 0.003x^{2.245}$	0.84	$y = 0.006x^{2.118}$	0.760
K	Closed canopy	$y = 0.042x^{2.18}$	0.853	$y = 0.059x^{2.46}$	0.922	$y = 0.016x^{2.447}$	0.908	$y = 0.013x^{2.455}$	0.874
	Open canopy	$y = 0.069x^{2.304}$	0.784	$y = 0.028x^{2.98}$	0.953	$y = 0.022x^{2.463}$	0.881	$y = 0.027x^{2.131}$	0.797
C	Closed canopy	$y = 2.642x^{2.312}$	0.847	$y = 29.886x^{2.339}$	0.903	$y = 3.301x^{2.469}$	0.930	$y = 1.202x^{2.913}$	0.89
	Open canopy	$y = 5.57x^{2.422}$	0.783	$y = 17.987x^{2.567}$	0.901	$y = 4.074x^{2.511}$	0.909	$y = 6.491x^{2.313}$	0.876
Ca	Closed canopy	$y = 0.051x^{2.208}$	0.814	$y = 0.038x^{2.383}$	0.859	$y = 0.0417x^{2.311}$	0.876	$y = 0.008x^{3.145}$	0.888
	Open canopy	$y = 0.04x^{2.666}$	0.824	$y = 0.015x^{2.939}$	0.923	$y = 0.069x^{2.273}$	0.886	$y = 0.072x^{2.219}$	0.869
Mg	Closed canopy	$y = 0.011x^{2.352}$	0.817	$y = 0.019x^{2.345}$	0.902	$y = 0.024x^{2.454}$	0.868	$y = 0.005x^{2.858}$	0.881
	Open canopy	$y = 0.019x^{2.535}$	0.816	$y = 0.008x^{2.874}$	0.937	$y = 0.019x^{2.512}$	0.914	$y = 0.022x^{2.394}$	0.878
Na	Closed canopy	$y = 0.008x^{2.2978}$	0.833	$y = 0.011x^{2.344}$	0.896	$y = 0.003x^{2.524}$	0.8481	$y = 0.002x^{2.831}$	0.843
	Open canopy	$y = 0.01x^{2.523}$	0.845	$y = 0.003x^{3.085}$	0.961	$y = 0.007x^{2.32}$	0.864	$y = 0.007x^{2.404}$	0.868

## 4.5 Discussion

Physiological changes in the plant largely determine its nutritional requirements, which are continuously changing as a response to environmental variables (Attiwill et al., 1993). Resources within a tree are, however, allocated to one function or organ at a time and are not available to another function or organ simultaneously (Kollman et al., 2004; Weiner, 2004); allocation of such resources thus plays a significant role in plant allometry. In natural forests, allocation of any resource to a plant organ is likely to be species-specific and can be influenced by (in addition to the species) the life stage of the plant, competition for resources as a result of population densities, and could also respond to changes in climatic or seasonal weather conditions (Pugliese, 1988; Enquist et al., 1998). This probably differs from resource allocation in plantations where stands are evenly spaced and of a similar age, minimizing and homogenizing the effects of competition and age-related differences in resource allocation. These traits make plantation nutrient fluxes easier to model, track, and replenish, whereas nutrient distributions in natural forests are much more complex due to heterogeneity in stand age structure, spacing, and environmental influences.

While consideration has been given to *E. camaldulensis* nutrient dynamics as a response to differences in stem densities (Harrison et al., 2000), no studies have to date been done that explicitly investigate the effect of stem densities and tree age on nutrient dynamics of *A. mearnsii*. Where *E. camaldulensis* were planted further away from each other in a plantation (between a spacing of 4.5 m<sup>2</sup> to 12 m<sup>2</sup>) a significant decrease in foliar N and P of up to 31% and 29 % respectively has been reported (Harrison et al., 2000). The same study also reported a decrease in foliar P as a result of age, although the total P in the study site increased significantly as a result of accumulation of P in the thicker stem material. The current study does report marginally higher levels of foliar N in *E. camaldulensis* trees Closed canopy stands but not in the adult life stage. The current study also reports marginally lower foliar P in adult *E. camaldulensis* trees, but this was also non-significant.

The decrease in aboveground %P with increasing stand age was also reported by Leite et al. (2011); the study reports reduction of %P throughout all the aboveground biomass components of the tree, in addition to reductions of %K, %Ca, and %Mg in stem material, citing potential export of the cations from the stemwood. During this study, I found no significant differences in %N or %P for *E. camaldulensis* as a result of the size or age of the trees tested. There was, however, significant variation in the cation dynamics. Foliar %K was higher in the juvenile stage than in the sapling and adult stages for both closed and Open canopy trees, while foliar Mg was highest in foliage of Closed canopy adult trees.



*Acacia mearnsii* nutrient data presented here correlated well with previous findings by Dovey (2005), showing significantly greater %N in foliage than in the other biomass components. The current study, however, reports significantly higher %N values at the juvenile stage for Open canopy trees (up to 4% foliar nutrient content), which decreases significantly towards the adult stage. Bark %N in adult trees were similar to those reported by Dovey (2005), having 1-1.5% N concentration. The cations, K, Ca, and Mg were all more concentrated in the foliage of *A. mearnsii* in the adult stages than in the younger plants. It is worth noting that the study done by Dovey (2005) was done in the KwaZulu Natal province of South Africa, which is a summer rainfall area with soils that are inherently more fertile than Fynbos soils (Fey, 2010). It is thus interesting that foliar cation concentrations in the current study are marginally higher than those previously reported elsewhere. However, stem cation concentrations remained low throughout, suggesting that cations are actively transported to the photosynthetically active material and are not readily stored in the stemwood of *A. mearnsii*. It has been reported that the little investment in nutrients for stemwood may provide invasive alien plant species in the CFR with a competitive advantage over native species, as this could allow for faster growth and greater investment in photosynthetically active biomass components (Redmond et al., 2019). The current study does not consider aboveground nutrient dynamics of native plant species, but results here show that stemwood of both invasive species tested is largely devoid of nutrients. More importantly, this study does not compare stem nutrient levels of invasive alien trees to those of native species. It is thus suggested that concentrations of nutrients in biomass of native species be determined for the CFR to test this theory and its applicability as an indicator for the invasive potential of a species.

The direct impact of invasive alien trees on affected ecosystems could, however, be strongly related to its foliar nutrient relations. Foliar nutrient concentrations and their ratios within *A. mearnsii* and *E. camaldulensis* have variable implications for ecosystem functioning, mainly as a result of their litter, and are dependent on the plant's nutrient resorption efficiency (Van der Colff et al., 2017). Railoun (2018) reported that the N resorption efficiency of *A. mearnsii* is lower than that of native riparian species *Metrosideros angustifolia* and *Brabejum stellatifolium*. Railoun (2018) also found relatively greater inputs of N into soil from *A. mearnsii* as a result of leaf litter, than native riparian species such as *B. stellatifolium* and *M. angustifolia*. This is partly explained by the high foliar N concentration of *A. mearnsii* and also its tendency to drop leaves twice a year (summer and autumn), rather than once, as is the case with native riparian species (Railoun, 2018). This deposition has great implications for the impact of invasion on local nutrient cycles and is discussed more in-depth in Chapter 5 of this study. However, if nitrogen resorption efficiency in plants is related to soil nitrogen

availability, it is possible that increased levels of soil N could, over time, lead to reduced levels of N resorption efficiency and lower the competitive ability of plants when soil N is reduced to previous, lower levels. This is also an important area of study that could add significant value to the long-term success of post-clearing rehabilitation. More importantly, the deposition of excess N into soils under invasive stands has also been shown to promote secondary invasions (Nsikani et al., 2018), which also suggests the need for efforts at reducing the inputs of N into soil as a result of litterfall.

A second attribute of leaves, litter decomposition rate, could also be related to the C:N ratio of the material (Killingbeck, 1996). In the current study, I report that C:N ratios of *A. mearnsii* foliage (between 17 and 20) are significantly lower than that of *E. camaldulensis* foliage between 27 and 32). In fact, throughout all life stages, *A. mearnsii* had lower foliar C:N ratios than *E. camaldulensis*, with the lowest *A. mearnsii* C:N ratio being at the juvenile stage (18.46) and for *E. camaldulensis* at the adult stage (28.84). This is likely due to the non-leguminous nature of *E. camaldulensis* and thus lower concentrations of foliar N. Railoun (2018) also reported that *A. mearnsii* litter has significantly lower C:N ratios than the native *B. stellatifolium* and *M. angustifolia*. The low C:N ratio of *A. mearnsii* has been associated with one way in which nutrient cycles in invaded Fynbos riparian ones could be altered by the species. As is reported by Killingbeck (1996) and later by Tye (2014), low C:N ratios are associated with quicker decomposition of litter and thus faster release of nutrients into the soil and aquatic environments. Thus, whereas species like *A. mearnsii* have been shown to drop leaves more than once a year, and containing greater concentrations of nutrients such as nitrogen, low C:N ratios could signify an increased rate of decomposition in the soil, leading to spikes in soil available N, and encouraging establishment of secondary invaders (Nsikani et al., 2018).

*Eucalyptus camaldulensis*' foliar N:P ratios were consistently low (compared to those of *A. mearnsii*) between life stages and stem densities in this study (between 17 and 21), while *A. mearnsii* N:P ratios showed a decrease in foliar N:P ratio from juvenile plants (approx. 64) to sapling (54) and to adult (28). The ecological implications of foliar N:P ratios are still poorly understood, and it is generally used as an indicator of soil nutrient availability (Koerselman & Meulman, 1996). It is suggested that high foliar N:P ratios are correlated with P-limited soils, while low N:P ratios correlate with N-limited soils (Tian et al., 2018). Schreeg et al. (2014) also showed how the N:P ratio of new leaves, old leaves, stems, and roots respond to nitrogen addition to soil (increased N:P ratio) and to phosphorus addition (decrease in N:P ratio). Schreeg et al. (2014) also showed that increasing both soil N and P increases foliar N:P ratios. It is also possible that foliar N:P ratios are related to growth stage and phenology of plants and respond to differences in storage and mobilization patterns (Schreeg et al., 2014). The current

study used data compiled from summer and winter to minimize the sources of variation in this regard. Interestingly, the current study reports relatively high N:P ratios for *A. mearnsii* at the adult stage (approximately 28), while the juvenile stage had foliar N:P ratios of approximately 64, about three times more than global averages (Tian et al., 2017). This can be attributed to significantly higher %N in juvenile foliage of *A. mearnsii* than in the adult stage and comparatively lower P percentages.

Results from the current study also suggest that N, P, and cation percentages of aboveground biomass of the two invasive species decrease with increasing tree biomass. Higher nutrient concentrations in juveniles could be related to increased growth rates necessary to overcome light limitations below the canopy (Rozendaal et al., 2006). High foliar %P recorded for *E. camaldulensis* during this study were not expected and requires further studying, as no studies to date have attempted to determine foliar nutrients of invasive stands of *E. camaldulensis*. It has been shown previously that *Eucalyptus* species also benefit from root associations with mycorrhiza, which play an important role in acquisition of phosphates (Malajczuk et al., 1975). While this could also lead to local soil enrichment of phosphates, it demonstrates another way in which *Eucalyptus* trees are able to thrive in low-nutrient environments such as Fynbos soils. Milner et al. (2011) reported on foliar nutrients of a range of *Eucalyptus* species found in New Zealand (not including *E. camaldulensis*). The study showed foliar N concentrations which ranged between 1.14% and 1.92% of foliar biomass across the range of species, and foliar %P of between 0.13% and 0.19%. While the numbers for %P are slightly lower than averages for this study, it is considerably higher than those for *A. mearnsii* reported during this study, and those reported by Dovey (2005) from plantations. Additionally, the effect of canopy position of foliage has been reported on for *E. nitens* (Medhurst & Beadle, 2005), which showed that photosynthetic rates of foliage increased significantly as a result of thinning, and with it, foliar N and P concentrations. The study showed the most notable increases in the middle and lower canopy areas, as the upper canopy was not limited by light availability prior to the thinning experiment. It is also reported by Milner et al. (2011) that different heights on the canopy, combined with different levels of access to sunlight, could influence foliar N and P in various *Eucalyptus* species, with the lower shaded areas having the lowest levels of these nutrients. Foliar P thus appears to be moderately related to light availability and strongly related to genus/ functional type, while other factors such as soil available P could also play a role. These patterns are yet to be tested in the Fynbos.

All these variations in nutrient allocation as a function of stem densities, life stage, and biomass components thus justify the consideration of these variables in its modelling. The increased resolution presented through allometric models developed here allows for the

determination of nutrient stocks as discussed above, by only measuring the stem basal diameter. Models presented could be used effectively to predict the impact of invasion by *A. mearnsii* or *E. camaldulensis* on an ecosystem (nutrient export in biomass or nutrient deposition through litterfall and/or nitrogen fixing). The models could be used effectively in Closed canopy and Open canopy sites with great accuracy. In conjunction with biomass models presented in chapter 3 of this study, these nutrient models could also be useful in remote sensing applications and further inform management planning for these sites. Stem densities are thus an important influence on these models, but their effect could be minimised by a sufficiently large sample size.

This study shows that the establishment of invasive alien plants could catalyse significant changes to nutrient cycles in Fynbos riparian zones. One such impact is potentially rapidly decomposing nutrient-rich foliage that could impact on soil and nearby aquatic ecosystems, and potentially encourage secondary invasions (Nsikani et al., 2018; Railoun, 2018). The impact of invasion on local nutrient cycles could make affected ecosystems vulnerable to perpetual invasion. It is thus important to direct management of biomass of invaded sites to further prevent issues such as excessive nutrient influxes into affected ecosystems. Additionally, the potential exports of nutrients not sequestered by invasive species could significantly affect ecosystem recovery post-clearing.

## 4.6 Conclusions

This study has shown that nutrients within the aboveground biomass of *A. mearnsii* and *E. camaldulensis* both vary with growth stage and, to a lesser extent, stem densities. The study also confirms that modelling of the nutrient stocks of the invasive trees requires inclusion of a wide range of stem diameters, and that the growth form of trees from different densities warrant separate nutrient modelling in aboveground biomass components. As expected, *A. mearnsii* had greater concentrations of N in its aboveground biomass. However, *E. camaldulensis* had significantly higher concentrations of P in its foliage, which warrants further studies.

This study demonstrated the significant variation in nutrient allocation within trees to different aboveground biomass components and in relation to growth stage, season, and stem densities. The study also highlights the high-nutrient levels in foliage and their potential effects on soil nutrient status upon abscission, especially in stands of the leguminous *A. mearnsii*, its implications for rehabilitation of such ecosystems post-clearing, and the potential impacts of nutrient-rich litter on the nutrient status of affected river systems such as the Breede River and

its aquatic biodiversity. Moreover, this dataset, including nutrient models, could be a useful tool in decision-making processes around biomass products and their potential uses and the implications of these for the nutrient cycles in the ecosystems these trees have invaded.

#### 4.7 References

- Attiwill, P. M., & Adams, M. A. (1993). Nutrient cycling in forests. *New phytologist*, 124(4), 561-582.
- Beets, P. N., Kimberley, M. O., Oliver, G. R., Pearce, S. H., Graham, J. D., & Brandon, A. (2012). Allometric equations for estimating carbon stocks in natural forest in New Zealand. *Forests*, 3(3), 818-839.
- Chapin, F. S., Vitousek, P. M., & Van Cleve, K. (1986). The nature of nutrient limitation in plant communities. *The American Naturalist*, 127(1), 48-58.
- Dovey, S. B. (2005). *Above-ground allometry, biomass and nutrient content of Acacia mearnsii across four ages and three sites in the KwaZulu-Natal Midlands* (MSc thesis, University of KwaZulu-Natal).
- Ericsson, T., Rytter, L., & Vapaavuori, E. (1996). Physiology of carbon allocation in trees. *Biomass and Bioenergy*, 11(2-3), 115-127.
- Fey, M. (2010). *Soils of South Africa*. Cambridge University Press.
- Jacobs, S., Naude, M., Slabbert, E., Kambaj, O., Fourie, M., Esler, K., & Cowan, D. (2013). Identifying relationships between soil processes and biodiversity to improve restoration of riparian ecotones invaded by invasive acacias. *Report to the Water Research Commission. Report No. 1927/1*
- Killingbeck, K. T. (1996). Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology*, 77(6), 1716-1727.
- Koerselman, W., & Meuleman, A. F. (1996). The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. *Journal of applied Ecology*, 33(6), 1441-1450.
- Kollman, J., Dietz, H., & Edwards, P. J. (2004). Allocation, plasticity and allometry, 6(2002), 205-206.
- Kollmann, J., Dietz, H., & Edwards, P. J. (2004). Allocation, plasticity and allometry. *Perspectives in Plant Ecology, Evolution and Systematics*, 4(6), 205-206.
- Lambert, M. J. (1976). Preparation of plant material for estimating a wide range of elements. Forestry Commission of New South Wales. Sydney
- Leite, F. P., Silva, I. R., Novais, R. F., Barros, N. F. D., Neves, J. C. L., & Villani, E. M. D. A. (2011). Nutrient relations during a *Eucalyptus* cycle at different population densities. *Revista Brasileira de Ciência do Solo*, 35(3), 949-959.
- Medhurst, J. L., & Beadle, C. L. (2005). Photosynthetic capacity and foliar nitrogen distribution in *Eucalyptus nitens* is altered by high-intensity thinning. *Tree Physiology*, 25(8), 981-991.
- Millner, J. P., & Kemp, P. D. (2012). Foliar nutrients in *Eucalyptus* species in New Zealand. *New Forests*, 43(2), 255-266. DOI 10.1007/s11056-011-9279-3
- Mohler, C. L., Marks, P. L., & Sprugel, D. G. (1978). Stand structure and allometry of trees during self-thinning of pure stands. *The Journal of Ecology*, 599-614.

- Müller, I., Schmid, B., & Weiner, J. (2000). The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspectives in plant ecology, evolution and systematics*, 3(2), 115-127.
- Naudé, M. (2012). *Fynbos riparian biogeochemistry and invasive Australian acacias* (MSc thesis, Stellenbosch: Stellenbosch University).
- Nsikani, M. M., van Wilgen, B. W., & Gaertner, M. (2018). Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N<sub>2</sub>-fixing woody species: implications for ecological restoration. *Restoration ecology*, 26(2), 235-244.
- Pretzsch, H., Grote, R., Reineking, B., Rötzer, T. H., & Seifert, S. T. (2008). Models for forest ecosystem management: a European perspective. *Annals of botany*, 101(8), 1065-1087.
- Railoun, M. Z. (2018). *Impacts of the invasive tree Acacia mearnsii on riparian and instream aquatic environments in the Cape Floristic Region, South Africa* (MSc thesis, Stellenbosch: Stellenbosch University).
- Redmond, M. D., Morris, T. L., & Cramer, M. C. (2019). The cost of standing tall: wood nutrients associated with tree invasions in nutrient-poor fynbos soils of South Africa. *Ecosphere*, 10(9), e02831.
- Rozendaal, D. M. A., Hurtado, V. H., & Poorter, L. (2006). Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Functional Ecology*, 20(2), 207-216.
- Schreeg, L. A., Santiago, L. S., Wright, S. J., & Turner, B. L. (2014). Stem, root, and older leaf N: P ratios are more responsive indicators of soil nutrient availability than new foliage. *Ecology*, 95(8), 2062-2068.
- Schulze, J. (2004). How are nitrogen fixation rates regulated in legumes? *Journal of Plant Nutrition and Soil Science*, 167(2), 125-137.
- Slabbert, E., Jacobs, S. M., & Jacobs, K. (2014). The soil bacterial communities of South African Fynbos riparian ecosystems invaded by Australian Acacia species. *PloS one*, 9(1).
- Snook, R. D. (1992). Handbook of inductively coupled plasma mass spectrometry. *Chromatographia*, 34(9), 546-546.
- Sterner, R. W., & Elser, J. (2008). Ecological stoichiometry: overview. In *Encyclopedia of Ecology, Five-Volume Set* (pp. 1101-1116). Elsevier.
- Tetemke, B. A., Birhane, E., Rannestad, M. M., & Eid, T. (2019). Allometric Models for Predicting Aboveground Biomass of Trees in the Dry Afromontane Forests of Northern Ethiopia. *Forests*, 10(12), 1114.
- Tian, D., Yan, Z., Niklas, K. J., Han, W., Kattge, J., Reich, P. B., ... & Wright, I. J. (2017). Global leaf nitrogen and phosphorus stoichiometry and their scaling exponent. *National Science Review*, 5(5), 728-739.
- Tye, D. R. (2014). *Nutrient and biomass allocation strategies in an invasive Australian Acacia and a co-occurring native Acacia in South Africa* (Doctoral dissertation).
- Van der Colff, D., Dreyer, L. L., Valentine, A., & 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, 358-364.
- Vitousek, P. (1982). Nutrient cycling and nutrient use efficiency. *The American Naturalist*, 119(4), 553-572.
- Weiner, J. (2004). Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 6(4), 207-215.



## Chapter 5: Nutrient export in biomass a potential stressor on riparian zones invaded by *Acacia mearnsii* and *Eucalyptus camaldulensis*

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### 5.1 Abstract

Large-scale nutrient uptake and storage in invasive tree biomass in Fynbos riparian zones of South Africa creates an opportunity for large-scale export of nutrients when the trees are cleared and removed to be replaced with indigenous vegetation. This potential nutrient export has been shown to be beneficial in some cases where it may have initially been enriched by the presence of invasive alien trees, but could also lead to reductions in other nutrients not enriched in this manner. Nutrient export in invasive biomass has not yet been quantified despite country-wide clearing and removal efforts, with variable outcomes. This study reports on the quantities of various elements contained in the aboveground biomass of *Acacia mearnsii* and *Eucalyptus camaldulensis* in riparian zones of the Breede River Catchment, Western Cape and thus provides estimates of the potential for nutrient export when these sites are cleared. Biomass data were compiled by destructive sampling of trees and stem density counts within 10x25 m<sup>2</sup> plots each of *A. mearnsii* and *E. camaldulensis* at high density (>75% canopy cover; Closed canopy) and low density (<25% canopy cover; Open canopy). Given the significant amounts of *A. mearnsii* biomass at some places (110.996 ± 55.1 t ha<sup>-1</sup> at a site at Bainskloof), nutrient exports are of potential significance (1208.48 ± 530.75 kg ha<sup>-1</sup> of N per and 45.97 ± 20.21 kg of P per ha). Of the total biomass, C consistently made up more than 50%, amounting to up to 63.45 ± 31.6 t ha<sup>-1</sup> at the Closed canopy site at Bainskloof. Nutrients are generally concentrated in the leaf and bark material, while the stem material, due to its bulk, also contributes significantly to the total nutrient pool for both invasive species. *Eucalyptus camaldulensis* could have a significantly greater impact on soil nutrient reserves such as N, P, and cations. This is due to the non- N-fixing nature of the species, strong accumulation of P in foliage, and, on average, more standing biomass across sites. This study shows that, in addition to nutrient depositions due to presence of invasive alien trees, removal from riparian zones has the potential for large-scale removal of other nutrients and that this could significantly disrupt local nutrient cycles. It is unclear how these disruptions may affect ecosystem recovery post-clearing and how nutrient reserves may be replenished, especially where nutrient enrichment is likely an issue. It is suggested that new biomass management practices consider and account for both these possibilities.



## 5.2 Introduction

Plant biomass represents a relatively stable pool of nutrient utilization and storage, as plants are responsible for uptake and storage of large amounts of nutrients in their biomass. Whereas the storage capacity of plants is an important ecosystem function in the form of carbon sequestration, the ability of plants to assimilate and store nutrients can lead to significant nutrient exports in cases where the biomass is removed. For instance, significant amounts of nutrients accumulated in biomass have previously led to disruptions in forest nutrient cycles and large-scale soil nutrient deficiencies when removed (Hall, 2002; Kering et al., 2012; Madalcho, 2016; Masters et al., 2016). The *modus operandi* for biomass removal from forestry plantations was previously largely limited to stem material; however, increased demand for a variety of biomass products have led to increased use of plant biomass and total export thereof (Ge et al., 2015). Whole-tree removal has thus been identified as a detrimental management activity for nutrient cycling in forestry scenarios (Tew et al., 1986; Dovey, 2009; Ge et al., 2015).

Similarly, emerging markets for biomass Value-Added Products (VAPs) such as wood chips, biochar, wood pellets, firewood, and wood bricks have begun to drive discussions of capitalizing on invasive biomass in South Africa (Mudavanhu et al., 2016; Vundla et al., 2016., Stafford & Blignaut, 2017; Stafford et al., 2018). The country, well-known as being water-stressed and for its high levels of biodiversity and endemism, is faced with large-scale water and biodiversity losses due to invasion from exotic tree species (Le Maître et al., 2000; Scott-Shaw et al., 2017), especially those from the genera *Acacia* and *Eucalyptus*. In the Western Cape, *Acacia mearnsii* and *Eucalyptus camaldulensis* are frequently targeted for clearing, largely due to their proximity to riparian zones and their impact on the water supply to towns and irrigation schemes. Clearing of biomass does, however, often have unintended consequences. One of the most common methods of alien clearing in riparian zones in South Africa is by fell and burn, whereby biomass is cleared and removed from riparian zones to prevent flooding and obstruction of waterways (Blanchard & Holmes, 2008). The biomass is then piled and burned during the cool winter months (Jacobs et al., 2017). Pile burning, due to extremely high temperatures, potentially affects seed banks of native species (Cilliers et al., 2004; Blanchard & Holmes, 2008), and promotes erosion (Neary et al., 1999; Euston-Brown, 2000; Doerr et al., 2009).

The effects of invasion by Australian acacias on soils in the Fynbos biome has been widely reported on, and it is widely understood that the presence of these leguminous trees lead to elevated soil nutrients such as nitrogen and phosphorus, through their root associations with rhizobial bacteria and mycorrhizal fungi, respectively (Witkowski et al., 1991; Tye & Drake,

2012; Fourie, 2014; Slabbert et al., 2014). It is also known that the elevated soil nutrient status may have a strong impact on ecosystem recovery after clearing (Holmes et al., 2008; Naudé 2012; Nsikani et al., 2018), and that these nutrients are prone to be leached out into nearby river systems (Tye & Drake, 2012; Railoun, 2018). However, nutrients accumulated by tree biomass and not enriched in the soil as a result of the invasion has not yet been quantified, and its export in cleared biomass may lead to local depletions. While acknowledging the need for effective alien clearing and the removal of biomass (including litter), it is important to explore the possibility that these management activities could have significant long-term effects on ecosystem functioning that are not related to nutrient accumulation/deposition. The studies mentioned above studies have been largely confined to leguminous species, and excluded the potential effect on soil nutrient status of others such as *Eucalyptus*, *Pinus*, and *Hakea* spp. Similarly, none of these studies have explicitly attempted to quantify nutrient stocks available in aboveground biomass of invaded sites. Plant biomass is an important conduit for the flow and temporary storage of nutrients; taking it up and releasing it slowly back into soil through litterfall or through mortality of tree components or entire trees (Attiwill et al., 1993; Schulze et al., 2004). While research has shown the potential for nutrient accumulation and its effect on ecosystem recovery, it is now important to quantify possible losses of other critical elements through activities such as clearing and removal to make management decisions for possibilities for recovery of such ecosystems *post facto*.

#### Hypotheses:

- Large-scale nutrient export through removal of invasive biomass is a potential stressor for ecosystem recovery after alien tree removal.
- The non-leguminous *Eucalyptus camaldulensis* has a greater potential for nutrient export than the leguminous *A. mearnsii*.

### 5.3 Materials and methods

This study was conducted in four riparian sites of the Breede River Catchment in the Western Cape, two of which each had stands of *Eucalyptus camaldulensis*, and two of *Acacia mearnsii* (Figures 5.1 and 5.2). The first *A. mearnsii* site was at Bainskloof near Wellington, along the Wit River, which is a tributary of the Breede River (33°32'24.98"S; 19°10'36.26"E) (Figure 5.1 A). The first *E. camaldulensis* site was on a private farm near the town of Wolseley (33°26'18.35"S; 19°13'36.26"E) (Figure 5.1 B) and the second one on a dairy farm between

Worcester and Robertson, below the Brandvlei dam (33°46'2.79"S; 19°32'13.11"E). The second *E. camaldulensis* site is adjacent to the first *A. mearnsii* site, which is situated on the same farm but forms a distinct stand to the south of the *E. camaldulensis* stand (33°46'3.34"S; 19°31'48.05"E) (Figure 5.2).

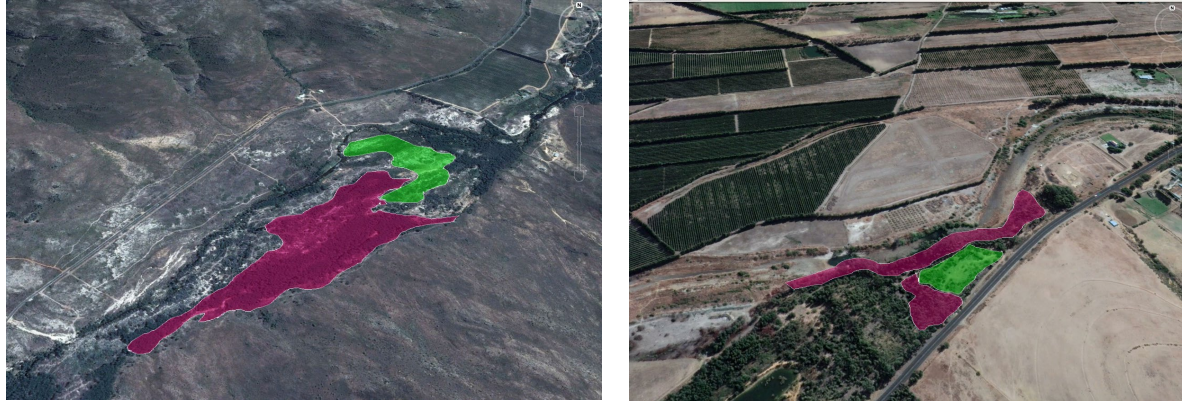


Figure 5.1: Google Earth images of a site invaded by *Acacia mearnsii* at Bainskloof (A) and a second site invaded by *Eucalyptus camaldulensis* at Wolseley (B). Green shows area of low-density invasion (Open canopy) and red shows areas of high-density invasion (Closed canopy). Images are dated to 2016.



Figure 5.2: Google Earth image of the invaded site at Alfalfa, where both *Acacia mearnsii* and *Eucalyptus camaldulensis* occur. Densities are colour-coded: red shows high-density invasion (Closed canopy) and green shows low-density invasion (Open canopy). Plots are marked as Closed canopy *A. mearnsii* (AM-D), Open canopy *A. mearnsii* (AM-F), Closed canopy *E. camaldulensis* (EC-D) and Open canopy *E. camaldulensis* (EC-F). Image is dated to 2016.



Trees of *E. camaldulensis* and *A. mearnsii* were harvested and all aboveground components separated from each other. These components included stem, leaves, bark, and branches. For biomass determinations, six adult samples of each species were harvested in sites of high density (Closed canopy) and six each at sites of low density (Open canopy). Of these, three samples each of juvenile, sapling, and adult plants of both species, from two sites each, and from the two density extremes were sent for nutrient analyses. Sampling was done during the summer (all age groups) and winter (adults).

Nutrients were quantified through combustion of biomass by a commercial laboratory, Bemlab, located in Somerset West, South Africa. Dry samples were cut into smaller pieces using a band saw, after which they were crushed with a Netzsch Condux LV15M toothed disk mill to allow for further processing. An Ultra Centrifugal Retsch ZM200 mill was used to mill the crushed samples into a powder using a screen of 2 mm and finally 0.5 mm aperture size. Samples were stored in 15 ml plastic vials for laboratory analyses. Nutrient analyses were done using the following methods: Phosphorus (P), Potassium (K), Magnesium (Mg), Calcium (Ca), and Sodium (Na) were determined using the Ash method (Lambert, 1976), which includes treating the ashed sample with a 50% solution of hydrochloric acid (HCl) and measuring the contents using a Perkin Elmer Optima 7300 DV ICP-OES (Inductively Coupled Plasma- Optical Emission Spectrometry) spectrometer. Carbon (C) and Nitrogen (N) contents were determined through Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Snook, 1992) using a LECO-CNS 2000 analyser.

## Statistics

Normal distribution within the datasets was confirmed with a Shapiro-Wilks test for normality. Where normal distribution was confirmed, a factorial Analysis of Variance (ANOVA) was employed to determine the differences. This was done in most instances with stem densities and average stem diameter as a factor of the two stem density extremes (Closed canopy and Open canopy), as well as for determination of overall nutrients percentages of biomass between the two invasive species (*A. mearnsii* and *E. camaldulensis*) and their aboveground biomass components (stem, leaves, bark, and branches). Data were not normally distributed for stem densities of *A. mearnsii*, due to high stem densities at one site. For this data, a Kruskal Wallis test was done. In other instances where cumulative values (total) formed part of an ANOVA, no statistics are reported due to the inherent skewing of data. Where significant differences were found a post-hoc Fisher's LSD test was used throughout.

