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Nutrient and biomass allocation strategies in an invasive Australian *Acacia* and a co-occurring native *Acacia* in South Africa

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DECLARATION

I **Donovan Tye** declare that this dissertation is my own, original and unaided work. It is being submitted for the Degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.



(Signature of Candidate)

08/10/2013

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ABSTRACT

The introduction of alien invasive plants can have fundamental impacts on ecosystem functioning, such as the cycling of limiting nutrients between plant and soil pools. Such impacts are primarily mediated through differences in physiological traits between the invasive and native species. In South Africa, invasions by Australian acacias (Fabaceae) are causing widespread ecosystem-level disturbances, including changes to N cycling through increased bioavailable N inputs. To assess potential alterations to nutrient dynamics, I compared aboveground biomass and N and P standing stocks of a native (*Acacia ataxacantha*) and an invasive Australian acacia (*A. dealbata*) which is displacing *A. ataxacantha* in a riparian woodland in eastern South Africa. This entailed the development of species-component specific allometric models for biomass prediction, determination of novel N contributions through symbiotic N₂-fixation, investigation of seasonal N and P allocation in aboveground components and the evaluation of nutrient conservation strategies in these species. Stem diameter was a good predictor of aboveground biomass in both acacias (accounting for 90-98% of the variation in biomass across species) which differed primarily in terms of higher leaf biomass allocation in the invasive acacia. N isotope analyses showed that *A. dealbata* trees derived more than 70% of their leaf N through N₂-fixation, while *A. ataxacantha* (a non-nodulating acacia) did not fix N. Seasonal N and P concentrations, analysed in aboveground components over a nine months period, were generally higher with more pronounced seasonal fluctuations in the deciduous *A. ataxacantha* than in the evergreen *A. dealbata*. Mature leaf N concentrations were considerably higher than the global averages, while green leaf P concentrations were low. Inefficient N-resorption was found in both acacias, most notably in the invasive (mean = 27% N resorbed), however P was tightly conserved (74 – 88% of leaf P resorbed). Weak N conservation in the invasive acacia was attributed to both a high N₂-fixing capacity and a long leaf lifespan, both of which have been hypothesised to carry evolutionary trade-offs in nutrient conservation and allocation. Theoretical considerations and the data available suggest that phosphorus is the limiting nutrient in this system. Standing stock models suggested that N input rates via litterfall were >4-fold higher in *A. dealbata* relative to *A. ataxacantha*, and that novel N inputs by *A. dealbata* are higher than total N inputs by *A. ataxacantha*. Overall, this study has provided considerable evidence that invasions by the alien invasion *A. dealbata* will increase N cycling rates and inputs considerably, and to a lesser extent, increase rates of P cycling. Invasive

Australian acacias successfully compete for resources in non-native environments, allowing them to proliferate at remarkable rates under variety resource limitations.

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CHAPTER 1: INTRODUCTION AND STUDY OUTLINE

1.1. Motivation for Study

The introduction of alien invasive plants can have important impacts on ecosystem functioning, such as the cycling of nutrients between plant and soil pools (Vitousek, 1990; Vitousek *et al.*, 1997b; Levine *et al.*, 2003). These impacts are primarily mediated through differences in physiological traits between the alien invasive and indigenous species, with clear alterations expected with the introduction of novel traits. Consequently, a substantial body of research has focused on alien plant invasions where the invader represents a new functional type in the non-native ecosystem due to a novel trait (Vitousek and Walker, 1989; Stock *et al.*, 1995; Yelenik *et al.*, 2004). These include dramatic examples of invader impacts on nitrogen cycling, such as the work on *Myrica Fava* in Hawaii by Vitousek and Walker (1989), invasive Australian *Acacia* invasions in the Cape Floristic Region in South Africa and invasive broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*) in riparian systems in New Zealand (Drake, 2011). Such examples clearly demonstrate the potential for invasive N₂-fixing plants to alter N dynamics in non-native environments. However, it is less clear how introductions of exotic members of a functional group already present in a system impact ecosystem functioning.

Within-functional group comparisons of native and exotic species demonstrate considerable variation in functional trait characteristics between members (e.g. Hooper and Vitousek, 1998; Tye and Drake, 2012). In fact, it stands to reason that even relatively minor differences in the physiological traits involved in nutrient cycling (N₂-fixation, leaf-lifespan) can have profound impacts when the alien invasive species outcompetes one more dominant resident species.

In South Africa, invasions by Australian acacias (1012 species in the subgenus *Phyllodeneae* DC native to Australia; Miller *et al.*, 2011; Richardson *et al.*, 2011) are causing widespread ecosystem-level disturbances, including simultaneous changes in native communities, soil moisture regimes, and nutrient cycling (Witkowski, 1991; Stock *et al.*, 1995; Dye and Jarmain, 2004; Yelenik *et al.*, 2004). In addition to ecological impacts, *Acacia* invasions have been shown to have a range of socio-economic impacts related to the delivery of ecosystem services and the benefits to

society (Le Maitre *et al.*, 2011). Many Australian acacias were deliberately introduced into the country in the early 19th century as a source of fuel wood and tannin industry (for a full history of introductions see Le Roux *et al.* 2011). The subsequent spread of the species (and their alteration of ecosystem structure, function and composition), has been related to several key traits, including fast growth rates, strong competitive interactions with native flora, high outputs of persistent seeds, and symbiotic nitrogen fixation (Yelenik *et al.*, 2004; Sprent, 2009; Morris *et al.*, 2011a). These life history traits contribute to the high invasiveness of these acacias, especially if they are novel in the invaded system. In efforts to mitigate the spread and impacts of Australian acacias, a number of control and restoration initiatives have been implemented in South Africa. The results of these management interventions have been mixed, with many efforts producing unplanned and undesirable outcomes (Holmes *et al.*, 2008; Le Maitre *et al.*, 2011). At present, there are 15 known invasive Australian *Acacia* species in southern Africa (Richardson and Rejmánek, 2011).

The potential for N₂-fixing plant invasions to alter nutrient pools (most frequently nitrogen) at the ecosystem level has been identified in a variety of ecosystems in many parts of the world (Boring and Swank, 1984; Vitousek and Walker, 1989; Stock *et al.*, 1995; Ehrenfeld, 2003; Rice *et al.*, 2004; Drake, 2011). These, and other, changes in ecosystem functioning which are associated with invader impacts are of particular concern because of the long-term nature of such impacts and the potential of these shifts to further facilitate exotic plant invasions. However, the extent to which Australian acacias can alter nutrient cycling in non-native systems is not well understood. Even less is known about how nutrient acquisition, allocation and conservation strategies of Australian acacia compare with co-occurring, and often competing, native *Acacia* in South Africa.

1.2. Study Aim and Objectives

This study aims to estimate the potential extent to which an invasive N₂-fixing Australian *Acacia* alters nitrogen (N) and phosphorus (P) dynamics regimes in riparian woodlands with resident African acacias. The Komati Wildlife Reserve, located in the upper Komati River catchment in Mpumalanga Province in eastern South Africa, provided a suitable setting for this within-functional group comparison. Within the

reserve, native acacia species growing alongside the Komati River are being displaced by mono-specific stands of *Acacia dealbata* (Link), a highly invasive and globally widespread species native to south-eastern Australia (Richardson and Rejmánek, 2011). Although at least four species of native acacia grown in the Komati riparian area, I chose *Acacia ataxacantha* (a widespread African *Acacia*) for comparisons due to its high abundance in the riparian zone, similar thicket-forming growth habit and the fact that it co-occurs with *A. dealbata*. Also of interest was the N₂-fixing status *Acacia ataxacantha* (DC), a member of a small group of Africa acacias (*Monacantha* group) reported to not form root nodules with symbiotic N₂-fixing bacteria (Harrier *et al.*, 1997; Sprent, 2009), yet few studies have tested for *in situ* N₂-fixation capacity in this species (Cramer *et al.*, 2007; Cramer *et al.*, 2010). Previous work in this system showed that both native and invasive acacias (see Miller *et al.*, 2011 and for recent taxonomic revisions) fix nitrogen, with higher rates of N₂-fixation in invasive species (Tye and Drake, 2012). High fixation rates likely support the high growth rates which are characteristic of invasive Australian acacia, and contribute to their global invasiveness (Sprent, 2009; Morris *et al.*, 2011a). Subsequent alterations of the N and P cycle may further reduce the competitive advantage of native flora, and increase the vulnerability of this system to further invasions.

Here I addressed three key objectives: in Chapter 2, I developed species-specific allometric equations for predicting aboveground component biomass. Concepts important to biomass estimation and the development and evaluating allometric models are integrated and discussed. Chapter 3 describes the evaluation of *in situ* N₂-fixation capacity through the quantification the contribution of N derived through symbiotic N₂-fixation in each species. Seasonal N and P allocation and conservation strategies in aboveground components were considered in Chapter 4. I then used these data collectively in Chapter 5 to model N and P stocks in aboveground components stocks for the respective species, and concluded with a synthesise of the potential alterations to N and P cycling which might follow *A. dealbata* invasions. Because N and P are the two most common limiting nutrients in terrestrial ecosystems (Vitousek *et al.*, 2010), insights into the competitive advantage of *A. dealbata* in non-native landscapes were gained in this study.

1.3. References

- Boring, L. R. and Swank, W. T. (1984) The role of black locust (*Robinia Pseudo-Acacia*) in forest succession. *Journal of Ecology* 72(3):749-766.
- Cramer, M. D., Chimphango, S. B. M., Van Cauter, A., Waldram, M. S., and Bond, W. J. (2007) Grass competition induces N₂ fixation in some species of African *Acacia*. *Ecology* 95:1123-1133.
- Cramer, M. D., van Cauter, A., and Bond, W. J. (2010) Growth of N₂-fixing African savanna *Acacia* species is constrained by below-ground competition with grass. *Journal of Ecology* 98(1):156-167.
- Drake, D. C. (2011) Invasive legumes fix N₂ at high rates in riparian areas of an N-saturated, agricultural catchment. *Journal of Ecology* 99(2):515-523.
- Dye, P. and Jarman, C. (2004) Water use by black wattle (*Acacia mearnsii*): implications for the link between removal of invading trees and catchment streamflow response. *South African Journal of Science* 100(1):40-44.
- Ehrenfeld, J. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6(6):503-523.
- Harrier, L. A., Whitty, P. W., Sutherland, J. M., and Sprent, J. I. (1997) Phenetic investigation of non-nodulating African species of *Acacia* (Leguminosae) using morphological and molecular markers. *Plant Systematics and Evolution* 205(1):27-51.
- Holmes, P. M., Esler, K. J., Richardson, D. M., and 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.
- Hooper, D. U. and Vitousek, P. M. (1998) Effects of plant composition and diversity on nutrient cycling. *Ecological Monographs* 68(1):121-149.
- Le Maitre, D. C., Gaertner, M., Marchante, E., Ens, E. J., Holmes, P. M., Pauchard, A., O'Farrell, P. J., Rogers, A. M., Blanchard, R., and Blignaut, J. (2011) Impacts of invasive Australian acacias: implications for management and restoration. *Diversity and Distributions* 17(5):1015-1029.
- Le Roux, J. J., Brown, G. K., Byrne, M., Ndlovu, J., Richardson, D. M., Thompson, G. D., and Wilson, J. R. (2011) Phylogeographic consequences of different introduction histories of invasive Australian *Acacia* species and *Paraserianthes lophantha* (Fabaceae) in South Africa. *Diversity and Distributions* 17(5):861-871.
- Levine, J. M., Vila, M., Antonio, C., Dukes, J. S., Grigulis, K., and Lavorel, S. (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:775-781.
- Miller, J. T., Murphy, D. J., Brown, G. K., Richardson, D. M., and González-Orozco, C. E. (2011) The evolution and phylogenetic placement of invasive Australian *Acacia* species. *Diversity and Distributions* 17(5):848-860.
- Morris, T. L., Esler, K. J., Barger, N. N., Jacobs, S. M., and Cramer, M. D. (2011) Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions* 17:898-910.
- Rice, S. K., Westerman, B., and Federici, R. (2004) Impacts of the exotic, nitrogen-fixing black locust (*Robinia pseudoacacia*) on nitrogen-cycling in a pine-oak ecosystem. *Plant Ecology* 174(1):97-107.

- Richardson, D. M. and Rejmánek, M. (2011) Trees and shrubs as invasive alien species – a global review. *Diversity and Distributions* 17(5):788-809.
- Sprent, J. I. (2009) *Legume nodulation: a global perspective*. Wiley-Blackwell, Chichester, UK.
- Stock, W. D., Wienand, K. T., and Baker, A. C. (1995) Impacts of invading N₂-fixing *Acacia* species on patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies and ¹⁵N natural abundance values. *Oecologia* 101(3):375-382.
- Tye, D. and Drake, D. (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.
- Vitousek, P. M. (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57(1):7-13.
- Vitousek, P. M., D'antonio, C. M., Loope, L. L., Rejmanek, M., and Westbrooks, R. (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* 21(1):1-16.
- Vitousek, P. M., Porder, S., Houlton, B. Z., and Chadwick, O. A. (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications* 20(1):5-15.
- Vitousek, P. M. and Walker, L. R. (1989) Biological invasion by *Myrica Faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. *Ecological Monographs* 59(3):247-265.
- Witkowski, E. T. F. (1991) Effects of invasive alien acacias on nutrient cycling in the coastal lowlands of the cape fynbos. *Journal of Applied Ecology* 28(1):1-15.
- Yelenik, S. G., Stock, W. D., and Richardson, D. M. (2004) Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. *Restoration Ecology* 12(1):44-51.

CHAPTER 2: BIOMASS ESTIMATION OF AN INVASIVE AND A COMPETING NATIVE ACACIA IN A SOUTHERN AFRICAN RIPARIAN SYSTEM

2.1. Abstract

Species-specific allometric relationships which relate the biomass of individual tree components to easily obtainable measurements are essential for comparative assessments of standing biomass and biomass allocation strategies. To explore allometric differences between an invasive Australian acacia and a native African acacia, we developed biomass regression equations for the invasive *Acacia dealbata* and the native *Acacia ataxacantha* co-occurring in a riparian system located in a grassland-savanna ecotone in eastern South Africa. Destructive harvesting was employed to obtain weights of aboveground components, which were related to stem diameter (D) and tree height (H). The predictive performance of five different candidate models using different predictors were assessed using second-order Akaike information criterion (AIC). Models used natural log transformation of the common allometric equation form $y = ax^b$, where y is the component biomass, x is the predictor variable and a and b are regression coefficients. Top ranked models were those using either D_{10} (stem diameter at 10 cm above the ground) or a combination of D_{10} and H, accounting for 91–99% percent of the variability in biomass across species. A model using H alone as a predictor was consistently the lowest ranked model in terms of predictive power. This finding, in combination with the potential measurement error associated with H, lead to the recommendation of the model using D_{10} alone as a predictor. Parameter estimates for this model and the best performing model for each species-component combination are presented. Overall, the two acacias had similar allometries. However, notable component level differences included higher allocation to leaves by the invasive *A. dealbata* and a high percentage of attached dead wood on *A. ataxacantha*. These allometric differences may contribute to the fast growth rates which are an important trait contributing to the invasiveness of many Australian acacias. The equations developed can be used to compare carbon and nutrient standing stocks in these species, and assess the potential alterations to ecosystem functioning as a consequence of the invasion.

2.2. Introduction

Reliable estimates of tree biomass are essential for determining primary production, fuel inventories, carbon budgets, water demands, and nutrient stocks and fluxes (Parresol, 1999; Keller *et al.*, 2001; Bond-Lamberty *et al.*, 2002; Sawadogo *et al.*, 2010; Shackleton and Scholes, 2011). The most common method used for estimating aboveground biomass (ABG) is through the use of allometric equations, whereby accurate estimates of biomass can be attained using easily measured structural dimensions such as tree height and stem diameter (Zianis and Mencuccini, 2004). Applying allometric equations to inventory data permits broad-scale biomass estimates, for which application into projects aimed at assessing carbon budgets and nutrient stocks is typically more suitable (e.g. Brown and Lugo, 1984; Brown *et al.*, 1989; Ketterings *et al.*, 2001; Montagu *et al.*, 2005). In addition to total aboveground biomass, allometric equations are routinely developed for separate plant components, such as branches, foliage, bole (trunk), and bark (e.g. Alaback, 1986; Bond-Lamberty *et al.*, 2002; Caldeira *et al.*, 2002; Kaonga and Bayliss-Smith, 2010). Such species-component-specific predictive equations provide the opportunity for comparisons in biomass allocation strategies among species and life forms. Furthermore, estimates of component biomass can be used in conjunction with data on biochemical concentrations to gain further insights into nutrient allocation.

Field methods used for developing allometric equations fall into two broad categories: non-destructive or destructive methods. Non-destructive methods do not require trees to be harvested, but rather employ the use of fractal allometry theory and self repeating branching models to develop predictive equations (West *et al.*, 1999; van Noordwijk and Mulia, 2002; Santos-Martin *et al.*, 2010). On the other hand, destructive methods normally entail harvesting individual trees, separating and determining the fresh/wet weight of individual components, and developing allometric equations (Whittaker and Marks, 1975). Both types of methods have been used successfully in a number of ecosystems, however, destructive methods are often used in conjunction with non-destructive methods for model validation purposes as they have been shown to provide reliable empirical estimates (Brown *et al.*, 1989). Regression modelling is generally employed to develop allometric equations which describe the relationship between predictor variables and the respective component

mass. Decisions around which predictors variables (typically structural variables) to use and the type of equation are required during regression model selection. A variety of regression models have been employed in plant allometric equations, including both linear and non-linear (logarithmic, exponential and power) equations, each of which has their advantages and limitations (reviewed by Parresol, 1999).

In South Africa, several introduced *Acacia* trees from Australia are invading river courses, rangelands, and other disturbed areas (Richardson *et al.*, 1997). Considering their global recognition as some of the most prolific invasive species and the extent of their invasion in South Africa (Richardson and Rejmánek, 2011), the development of predictive biomass equations should prove beneficial to research and monitoring of the ecological impacts associated with the Australian acacias. Although an abundance of both general and species-specific allometric equations for woody plants are available in the literature, reliable equations for estimating component biomass in many tropical and sub-tropical acacias are lacking. To the best of our knowledge, equations derived for invasive stands of acacias in non-commercial settings in southern Africa riparian settings are not yet available.