Where no significant differences were found, no further tests were conducted. Statistical analyses were done using Statistica® 13.

## 5.4 Results

### Stem densities

There were significant differences in stem densities between the two *Acacia mearnsii* sites (Kruskal-Wallis ANOVA;  $H_{[1;46]} = 12.6$ ;  $P < 0.001$ ; Figure 5.3 A). The Closed canopy site at Bainskloof had the highest stem densities ( $31320 \pm 17752$  stems  $ha^{-1}$ ) and the Open canopy site had a density of  $5200 \pm 4643$  stems  $ha^{-1}$ . The Closed canopy site at Alfalfa had a stem density of  $4440 \pm 2433$  stems  $ha^{-1}$  and the Open canopy site had a density of  $1160 \pm 173$  stems  $ha^{-1}$ .

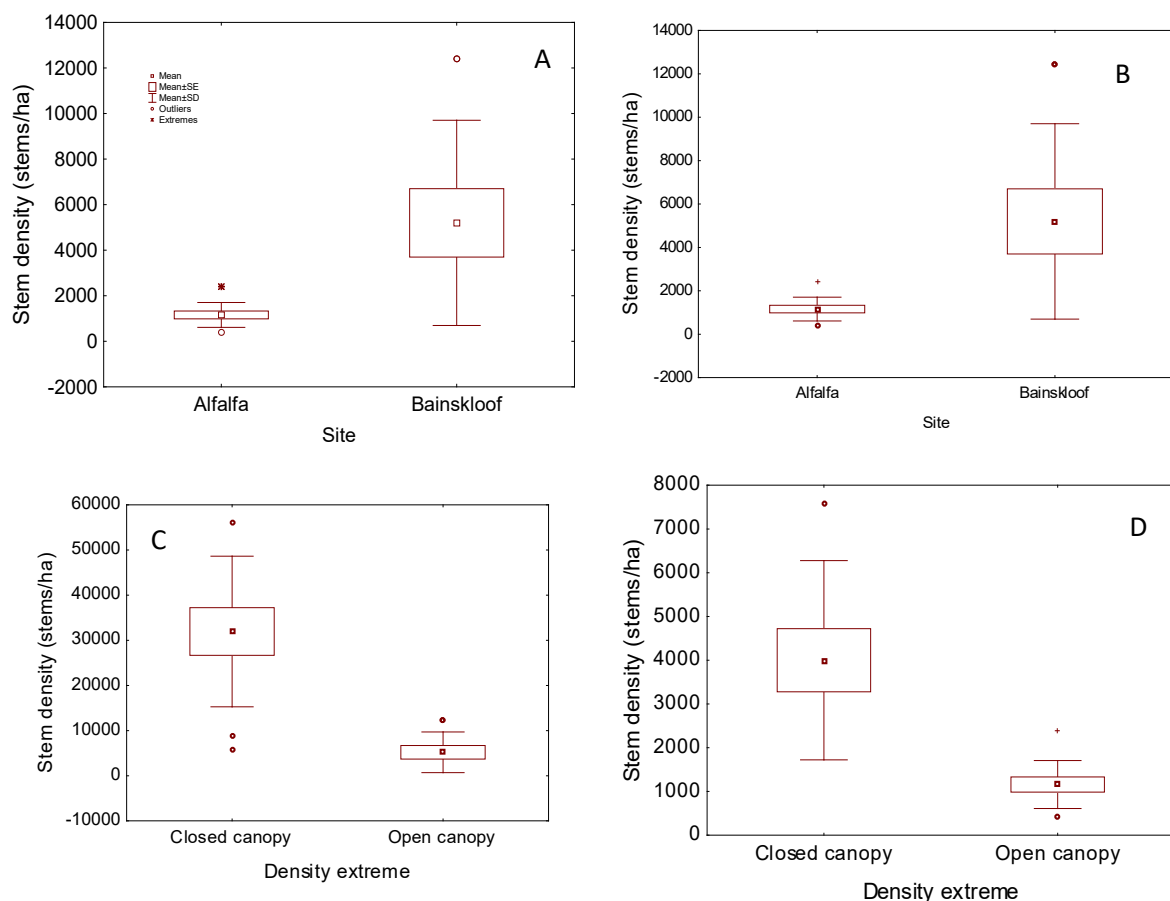


Figure 5.3: Differences in stem densities between the two Closed canopy *A. mearnsii* sites (A), and between the two Open canopy *A. mearnsii* sites (B). Differences in stem densities between the two density extremes are shown also shown for Bainskloof (C) and Alfalfa (D). Boxes denote mean  $\pm$  SE and error bars denote mean  $\pm$  SD.

There were significant differences in *A. mearnsii* stem basal diameter between sites (ANOVA;  $F_{[1,36]} = 4.85$ ;  $P < 0.001$ ; Fig. 5.4) but none between stem densities within sites ( $F_{[1,36]} = 0.26$ ;  $P = 0.612$ ) and no significant interaction between the two independent variables ( $F_{[1,36]} = 1.21$ ;  $P = 0.279$ ). Between sites, both densities at Alfalfa had significantly larger stem diameters than those at Bainskloof (LSD;  $P < 0.05$ ). The Closed canopy site at Bainskloof had an average stem basal diameter of  $4.58 \pm 1.94$  cm and the Open canopy Bainskloof site an average of  $3.44 \pm 1.45$  cm, while the sites at Alfalfa had  $7.17 \pm 2.41$  cm and  $7.58 \pm 2.76$  cm stem diameter at Closed canopy and Open canopy sites, respectively.

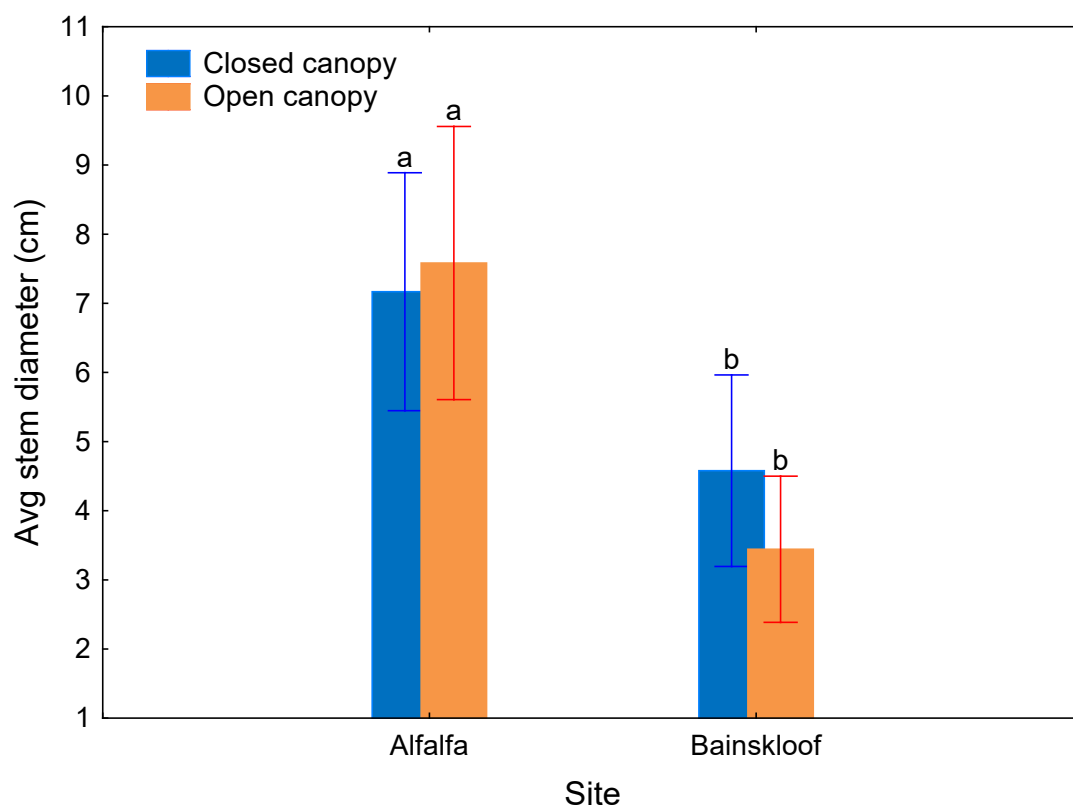


Figure 5.4: Stem diameters of *Acacia mearnsii* measured at Bainskloof and Alfalfa, and at both density extremes (Closed canopy and Open canopy). Letters denote significant differences (LSD;  $P < 0.05$ ); error bars denote 0.95 confidence intervals.

For average stem densities of *Eucalyptus camaldulensis*, there was significant interaction between site and stem density extremes (ANOVA;  $F_{[1,36]} = 4.76$ ;  $P = 0.036$ ; Fig 5.5 A). The Open canopy site at Wolseley had the greatest stem densities ( $6481 \pm 2956$  stems  $\text{ha}^{-1}$ ) which was significantly more than the Open canopy site at Alfalfa ( $2720 \pm 920$ ; LSD;  $P = 0.002$ ). The two Closed canopy sites did not have significantly different stem densities, but

both had significantly higher stem densities than the Open canopy site at Alfalfa and significantly lower stem densities than the Open canopy site at Wolseley (LSD  $P < 0.05$ ).

The high density of trees at the Wolseley Open canopy site could be correlated with the relatively small tree sizes found in the area (Fig 5.5 B). In this instance, the Wolseley Open canopy site had the smallest stem basal diameters ( $3.24 \pm 1.48$  cm), and was significantly smaller than any of the other sites (LSD;  $P < 0.05$ ). The stem diameter of the two Closed canopy *E. camaldulensis* sites did not differ significantly (LSD;  $P = 0.73$ ), neither did the average stem densities ( $P = 0.776$ ).



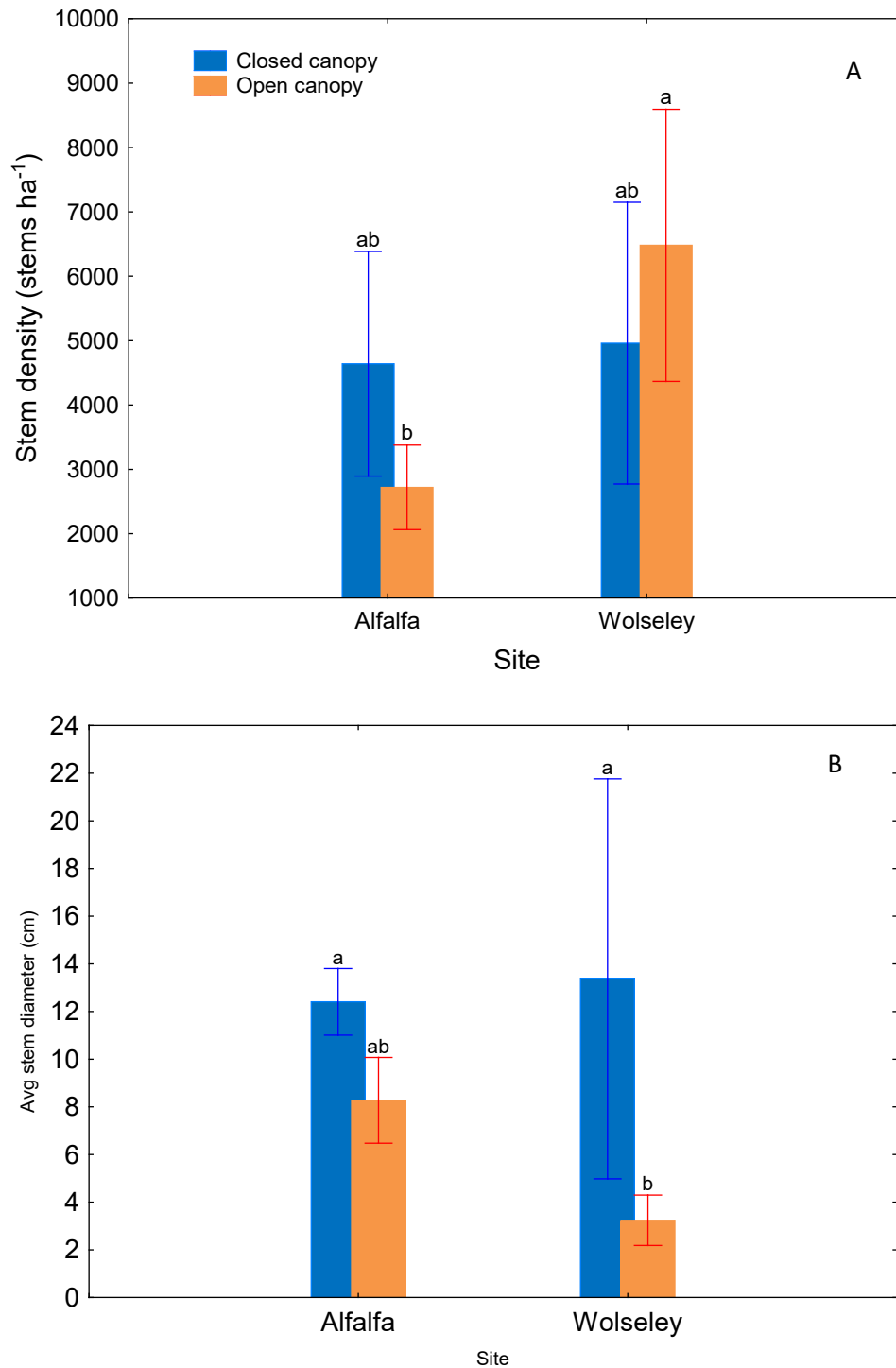


Figure 5.5: Differences in *E. camaldulensis* stem densities (A) and stem diameters (B) measured at both sites (B). Letters denote significant differences (LSD;  $P < 0.05$ ); error bars denote 0.95 confidence intervals.

## Differences in carbon, nitrogen, phosphorus, and total cation percentages in aboveground biomass between *Acacia mearnsii* and *Eucalyptus camaldulensis*

There were significant differences in carbon percentages of biomass components between the two invasive species (ANOVA;  $F_{[3; 346]}=4$ ;  $P=0.008$ , Figure 5.6). *A. mearnsii* bark C ( $57.134 \pm 0.33\%$ ) was significantly more than that of *E. camaldulensis* ( $56.7 \pm 0.46\%$ ; LSD:  $P<0.001$ ). The lowest C allocation was for leaves in both species. There were no additional significant differences (LSD;  $P\geq 0.05$ )

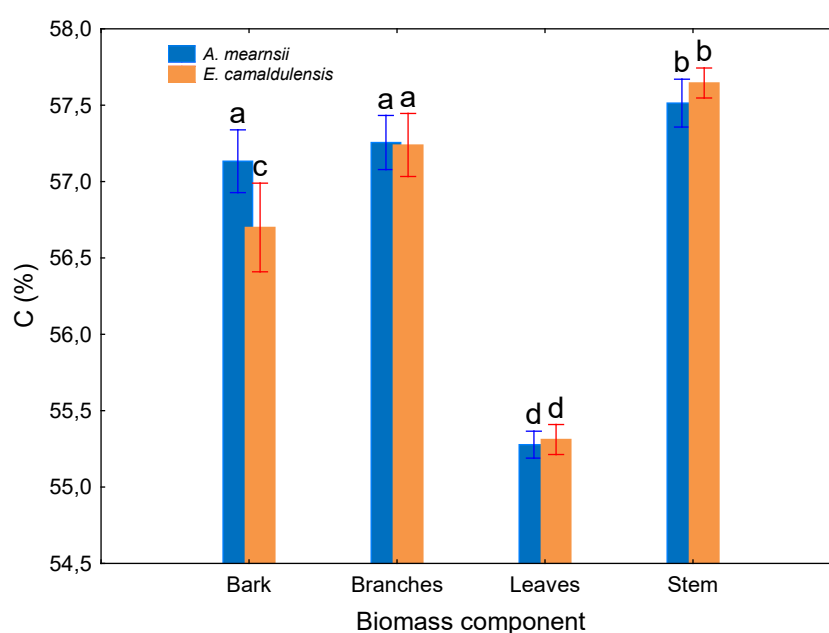


Figure 5.6: Differences in % carbon between biomass components of *A. mearnsii* and *E. camaldulensis*. Letters denote significant differences (LSD;  $P<0.05$ ); error bars denote 0.95 confidence intervals.

There were also significant differences and interaction between species and biomass components for nitrogen (ANOVA;  $F_{[3; 346]}=52.975$ ;  $P<0.001$ ; Figure 5.7 A). Nitrogen contents of all *E. camaldulensis* biomass components were less than for *A. mearnsii*. Only the differences in stem %N were non-significant ( $P=0.06$ ), while all other differences were significant ( $P<0.05$ ). The highest %N contents were reported for foliage of *A. mearnsii* ( $3.12 \pm 0.24\%$ ). For P, there was significant interaction between species and biomass component (ANOVA;  $F_{[3; 346]}=66.861$ ;  $P<0.001$ ; Figure 5.7 B). Phosphorus content of *E. camaldulensis* leaves ( $0.21 \pm 0.04\%$ ) was significantly higher than that of *A. mearnsii* leaves ( $0.08 \pm 0.01$ ;

LSD;  $P < 0.001$ ). *Acacia mearnsii* bark had significantly higher %P ( $0.077 \pm 0.03\%$ ) than *E. camaldulensis* bark ( $0.043 \pm 0.003\%$ ;  $P < 0.001$ ).

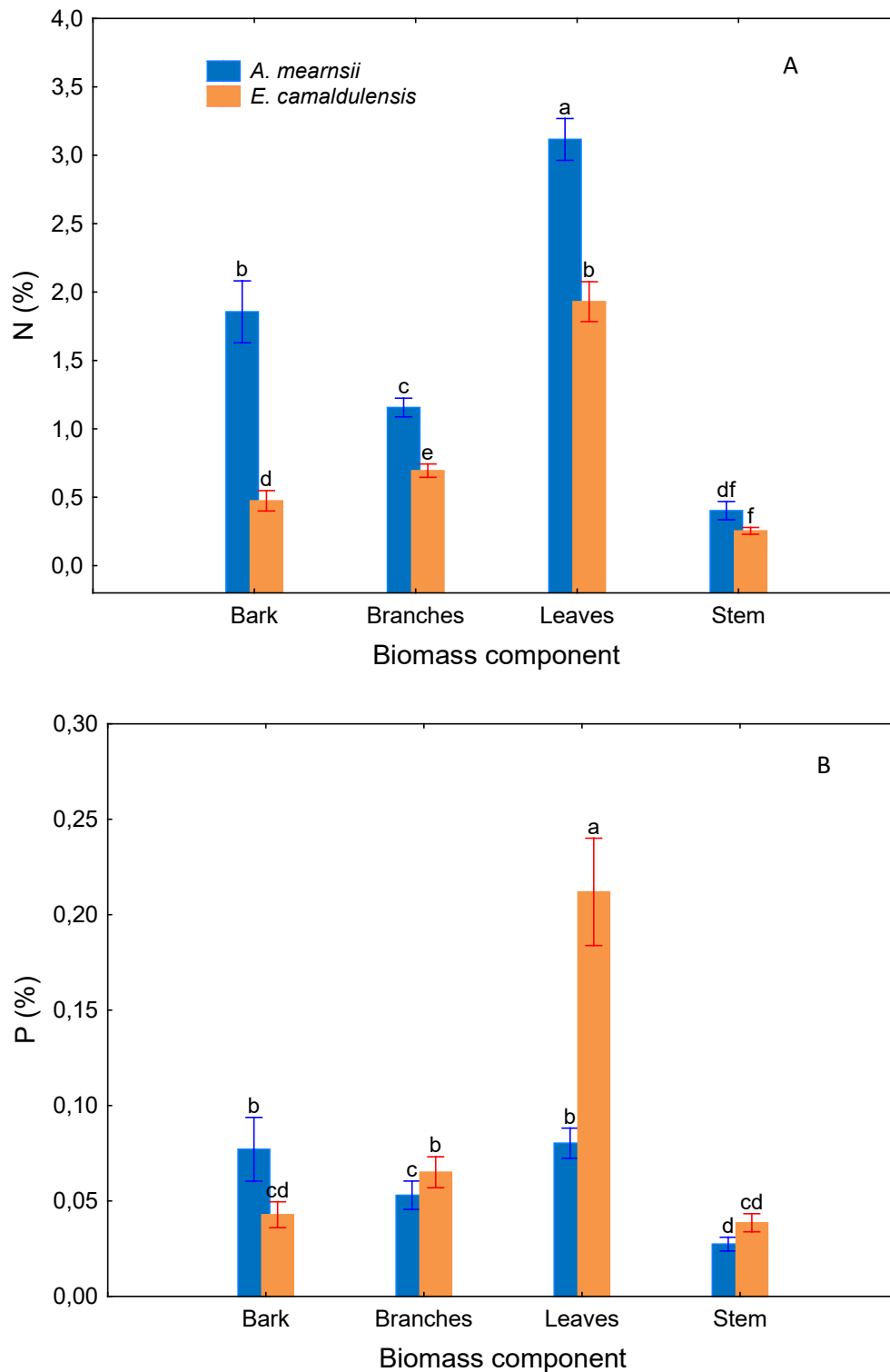


Figure 5.7: Differences in % nitrogen (A) and % phosphorus (B) between biomass components of *A. mearnsii* and *E. camaldulensis*. Letters denote significant differences (LSD;  $P < 0.05$ ); error bars denote 0.95 confidence intervals.

There were also significant differences and interaction between species and biomass components for total cations (ANOVA;  $F_{[3; 346]}=19.273$ ;  $P<0.001$ ; Figure 5.8). Foliage of *E. camaldulensis* had significantly more total cations than that of *A. mearnsii* (LSD;  $P<0.001$ ). Total cations in bark and branch material of *E. camaldulensis* were also significantly more than in those components of *A. mearnsii* ( $P<0.05$ ), while the difference in stem cations between the two species was non-significant ( $P=0.213$ ).

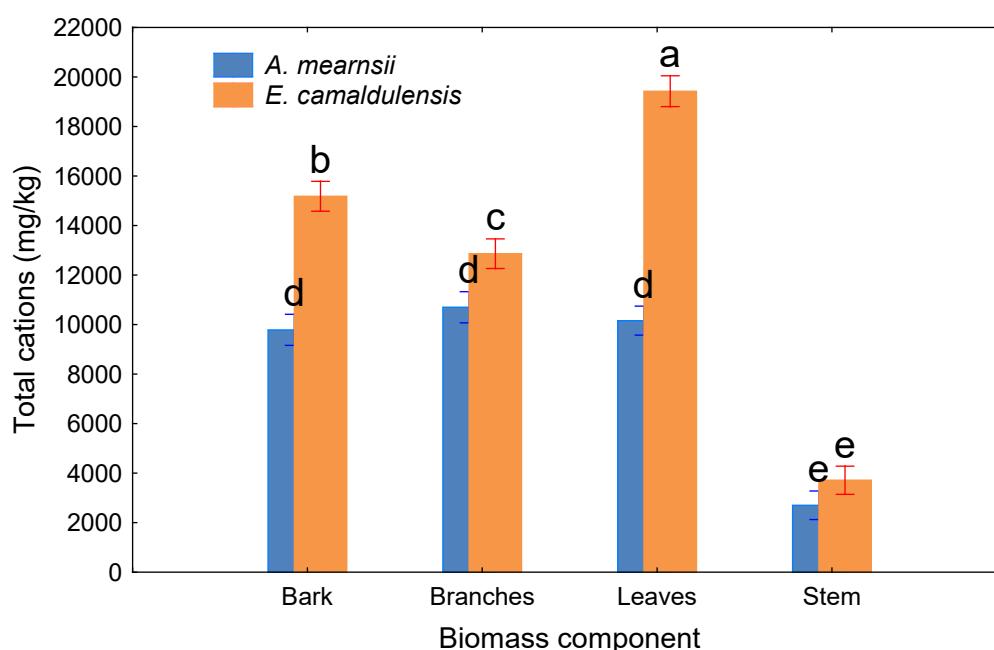


Figure 5.8: Differences in total cations between biomass components of *A. mearnsii* and *E. camaldulensis*. Letters denote significant differences (LSD;  $P<0.05$ ); error bars denote 0.95 confidence intervals.

As a result of high stem densities, the Closed canopy site at Bainskloof supported the greatest amounts of biomass ( $110.996 \pm 55.1 \text{ t ha}^{-1}$ ; Table 5.1). Closed canopy sites of *A. mearnsii* typically have more biomass than Open canopy ones (Table 5.1). The Closed canopy site at Bainskloof also had more biomass than the Closed canopy site at Alfalfa ( $33.729 \pm 9.5 \text{ t ha}^{-1}$ ), while the Open canopy site at Alfalfa had more biomass ( $14.423 \pm 8.3 \text{ t ha}^{-1}$ ) than the Open canopy site at Bainskloof ( $13.026 \pm 9.9 \text{ t ha}^{-1}$ ). As shown in table 5.2, the Closed canopy site at Alfalfa had the greatest amounts of *E. camaldulensis* biomass per hectare found during this study ( $92.012 \pm 19.4 \text{ t ha}^{-1}$ ), while the Open canopy site at Wolseley had the lowest amount of biomass available ( $2.751 \pm 1.4 \text{ t ha}^{-1}$ ). The Closed canopy site at Wolseley had  $78.144 \pm 84.7 \text{ t ha}^{-1}$  of aboveground biomass.

Table 5.1: Amounts of *A. mearnsii* biomass available ( $\text{t ha}^{-1}$ ; Mean  $\pm$  SD) at the different sites for the various biomass components and at Closed canopy sites and for Open canopy trees.

Biomass component	Site	Density	Biomass ( $\text{t ha}^{-1}$ )
Stem	Bainskloof	Closed canopy	$49.514 \pm 32.7$
		Open canopy	$3.094 \pm 2.9$
	Alfalfa	Closed canopy	$18.0389 \pm 6,1$
		Open canopy	$5.392 \pm 4.5$
Leaves	Bainskloof	Closed canopy	$14.943 \pm 9$
		Open canopy	$4.527 \pm 3.1$
	Alfalfa	Closed canopy	$4.427 \pm 1.7$
		Open canopy	$3.407 \pm 1.7$
Branches	Bainskloof	Closed canopy	$30.846 \pm 12.5$
		Open canopy	$1.801 \pm 1.9$
	Alfalfa	Closed canopy	$6.229 \pm 1.6$
		Open canopy	$3.219 \pm 2.1$
Bark	Bainskloof	Closed canopy	$15.693 \pm 9.4$
		Open canopy	$3.604 \pm 2.4$
	Alfalfa	Closed canopy	$5.035 \pm 1.4$
		Open canopy	$2.405 \pm 1.1$
Total	Bainskloof	Closed canopy	$110.996 \pm 55.1$
		Open canopy	$13.026 \pm 9.9$
	Alfalfa	Closed canopy	$33.729 \pm 9.5$
		Open canopy	$14.423 \pm 8.3$

Table 5.2: Amounts of *E. camaldulensis* biomass available ( $\text{t ha}^{-1}$ ; Mean  $\pm$  Std. Err.) at the different sites for the various biomass components and at Closed canopy sites and for Open canopy trees.

Biomass component	Site	Density	Biomass ( $\text{t ha}^{-1}$ )
Stem	Wolseley	Closed canopy	$53.587 \pm 58.2$
		Open canopy	$1.927 \pm 1$
	Alfalfa	Closed canopy	$60.822 \pm 13.1$
		Open canopy	$43.063 \pm 14.7$
Branches	Wolseley	Closed canopy	$10.324 \pm 11.1$
		Open canopy	$0.177 \pm 0.1$
	Alfalfa	Closed canopy	$12.772 \pm 0.4$
		Open canopy	$2.409 \pm 0.8$
Leaves	Wolseley	Closed canopy	$7.778 \pm 8.41$
		Open canopy	$0.334 \pm 0.2$
	Alfalfa	Closed canopy	$2.409 \pm 0.8$
		Open canopy	$5.449 \pm 1.3$
Bark	Wolseley	Closed canopy	$6.456 \pm 7.0$
		Open canopy	$0.313 \pm 0.2$
	Alfalfa	Closed canopy	$8.01 \pm 2.5$
		Open canopy	$4.294 \pm 3.6$
Total	Wolseley	Closed canopy	$78.144 \pm 84.7$
		Open canopy	$2.751 \pm 1.4$
	Alfalfa	Closed canopy	$92.012 \pm 19.4$
		Open canopy	$55.215 \pm 39.3$

Tables 5.3 and 5.4 show the stocks of nutrients ( $\text{kg ha}^{-1}$ ) and carbon ( $\text{t ha}^{-1}$ ) likely to be removed through clearing of *A. mearnsii* and *E. camaldulensis* based on the data from the current study sites. Most of the sites invaded by *Acacia mearnsii* had low to moderate amounts of carbon stored in aboveground biomass components (Table 5.3). The site with the most biomass (Closed canopy site at Bainskloof) had the highest amounts of all nutrients tested. In particular, the site is estimated to have  $63.448 \pm 31.6 \text{ t ha}^{-1}$  of C. The lowest amount of C was found in the Open canopy site at Bainskloof ( $7.346 \pm 5.62 \text{ t ha}^{-1}$ ). Likewise, the Closed canopy site at Bainskloof had up to  $1208.483 \pm 530.75 \text{ kg ha}^{-1}$  of N and  $45.971 \pm 20.21 \text{ kg ha}^{-1}$  of P in its aboveground biomass. Stem mass had the greatest amounts of C, while leaf mass had the greatest amounts of all other nutrients tested.

The *Eucalyptus camaldulensis* site with the highest amounts of biomass (Alfalfa Closed canopy) is also the site with the highest total amounts of C in its aboveground biomass ( $52.613 \pm 11.1 \text{ t ha}^{-1}$ ; Table 5.4). The lowest amount of C was recorded at the Wolseley Open canopy site ( $1.577 \pm 0.81 \text{ t ha}^{-1}$ ). All other nutrients followed the same trends in terms of site differences. As with *A. mearnsii*, stem material of *E. camaldulensis* also stored the highest amounts of C. However, the highest total amounts of N were found in leaves in Closed canopy sites and in stem material in Open canopy trees. Additionally, the highest amounts of P were recorded in stem material for all sites, while the other nutrients showed variations between biomass components.



Table 5.3: Total amounts of nutrients in biomass of *A. mearnsii* at Bainskloof and Alfalfa sites, broken up into Closed canopy and Open canopy plots. All values are presented as kg ha<sup>-1</sup>; C is presented as t ha<sup>-1</sup>

Site	Density	Biomass component	C (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	P (kg ha <sup>-1</sup> )	Ca (kg ha <sup>-1</sup> )	K (kg ha <sup>-1</sup> )	Mg (kg ha <sup>-1</sup> )	Na (kg ha <sup>-1</sup> )
Alfalfa	Closed canopy	Bark	2.870 ±0.81	60.064 ± 17.01	2.316 ±0.66	34.588 ± 9.8	3.172 ±0.9	7.854 ±2.22	8.735 ±2.47
		Branches	3.560 ±0.92	74.397 ± 19.28	2.928 ± 0.76	23.483 ±6.09	7.350 ± 1.91	7.724 ± 2	5.814 ± 1.51
		Leaves	2.448 ±0.91	137.987 ± 35.35	4.530 ±1.68	29.398 ±10.96	15.186 ±5.66	10.493 ±3.91	9.714 ±3.62
		Stem	10.421 ±3.52	85.138 ± 28.75	3.247±1.1	16.955 ±5.73	6.674 ±2.25	4.509 ±01.52	9.387 ±3.16
		<b>Total</b>	<b>19.300 ±5.42</b>	<b>343.764 ± 96.28</b>	<b>13.02 ±3.61</b>	<b>104.425 ±28.28</b>	<b>32.382 ±9.33</b>	<b>30.580 ±8.34</b>	<b>33.650 ±9.44</b>
	Open canopy	Bark	1.369 ±0.63	35.673 ± 16.39	1.708 ±0.78	13.832 ±6.35	1.323 ±0.61	3.199 ±1.47	4.092 ±1.88
		Branches	1.845 ±1.18	38.632 ± 24.78	1.417 ±0.91	11.718 ±7.52	5.505 ±3.53	3.670 ±2.35	2.872 ±1.84
		Leaves	1.877 ±0.91	90.756 ± 44.21	3.339 ±1.63	18.533 ±9.03	10.322 ±5.03	5.757 ±2.8	8.064 ±3.93
		Stem	3.088 ±2.03	20.976 ± 13.79	1.726 ±1.13	4.637 ±3.05	4.637 ±3.05	1.833 ±1.21	2.283 ±1.5
		<b>Total</b>	<b>8.180 ±4.73</b>	<b>186.038 ± 89.49</b>	<b>8.189 ±4.42</b>	<b>54.624 ±38.88</b>	<b>21.788 ±12.13</b>	<b>14.460 ±7.77</b>	<b>17.310 ±9.09</b>
Bainskloof	Closed canopy	Bark	8.945 ±5.37	187.216 ± 112.42	7.219 ±4.34	107.810 ±64.74	9.887 ±5.93	24.481 ±14.7	27.227 ±16.35
		Branches	17.634 ±7.13	368.297 ± 148.99	14.448 ±5.9	116.072 ±47.21	37.279 ±14.2	38.082 ±15.6	28.721 ±11.69
		Leaves	8.263 ±4.98	523.954 ± 235.77	15.392 ±9.28	99.224 ±59.83	51.256 ±30.9	35.416 ±21.35	32.786 ±19.77
		Stem	28.607 ±18.59	233.706 ± 151.85	8.913 ±5.79	46.543 ±30.24	18.320 ±11.9	12.379 ±8.04	25.768 ±16.74
		<b>Total</b>	<b>63.448 ±31.6</b>	<b>1208.48 ± 530.75</b>	<b>45.97 ±20.21</b>	<b>369.65 ±165.02</b>	<b>116.74±50.34</b>	<b>110.36 ±48.04</b>	<b>114.503 ±52.3</b>
	Open canopy	Bark	2.052 ±1.37	52.566 ± 36.02	2.486 ±1.74	20.120 ±14.09	2.174 ±1.35	4.766 ±3.21	5.897 ±4.2
		Branches	1.028 ±1.1	25.152 ± 24.9	0.923 ±0.91	7.218 ±7.23	3.459 ±3.45	2.324 ±02.31	1.894 ±1.87
		Leaves	2.495 ±1.71	120.591 ± 82.6	4.436 ±3.04	24.625 ±16.87	13.716 ±9.39	7.650 ±5.24	10.715 ±7.3
		Stem	1.772 ±1.68	12.037 ± 11.39	0.990 ±0.93	2.661 ±2.52	2.661 ±2.52	1.052 ±0.99	1.310 ±1.23
		<b>Total</b>	<b>7.346 ±5.62</b>	<b>210.245 ± 147.79</b>	<b>8.835 ±6.31</b>	<b>48.720 ±25.75</b>	<b>22.010 ±15.9</b>	<b>15.792 ±11.19</b>	<b>19.815 ±13.98</b>

Table 5.4: Total amounts of nutrients in biomass of *E. camaldulensis* at Wolseley and Alfalfa sites, broken up into Closed canopy and Open canopy plots. All values are presented as kg ha<sup>-1</sup>; C is presented as t ha<sup>-1</sup>

Site	Density	Biomass component	C (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	P (kg ha <sup>-1</sup> )	Ca (kg ha <sup>-1</sup> )	K (kg ha <sup>-1</sup> )	Mg (kg ha <sup>-1</sup> )	Na (kg ha <sup>-1</sup> )
Wolseley	Closed	Bark	3.696±3.99	32.536±35.11	3.873±4.18	34.343±37.07	18.075±19.51	23.885±25.78	3.974±4.29
		Branches	4.438±4.8	58.254±63.01	6.222±6.73	40.677±44	26.288±28.44	16.955±18.34	5.815±6.39
		Leaves	5.733±6.19	208.76±225.39	13.422±14.49	77.433±83.6	74.336±80.26	25.088±27.09	18.554±20.03
		Stem	30.83±33.48	136.646±148.42	42.869±46.56	50.907±55.29	72.342±78.57	20.363±22.12	10.744±11.67
		<b>Total</b>	<b>44.697±48.47</b>	<b>436.197±471.94</b>	<b>66.387±71.72</b>	<b>203.361±219.96</b>	<b>191.042±206.78</b>	<b>86.292±93.33</b>	<b>39.088±42.28</b>
	Open	Bark	0.179±0.09	1.485±0.78	0.203±0.11	1.636±0.86	1.013±0.53	0.966±0.51	0.238±0.12
		Branches	0.191±0.1	2.174±1.1	0.267±0.13	1.72±0.87	1.363±0.69	0.785±0.4	0.279±0.14
		Leaves	0.097±0.05	3.249±1.7	0.335±0.18	1.407±0.74	0.803±0.42	0.484±0.25	0.261±0.13
		Stem	1.11±0.57	4.626±2.36	1.542±0.79	1.85±0.95	2.641±1.35	0.829±0.42	0.552±0.28
		<b>Total</b>	<b>1.577±0.81</b>	<b>11.535±5.95</b>	<b>2.348±0.21</b>	<b>6.613±3.41</b>	<b>5.82±2.99</b>	<b>3.064±0.16</b>	<b>1.33±0.6</b>
Alfalfa	Closed	Bark	4.586±0.92	40.372±8.16	4.806±0.97	42.615±8.61	22.874±5.02	29.638±5.99	4.932±1
		Branches	5.939±1.16	77.955±15.35	8.326±1.64	54.433±10.72	35.179±6.92	22.689±4.47	7.782±1.53
		Leaves	7.093±1.44	258.246±52.34	16.603±3.37	95.789±19.41	91.957±18.64	31.036±6.29	22.952±4.65
		Stem	34.996±7.57	155.095±33.15	48.657±10.52	57.78±12.49	82.109±17.75	23.112±4.99	12.866±3.57
		<b>Total</b>	<b>52.613±11.1</b>	<b>531.669±109.35</b>	<b>78.393±16.49</b>	<b>250.618±51.22</b>	<b>232.1119±40.88</b>	<b>106.475±21.74</b>	<b>48.531±10.51</b>
	Open	Bark	2.453±0.78	20.396±6.5	2.791±0.89	22.457±7.16	13.912±4.44	13.268±4.23	3.274±1.04
		Branches	1.379±0.46	15.685±5.22	1.928±0.64	12.408±4.13	9.83±3.27	5.662±1.89	2.013±0.67
		Leaves	1.328±0.44	44.357±14.78	4.578±1.53	19.203±6.4	10.963±3.65	6.602±2.2	3.557±0.12
		Stem	24.797±8.47	103.35±35.32	34.45±11.76	41.34±14.11	58.996±20.14	18.517±6.32	12.337±4.2
		<b>Total</b>	<b>29.956±10.15</b>	<b>183.789±61.79</b>	<b>43.746±14.81</b>	<b>95.409±31.8</b>	<b>93.701±31.5</b>	<b>44.049±14.64</b>	<b>21.182±7.11</b>

## 5.5 Discussion

Changes in soil N and P as a result of invasion in riparian zones could be a significant driver of the trajectory of recovery post-clearing, but this is mostly as a result of enrichment, and not depletion (Holmes et al., 2008). This chapter discusses the potential for nutrient export through cleared invasive biomass and shows that substantial amounts of nutrients could be lost through alien tree removal. This study reports on the potential for nutrient export in biomass of *A. mearnsii* and *E. camaldulensis*. With the exception of N, *E. camaldulensis* has a greater capacity for nutrient export than *A. mearnsii*, including export of P. It is reported here that up to 1200 kg ha<sup>-1</sup> of N could be exported for *A. mearnsii*, along with up to 45 kg ha<sup>-1</sup> of P. Nitrogen fixation and accumulation of P through associations with rhizobium and mycorrhiza, respectively, have not been accounted for in these estimates and the results only shown values of what was present in AGB. For the non-leguminous *E. camaldulensis*, however, nitrogen fixation is not a factor in the nutrient balance of sites it invades. The potential for export here thus reflects the majority of nutrients stored and utilised in the aboveground biomass (bar nutrients lost through litterfall and seed and flower production). In fact, *E. camaldulensis* may have a disproportionately large impact on soil P reserves. Whereas the maximum amount of N exported through *E. camaldulensis* AGB in this study is 533 kg ha<sup>-1</sup> (less than half of the maximum for *A. mearnsii*), P export in *E. camaldulensis* AGB (78 kg ha<sup>-1</sup>) is 1.7 times more than the maximum for *A. mearnsii* (45 kg ha<sup>-1</sup>). For comparison, Coetsee et al. (2015) studied fynbos and forest topsoil at various depths between 0 and 100 cm and reported availability of between 2.19 and 1.19 g m<sup>-2</sup> of P (or between 21.9 and 11.9 kg ha<sup>-1</sup>). It is, however, still unclear whether these values are reflected in soils affected by either *A. mearnsii* or *E. camaldulensis* invasion and what the net impact of these species could be on P or other soil nutrients.

An aspect of nutrients in invasive stands that warrants further discussion is the potential enrichment of elements such as nitrogen and phosphorus by invasive alien plant species to soils they colonize. For instance, in the case of *A. mearnsii*, the net exports of N and P projected here may not reflect the potential of the species to sequester these elements and contribute to the overall pools in the soil and thus increase its availability through its association with root microbes and the formation of root nodules (Witkowski, 1991; Naude, 2012) (due to litterfall, symbiotic microbes, nutrient resorption, etc.). Witkowski (1991) also reported elevated concentrations of N in *A. saligna* litter when compared to *Leucospermum parile* in Fynbos vegetation and the same for *A. cyclops* when compared to *Pteroclastrus tricuspidatus* in Strandveld vegetation. Interestingly, the study did not report any significant differences in foliar P concentration between *A. saligna* and *L. parile*, while *P. tricuspidatus*

had significantly higher foliar P concentrations than *A. cyclops* (Witkowski, 1991). The *Acacia* species in both instances had higher concentrations of foliar N than the native Fynbos and Strandveld species, which was also reflected in its litter. Witkowski (1991) showed that the presence of invasive *Acacia* species in either Fynbos or Strandveld could increase soil N status through litterfall. Leaf litter in *E. camaldulensis* forests in Australia and Morocco were recorded by Briggs & Maher (1983), who showed that litterfall from the species could amount to approximately 500 g m<sup>-2</sup>. It is thus possible that a significant portion of the foliar nutrients reported in this study for *E. camaldulensis* (dependent on resorption efficiency) could further enrich soils with excess nutrients. Hence, while these nutrients are likely exported in large quantities through removal of invasive biomass, it is still possible that soil nutrients in affected sites are elevated further. If not utilised, these nutrients could be leached out of the soil profile into nearby river systems, possibly causing eutrophication (Tye and Drake, 2012) and affecting aquatic biodiversity (Chamier et al., 2012). It has also been suggested that the inputs of excess N and P to riparian zones in nutrient-poor soils could encourage secondary invasions (Nsikani et al., 2018; Railoun, 2018). To counter the effect of soil nutrient enrichment after invasion by *Acacia saligna* (another legume) it is proposed by Nsikani et al. (2018) that leaf litter be removed from previously invaded areas to assist with the return of soil properties pre-invasion. This suggestion is based on the changes in soil nitrate (NO<sub>3</sub><sup>-</sup>), pH, and available P and the promotion of the altered soil conditions to secondary plant invasions.

However, N and P are not the only nutrients utilised by invasive trees and foliage, seeds, and flowers are not the only points of nutrient accumulation. Removing litter after removal of standing biomass does not account for the wider range of resources allocated to the growth and development of invasive alien trees that are not enriched in post-clearing landscapes. In Fynbos sites invaded by *Eucalyptus* and *Acacia* species, it is important to determine inherent nutrient budgets and the sources of nutrient deposition. The approach to managing nutrient stocks in these instances thus needs to consider both enrichment and export, and develop methodologies to discriminately remove certain elements while retaining others (for instance, focusing on separating biomass components and removing photosynthetically active material; or making use of differential volatilization temperatures of elements). For effective recovery of affected and historically low-nutrient ecosystems such as the Fynbos, it is unlikely that a blanket approach of removal of standing biomass and litter of invasive trees will result in the intended outcome. A more nuanced approach to nutrient management could significantly improve ecosystem recovery, especially as it has been shown that the seedbanks of sites once invaded by aliens are generally diverse enough to drive recovery of the affected site, without much need for re-planting (Vosse et al., 2008). This supports a possibility of spontaneous succession in such areas, but which is often case-specific and could ultimately

also be directly related to the method of clearing (Blanchard & Holmes, 2008) and the treatment of the biomass.

Alien biomass removal is an important activity in South Africa due to the threat that invasions pose to local biodiversity and the high consumption of water resources. Alien clearing is also an important driver of rural economies, as jobs are continuously created through clearing and through secondary activities such as value adding to biomass products including firewood, wood chips, biochar, wood pellets, and wood bricks. These incentives for alien clearing result in large-scale removal of biomass and, inevitably, export of large amounts of nutrients that, until now, has not been accounted for in any invaded site in South Africa. However, quantification of nutrient export through invasive biomass removal could be critical in informing rehabilitation of affected sites post-clearing. The economic potential in the trade of invasive biomass is deemed as a viable long-term solution to the issue of sustainable alien tree removal (Vundla et al., 2017). Alien clearing, however, also necessitates additional activities such as follow-up clearing and revegetation of the cleared area to ensure minimal further damage to ecosystem integrity and to the prospects of recovery of ecosystem function. Failure to rehabilitate cleared riparian zones properly could lead to severely reduced hydrological functioning of a catchment and prevent the re-establishment of native vegetation. Up until now, research on riparian zone invasion has led to concerted efforts in alien clearing, follow-up clearing, and, in many cases, active re-planting of native vegetation, whereas others have been left for spontaneous succession (Reinecke et al., 2008). However, as noted by Holmes et al. (2008), it is critical for the rehabilitation success of previously disturbed ecosystems that the remaining stressors on that ecosystem are identified, quantified, and addressed. Nutrient changes in soil as a result of invasion by *Acacia* species in South Africa has been widely documented on, especially from a P and N perspective (Witkowski, 1991; Naude, 2012; Nsikani et al., 2018). However, no studies have explicitly accounted for export of these and other nutrients not enriched in soils by IAPs. This makes studies such as the current work important in driving consideration of this aspect of nutrient changes due to invasion. The potential threat of nutrient export in invasive plant biomass to riparian zone recovery post-clearing is one such stressor that, having been identified, should be quantified and, if necessary, be addressed.

The current study is the first account of standing nutrient stocks and biomass of *A. mearnsii* and *E. camaldulensis* in invasive stands. This data could greatly enhance our understanding of the impact of plant invasion on local ecosystems but needs to be further supported by soil data. This study is also the first documentation of the carbon storage potential of invasive alien stands in South Africa. While it is not intended as an argument for keeping stands of IAPs, this

data could be important in informing the potential loss of carbon sequestering potential as a result of alien clearing, and in informing management of biomass post-clearing.

## 5.6 Conclusions

Concentrations of P and total cations were all greater in *E. camaldulensis* biomass than in *A. mearnsii* biomass and are thus the opposite of what was expected for this study. With appreciation for the significantly greater biomass growth of *E. camaldulensis*, and its inability to facilitate nitrogen fixation, its impact on soil nutrient depletion could be much more severe than that of *A. mearnsii*. Nutrient export from ecosystems in invasive biomass and possible reduction in restoration potential of a cleared site suggests the need for an in-depth study on the effect of nutrient exports on riparian zone recovery. Large volumes of nutrients are exported in alien biomass, are generally unaccounted for, and are lost to local nutrient cycles. While it has been shown that nutrient enrichment could promote the secondary invasion, the effects of export of other nutrients from Fynbos ecosystems are not yet understood. Retention of nutrients in ecosystems may be greatly improved by allowing the decomposition of biomass products on the unprotected soil in the form of wood chips or untreated biomass. This could improve recovery of the ecosystem by enhancing soil functioning and providing favourable conditions for new growth but needs to be tested first. Before this happens, however, it is suggested that the direct impact of alien tree removal on soil nutrients is determined and if, in fact, nutrient export could significantly impact recovery of natural ecological processes in affected ecosystems.

## 5.7 References

- Attiwill, P. M., & Adams, M. A. (1993). Nutrient cycling in forests. *New phytologist*, 124(4), 561-582.
- Blanchard, R., & Holmes, P. M. (2008). Riparian vegetation recovery after invasive alien tree clearance in the Fynbos Biome. *South African Journal of Botany*, 74(3), 421-431.
- Chamier, J., Schachtschneider, K., Le Maître, D. C., Ashton, P. J., & 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.
- Cilliers, C. D., Esler, K. J., & Boucher, C. (2004). Effects of alien plant management and fire on soil seed banks and regeneration in the Cape Peninsula National Park, South Africa. *South African Journal of Botany*, 70(5), 705-712.
- Coetsee, C., Bond, W. J., & Wigley, B. J. (2015). Forest and fynbos are alternative states on the same nutrient poor geological substrate. *South African Journal of Botany*, 101, 57-65.

- Doerr, S. H., Shakesby, R. A., & MacDonald, L. H. (2009). Soil water repellency: a key factor in post-fire erosion. In *Fire effects on soils and restoration strategies* (pp. 213-240). CRC Press.
- Dovey, S. B. (2014). Current carbon stock estimation capability for South African commercial forest plantations.
- Du Toit, B., Dovey, S. B., & Smith, C. W. (2008). Effects of slash and site management treatments on soil properties, nutrition and growth of a *Eucalyptus grandis* plantation in South Africa. *Site Management and Productivity in Tropical Plantation Forests*, 63.
- Du Toit, B., Dovey, S. B., Fuller, G. M., & Job, R. A. (2004). Effects of harvesting and site management on nutrient pools and stand growth in a South African eucalypt plantation. *Site Management and Productivity in Tropical Plantation Forests*, 31.
- Du Toit, B., Dovey, S., Seifert, T., Muyambo, P., & Malherbe, D. (2016). *Carbon sequestration in South African plantation forests*. Progress report number 1: Techniques to estimate net carbon sequestration carbon at the Tier 2 level using available data from local studies and international literature.
- Esler, K. J., Jacobsen, A. L., & Pratt, R. B. (2018). The biology of Mediterranean-type ecosystems. Oxford University Press.
- Euston-Brown, D. (2000). The influence of vegetation type and fire severity on catchment stability after fire: a case study from the Cape Peninsula. *South Africa'(Final report, Fynbos Working for Water Programme, Cape Town, South Africa.*
- Fourie, M. (2014). Investigating soil nitrogen dynamics in natural, invaded and cleared fynbos riparian ecotones and implications for riparian functioning (*MSc thesis*, Stellenbosch: Stellenbosch University).
- Ge, X., Tian, Y., Tang, L., 2015. Nutrient distribution indicated whole-tree harvesting as a possible factor restricting the sustainable productivity of a poplar plantation system in China. *PLoS One* 10, 1–14. doi:10.1371/journal.pone.0125303
- Hall, J.P., 2002. Sustainable production of forest biomass for energy. *For. Chron.* 78, 391–396. doi:10.5558/tfc78391-3
- Holmes, P. M., Esler, K. J., Richardson, D. M., & Witkowski, E. T. F. (2008). Guidelines for improved management of riparian zones invaded by alien plants in South Africa. *South African Journal of Botany*, 74(3), 538-552.
- Jacobs, S., Cogill, L., Jacobs, K., Juba, R., Maubane, T., Slabbert, E., & Smart, R. (2017). Assessing the impact of selected methods of removal of invasive alien *Acacia mearnsii* and *Eucalyptus camaldulensis* on fynbos riparian ecosystem function. *Report to the Water Research Commission*. WRC Report No. 2343/1/16
- Kering, M.K., Butler, T.J., Biermacher, J.T., Guretzky, J.A., 2012. Biomass Yield and Nutrient Removal Rates of Perennial Grasses under Nitrogen Fertilization. *Bioenergy Res.* 5, 61–70. doi:10.1007/s12155-011-9167-x
- Lambert, M. J. (1976). Preparation of plant material for estimating a wide range of elements. Forestry Commission of New South Wales. Sydney
- Le Maître, D. C., Versfeld, D. B., & Chapman, R. A. (2000). Impact of invading alien plants on surface water resources in South Africa: A preliminary assessment. *WaterSA*, 26 (3)
- Masters, M.D., Black, C.K., Kantola, I.B., Woli, K.P., Voigt, T., David, M.B., DeLucia, E.H., 2016. Soil nutrient removal by four potential bioenergy crops: *Zea mays*, *Panicum virgatum*, *Miscanthus x giganteus*, and prairie. *Agric. Ecosyst. Environ.* 216, 51–60. doi:10.1016/j.agee.2015.09.016
- Mudavanhu, S., Blignaut, J., Nkambule, N., Morokong, T., & Vundla, T. (2016). A cost-benefit analysis of using Rooikrans as biomass feedstock for electricity generation: A case study of the De Hoop nature reserve, South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 788-813.



- Muyambo, P. (2017). Modelling aboveground biomass and nutrient export in South African *Pinus elliottii* (Doctoral dissertation).
- Naudé, M. (2012). Fynbos riparian biogeochemistry and invasive Australian *Acacias* (MSc thesis, Stellenbosch: Stellenbosch University).
- Neary, D. G., Klopatek, C. C., DeBano, L. F., & Ffolliott, P. F. (1999). Fire effects on belowground sustainability: a review and synthesis. *Forest ecology and management*, 122(1-2), 51-71.
- Nsikani, M. M., van Wilgen, B. W., & Gaertner, M. (2018). Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N<sub>2</sub>-fixing woody species: implications for ecological restoration. *Restoration ecology*, 26(2), 235-244.
- Railoun, M. Z. (2018). Impacts of the invasive tree *Acacia mearnsii* on riparian and instream aquatic environments in the Cape Floristic Region, South Africa (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Reinecke, M. K., Pigot, A. L., & King, J. M. (2008). Spontaneous succession of riparian fynbos: is unassisted recovery a viable restoration strategy?. *South African Journal of Botany*, 74(3), 412-420.
- Sankaran, K. V., Mendham, D. S., Chacko, K. C., Pandalai, R. C., Pillai, P. K. C., Grove, T. S., & O'Connell, A. M. (2008). Impact of site management practices on growth of eucalypt plantations in the monsoonal tropics in Kerala, India. *Site Management and Productivity in Tropical Plantation Forests*, 23.
- Scott-Shaw, B. C., Everson, C. S., & Clulow, A. D. (2017). Water-use dynamics of an alien-invaded riparian forest within the Mediterranean climate zone of the Western Cape, South Africa.
- Slabbert, E., Jacobs, S. M., & Jacobs, K. (2014). The soil bacterial communities of South African Fynbos riparian ecosystems invaded by Australian *Acacia* species. *PloS one*, 9(1).
- Snook, R. D. (1992). Handbook of inductively coupled plasma mass spectrometry. *Chromatographia*, 34(9), 546-546.
- Stafford, W. H., Von Maltitz, G. P., & Watson, H. K. (2018). Reducing the costs of landscape restoration by using invasive alien plant biomass for bioenergy. *Wiley Interdisciplinary Reviews: Energy and Environment*, 7(1), e272.
- Stafford, W., & Blignaut, J. (2017). Reducing landscape restoration costs: Feasibility of generating electricity from invasive alien plant biomass on the Agulhas Plain, South Africa. *Ecosystem Services*.
- Stafford, W., Blignaut, J., 2017. Reducing landscape restoration costs: Feasibility of generating electricity from invasive alien plant biomass on the Agulhas Plain, South Africa. *Ecosystem Serv.* 27, 224–231. doi:10.1016/j.ecoser.2017.04.008
- Tew, D. T., Morris, L. A., Allen, H. L., & Wells, C. G. (1986). Estimates of nutrient removal, displacement and loss resulting from harvest and site preparation of a *Pinus taeda* plantation in the Piedmont of North Carolina. *Forest Ecology and Management*, 15(4), 257-267.
- Tye, D. R., & 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.
- Vosse, S., Esler, K. J., Richardson, D. M., & Holmes, P. M. (2008). Can riparian seed banks initiate restoration after alien plant invasion? Evidence from the Western Cape, South Africa. *South African Journal of Botany*, 74(3), 432-444.
- Vundla, T., Blignaut, J., Nkambule, N., Morokong, T., & Mudavanhu, S. (2016). The opportunity cost of not utilising the woody invasive alien plant species in the Kouga, Krom and Baviaans catchments in South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 814-830.
- 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*, 1-15.

Witkowski, E. T. F. (1991). Growth and competition between seedlings of *Protea repens* (L.) L. and the alien invasive, *Acacia saligna* (Labill.) Wendl. in relation to nutrient availability. *Functional Ecology*, 101-110.

## CHAPTER 6: Value-added products from invasive biomass; allelopathic potential of various biomass components on growth of wheat and canola

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### 6.1 Abstract

One option for encouraging the use of invasive biomass as a mechanism to reduce the net costs of clearing of invasive alien trees is to repurpose biomass for agricultural use as soil amendment, most commonly through wood chips or biochar. However, this poses a risk because of the allelopathic potential of mulch from invasive species, especially *Eucalyptus* species. This study considers the potential for the use of two major invaders in the Western Cape riparian zones, *Eucalyptus camaldulensis* (Red gum) and *Acacia mearnsii* (Black wattle) in the cultivation and growth of two locally important agricultural crops, wheat (*Triticum aestivum*), and canola (*Brassica napus*), as these are relevant to agriculture in the Western Cape. The allelopathic potential of each of the aboveground biomass components (stem, branches, leaves, and bark) was tested in an aqueous solution of various dose concentrations. Germination rate and root and shoot growth were recorded during a two-week experiment where no additional fertilizers were added. Seeds were grown in petri dishes containing sheets of filter paper soaked in 4 ml of the solution. At the highest dose, 0.30 g ml<sup>-1</sup>, for most biomass components of both invasive species, germination and root and shoot growth are diminished significantly for both crop species. Low dosages of leachate, however, often show a positive response in shoot growth. Material from stems and bark of *E. camaldulensis* had no significant effect on germination of wheat, while leaf and branch material at 0.30 g ml<sup>-1</sup> resulted in less than 30% germination. Canola seeds are more sensitive to leachate applications, showing an 80% reduction in germination when treated with the 0.075 g ml<sup>-1</sup> (low dosage) leaf and bark leachates of *E. camaldulensis*. Canola seeds were, however, not sensitive to stem material, reaching 100% germination at 0.30 g ml<sup>-1</sup> and also the greatest shoot growth (>80 cm). The potential of wood chips of both the invasive species to affect growth of wheat and canola seeds was also tested in a pot experiment using fresh biomass material for chip production. Chips were stored in piles of 1 m<sup>3</sup> and sampled fresh (0 months), and again after 6 and 12 months of storage. Chips were added to a growth medium consisting of a 50/50 mixture of river sand and commercial potting soil (500 g to 2.5 kg soil). The crops were planted in 30 cm<sup>3</sup> pots and treated with *E. camaldulensis* and *A. mearnsii* chips. Both crop species responded significantly better to the presence of chips of any age than to the control. In most cases, growth was best in the fresh chip treatment. Findings from this study suggest that fresh stemwood is preferable when applying wood chips as mulch but advise against the mixture of the biomass material, especially with leaves and bark, due to the greater allelopathic potential of these components.

## 6.2 Introduction

The Western Cape of South Africa is renowned for its high levels of plant biodiversity, specifically in the Cape Floristic Region (CFR). This area is also subject to intense invasion by various tree species which costs millions of Rands annually to manage (de Wit et al., 2001). Seasonal rainfall in the region makes invaded riparian zones strategic management areas where aliens are cleared to reduce water losses through evapotranspiration but also as a measure to maintain local biodiversity and ecosystems service benefits. A possible avenue for recouping a portion of the funds used for management of invasive alien trees is the production of Value-Added Products (VAPs) from the cleared biomass (Stafford & Blignaut 2017; Stafford et al., 2018). One such VAP is wood chips, which could be produced from the aboveground biomass to assist on agricultural land with mulches for improving soil structure, lowering temperatures, and enhancing water relations, nutrient cycles, and soil health (Chalker-Scott, 2007; Mulumba & Lal, 2008). This could lower inputs from irrigation and promote sustainable farming practices; chipping of invasive biomass for mulching is therefore actively pursued in agriculture. Because of its benefits to soil productivity and function, mulching in most forms (chips, straw, and sawdust) has been shown to significantly improve crop growth (Foshee et al., 1996; Smith et al., 2000; Kumar & Lal, 2012). However, this may be case-specific, as it has also been shown to reduce growth of some crops (Johnson et al., 2004) and is widely used in suppression of weeds due to the allelopathic potential of mulches of some species (Rathinasabapathi et al., 2005; Murungu et al., 2011; Wang et al., 2012).

Allelopathy refers to the direct or indirect suppression of a neighbouring plant (receiver) by broad-spectrum chemicals released by a donor plant to increase its competitive ability (Rice, 1979). This suggests that the effects may also be detrimental to non-target plants and has led to concerns over the use of mulch in the presence of crops. Seigler (2006) reported on a wide range of groups of isolated compounds based on their molecular makeup and thus their mode of activity. These compound groups include acetylenic compounds, waxes, polyketides, terpenoids, phenolic compounds, and even tannins (Gross, 1999). Allelopathic compounds isolated from *E. camaldulensis* include *p*-cymene,  $\alpha$ -phellandrene, cuminal, phellandral, cineole,  $\alpha$ -pinene,  $\beta$ -pinene, and geraniol (Del Moral & Muller, 1970). These substances have been found to generally be concentrated in the foliage but may be available in limited concentrations in the bulk of the tree.

The wide range of allelopathic compounds and their variable effect on receiver plants (and animals) is an important consideration in the study of allelopathy and its role in competition, but testing is not always feasible due to lack of access to appropriate facilities. An indirect approach which considers allelopathy of a leachate produced from the biomass

has been used widely, with varying results. Achmed et al. (2008) tested the inhibition of leaf litter extract of *E. camaldulensis* on root and shoot growth of five crop species and found significant decreases in shoot length, while root length showed variable results. In other studies, leachates from both fresh and dried eucalypt leaves (*E. globulus*) have been shown to inhibit the growth of some plants, while promoting the growth of others (Babu & Kandasamy, 1997; El-Khawas & Shehata, 2005). Del Moral & Muller, (1970), on the other hand, showed that some biomass components of *Eucalyptus* spp. do not affect receiver plants negatively at all. In *Acacia mearnsii* biomass, allelopathic properties have also been detected when tested on crop species and some grasses (Swaminatham et al., 1989; Gross 1999, Fatunbi et al., 2009; Mehmood et al., 2011). A seedling emergence study by Fatunbi et al. (2010) suggested that the leaves of *A. mearnsii* have high allelopathic potential; higher than bark, branches, and stems. Differential allelopathic potential in different biomass components of *Acacia* species has also been reported by Kamel & Hammad (2015), who found greater inhibition in growth of wheat and canola seedlings from leaf extract of *A. saligna* than stem leachates. *Acacia* bark material has been suggested to significantly inhibit crop growth in high enough doses due to high tannin contents (up to 30%; Duke, 1983). All these studies confirm some level of allelopathy in both *E. camaldulensis* and *A. mearnsii* but mainly in leaves and bark, sometimes in branches, and never in stem material. Common practice to promote leaching of allelopathic compounds from wood is to store chips in piles for at least one year to allow leaching and/or degradation of allelopathic substances in wood. No research has yet been published on this practice to suggest that it is, in fact, necessary to store wood chips before use. Apart from storage of chips to allow for the elimination of its previous allelopathic potential, allelopathy may also be context-specific and requires testing before biomass is used in any way.

In this chapter I will discuss the susceptibility of two receiver crop species, *Triticum aestivum* (wheat) and *Brassica napus* (canola), to different dosages of leachate from various biomass components of *Eucalyptus camaldulensis* and *Acacia mearnsii*. No explicit determination of active allelopathic compounds was done; rather, allelopathic potential of extracts from biomass components from known antagonistic and strong invasive species were applied in low to high doses. These tests aim to address the current perceptions of allelopathic effects on crops by invasive species.

The hypotheses tested here are thus:

- Allelopathic potential of *A. mearnsii* and *E. camaldulensis* is limited to the photosynthetically active biomass components

- The allelopathic effect of leachate from biomass components on crops is dose-dependent
- Where allelopathic potential is discovered, the effective dose of the same treatment differs between receiver species
- Any negative influence of wood chips on crop growth is eliminated through long-term storage

### 6.3 Materials and methods

Trees of *A. mearnsii* and *E. camaldulensis* were cut and chipped at the Alfalfa Dairy farm between Worcester and Robertson in the Western Cape (Coordinates: 33°46'4.16"S; 19°31'59.93"). The site was chosen because it had large stands of both species and was easily accessible. To test the possible negative effects of the two invasive species on germination, fresh trees were cut, and the biomass material separated per the various aboveground components: leaves, stem, bark, and branches. Experiments were done using a method adapted from Zhang et al. (2010), where the allelopathic potential of various aliquot doses of leachate is tested on crop germination and growth. Leachate was created from the various fresh aboveground biomass components by breaking it down into small pieces, selecting a 30 g subsample, and soaking it in 100 ml distilled water for three (3) days at room temperature. The supernatant solution is then transferred from the storage containers using a Whatmann No.7 filter paper, to remove plant material. This is the 0.30 g ml<sup>-1</sup> solution. This solution was then diluted to two more doses of 0.15 g ml<sup>-1</sup>, and 0.075 g ml<sup>-1</sup> each with the addition of distilled water. These solutions were tested against a control treatment of pure distilled water, also filtered through the Whatmann No. 7 Filter paper. According to Zhang et al (2010), a concentration of 2% leachate (0.2 gml<sup>-1</sup>) is similar to a natural environment where *Eucalyptus* leaf litter decomposes naturally (annual litter production per square meter). No analyses were done on leachate contents, and concentrations are purely based on an aqueous extract of the biomass material. Petri dishes were prepared containing sheets of filter paper soaked in 4 ml of the solution. Seeds of canola and wheat were placed on top of the filter paper and allowed to grow for two weeks (Zhang et al., 2010). Measurements were then made on the root and shoot lengths of the crops. Petri dishes containing 5 seeds each were used as experimental unit.