Acacia dealbata (silver wattle) was introduced into South Africa during the early 19th century as a fast-growing source of poles and fuel wood (Kull and Rangan, 2008), but subsequently escaped commercial control and aggressively invaded grasslands, roadsides and watercourses (Bromilow 2001). This medium-sized evergreen tree reaches heights between 4 and 20 m, and typically grows in dense clumps. Within the species' native range in south-eastern Australia, *A. dealbata* occurs across wide range of soil types and rainfall (600 – 1000 mm MAP) at altitudes ranging from 50 to 1000 m. *A. dealbata* is closely related to the fast-growing *A. mearnsii* (black wattle), and both species have been reported to have an “extremely high invasive potential” by Wilson *et al.* (2011). Invasive stands of *A. dealbata* typically regenerate through seeds (they produce copious amounts of seeds), but the species also has a high capacity for vegetative regeneration from rhizomes after disturbance (e.g. fire, cutting).

A. ataxacantha (flame thorn) is a common, widespread tree occurring in subtropical Africa from Senegal in the west to Sudan in the north-east, extending southwards into South Africa where it is abundant in the northern and eastern parts of

the country. It grows under a wide variety of soil types and MAP; however, tends to be restricted to watercourses in drier regions where it often forms dense, impenetrable thickets. Under higher rainfall conditions, the species is often found under forest edge and riverine thicket situations (Schmidt *et al.*, 2007).

This study develops allometric equations for estimating aboveground component biomass for invasive *A. dealbata* (silver wattle) and a co-occurring native acacia, *A. ataxacantha* (flame thorn) in the Komati River catchment in eastern South Africa. The upper Komati River riparian zone represents a typical *A. dealbata* invasion in southern Africa, and native acacias (of which *A. ataxacantha* is the most abundant) are important resident species. This work contributes to the understanding of biomass allocation in acacias, providing insights into allocation strategies which may support high growth rates which are a common trait of invasive Australian acacias.

2.3. Methods and Materials

2.3.1. Site description

Field sampling was conducted on a farm on the boundary of the Komati Gorge Wildlife Reserve in Mpumalanga, South Africa (henceforth referred to as the 'Komati site'; coordinates 25°53'32"S, 30°17'53"E). The surrounding landscape is largely comprised of grassland plains and undulating hills that occur in the transition zone between Highveld grassland and eastern Escarpment, containing elements of both (Mucina and Rutherford, 2006). Underlying granites and sandstones of the area yield low nutrient soils, although the riparian zone of the bottom slopes of the region typically has higher soil nutrient concentrations. Mean Annual Precipitation (MAP) at the Komati site is 910 mm, with early summer rainfall and Mean Annual Temperature (MAT) of 16 °C. It is located in the KaNgwane Montane Grassland vegetation type (Mucina and Rutherford, 2006), with dominant woody species growing in the riparian zone including *Diospyrus lysoides*, *Ziziphus mucronata*, *Euclea divinorum*, *Acacia. caffra*, *A. ataxacantha*, and *A. dealbata*. The most abundant native acacia at the Komati site was *A. ataxacantha*. The alien invasive *A. dealbata* has established in the region and a control programme is currently being implemented within the Komati River catchment. These invasive acacias grow intermixed with native species, although dense, mono-specific stands of these species occur in patches (*pers. obs.* Tye).

2.3.2. Field data collection and dry weight estimation

Destructive-sampling of trees took place during the wet season months of November and December 2011. Aboveground biomass relations were based on 10 trees of each species, spanning a size range representative of the dominant size classes growing in along the riparian zone. Measurements collected prior to harvesting included diameter at 10 cm above the ground (D_{10}), diameter at 50 cm (D_{50}), diameter at breast height (1.3 m; DBH) and tree height (H). Trees were cut down at ground level using a mechanical chain saw and separated into bole (main stem and branches > 2 cm in diameter), branches (diameter < 2 cm), dead wood (attached dead wood), and leaves. When inflorescences were present they were incorporated into the leaf component, of which they constituted a minor proportion. Fresh weights of all aboveground components were recorded in the field using a hand scale. Representative subsamples of each component from each tree were taken for moisture content determination. Stem disks (c. 5 cm thick) were taken from the bole component for moisture analysis.

Subsamples were returned to the laboratory in sealed polythene bags and kept in cold storage. After recording the fresh weights, samples were dried in a Labcon 2029K drying oven at 80°C until constant weight was achieved. Component dry weights were calculated using respective subsample dry:fresh weight ratios and the component fresh weight field measure (Mohan Kumar *et al.*, 1998).

2.3.3. Model selection for allometric equations

Our dataset contained three basic measures of trunk diameter, D_{10} , D_{50} and DBH, all of which have been routinely been used as predictor variables for biomass, often in combination with H. Of the three diameter measures, D_{10} was chosen for use in model selection based on preliminary regression analyses with component biomass, which suggested that D_{10} was the most reliable diameter measure for dry biomass estimation for acacias.

To investigate the relationship between aboveground biomass (kg.tree^{-1}) and the predictor variables D_{10} and H, we restricted our analysis to the most common allometric form, the power equation (Parresol, 1999; Nickless *et al.*, 2011b):

$$y = ax^b \quad (\text{Eqn 2.1})$$

where y is the component mass, x is the predictor variable, and a and b are regression coefficients. The power equation, or power function, is commonly used in allometric equations as it best models the heteroscedasticity (unequal error variance) which is common in allometric data (Parresol, 1999). The ln-transformed form of this power function, $\ln(y) = \ln(a) + b\ln(x)$, was used to stabilise variance and follows the logarithmic form of the simple linear equation:

$$\ln(y) = \beta_0 + \beta_1 \ln(x) \quad (\text{Eqn 2.2})$$

where β_0 and β_1 are the log regression parameters. Using this linear equation allows for linear regression techniques to be applied (Nickless *et al.*, 2011b). We selected five commonly used model formations as candidate models for evaluation, with predictor variables ranging from D to H alone, with differing combinations of D and H. This technique was used to determine whether the incorporation of H improved the model performance. The model forms evaluated were:

$$\ln(y) = \beta_0 + \beta_1 \ln(H) \quad (\text{Model 1})$$

$$\ln(y) = \beta_0 + \beta_1 \ln(D_{10} \times H^2) \quad (\text{Model 2})$$

$$\ln(y) = \beta_0 + \beta_1 \ln(D_{10} \times H) \quad (\text{Model 3})$$

$$\ln(y) = \beta_0 + \beta_1 \ln(D_{10}) \quad (\text{Model 4})$$

$$\ln(y) = \beta_0 + \beta_1 \ln(D_{10}^2 \times H) \quad (\text{Model 5})$$

Akaike Information Criterion (AIC) was used for selection of best-performing model (hypothesis with the most support) on a component and species basis. For a detailed description of AIC refer to Burnham and Anderson (2002). Summarised briefly, AIC is an information-theoretic criterion for model selection based on the statistical likelihood function. The selection of a “best” model out of a set of candidate models can be defined as the one which loses the minimum amount of information when compared to the theoretically *true model*. Thus, the candidate model with the minimum AIC value is considered closest to the true model and selected as the best approximation. It is important to note that AIC values are only comparable if the candidate models are fitted to the same dataset, and the relative difference between AIC values and the minimum AIC value scored by a candidate model is used to rank

model performance, expressed as ΔAIC (delta AIC). We calculated and the second-order AIC (AICc) in this study as recommended for data with low sample sizes (Burnham and Anderson, 2002). In the case of numerous models achieving delta AICc values of less than 2, the models Akaike weight (w_i) proved useful in best model selection, indicating the weight of evidence (ranging from 0, no evidence, to 1, full confidence) in favour of the respective model being closest to the true model for the situation at hand (Burnham and Anderson, 2002). Models with delta AICc values in the range of 0 – 2 and $w_i > 0.3$ were considered to have essentially equal empirical support for being the K-L (Kullback-Leibler) best model given the data (Burnham and Anderson, 2002). When multiple models fell into this category for a specific species-component combination, then the most parsimonious (i.e. fewest variables required for calculations or simplest form for those parameters) was selected as the best performance model. Fitting of the five models listed above was carried out using the ‘AICcmodavg’ (Mazerolle, M.K, version 1.24, 2002) for R open-source statistical software (<http://www.r-project.org>). Slopes of the regression lines were compared using a t-test for comparison of two slopes. All additional statistics were performed in R.

2.4. Results

2.4.1. Data used for development of allometric equations

A total of 20 acacias (10 of each species) from a range of size classes were harvested for biomass determination (Table 2.1). The range in D_{10} was wider for *A. ataxacantha* (2.5 cm - 17.9 cm) than for *A. dealbata* (1.8 cm – 13.5 cm), while H varied more widely for *A. dealbata* (1.6 m – 9.4 m) than for *A. ataxacantha* (4.6 m – 8 m). These ranges were representative of the respective stands growing at the Komati site. Total aboveground tree biomass ranged from 0.3 to over 89 kg.tree⁻¹ (Table 2.1).

The relationship between D_{10} and H was stronger in *A. dealbata* trees ($R^2 = 0.95$, $P < 0.001$) than in *A. ataxacantha* trees ($R^2=0.80$, $P < 0.001$; Figure 2.1a). Furthermore, the slope of this relationship differed between the two species ($t = 6.31$, $P < 0.01$). For a given D_{10} , *A. ataxacantha* tended to be taller than *A. dealbata* at diameters less than 6 cm ($D_{10} > 6$ cm, Figure 2.1a). For stems with D_{10} higher than 6cm, *A. dealbata* trees were taller.

Table 2.1 Descriptive statistics on tree data used in developing allometric equations. All data were collected from the Komati Gorge Reserve study site. D10 is bole diameter at 10 cm above the ground, H is tree height, and AGB is aboveground biomass per tree. Maximum, minimum and mean values indicated for each of D, H and AGB.

Species	N	D ₁₀ (cm)			H (m)			AGB (kg)		
		Min.	Max.	Mean	Min.	Max.	Med.	Min.	Max.	Mean
<i>A. ataxacantha</i>	10	2.5	17.9	10.7	4.6	8.0	6.1	0.9	89.8	31.8
<i>A. dealbata</i>	10	1.8	13.5	6.6	1.6	9.4	5.8	0.3	57.3	12.5

Plots of AGB against D₁₀ and H demonstrated exponential relationships between these variables for both species (Figure 2.1b and c), although D₁₀ was a stronger standalone predictor of AGB than H. Aboveground biomass was generally higher in *A. dealbata* trees for a given D₁₀, while the opposite trend was observed for a given H.

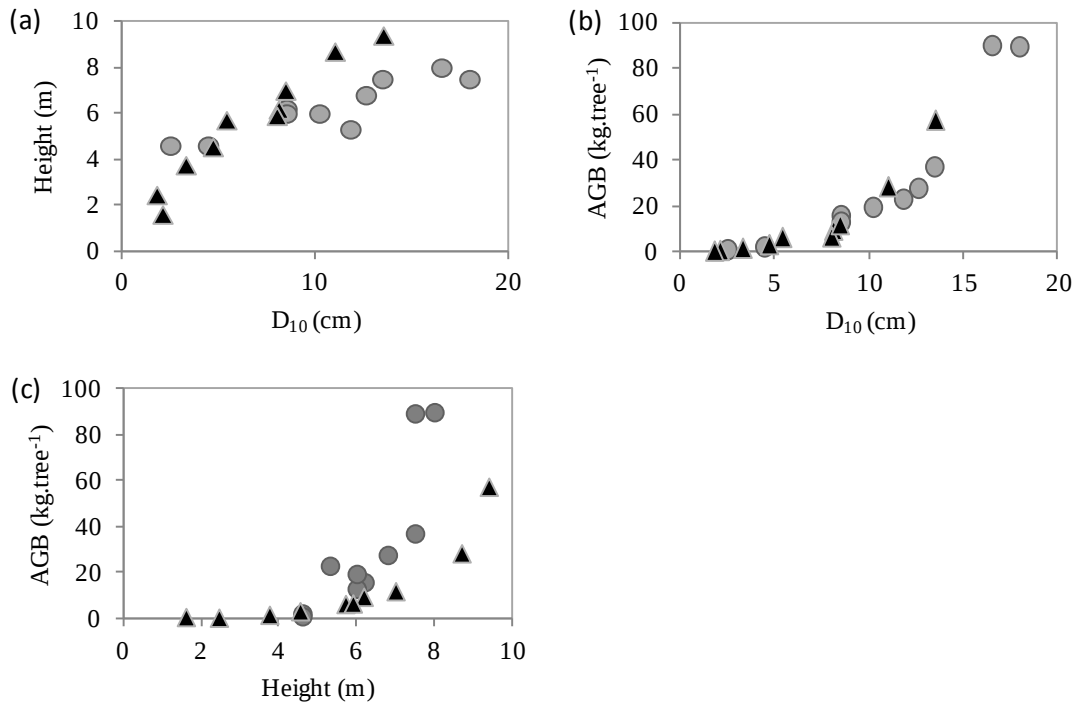


Figure 2.1 Relationship between aboveground biomass (AGB) and tree size variables for *A. ataxacantha* (circles) and *A. dealbata* (triangles). (a) H v. D₁₀, (b) AGB v. D₁₀, (c) AGB v. H.

2.4.2. Comparison of allometric model performance

Model selection techniques revealed no consistent best-performing model relating ln(component mass) to ln-transformed predictor variables D₁₀ and H, or linear

combinations of these two variables (Appendix A, Table 2.4). Instead, numerous models tended to have low delta AICc values (less than 7), similar coefficient of determination values (R^2) and comparable MSE estimates. The lack of a clear, consistent best performance models was not surprising considering the similarity in model forms and the reasonable correlation between D_{10} and H. For models that performed equally, the most parsimonious model (simplest form of the predictor variable calculation) was select as the best-performing model.

Best-performing models for the different species-component categories explained 91 – 99% and 95 – 98% of the variation in biomass for *A. ataxacantha* and *A. dealbata* respectively (Table 2.2). Despite the lack of a consistent best-performing model among the species-component combinations, the candidate model which used H alone as a predictor (Model 1) consistently gave the poorest fit, accounting for lowest, only 73-91%, of the variation in component biomass (Appendix A, Table 2.4). A clear improvement in the performance of the models was observed as diameter was introduced, and all best-performing models incorporated D_{10} as a predictor. Candidate models using simple combinations of D_{10} and H (models 2, 3, and 5) were more frequently selected as best performing models for *A. ataxacantha* components than for *A. dealbata* components (Table 2.2).

Table 2.2 Best performance biomass prediction models based on AICc selection procedure. All regressions are significant with $P < 0.01$, $N = 10$. Total wood is the sum of bole, branch and dead wood components.

Component	Model no.	β_0	β_1	Δ AICc	w_i	MSE	R^2
<i>A. ataxacantha</i>							
AGB	3	2.185 (0.283)	1.859 (0.069)	0.00	0.55	0.026	0.989
Leaf	3	0.091 (0.732)	1.615 (0.178)	0.00	0.31	0.177	0.911
Total wood	3	2.068 (0.301)	1.875 (0.073)	0.00	0.54	0.030	0.988
Bole	2	0.22 (0.28)	1.515 (0.047)	0.00	0.61	0.019	0.992
Branch	4	2.247 (0.543)	2.327 (0.236)	0.19	0.31	0.186	0.924
Dead wood	5	1.545 (0.699)	1.095 (0.109)	0.00	0.34	0.208	0.927
<i>A. dealbata</i>							
AGB	4	4.408 (0.266)	2.378 (0.146)	0.00	0.60	0.089	0.971
Leaf	3	1.953 (0.287)	1.49 (0.082)	0.41	0.37	0.092	0.976
Total wood	4	4.235 (0.274)	2.339 (0.15)	0.00	0.74	0.095	0.968
Bole	4	3.882 (0.288)	2.323 (0.157)	0.00	0.81	0.105	0.965
Branch	5	2.455 (0.374)	0.902 (0.07)	0.00	0.34	0.163	0.954

2.4.3. Models using diameter alone

Models using a single predictor variable reduce the chance of error that is unaccounted for in the predictive models, through occurrences of multicollinearity, which is already proven in the case of height and stem diameter. Biomass equations using D_{10} alone as a predictor explained 90 - 98% of the variation in component biomass (Table 2.3). This strong relationship between tree diameter and component biomass can be observed graphically with scatter plots of the respective ln-transforms (Figure 2.2). Interestingly, AGB as a whole was better explained by $\ln(D_{10})$ than the any of the individual components in both acacias (Table 2.3). The only component

where the relationship with D_{10} differed significantly between species was leaf biomass ($t = 2.578$, $P < 0.05$, Figure 2.2b).