A complementary mulching experiment was also conducted to determine the possible effects of wood chips from *A. mearnsii* and *E. camaldulensis* on the canola and wheat crops. This experiment was conducted in a tunnel under 150 µm polyethylene. Airflow in the tunnel



was enhanced with built-in fans and pots were placed at least 30 cm apart in a 30 m<sup>2</sup> space. Pots were placed randomly and rotated every 2 weeks to account for any potential differences in sunlight availability and irrigation bias. Wheat and canola plants were planted out from seed and were thus germinated as part of the trial. Wood chips were sourced from freshly cut trees of both invasive species at the Alfalfa site near Robertson, as both species of interest occur in the region. These were the chips sampled at time 0. The remainder of the chips were then piled and allowed to decompose; chips from the same piles were then collected after 6 and 12 months respectively (Fig 6.1). Piles for both species amounted to 3 piles each, from which 5 samples were taken, amounting to 15 samples per species, which were used in pot experiments. This experiment tested the effect of the chips on the germination and growth of wheat and canola seeds, compared to a control of no chips.



Figure 6.1: Wood chips production from *Eucalyptus camaldulensis* (A) and *Acacia mearnsii* (B) and the resultant chip piles of the two invasive species stored for 12 months (C, *E. camaldulensis* and D, *A. mearnsii*)

The soil used for growth was composed of a 50-50 mixture of river soil and commercially available potting mix (DoubleGrow® all-purpose organic potting soil); no additional fertilizer was added. The potting mix was used to improve repeatability of trials, and to minimise sources of variation in aspects of soil structure and organic matter content. Ten 30 cm<sup>3</sup> pots were used per treatment where 25 seeds each were planted and treated with *E.*



*camaldulensis* chips and ten treated with *A. mearnsii* chips, with another five pots with no chips planted as a control treatment. After three months, 10 specimens per pot were selected to determine shoot length and overall dry mass, for both wheat and canola plants, sourced from the Welgevallen Experimental Farm. Root length was not determined due to entanglement in most samples. Rather, the roots and shoots were weighed, and the dry mass ratio calculated. The wheat used in the experimentation was from South African cultivar 027 and canola from cultivar South African 555 TT. The pots were watered weekly with municipal water for the duration of the experiment.

## Statistical analysis

All analyses were done using Statistica 13 (Tibco). Normality was tested using a Shapiro-Wilks test and homoscedasticity was tested with Levene's Test. Tests showed that the data were normally distributed and a factorial ANOVA (Analysis of Variance) was done to detect differences in root and shoot length of crops. Where the effect of invasive species is included in testing allelopathic potential of biomass components and leachate dosages, a three-factor ANOVA was done. Where differences were detected, a Fisher LSD test was used to determine sources of variation.

## 6.4 Results

Plant components of both *A. mearnsii* and *E. camaldulensis* were tested separately for their individual effects on germination growth of wheat and canola seeds (Figures 6.2 and 6.3). Germination of canola seeds was dose-dependent for most biomass components and was most sensitive to leachate of leaf material (Fig 6.2 A). Germination in stem leachate was consistently above 90% at all dosage levels for both *A. mearnsii* and *E. camaldulensis*. In the distilled water treatment (0 g ml<sup>-1</sup>) germination was generally high, while germination of canola in leachate of *E. camaldulensis* and *A. mearnsii* leaves at 0.3 g ml<sup>-1</sup> was 0%. Wheat germination in high concentrations of stem leachate of both *E. camaldulensis* and *A. mearnsii* remained at or close to 100%, while leachate from leaf material of both invasive species supported 20% germination at a dosage of 0.3 g ml<sup>-1</sup> (Fig 6.2 B).

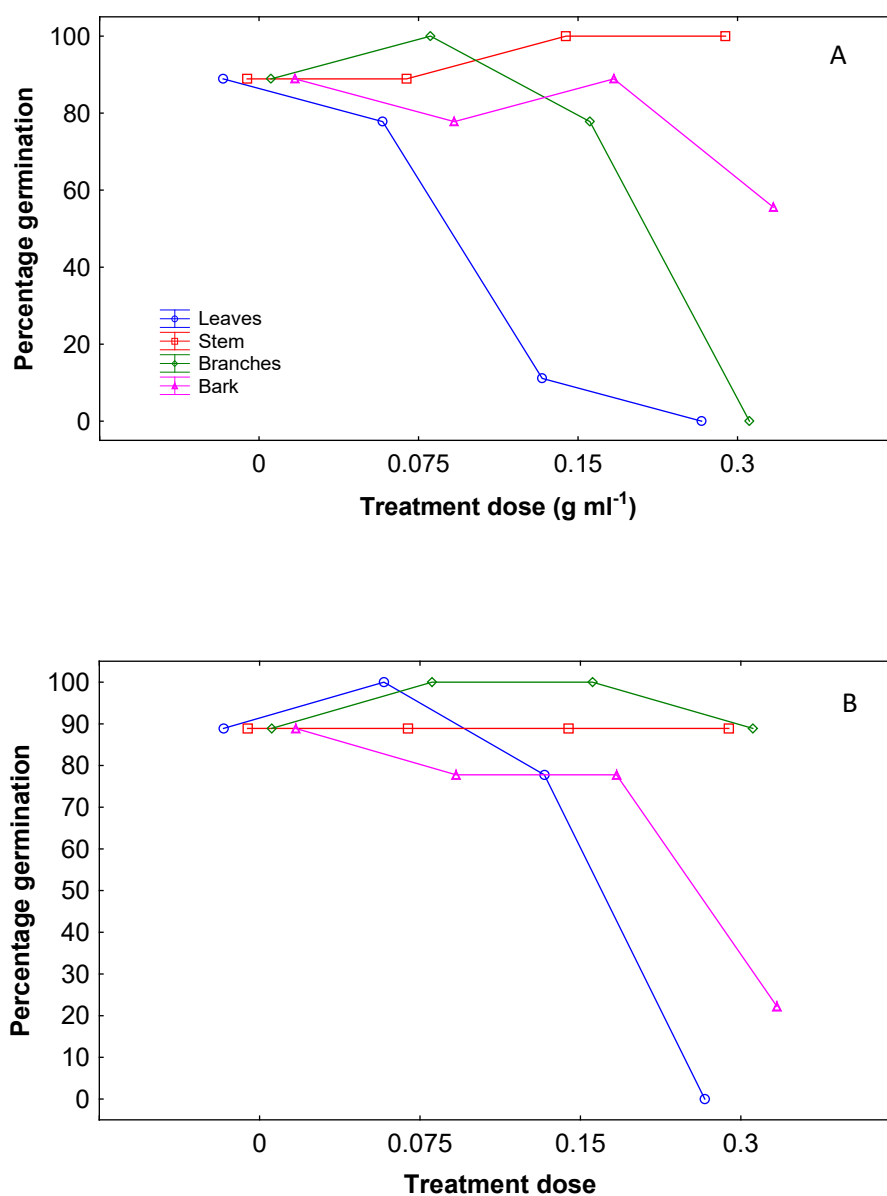


Figure 6.2: Total overall germination rates of canola seeds ex situ when treated with different aliquot doses of various biomass components of *Eucalyptus camaldulensis* (A) and *Acacia mearnsii* (B).

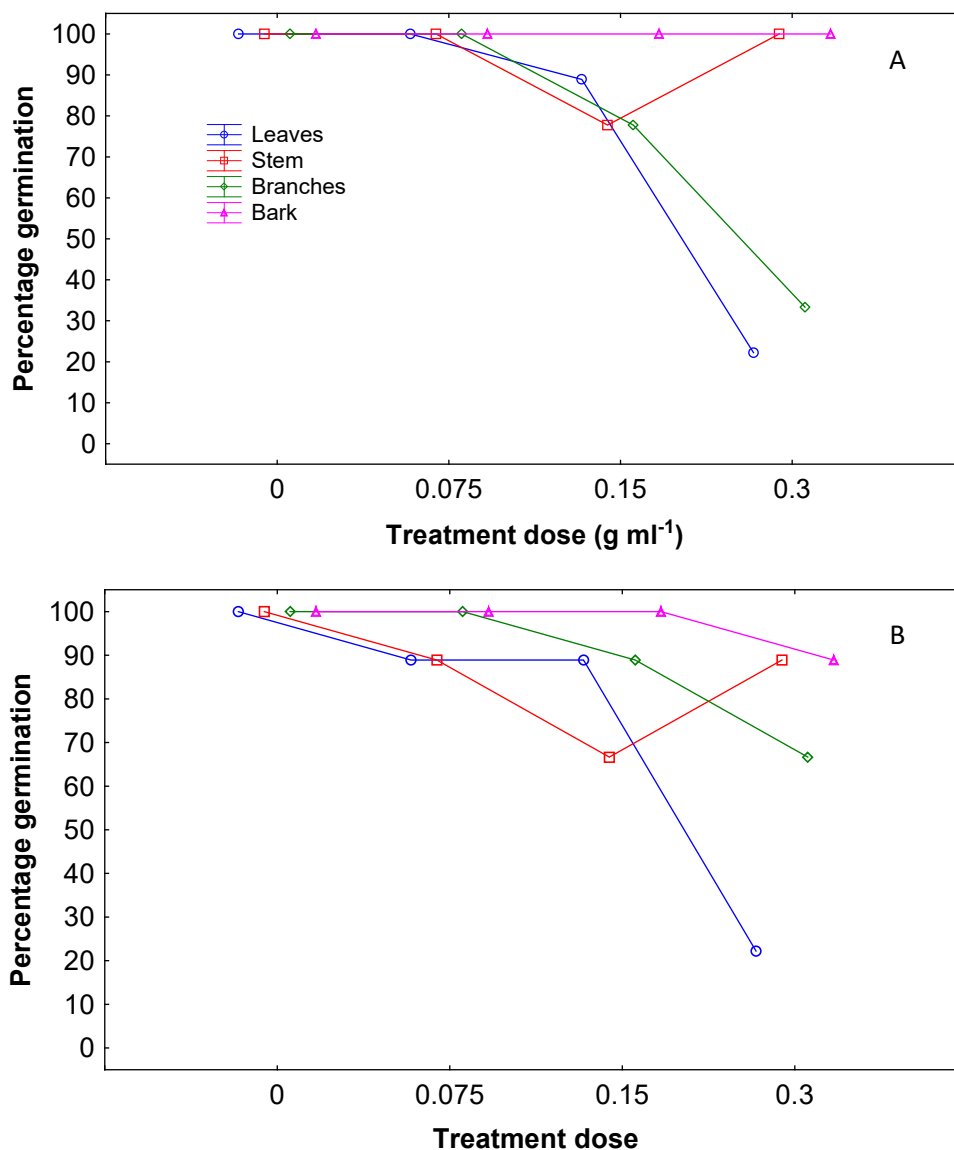


Figure 6.3: Germination rates of wheat seeds when treated with different aliquot concentrations of various biomass components of *Eucalyptus camaldulensis* (A) and *Acacia mearnsii* (B).

### Effect of different leachates concentrations on root and shoot development of wheat

For shoot length of wheat, there was no significant interaction between dose concentration, tree component, and tree species (ANOVA;  $F_{[6; 55]}=1.6$ ;  $P=0.151$ ) but there was significant interaction between tree component and concentration (ANOVA;  $F_{[6; 55]}=11.25$ ;  $P<0.001$ ; Figure 6.4 A, B). For root length of wheat, dose concentration, tree component, and tree species showed significant interaction (ANOVA;  $F_{[6; 55]}=4.74$ ;  $P<0.001$ ; Figure 6.4 C, D). There was an initial increase in root and shoot growth of wheat, from 106 mm to 137 mm for shoots

and 153 mm to 169 mm for roots at a 0.075 g ml<sup>-1</sup> dose of *A. mearnsii* leaf material (Figure 6.4). A significant reduction in both is then seen towards a 0.3 g ml<sup>-1</sup> dose. Shoot length is reduced to 44 mm, which is significantly lower than the control and all the other dose applications (LSD;  $P \leq 0.05$ ). This trend is similar for the root length of wheat when treated with *A. mearnsii* leaf leachates; a low and significantly different root length at 0.3 g ml<sup>-1</sup> dose application. Material from both stems and branches had no significant effect on root and shoot growth of wheat; none of the dose applications significantly differed from the control or from one another (LSD;  $P > 0.05$ ). Shoot length of wheat showed a consistent reduction with an increase in dose of *A. mearnsii* bark material. At the control, shoot length was at 107 mm and then reduced to 78 mm at 0.075 g ml<sup>-1</sup> dose. Both 0.075 g ml<sup>-1</sup> and 0.15 g ml<sup>-1</sup> dose had significantly longer shoots than at 0.3 g ml<sup>-1</sup> dose (LSD;  $P \leq 0.05$ ), which only produced shoots 23 mm long. Treatment with leaf leachate from *E. camaldulensis* (Fig. 6.4 B) did not have a significant stunting effect on wheat root growth at 0.075 g ml<sup>-1</sup> dose treatment ( $116.11 \pm 21.7$  mm), but was slightly higher than root length at the control treatment ( $106.89 \pm 28.42$  mm). This treatment and the 0.15 g ml<sup>-1</sup> dose yielded significantly longer roots than the 0.3 g ml<sup>-1</sup> dose treatment ( $8 \pm 13.17$  mm; LSD;  $P \leq 0.05$ ), which had the shortest roots. Shoot length did not differ significantly between 0 g ml<sup>-1</sup>, 0.075 g ml<sup>-1</sup> and 0.15 g ml<sup>-1</sup> dose treatments (LSD;  $P > 0.05$ ). When treated with a 0.3 g ml<sup>-1</sup> dose there was a significant decrease in shoot length to  $8 \pm 11.73$  mm, compared to  $106.89 \pm 21.7$  mm at the control. The 0.3 g ml<sup>-1</sup> dose treatment was significantly different to all the other treatments (LSD;  $P \leq 0.05$ ).

For both the root and shoot components of wheat seedlings, differences in dose of *A. mearnsii* leaf leachate resulted in a significant differences in length. Also, in both instances, an initial increase in length is observed at the 0.075 g ml<sup>-1</sup> level. At 0.15 g ml<sup>-1</sup>, shoot length of wheat was not significantly different from that of the control ( $P = 0.734$ , but was significantly shorter than at 0.075 g ml<sup>-1</sup> ( $P = 0.023$ ). In both instances, the 0.3 g ml<sup>-1</sup> concentration resulted in the lowest measurements. There were no significant differences in root or shoot lengths of wheat seedlings when grown in any of the treatments ( $P > 0.05$ ) (Figure 6.4 B). Roots of wheat samples grown in 0.15 g ml<sup>-1</sup> dose of the branch treatments were significantly shorter ( $88.43 \pm 52.73$  mm) than those grown in the control ( $153.44 \pm 57.64$  mm) while roots from all treatments were significantly longer than those grown at 0.3 g ml<sup>-1</sup> dose treatments ( $25 \pm 19.06$  mm) (LSD;  $P \leq 0.05$ ). Shoot length was only significantly affected by the 0.3 g ml<sup>-1</sup> treatment, which had significantly shorter shoots than the other treatments ( $75.67 \pm 16.67$  mm vs. a maximum of  $127 \pm 10.92$  mm at the 0.15 g/ml dose). Roots of wheat were the longest in the *A. mearnsii* stem 0.15 g ml<sup>-1</sup> treatment ( $197.83 \pm 62.54$  mm), which was significantly longer than any of the other non-stem treatments (LSD;  $P \leq 0.05$ ) (Fig 6.4 D). The shortest roots were recorded at 0.3 g ml<sup>-1</sup> dose treatment ( $27.78 \pm 9.28$  mm) and this was significantly different

from any of the other treatments (LSD;  $P \leq 0.05$ ). The longest shoots were recorded at the  $0.075 \text{ g ml}^{-1}$  treatment ( $141.22 \pm 18.81 \text{ mm}$ ) and were significantly different from all other treatments (LSD;  $P \leq 0.05$ ). At  $0.3 \text{ g ml}^{-1}$  treatment shoot length was the shortest ( $28.67 \pm 7.92 \text{ mm}$ ) and this was also significantly different from all the other treatments (LSD;  $P \leq 0.05$ ).

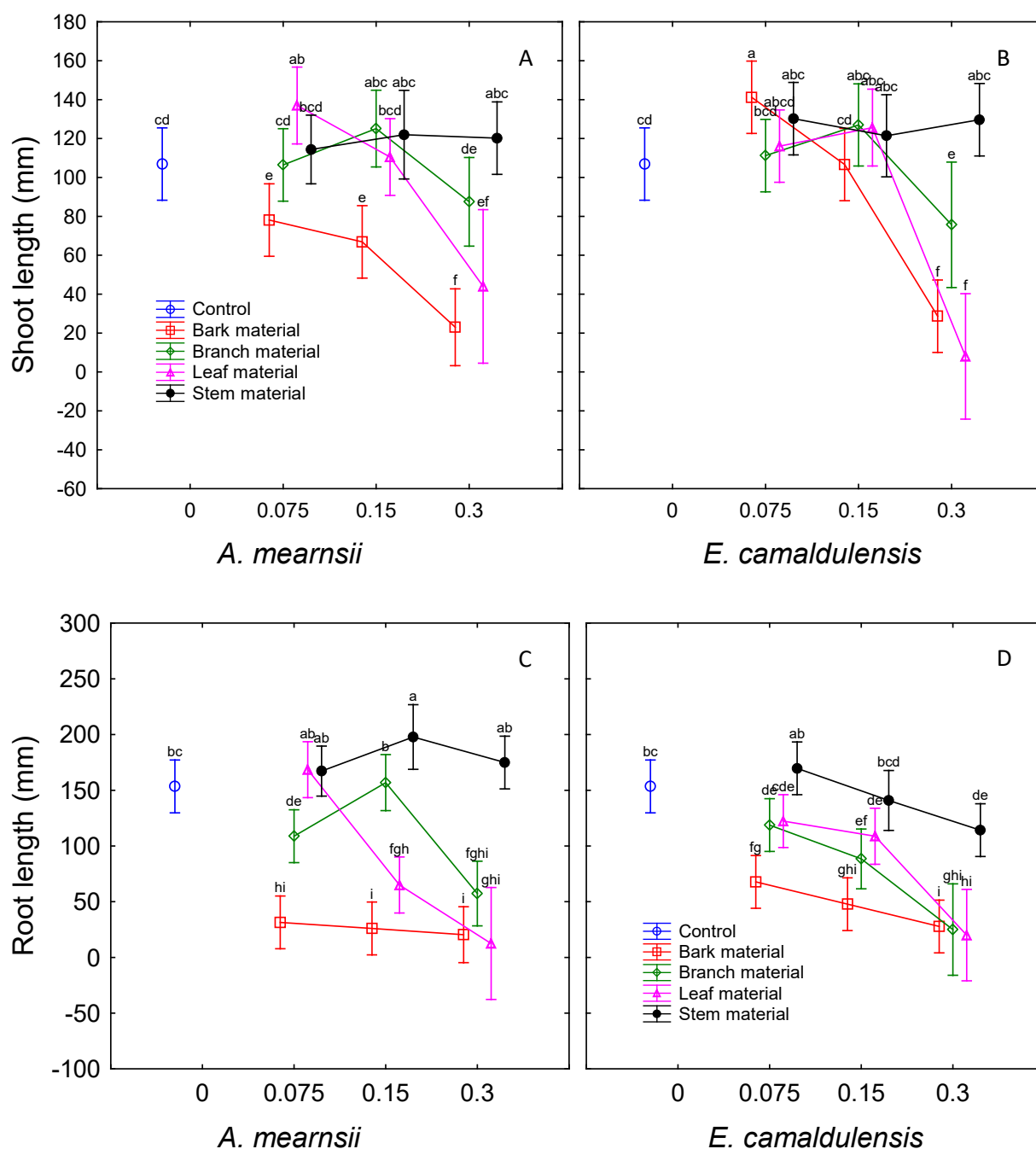


Figure 6.4: Shoot and root lengths of wheat grown with *A. mearnsii* leachates (A, C) and *E. camaldulensis* (B, D), using leachate solutions of various biomass components (leaves, stem, branches, and bark). Leachate dose concentrations were  $0 \text{ g ml}^{-1}$ ,  $0.3 \text{ g ml}^{-1}$ ,  $0.15 \text{ g ml}^{-1}$  and  $0.075 \text{ g ml}^{-1}$  and compared with a control of distilled water. Letters denote significant differences (LSD;  $P < 0.05$ ); error bars denote 0.95 confidence intervals. Lack of germination is denoted with a dash (-).

For root/shoot ratios of wheat seedlings, there was significant interaction between tree component and dose concentration (ANOVA;  $F_{[6; 55]}=2.4$ ;  $P=0.029$ ; Table 6.1). Table 6.1 shows that there were no significant differences between  $R_{R/S}$  for the different doses of *A. mearnsii* leaf leachate (LSD;  $P \geq 0.05$ ), although it declined as dose was increased. There was no germination at  $0.3 \text{ g ml}^{-1}$  dose of this treatment. Leachate from stem material resulted in a consistent decrease in  $R_{R/S}$  with dose increases. For bark material, there was a significant reduction in  $R_{R/S}$  with the  $0.075 \text{ g ml}^{-1}$  treatment, from  $1.224 \pm 0.95$  at the control, to  $0.115 \pm 0.06$  at  $0.075 \text{ g ml}^{-1}$ . The stem material of *E. camaldulensis* caused significant reduction in  $R_{R/S}$  at low doses. All the treatments promoted seedlings with significantly lower  $R_{R/S}$  than the control (LSD;  $P < 0.05$ ), while none of these treatments differed significantly from each other. When treated with leachate from branch material, there was also a significant reduction in  $R_{R/S}$  from the control to a  $0.075 \text{ g ml}^{-1}$  dose treatment (LSD;  $P=0.018$ ). There were no significant differences between the  $0.075 \text{ g ml}^{-1}$  dose and the  $0.15 \text{ g ml}^{-1}$  dose (LSD;  $P=0.716$ ) and there was no germination at  $0.3 \text{ g ml}^{-1}$  dose. For bark leachate, there was also no significant difference in  $R_{R/S}$  from the different treatments (LSD;  $P > 0.05$ ) and all the treatments had significantly lower ratios than the control (LSD;  $P < 0.05$ ).

Table 6.1: Root/shoot ratios (mean  $\pm$  standard error) of wheat seedlings germinated in leachate solutions of various biomass components (leaves, stem, branches, and bark) *A. mearnsii* and *E. camaldulensis*. Leachate dose concentrations were  $0.3 \text{ g ml}^{-1}$ ,  $0.15 \text{ g ml}^{-1}$  and  $0.075 \text{ g ml}^{-1}$  and compared with a control of distilled water. Letters denote where significant differences were found (LSD;  $P \leq 0.05$ ).

Invasive Species	Dose ( $\text{g ml}^{-1}$ )	Root/shoot ratio; Mean $\pm$ Std. Err.			
		Leaves	Stem	Branches	Bark
<i>A. mearnsii</i>	0	$1.224 \pm 0.95^{ab}$			
	0.075	$0.758 \pm 0.39^{3cd}$	$1.62 \pm 0.61^b$	$1.201 \pm 0.43^a$	$0.115 \pm 0.06^e$
	0.15	$0.655 \pm 0.41^d$	$1.111 \pm 0.32^a$	$1.114 \pm 0.45^{ac}$	$0.125 \pm 0.07^e$
	0.3	-	$0.565 \pm 0.19^d$	$0.783 \pm 0.7^{cd}$	-
<i>E. camaldulensis</i>	0	$1.224 \pm 0.95^{ab}$			
	0.075	$0.737 \pm 0.14^{cd}$	$0.680 \pm 0.29^f$	$0.569 \pm 0.26^d$	$0.231 \pm 0.08^e$
	0.15	$0.736 \pm 0.33^{acd}$	$0.288 \pm 0.15^e$	$0.475 \pm 0.33^d$	$0.300 \pm 0.024^e$
	0.3	-	$0.381 \pm 0.12^e$	-	$0.029 \pm 0.05^e$

## Effect of different leachates concentrations on canola root and shoot development

For shoot length of canola, there was significant statistical interaction between tree component, dose concentration, and tree species (ANOVA;  $F_{[4; 42]} = 7.88$ ;  $P < 0.0010$ ; Figure 6.5 A, C). Similarly, there was significant interaction between tree component, dose concentration, and tree species (ANOVA;  $F_{[4; 42]} = 3.92$ ;  $P = 0.005$ ; Figure 6.5 B, D).

Leaf material of *E. camaldulensis* (Fig 6.5 A) supported a growth increment for roots of canola seedlings when exposed to higher doses from  $33.5 \pm 18.89$  mm at a dosage of  $0 \text{ g ml}^{-1}$  to  $72 \pm 37.95$  mm at  $0.15 \text{ g ml}^{-1}$  dose. This trend is similar to the shoot growth of canola, which was also significantly longer at a  $0.075 \text{ g ml}^{-1}$  ( $58.57 \pm 26.32$  mm) dose than at  $0 \text{ g ml}^{-1}$  ( $32 \pm 10.81$  mm, LSD;  $P = 0.006$ ). Shoot growth at  $0.075 \text{ g/ml}$  dose was also significantly less than at  $0.15 \text{ g ml}^{-1}$  dose ( $97.5 \pm 7.91$  mm, LSD;  $P = 0.009$ ). When treated with leachate from stem material root growth is significantly enhanced by a  $0.075 \text{ g ml}^{-1}$  ( $50.38 \pm 24.46$  mm) dose when compared to the control ( $33.5 \pm 18.9$  mm, LSD;  $P = 0.04$ ). Leachate from branch material also only supported germination for doses  $0\text{-}0.15 \text{ g ml}^{-1}$  (Fig 6.5 C). Shoot growth of canola seedlings was significantly better at  $0.075 \text{ g ml}^{-1}$  ( $70.56 \pm 15.12$  mm) and  $0.15 \text{ g ml}^{-1}$  ( $63 \pm 22.93$  mm) doses than the control ( $32 \pm 10.81$  mm) (LSD;  $P \leq 0.05$ ). There was no significant difference in root length when treated with different doses between  $0 \text{ g ml}^{-1}$  and  $0.15 \text{ g ml}^{-1}$  doses. Using leachate from bark material resulted in a significant reduction in root length from the control ( $33.5 \pm 18.9$  mm) to the  $0.3 \text{ g ml}^{-1}$  dose ( $1.6 \pm 2.93$  mm) (LSD;  $P < 0.001$ ). Root length also differed significantly between dose applications of  $0 \text{ g ml}^{-1}$  and  $0.075 \text{ g ml}^{-1}$  ( $14.29 \pm 3.57$  mm) (LSD;  $P < 0.001$ ), which was significantly longer than at  $0.3 \text{ g ml}^{-1}$  dose (LSD;  $P = 0.03$ ). Shoot length significantly increased when treated with  $0.075 \text{ g ml}^{-1}$  dose ( $67.71 \pm 32.11$  mm) compared to the control ( $32 \pm 10.81$ ) (LSD;  $P = 0.004$ ).

There was no significant difference in shoot length between dose applications of  $0.15 \text{ g ml}^{-1}$  ( $32.5 \pm 22.17$  mm) and  $0.3 \text{ g ml}^{-1}$  ( $41.20 \pm 41.03$  mm) (LSD;  $P = 0.485$ ) but both treatments supported significantly less shoot growth than  $0.075 \text{ g ml}^{-1}$  dose (LSD;  $P \leq 0.05$ ). Treatment with leaf extract (Fig. 6.5 A) of *A. mearnsii* at  $0.075 \text{ g ml}^{-1}$  dose supported a significant increase in root growth for canola seedlings ( $58.78 \pm 22.22$  mm) when compared to the control ( $33.5 \pm 18.9$  mm) (LSD;  $P = 0.006$ ) (Figure 6.5A). Root growth at  $0.15 \text{ g ml}^{-1}$  ( $40.89 \pm 8.51$  mm) dose treatment was significantly less than at  $0.075 \text{ g ml}^{-1}$  dose (LSD;  $P = 0.015$ ). Shoot growth of canola also improved significantly when treated with a  $0.075 \text{ g/ml}$  dose ( $89.44 \pm 11.03$  mm) of *A. mearnsii* leaf leachate, being significantly longer than those in the control treatment ( $32 \pm 10.81$  mm) (LSD;  $P < 0.001$ ). The  $0.15 \text{ g ml}^{-1}$  dose treatment yielded samples with significantly longer shoots than the control (LSD;  $P = 0.002$ ). Treatment with stem material



(Fig. 6.5 B) produced significantly longer roots at 0.075 g ml<sup>-1</sup> (66.4 ± 27.57 mm) and 0.15 g ml<sup>-1</sup> (66.77 ± 22.53 mm) dose treatments than with the control (33.5 ± 18.9 mm) and the 0.3 g ml<sup>-1</sup> dose (37.33 ± 12.58 mm) (LSD; P≤0.05). There was no significant difference between root length of samples treated with the control and those treated with a 0.3 g ml<sup>-1</sup> dose of *A. mearnsii* stem leachate (LSD; P=0.665).

Leachate from branch material (Fig 6.5 C) also supported significant growth increase of roots when applied at 0.075 g ml<sup>-1</sup> (69.89 ± 36.64 mm) and 0.15 g ml<sup>-1</sup> (78.56 ± 29.71 mm) doses when compared to the control (33.5 ± 18.9 mm) and to samples grown at 0.3 g ml<sup>-1</sup> treatment dose (35 ± 26.63 mm) (LSD; P≤0.05). Shoot length of canola seedlings showed similar trends as roots. Maximum shoot length was recorded at the 0.15 g ml<sup>-1</sup> treatment (71.11 ± 9.43 mm) and was significantly longer than those at all the other treatments (LSD; P≤0.05). There was no significant difference in shoot lengths between treatments of 0.075 g ml<sup>-1</sup> (57.33 ± 21.68 mm) and 0.3 g ml<sup>-1</sup> (46.13 ± 11.28 mm) doses (LSD; P>0.05) but both these treatments resulted in significantly longer shoots than the control (LSD; P≤0.05). Figure 6.5 D shows that there was no significant difference in root or shoot lengths of canola seedlings when treated with different doses of *A. mearnsii* bark material.

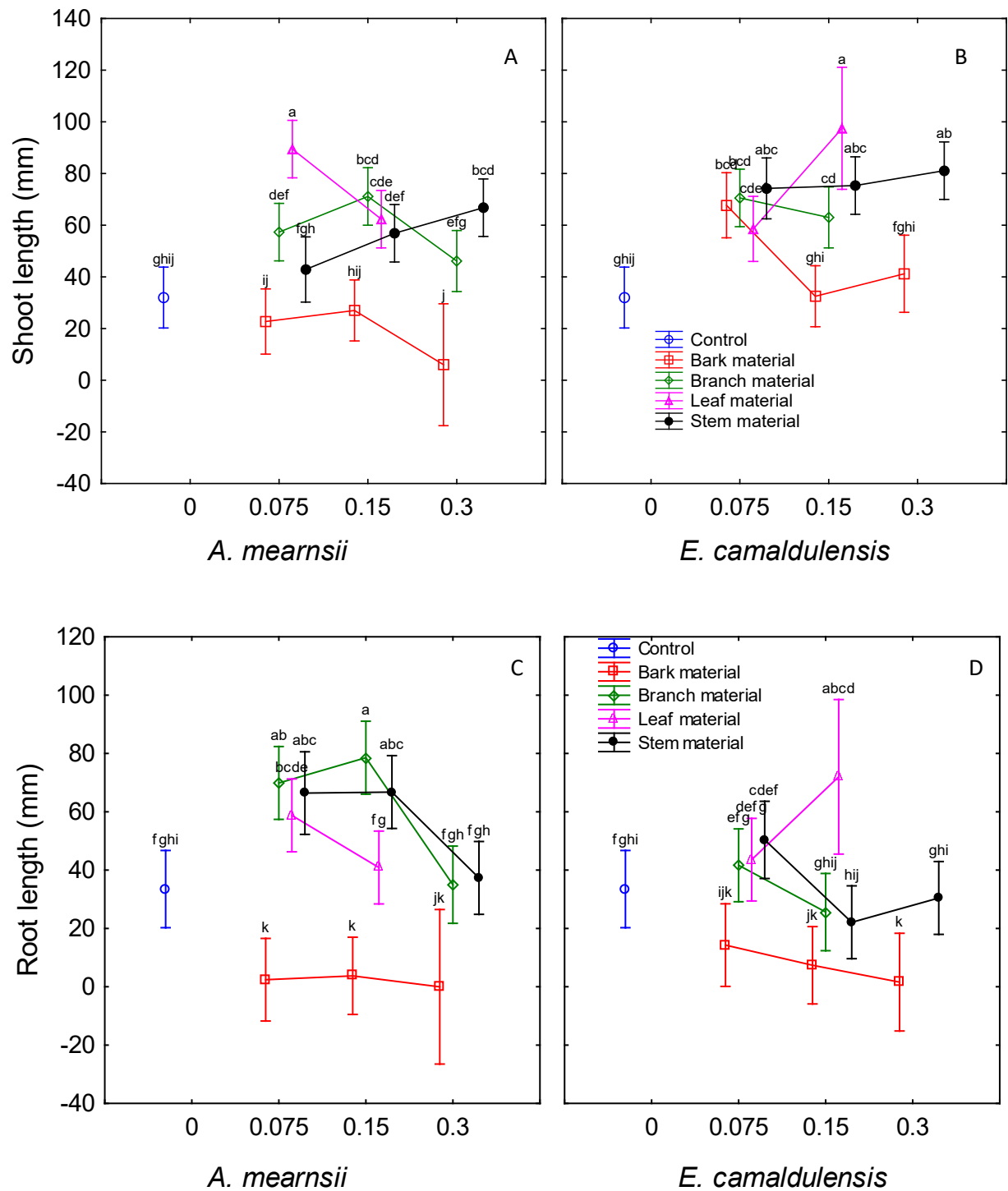


Figure 6.5: Shoot and root lengths of canola grown with *A. mearnsii* leachates (A, C) and *E. camaldulensis* (B, D), using leachate solutions of various biomass components (leaves, stem, branches, and bark). Leachate dose concentrations were 0 g ml<sup>-1</sup>, 0.3 g ml<sup>-1</sup>, 0.15 g ml<sup>-1</sup> and 0.075 g ml<sup>-1</sup> and compared with a control of distilled water. Letters denote significant differences (LSD; P<0.05); error bars denote 0.95 confidence intervals.

There was significant interaction between tree component and concentration (ANOVA;  $F_{[4; 48]} = 1.78$ ;  $P=0.014$ ; Table 6.2). Table 6.2 shows that leaf leachate of *A. mearnsii* at  $0.3 \text{ g ml}^{-1}$  resulted in a significantly lower wheat R/S ratio ( $0.26 \pm 0.13$ ) than the control ( $1.43 \pm 0.35$ ) and the application of  $0.075 \text{ g ml}^{-1}$  dosage (LSD;  $P \leq 0.05$ ). For *E. camaldulensis*, samples treated with  $0.075 \text{ g ml}^{-1}$  leaf leachate dose resulted in a higher wheat R/S ratio ( $3.23 \pm 0.28$ ) at  $0.3 \text{ g ml}^{-1}$  dosage than any other leaf treatment (LSD;  $P \leq 0.05$ ). There were no differences in R/S ratios between seedlings treated with any dosage of leachate from stem material of *A. mearnsii* (LSD;  $P \geq 0.05$ ), whereas there was a significant difference for *E. camaldulensis* at  $0.3 \text{ g ml}^{-1}$  when compared to the control (LSD;  $P < 0.001$ ). The strongest effect of leachate dose can be seen in the bark component of *E. camaldulensis* where the R/S ratio was as high as  $6.3 \pm 12.75$ , significantly higher than the control and any of the other bark treatments (LSD;  $P < 0.05$ ). Similar to this, *A. mearnsii* bark at high dosages ( $0.3 \text{ g ml}^{-1}$ ) also showed a relatively high R/S ratio ( $2.078 \pm 0.745$ ), which was also significantly higher than the control and other doses of *A. mearnsii* bark (LSD;  $P < 0.05$ ).

Table 6.2: Root/shoot ratios (Mean $\pm$ SE) of canola seedlings germinated in leachate solutions of various biomass components (leaves, stem, branches, and bark) *A. mearnsii* and *E. camaldulensis*. Leachate dose concentrations were  $0 \text{ g ml}^{-1}$ ,  $0.3 \text{ g ml}^{-1}$ ,  $0.15 \text{ g ml}^{-1}$  and  $0.075 \text{ g ml}^{-1}$  and compared with a control of distilled water. Letters denote where significant differences were found (LSD;  $P \leq 0.05$ ). Lack of germination is denoted with a dash (-).

Invasive Species	Dose (g ml <sup>-1</sup> )	Root/shoot ratio; Mean $\pm$ SE			
		Leaves	Stem	Branches	Bark
<i>A. mearnsii</i>	0			$1.43 \pm 0.35^a$	
	0.075	$1.24 \pm 0.32^a$	$1.5 \pm 0.28^a$	$0.98 \pm 0.22^b$	$0.8 \pm 0.133^b$
	0.15	$0.62 \pm 0.38^b$	$1.72 \pm 0.51^a$	$1.14 \pm 0.57^a$	$0.62 \pm 0.79^b$
	0.3	$0.26 \pm 0.13^b$	$1.65 \pm 0.7^a$	$0.84 \pm 0.6^b$	$2.08 \pm 2.37^c$
<i>E. camaldulensis</i>	0			$1.43 \pm 0.35^a$	
	0.075	$1.03 \pm 0.22^a$	$1.32 \pm 0.22^a$	$1.04 \pm 0.28^b$	$0.49 \pm 0.22^d$
	0.15	$0.87 \pm 0.25^b$	$1.18 \pm 0.22^{ab}$	$0.69 \pm 0.38^{cd}$	$0.46 \pm 0.16^d$
	0.3	$3.23 \pm 0.28^c$	$0.9 \pm 0.34^b$	$0.41 \pm 0.35^d$	$6.3 \pm 12.75^e$

## The effect of wood chips of *Acacia mearnsii* and *Eucalyptus camaldulensis* on growth of wheat and canola

Wheat grown under *A. mearnsii* wood chips grew best (shoots) under the fresh chips (0 months;  $25.53 \pm 31.12$  g) and the worst at the control of no chips ( $8.44 \pm 3.45$  g) (Table 6.3). The 12-month-old *A. mearnsii* chips also had significantly less growth ( $9.96 \pm 4.42$  g) than the fresh and 6-month-old chips ( $13.68 \pm 4.93$  g). For *E. camaldulensis*, the most shoot growth was with 12-month-old chips ( $15.73 \pm 16.1$  g), which was significantly more than the control (LSD;  $P < 0.001$ ) and significantly less than the best growth from *A. mearnsii* fresh chips ( $P < 0.001$ ). Shoot growth for canola under *A. mearnsii* was the lowest with the 12 month old chips ( $1.12 \pm 0.51$  g) and the highest under the 6-month-old chips ( $1.88 \pm 2.18$  g) (Table 6.4). The greatest growth under *A. mearnsii* was, however, not significantly different from the control ( $1.1 \pm 1.01$  g), which showed the least growth overall. Canola grown with the fresh chips of *E. camaldulensis* showed the overall best shoot growth ( $4.19 \pm 3.32$  g), followed by the 6-month-old chips ( $2.09 \pm 1.68$  g). At 12 months old, the *E. camaldulensis* chips had the least canola shoot growth ( $0.86 \pm 0.69$  g).

Chip treatments of both invasive species showed an increased  $R_{R/S}$  for wheat as treatment age increased (Table 6.3) and was the least for chip treatments of both invasive species at time 0 ( $0.55 \pm 0.44$  for *A. mearnsii* and  $0.76 \pm 0.38$  for *E. camaldulensis*). These were the only ones below 1 and were also significantly lower than the  $R_{R/S}$  of the control, which had the overall highest  $R_{R/S}$  ( $1.87 \pm 1.01$ ). Root/shoot ratios (Table 6.3) for canola were all similar and mostly below 1 for all the treatments, including the control and except for the 6-month old chips treatment of both invasive species: plants grown with *E. camaldulensis* chips had a higher R/S ratio ( $1.08 \pm 0.85$ ) and those grown with *A. mearnsii* had a lower R/S ratio ( $0.42 \pm 0.32$ ).

Table 6.3: Shoot biomass, root biomass, and root/shoot ratios (mean  $\pm$  standard error) of wheat seedlings germinated and grown under mulch from the biomass of *A. mearnsii* and *E. camaldulensis*. Letters denote where significant differences were found (LSD;  $P \leq 0.05$ ).

Chip treatment	Shoot biomass (g)	Root biomass (g)	Root/shoot ratio
Control	$8.44 \pm 3.45^b$	$15.04 \pm 7.3^a$	$1.87 \pm 1.01^a$
<i>Acacia</i> 0	$25.53 \pm 31.12^a$	$11.41 \pm 9.08^{ab}$	$0.55 \pm 0.44^c$
<i>Acacia</i> 6	$13.68 \pm 4.93^{ab}$	$13.04 \pm 1.96^{ab}$	$1.0 \pm 0.34^{bc}$
<i>Acacia</i> 12	$9.96 \pm 4.42^b$	$16.04 \pm 12.97^a$	$1.53 \pm 0.73^a$
<i>Eucalyptus</i> 0	$9.55 \pm 2.78^b$	$7.36 \pm 5.12^b$	$0.76 \pm 0.38^{bc}$
<i>Eucalyptus</i> 6	$8.27 \pm 2.62^b$	$11.71 \pm 7.27^{ab}$	$1.41 \pm 0.7^{ab}$
<i>Eucalyptus</i> 12	$15.73 \pm 16.1^{ab}$	$13.34 \pm 9.04^{ab}$	$1.18 \pm 1.14^{abc}$

Table 6.4: Shoot biomass, root biomass, and root/shoot ratios (mean  $\pm$  standard error) of canola seedlings germinated and grown under mulch from the biomass of *A. mearnsii* and *E. camaldulensis*. Letters denote where significant differences were found (LSD;  $P \leq 0.05$ ).

Chip treatment	Shoot biomass (g)	Root biomass (g)	Root/shoot ratio
Control	1.1 $\pm$ 1.01 <sup>b</sup>	0.7 $\pm$ 0.7 <sup>b</sup>	0.58 $\pm$ 0.22 <sup>b</sup>
<i>Acacia</i> 0	1.79 $\pm$ 0.7 <sup>b</sup>	1.15 $\pm$ 0.54 <sup>b</sup>	0.64 $\pm$ 0.16 <sup>b</sup>
<i>Acacia</i> 6	1.88 $\pm$ 2.18 <sup>b</sup>	1.24 $\pm$ 1.3 <sup>b</sup>	0.7 $\pm$ 0.22 <sup>b</sup>
<i>Acacia</i> 12	1.12 $\pm$ 0.51 <sup>b</sup>	0.49 $\pm$ 0.47 <sup>b</sup>	0.42 $\pm$ 0.32 <sup>b</sup>
<i>Eucalyptus</i> 0	4.19 $\pm$ 3.32 <sup>a</sup>	2.69 $\pm$ 1.99 <sup>a</sup>	0.66 $\pm$ 0.19 <sup>b</sup>
<i>Eucalyptus</i> 6	2.09 $\pm$ 1.68 <sup>b</sup>	1.11 $\pm$ 0.44 <sup>b</sup>	0.63 $\pm$ 0.51 <sup>b</sup>
<i>Eucalyptus</i> 12	0.86 $\pm$ 0.69 <sup>b</sup>	0.75 $\pm$ 0.44 <sup>b</sup>	1.08 $\pm$ 0.85 <sup>a</sup>

## 6.5 Discussion

In South Africa, the potential for allelopathic effects of invasive plant material on agricultural crops serves to caution against its use as a mulching agent to protect soils and help improve its functioning. This chapter sought to determine if biomass of invasive *Eucalyptus camaldulensis* and *Acacia mearnsii* trees could be used in wood chips as mulch for agricultural purposes, where the entire tree is used in the chipping process. To test for allelopathy in the various biomass components of *E. camaldulensis* and *A. mearnsii*, petri dish experiments were conducted where germination and root and shoot growth of wheat and canola seedlings were recorded. Results from these germination experiments confirm previous findings that both *E. camaldulensis* and *A. mearnsii* possess significant allelopathic potential in their leaf and bark material. The results also confirm that receiver species from different guilds respond differently to the treatments from the two species.

Studies by Fatunbi et al. (2009) and others (Kholi & Singh, 1991; Lisanework & Michelsen, 1993; Hoque et al., 2003; El-Khawas & Shehata, 2005; Jayakumar & Manikandan, 2005; Oyun, 2006) suggest that a more important factor to be considered in allelopathy is the actual dose of an extract. At low concentrations, bark and leaf components may be harmless to receiver plants and may have an added advantage of higher nutrient contents. Their findings also hint at a differential influence of possible allelopathic compounds to receiver species of different guilds (Schumann et al., 1995). In medical science, the phenomena of dose-dependency and variations thereof are often termed “hormesis”, referring to the stimulatory effect of a known poison at low doses and a significant inhibitory effect at higher doses. This

possibility has been speculated on by some researchers (Duke et al., 2006; Abbas et al., 2017), but many species, including *Eucalyptus*, still require further investigation. The petri dish experiments from the current study support the possibility of the hormesis effect in allelopathy. It is possible that the allelopathic compounds present at low levels have a stimulatory effect on plant growth or that it simply has no effect on plant development. Dose is, however, not only related to the concentration of a specific compound but may also refer to amount of time a receiver plant is exposed to the compound (Duke et al., 2005). This suggests that an allelopathic compound produced in high volumes can only be effective if absorbed long enough by the receiver, or if it is available for uptake for long enough. In reality, this distinction is important where soil type and texture drive the availability and persistence of allelochemicals and thus could be instrumental in its effect on receiver plants.

Historically, wood chips have been applied as weed suppressant and general pest management in agriculture and not as a means to improve crop growth directly (Duryea et al., 1999; Rathinasabapathi et al., 2005; Ferguson et al., 2008). The use of wood chips may thus be more beneficial after germination and establishment of the crop, suggesting positive results could be skewed towards perennial crops. In this instance, biochar might be a viable alternative to wood chips for annual crops and should be explored. During this study it is reported that canola shoot growth responded best to fresh *E. camaldulensis* chips, while wheat shoot growth responded best to 12-month old *E. camaldulensis* chips.

Similarly, fresh *A. mearnsii* chips performed best for wheat growth; however, none of the *A. mearnsii* chip treatments had any significant positive or negative effect on canola growth. The species used to make wood chips and the crop species are thus important considerations when applying wood chips. These findings should also lead to questions about the (allelopathic) bark component on chipped wood and its impact on receiving plants. Bark comprises less than 15% of an adult tree of *A. mearnsii* and *E. mearnsii* (chapter 3 of this study). Of the total volume of a stem, less than 30% constitutes the bark component. Considering the hormesis effect, this bark allocation might thus either be too little to have any significant impact on crop growth, or it might be enough to enhance growth rate of crops.

Although this study is done on agricultural crops, the findings here may be relevant more broadly to aspects of rehabilitation in landscapes where invasive alien trees have been removed. As discussed in chapter 5, the large amounts of nutrients potentially exported from invaded sites through biomass removal could significantly affect local nutrient cycles. Wood chips could be used in veld rehabilitation as an effective mulching agent, to protect soils and maintain healthy soil environments (Williams, 2020), while they have also been shown to significantly improve establishment and survival of trees in passive restoration sites (Cahill et

al., 2005). Wood chips have been used as soil amendment in forestry plantations before (Bulmer, 2000) where they were outperformed by sawdust in producing vigorous trees. The use of wood chips has also been documented in sites restored after mining (Kramer et al., 2000a, b; Eldridge et al., 2011), and in agriculture (Davis et al., 2000). Wood chips are thus useful for a wide range of applications. It is suggested here that wood chips may be beneficial where they are produced from invasive alien tree biomass and used as a soil amendment. Hence, while this study is agriculturally relevant, it also proposes a highly beneficial pathway for the use of wood chips in ecosystem rehabilitation, especially where invasive biomass is removed at a large scale.

## 6.6 Conclusions and recommendations

This study is the first to document the effect of age of wood chips of *Acacia mearnsii* and *Eucalyptus camaldulensis* on its potential allelopathic effects and its implications for agricultural use in South Africa. This study also documents the allelopathic potential of all aboveground biomass components of the two invasive species to determine the most likely sources of allelopathic compounds. Through this work, it is shown that the photosynthetically active biomass components (leaves and bark) had the greatest allelopathic effect on both germination and shoot development. There were also dose-dependent but differential effects of leachates on development of the two crop species for both *A. mearnsii* and *E. camaldulensis* biomass components. Stem material from either *E. camaldulensis* or *A. mearnsii* did not negatively affect wheat germination and initial growth. This is a critical stage of plant development and has significant bearing on its growth trajectory. In this case, it is possible that dose of the allelopathic compounds in the stem material is too low to affect growth negatively. Wood chips from different invasive species have different effects on different crops, which can be related to its storage time before being used. For instance, fresh *E. camaldulensis* chips performed best for canola while fresh *A. mearnsii* chips performed best with wheat. This suggests a species-specific effect, which is also in line with the findings from the petri dish experiment. Additionally, extended storage periods may lead to extensive nutrient losses from wood chips through severe weathering, leaving chips devoid of nutrients. Nutrient contents of wood chips were not determined during this study and there is great potential for further research in this area. It should be noted that the outcomes of this study may have been different if it had not been done in a greenhouse. It is thus suggested that these experiments be done under field conditions. As a practical outcome, it is also suggested that the chips be tested against the intended crops before use due to the differential effect of chip species on different crops. Furthermore, given the impact of leaf material on germination



in petri dishes, risk may be lowered by excluding leaf material from mulch mixtures applied to agricultural crops, until more extensive experiments under field conditions can be carried out.

## 6.7 References

- Aslan-Tutak, F., & Adams, T. L. (2015). A study of geometry content knowledge of elementary preservice teachers. *International Electronic Journal of Elementary Education*, 7(3), 301–318.
- Babu, R. C., & Kandasamy, O. S. (1997). Allelopathic Effect of *Eucalyptus globulus* Lahill . on *Cyperus rotundus* L . and *Cynodon dactylon* L . Pers . *Agronomy and Crop Science*, 179, 123–126.
- Blanco, J. A. (2007). The representation of allelopathy in ecosystem-level forest models. *Ecological Modelling*, 209(2–4), 65–77.
- Borbély, M. and Dávid, I. (2008): Changeability of allelopathy depending on several factors. *Cereal Research Communications*, 36, 1383–1386.
- Bulmer, C. (2000). Reclamation of forest soils with excavator tillage and organic amendments. *Forest Ecology and Management*, 133(1-2), 157-163.
- Cahill, A., Chalker-Scott, L., & Ewing, K. (2005). Wood-chip mulch improves woody plant survival and establishment at no-maintenance restoration site (Washington). *Ecological restoration*.
- Corbett, B. F., & Morrison, J. A. (2017). The Allelopathic Potentials of the Non-native Invasive Plant *Microstegium vimineum* and the Native *Ageratina altissima* : Two Dominant Species of the Eastern Forest Herb Layer. *Northeastern naturalist*, 19(2), 297-312.
- Davis, J. G., Whiting, D., & Wilson, C. R. (2000). Choosing a soil amendment. Gardening series. Basics; no. 7.235.
- Díaz, S., Kattge, J., Cornelissen, J. H. C., Wright, I. J., Lavorel, S., Dray, S., ... Gorné, L. D. (2015). The global spectrum of plant form and function. *Nature*, 1–17. <http://doi.org/10.1038/nature16489>
- Duke, S. O., Cedergreen, N., Velini, E. D., & Belz, R. G. (2006). Hormesis: is it an important factor in herbicide use and allelopathy? *Outlooks on Pest Management*, 17(1), 29-33.
- Eldridge, J. D., Redente, E. F., & Paschke, M. (2012). The use of seedbed modifications and wood chips to accelerate restoration of well pad sites in western Colorado, USA. *Restoration Ecology*, 20(4), 524-531.
- El-Khawas, S. A., & Shehata, M. M. (2005). The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on monocot (*Zea mays* L.) and dicot (*Phaseolus vulgaris* L.) plants. *Biotechnology*, 4(1), 23-34.
- Ferguson, J. J., & Rathinasabapathi, B. (2003). Allelopathy: How plants suppress other plants. *EDIS*, 2003(18).
- Hershey, D. R. (1994). Allelopathy misconceptions. *The American Biology Teacher*, 56(5), 261-262.
- Hierro, J. L., & Callaway, R. M. (2003). Allelopathy and exotic plant invasion. *Plant and soil*, 256(1), 29-39.
- Inderjit, & Duke, S. O. (2003). Ecophysiological aspects of allelopathy. *Planta*, 217(4), 529–539. <http://doi.org/10.1007/s00425-003-1054-z>

- Inderjit, A., & Moral, R. (2017). Is Separating Resource Competition from Allelopathy Realistic ? Published by : Springer on behalf of New York Botanical Garden Press, 63(3), 221–230.
- Josic, D., Delic, D., Rasulic, N., Stajkovic, O., Kuzmanovic, D., Stanojkovic, A., & Pivic, R. (2012). Indigenous pseudomonads from rhizosphere of maize grown on pseudogley soil in serbia. *Bulgarian Journal of Agricultural Science*, 18(2), 197–206. <http://doi.org/10.1007/sl>
- Kramer, P. A., Zabowski, D., Scherer, G., & Everett, R. L. (2000a). Native plant restoration of copper mine tailings: I. Substrate effect on growth and nutritional status in a greenhouse study. *Journal of Environmental Quality*, 29(6), 1762-1769.
- Kramer, P. A., Zabowski, D., Scherer, G., & Everett, R. L. (2000b). Native plant restoration of copper mine tailings: II. Field survival, growth, and nutrient uptake. *Journal of environmental quality*, 29(6), 1770-1777.
- Levine, J. M. (2015). A trail map for trait-based studies. *Nature*, 1–2. <http://doi.org/10.1038/nature16862>
- Meiners, S. J., Kong, C. H., Ladwig, L. M., Pisula, N. L., & Lang, K. A. (2012). Developing an ecological context for allelopathy. *Plant Ecology*, 213(8), 1221-1227.
- Muller, C. H. (1969). Allelopathy as a factor in ecological process. *Vegetatio*, 18(1–6), 348–357. <http://doi.org/10.1007/BF00332847>
- Patnaik, P. K. (2000). on potential of Eucalyptus leaf litter leachates Allelopathic growth of finger millet germination and seedling, 78(1), 69–78.
- Puri, A. V, Review, S. B., & Nov, A. (2017). Placentation in Angiosperms Published by: Springer on behalf of New York Botanical Garden Press Stable URL: <http://www.jstor.org/stable/4356460>, 18(9), 603–651.
- Rejmánek, M., & Richardson, D. M. (2011). Eucalypts. in: Simberloff, D., and Rejmánek M., (eds) *Encyclopedia of Biological Invasions* (3), 203–209. University of California Press.
- Rice, E. L. (2016). Allelopathy: An Update Author ( s ): Elroy L . Rice Published by : Springer on behalf of New York Botanical Garden Press Stable, 45(1), 15–109.
- Seigler, D. S. (2006). Basic pathways for the origin of allelopathic compounds. In *Allelopathy* (pp. 11-61).
- Smith, M. W., Carroll, B. L., & Cheary, B. S. (2000). Mulch improves pecan tree growth during orchard establishment. *HortScience*, 35(2), 192-195.
- Stafford, W. H., Von Maltitz, G. P., & Watson, H. K. (2018). Reducing the costs of landscape restoration by using invasive alien plant biomass for bioenergy. *Wiley Interdisciplinary Reviews: Energy and Environment*, 7(1), e272.
- Stafford, W., & Blignaut, J. (2017). Reducing landscape restoration costs: Feasibility of generating electricity from invasive alien plant biomass on the Agulhas Plain, South Africa. *Ecosystem Services*.
- Swaminathan, C., Rai, R. V., & Suresh, K. K. (1989). Allelopathic proclivities of *Acacia nilotica* (L.) Willd. ex Del. *Journal of Tropical Forest Science*, 56-60.
- Wang, L., Gruber, S., & Claupein, W. (2012). Effects of woodchip mulch and barley intercropping on weeds in lentil crops. *Weed research*, 52(2), 161-168.
- Williams, D (2020). Restoring a Forest Using Wood Chips. Capitol Land Trust. <https://capitolandtrust.org/restoring-forest-wood-chips/> (accessed 18 July 2020)

Zhang, C., & Fu, S. (2010). Allelopathic effects of leaf litter and live roots exudates of *Eucalyptus* species on crops. *Allelopathy Journal*, 26(1), 91-100.

## Chapter 7: Value-added products: the effect of biochar application on crop health is dependent in the addition of sufficient amounts of fertilizer

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### 7.1 Abstract

Clearing of invasive alien trees in riparian zones of South Africa is expensive, and in many cases, ineffective due to a perpetual lack of resources, even as invasion keeps spreading. To recover some of the costs of alien tree clearing, the utilizable biomass can undergo several value-adding processes, of which biochar has great potential in areas where invasion is problematic. Biochar can be used as a soil amendment to improve soil functioning and is gaining popularity for use in agriculture. Biochar's production process, however, potentially depletes it of any nutritional value and this may impede crop growth if not quantified and managed. This study reports on ex-situ experimentation of biochar developed from *Acacia mearnsii* and *Eucalyptus camaldulensis* biomass on the growth of wheat (*Triticum aestivum*) and canola (*Brassica napus*). Treatments of 0, 25, 50, and 150 g of biochar were applied to 1 kg of potting soil with and without an NPK fertilizer in six treatments of 5 replicates for each biochar type and for each crop species. Biochar from both invasive species improved crop growth in the presence of fertilizer but growth peaked at different stages for the different crops when treated with the two biochar types. Basic chemical analyses were done, including analyses of biochar pH, EC, C, N, P, Ca, Mg, K, and Na. The pH of *E. camaldulensis* biochar was between 9.6 and 9.8 and was generally higher than that of *A. mearnsii* biochar (9.4-9.5), while electrical conductivity had a higher maximum but wider range in *E. camaldulensis* than in *A. mearnsii* (650 - 1090 and 733 - 906  $\mu\text{S cm}^{-1}$ , respectively). Total C and N contents of *A. mearnsii* (43.4 - 73.2% and 0.2 - 0.35%, respectively) were higher than *E. camaldulensis* biochar (44.4 - 50.2% and 0.14 - 0.16%, respectively) while the latter had more available P (6.1 - 9.0  $\text{mg kg}^{-1}$  in *E. camaldulensis* vs. 4.4 - 5.2  $\text{mg/kg}$  in *A. mearnsii*). Similarly, Mg and K were higher in *E. camaldulensis* biochar (65.9 - 78.6  $\text{mg L}^{-1}$  and 125.8 - 202.5  $\text{mg L}^{-1}$ , compared to 20.5 - 37.6  $\text{mg L}^{-1}$  and 69.6 - 76.5  $\text{mg L}^{-1}$  in *A. mearnsii*), while Ca and Na were more in the *A. mearnsii* biochar (168.1 - 201.2  $\text{mg L}^{-1}$  and 48.2 - 55.6  $\text{mg L}^{-1}$  compared to 144.2 - 178.6  $\text{mg/L}$  and 22.8 - 32.1  $\text{mg L}^{-1}$  respectively in *E. camaldulensis*). These results show that the effect of biochar on crop growth depends on biochar type, biochar volume, crop type and fertilizer addition. Other factors not tested here and but likely to influence success of biochar in enhancing crop production are soil type and biochar production process.

## 7.2 Introduction

Soil is an important storage mechanism for carbon in the biosphere, which, if lost, could significantly affect the global carbon balance, and have serious implications for the rates of global climate change (Lal & Pimentel, 2008). Agricultural expansion and large-scale soil degradation have led to the release of soil carbon from topsoil layers, and have been estimated to have contributed approximately 60% of the total carbon released to the atmosphere (Lal, 2004). To reverse the adverse effects of global climate change, soil thus represents a potentially critical component, and biochar could likely provide long-term stability of soil carbon. The term biochar (black carbon) refers to *semi-pyrolyzed* biomass produced under low oxygen conditions and temperatures exceeding 250°C (Lehman et al., 2011), producing a product similar to charcoal. This can be done on-site where biomass is cleared, or at specialised facilities, leading to great variations in biochar physical properties. Black carbon does not decompose easily, and thus represents a large portion of underground carbon (Kuhlbusch et al., 1998). Unlike charcoal, however, biochar's major intended use is not heat generation, but rather for soil application, water purification, and for cosmetic use. Fundamentally, the difference between charcoal and biochar may thus be related to its intended use, more than it is related to its production process.

In agriculture, biochar can be applied to soils to improve crop growth. Due to its overall high porosity, biochar has the potential to retain large volumes of water in soil, making it available to plant roots for longer and may even assist in controlling nutrient release (Day et al., 2005). It also serves as a good substrate for microbial activity, contributing significantly to the overall functioning of soil (Lehman et al., 2011). Biochar has also been used successfully as a nutrient filter, which has sparked interest in the product as a chemical filter to remove pollutants from water (Laird et al., 2010; Angst et al., 2013). It has, for instance, been used to reduce N<sub>2</sub>O and mineral N from soil as a result of excess animal manure on livestock farms (Angst et al., 2013), and also been shown to lead to a reduction of nutrients leached from pig manure (Laird et al., 2010). Biochar has also been used in denitrifying bioreactors, successfully removing nitrate and phosphate from polluted water, through the increased activity of anaerobic bacteria (Bock et al., 2015). Because of its wide range of potential uses, the product has been earmarked as a potential pathway to reduce and effectively utilize biomass of invasive plant species as this biomass could be used in its production (Kumar et al., 2013; Liao et al., 2013; Li et al., 2015; Stafford & Blignaut, 2017; Stafford et al., 2018). Invasive biomass in South Africa's riparian zones and its cost of clearing present an ideal opportunity for value-adding to reduce net costs of invasive alien tree management.

However, most of the great variety of work on biochar done in the country remains unpublished and thus not easily accessible. This could be due to difficulties in standardizing methodologies, or acquiring comparable materials in terms of its production process. However, in some instances, this may create a barrier for continued development of a highly promising agricultural product. Biochar could have secondary advantages of offsetting the cost of removing invasive alien plants, when used as stock for biochar reactors, and promoting carbon storage in soil. This study is aimed at highlighting the information currently available on biochar effects on crop growth through a short literature review. Additionally, the study aimed to address some of the variation in results reported, through a small-scale investigation of biochars from the general aboveground biomass of two species of invasive alien trees, produced under similar pyrolysis conditions, in the same growth medium, on two locally important crop species, using varying levels of biochar application.

### **Biochar type and production process**

Biochar can be produced from any high-carbon source that can be pyrolyzed at relatively low temperatures (400°C - 800°C). The primary input to biochar is thus not limited to woody biomass, as other materials such as cow manure (Uzoma et al., 2011), green waste (e.g. pruned grass; Beesley et al., 2010; Chan et al., 2008), peanut hulls (Gaskin et al., 2010), and even coconut shells (Saranya et al., 2011) have been used successfully in biochar production.

Locally, Burger (2018) made a case for the use of biochar made from bamboo as one of the most financially justifiable options for value-adding. Similarly, Aghoghovwia (2018) documented various properties of six types of biochar (maize stover, grape pips, grape skins, pine wood, rubber tyres, and sugarcane piths), and demonstrated the importance of differences in biochar feedlot type on the properties of the resultant product. The study noted significant differences in elements such as carbon, which ranged between 27.9% and 80.31% for sugarcane piths and pine wood as the respective minimum and maximum. Total nitrogen was as little as 0.2% in maize stover and as high as 1.27% in grape pip material. The study also reported little to no correlation in biochar properties from different components of the same plant, as biochar from grape pips and skins were significantly different on most accounts; most notably an almost six-fold difference in ash content (5.91% for pips and 30.43% for skins). To support this, a study done by Botha (2018) also showed significant elemental differences between *Pinus* and *Eucalyptus* biochar, reporting clear differences between the feedlot types in, amongst others, pH, electrical conductivity, and ash content, while the study also reported significant differences in most elements tested between the two feedlot types.

An apparent point of concern in both these studies could be that the biochars used were produced under different pyrolysis conditions, as the *Pinus* biochar was produced at 450°C for and the *Eucalyptus* biochar at 900°C. Pine biochar produced at 450°C was also used in separate studies by Olivier (2011) and by Moller (2012) and was produced by a small-scale commercial producer in both cases. Due to the similarities in the description of the biochar used in both studies mentioned above, it is likely that they made use of material from the same source and were all unable to effectively control for the potential effects of pyrolysis regimes. This is potentially a critical aspect of biochar production and its effect on crops, as pyrolysis temperature, at least, has been shown to significantly alter the structure of biochar with implications for its physicochemical properties (Chan et al., 2009) and, as a result, its interaction with the soil.