Table 2.3 Parameter estimates (with standard errors) for regression equations relating D_{10} to component biomass of *A. ataxacantha* and *A. dealbata*. All regressions significant at $P < 0.01$

Component	N	β_0	β_1	MSE	R^2
<i>A. mearnsii</i>					
AGB	10	4.408 (0.266)	2.378 (0.146)	0.089	0.971
Leaf	10	2.358 (0.388)	2.64 (0.213)	0.191	0.951
Total wood	10	4.235 (0.274)	2.339 (0.150)	0.095	0.968
Bole	10	3.882 (0.288)	2.323 (0.157)	0.105	0.965
Branch	10	2.694 (0.394)	2.503 (0.216)	0.196	0.944
<i>A. ataxacantha</i>					
AGB	10	4.374 (0.283)	2.391 (0.123)	0.050	0.979
Leaf	10	1.995 (0.559)	2.077 (0.242)	0.196	0.902
Total wood	10	4.277 (0.295)	2.411 (0.128)	0.055	0.978
Bole	10	3.773 (0.362)	2.387 (0.157)	0.082	0.967
Branch	10	2.247 (0.543)	2.327 (0.236)	0.186	0.924
Dead wood	10	2.830 (0.576)	2.506 (0.250)	0.209	0.926

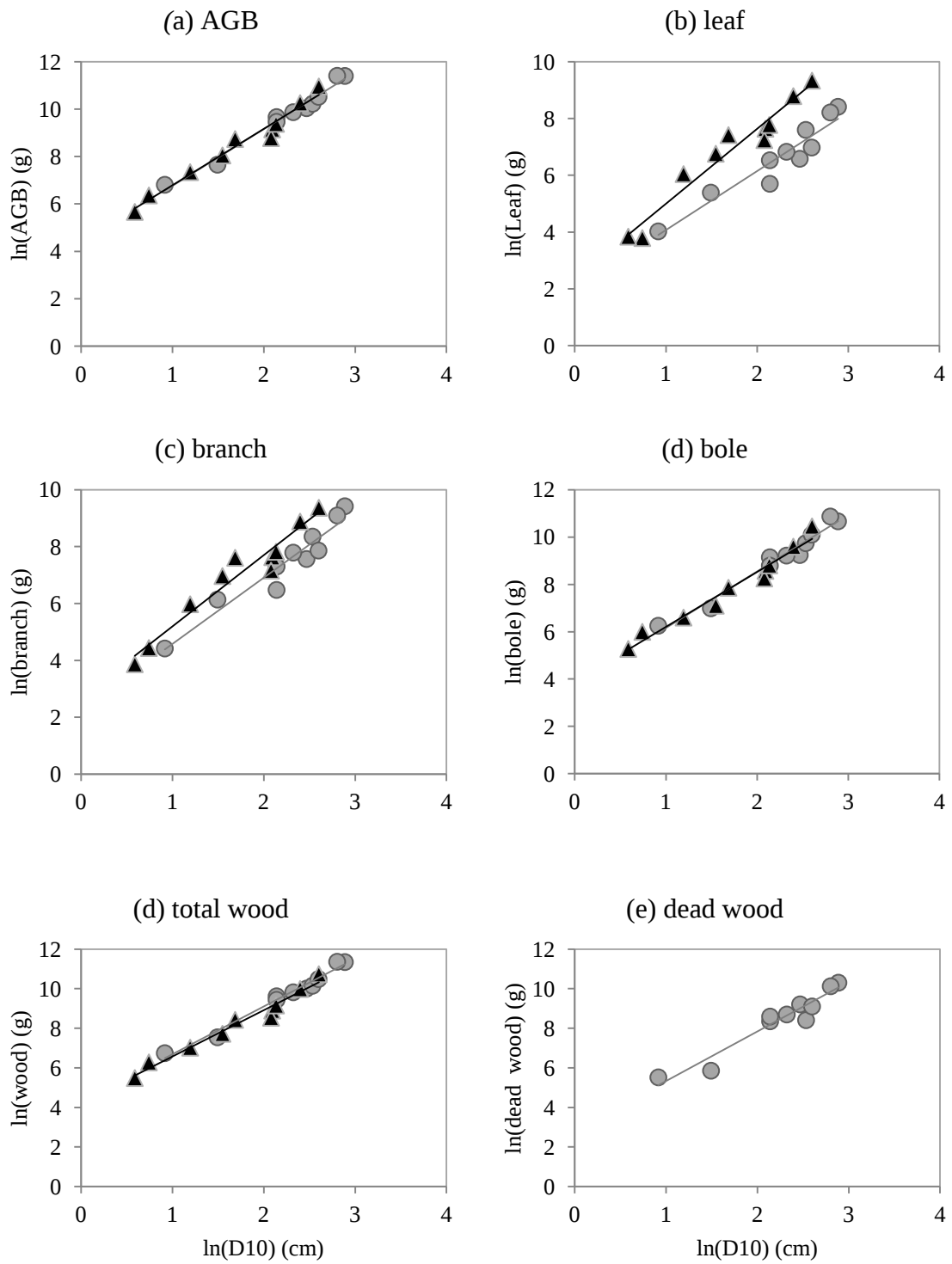


Figure 2.2 Species-component specific relationships between ln-transformed biomass and D_{10} . (a) aboveground biomass (AGB) , (b) leaf, (c) branch, (d) bole, (e) dead wood, and (f) total wood. Solid lines are linear regressions for *A. ataxacantha* (light, circles) and *A. dealbata* (dark, triangles). See Table 2.3 for details of the relationships.

2.4.4. Variations in biomass partitioning between species

Although the relationships (slopes Figure 2.2) were similar for AGB between acacias, there were differences in the biomass partitioning between species (Figure 2.3). For example, mean percentage of leaf biomass was more than four times higher in *A. dealbata* (~ 21% of total biomass) than in *A. ataxacantha* (~5% of total biomass). This could be partially explained by the high percentage on attached dead branches (16 – 46%) on *A. ataxacantha*, however, the average leaf:live branch ratio of *A. ataxacantha* is less than half that of *A. dealbata* (Figure 2.3). Back-transformed biomass predictions (Figure 2.4) indicate higher (5 - 20%) aboveground biomass of *A. dealbata* trees relative to *A. ataxacantha* for a given diameter.

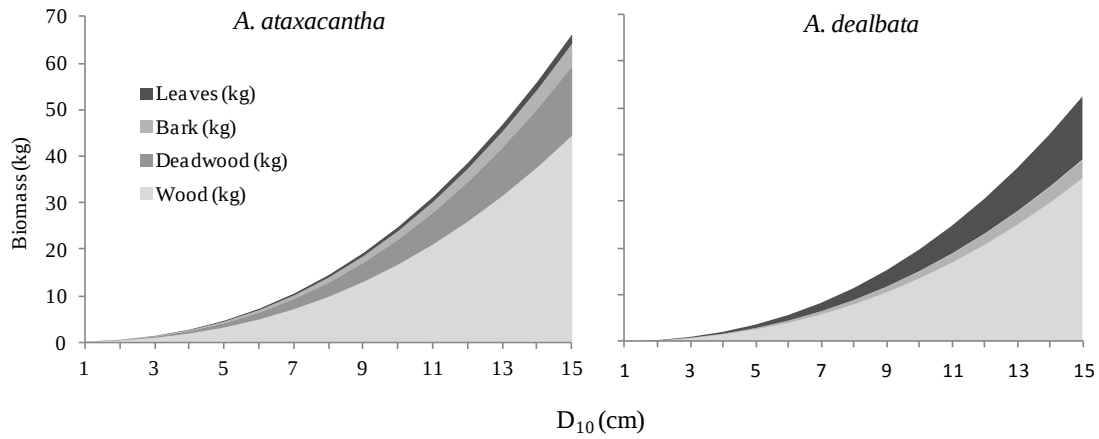


Figure 2.3 Total aboveground and component biomass in relation to stem diameter (D_{10}) for *Acacia* species. Values estimated using our allometric equations using D_{10} as a predictor (model 4).

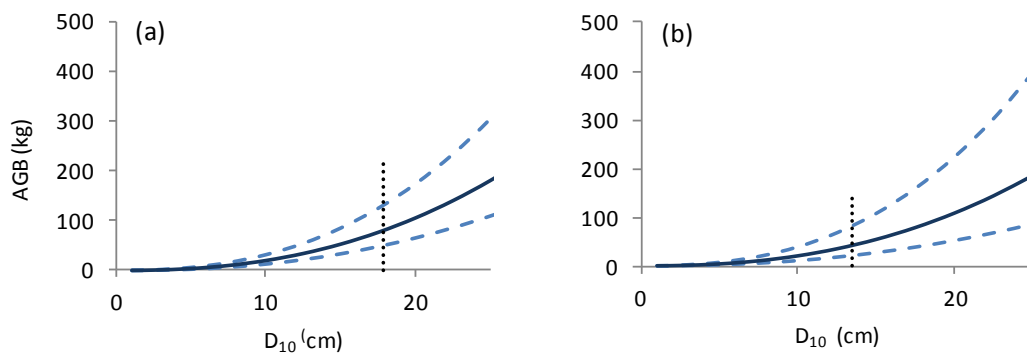


Figure 2.4 Best estimate (solid line) and upper and lower 95% confidence limits for AGB in (a) *A. dealbata*, and (b) *A. ataxacantha*, as a function of D_{10} .

2.5. Discussion

The purpose of this study was to develop allometric equations for estimating total aboveground biomass as well as individual component biomass of an alien invasive and a native *Acacia* co-occurring in a riparian setting in South Africa. The study species accounted for the majority of aboveground acacia tree biomass at the Komati study site (*pers. obs.* D. Tye) and share a similar thicket-forming growth habit. Although allometric equations do exist for several *Acacia* species in southern Africa (e.g. Rutherford, 1979; Nickless *et al.*, 2011b), the development of species-site specific equations is often recommended for increase measurement accuracies (Dudley and Fownes, 1991). This may be particularly important for widespread African *Acacia* species growing under a range of selective pressures imposed by different environments. For example, the architecture of *Acacia karroo* exhibits considerable variation among populations growing in forests, arid-shrublands and savannas, suggesting factors such fire and herbivory play an important role in tree-life history traits (Archibald and Bond, 2003). These equations contribute to the growing number of species-specific allometric equations which have broad applications in both forestry and forest ecology.

2.5.1. Allometric model selection using AIC

Numerous model forms have been proposed in the literature to best describe the relationship between tree structural variables and aboveground biomass (reviewed by Parresol, 1999). Commonly used forms include simple linear, multiple regressions, additive error, quadratic equations, and even more complex polynomials. Furthermore, the choice of which structural variables to include in the model is extensive, with published models incorporating predictors such as H, D (at various heights), height of first branching, canopy area and wood density (Fownes and Harrington, 1992; Bond-Lamberty *et al.*, 2002; Montagu *et al.*, 2005; Williams *et al.*, 2005). To enhance the application of a predictive model, the choice of which structural predictor(s) to use should be based on practicality and reliability of obtaining the measure for the species of interest. Thus, measures of D and H tend to be the most common variables incorporated into biomass equations (Brown *et al.*, 1989; Parresol, 1999).

We examined a number of candidate models to identify one that is empirically best supported by our data. All candidate models were highly significant, with biomass positively related to predictor variables D (using diameter at 10 cm above the ground, D_{10}) and H. Although there were inconsistencies in the ranking of the model forms among aboveground components (Table 2.1), the model using H alone as a predictor (Model 1) explained the least amount of variation in biomass for each species-component combination. Model 1 was also ranked as least appropriate using the AICc model selection procedures employed. We found that models incorporating a combination of D and H as a predictor variable were overall top performing models, most notably when D was squared (Model 5). This was in agreement with numerous published works on tree allometric equations which have identified the use of D^2H as a suitable predictor in the power equation (Niklas, 2004; Zianis, 2008; Nickless *et al.*, 2011a). We also noted that the most simple and arguably most frequently used model, based solely on stem diameter (Model 4), performed excellently throughout our analyses. This model generally exhibited high R^2 values ($R^2 > 0.9$), low delta AICc values (average ~ 3.5) and good model support (w_i values). This was most notable for *A. dealbata* where it was ranked best model for AGB and the major wood components.

Our data on the biomass partitioning among aboveground components provided insights into observed model ranking patterns. For example, in both species the top-ranked model for total wood mass (including branch and dead wood) was also the top ranked model for AGB, which could be expected with total wood biomass accounting for 79% - 95% of AGB. Interestingly, the tendency of *A. ataxacantha* trees to have substantial amounts of attached dead wood did not greatly reduce the predictive performance of the models for this species. Why *A. ataxacantha* tends to have a high proportion of attached senesced wood whereas *A. dealbata* tends to have very little, or none, is itself an interesting question. It is possible that it is an artefact of plant age (*A. ataxacantha* tends to have slower growth rates, i.e. stems comparatively older), yet this seems unlikely considering the range of stem diameters included in this analysis. Previous reports of proportionally low (less than 1%) attached dead wood in *A. mearnsii* (black wattle), a close relative of *A. dealbata*, also suggest that plant age is not a major factor here (Caldeira *et al.*, 2002). It has also been argued by Lambers *et*

al. (2008b), tissue senescence should not be viewed as “getting old and wearing out”, but rather as a hormonally controlled developmental process, which it is affected by numerous environmental factors (e.g., irradiance level, photoperiod, and nutrient supply). Typically, *A. ataxacantha* is a forest edge species, where shading and reduced photosynthate production could explain relatively high branch senescence. However, *A. dealbata* has a similar thicket forming growth habit to that of *A. ataxacantha*, and light limitation would also be expected to limit photosynthate production in lower canopy branches, yet no senesced branches were observed on the sampled *A. dealbata* trees. The longer leaf lifespan and lower specific leaf area (SLA) of the evergreen *A. dealbata* leaves relative to deciduous *A. ataxacantha* suggests a lower photosynthetic capacity and quite possibly lower light and nutrient requirements of the shaded leaves (Lambers *et al.*, 2008b). This, in combination with a relatively higher leaf:branch mass ratio in *A. dealbata*, may reduce branch senescence in this species.

Allometric similarities

The two *Acacia* species included in this analysis have, for the most part, similar allometries. Such allometric generality has been observed in numerous studies aimed at developing allometric equations for co-occurring woody species (Brown *et al.*, 1989; Ketterings *et al.*, 2001; Williams *et al.*, 2005; Nickless *et al.*, 2011b), although it was somewhat surprising here as these two *Acacia* have evolved in different landscapes and have visibly different architecture. The greatest difference in biomass allocation between the two species was observed between the leaf components, which was approximately 4 times higher, for a given diameter, in *A. dealbata* trees. Australian acacias in South Africa (e.g.: *A. mearnsii*, *A. dealbata*, *A. decurrens*) are reported to have high water losses via transpiration (Dye and Jarmain, 2004), and this relatively high leaf biomass component is likely to contribute to high stand-level water losses. High leaf biomass may also contribute to high growth rates as well as high N₂-fixation capacities (these come at a carbon expense) which are typical traits in many invasive Australian acacias (Le Maitre *et al.*, 2011; Morris *et al.*, 2011a; Richardson and Rejmánek, 2011; Tye and Drake, 2012).

2.5.2. Evaluation of tree height in biomass equations

The incorporation of height as a predictor variable in allometric equations has yielded mixed results in model performance (Dudley and Frownes, 1991; Bond-Lamberty, 2001; Montagu *et al.*, 2005; Williams *et al.*, 2005). . Typically, parameter estimates are derived without taking into account measurement error of predictor variables, or otherwise assume equal measurement error across these variables. However, this assumption is rarely met, as predictive variables, or estimators, typically have differing measurement precision (Parresol, 1999). We compared the performance of models incorporating H with a model which used D alone as a predictor variable for estimating component biomass (Model 4). We found that the inclusion of height in the predictor did not greatly improve model performance, and in the some cases resulted in a weaker predictive capacity of the model compared to models based on D alone. This raises the question - is the inclusion of height into these equations justified?

It should be noted that, in this study, height was measured directly after the tree was felled, and thus the accuracy of height measures presented here is considered to be greater than if obtained using traditional forest survey techniques, such as clinometers measurements. For example, Brown (1995) reported measurements errors in the range of 10-15% in mature forest stands (trees typically taller than 20m). Consequently, the gains in model accuracy with the addition of H should be critically assessed when choosing best-performance models for field applications. Our results indicate that although models using combinations of H and D₁₀ were selected as the top-performing model approximately 64% of the time (Table 2.2), models using D₁₀ alone as a predictor explained 90 - 98% of the variation in component biomass. Statistically (and logically), it is more appropriate use a predictor variables with minimal least associated measurement error. We chose stem diameter as a more precise predictor, which is measurement errors reported at f 3% (Gregoire *et al.*, 1989). Furthermore, from a practical standpoint, height measures are more time-consuming to obtain than measures of diameter, making stand inventories more costly when height is included. The uncertainty in defining (and measuring) tree height measures in a multi-form species such as *A. ataxacantha* in densely wooded areas may also increase the measurement error. Thus, although we were able to detect slight improvement in the precision (mostly in *A. ataxacantha* components), of equations using H measures in

addition to D_{10} as a predictor the amount of variation accounted for by the addition of height in this instance was less than 4%. This suggests that equations requiring measurements of tree height may have limited applicability and offer minor improvement in the precision of biomass estimates of *Acacia*. With this in consideration, we support the recommendations of Montagu (2005) and Williams *et al.* (2005) by advocating the use of a model which uses a single, low measurement error, predictor (D_{10}), and have included these equations for this reason.

2.5.3. *The error involved in extrapolation.*

Graphical representations of allometric estimates along with their prediction intervals show widening asymmetrical confidence intervals as one extrapolates beyond the range of predictor measures from which the allometric equations were developed (Figure 2.4). This asymmetrical nature of the prediction interval is a consequence of the lognormal distribution assumed to underlie the data. Although the data used in this study were typical of published site-specific allometry with unique species (often between 5 and 20 samples), with such sample sizes the variance estimates associated with the regressions are inherently unstable, and are often sensitive to single data points. When ln-transformed equations are used, the uncertainty in regression variance estimates impact on the back-transformed prediction intervals. Caution should be taken when applying these equations outside of the range of diameters from which they were developed.

2.5.4. *Applications of allometry in invasion monitoring in riparian systems*

In the context of invasive N_2 -fixing acacias, biomass estimates should facilitate research on potential alterations to native ecosystem functioning. This is supported by studies which have developed allometric equations for the estimation of ecosystem function traits, such as nutrient cycling (Keller *et al.*, 2001) and primary productivity (Haase and Haase, 1995). There has been, however, a lack of empirical studies on species-specific biomass and general vegetation structural attributes for invasive legumes in southern Africa. Although a core objective of this study was to develop reliable allometric equations for estimating biomass of the invasive *A.*

dealbata, the equations developed here can be applied to a large body of research dealing with the acacia invasions.

In addition to carbon accounting and water-use monitoring, allometry provides the basis for estimates in nutrient stocks and potential fluxes, for which estimates of biomass are more important than population density (Keller *et al.*, 2001). For example, calculating the potential inputs of bioactive N in landscapes invaded by efficient N₂-fixing *Acacia* species will require accurate estimates of standing biomass, nutrient concentration and N₂-fixation rates.

2.6. Conclusions

The value of species-specific allometric models has been recognised in a wide variety of ecosystems. (Dudley and Fownes, 1992; Bond-Lamberty *et al.* 2001; Sawadogo *et al.*, 2010). We have developed equations which will improve our capacity to accurately estimate and monitor biomass, fuel loads and nutrient standing stocks associated with two co-occurring native and alien invasive *Acacia* in South Africa. The use of easily measurable predictor variables makes the equations presented here useful for both land managers and researchers. Additionally, the development of equations based solely on stem diameter avoids the measurement error typically associated with field estimates of tree height, with minimal loss to the predictive capacity of the models. Ecological and economic impacts associated with the invasive acacias in South Africa and the breakdown of each species into morphological components make this a useful dataset, providing insights into biomass allocation in phylogenetically related but architecturally divergent acacia trees. Furthermore, allometric equations of invasive trees growing in non-native environments provide opportunities for further work on comparing biomass production with native stands. However, care should be taken when applying the allometric models developed here to trees with structural characteristics outside of the range from which these models were developed.

2.7. References

- Alaback, P. B. (1986) Biomass regression equations for understory plants in coastal Alaska: Effects of species and sampling design on estimates. *Northwest Science* 60(2):90-103.
- Archibald, S. and Bond, W. J. (2003) Growing tall vs growing wide: tree architecture and allometry of *Acacia karroo* in forest, savanna, and arid environments. *Oikos* 102(1):3-14.