According to Van Zwieten et al., (2010), the temperature of the pyrolysis process may be largely responsible for the amount of carbon lost during pyrolysis, and this affects the structure of the resultant biochar. The amount of carbon lost during the process has also been linked to the increased/decreased creation of micropores in biochar, affecting the resultant surface area of the biochar (Bagreev et al., 2001). Micropores are not necessarily important for the functioning of biochar in soils and do not affect its microbial dynamics as much as macropores (Saito 1990; Pietikäinen et al. 2000). However, micropores may affect adsorption and release of molecules in the soil, directly affecting availability of elements to be taken up by plants. It has also been shown that biochar produced at low heat produce less ash than high-heat pyrolysis, which, in turn, affects the availability of elements found abundantly in ash, like potassium and calcium, and has an indirect but significant effect on the pH and electrical conductivity of the product (Rerah et al., 2014).

Uras et al. (2012) aimed to eliminate the potential variation of differing pyrolysis regimes on physicochemical properties of biochar from black wattle biomass, vine prunings, and sugar cane bagasse, by controlling the pyrolysis temperature (450°C), heating rate (17°C per minute), time (1 hour), and pressure (8 kPa). The study reported much less variation in most elemental properties, except for pH, which was significantly lower in sugar cane bagasse biochar, while ash content and the C:N ratio were significantly higher in the sugar cane bagasse biochar than for the other two feedlot types (12.1 and 125.3, respectively). Of the three types of biochar, black wattle biomass had the least ash (4.8%), and the highest concentrations of Na (1140 mg/kg), Cl (460 mg/kg), and  $\text{PO}_4^{3-}$  (1033 mg/kg).

The importance of pyrolysis regime thus should not be understated. However, in the current state of biochar production in South Africa, precision and overall control of this variable is only likely where significant industry growth could increase production scale, justifying the



investment into more specialised equipment. The inability to effectively control pyrolysis regime is a reality faced frequently by small-scale producers, where the process is dependent on feedlot availability, feedlot moisture contents, and atmospheric conditions, especially where open kilns are used. In most cases it is thus unlikely that a constant pyrolysis temperature regime could be achieved and maintained in current production systems. However, for research purposes, and for other applications where consistency is important, it is critical that this aspect of biochar production be standardized.

### **Treatment: Biochars and fertilizers**

In cases where biochar addition improves crop growth, it is likely as a result of a significant improvement in the soil's ability to retain nutrients, as soils receiving a mixture of biochar and an NPK fertilizer has been shown to produce significantly greater crop yields than controls (Chan et al., 2008; Steiner et al., 2008). The absorptive properties of biochar also suggest that nutrients absorbed by the material may be less available for plant uptake and could impede plant growth if not monitored. Sika & Hardie (2014) expressed concern over the ability of biochar to affect nitrogen use efficiency in plants, as they reported that biochar not only reduced leaching of ammonium and nitrate from soils, but also reduced their overall availability in the soil, with up to 79% of nitrates lost as a result of biochar filtration. Counteracting the filtering effect of pure biochar through the addition of an N-based fertilizer to biochar-treated soil was shown to significantly improve growth of green beans (Olivier, 2011). The success of biochar addition to soils may thus be directly dependent on the sufficient availability of nutrients and could impact crops negatively if applied without an appropriate fertilizer.

Further quantification of the filtration capacity of biochar is provided by Israel (2015), who suggested that, although biochar reduces compounds such as nitrates, its efficiency is quickly reduced, leading to further leaching. The study attributed this loss of filtering capacity of biochar to the physical filtering process of the product, rather than microbial activity. The final intended use of biochar may thus play an important role in its preparation; to promote leaching and thus increase nutrient availability to plants, one might ensure sufficient amounts of an appropriate nutrient source are added to the biochar, and where improved filtering is the objective, inoculation with appropriate microbial communities could increase denitrification.

### 7.3 Case study: Is biochar from invasive alien tree species in South Africa potentially useful for crop growth?

As mentioned previously, the effect of biochar on crop growth may differ significantly between different feedlot types. For instance, biochar produced from labile feedlots are likely more easily altered in the soil by soil physical characteristics such as moisture content (Lehmann et al., 2009) and could have differential effects on plant growth. The great variation in feedlot materials coupled with the variation in production processes thus results in great variations in the physical properties of the biochar produced and largely influences the effect it will have on the soil and resultant crop growth.

Research is thus still required to quantify the effects of different feedlot types on different receiver plant species. Biochar produced from *Acacia mearnsii* and *Eucalyptus camaldulensis* could be used successfully in agriculture in South Africa as these, and other species in the two genera are widespread in the country. Exploring the opportunities and considerations for biochar production from these species could significantly assist with maintaining high quality soil and assist with soil carbon storage. In chapter 5, I discussed allelopathy of *Acacia mearnsii* and *Eucalyptus camaldulensis* to wheat (*Triticum aestivum*) and canola (*Brassica napus*) and its implications for the use of wood chips produced from the two species. This section describes the results of an experiment conducted to determine the effect of *A. mearnsii* and *E. camaldulensis* biochar on the growth of wheat and canola and the importance of fertilizer.

### 7.4 Materials and methods

An experiment on the efficacy of biochar in agriculture was conducted under similar conditions to the experiment previously described for wood chips (Chapter 6). The objective, however, was not to establish allelopathic potential, but rather to establish whether biochar can and should be used in conjunction with an organic NPK fertilizer (3:1:5) to maximize crop growth. This experiment was conducted in a 150 µm polyethylene greenhouse tunnel at the Welgevallen Experimental Farm, Stellenbosch University in Stellenbosch for two months under the setup explained in Table 6.3.1 (adapted from Carter et al., 2013). Airflow in the tunnel was enhanced with built-in fans and planting bags were placed randomly and rotated every 2 weeks. The experiments set out in Table 7.1 were repeated for biochar of both *Acacia mearnsii* and *Eucalyptus camaldulensis* and for both wheat and canola. Samples were watered weekly using municipal water. Initial planting densities were 25 seeds per bag. Plants were then limited to 10 per planting bag after initial thinning at five weeks old. Shoot and root

length of the crops were measured after the two-month trial. The treatments consisted of incremental amounts of biochar of *A. mearnsii* and *E. camaldulensis* of 25 g, 50 g, and 150 g each, tested against a control of no biochar and fertilizer (0), a treatment with only fertilizer (F), and a treatment with only biochar (B50).

Table 7.1: Experimental setup to test the effect of biochar and fertilizer effects on growth of wheat and canola seedlings ex-situ

Treatment	Name	Biochar mass (g)	Potting soil (kg)	Replicates
<b>0</b>	Non-fertilized soil with no biochar	0	1	5
<b>B50</b>	Non-fertilized soil with biochar	50	1	5
<b>F</b>	Fertilized soil with no biochar	0	1	5
<b>F+B25</b>	Fertilized soil with low biochar dose	25	1	5
<b>F+B50</b>	Fertilized soil with medium biochar dose	50	1	5
<b>F+B150</b>	Fertilized soil with high biochar dose	150	1	5

The nutrient status of the biochar was also determined. Methods typically used for nutrient determinations of soil were used and are described here. Biochar samples were crushed and sieved with a 2 mm mesh sieve. The pH of the biochar was determined by placing 10 g of biochar in 10 ml of distilled water. After shaking the solution for 30 minutes at 120 rpm on a mechanical shaker, the solution was allowed to rest for another 30 min. After this, a probe was used to measure the pH of the supernatant solution (Robertson et al., 1999). Similarly, electrical conductivity (EC) was measured in an aqueous solution. For EC, 10 g of biochar was placed in 50 ml of water, placed on a mechanical shaker for one hour at 60 rpm. The EC of the suspension was measured with a conductivity cell (Robertson et al., 1999). Available P in the biochar was determined using the Bray 2 extraction method. Elemental analyses of the biochar were done using a thermal gravimetric analysis (TGA) at the Central Analytical Facilities, Stellenbosch University. It should, however, be noted that, due to insufficient availability of the specific biochar material, all samples for chemical analyses were retrieved from two large, 50 kg bags, albeit from varying depths. Interpretation of results in this part of the study should thus be conservative due to the high likelihood of pseudoreplication. Instead, these results seek to highlight some of the key aspects of biochar that might affect soil and crop growth, as influenced by fertilizer availability.

## Statistical analysis

Normality was tested using a Shapiro-Wilks test. The normality test showed normal distribution in some cases and non-normal distribution in others. Data that tested normal were subjected to a one-way ANOVA (Analysis of Variance) to detect differences. Where differences were detected, a Fisher LSD test was used to determine sources of variation. Non-normal data were analysed using a Kruskal-Wallis test. All analyses were done using Statistica 13 (Tibco). Because of possible pseudo-replication in sapling of biochar, no statistical analyses were done on nutrient analyses, and data are only presented as means values.

## 7.5 Results

The small-scale study on the different properties of biochars produced from *A. mearnsii* and *E. camaldulensis* biomass showed differences between the biochar types (Table 7.2). The pH of *E. camaldulensis* biochar (9.6 - 9.8) was higher than that of *A. mearnsii* (9.4 - 9.5). Electrical conductivity in *E. camaldulensis* samples (650 – 1090  $\mu\text{S}/\text{cm}$ ) was also higher than that of *A. mearnsii* biochar samples (733 – 906  $\mu\text{S}/\text{cm}$ ).

Table 7.2 also shows that *E. camaldulensis* biochar has more available P (6.1 - 9.0 mg/kg) than *A. mearnsii* biochar (4.4 - 5.2 mg/kg). Biochar from *E. camaldulensis* had less total nitrogen than that of *A. mearnsii*. The C/N ratio between the two types of biochars then also differed, with *E. camaldulensis* having a higher C/N ratio than *A. mearnsii*. Cation data showed variable results. Magnesium (Mg) and Potassium (K) content in an extract of *E. camaldulensis* were more than that of *A. mearnsii* biochar while sodium (Na) and calcium (Ca) were both lower in *E. camaldulensis* than in *A. mearnsii* biochar extracts.

Table 7.2 Selected properties of biochars made from *Acacia mearnsii* and *Eucalyptus camaldulensis* (min-max).

Variable	<i>A. mearnsii</i>	<i>E. camaldulensis</i>
pH	9.4 - 9.5	9.6 - 9.8
EC ( $\mu\text{S}/\text{cm}$ )	733 - 906	650 - 1090
C (%)	43.4 - 73.2	44.4 - 50.2
N (%)	0.2 - 0.35	0.14 - 0.16
C/N	198 - 221	299 - 326
Available P (mg/kg)	4.4 - 5.2	6.1 - 9.0
K (mg/L)	69.6 - 76.5	125.8 - 202.5
Na (mg/L)	48.2 - 55.6	22.8 - 32.1
Ca (mg/L)	168.1 - 201.2	144.2 - 178.6
Mg (mg/L)	20.5 - 37.6	65.9 - 78.6

There was significant interaction for the effect of treatment and biochar type for wheat shoot growth (ANOVA;  $F_{[5, 25]}=1.96$ ;  $P<0.04$ ; Figure 7.1 A) and for root growth (ANOVA;  $F_{[5, 25]}=11.8$ ;  $P<0.001$ ; Fig 7.1 B). Biochar treatments of both invasive species significantly improved shoot growth of wheat seedlings (Fig. 7.1 A), while significantly reducing root growth (Fig 7.1 B). There was also significantly better shoot growth with the addition of fertilizer (from 234 mm to 259 mm). Shoot growth with *A. mearnsii* biochar was the highest when used with a fertilizer (359 mm), whereas for *E. camaldulensis* the most shoot growth was at treatment F+B150 (313 mm). The B50 treatment of *A. mearnsii* biochar showed the least shoot growth of all the biochar treatments on wheat (231 mm), which was similar to growth at the F+B25 treatment for *E. camaldulensis* (LSD;  $P=0.665$ ). Root growth was significantly reduced with the addition of biochar of both invasive species, with the exception of the F+B150 treatment of *E. camaldulensis*.

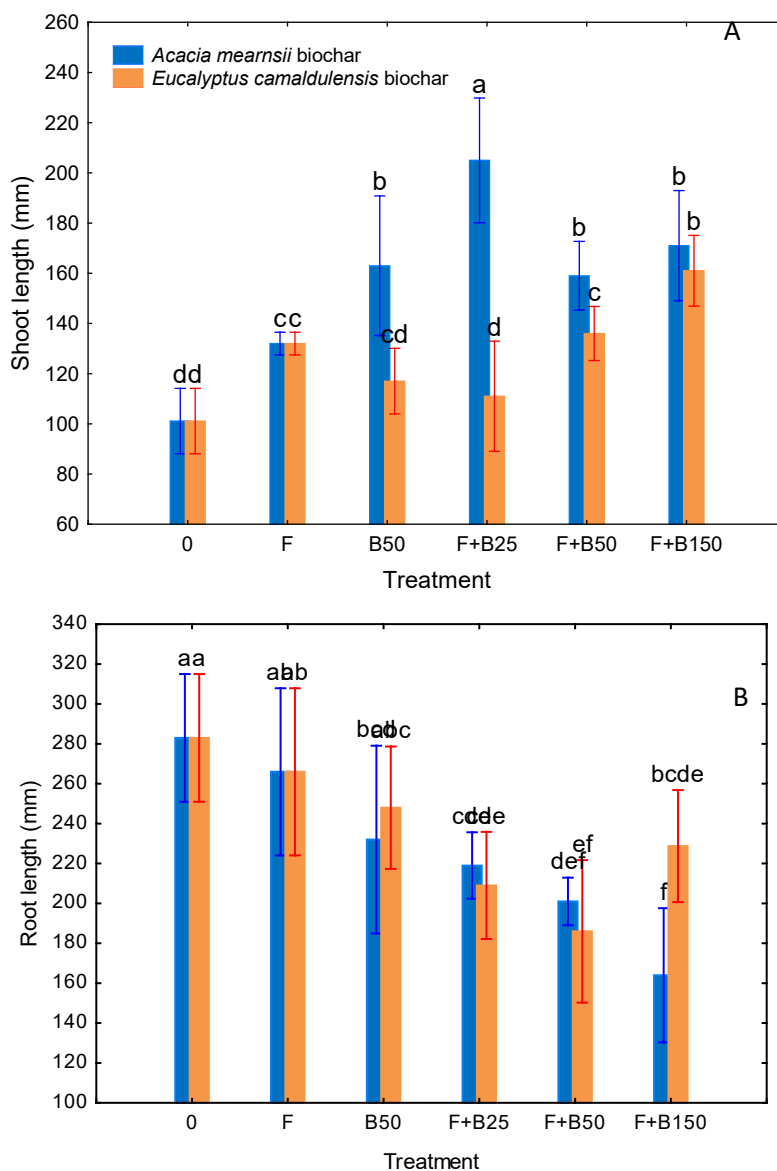


Figure 7.1: Shoot (A) and root (B) length of wheat when grown under different biochar and fertilizer treatments of *A. mearnsii* and *E. camaldulensis*. Letters denote significant differences (LSD;  $p < 0.05$ ); error bars denote 0.95 confidence intervals. Treatments are as follows: 0=Non-fertilized soil with no biochar; B50=Non-fertilized soil with biochar; F=Fertilized soil with no biochar; F+B25=Fertilized soil with low biochar dose; F+B50=Fertilized soil with medium biochar dose; F+B150=Fertilized soil with high biochar dose.

Similarly, there was significant interaction for root/shoot ratios between biochar type and treatment (ANOVA;  $F_{[5; 25]}=2.1$ ;  $P=0.04$ ). The root/shoot ratios decline consistently with the addition of biochar and fertilizer, except in the case of the F+B125 treatment of *E. camaldulensis* (Fig 7.2) The only treatments with a R/S ratio of more than 1 were the control (1.27), F (1.04) and the B50 treatment of *A. mearnsii* (1.03). Wheat treated with the F+B150 treatment of *A. mearnsii* biochar had the lowest R/S ratio of 0.5.

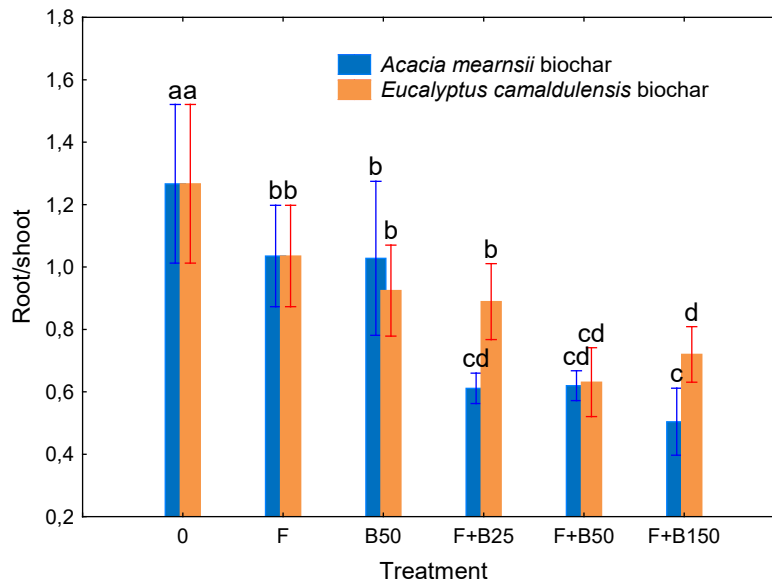


Figure 7.2: Root/shoot (R/S) ratio of wheat when grown under different biochar and fertilizer treatments of *A. mearnsii* and *E. camaldulensis*. Letters denote significant differences (LSD;  $P < 0.05$ ); error bars denote 0.95 confidence intervals. Treatments are as follows: 0=Non-fertilized soil with no biochar; B50=Non-fertilized soil with biochar; F=Fertilized soil with no biochar; F+B25=Fertilized soil with low biochar dose; F+B50=Fertilized soil with medium biochar dose; F+B150=Fertilized soil with high biochar dose.

There was also significant difference between treatments and biochar type for shoot length of canola (ANOVA;  $F_{[5, 25]}=11.8$ ;  $P < 0.001$ ), and for root length (ANOVA;  $F_{[5,25]}=4.13$ ;  $P=0.002$ ; Figure 7.3 B). Similar to wheat, canola shoot length increased significantly with increasing biochar applications of both invasive species (Fig 7.3 A). The most shoot growth of canola was recorded at the F+B25 treatment of *A. mearnsii* (205 mm) and the lowest at the same treatment of *E. camaldulensis* (111 mm). The latter was, however, greater than the control (101 mm) but not significantly different (LSD;  $P=0.362$ ). Canola treated with the B+150 treatment of *E. camaldulensis* showed the best growth for this biochar type and was significantly better than any of the other *E. camaldulensis* treatments (LSD;  $P < 0.05$ ). Root growth was significantly affected by the different treatments, but differential results were recorded. The F treatment resulted in the greatest canola root growth (257 mm) while the B50 treatment of *A. mearnsii* resulted in the least root growth (88 mm) (Fig 7.3 B). Root growth at the F+B50 and F+B150 treatments were more stable and did not show any significant differences for both biochar types.



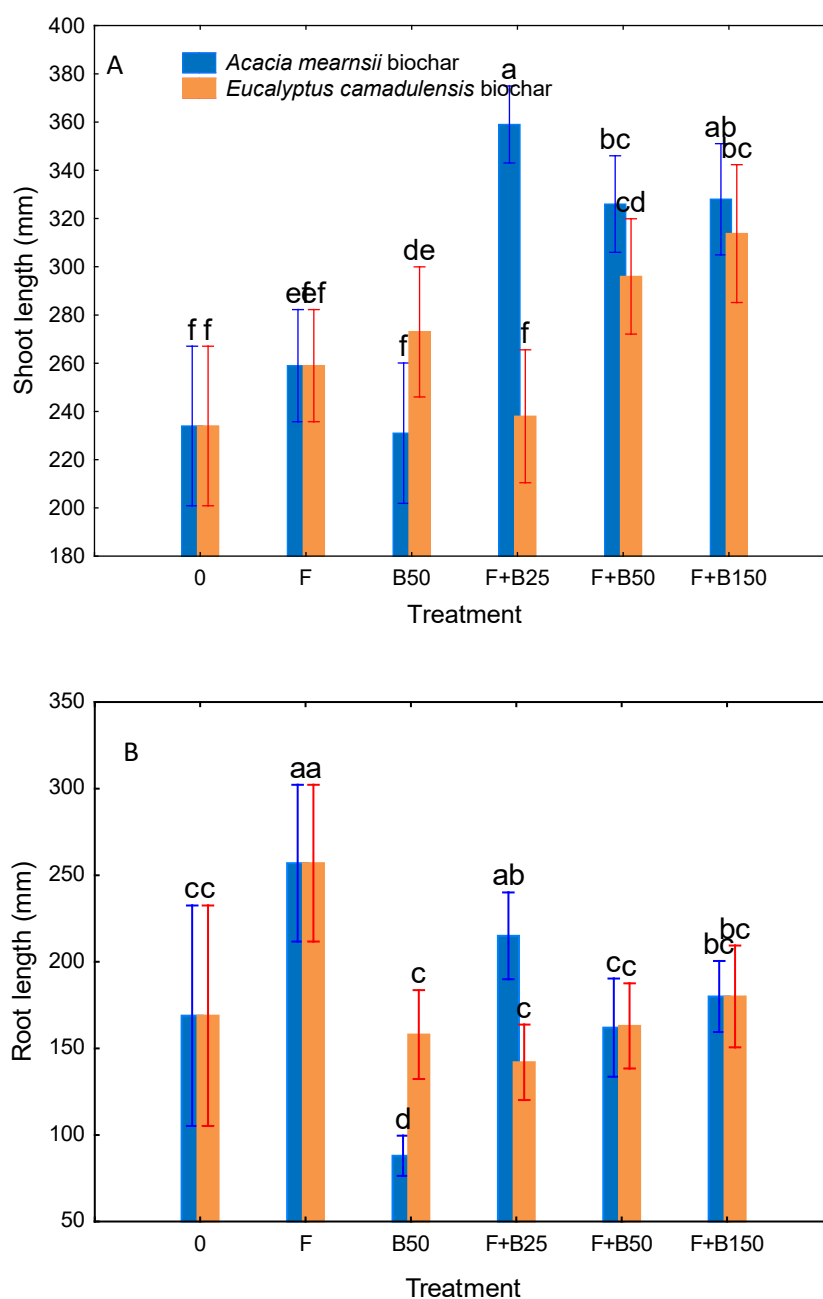


Figure 7.3: Shoot (A) and root (B) length of canola when grown under different biochar and fertilizer treatments of *A. mearnsii* and *E. camaldulensis*. Letters denote significant differences (LSD;  $P < 0.05$ ); error bars denote 0.95 confidence intervals. Treatments are as follows: 0=Non-fertilized soil with no biochar; B50=Non-fertilized soil with biochar; F=Fertilized soil with no biochar; F+B25=Fertilized soil with low biochar dose; F+B50=Fertilized soil with medium biochar dose; F+B150=Fertilized soil with high biochar dose.

For canola, there was significant interaction between biochar type and treatment (ANOVA;  $F_{[5; 25]} = 2.57$ ;  $P = 0.03$ ). Root/shoot ratios of canola showed a decline with an increase in *E. camaldulensis* biochar addition but not for *A. mearnsii* biochar (Fig 7.4). The ratio,

however, stabilized around 1 for *A. mearnsii* biochar treatments at F+ B25, F+B50, and F+B150.

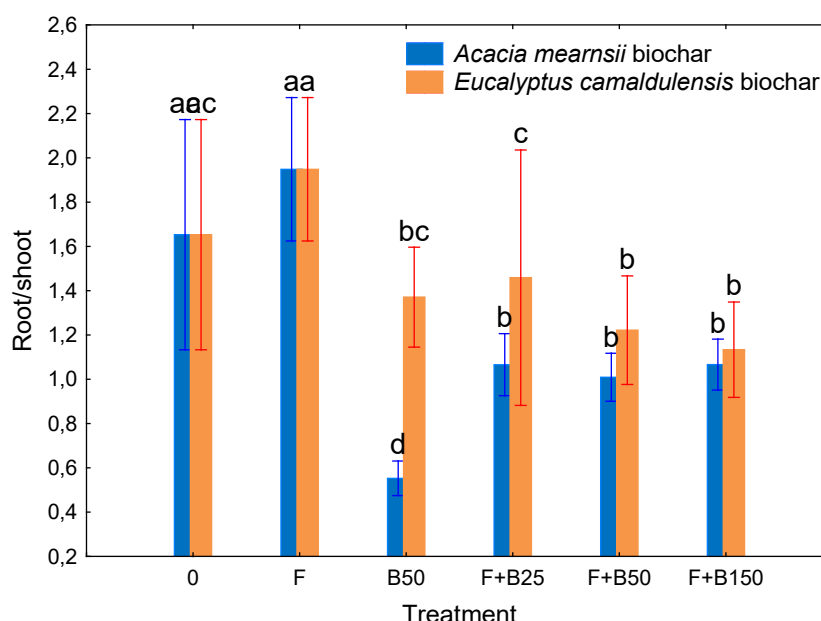


Figure 7.4: Root/shoot (R/S) ratio of canola when grown under different biochar and fertilizer treatments of *A. mearnsii* and *E. camaldulensis*. Letters denote significant differences (LSD;  $P < 0.05$ ); error bars denote 0.95 confidence intervals. Treatments are as follows: 0=Non-fertilized soil with no biochar; B50=Non-fertilized soil with biochar; F=Fertilized soil with no biochar; F+B25=Fertilized soil with low biochar dose; F+B50=Fertilized soil with medium biochar dose; F+B150=Fertilized soil with high biochar dose.

## 7.6 Discussion

Biochar addition significantly improved shoot growth of both wheat and canola crops in most instances. The treatment that performed the best for both crops is the F+B25 (Fertilizer and 25 g of biochar per kg soil) treatment using *A. mearnsii* biochar. For wheat growth, the B50 treatment of *A. mearnsii* (50 g of biochar only) showed the least shoot growth and this correlates with findings by Chan et al. (2007) and Steiner et al. (2008), who showed that biochar addition with fertilizer would result in the best growth, and also agrees with Glaser et al. (2002), who suggested best results at low volume biochar additions. This is, however, not the case with biochar from *E. camaldulensis*, which produced the best shoot growth of wheat and canola at the F+B150 treatment (fertilizer and 150 g of biochar per kg soil). This was below the maximum growth recorded for the *A. mearnsii* biochar, suggesting in both cases that even greater additions of *E. camaldulensis* may be necessary to achieve similar results to the F+B25 treatment of *A. mearnsii* chips.

The root/shoot ratios for both crops decreased significantly with the addition of greater amounts of biochar and were below 1 for all wheat samples where biochar was applied with fertilizer. This suggests that the plants were able to allocate more energy/ resources to aboveground growth relative to belowground growth as the substrate provided sufficient access to belowground resources. The B50 treatment of *A. mearnsii* was the only treatment with a root/shoot ratio of below 1 in canola seedlings, while the ratio stabilized with further biochar and fertilizer additions. Root/shoot ratios of canola continued declining with increased biochar additions of *E. camaldulensis* biochar, again suggesting that further biochar increments may lead to more desirable aboveground growth relative to belowground growth. The B50 treatment in most cases showed the weakest overall growth of roots and shoots of both canola and wheat (more severe), and potentially points to the effects of the low-nutrient soil environment created (figure 7.5).

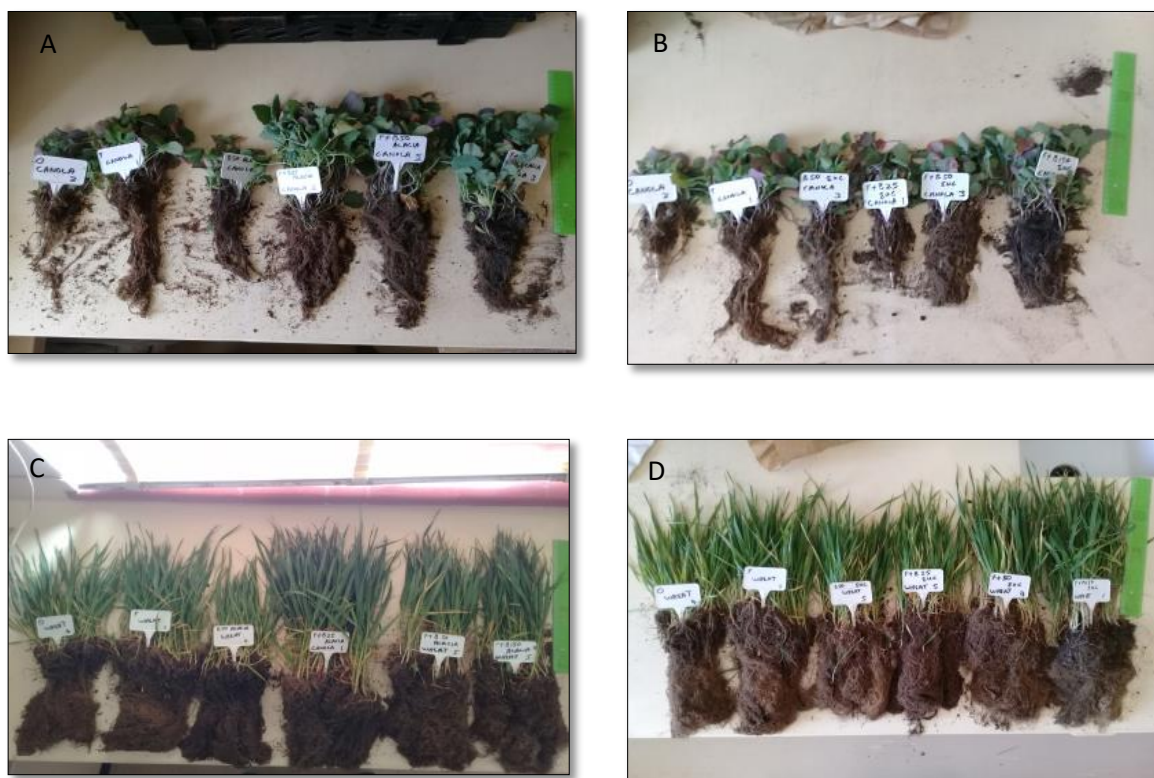


Figure 7.5: Effects of the different treatments of biochar on the root and shoot development of canola and wheat seedlings. Biochar of *A. mearnsii* are shown in A and C and biochar from *E. camaldulensis* shown in B and D. Treatments shown from left to right for both species is 0=Non-fertilized soil with no biochar; F=Fertilized soil with no biochar; B50=Non-fertilized soil with biochar; F+B25=Fertilized soil with low biochar dose; F+B50=Fertilized soil with medium biochar dose; F+B150=Fertilized soil with high biochar dose.

This study confirmed findings by Chan et al. (2007), who reported a clear increase in radish yields when treated with biochar and a nitrogen-based fertilizer, while biochar-only applications did not improve yields. The study also reported that increased biochar application

(up to 100 t ha<sup>-1</sup>) in the presence of fertilizer led to improved radish yield. Moreover, Chan et al. (2007) reported a significant increase in soil pH as a result of biochar application. Chan et al. (2007) also showed a small reduction in uptake of N by radish when biochar is applied to soil, both in the presence and absence of fertilizer. Additionally, Chan et al. (2007) reported an increase in P (Colwell) in soil when treated with increasing amounts of biochar, both in the presence and absence of fertilizer.

The study on the elemental composition of the biochars follows on from work done by Tshoke (2018; unpublished), who reported a range of effects of biochar on soil chemistry. Of these, the study reported a significant increase in soil pH and decrease in EC when treated with biochars of both *A. mearnsii* and *E. camaldulensis*. The high pH of biochar and its potential effects on soil pH in an agricultural or rehabilitation setting needs to be considered in future studies of nutrient availability and uptake by plants adapted to low-pH environments such as the Fynbos. The study also showed a significant reduction in plant available N in the form of nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) where biochar was applied compared to a control, while available P was increased in soils containing biochar. Findings of the current study show that some properties of biochar are reflected in soil samples containing biochars of similar origin and that the filtering and storage capacity of biochars in already nutrient-poor soils could significantly inhibit plant growth if not quantified and controlled for during planting. The current study used the same source material as the one done previously by Tshoke (unpublished). This is an important consideration because superficial differences between the effects of different biochars that should be attributed to differences in its production process could be mistaken for generic effects of biochar. This is because biochars from different sources and produced from varying production processes could have different effects on soil.

Mineral elements present in biochars can be directly related to what was originally present in the feedlot. In chapter 4 of this study, I explored the differences in amounts of various elements in the aboveground components of *A. mearnsii* and *E. camaldulensis* and reported significant differences in elemental composition of the two species. Some of these results are reflected in the biochar elemental analyses in this study, which reported more C, N, Ca, and Na in *A. mearnsii*, and more available P, Mg, and K in *E. camaldulensis*. The level of variation in the production processes of these biochars is, however, unknown and these results can only be used as a guide. In cases where the production process was kept similar between feedlot types, it has been shown that biochars produced from organic waste such as manure could have significantly higher nutrient concentrations than woody biomass (Chan & Xu, 2009). In areas where excess manure is available, it could potentially be considered as an augmentation to woody plant biomass, either in the feedlot or after production. This is already done in some



instances in South Africa but a lack of appropriate documentation of processes presents an important knowledge sharing gap in biochar production, use, and effectiveness (Figure 7.6).



Figure 7.6 Biochar produced in an open kiln before it is enriched with animal manure (A) and during the process of enrichment (B) by a small-scale farmer near Genadendal, Western Cape.

As mentioned before, differences in pyrolysis temperature of biochars from the same feedlot can also result in varying physicochemical properties (Trompowsky et al., 2005; Chan et al., 2008). Production temperature was not controlled for during this study and it is suggested that future studies on *A. mearnsii* and *E. camaldulensis* biochar consider this in its design. A major constraint to control of production process is the current dominant method of production, which relies on open kilns and varying quantities and quality of available feedlot. In many cases it is thus unlikely that a constant pyrolysis temperature regime could be achieved and maintained in current production systems.

The current study, however, confirmed that biochar has varying effects on crop productivity that could be attributed to crop type, biochar feedlot type, and application rate. The results here also confirm a fertilizer as a necessity with biochar application, the effects of which are also strongly related to biochar application rate and crop species. A meta-analysis (MA) of biochar effect on crop productivity was done by Jefferey et al. (2011) that considered findings from studies on different classes of soil texture, soil pH, feedstock, biochar application rate, fertilizer addition, crop type, and experiment type (pot vs. field). The study reported varied results for most variables and concluded an average of 10% increase in crop productivity. However, the study cautions against generalizations on the effect of biochar on crop health, especially given the large variation in effects with different application rates and types of biochar. Despite the MA by Jefferey et al. (2011), there is still uncertainty in the market about biochar as a potential soil amendment product. In South Africa, a similar approach to collating all available knowledge on biochar effect on crop productivity may very well be the catalyst

required to identify significant gaps and guide any future research. Additionally, standardization of biochar production methods will be a key first step in minimizing the variation not related to the treatment.

## 7.7 Conclusions

Biochar production in South Africa is potentially a viable option for value adding to invasive biomass in order to reduce the net costs of alien tree clearing and management. Unavoidable differences in feedlot and production process, however, necessitate case-specific studies that consider these variables and also the target species for cultivation. The feedlot type and the target crop species are also important considerations in the amount of biochar to be added. It is accepted that the low-nutrient nature of biochar and the adsorption of certain elements, the presence of biochar could potentially stunt crop growth if not augmented with an appropriate nutrient source. This study used a commercial NPK fertilizer but other high-nutrient sources like animal manure could be used successfully.

## 7.8 References

- Aghoghovwia, M. P. (2018). *Effect of different biochars on inorganic nitrogen availability* (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Angst, T. E., Patterson, C. J., Reay, D. S., Anderson, P., Peshkur, T. A., & Sohi, S. P. (2013). Biochar diminishes nitrous oxide and nitrate leaching from diverse nutrient sources. *Journal of environmental quality*, 42(3), 672-682.
- Bagreev, A., Bandosz, T. J., & Locke, D. C. (2001). Pore structure and surface chemistry of adsorbents obtained by pyrolysis of sewage sludge-derived fertilizer. *Carbon*, 39(13), 1971-1979.
- Beesley, L., Moreno-Jiménez, E., & Gomez-Eyles, J. L. (2010). Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. *Environmental Pollution*, 158(6), 2282–2287. <https://doi.org/10.1016/j.envpol.2010.02.003>
- Bock, E., Smith, N., Rogers, M., Coleman, B., Reiter, M., Benham, B., & Easton, Z. M. (2015). Enhanced Nitrate and Phosphate Removal in a Denitrifying Bioreactor with Biochar. *Journal of Environment Quality*, 44(2), 605. <https://doi.org/10.2134/jeq2014.03.0111>
- Botha, O. G. (2016). *The effect of composted biochar on compost properties and mineralisation* MSc thesis, Stellenbosch: Stellenbosch University).
- Burger, M. D. (2018). *Evaluating different value adding processing systems for bamboo developments* (MSc thesis, Stellenbosch: Stellenbosch University).
- Carter, S., Shackley, S., Sohi, S., Suy, T.B., and Haefele, S. (2013). The impact of biochar application on soil properties and plant growth of pot grown lettuce (*Lactuca sativa*) and cabbage (*Brassica chinensis*). *Agronomy*, 3, 404-418. doi:10.3390/agronomy3020404

- Chan, K. Y., & Xu, Z. (2009). Biochar: nutrient properties and their enhancement. *Biochar for environmental management: science and technology*, 1, 67-84.
- Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., & Joseph, S. (2008). Agronomic values of greenwaste biochar as a soil amendment. *Soil Research*, 45(8), 629-634.
- Day D, Evans RJ, Lee JW, Reicosky D (2005) Economical CO<sub>2</sub>, SO<sub>x</sub>, and NO<sub>x</sub> capture from fossil-fuel utilization with combined renewable hydrogen production and large-scale carbon sequestration. *Energy* 30:2558–2579
- Gaskin, J. W., Speir, R. A., Harris, K., Das, K. C., Lee, R. D., Morris, L. A., & Fisher, D. S. (2010). Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. *Agronomy Journal*, 102(2), 623–633. <https://doi.org/10.2134/agronj2009.0083>
- Glaser B, Lehmann J, Zech W (2002) Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – a review. *Biol Fert Soils* 35:219–230
- Kuhlbusch, T. A. (1998). Black carbon and the carbon cycle. *Science*, 280(5371), 1903-1904.
- Kumar, S., Masto, R. E., Ram, L. C., Sarkar, P., George, J., & Selvi, V. A. (2013). Biochar preparation from *Parthenium hysterophorus* and its potential use in soil application. *Ecological Engineering*, 55, 67–72. <https://doi.org/10.1016/j.ecoleng.2013.02.011>
- Laird, D., Fleming, P., Wang, B., Horton, R., & Karlen, D. (2010). Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma*, 158(3–4), 436–442. <https://doi.org/10.1016/j.geoderma.2010.05.012>
- Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *science*, 304(5677), 1623-1627.
- Lal, R., & Pimentel, D. (2008). Soil erosion: a carbon sink or source?. *Science*, 319(5866), 1040-1042.
- Lehmann, J., Czimczik, C., Laird, D., & Sohi, S. (2009). Stability of biochar in soil. *Biochar for environmental management: science and technology*, 183-206.
- Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota—a review. *Soil biology and biochemistry*, 43(9), 1812-1836.
- Li, M., Lou, Z., Wang, Y., Liu, Q., Zhang, Y., Zhou, J., & Qian, G. (2015). Alkali and alkaline earth metallic (AAEM) species leaching and Cu (II) sorption by biochar. *Chemosphere*, 119, 778–785. <https://doi.org/10.1016/j.chemosphere.2014.08.033>
- Liao, R., Gao, B., & Fang, J. (2013). Invasive plants as feedstock for biochar and bioenergy production. *Bioresource Technology*, 140, 439–442. <https://doi.org/10.1016/j.biortech.2013.04.117>
- Moller, L. (2012). *Soil yeasts, mycorrhizal fungi and biochar: their interactions and effect on wheat (Triticum aestivum L.) growth and nutrition* (MSc thesis, Stellenbosch: Stellenbosch University).
- Olivier, C. F. (2011). *An investigation into the degradation of biochar and its interactions with plants and soil microbial community* (MSc thesis, Stellenbosch: Stellenbosch University).
- Pietikäinen, J., Kiikkilä, O., & Fritze, H. (2000). Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos*, 89(2), 231-242.
- Rehrah, D., Reddy, M. R., Novak, J. M., Bansode, R. R., Schimmel, K. A., Yu, J., Watts, D.W., & Ahmedna, M. (2014). Production and characterization of biochars from agricultural by-products for use in soil quality enhancement. *Journal of Analytical and Applied Pyrolysis*, 108, 301-309.



- Saito, M. (1990). Charcoal as a micro-habitat for VA mycorrhizal fungi, and its practical implication. *Agriculture, Ecosystems & Environment*, 29(1-4), 341-344.
- Saranya, K., Krishnan, P. S., Kumutha, K., & French, J. (2011). Potential for biochar as an alternate carrier to lignite for the preparation of biofertilizers in India. *International Journal of Agriculture, Environment and Biotechnology*, 4(2), 167-172.
- Sika, M. P., & Hardie, A. G. (2014). Effect of pine wood biochar on ammonium nitrate leaching and availability in a South African sandy soil. *European journal of soil science*, 65(1), 113-119.
- Stafford, W. H., Von Maltitz, G. P., & Watson, H. K. (2018). Reducing the costs of landscape restoration by using invasive alien plant biomass for bioenergy. *Wiley Interdisciplinary Reviews: Energy and Environment*, 7(1), e272.
- Stafford, W., & Blignaut, J. (2017). Reducing landscape restoration costs: Feasibility of generating electricity from invasive alien plant biomass on the Agulhas Plain, South Africa. *Ecosystem Services*, 224–231. <https://doi.org/10.1016/j.ecoser.2017.04.008>
- Steiner, C., Rademakers, L., & Blum, W. E. (2008). Biochar carbon sequestration. *University of Georgia, Biorefining and Carbon Cycling Program, Athens, GA, 30602*.
- Tshoke, T., (2018). The effect of invasive alien plant derived biochar on the physiological properties of soil and its implications for agriculture. Unpublished.
- Uzoma, K. C., Inoue, M., Andry, H., Fujimaki, H., Zahoor, A., & Nishihara, E. (2011). Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil use and management*, 27(2), 205-212.
- Van Zwieten, L., Kimber, S., Morris, S., Chan, K. Y., Downie, A., Rust, J., Joseph, S., & Cowie, A. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and soil*, 327(1-2), 235-246.

## Chapter 8: Synthesis; Are we ignoring critical blind spots in invasive alien tree management?

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### 8.1 Introduction

Invasion by alien trees in South Africa poses a significant threat to the country's water resources, biodiversity, and agricultural land. Under the South African legislation, specifically NEMA (National Environmental Management Act), CARA (Conservation of Agricultural Resources Act), and the NWA (Nation Water Act), invasive alien trees such as *Acacia mearnsii* and *Eucalyptus camaldulensis* are to be removed from riparian zones and may not be propagated outside of woodlots dedicated for forestry purposes (Van der Linde, 2006).

Through this study, information is presented on the biomass and nutrients available in the aboveground biomass components of *Acacia mearnsii* and *Eucalyptus camaldulensis* of various affected ecosystems in the Breede River catchment. These data are only directly relevant to the sites investigated and at the time of sampling (2016-2017; trees will keep growing, and clearing operations will remove these stands in the future). The information generated through these datasets, however, will lead to much greater understanding of the impact of invasive alien trees on natural ecosystems in these and other sites. Additionally, the models developed here are suitable for a wide range of uses, including planning for biomass use in activities such as production of biochar, wood chips, and electricity, while insights into the impact of these activities on nutrient cycles (including potential carbon sequestration) are discussed. While, admittedly, this work only illustrates some the complexity of invaded systems, it should contribute significantly towards the discussion of invasive alien trees as part of current socio-economic, ecological and agricultural systems and towards shifting our focus from alien tree removal for the sake of removal to decision-making based on proper conceptualization of the current system and the desired system.

### 8.2 Study conceptualization

Effectively, this study takes a reductionist approach to understanding biomass and nutrient dynamics in invasive alien trees through the separation of tree components into leaf, branches, stem, and bark material, and by presenting aboveground biomass and nutrients as a sum of all these components (Figure 8.1). Although an attempt is made to observe the interconnectedness and internal flows of resources, the scope of this study only ventures as far as seasonality and tree life stage, and does not explicitly track exchanges of resources

between biomass components as a result of the changing phenological stages of trees. A major drawback of this approach is that the importance of root storage on nutrient cycles is not considered here. However, as management of invasive trees is generally aimed at removing aboveground biomass, the information generated during this study serves an important function in improved understanding of the implications of these management activities on ecosystem functioning and the possibilities for effective use of the cleared biomass and for ecosystem recovery.

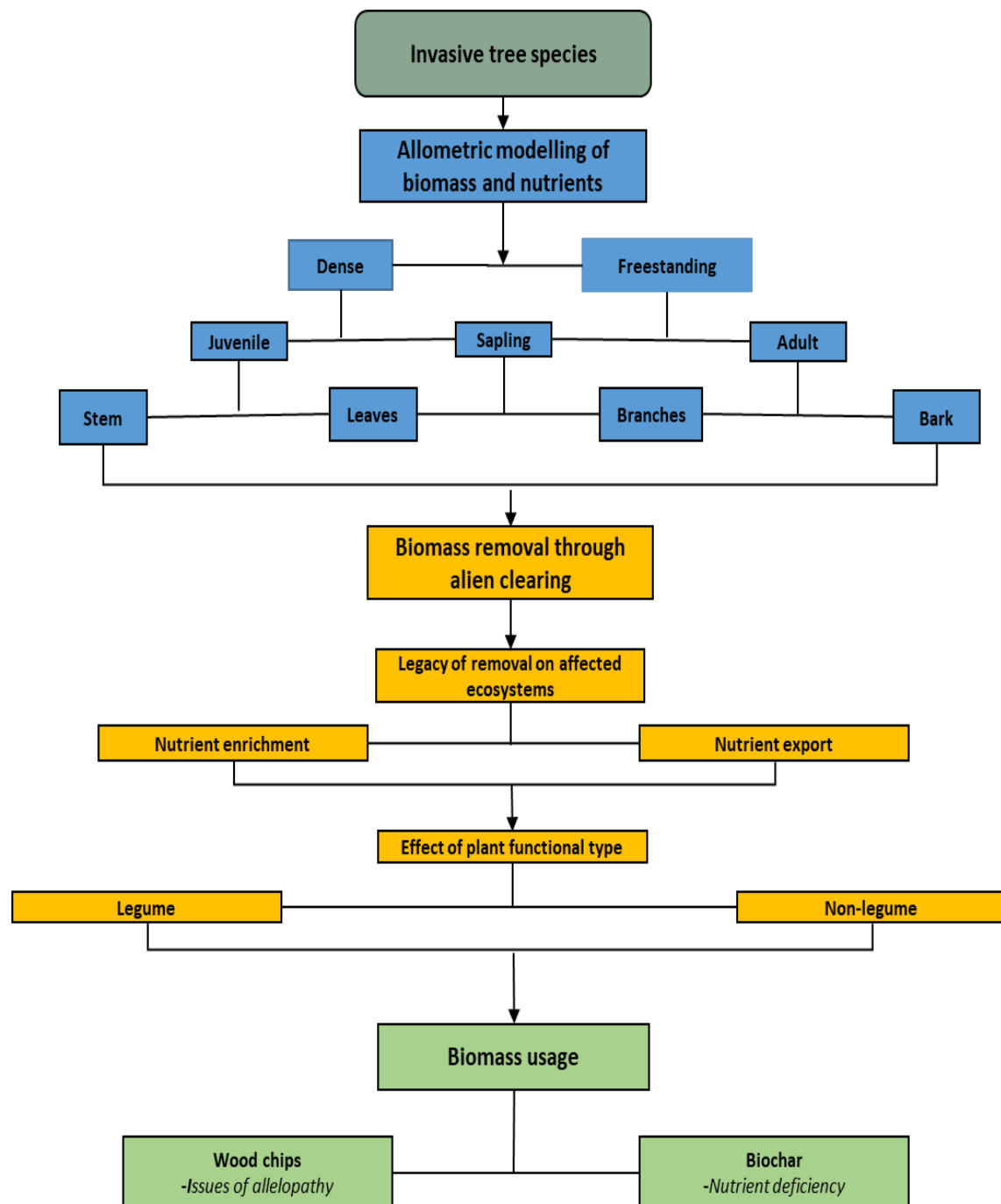


Figure 8.1: Framework used throughout this study to document biomass and nutrients in invasive stands, the potential for use of biomass products, and the potential implications for ecosystem recovery post-clearing.

The experimental layout of this study proved to be effective in modelling aboveground nutrients and biomass of the invasive *Acacia mearnsii* and *Eucalyptus camaldulensis* at different densities. The modelling exercise allowed for the determination of the total aboveground biomass in the sites investigated and the bulk volumes of nutrients in the biomass. It is widely accepted that leguminous plants contribute to soil nitrogen pools; this has

also been shown for invasive *Acacia* species in South Africa. However, little work has been done to explicitly account for the potential export of nitrogen and other nutrients in cleared biomass of both leguminous and non-leguminous species.

This study determined nutrient contents of all aboveground biomass components and reports on the potential for large-scale nutrient export for both functional types. It should be noted that it did not account for litterfall as a means of nutrient deposition. This aspect of nutrient dynamics in similar invasive stands has been reported on by Railoun (2018) who has also provided a useful comparison with native plant species. Likewise, this study did not consider nutrient stocks as a function of tree height, but rather attempted to define nutrients more broadly by growth stage. Redmond et al. (2019) suggested that low stem nutrient concentrations may be an evolutionary adaptation of invasive alien species to assist with rapid height growth. There is potential to further test this theory using the two species from this study.

### 8.3 Main findings and discussion

Through this study, a set of results have been produced as outlined below:

Chapter 3: The aboveground biomass allometry of the invasive *Eucalyptus camaldulensis* and *Acacia mearnsii*

- Stem density has a more pronounced impact on growth form of *A. mearnsii* than *E. camaldulensis*.
- Biomass allocation changes with life stage for both *A. mearnsii* and *E. camaldulensis*. Biomass allocation patterns were unique between the two species and did not follow any specific trends.

Chapter 4: Nutrient allocation of invasive *Eucalyptus camaldulensis* and *Acacia mearnsii* aboveground biomass components in riparian zones of the Western Cape, South Africa

- Consideration of open and Closed canopy trees is necessary to increase accuracy of allometric modelling of nutrients in *A. mearnsii* and in *E. camaldulensis*.

- *Eucalyptus camaldulensis* stores greater concentrations of P and total cations in its foliage than *A. mearnsii*, while *A. mearnsii* stores greater concentrations of N in its foliage.
- High concentrations of stem C and low concentrations of stem N in *A. mearnsii* lead to high C:N ratios and this is more pronounced in Open canopy sites
- Leaves of juvenile *A. mearnsii* trees had the highest N:P ratios, which were more than twice that of adult *A. mearnsii* trees.

#### Chapter 5: Nutrient export in biomass a potential stressor on riparian zones invaded by *Acacia mearnsii* and *Eucalyptus camaldulensis*

- Due to its comparatively greater concentrations of nutrients in photosynthetically active biomass components, *E. camaldulensis* removal could lead to greater net export of nutrients than removal of *A. mearnsii* and could have a more severe impact on soil nutrient reserves. However, the site with the greatest biomass and nutrient standing stocks recorded here is a high-density *A. mearnsii* site.
- Significant amounts of carbon are contained in aboveground biomass of invasive species. The removal of invasive trees could lead to significant reduction of carbon sequestering capacity where rehabilitation does not account for the carbon sequestering capacity lost through alien tree removal.
- While large-scale nutrient export through biomass removal is highly likely, nutrient accumulation as a result of invasion is still a significant threat to affected ecosystems, especially in naturally nutrient-poor systems such as the Fynbos. Nutrient budgets for relevant sites need to be developed to account for both aspects of nutrient changes as a result of invasion.

#### Chapter 6: Value-added products from invasive biomass: allelopathic potential of various biomass components on growth of wheat and canola

- Allelopathic influence of both species has been shown to be confined to the photosynthetically active biomass components and is dose-dependent.
- Wood chips' influence on crop development/ growth is specific to the type of chips used, and the receiver species.
- Wood chips could significantly increase soil nutrient storage and can possibly assist with ecosystem recovery post-clearing.

Chapter 7: Value-added products: the effect of biochar application on crop health is dependent in the addition of sufficient amounts of fertilizer

- Biochar should not be applied to soil without an appropriate fertilizer, due to the nutrient sequestering nature of the product.
- The production process of biochar need to be standardized to make more accurate recommendations.
- Biochar could also be used to increase soil functioning but needs more studies to produce more generalizable results.

Through this study I have quantified the effect that differences in stem densities has on the growth form of invasive alien trees. This ground-truthing addresses a possible shortcoming of remote sensing applications; by measuring actual canopy cover and presenting the data as a theoretical 100% canopy cover, these models may not account for the differences in tree shape, and the implications for differences in percentages of biomass allocation to various aboveground components. For instance, it is reported here that Open canopy *A. mearnsii* trees (canopy cover of less than 25%) have an adult foliar allocation of greater than 20% of their total aboveground biomass, while trees in Closed canopy stands on average allocate less than 10% of their total aboveground biomass to foliage. This is likely due to the differences in canopy width between trees grown in different densities, and also differences in height of lowest active branches (plants in Closed canopy stands typically have minimal foliar growth lower down the stem). It is thus suggested that variation in tree growth form as a response to stem densities/canopy cover be considered in future modelling exercises, especially in invasive stands.

As noted before, nutrient deposition by invasive *Acacia* species is widely reported on in South Africa (Witkowski , 1991; Tye & Drake, 2012; Jacobs et al., 2013). It is also likely that invasive *Eucalyptus* species promote mycorrhizal activity in soil and hence lead to local enrichment of nutrients such as phosphorus. Additionally, nutrients accumulated in leaf litter either from mineral or atmospheric sources are likely to further lead to soil nutrient enrichment through litterfall (Nsikani et al., 2018 Railoun, 2018). Nutrient deposition in this manner has already been shown to promote secondary invasion in cleared landscapes (Nsikani et al., 2018). Excess soil nutrients may also affect neighbouring water bodies and aquatic biodiversity negatively (Tye & Drake 2012). As shown here, unintended consequences of alien tree removal could be largely underestimated or even completely discounted. One such consequence these results emphasise is large-scale export of nutrients in an already nutrient-poor Fynbos ecosystem. This could severely impact local nutrient cycles and may not be



readily replaced by atmospheric or mineral sources. Additionally, the loss of carbon sequestering potential of cleared sites has not yet been reported on for invasive stands but may be an important and unavoidable result of alien tree removal. The current study shows that up to  $63 \text{ t ha}^{-1}$  of C could be stored in aboveground biomass of heavily invaded *A. mearnsii* sites. This amount of storage capacity could thus be lost, while the total amounts of C countrywide lost through clearing could be up to 87.12 million tonnes, as per the estimation by Le Maître (2016) of 1.32 million condensed hectares of *A. mearnsii* invasion in South Africa ( $63 \text{ t ha}^{-1} \times 1.32 \text{ million ha}$ ). These assertions, however, do not suggest that clearing of invasive alien trees in South African riparian zones is not necessary. Rather, they call for a revision of how the process is viewed within the context of its externalities. Whereas some nutrients may be significantly enriched in post-clearing landscapes, others may be severely depleted.

The potential usefulness of invasive biomass in agriculture is highlighted here through experimentation with wood chips and biochar. By using invasive biomass in this manner, one not only provides economic incentive for further alien clearing, but aspects of local legislation are also addressed, namely i) the protection of soil from erosion (through mulching), and ii) the protection of hydrological systems from degradation (CARA). Additionally, if managed properly, large volumes of carbon could be returned to the soil to help rebuild rapidly diminishing soil reserves, and help realise the climate change mitigation potential of agriculture. While this aspect of the study focuses on agricultural landscapes, I suggest follow-up studies on the efficacy of these practices in post-clearing landscapes with appreciation for the significant export of nutrients from such sites. Development of these Value-Added Products also creates markets and revenue opportunities for those involved that are not limited to clearing and follow-up clearing. While alien tree removal has been shown as the most cost effective way of catchment management (Morokong et al., 2017), developing of various VAPs greatly increases its cost-effectiveness and involves the private sector in alien tree removal (Nkambule et al., 2017). The net benefit of turning IAP biomass into VAPs in KZN, for instance, could be between R96 million and R140 million (depending on the possibility of a co-financing agreement of 20% from the private sector) at clearing rates of 21,581 ha and 31,520 ha between 2008 and 2030. This translates to a net benefit of approximately 60% of the total financial benefit of alien tree removal (alongside benefits such as water savings, increase in grazing potential and increases in crop production) (Nkambule et al., 2017). Similar analyses such as this one is needed for the CFR, that also provides detail on the production costs of various VAPs and its market value.

An issue not discussed in great detail in this dissertation is that of an ecosystem with compromised ecological function as a driver of invasion. Most of our understanding of invaded systems stems from the idea of invasion as the first step in ecosystem degradation. This

assertion allows for a narrative depicting invasive alien trees as drivers of ecological change, and not necessarily as a result of change. This has been discussed by Davis et al. (2000), who suggested that a plant community could be susceptible to invasion when there is an excess of unused resources available. Davis et al. (2000) postulated that disturbance to an ecosystem could reduce plant cover and result in lowered use of resources such as light, water, and nutrients. As resource availability fluctuates over time thus, so does the invasibility of an ecosystem (Myser, 1993; Huston, 1994; Davis et al., 2000). Therefore, the importance of maintaining healthy, resilient ecosystems is often overlooked as a critical tool for preventing invasion of new areas and thus further spread of invasive plant species. Larson (2007) also argues that invasion may not be the main driver of ecological change in areas where it occurs, and that invasion is often a product of a prior anthropogenic influence. This view also supports the proposition of Kennedy (2002), who suggested that a healthy and biodiverse ecosystem could act as its own defence against invasion. Similarly, Scorer et al. (2017) showed that disturbed grasslands in high-lying areas of the Eastern Cape are more prone to invasion by *A. mearnsii* than intact grasslands. In this context (Scorer et al., 2017), the cause of disturbance can be linked to intensive agriculture. It is thus highly likely that an ecosystem's disturbance history has a significant influence on its invasibility, not less so than the disturbance caused by the invasion itself. Hence, while alien tree removal is important, protecting intact ecosystems may be as important in managing IAPs and preventing further spread. It is also recommended that ecosystems vulnerable to invasion are identified and rehabilitated before IAPs could establish. Lastly, cleared ecosystems need to be managed effectively to prevent reinvasion by the same species (through its seedbank) or colonization of other secondary invaders.

To maximise the potential of a previously invaded site to become fully functional, biologically diverse, and ecologically resilient, an IAP management program requires four steps: clearing, follow-up clearing, rehabilitation, and maintenance (Figure 8.2). Clearing without follow-up clearing is highly likely to result in re-invasion or invasion by opportunistic species due to increased availability of space, light, high numbers of propagules in the soil, and alterations to soil nutrient availability (Holmes et al., 1987; Holmes et al., 2008; Nsikani et al., 2018). Similarly, in the absence of rehabilitation, clearing and follow-up clearing are not likely to prevent reinvasion (Holmes et al., 2008). This is because ecosystem function is compromised and needs to be re-established through a targeted intervention program that systematically introduces critical ecosystem components such as soil protection features and plant cover (Holmes et al., 2020). This study has shown that biomass in the form of wood chips can be used in agricultural soil to enhance growth of crops. There is scope for this to be tested in post-clearing landscapes, where a fraction of the nutrients in biomass material could

be returned to the soil over time. Lastly, maintenance allows for the opportunity to upgrade previous work done, to troubleshoot potential issues effectively, and to introduce and encourage establishment of later-successional species, in turn increasing the resilience of such a site and significantly reducing its potential for reinvasion. This study only went as far as discussing removal of biomass, management of biomass, and some aspects of soil rehabilitation. It is important that other aspects of IAP management be refined further, including follow-up clearing and long-term rehabilitation programs aimed at ecological and socioeconomic resilience to improve the impact of ongoing IAP clearing initiatives.

This framework assumes that the objective of an alien clearing program is to achieve a fully functional, biologically diverse, and ecologically resilient ecosystem. The importance of this approach, however, is that it has far-reaching consequences. Firstly, it requires a shift from indicators such as hectares cleared and person days administered, to indicators based on ecosystem function, and development of social and economic capital. It also requires a multi-year investment into a site, rather than a few months, and more investment into upskilling of teams to conduct alien clearing, biomass management, soil stabilization, follow-up clearing, nursery management for propagation of indigenous plants, and conducting monitoring and evaluation programs. The view of Larson (2007) that invasion should not be seen as independent of social systems is thus supported here, along with the call for greater interdisciplinarity in management of invasive species (Vaz et al., 2017).

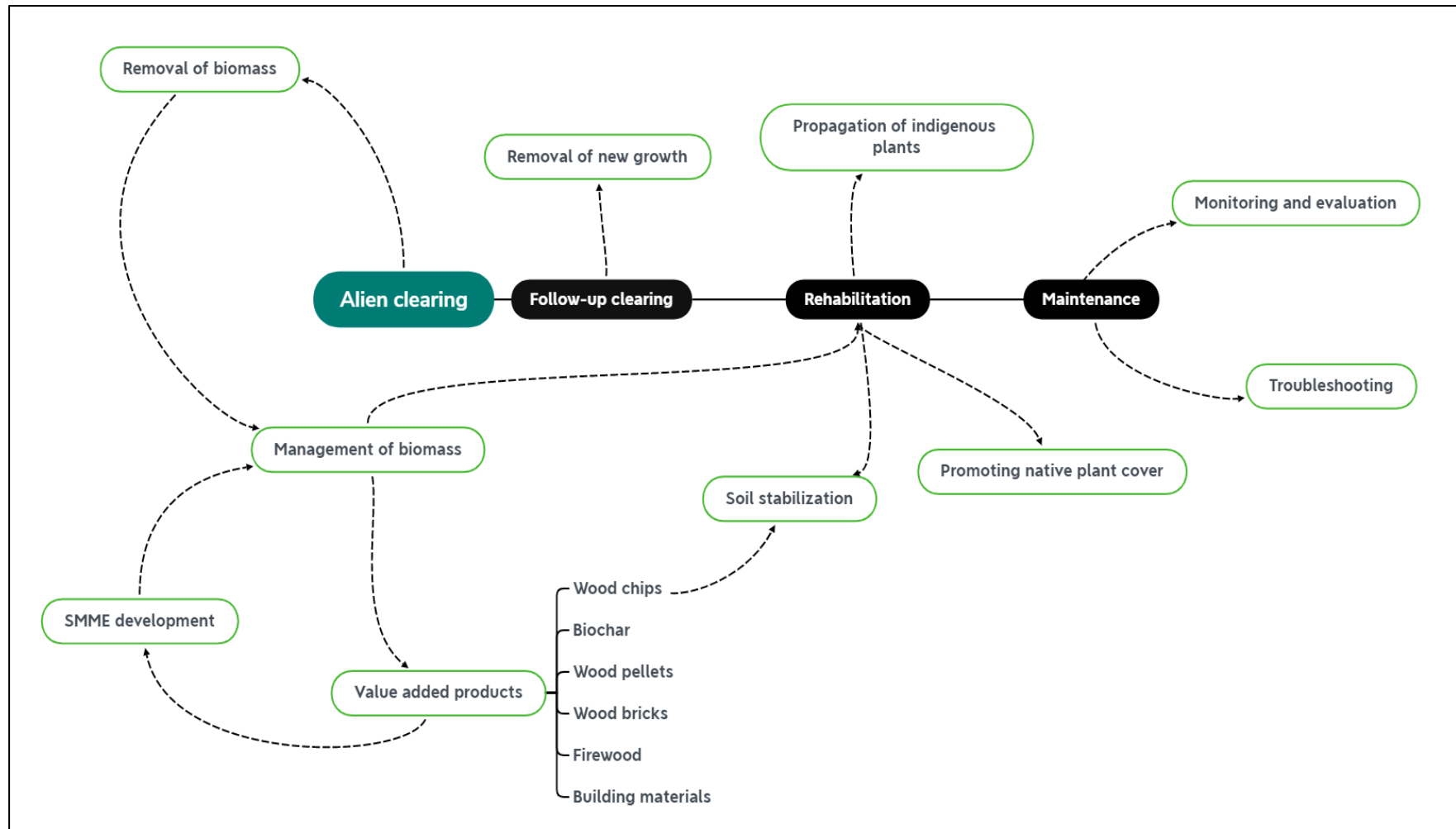


Figure 8.2: Steps necessary in management of sites invaded by invasive alien trees to ensure ecological resilience, climate adaptation, restoration of hydrological functionality, and promote the development of social and economic capital.

Invasive alien trees do not exist in isolation and should not be treated as such. This dissertation shows that invasion needs to be discussed as an important part of social, economic, agricultural, and hydrological systems to holistically approach its management. In a South African context, Gaertner et al. (2012) suggested a framework for rehabilitation of sites post-clearing, which relies heavily on the understanding of the specific ecosystem from the perspectives of rehabilitation practitioners and ecologists, within the confines of ecological and economic feasibility, showing that alien clearing should realistically only be done where resources are allocated for effective removal of biomass, follow-up clearing, and rehabilitation. It is important that the framework for rehabilitation post-clearing by Gaertner et al. (2012) is supported by similar frameworks for other aspects of invasion, including issues such as biomass management, control of secondary invasion, and implementation of programs for prevention of invasion of new systems. Additionally, more in-depth consideration of the socio-economic value of invasive alien trees should be encouraged, to further inform management decisions. There are undoubtedly many more blind spots in our understanding of invasion in South Africa. In the wake of global climate change, increasingly more unpredictable rainfall patterns locally, and soaring local unemployment rates, management of invasive alien trees needs to evolve past simply clearing, follow-up clearing and ecosystem rehabilitation, and consider invasion as a part of many complex systems.