- Bond-Lamberty, B., Wang, C., and Gower, S. T. (2002) Aboveground and belowground biomass and sapwood area allometric equations for six boreal tree species of northern Manitoba. *Canadian Journal of Forest Research* 32(8):1441.
- Bromilow, C. (2001) *Problem Plants of Southern Africa*. Briza Publications, Pretoria.
- Brown, I. F., Martinelli, L. A., Thomas, W. W., Moreira, M. Z., Ferreira, C. A. C., and Victoria, R. A. (1995) Uncertainty in the biomass estimation methods for tropical forest with application to forest inventory data. *Forest Science* 35:88-902.
- Brown, S., Gillespie, A. J. R., and Lugo, A. E. (1989) Biomass estimation methods for tropical forests with applications to forest inventory data. *Forest Science* 35(4):881-902.
- Brown, S. and Lugo, A. E. (1984) Biomass of tropical forests: a new estimate based on forest volumes. *Science* 223:1290-1293.
- Burnham, K. P. and Anderson, D. R. (2002) *Model selection and multimodel inference: a practical information-theoretic approach*. Second Edition edition. Springer Science and Business Media, New York.
- Caldeira, M. V. W., Schumacher, M. V., and Spathelf, P. (2002) Quantification of nutrient content in above-ground biomass of young *Acacia mearnsii* De Wild., provenance Bodalla. *Ann. For. Sci.* 59(8):833-838.
- Dudley, N. S. and Fownes, J. H. (1991) Preliminary biomass equations for eight species of fast-growing tropical trees. *Journal of Tropical Forest Science* 5(1):68-73.
- Dye, P. and Jarman, C. (2004) Water use by black wattle (*Acacia mearnsii*): implications for the link between removal of invading trees and catchment streamflow response. *South African Journal of Science* 100(1):40-44.
- Fownes, J. H. and Harrington, R. A. (1992) Allometry of woody biomass and leaf area in five tropical multipurpose trees. *Journal of Tropical Forest Science* 4:317-330.
- Gregoire, T. G., Zedaker, S. M., and Nicholas, N. S. (1989) Modeling relative error in stem basal area estimates. *Canadian Journal of Forest Research* 20:496-502.
- Haase, R. and Haase, P. (1995) Above-ground biomass estimates for invasive trees and shrubs in the Pantanal of Mato Grosso, Brazil. *Forest Ecology and Management* 73(1-3):29-35.
- Kaonga, M. L. and Bayliss-Smith, T. P. (2010) Allometric models for estimation of aboveground carbon stocks in improved fallows in eastern Zambia. *Agroforestry Systems* 78(3):217-232.
- Keller, M., Palace, M., and Hurtt, G. (2001) Biomass estimation in the Tapajos National Forest, Brazil: Examination of sampling and allometric uncertainties. *Forest Ecology and Management* 154(3):371-382.
- Ketterings, Q. M., Coe, R., van Noordwijk, M., Ambagau, Y., and Palm, C. A. (2001) Reducing uncertainty in the use of allometric biomass equations for predicting above-ground tree biomass in mixed secondary forests. *Forest Ecology and Management* 146(1-3):199-209.
- Lambers, H., Raven, J. A., Shaver, G. R., and Smith, S. E. (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution* 23(2):95-103.

- Le Maitre, D. C., Gaertner, M., Marchante, E., Ens, E. J., Holmes, P. M., Pauchard, A., O'Farrell, P. J., Rogers, A. M., Blanchard, R., and Blignaut, J. (2011) Impacts of invasive Australian acacias: implications for management and restoration. *Diversity and Distributions* 17(5):1015-1029.
- Le Maitre, D. C., Van Wilgen, B. W., Chapman, R. A., and McKelly, D. H. (1996) Invasive plants and water resources in the Western Cape Province, South Africa: modelling the consequences of a lack of management. *Journal of Applied Ecology* 33:161-172.
- Mohan Kumar, B., Jacob George, S., Jamaludheen, V., and Suresh, T. K. (1998) Comparison of biomass production, tree allometry and nutrient use efficiency of multipurpose trees grown in woodlot and silvopastoral experiments in Kerala, India. *Forest Ecology and Management* 112(1-2):145-163.
- Montagu, K. D., Duttmer, K., Barton, C. V. M., and Cowie, A. L. (2005) Developing general allometric relationships for regional estimates of carbon sequestration: an example using *Eucalyptus pilularis* from seven contrasting sites. *Forest Ecology and Management* 204:113-127.
- Morris, T. L., Esler, K. J., Barger, N. N., Jacobs, S. M., and Cramer, M. D. (2011) Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions* 17:898-910.
- Mucina, L. and Rutherford, M. C. (2006) *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute Pretoria, Pretoria, South Africa.
- Nickless, A., King, S., Archibald, S., Scholes, R. J., Portman, J., and Goosen, J. (2011a) A ground-based Lidar approach to measuring tree biomass in southern Africa. in Fifth Natural Forests and Woodlands Symposium, Richards Bay.
- Nickless, A., Scholes, R. J., and Archibald, S. (2011b) A method for calculating the variance and confidence intervals for tree biomass estimates obtained from allometric equations. CSIR, Pretoria.
- Niklas, K. J. (2004) Plant allometry: is there a grand unifying theory? *Biological Reviews* 79(04):871-889.
- Parresol, B. (1999) Assessing Tree and Stand Biomass: A Review with Examples and Critical Comparisons. *Forest Science* 45(4):573-593.
- Richardson, D. M. and Rejmánek, M. (2011) Trees and shrubs as invasive alien species – a global review. *Diversity and Distributions* 17(5):788-809.
- Richardson, D. M., Macdonald, I. A. W., Hoffmann, H. H., and Henderson, L. (1997) Alien plant invasions. Pp 421-446 in R.M. Cowling, D.M. Richardson, and S.M. Peirce, editors. *Vegetation of Southern Africa*. Cambridge University Press, Cambridge, UK.
- Rutherford, M. (1979) *Aboveground biomass subdivisions in woody species of the savanna ecosystem project study area, Nylsvley*. Cooperative Scientific Programmes: CSIR.
- Santos-Martin, F., Navarro-Cerrillo, R., Mulia, R., and van Noordwijk, M. (2010) Allometric equations based on a fractal branching model for estimating aboveground biomass of four native tree species in the Philippines. *Agroforestry Systems* 78(3):193-202.
- Sawadogo, L., Savadogo, P., Tiveau, D., Dayamba, S., Zida, D., Nouvellet, Y., Oden, P., and Guinko, S. (2010) Allometric prediction of above-ground biomass of

- eleven woody tree species in the Sudanian savanna-woodland of West Africa. *Journal of Forestry Research* 21(4):475-481.
- Shackleton, C. M. and Scholes, R. J. (2011) Above ground woody community attributes, biomass and carbon stocks along a rainfall gradient in the savannas of the central lowveld, South Africa. *South African Journal of Botany* 77(1):184-192.
- Tye, D. and Drake, D. (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 Noordwijk, M. and Mulia, R. (2002) Functional branch analysis as tool for fractal scaling above- and belowground trees for their additive and non-additive properties. *Ecological Modelling* 149(1-2):41-51.
- West, G. B., Brown, J. H., and Enquist, B. J. (1999) A general model for the structure and allometry of plant vascular systems. *Nature* 400(6745):664.
- Whittaker, R. H. and Marks, P. L. (1975) Methods of assessing terrestrial productivity. *Primary productivity of the biosphere* 14:55-118.
- Williams, R. J., Zerihun, A., Montagu, K. D., Hoffman, M., Hutley, L. B., and Chen, X. (2005) Allometry for estimating aboveground tree biomass in tropical and subtropical eucalypt woodlands: towards general predictive equations. *Australian Journal of Botany* 53(7):607-619.
- Wilson, J. R., Gairifo, C., Gibson, M. R., Arianoutsou, M., Bakar, B. B., Baret, S., Celesti-Grapo, L., DiTomaso, J. M., Dufour-Dror, J. M., and Kueffer, C. (2011) Risk assessment, eradication, and biological control: global efforts to limit Australian acacia invasions. *Diversity and Distributions* 17(5):1030-1046.
- Zianis, D. (2008) Predicting mean aboveground forest biomass and its associated variance. *Forest Ecology and Management* 256(6):1400-1407.
- Zianis, D. and Mencuccini, M. (2004) On simplifying allometric analyses of forest biomass. *Forest Ecology and Management* 187(2-3):311-332.

2.8. Appendix A

Table 2.4 Parameter estimates (with standard errors) for all regression models evaluated using AICc in order of component and model performance.

Component	Model no.	β_0	β_1	Δ AICc	wi	MSE	R ²
<i>A. dealbata</i>							
AGB	4	4.408 (0.266)	2.378 (0.146)	0.00	0.60	0.089	0.971
AGB	5	4.213 (0.299)	0.850 (0.056)	1.50	0.28	0.104	0.966
AGB	3	4.132 (0.342)	1.315 (0.098)	3.76	0.09	0.130	0.958
AGB	2	4.067 (0.402)	0.901 (0.078)	6.59	0.02	0.173	0.944
AGB	1	4.009 (0.563)	2.805 (0.336)	12.65	0.00	0.317	0.897
Bole	4	3.882 (0.288)	2.323 (0.157)	0.00	0.81	0.105	0.965
Bole	5	3.713 (0.358)	0.826 (0.067)	3.52	0.14	0.149	0.950
Bole	3	3.647 (0.414)	1.274 (0.118)	5.99	0.04	0.190	0.935
Bole	2	3.598 (0.482)	0.869 (0.093)	8.64	0.01	0.248	0.916
Bole	1	3.575 (0.649)	2.687 (0.387)	13.91	0.00	0.421	0.857
Branch	5	2.455 (0.374)	0.902 (0.07)	0.00	0.34	0.163	0.954
Branch	3	2.350 (0.385)	1.401 (0.110)	0.12	0.32	0.165	0.953
Branch	2	2.259 (0.414)	0.964 (0.080)	1.18	0.19	0.183	0.948
Branch	4	2.694 (0.394)	2.503 (0.216)	1.88	0.13	0.196	0.944
Branch	1	2.149 (0.532)	3.032 (0.318)	5.52	0.02	0.283	0.919
Leaf	2	1.841 (0.287)	1.028 (0.055)	0.00	0.45	0.088	0.977
Leaf	3	1.953 (0.287)	1.490 (0.082)	0.41	0.37	0.092	0.976
Leaf	5	2.080 (0.311)	0.956 (0.058)	2.45	0.13	0.112	0.971
Leaf	1	1.685 (0.385)	3.258 (0.230)	5.19	0.03	0.148	0.962
Leaf	4	2.358 (0.388)	2.640 (0.213)	7.75	0.01	0.191	0.951
Wood	4	4.235 (0.274)	2.339 (0.150)	0.00	0.74	0.095	0.968
Wood	5	4.054 (0.327)	0.834 (0.061)	2.69	0.19	0.124	0.958

Table 2.4 continued

Component	Model no.	β_0	β_1	Δ AICc	wi	MSE	R ²
Wood	3	3.981 (0.377)	1.288 (0.108)	5.10	0.06	0.158	0.947
Wood	2	3.924 (0.441)	0.881 (0.085)	7.85	0.01	0.208	0.930
Wood	1	3.884 (0.604)	2.732 (0.361)	13.47	0.00	0.365	0.878
<i>A. ataxacantha</i>							
AGB	3	2.185 (0.283)	1.859 (0.069)	0.00	0.55	0.026	0.989
AGB	5	3.118 (0.257)	1.050 (0.040)	0.60	0.40	0.028	0.988
AGB	2	0.926 (0.444)	1.498 (0.075)	5.91	0.03	0.048	0.980
AGB	4	4.374 (0.283)	2.391 (0.123)	6.43	0.02	0.050	0.979
AGB	1	-2.690 (1.952)	6.827 (1.069)	27.16	0.00	0.400	0.836
Bole	2	0.220 (0.280)	1.515 (0.047)	0.00	0.61	0.019	0.992
Bole	3	1.529 (0.252)	1.871 (0.061)	0.97	0.37	0.021	0.991
Bole	5	2.491 (0.303)	1.053 (0.047)	7.19	0.02	0.039	0.984
Bole	4	3.773 (0.362)	2.387 (0.157)	14.66	0.00	0.082	0.967
Bole	1	-3.700 (1.664)	7.048 (0.912)	27.28	0.00	0.291	0.882
Branch	2	0.220 (0.28)	1.515 (0.047)	0.00	0.61	0.019	0.992
Branch	4	2.247 (0.543)	2.327 (0.236)	0.19	0.31	0.186	0.924
Branch	3	0.166 (0.767)	1.797 (0.186)	0.63	0.25	0.194	0.921
Branch	2	-1.015 (0.981)	1.442 (0.165)	2.46	0.10	0.233	0.905
Branch	1	-4.273 (2.448)	6.450 (1.341)	12.40	0.00	0.629	0.743
Dead wood	5	1.545 (0.699)	1.095 (0.109)	0.00	0.34	0.208	0.927
Dead wood	4	2.830 (0.576)	2.506 (0.250)	0.05	0.33	0.209	0.926
Dead wood	3	0.597 (0.824)	1.933 (0.200)	0.75	0.24	0.224	0.921
Dead wood	2	-0.666 (1.061)	1.550 (0.179)	2.71	0.09	0.272	0.904
Dead wood	1	-4.131 (2.66)	6.912 (1.457)	12.74	0.00	0.742	0.738

Table 2.4 continued

Component	Model no.	β_0	β_1	Δ AICc	wi	MSE	R ²
Leaf	3	0.091 (0.732)	1.615 (0.178)	0.00	0.31	0.177	0.911
Leaf	5	0.903 (0.648)	0.912 (0.101)	0.11	0.30	0.179	0.910
Leaf	2	-1.005 (0.891)	1.302 (0.150)	0.82	0.21	0.192	0.904
Leaf	4	1.995 (0.559)	2.077 (0.242)	1.05	0.18	0.196	0.902
Leaf	1	-4.161 (2.081)	5.942 (1.140)	9.44	0.00	0.454	0.772
Wood	3	2.068 (0.301)	1.875 (0.073)	0.00	0.54	0.030	0.988
Wood	5	3.010 (0.274)	1.058 (0.043)	0.63	0.40	0.032	0.987
Wood	2	0.798 (0.460)	1.511 (0.078)	5.37	0.04	0.051	0.979
Wood	4	4.277 (0.295)	2.411 (0.128)	6.04	0.03	0.055	0.978
Wood	1	-2.854 (1.971)	6.889 (1.080)	26.11	0.00	0.408	0.836

CHAPTER 3: N-ACQUISITION STRATEGIES IN ALIEN INVASIVE AND NATIVE ACACIAS

3.1. Abstract

Invasive Australian acacias in South Africa can increase ecosystem N availability through biological N₂-fixation. Little is known, however, of how N₂-fixation rates in alien acacias compare with that of indigenous acacias which are often out-competed by invasive acacias. We examined N₂-fixation in the alien invasive *Acacia dealbata* and a co-occurring native species (*A. ataxacantha*) in riparian woodland along the Komati River in eastern South Africa. Foliar samples were obtained from acacias and non-fixing reference plants (non-fixers) and analysed for ¹⁵N isotope abundance and N concentrations. Most reference plants had $\delta^{15}\text{N}$ values near 0‰ (range -1.7 to +1.8‰) and N concentrations between 1.4 and 2.4%. The invasive acacia had significantly lower ¹⁵N abundance ($\delta^{15}\text{N} = -1.6 \pm 0.18\text{‰}$; mean $\pm$ S.E.) and elevated %N (%N = $2.4 \pm 0.09\%$) relative to reference species, suggestive of symbiotic N₂-fixation. In contrast, the native *A. ataxacantha* had higher ¹⁵N isotope values ($\delta^{15}\text{N} = 0.6 \pm 0.27\text{‰}$) than reference species. This unexpected result may be a consequence of rooting depth (access to different N pools), mycorrhizal associations and/or reduced discrimination of ¹⁵N in this species. These data confirm that invasive Australian acacias are capable of high rates of N₂-fixation (mean of 70% of leaf N derived from the atmosphere) in non-native habitats, while *A. ataxacantha* does not fix N. Furthermore, the ¹⁵N enriched signature in the non-fixing acacia relative to reference species suggested that N contributions from N₂-fixation may be considerably underestimated in *A. dealbata*. N₂-fixation is an important attribute increasing the invasiveness of Australian acacias which are thus potentially a major novel source of bioactive N in invaded landscapes

3.2. Introduction

Biological N₂-fixation is the world's largest natural source of bioactive nitrogen (N) (Belsky, 1994; Sprent and Parsons, 2000). Plants capable of biological N₂-fixation (N₂-fixation) through symbiotic association with N₂-fixing bacteria contribute to the long-term maintenance, or increase, in ecosystem N stocks in both

aquatic and terrestrial environments globally (Chapin *et al.*, 2002). Many invasive plant species are capable of N₂-fixation and are therefore able to transform ecosystems through their effects on nutrient cycling and ecosystem functioning (Vitousek *et al.*, 1997b; Ehrenfeld, 2003; Levine *et al.*, 2003). However, the potential for invasive plants to alter nutrient cycling in non-native environments is largely dependent on how they differ from the dominant native species in their nutrient-acquisition and nutrient-use strategies (Ehrenfeld, 2003). With the ever-increasing population and distribution of invasive N₂-fixers globally (Richardson and Rejmánek, 2011), the need to better understand this potential is growing.

Quantifying ecological impacts of invasive N₂-fixers requires an understanding of the relative contribution of N₂-fixation to the N-cycle in specific ecosystems. Exotic N₂-fixers may not necessarily form symbiotic relationships with rhizobia found in non-native soils (Sprent and Parsons, 2000; Giller, 2001), thus site-specific assessments of N₂-fixation are often necessary to quantify local-scale inputs. Ecosystems most impacted by N₂-fixer invasions are those which do not have native N₂-fixing species with comparable N₂-fixing capacities. Differences in N₂-fixation strategy (facultative vs. obligate, Menge *et al.*, 2009) between native and invasive N₂-fixers will also influence nutrient cycling impacts (Drake, 2011; Tye and Drake, 2012).

The legume genus *Acacia* (sub family Mimosoideae) is widely distributed throughout the world, particularly in Africa, Asia and Australia. Acacias are generally capable of forming symbiotic associations with N₂-fixing rhizobia bacteria in root nodules (Sprent and Parsons, 2000; Sprent, 2009), and their ability to fix N₂ means they can meet much of their N requirement directly from symbiosis (Sprent, 2007). In South Africa, invasions by Australian *Acacia* trees (a majority of which are in the subgenus *Phyllodeneae*, see Miller *et al.*, 2011 for full taxonomic details and recent revisions) are causing widespread ecosystem disturbances (Le Maitre *et al.*, 2011). The range of recognised ecological impacts associated with Australian acacias has expanded considerably over the last decade as impacts have become more severe. Consequently, a variety of interventions have been initiated to control the spread and mitigate the impacts of their invasion (Nel *et al.*, 2004; Yelenik *et al.*, 2004; Le Maitre *et al.*, 2011). From an invasion biology perspective, key traits attributed to the success of Australian acacias as invaders include high growth rate (Morris *et al.*, 2011b), high

biomass accumulation capacity (Yelenik *et al.*, 2007; Morris *et al.*, 2011b), prolonged viability of mass produced seeds (Yelenik *et al.*, 2004) and high N₂-fixation capacity (Sprent, 2009). Considering that N limits primary production in many southern African ecosystem (Scholes *et al.*, 2003), it seems reasonable to assume that the traits which enhance the invasiveness of woody species (such as high growth rates and biomass accumulation) are facilitated by adaptive N acquisition strategies, stressing the importance of N₂-fixation as a key trait of invasive acacias. Furthermore, due to their ability to increase N availability in soils, acacias are used in mixed-species plantations across the world (Wiersum, 1985; Caldeira *et al.*, 2002; Forrester *et al.*, 2007; Moyo and Fatunbi, 2010), facilitating the introduction and spread of these species.

Estimation of N₂-fixation rates in woody perennials has been achieved through a number of techniques in forestry and agricultural settings (e.g. Peoples *et al.*, 1989; Werner and Newton, 2005). These include the acetylene reduction technique (Hardy *et al.*, 1968), dry matter yield, total N balance, xylem sap based procedures, nodule observations and ¹⁵N methodologies. Details of these methods have been described in several publications (see review by Hardarson and Danso, 1993). The ¹⁵N natural abundance method has gained popularity in natural and field systems as it only requires the collection of plant material for isotopic analysis, does not disturb soil processes taking place in these environments and gives an integrated estimate of percentage N fixed (Högberg, 1997). ¹⁵N isotope techniques theoretically allow the identification of N derived from soil and from the atmosphere by exploiting the natural enrichment of plant-available N in the soil relative to that obtained through atmospheric nitrogen fixation (Shearer and Kohl, 1986). Calculation of the proportion of N derived from the atmosphere is based on the principle that with increasing N₂-fixation, the abundance of ¹⁵N in the N₂-fixing plant declines. This is because N assimilated from the soil is effectively diluted by fixed atmospheric N₂ which typically has lower ¹⁵N abundance (Shearer and Kohl, 1986; Högberg, 1997; Boddey *et al.*, 2000). Relative N contributions by native vs. invasive acacias can thus be estimated for particular ecosystems using $\delta^{15}\text{N}$, standing stocks, and annual growth rates, and can allow the estimation of relative changes in N cycling at large scales.

Here, we investigate N₂-fixation of an invasive Australian *Acacia* co-occurring with an African species in the Komati River Catchment in eastern South Africa.

Acacia dealbata is an invasive Australian acacia (native to southeastern Australia) which has aggressively invaded grasslands, disturbed watercourses and roadsides in many parts of southern Africa (Richardson *et al.*, 1997). The native *A. ataxacantha* is one of few *Acacia* species reported not capable of forming symbiotic root nodules, and is thought to be a non-N₂-fixing (non-fixing) species (Harrier *et al.*, 1997; Cramer *et al.*, 2007). However, nodulation is not an ultimate requirement of N₂-fixation (Sprent and Parsons, 2000), and field assessments of *A. ataxacantha* have not been carried out throughout its range. The objectives of this study were to answer three key questions regarding the N₂-fixation capacity of the study species using the ¹⁵N natural abundance technique. These were (1) is the native *A. ataxacantha* fixing N in the Komati riparian zone, and if so, at what rates per unit biomass? (2) At what rates per unit biomass is the invasive *A. dealbata* fixing N? (3) Is N₂-fixation capacity related to plant structural characteristics, such as stem diameter and height? Insights gained from this study will aid in predictions around changes in N contribution/availability at ecosystem scales associated with invasion by *A. dealbata*. Furthermore, knowledge around N₂-fixing capacity is critical to understanding the nutrient economy of these species, and aid in the interpretation of differences in nutrient allocation and conservation strategies.

3.3. Material and Methods

The Komati study site was located along the upper Komati River in the mountain grasslands of the Drakensberg escarpment in Mpumalanga province in eastern South Africa (25° 892S, 30° 298E). The region is characterised by undulating hills, rocky cliffs and patches of acacia woodlands in the lowland regions. The area receives relatively high rainfall for the region, with mean annual rainfall of 910 mm, ranging between 800 and 1250 mm. Mean annual temperature for the region is 16 °C (Mucina and Rutherford, 2006)

Foliar samples were collected in mid wet season (Early December 2011), when the leaves on the trees were fully expanded. Fifteen trees were selected for sampling from each species, and a representative selection of leaves from the canopy without and visible signs of disease were taken from each tree and stored in paper bags. Samples were collected from 15 trees of each acacia species. Each acacia sample was paired with a reference plant sample comprised of foliar material collected and pooled

from three or more non-fixing reference plants growing within a 5 m radius of the acacia. Reference species included *Ziziphus mucronat*, *Diospyros lycioides*, *Euclea divinorum*, *Rhus spp* and *Gymnosporia spp*. Samples were returned to the laboratory where they were ground in a rotary mill, weighed to ~1.2 mg using an ultramicrobalance (Mettler Toledo XP6), and prepared for C and N elemental compositions and isotope analysis using a Thermo Delta V Advantage continuous flow isotope ratio mass spectrometer. Stable isotopic data are reported in standard delta notation ($\delta^{15}\text{N}$, relative to atmospheric N_2 reference standard). Analytical precision was $\leq \pm 0.2\text{‰}$ for nitrogen stable isotopes, and $< \pm 0.5\%$ of the sample value for %N.

The fractional contribution of biologically fixed N (%Ndfa) in N_2 -fixing species was estimated using the following equation (Amarger *et al.*, 1979)

$$\%Ndfa = 100 \frac{(\delta^{15}\text{N}_{ref} - \delta^{15}\text{N}_{fixingplant})}{\delta^{15}\text{N}_{ref} - \beta} \quad (\text{Eqn 3.1})$$

where $\delta^{15}\text{N}_{ref}$ is the ^{15}N natural abundance of N derived from the soil (estimated using neighbouring non-fixing reference plant), $\delta^{15}\text{N}_{fixingplant}$ is the $\delta^{15}\text{N}$ of the potentially N_2 -fixing acacia and β is the $\delta^{15}\text{N}$ value of nodulated acacias reliant solely on N derived from the atmosphere. We used a β value of -2.22, calculated as the lower 10% percentile of the range of $\delta^{15}\text{N}$ values observed in *A. dealbata*, a known N_2 -fixing species. A similar approach was applied in previous ^{15}N natural abundance studies in the Komanti study site (Tye and Drake, 2012), and yields conservative estimates of %N fixed.

Differences between foliar $\delta^{15}\text{N}$ values in an acacia and associated reference plants were tested for using a Wilcoxon Matched Pairs test. Interspecific differences in foliar $\delta^{15}\text{N}$ and C:N ratios between *Acacia* species were assessed using a Mann-Whitney U test. A one-way ANOVA was used to test for differences in foliar %N among acacias and reference plant groups, followed by a Tukey's HSD *post hoc* test for means comparison when significant differences were found. We used an Arcsine transform of %N in statistical analyses. Non-parametric tests were used for data that were not normally distributed. Data were processed using Statistica v8.0 (Statsoft 2007), and significance was determined at $P < 0.05$.

3.4. Results

Leaf $\delta^{15}\text{N}$ values of the non-fixing reference species ranged from -1.7 to 1.8‰, and the values associated with *Acacia* species varied from -2.3 to +2.3 (-0.1 to +2.3‰ in all but one tree in *A. ataxacantha*) and from -2.4 to +0.1‰ in *A. dealbata* (Figure 3.1). Between the two *Acacia* species, there were differences in leaf $\delta^{15}\text{N}$ (Mann-Whitney U test, $U = 16$, $P < 0.001$), with depleted leaf $\delta^{15}\text{N}$ in *A. dealbata* (mean = -1.6‰) relative to the generally enriched values of *A. ataxacantha* (mean = 0.6‰). Overall, both acacias differed significantly from their associated reference species in terms of leaf $\delta^{15}\text{N}$ ($P < 0.01$ for both species, Wilcoxon Matched Pairs test). The magnitude of the difference averaged 1.0‰ (± 0.23 S.E.) and -1.07 (± 0.19 S.E.) for *A. ataxacantha* and *A. dealbata*, respectively. Surprisingly, 14 of the 15 ($N=15$) *A. ataxacantha* plants sampled had higher leaf $\delta^{15}\text{N}$ (mean = 0.6‰) than associated reference plants (mean = -0.4‰) that were used to estimate soil available $\delta^{15}\text{N}$. This suggested that *A. ataxacantha* is not fixing N in the Komati study site, and also suggests markedly different N sources or internal N-cycling. Conversely, *A. dealbata* had significantly lower leaf $\delta^{15}\text{N}$ (mean = -1.6‰) than associated reference plants (mean = -0.5), a pattern indicative of N_2 -fixing species. We found no difference in leaf $\delta^{15}\text{N}$ of the reference plants (incorporation five species) associated with the two *Acacia* species, though leaf $\delta^{15}\text{N}$ in reference plants associated with *A. dealbata* was slightly, but not significantly, lower than those associated with *A. ataxacantha*. As only *A. dealbata* had leaf $\delta^{15}\text{N}$ lower than reference plants, %Ndfa was calculated for *A. dealbata* alone. This translated to an average of 70% leaf N from fixation ($\pm 7\%$ SE), ranging between 0 and 100%. Negative estimates were set to zero and estimates above 100% were rounded to 100%.

Comparison of leaf-N concentrations between putative N_2 -fixers and reference species yielded significant differences in most cases (ANOVA, $df = 56$, $F = 24.759$, $P < 0.001$). Leaves from *A. ataxacantha* had higher N concentrations (1.9 to 3.6%) than leaves from *A. dealbata* (1.9 to 3.2%), and both acacias had substantially higher leaf-N than their associated reference plants (Tukey's post hoc test, Figure 31b). Reference plant leaf N concentrations did not differ significantly between acacias, however, mean leaf N concentration in reference plants associated with *A. dealbata* was 10% greater than those associated with *A. ataxacantha* (Table 3.1), suggesting possible N

enrichment by *A. dealbata*. Leaf C:N mass ratios showed significant species interactions between acacias, with higher C:N ratios in leaves of *A. dealbata* than those of *A. ataxacantha* (Mann-Whitney U test, $Z = -3.67$, $P < 0.001$). Reference plants had lower C:N ratios than their associated legumes.

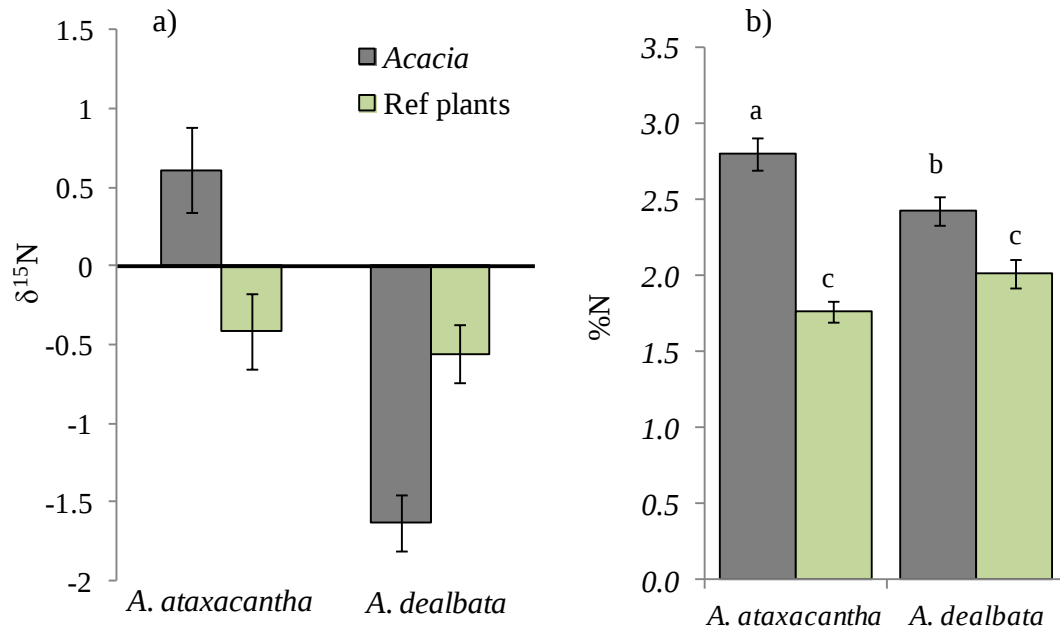


Figure 3.1 Foliar $\delta^{15}\text{N}$ (a) and N concentration (b) in *A. dealbata* and *A. ataxacantha* with associated reference plants. Fully expanded mature leaves collected during December 2011 from the Komati study site. Values are means, error bars indicate SE.

Table 3.1 Nitrogen (%) and $\delta^{15}\text{N}$ (‰) in leaves of potential N_2 -fixing *Acacia* and non-fixing reference plants growing in the Komati study site. Replicate values represent independent composite leaf samples from different trees. All values are means [$\pm$ standard error].

Species	n	Range of $\delta^{15}\text{N}$ (‰)	Mean $\delta^{15}\text{N}$ (‰) [S.E.]	Range of %N	Mean %N [S.E.]	Mean C:N [S.E.]
Acacias						
<i>A. ataxacantha</i>	15	-2.3 to 2.3	0.6 [0.27]	1.9 to 3.6	2.8 [0.11]	21.1 [0.91]
<i>A. dealbata</i>	15	-2.4 to 0.1	-1.6 [0.18]	1.9 to 3.2	2.4 [0.09]	27.0 [0.94]
Reference trees						
Ref - a	15	-1.7 to 1.8	-0.4 [0.24]	1.4 to 2.2	1.8 [0.07]	32.8 [1.36]
Ref - d	15	-1.7 to 0.6	-0.6 [0.19]	1.5 to 2.4	2.0 [0.09]	28.8 [1.37]

The divergence between leaf N properties in acacias can be seen in a bi-plot comparing %N and $\delta^{15}\text{N}$ (Figure 3.2). The N_2 -fixing *A. dealbata* shows slight separation out with relatively depleted $\delta^{15}\text{N}$ values and greater N concentrations.

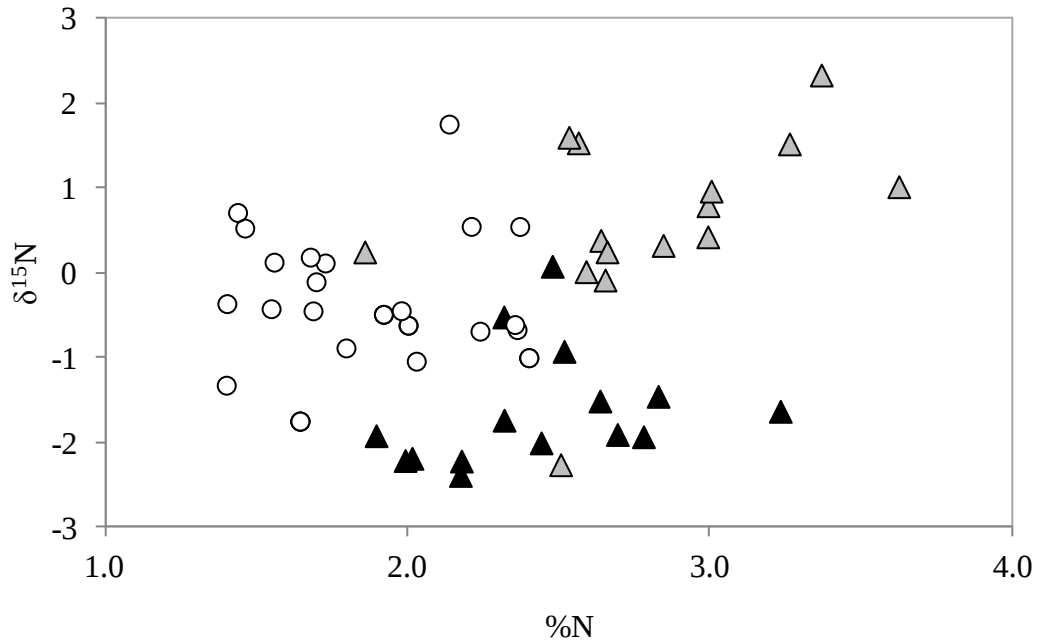


Figure 3.2 Leaf N concentrations and $\delta^{15}\text{N}$ for ▲ - *Acacia dealbata*; △ - *Acacia ataxacantha*; ○ - all reference plants (composite samples representing 3-5 species/individuals each).

We found no relationship between foliar $\delta^{15}\text{N}$ values and plant age (of which basal diameter was used as a proxy) or aboveground biomass in either *Acacia* species. Leaf-N concentrations also did not vary systematically with $\delta^{15}\text{N}$ values across *Acacia* species or across reference plants (Figure 3.2).

3.5. Discussion

We found interspecific differences between native and alien invasive *Acacia* species leaf-N traits analysed in this study. The most striking of these was high N_2 -fixation capacity of the invasive *A. dealbata* in comparison to the evident non-fixing strategy of the native *A. ataxacantha*. The lack of N_2 -fixation in *A. ataxacantha* supports previous findings (e.g., Hernandez-Lucas *et al.*, 1995; Cramer *et al.*, 2007;

Cramer *et al.*, 2010) and demonstrates how phylogenetically related species co-occurring the same environment can employ divergent nutrient acquisition strategies. Furthermore, this suggests novel N-inputs in the t the Komati study site may be considerable increased with the displacement of *A. ataxacantha* by *A. dealbata*.

The range of $\delta^{15}\text{N}$ values found in this study (-2.4 to 2.3‰) was within the range typically found in other woody perennials growing in grassland or savanna systems (Aranibar *et al.*, 2003; Cramer *et al.*, 2007; Jacobs *et al.*, 2007), and are similar to previously reported values for fixing and non-fixing species growing in the upper Komati River catchment (Tye and Drake, 2012). This was a relatively narrow range considering that co-occurring species can vary as much as 10‰ in the tissue $\delta^{15}\text{N}$ (Handley and Scrimgeour, 1997; Dawson *et al.*, 2003). Nonetheless, our results show a clear divergence in foliar $\delta^{15}\text{N}$ values between the native and invasive *Acacia* species. This suggests that the two species of *Acacia* are utilising N from different sources for their internal N stocks, however, we acknowledge that caution should be used when using $\delta^{15}\text{N}$ values as indicators of N source, and discuss additional potential sources of variation.