## 8.4 References

- Blanchard, R., & Holmes, P. M. (2008). Riparian vegetation recovery after invasive alien tree clearance in the Fynbos Biome. *South African Journal of Botany*, 74(3), 421-431.
- Davis, M. A., Grime, J. P., & Thompson, K. (2000). Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*, 88(3), 528-534.
- Gaertner, M., Larson, B. M., Irlich, U. M., Holmes, P. M., Stafford, L., van Wilgen, B. W., & Richardson, D. M. (2016). Managing invasive species in cities: A framework from Cape Town, South Africa. *Landscape and Urban Planning*, 151, 1-9.
- Geldenhuys, C. J., Atsime-Edda, A., & Meguro, M. W. (2017). Facilitating the recovery of natural evergreen forests in South Africa via invader plant stands. *Forest Ecosystems*, 4(1), 21.
- Henderson, L., & Wilson, J. R. (2017). Changes in the composition and distribution of alien plants in South Africa: An update from the Southern African Plant Invaders Atlas. *Bothalia-African Biodiversity & Conservation*, 47(2), 1-26.
- Holmes, P. M., Esler, K. J., Gaertner, M., Geerts, S., Hall, S. A., Nsikani, M. M., Richardson, D. M., & Ruwanza, S. (2020). Biological invasions and ecological restoration in South Africa. In *Biological Invasions in South Africa* (pp. 665-700). Springer, Cham.
- Holmes, P. M., Esler, K. J., Richardson, D. M., & Witkowski, E. T. F. (2008). Guidelines for improved management of riparian zones invaded by alien plants in South Africa. *South African Journal of Botany*, 74(3), 538-552.

- Holmes, P. M., Macdonald, I. A. W., & Juritz, J. (1987). Effects of clearing treatment on seed banks of the alien invasive shrubs *Acacia saligna* and *Acacia cyclops* in the southern and south-western Cape, South Africa. *Journal of Applied Ecology*, 1045-1051.
- Huston, M.A. (1994). *Biological Diversity*. Cambridge University Press, Cambridge, UK.
- Jacobs, S., Naude, M., Slabbert, E., Kambaj, O., Fourie, M., Esler, K., Jacobs, K., Mantlana, B., Rozanov, A., & Cowan, D. (2013). Identifying relationships between soil processes and biodiversity to improve restoration of riparian ecotones invaded by exotic acacias. *Report to the Water Research Commission. Report No. 1927/1/13*
- Johannsmeier, M. F. (2016). Beeplants of South Africa. Sources of nectar, pollen, honeydew and propolis for honeybees. South African National Biodiversity Institute.
- Kennedy, T. A., Naeem, S., Howe, K. M., Knops, J. M., Tilman, D., & Reich, P. (2002). Biodiversity as a barrier to ecological invasion. *Nature*, 417(6889), 636.
- Larson, B. M. (2007). Who's invading what? Systems thinking about invasive species. *Canadian Journal of Plant Science*, 87(5), 993-999.
- Le Maître, D. C., Forsyth, G. G., Dzikiti, S., & Gush, M. B. (2016). Estimates of the impacts of invasive alien plants on water flows in South Africa. *Water SA*, 42(4), 659-672.
- Morokong, T., Blignaut, J., Nkambule, N. P., Mudhavanhu, S., & Vundla, T. (2016). Clearing invasive alien plants as a cost-effective strategy for water catchment management: The case of the Olifants River catchment, South Africa. *South African Journal of Economic and Management Sciences*, 19(5), 774-787.
- Myster, R.W. (1993) Tree invasion and establishment in old field at Hutcheson Memorial Forest. *The Botanical Review*, 59, 251-572.
- Nkambule, N. P., Blignaut, J. N., Vundla, T., Morokong, T., & Mudavanhu, S. (2017). The benefits and costs of clearing invasive alien plants in northern Zululand, South Africa. *Ecosystem Services*, 27, 203-223.
- Nsikani, M. M., van Wilgen, B. W., & Gaertner, M. (2018). Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N<sub>2</sub>-fixing woody species: implications for ecological restoration. *Restoration ecology*, 26(2), 235-244.
- Railoun, M. Z. (2018). Impacts of the invasive tree *Acacia mearnsii* on riparian and instream aquatic environments in the Cape Floristic Region, South Africa (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Shackleton, C. M., McGarry, D., Fourie, S., Gambiza, J., Shackleton, S. E., & Fabricius, C. (2007). Assessing the effects of invasive alien species on rural livelihoods: case examples and a framework from South Africa. *Human Ecology*, 35(1), 113-127.
- Tye, D. R., & 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.
- Van der Linde, M. (Ed.). (2006). *Compendium of South African environmental legislation*. PULP.
- Van der Vyver, M. L., & Cowling, R. M. (2019). Aboveground biomass and carbon pool estimates of *Portulacaria afra* (Spekboom)-rich subtropical thicket with species-specific allometric models. *Forest Ecology and Management*, 448, 11-21.
- Van der Vyver, M. L., Cowling, R. M., Mills, A. J., & Difford, M. (2013). Spontaneous return of biodiversity in restored subtropical thicket: *Portulacaria afra* as an ecosystem engineer. *Restoration ecology*, 21(6), 736-744.
- Vaz, A. S., Kueffer, C., Kull, C. A., Richardson, D. M., Schindler, S., Muñoz-Pajares, A. J., Vicente, J. R., Martins, J., Hui, C., Kuhn, I., & Honrado, J. P. (2017). The progress of interdisciplinarity in invasion science. *Ambio*, 46(4), 428-442.

Witkowski, E. T. F. (1991). Growth and competition between seedlings of *Protea repens* (L.) L. and the alien invasive, *Acacia saligna* (Labill.) Wendl. in relation to nutrient availability. *Functional Ecology*, 101.



## Appendix A: Biomass models

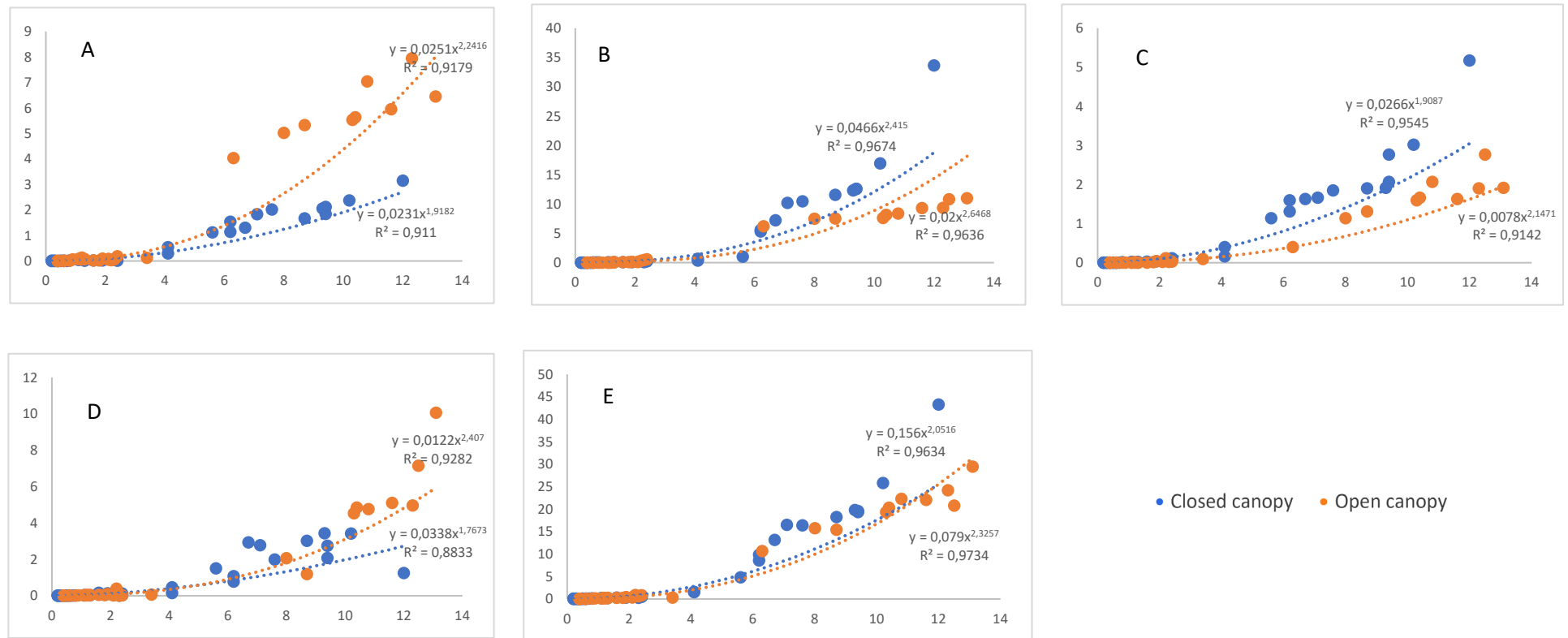


Figure A1: Biomass models for *A. mearnsii* leaves (A), Stem (B), bark (C), branches (D) and total (E) at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and biomass (kg) is shown on the y-axis

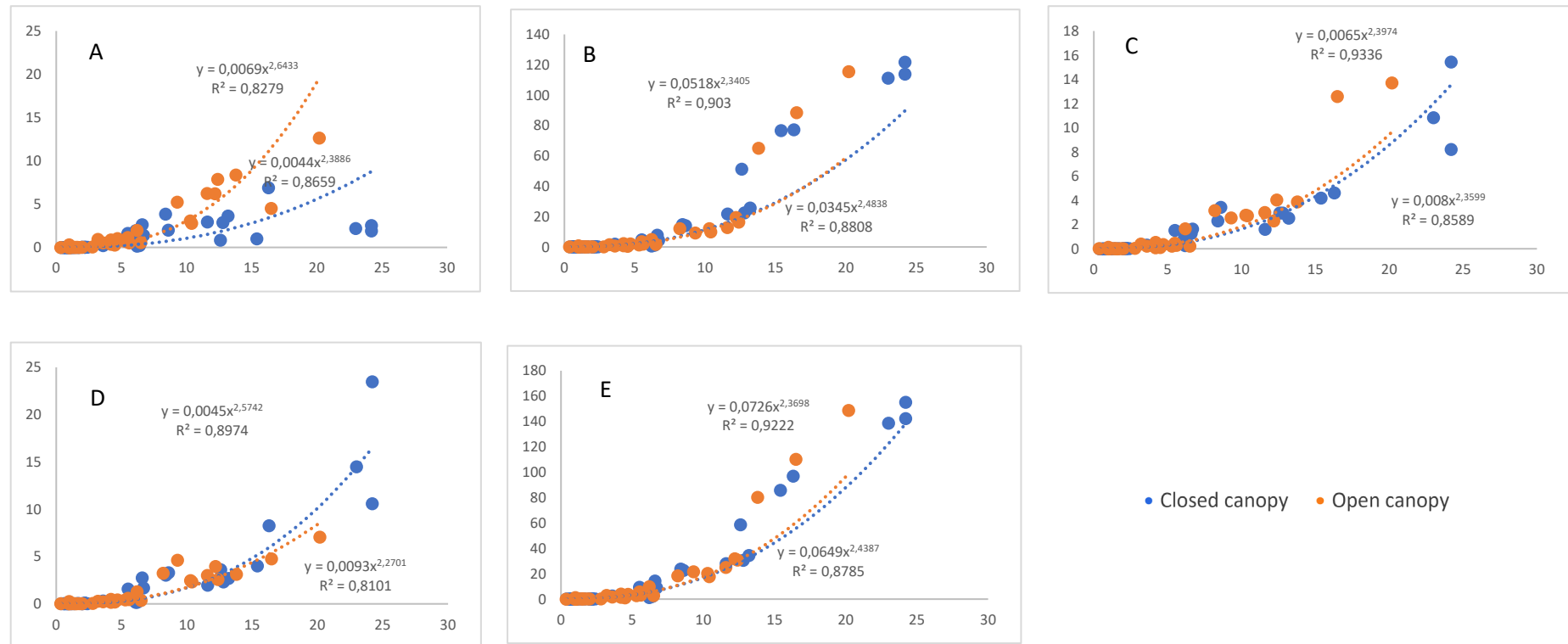


Figure A2: Biomass models for *E. camaldulensis* leaves (A), Stem (B), bark (C), branches (D) and total (E) at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and biomass (kg) is shown on the y-axis

## Appendix B: nutrient models

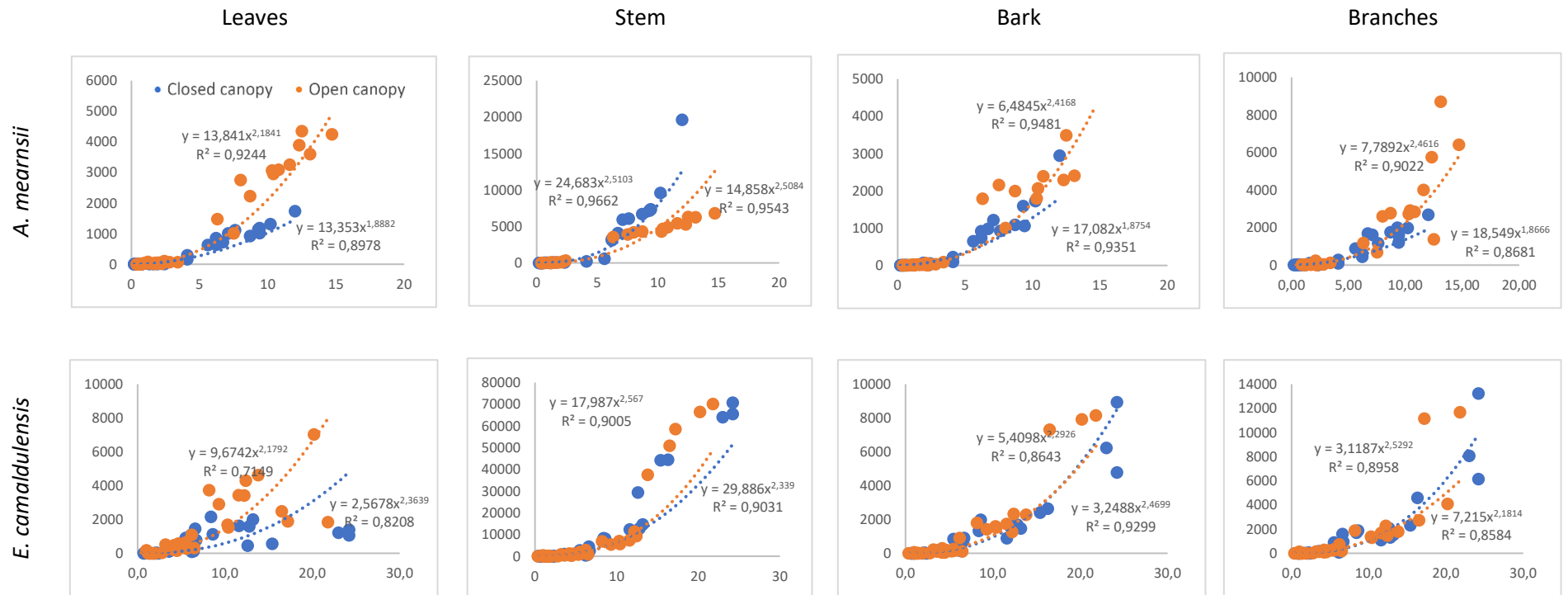


Figure B1: Carbon models for *A. mearnsii* and *E. camaldulensis* at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and mass (g) is shown on the y-axis

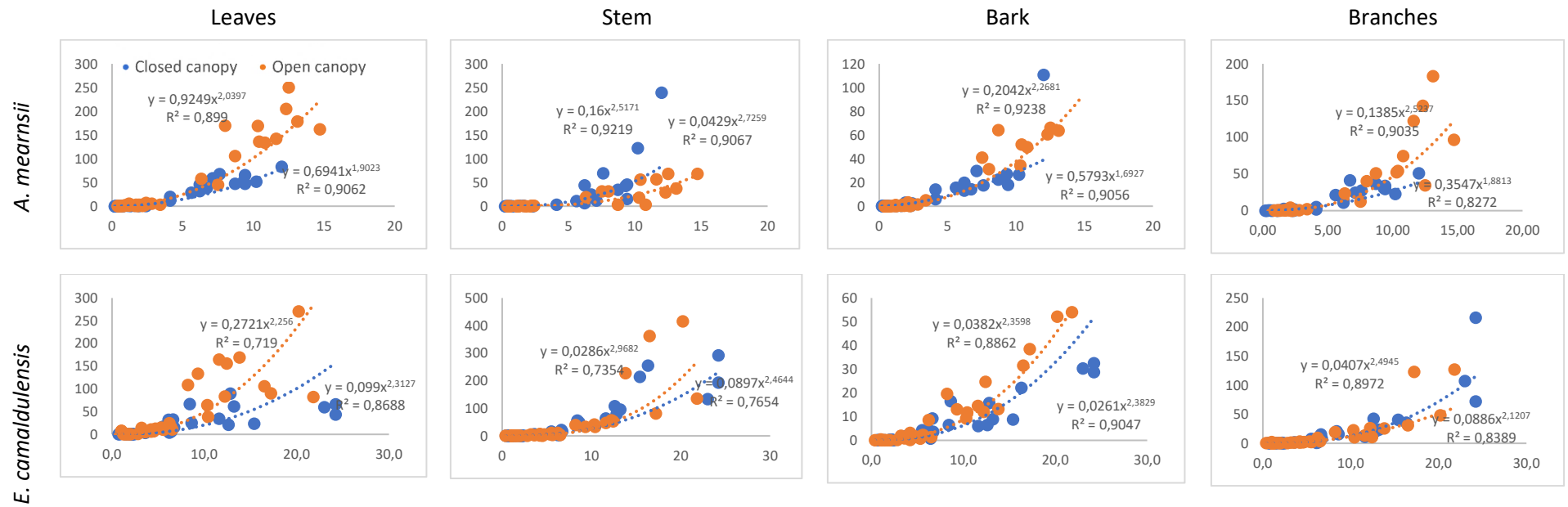


Figure B2: Nitrogen (N) models for *A. mearnsii* and *E. camaldulensis* at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and mass (g) is shown on the y-axis

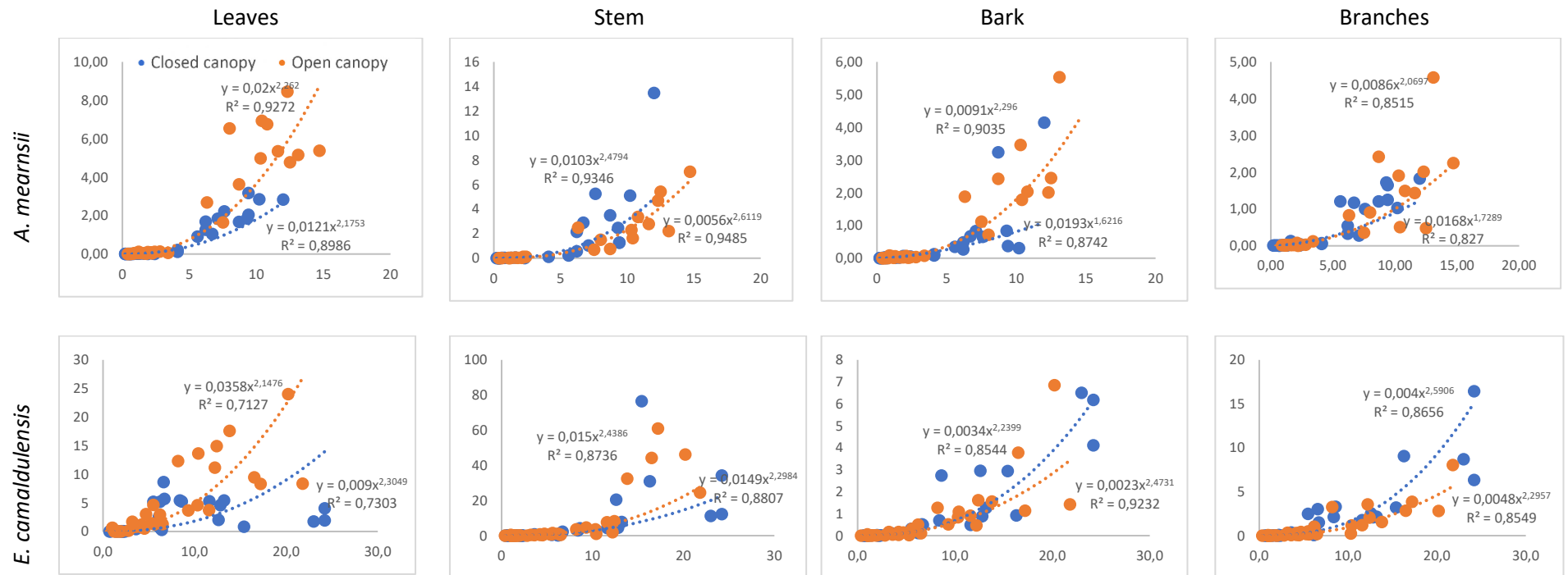


Figure B3: Phosphorus (P) models for *A. mearnsii* and *E. camaldulensis* at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and mass (g) is shown on the y-axis

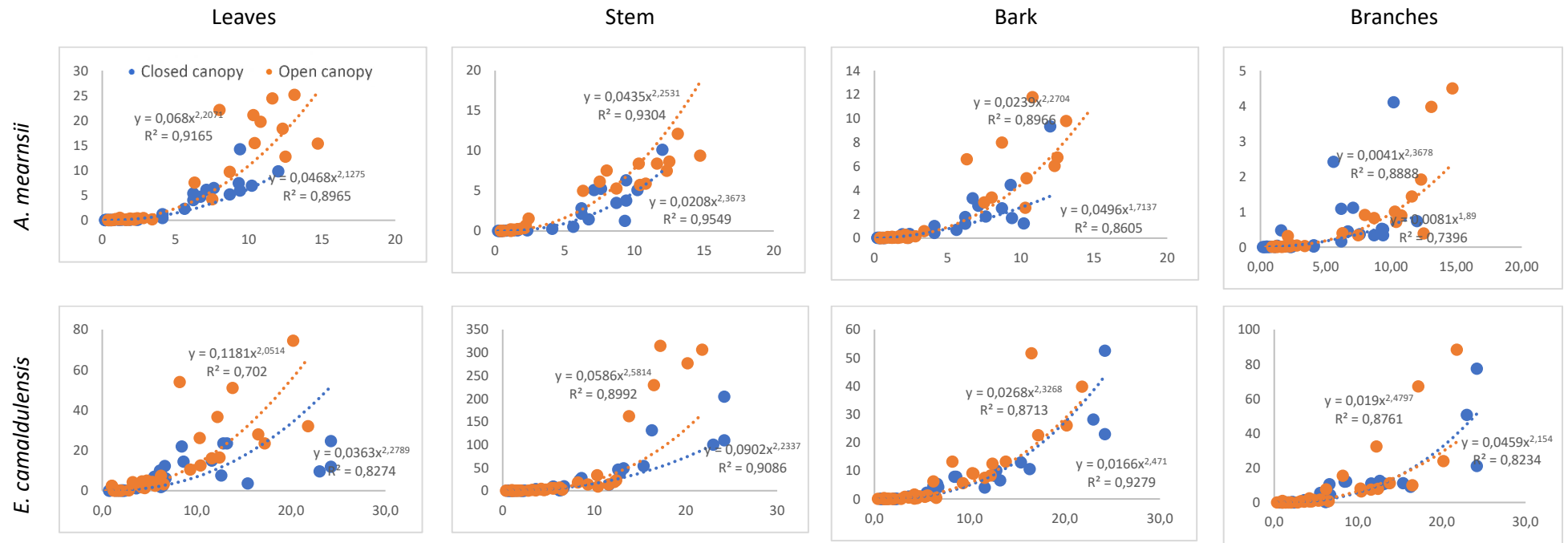


Figure B4: Potassium (K) models for *A. mearnsii* and *E. camaldulensis* at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and mass (g) is shown on the y-axis

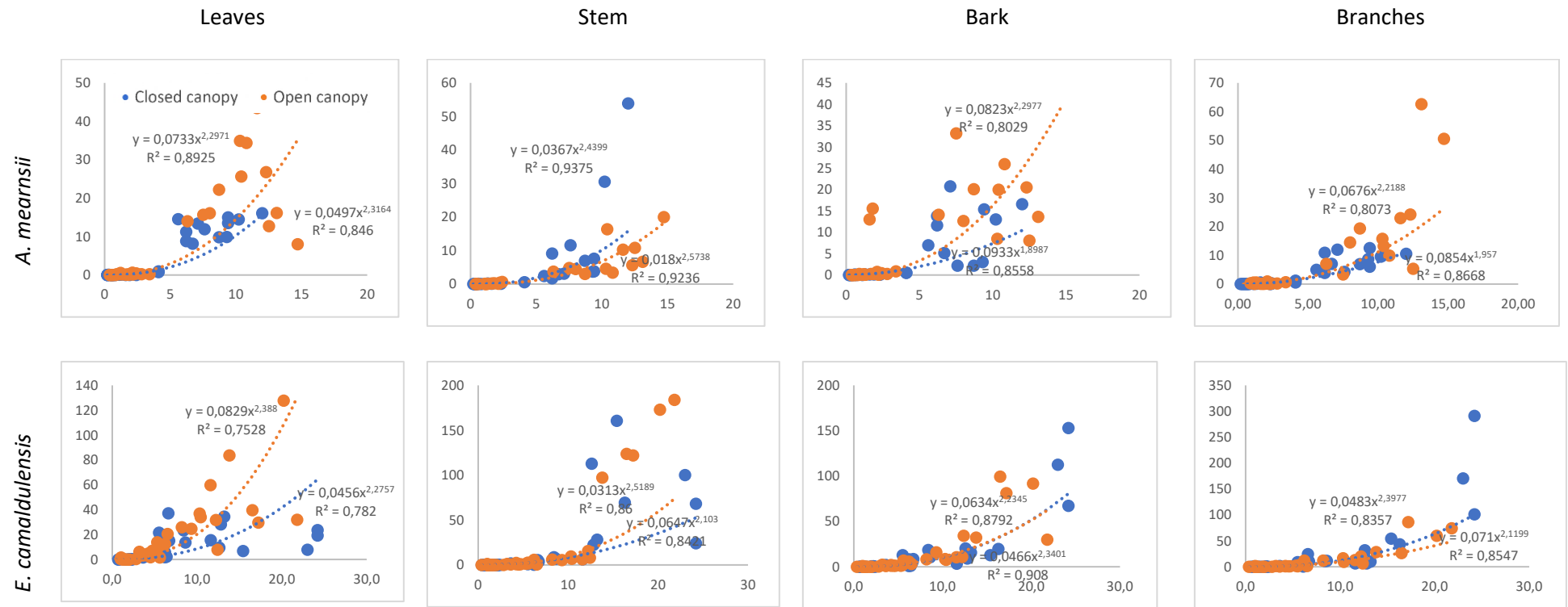


Figure B5: Calcium (Ca) models for *A. mearnsii* and *E. camaldulensis* at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and mass (g) is shown on the y-axis



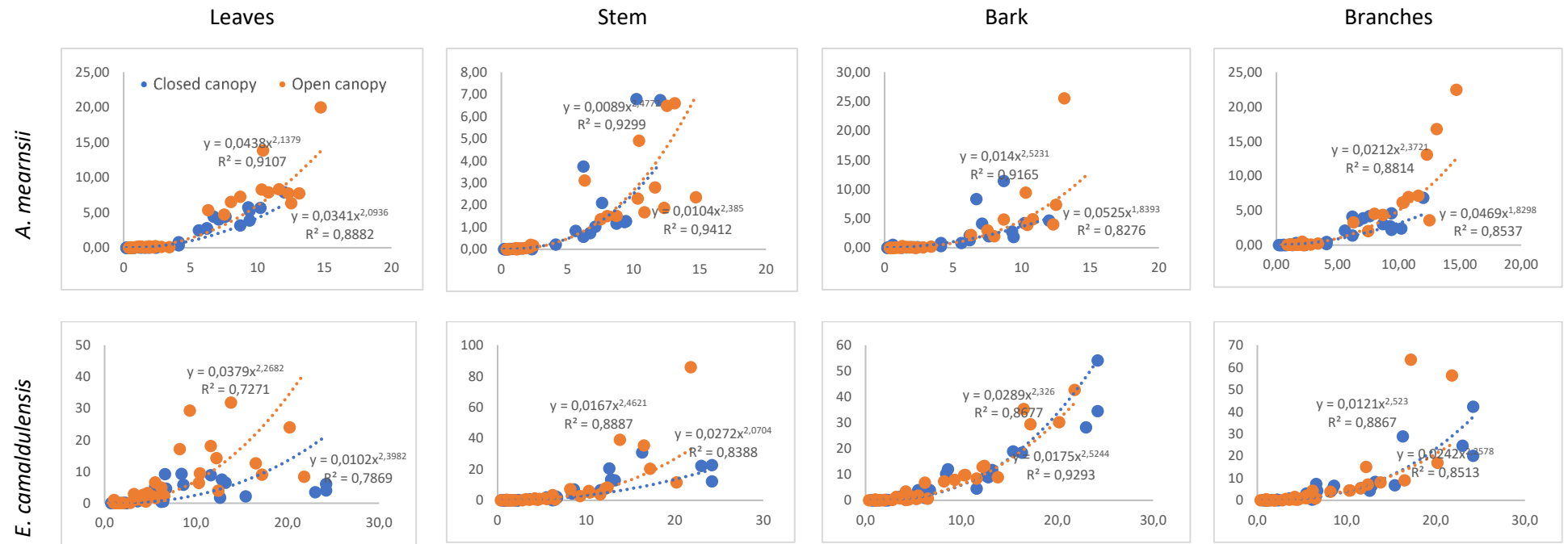


Figure B6: Magnesium (Mg) models for *A. mearnsii* and *E. camaldulensis* at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and mass (g) is shown on the y-axis

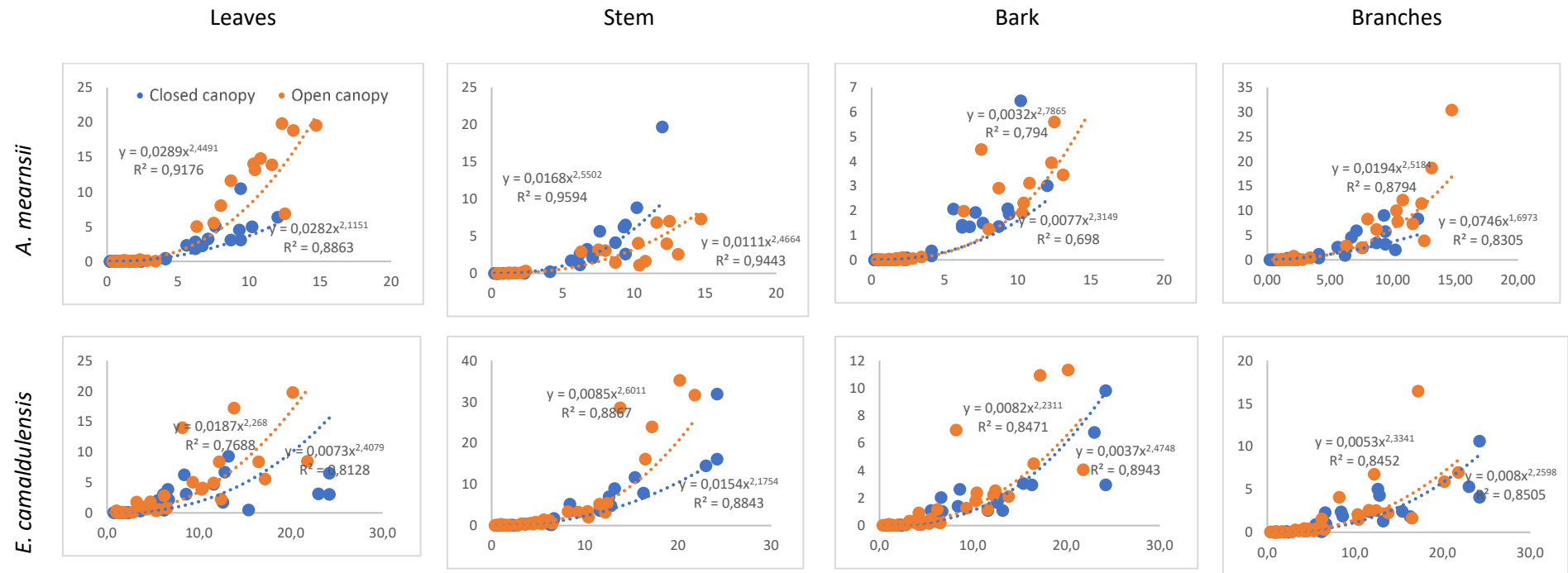


Figure B7: Sodium (Na) models for *A. mearnsii* and *E. camaldulensis* at the two density extremes: open canopy (orange) and closed canopy (blue). Basal diameter (BD; cm) is shown on the x-axis and mass (g) is shown on the y-axis