3.5.1. Foliar $\delta^{15}\text{N}$ in acacias and reference plants

The pattern of ^{15}N enrichment in *A. ataxacantha* relative to reference plants was not expected. As leaf N isotope composition is primarily determined by the isotope ratio of the external N source and the physiological mechanisms within the plant (Evans, 2001), we explore several possible explanations for this pattern. Firstly, many southern African *Acacia* are deep rooted species which is likely an adaptation to overcome seasonal water limitation in semi-arid and arid environments (Handley and Raven, 1992; Belsky, 1994). Deep-rooted species can often access different N pools than surrounding reference species, even when taking up the same form of N (e.g. NH_4). In general, $\delta^{15}\text{N}$ increases with depth in the soil profile (Shearer and Kohl, 1986; Nadelhoffer and Fry, 1994; Högberg, 1997), a pattern postulated to be a combined result of the microbial decomposition of ^{15}N -enriched organic matter present in deeper soils, the isolation of deep soil N from atmospheric inputs and the re-deposition of fresh litter which tends to be slightly depleted in ^{15}N relative to soil N (reviewed by Nadelhoffer and Fry, 1994; Högberg, 1997). This is important because it means that

deeper-rooted species may be taking up N with an enriched $\delta^{15}\text{N}$ signature relative to shallow rooted species. A similar pattern was observed in a field experiment in Hluhluwe-iMfolozi, where deeper rooted acacia species tended to have more positive $\delta^{15}\text{N}$ values than those with shallower roots (Cramer *et al.*, 2007). In our study, however, we sampled plants from a range of size classes, including smaller plants which are likely to have obtained most of their nutrient from shallow soils. We also used a composite of reference plant species which was likely to have included some deeper rooted plants, yet the pattern of foliar $\delta^{15}\text{N}$ enrichment in *A. ataxacantha* relative to reference plants was almost consistent. We thus argue that rooting depth alone is unlikely to account for the ^{15}N enriched signal of *A. ataxacantha* relative to non-fixing reference plants, though it was probably a contributing factor.

A second possible explanation for the observed pattern is that the degree of isotopic fractionation taking place during N uptake and assimilation from the soil is low for *A. ataxacantha* relative to that of reference plants. Plant tissues are typically depleted in ^{15}N relative to the soils as biological processes involved in plant nutrient cycling generally discriminate against ^{15}N , producing products that are ^{15}N -depleted (Handley and Raven, 1992; Nadelhoffer and Fry, 1994; Evans, 2001). Isotopic fractionations can take place at the uptake, transport and assimilation steps of N cycling in plants, and have been shown to vary among plant species utilising different strategies at each of the respective steps (Evans, 2001). For example, foliar $\delta^{15}\text{N}$ values differ among plants that are associated with different types of mycorrhizal fungi which facilitate N acquisition from the soil (see reviews by Nadelhoffer and Fry, 1994; Boddey *et al.*, 2000). This was demonstrated by Craine (2009) in a global meta-analysis of the patterns of foliar $\delta^{15}\text{N}$ in relation to nutrient concentrations, climate, and mycorrhizal fungi. Craine (2009) reported that relative to non-mycorrhizal plants, foliar $\delta^{15}\text{N}$ values from arbuscular mycorrhizal (AM), ectomycorrhizal (ECM), and ericoid mycorrhizal (EM) plants were on average depleted by 2‰, 3.2‰, and 5.9‰, respectively. However, these differences may be extreme examples and are dependent on both the degree of plant reliance on the fungi for N (Craine *et al.*, 2009) and the ratio of N supply to N demand. As mycorrhizal associations are found in most plants under natural conditions (Högberg *et al.*, 1999; Chapin *et al.*, 2002), could this explain the observed patterns in foliar $\delta^{15}\text{N}$ values in our study? To address this, we

considered the known mycorrhizal associations and the N demand of the species. Taxonomic studies indicate that *A. ataxacantha* would typically only associate with AM (Sprent, 2009), suggesting minimal fractionation during N uptake. Non-fixing reference species growing in sub-tropical woodland are also likely to have AM, although some may have a combination of AM and ECM associations (Högberg, 1982; Högberg, 1992). It is therefore unlikely that that mycorrhizal association alone would account for elevated leaf $\delta^{15}\text{N}$ in *A. ataxacantha*.

Thirdly, it seems logical that resorption of N from leaves may result in ^{15}N enrichment of residual leaf-N due to metabolic fractionations through the preferential resorption of the light isotope. Positive correlation between NRE and the difference between $\delta^{15}\text{N}$ in green and senesced leaves was observed in salt marsh grasses (*Spartina* spp.) in fertilized and natural salt marshes in New England (Drake *et al.*, 2008). However, we were unaware of any studies on woody plants which explicitly found ^{15}N enrichment resulting from nutrient resorption. Instead, numerous studies have reported relatively depleted $\delta^{15}\text{N}$ values in the leaf litter of N_2 -fixing acacias, suggesting nutrient resorption has little influence on $\delta^{15}\text{N}$ values of senescing leaves, and that the relatively high leaf $\delta^{15}\text{N}$ in *A. ataxacantha* is not primarily driven by N resorption. Furthermore, seasonal allocation data indicated that nutrient resorption takes place late in the growing season (see Chapter 4), thus it is unlikely that the foliar $\delta^{15}\text{N}$ values observed here would have been altered significantly by nutrient resorption processes.

Confounding the interpretation of foliar $\delta^{15}\text{N}$ is the large number of possible fractionation steps and sources of variation in $\delta^{15}\text{N}$ of plant available N. We have therefore put forward multiple, perhaps competing interpretations for the pattern of foliar ^{15}N signals observed in this study. Ultimately, natural abundance $\delta^{15}\text{N}$ of plant tissue is some function of all the source and metabolic effects to which its N pools have been subjected (Handley and Raven, 1992; Robinson *et al.*, 1998), and it is therefore likely to represent a combination of the above effects. Nevertheless, this does bring into question the use and selection of reference plants for %Ndfa calculations, and suggests that we may well have underestimated N_2 -fixation in *A. dealbata*. For instance, when we *corrected* reference plant $\delta^{15}\text{N}$ using the average difference (1.02‰) between *A. ataxacantha* and $\delta^{15}\text{N}$ in paired reference plants, %Ndfa in *A.*

dealbata ranged between 10 and 100%, with a mean of 79%, compared to the average of 70% calculated using the pair reference plants.

3.5.2. *Acacia ataxacantha* as a non-fixing species

Why does *A. ataxacantha* not fix nitrogen? This question is somewhat puzzling considering *A. ataxacantha* is often found growing in similar landscapes to other African acacias with high N₂-fixation capacity. *A. ataxacantha* is one of the few African species observed not to nodulate under a range of natural and experimental conditions. For example, Masutha *et al.* (1997) surveyed *A. ataxacantha* trees from six different ecological zones within South Africa and found no evidence of nodulation. Furthermore, Masutha *et al.* (1997) failed to induce nodulation of *A. ataxacantha* using a promiscuous marker strain (*Rhizobium* NGR234) known to nodulate over 75 genera of legumes, and ultimately concluded that *A. ataxacantha* is a non-nodulating *Acacia*. Similar experimental conclusions were reported by Hernandez-Lucas *et al.* (1995) and Harrier (1997). This lack of ability to form N₂-fixing root nodules is rare (estimated <10% of species) in the subfamily Mimosoideae (Allen and Allen, 1981).

Non-nodulating African *Acacia* species belong to the section *Monacantha* of the subgenus *Aculeiferum*. Section *Monacantha* is pan tropical, including species from Southern and Central America. Most of the African species of section *Monacantha* are scrambling shrubs or small trees. The phylogenetic distinctness of the non-nodulating group of acacias suggests that single event lead to the loss of nodulation, rather than them never having had it (Harrier *et al.*, 1997; Sprent, 2009). Interestingly, the non-nodulating character of these species has not been used as a taxonomic classification character for this group, but rather classification has been based upon morphological characters and molecular markers (e.g. Harrier *et al.*, 1997). Many of these morphological characters are considered adaptations to forest edge environments where many of these species typically occur (Harrier *et al.*, 1997). This suggests that the cost of the physiological investment required for nodule formation in low light environments where nutrient cycling is tight might be too high, i.e. there may be active selection against nodulation in these species. However, it is difficult to assume, and to test for that matter, that lack of nodulation infers lack of N₂-fixation (J. Sprent, pers. comm.).

A general ability of African *Acacia* is their capability of forming arbuscular mycorrhizas (AM, formally known as VAM), which is likely to facilitate uptake of N and P from soil pools. Symbiosis with AM is common in African trees, and is by no means limited to acacias. However, their Australian counterparts (of the subgenus *Phyllodineae*) are capable of forming both AM and ectomycorrhizas (ECT), even when growing in African soils (Sprent, 2007). *Phyllodineae* is the only sub-genus of *Acacia* that has members with both AM and ECT, a feature that makes them very good for land reclamation and also becoming invasive. Within *Phyllodineae*, nodulation seems to be a generic characteristic.

3.5.3. Estimation of N_2 fixation in *A. dealbata*

Our estimates of percentage contribution of N_2 -fixation in *A. dealbata* foliage (~70%) were similar to those reported of this and closely related species (May and Attiwill, 2003; Tye and Drake, 2012), and rank relatively high in comparisons to estimates for African acacias (Schulze *et al.*, 1991; Brockwell *et al.*, 2005; Pule-Meuelenberg and Dakora, 2010). This estimate suggests that *A. dealbata* fixes approximately three times more N than it takes up from the soil in the Komati study site. Considering its thicket forming growth habit, this represents an important novel source of N in the Komati study site. Previous ^{15}N natural abundance studies done at the Komati study, yielded a mean $\delta^{15}N$ for *A. mearnsii* (a closely related Australian species) of -1.1‰, however, these were based branch-tip samples rather than green foliage. Woody material typically has lower ^{15}N abundance relative to leaves (Boddey *et al.*, 2000). This pattern has been observed in other ^{15}N natural abundance studies of *A. dealbata* (May and Attiwill, 2003).

The use of ^{15}N natural abundance techniques to estimate contributions of atmospheric N_2 -fixation to N budgets has been demonstrated a wide variety of ecosystems, including grasslands and savannas, and has been reviewed in a number of key works (Shearer and Kohl, 1986; Vitousek *et al.*, 1989; Lajtha and Marshall, 1994; Boddey *et al.*, 2000; Dawson *et al.*, 2003). Although the ^{15}N natural abundance method has limited quantitative estimation ability, its suitability as a qualitative measure in natural settings has been well accepted (Boddey *et al.*, 2000; Dawson *et al.*, 2003). Comparisons of estimates obtained using the ^{15}N natural abundance method with those from other frequently used methods support its use with both African and Australian

Acacia species (Ndoye *et al.*, 1995; Hosseini Bai *et al.*, 2012), however it is likely to underestimate total contributions of N₂-fixation (see section 3.5.1).

The generally depleted ¹⁵N signal of the reference plants observed in the Komati study site suggests that the soil N pool has low ¹⁵N enrichment. A general suggestion for the application of the δ¹⁵N method of estimating N₂-fixation is that non-fixing reference species should differ by at least 5‰ than N derived from biological N₂-fixation for the putative N₂-fixing species, that is, the *B* value (Högberg, 1997). As the differences between reference plants and our *B* value for *A. dealbata*, the value of *B* becomes a potentially important source of error (Boddey *et al.*, 2000). The *B* value will also be more important if the N₂-fixing species has a high reliance on N₂-fixation. Additional potential sources of error include interspecific differences in N isotope fractionation between reference and N₂-fixing species, as demonstrated here in the relatively enriched *A. ataxacantha* leaf δ¹⁵N vs. reference plants used for %Nd_fa. We have already discussed the possible influence of inherent differences in isotopic fractionations between N₂-fixing and reference species, but for more information on fractionations associated with internal processes involved in N cycling within plants, see reviews by Handley and Raven (1992) and Boddey (2000). Variation among δ¹⁵N values in non-fixing species growing at a single site can be considerable (Schulze *et al.*, 1991; Tye and Drake, 2012). Such variation can either be a consequence of microscale variation in the soil N pools or difference in plant N metabolism. However, between-species differences have also been noted (Tye, 2010). We feel our use of a composite sample of non-fixing reference plant leaves from diverse selection of plant species provided a more fair baseline δ¹⁵N signature of plant available N in the soil at the microhabitat scale than use of single species or plants.

3.5.4. Concluding remarks

The alien invasive and the native acacia had distinct foliar δ¹⁵N values (Figure 3.1), suggesting a lack of N₂-fixation in the native *A. ataxacantha* and a high fixation capacity (70% of its leaf N) in *A. dealbata*. Therefore, the loss of ability to form N₂-fixing root nodules in *A. ataxacantha* appears to be accompanied by a loss of ability for symbiotic relationships with N₂-fixing bacteria. Despite being a non-fixing species, *A. ataxacantha* had the highest leaf N concentrations of all species measured suggesting the development of an effective N-acquisition strategy. Nitrogen 15

enrichment in *A. ataxacantha* relative to non-fixing reference plants was interpreted as a combined consequence of separation of rooting depth, weaker discrimination against ^{15}N or possible discrimination against ^{14}N occurring during N uptake (possibly as a consequence of a high reliance of AM mycorrhizae) and differences in internal fractionations. An enriched ^{15}N signature in a non-fixing acacia relative to reference plants also suggests that we may be underestimating %Ndfa in *A. dealbata* by as much as 9%. We found no relationship between N_2 -fixation capacity and tree structural dimensions. We can conclude that invasion by the N_2 -fixing *A. dealbata* will result in increased bioactive N inputs in landscapes where native acacias such as *A. ataxacantha* are not fixing N, and may even contribute to N excess at catchment system scales.

3.6. References

- Allen, O. N. and Allen, E. K. (1981) *The Leguminosae, a source book of characteristics, uses, and nodulation*. University of Wisconsin Press, Wisconsin, USA.
- Amarger, N., Mariotti, A., Mariotti, F., Durr, J., Bourguignon, C., and Lagacherie, B. (1979) Estimate of symbiotically fixed nitrogen in field grown soybeans using variations in ^{15}N Natural abundance. *Plant and Soil* 52(2):269-280.
- Aranibar, J. N., Macko, S. A., Anderson, I. C., Potgieter, A. L. F., Sowry, R., and Shugart, H. H. (2003) Nutrient cycling responses to fire frequency in the Kruger National Park (South Africa) as indicated by Stable Isotope analysis. *Isotopes in Environmental and Health Studies* 39(2):141-158.
- Belsky, A. J. (1994) Influences of trees on savanna productivity: tests of shade, nutrients, and tree-grass competition. *Ecology* 75(4):922-932.
- Boddey, R., Peoples, M., Palmer, B., and Dart, P. (2000) Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. *Nutrient cycling in agroecosystems* 57(3):235-270.
- Brockwell, J., Searle, S. D., Jeavons, A. C., and Waayers, M. (2005) Nitrogen fixation in acacias: an untapped resource for sustainable plantations, farm forestry and land reclamation. Monograph No. 115. ACIAR.
- Caldeira, M. V. W., Schumacher, M. V., and Spathelf, P. (2002) Quantification of nutrient content in above-ground biomass of young *Acacia mearnsii* De Wild., provenance Bodalla. *Ann. For. Sci.* 59(8):833-838.
- Chapin, F. S., Matson, P. A., and Mooney, H. A. (2002) *Principles of terrestrial ecosystem ecology*. Springer, New York, USA.
- Craine, J. M., Elmore, A. J., Aida, M. P. M., Bustamante, M., Dawson, T. E., Hobbie, E. A., Kahmen, A., Mack, M. C., McLauchlan, K. K., Michelsen, A., Nardoto, G. B., Pardo, L. H., Peñuelas, J., Reich, P. B., Schuur, E. A. G., Stock, W. D., Templer, P. H., Virginia, R. A., Welker, J. M., and Wright, I. J. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist* 183(4):980-992.

- Cramer, M. D., Chimphango, S. B. M., Van Cauter, A., Waldram, M. S., and Bond, W. J. (2007) Grass competition induces N₂ fixation in some species of African *Acacia*. *Ecology* 95:1123-1133.
- Cramer, M. D., van Cauter, A., and Bond, W. J. (2010) Growth of N₂-fixing African savanna *Acacia* species is constrained by below-ground competition with grass. *Journal of Ecology* 98(1):156-167.
- Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., and Tu, K. P. (2003) Stable isotopes in plant ecology. *Annual review of ecology and systematics* 33:507-559.
- Drake, D. C. (2011) Invasive legumes fix N₂ at high rates in riparian areas of an N-saturated, agricultural catchment. *Journal of Ecology* 99(2):515-523.
- Drake, D. C., Peterson, B. J., Deegan, L. A., Harris, L. A., Miller, E. E., and Warren, R. S. (2008) Plant nitrogen dynamics in fertilized and natural New England salt marshes: a paired ¹⁵N tracer study. *Marine Ecology Progress Series* 354:35-46.
- Ehrenfeld, J. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6(6):503-523.
- Evans, R. D. (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science* 6(3):121-126.
- Forrester, D. I., Schortemeyer, M., Stock, W. D., Bauhus, J., Khanna, P. K., and Cowie, A. L. (2007) Assessing nitrogen fixation in mixed-and single-species plantations of *Eucalyptus globulus* and *Acacia mearnsii*. *Tree physiology* 27(9):1319-1328.
- Giller, K. E. (2001) *Nitrogen fixation in tropical cropping systems*. CABI Publishing, Wallingford, USA.
- Handley, L. L. and Raven, J. A. (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell & Environment* 15(9):965-985.
- Handley, L. L. and Scrimgeour, C. M. (1997) Terrestrial plant ecology and ¹⁵N natural abundance: The present limits to interpretation for uncultivated systems with original data from a Scottish old field. *Advances in Ecological Research* Volume 27:133-212.
- Hardarson, G. and Danso, S. K. A. (1993) Methods for measuring biological nitrogen fixation in grain legumes. *Plant and Soil* 152(1):19-23.
- Hardy, R. W. F., Holsten, R. D., Jackson, E. K., and Burns, R. C. (1968) The acetylene-ethylene assay for N₂ fixation: laboratory and field evaluation. *Plant physiology* 43(8):1185-1207.
- Harrier, L. A., Whitty, P. W., Sutherland, J. M., and Sprent, J. I. (1997) Phenetic investigation of non-nodulating African species of *Acacia* (Leguminosae) using morphological and molecular markers. *Plant Systematics and Evolution* 205(1):27-51.
- Hernandez-Lucas, I., Segovia, L., Martinez-Romero, E., and Pueppke, S. (1995) Phylogenetic relationships and host range of *Rhizobium* spp. that nodulate *Phaseolus vulgaris* L. *Appl. Environ. Microbiol.* 61(7):2775-2779.
- Högberg, P. (1982) Mycorrhizal associations in some woodland and forest trees and shrubs in Tanzania. *New Phytologist* 92(3):407-415.
- Högberg, P. (1992) Root symbioses of trees in African dry tropical forests. *Journal of Vegetation Science* 3(3):393-400.

- Högberg, P. (1997) ^{15}N natural abundance in soil-plant systems. *New Phytologist* 137(2):179-204.
- Högberg, P., Hogberg, M. N., Quist, M. E., Ekblad, A., and Nasholm, T. (1999) Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *pinus sylvestris*. *New Phytologist* 142(3):569-576.
- Hosseini Bai, S., Sun, F., Xu, Z., Blumfield, T., Chen, C., and Wild, C. (2012) Appraisal of ^{15}N enrichment and ^{15}N natural abundance methods for estimating N_2 fixation by understorey *Acacia leiocalyx* and *A. disparimma* in a native forest of subtropical Australia. *Journal of Soils and Sediments* 12(5):653-662.
- Jacobs, S. M., Pettit, N. E., and Naiman, R. J. (2007) Nitrogen fixation by the savanna tree *Philenoptera violacea* (Klotzsch) Schrire (Apple leaf) of different ages in a semi-arid riparian landscape. *South African Journal of Botany* 73(1):163-167.
- Lajtha, K. and Marshall, J. D. (1994) Sources of variation in the stable isotopic composition of plants. in K. Lajtha and R.H. Michener, editors. *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publications, Oxford.
- Le Maitre, D. C., Gaertner, M., Marchante, E., Ens, E. J., Holmes, P. M., Pauchard, A., O'Farrell, P. J., Rogers, A. M., Blanchard, R., and Blignaut, J. (2011) Impacts of invasive Australian acacias: implications for management and restoration. *Diversity and Distributions* 17(5):1015-1029.
- Levine, J. M., Vila, M., Antonio, C., Dukes, J. S., Grigulis, K., and Lavorel, S. (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:775-781.
- Masutha, T. H., Muofhe, M. L., and Dakora, F. D. (1997) Evaluation of N_2 fixation and agroforestry potential in selected tree legumes for sustainable use in South Africa. *Soil Biology and Biochemistry* 29(5-6):993-998.
- May, B. and Attiwill, P. (2003) Nitrogen-fixation by *Acacia dealbata* and changes in soil properties 5 years after mechanical disturbance or slash-burning following timber harvest. *Forest Ecology and Management* 181(3):339-355.
- Morris, T. L., Esler, K. J., Barger, N. N., Jacobs, S. M., and Cramer, M. D. (2011) Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions* 17(5):898-910.
- Moyo, H. P. M. and Fatunbi, A. (2010) Utilitarian Perspective of the Invasion of Some South African Biomes by *Acacia mearnsii*. *Global Journal of Environmental Research* 4(1):6-17.
- Mucina, L. and Rutherford, M. C. (2006) *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute Pretoria, Pretoria, South Africa.
- Nadelhoffer, K. J. and Fry, B. (1994) Nitrogen isotope studies in forest ecosystems. Pp 23-44 in K. Lajtha and R. Michener, editors. *Stable isotopes in ecology and environmental science*. Wiley-Blackwell, Boston, MA, USA.
- Ndoye, I., Gueye, M., Danso, S. K. A., and Dreyfus, B. (1995) Nitrogen fixation in *Faidherbia albida*, *Acacia raddiana*, *Acacia senegal* and *Acacia seyal* estimated using the ^{15}N isotope dilution technique. *Plant and Soil* 172(2):175-180.
- Nel, J. L., Richardson, D. M., Rouget, M., Mgidi, T. N., Mdzeke, N., Le Maitre, D. C., van Wilgen, B. W., Schonegevel, L., Henderson, L., and Naser, S. (2004) A proposed classification of invasive alien plant species in South Africa: towards

- prioritizing species and areas for management action. *South African Journal of Science* 100(1/2):53-64.
- Peoples, M. B., Faizah, A. W., Rerkasem, B., and Herridge, D. F. (1989) Methods for evaluating nitrogen fixation by nodulated legumes in the field. *ACIAR Monograph* 11:1 - 76.
- Pule-Meuelenberg, F. and Dakora, F. D. (2010) Assessing the biological potential of N₂-fixing Leguminosae in Botswana for increased crop yields and commercial exploitation. *African Journal of Biotechnology* 6(4).
- Richardson, D. M., Macdonald, I. A. W., Hoffmann, H. H., and Henderson, L. (1997) Alien plant invasions. Pp 421-446 in R.M. Cowling, D.M. Richardson, and S.M. Peirce, editors. *Vegetation of Southern Africa*. Cambridge University Press, Cambridge, UK.
- Richardson, D. M. and Rejmánek, M. (2011) Trees and shrubs as invasive alien species – a global review. *Diversity and Distributions* 17(5):788-809.
- Robinson, D., Handley, L. L., and Scrimgeour, C. M. (1998) A theory for ¹⁵N/¹⁴N fractionation in nitrate-grown vascular plants. *Planta* 205(3):397-406.
- Scholes, M. C., Scholes, R. J., Otter, L. B., and Woghiren, A. J. (2003) Biogeochemistry: The cycling of Elements. Pp 130-148 in J.T.D. Toit, K.H. Rogers, and H.C. Biggs, editors. *The Kruger Experience: Ecology and Management of Savanna Heterogeneity*. Island Press, Washington, D.C., USA.
- Schulze, E. D., Gebauer, G., Ziegler, H., and Lange, O. L. (1991) Estimates of nitrogen fixation by trees on an aridity gradient in Namibia. *Oecologia* 88(3):451-455.
- Shearer, G. and Kohl, D. H. (1986) N₂-fixation in field settings: estimations based on natural ¹⁵N abundance. *Australian Journal of Plant Physiology* 13(6).
- Schmidt, E., Lotter, M. & McClelland, W. (2007) *Trees and Shrubs of Mpumalanga and the Kruger National Park*. Jacana Publishers, Cape Town.
- Sprent, J. I. (2007) Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. *New Phytologist* 174(1):11-25.
- Sprent, J. I. (2009) *Legume nodulation: a global perspective*. Wiley-Blackwell, Chichester, UK.
- Sprent, J. I. and Parsons, R. (2000) Nitrogen fixation in legume and non-legume trees. *Field Crops Research* 65(2-3):183-196.
- StatSoft, Inc. (2007). STATISTICA (data analysis software system), version 8.0. www.statsoft.com.
- Tye, D. and Drake, D. (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.
- Tye, D. R. C. (2010) Nitrogen fixation by exotic and indigenous *Acacia* trees in the Upper Komati catchment, South Africa. Honours thesis. University of the Witwatersrand, Johannesburg, South Africa.
- Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., and Tilman, D. G. (1997a) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7(3):737-750.
- Vitousek, P. M., D'antonio, C. M., Loope, L. L., Rejmanek, M., and Westbrooks, R. (1997b) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* 21(1):1-16.

- Vitousek, P. M., Shearer, G., and Kohl, D. H. (1989) Foliar ^{15}N natural abundance in Hawaiian rainforest: patterns and possible mechanisms. *Oecologia* 78(3):383-388.
- Werner, D. and Newton, W. (2005) *Nitrogen fixation in agriculture, forestry, ecology, and the environment*. Springer Netherlands.
- Wiersum, K. F. (1985) *Acacia mearnsii*. Multipurpose higland legume tree. *NFT Highlights* 85-02.
- Yelenik, S. G., Stock, W. D., and Richardson, D. M. (2004) Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. *Restoration Ecology* 12(1):44-51.
- Yelenik, S. G., Stock, W. D., and Richardson, D. M. (2007) Functional group identity does not predict invader impacts: differential effects of nitrogen-fixing exotic plants on ecosystem function. *Biological Invasions* 9(2):117-125.

CHAPTER 4: SEASONAL NUTRIENT ALLOCATION AND CONSERVATION STRATEGIES IN COMPETING INDIGENOUS AND ALIEN ACACIA IN A GRASSLAND RIPARIAN SYSTEM IN SOUTH AFRICA

4.1. Abstract

Nutrient allocation and conservation strategies of plant communities fundamentally influence ecosystem functioning through their role in nutrient cycling. Consequently, the introduction of novel strategies with the invasion of alien plants potentially carries ecosystem-scale implications. We compared nitrogen (N) and phosphorus (P) seasonal allocation patterns and conservation processes in an invasive Australian acacia (*Acacia dealbata*, an evergreen N₂-fixer) and a native acacia (*A. ataxacantha*, a deciduous non-N₂-fixing species) in a riparian woodland along the Komati River in eastern South Africa. This study is one component of a larger project looking at the potential implications to N and P cycling caused by invasive Australian *Acacia* species. We measured tissue N and P concentrations in aboveground components of five trees of each species across four seasons. Green-leaf and senesced-leaf N and P concentrations were used to calculate resorption efficiency and proficiency, two informative measures of nutrient conservation. The native acacia had higher N and P concentrations with more pronounced seasonal fluctuations in almost all aboveground components. Mature green leaf N concentrations (25 – 34 mg.g⁻¹) were considerably higher than the global average, while green leaf P concentrations (0.6 – 1.4 mg.g⁻¹) were slightly below the global average. N resorption efficiency (NRE) was low (ranging between 27 and 44%), while 74-88% P was resorbed from senescing leaves. Inefficient N resorption in the invasive acacia (~27%) was attributed to longer leaf lifespan and N₂-fixing capacity which have been hypothesised to carry evolutionary trade-offs in nutrient conservation and allocation. However, resorption proficiency was similar between species, suggesting phylogenetic or environmental controls over nutrient resorption. We found limited evidence of nutritional controls over N and P resorption. Overall, P is more tightly conserved than N in both non-N₂-fixer and the N₂-fixing species, suggesting P limitation in the Komati study site. We conclude that co-occurring native and alien invasive *Acacia* growing in the Komati

grassland-riparian environment differ in respect to their N and P allocation, seasonal dynamics and conservation. Such differentiation in competing native and invasive plants can lead to changes in nutrient cycling within the invaded ecosystem, and may well be a major contributing factor to the success of an invading species.

4.2. Introduction

A fundamental and vital activity of plants is the uptake, processing, and allocation of resources (Aerts and Chapin, 1999; Lambers *et al.*, 2008a; Craine, 2009). Processes involved influence the cycling of resources between plants, soil and the atmosphere, forming the basis of ecosystem nutrient dynamics (Chapin *et al.*, 2002). Consequently, nutrient allocation and conservation strategies of native plant communities are fundamental in regulating ecosystem nutrient cycling, and changes to plant communities through the introduction of alien invasive species have the potential to alter nutrient cycling processes (Vitousek, 1990; Hooper and Vitousek, 1998; Ehrenfeld, 2003). Of particular relevance for ecosystem functioning are the availability and cycling of essential growth limiting nutrient. Knowledge of allocation and conservation of essential nutrients in aboveground plant components is critical if we wish to better understand and predict the impacts on plant invasions on nutrient cycling.

Two essential nutrients which often limit plant growth in many ecosystems are nitrogen (N) and phosphorus (P), both individually and in combination (Vitousek and Howarth, 1991; Craine *et al.*, 2008; Vitousek *et al.*, 2010). Ancient landscapes with highly weathered soils (such as those in southern Africa and southern Australia) are in particular N and P limited (Scholes *et al.*, 2003; Lambers *et al.*, 2008b). To overcome N and P limitation, plants have developed a number of mechanisms which are exhibited in a wide array of traits. Included in these are mechanisms for facilitating nutrient uptake from the soil (such as mycorrhizal associations), symbiotic associations with N-fixing bacteria (e.g. legumes and *rhizobia*), mechanisms that increase the number of chemical forms that can be accessed (“nutrient mining”), and the effective nutrient-conserving strategies (i.e. resorption, internal cycling and allocation of N and P) (Aerts and Chapin, 1999; Lambers *et al.*, 2008b). These are considered as adaptive traits to overcome nutrient limitation, suggesting strong selective pressures on nutrient

acquisition and conservation strategies in plants growing in nutrient-limited landscapes.

It seems to be a general observation that nutrient contents in aboveground components vary among species, even when growing under similar environmental conditions (Tolsma *et al.*, 1987; Forrester *et al.*, 2007; Ratnam *et al.*, 2008; He *et al.*, 2011). Life history traits such as phenology, growth form and N-fixation capacity play an important role in nutrient allocation patterns (Aerts, 1996; Killingbeck, 1996; Aerts and Chapin, 1999; Ratnam *et al.*, 2008). With respect to the genus *Acacia* (see Miller *et al.*, 2011 for recent taxonomic reorganisations), the range of biogeographical histories and life history traits represented in this group leads to the expectation of a wide variety of seasonal nutrient allocation patterns. However, data documenting seasonal changes in nutrient allocation in woody perennials are sparse in the literature, and when available, are generally focused on a single plant component, of which leaves have received the most attention owing to their relative ease of sampling, typically high turnover rates, and importance in plant productivity (Ernst, 1975; Chapin and Kedrowski, 1983; Boerner, 1986; Gill *et al.*, 1987; Tolsma *et al.*, 1987; but for a quantification of seasonal N reallocation in leaves, roots, xylem and phloem see Drake *et al.*, 2006). Among aboveground components in acacias, leaves tend to have the highest concentrations of both N and P (Pereira *et al.*, 2000; Caldeira *et al.*, 2002), with peaks during the early growing season (Tolsma *et al.*, 1987; Fife *et al.*, 2008). High N and P concentrations in leaves are common as chlorophyll is relatively N-rich and ATP and other metabolic compounds concentrated in leaves are P-rich (Sterner and Elser, 2002). However, in comparison to non-legumes, acacias tend to have elevated leaf N concentrations which are generally thought to be due to the N₂-fixation strategies of many *Acacia* species (Vitousek, 1982; Craine, 2009). Structural compounds in woody material, such as lignins and cellulose, have high C, O and H contents but low N concentrations (Aerts and Chapin, 1999), explaining why woody components are generally N-poor. Markedly low concentrations of N and P are typical in dead branches relative to live branches, probably due to resorption of these essential nutrients into younger plant tissue (Caldeira *et al.*, 2002).

The commonly observed trend in leaf N and P translocation out of senesced leaves during drier months is consistent in many Africa acacias (Tolsma *et al.*, 1987).

This process, whereby nutrients are mobilised from senescing leaves and transported out of the leaves before abscission, is termed nutrient resorption. Nutrient resorption is generally thought to benefit plants by enabling them to re-use nutrients, thus acting as an important nutrient-conservation strategy, influencing processes such as nutrient uptake, productivity, and even competition (Pugnaire and Chapin, 1993; Killingbeck, 1996). This is especially true for perennials growing in nutrient-poor ecosystems, which highly depend on internal nutrient cycling (Aerts and Chapin, 1999). Two measures of resorption capacity are generally considered (after Killingbeck, 1996), resorption efficiency (percentage reduction in concentration in senesced leaves) and resorption proficiency (the minimum level to which species reduce nutrient in senescing leaves). Generally, plants are more efficient at P resorption than N resorption, with the global mean of 50% for N and 52% for P (Aerts, 1996). Higher P vs. N resorption efficiency has been observed in many *Acacia* species, and is hypothesized to be a consequence of P limitation in native environments (Tolsma *et al.*, 1987; Ratnam *et al.*, 2008; He *et al.*, 2011). Plants capable of symbiotic N₂-fixation tend to have lower N-resorption efficiencies and proficiencies than non-N-fixing species, while no difference in P resorption efficiency or proficiency exists between N₂-fixers and non-fixers (Killingbeck, 1996). Although there are conflicting results around N and P resorption between evergreen and deciduous tree species, evergreen species do tend to be more proficient at reducing P in their senescing leaves than are deciduous species (Killingbeck, 1996).

Nutritional controls over resorption efficiency are perhaps the most confounding issues around nutrient resorption in terrestrial plants (Killingbeck, 1996). Numerous studies have attempted to identify a relationship between environmental availability of nutrients and resorption, the findings of which have been reviewed and debated in a number of key works (see Aerts, 1996; Wright and Westoby, 2003; Kobe *et al.*, 2005). Although it is unlikely that a general trend exists, meta-analyses of nutritional controls on translocation processes have been plagued by the inconsistency in measures of environmental nutrient availability. Arguably the most widely used measure of environmental nutrient availability is mature leaf nutrient concentration, which Aerts (1996) used in his meta-analysis of nutrient resorption from a wide variety of studies and growth forms. He found leaf nutrient concentrations to be a very weak

predictor or nutrient resorption both within and across growth forms. Furthermore, Aerts (1996) reviewed the responsiveness of nutrient resorption and concluded that it was not very responsive to increase nutrient supply at the phenotypic level. Laboratory studies where plants are grown under elevated nutrient availability have reported mixed findings over the influence of nutrient availability and nutrient resorption (Lajtha and Klein, 1988). A large-scale field fertilization experiment, however, demonstrated significantly lower resorption efficiency in fertilized vs. unfertilized *Spartina* species (Drake et al. 2008).

In this study, we compare N and P seasonal allocation patterns and conservation mechanisms in aboveground components between an invasive Australian *Acacia* and a native African *Acacia* (now *Senegalia*) co-occurring in riparian woodland in eastern South Africa. Of particular interest, is the question of whether alien invasive and native acacias have similar or different nutrient conservation investments? Comparative data on seasonal nutrient allocation is critical for quantifying the potential impacts to N and P cycling associated with an invasion. We focus on the invasive *A. dealbata*, an evergreen species from south eastern Australian, and *A. ataxacantha*, a deciduous native African acacia. Broadly, our objectives were to (1) compare and contrast seasonal N and P allocation strategies in aboveground tissue components, (2) quantify and compare N and P resorption efficiency and proficiency, and (3) determine if nutrient resorption in these acacias are influenced by plant nutritional status. We interpret our findings using species differences in leafing phenology (evergreen vs. deciduous), N₂-fixation capacity (N₂-fixer vs. non-N₂-fixer) and biogeographical history of these species. We expected that as a deciduous, non-N₂-fixing African acacia, *A. ataxacantha* would have higher N and P concentrations in leaves and stronger N conservation (greater N-resorption efficiency) than *A. dealbata*. The insights gained regarding nutrient resorption efficiently were used to infer possible nutrient limitation at the study site.

4.3. Material and Methods

4.3.1. Study site

The study was conducted along a stretch of the Komati River riparian zone, in the surrounds of the Komati Wildlife Reserve (henceforth referred to as the 'Komati

site') in Mpumalanga Province, South Africa. Soils in the area are typically sandy with low nutrient availability, although bottom slopes soils typically have higher nutrient and clay content (Mucina and Rutherford, 2006). The region has a thirty year MAP of 910 mm, with early summer rainfall (September/October) with the majority of rainfall falling between the months of November and February (Figure 4.1). MAT is 16 °C, and frost days are common (3-20 days per year). The vegetation type is classified as KaNgwane Montane Grassland (Mucina and Rutherford, 2006), with common woody species including *Celtis africana*, *Diospirus lysoides*, *Ziziphus mucronata*, *Euclea divinorum*, *A. caffra*, *A.ataxacantha*, and the *A. dealbata*.

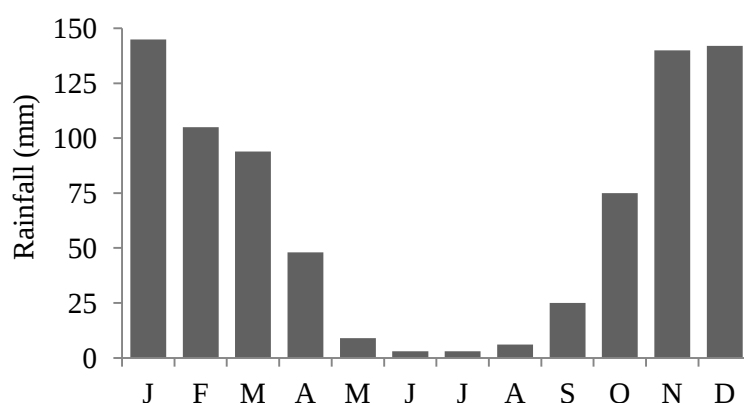


Figure 4.1 Average monthly rainfall for 30 year period for the KaNgwane Montane Grassland vegetation type. Data from Mucina and Rutherford (2006)

4.3.2. Plant sampling and analyses

Six trees were selected for sampling from each species. We collected samples from April 2011 to December 2011 at four sample intervals representing four sampling seasons; namely late wet season (April 2011), mid dry season (August 2011), late dry season (October 2011), and mid wet season (December 2011). Samples of the following tree components were collected when available: mature green leaves, senesced leaves, wood (xylem, typically sapwood), bark (phloem), dead branch, seed pods (pod and seeds combined) and inflorescences. Senesced leaves were identified based on colour (often light yellow) and could be removed by a gentle flick on the leaf. We collected senesced leaves directly from the branch and not the leaf litter to minimise effects of leaching and decomposition. Samples of the respective plant tissues were collected randomly at four cardinal points, at different canopy positions

and pooled per tree for analyses. Tissue samples were stored in paper bags and returned to the laboratory where they were oven-dried at 75°C for 36 hours, finely ground using rotary mill (MRC SW-2; 0.25 mm) and stored in plastic eppendorf tubes. Prepared samples were shipped to the Institute for Commercial Forestry Research (ICFR) in Scottsville, South Africa for N and P analysis using CHN analyser (combustion and infrared spectroscopy).

For each sample season, mean and standard error (SE) of N and P concentrations were calculated for each aboveground component for each species. Nitrogen and P content were calculated per unit mass (reported in milligram per gram dry mass), and leaf N:P ratios are reported on a mass basis. To identify seasonal changes in component nutrient concentrations, we used Kruskal-Wallis ANOVA tests. Between species tests on component nutrient concentrations were performed using Mann-Whitney U Tests. Dead or senesced branches attached to the trees were not sampled throughout the year as nutrient concentrations in dead branches were assumed to be relatively stable, and changes would be a result of leaching processes rather than translocation processes. Under this assumption, the April 2011 value represents a baseline dead branch value in this study.

Resorption parameters (efficiency and proficiency) were calculated per individual, and then pooled by species for comparisons. This provided insight into both the intraspecific and interspecific variation in resorption parameters. Resorption efficiency (percent N and P reduction between green and senesced leaves) was calculated using the following equation:

$$\text{resorption efficiency (RE)} = \frac{[\text{nutrient}]_{\text{green}} - [\text{nutrient}]_{\text{sen}}}{[\text{nutrient}]_{\text{green}}} \times 100 \quad (\text{Eqn 4.1})$$

where $[\text{nutrient}]_{\text{green}}$ is the mean N or P concentration in mature green leaves (leaves collected during mid wet season, Dec 2011), and $[\text{nutrient}]_{\text{sen}}$ is the senesced leaf N or P concentrations (calculated here as the lower 10th percentile of senesced leaf N or P concentrations pooled by individual). It was often difficult to judge the stage of leaf senescence, especially for the evergreen *A. dealbata*, and some of the “senesced” leaves collected had likely not completely undergone the process at

time of collection. We therefore chose to use the 0.10 quantile values for $[\text{nutrient}]_{\text{sen}}$ under the assumption that they represent leaves which had undergone complete, or near-complete, senescence. Resorption proficiency (terminal N and P content in senesced leaves) was determined using $[\text{nutrient}]_{\text{sen}}$ as defined as defined here. Interspecific differences nutrient resorption efficiency and proficiency were tested for using Mann-Whitney U Tests.

Relationships between green leaf N:P ratios, N and P content and resorption efficiency and proficiency were explored using Pearson correlation analyses. All statistical analysis was carried out using Statistica v.8.0 software (StatSoft, 2004) and significance was determined and $P < 0.05$.

4.4. Results

4.4.1. Annual N and P concentrations in aboveground components

Mean annual (data pooled across sampling seasons) N and P concentrations (mg.g^{-1}) varied significantly among aboveground components ($P < 0.05$ for N and P in both species, Kruskal-Wallis ANOVA; Figure 4.2, Table 4.1). Generally, N and P concentrations were higher in *A. ataxacantha* than in *A. dealbata*, however, interspecific differences in nutrient concentrations were not significant throughout all tissue types (Figure 4.2). The highest mean N and P concentrations were found in the inflorescences and leaves, with higher concentrations in *A. ataxacantha* than in *A. dealbata* in both components ($P < 0.05$ for both leaves and inflorescences, Mann-Whitney U Test; Figure 4.2). As expected, wood had the lowest mean N and P concentrations with little variation between species ($N \sim 7.3$ and $P \sim 0.1 \text{ mg.g}^{-1}$). Mean N concentrations were 50% and 100% higher in bark than in wood for *A. ataxacantha* and *A. dealbata* respectively ($P < 0.05$; Figure 4.2, Table 4.1), while P concentrations did not differ from that of wood in both species. Dead (senesced) branches were only observed on *A. ataxacantha* trees over the sampling period, and constituted a considerable biomass component for this species (see Chapter 2). Interestingly, mean annual N and P concentrations of attached dead branches on *A. ataxacantha* (8.50 mg N.g^{-1} and 0.15 mg P.g^{-1} , respectively) did not differ from live wood, suggesting little or no resorption of these nutrients from the sapwood of senescing branches. Seed pods collected from *A. dealbata* trees had mean N and P concentrations similar to mean

nutrient concentrations in senesced leaves of *A. dealbata* (~ 20 and 25 mg.g^{-1} for N and P respectively). Unfortunately, none of the *A. ataxacantha* trees sampled in this study produced seeds over the collection period. Senesced leaves of both *Acacia* species contained approximated 30% less N and P than green leaves ($P < 0.01$, Mann-Whitney U Test, Figure 4.2).

Variability in nutrient concentrations differed between species and nutrient. Mean standardised variation was higher in P concentrations (CV between 0.70 and 0.75) than in N concentrations (between 0.18 - 0.30) across species. Overall, N and P concentrations were more variable in *A. ataxacantha* (mean CV = 0.30 and 0.75 for N and P, respectively) than in *A. dealbata* (0.78 and 0.6, respectively for N and P, respectively). Observed variability in N and P concentrations at the component level was better explained through seasonal analyses.

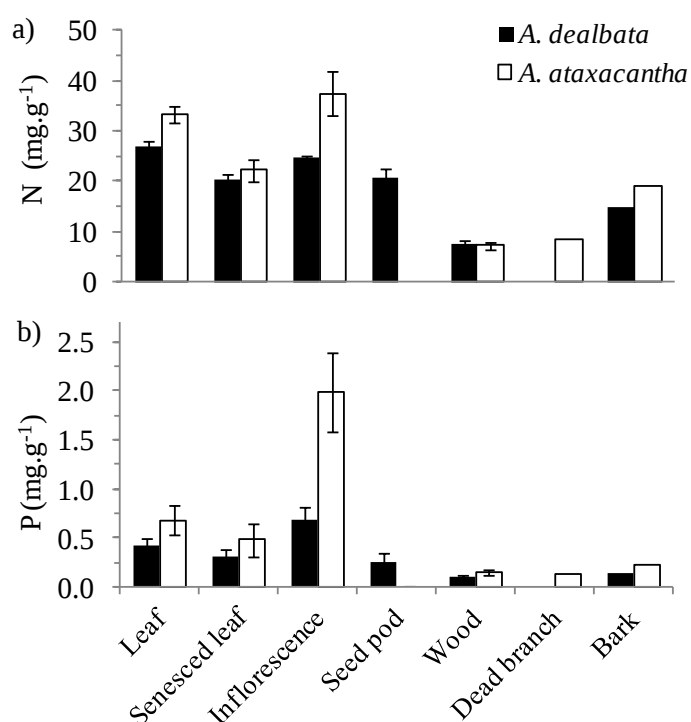


Figure 4.2 Mean annual (a) N and (b) P concentrations $\pm$ SE among aboveground components in *A. ataxacantha* (open bars) and *A. dealbata* (shaded bars) trees. Seed pods were only available for *A. dealbata* trees.

Table 4.1 Mean annual N and P concentrations (mg.g⁻¹) in the aboveground components of acacias collected from mixed riparian stands at Komati study site over the period April to December 2011. SE represents standard error of the mean; asterisks denote interspecific differences (Mann-Whitney U Tests, P < 0.05).

	<i>A. dealbata</i>		<i>A. ataxacantha</i>	
	N	Mean ± SE	N	Mean ± SE
Nitrogen (mg.g⁻¹)				
Leaf	20	26.87 ± 1.02	17	33.26 ± 1.76 *
Senesced leaf	13	20.31 ± 1.11	8	22.23 ± 2.27
Inflorescence	10	24.52 ± 0.56	6	37.45 ± 4.27 *
Seed pod ^a	4	20.70 ± 1.77	0	-
Wood ^b	20	7.46 ± 0.68	22	7.25 ± 0.83
Dead branch	0	-	6	8.50 ± 0.69
Bark	20	14.89 ± 0.41	23	19.09 ± 1.20 *
Phosphorus (mg.g⁻¹)				
Leaf	20	0.43 ± 0.06	17	0.68 ± 0.15
Senesced leaf	12	0.31 ± 0.06	8	0.48 ± 0.17
Inflorescence	10	0.68 ± 0.12	6	1.99 ± 0.40 *
Seed pod ^a	4	0.25 ± 0.10	0	-
Wood ^b	20	0.10 ± 0.01	22	0.15 ± 0.03
Dead branch	0	-	6	0.13 ± 0.02
Bark	20	0.15 ± 0.03	23	0.23 ± 0.05

^a Combination of seed pod and seed

^b Typically sapwood was sampled

4.4.2. Seasonal allocation patterns of N

Leaf N concentrations in *A. dealbata* showed little variation (<5 mg/g) throughout the sampling period apart from a small decrease subsequent to the April sample date (Figure 4.3a), while leaf N concentrations in *A. ataxacantha* showed greater seasonal variability (Figure 4.3b). New leaves of *A. ataxacantha* contained higher N concentrations (by ~4 mg.g⁻¹, Oct 08) than mature leaves (Dec 08), with concentrations tending to decline over the growing season (Figure 4.3b). Total defoliation of *A. ataxacantha* trees occurred around May 2011, and senesced leaves were only available in the late wet season (Apr 2011) and mid wet season (Dec 2011) sampling dates. Owing to its evergreen form leaves on *A. dealbata* senesced throughout the year at a more-or-less constant rate. Increasing N concentrations in

wood and bark tissue from late wet season to mid dry season was presumably due to retranslocation of nutrients from green leaves to overwintering components of the deciduous *A. ataxacantha*. This is supported by a decrease in wood and bark N during the leafing seasons (late dry season and wet season).

Flowering phenology differed between the two acacias. For *A. dealbata*, inflorescences were present over the late wet season, mid dry season and late dry season collection dates (Apr – Oct), with N concentrations gradually decreasing (maximum seasonal mean of 25 mg N.g⁻¹) toward the end of the flowering season. *A. ataxacantha* trees had inflorescences during the late dry season and wet season collections, with N concentrations increasing over the growing season to reach a maximum mean of ~40 mg.g⁻¹ (Figure 4.3b).

4.4.3. Seasonal allocation patterns of P

Phosphorus concentrations more variable across seasons (CV, *A. dealbata* = 0.57, *A. ataxacantha* = 0.88) than N concentrations (CV, *A. dealbata* = 0.13, *A. ataxacantha* = 0.26), with relatively higher variability in *A. ataxacantha*. For *A. dealbata*, P concentrations in plant tissue exhibited notable co-variation among components, with peaks in mid dry season (Aug 2011), and mid wet season (Dec 2011, Figure 4.3c). Components with the highest P concentrations were inflorescences and leaves in both species. *A. ataxacantha* mature leaf P concentrations increased three fold from late dry season to mid wet season, exhibiting an opposite trend to that of leaf N for this species. The relatively large difference between mid wet season and late wet season leaf-P (~1 mg.g⁻¹) in *A. ataxacantha* shows high P translocation from the leaves, which also supported by the relatively high bark-P concentration in late wet season (possibly indicating phloem loading).

A relatively larger allocation P to reproductive tissue and N was evident in both acacias. Unlike N concentrations in *A. dealbata* inflorescences which gradually declined as flowers matured, P concentrations more than doubled in inflorescences during the mid dry season months (Aug 2011), having the highest P concentrations of all the aboveground components (Figure 4.3c and d). Not all of the evidently mature individuals included in the sample produced seeds and flowers.

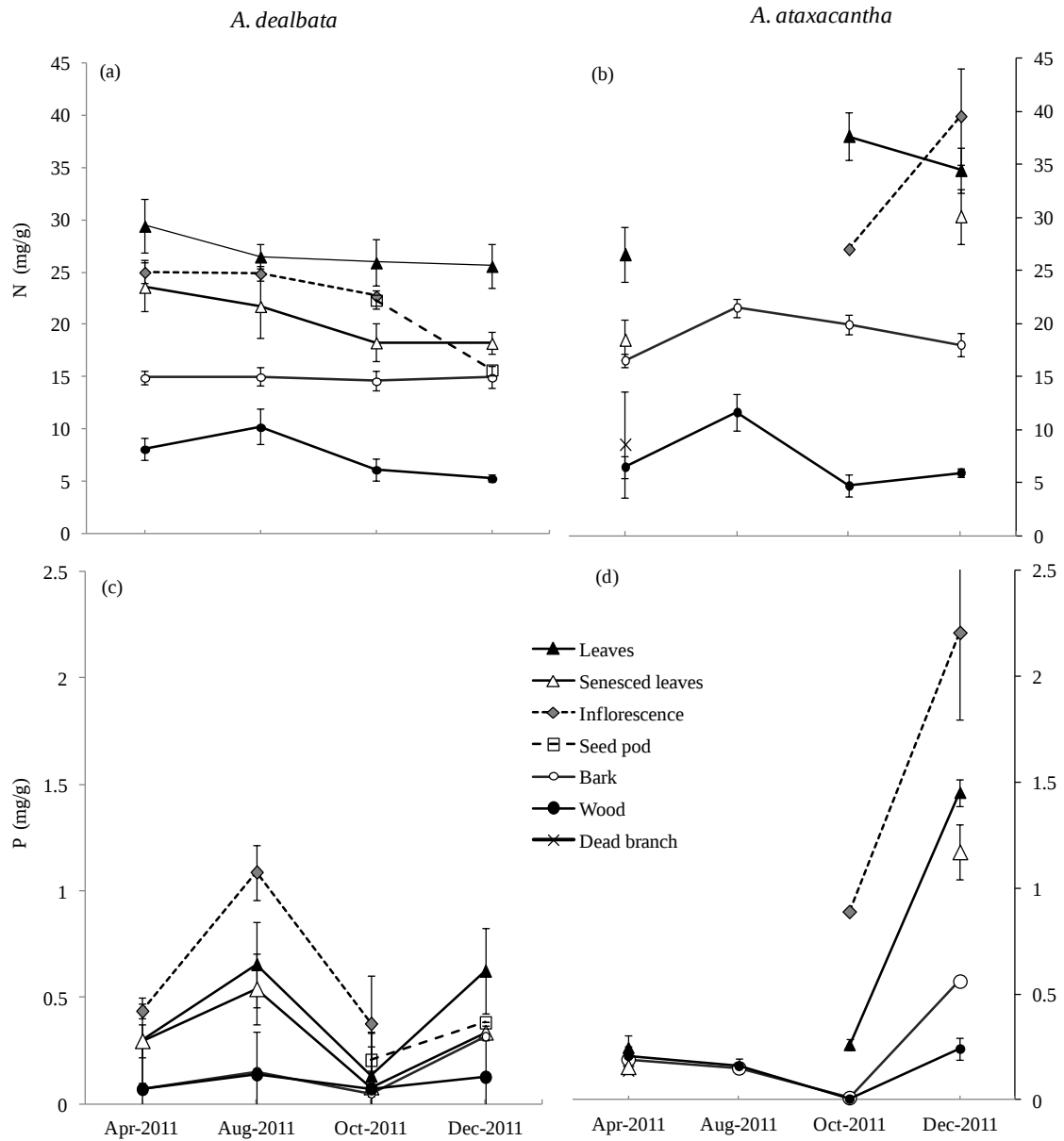


Figure 4.3 Seasonal variation in N (a and b) and P concentration (c and d) among aboveground components in *A. dealbata* and *A. ataxacantha* trees during 2011. Symbols followed by an asterisk (*) indicate that trees were leafless after this date.

4.4.4. Green leaf N and P concentrations and N:P ratios

Mean nutrient concentrations in mature green leaves (fully expanded leaves sampled during mid wet season, December 2008) varied substantially within species, but species effects were also apparent ($P < 0.05$, Mann-Whitney U Test; Table 4.2). Green leaf N concentrations varied from 27.0 to 36.0 mg.g^{-1} (mean 33.3 mg.g^{-1}) for *A.*

ataxacantha, and from 21.5 to 33.5 mg.g⁻¹ (mean 25.6 mg.g⁻¹) for *A. dealbata*, and were significantly higher than senesced leaf N concentrations in both species ($P < 0.05$, Wilcoxon Matched Pairs Test; Table 4.2). A similar pattern was observed in the P concentrations in mature leaves, which were significantly higher in *A. ataxacantha* (1.1 – 1.6 mg.g⁻¹, mean 1.4 mg.g⁻¹) than in *A. dealbata* (0.5 – 1.0 mg.g⁻¹, mean 0.6 mg.g⁻¹). Green leaf N and P concentrations were positively correlated in *A. dealbata* but unrelated in *A. ataxacantha* (Table 4.3)

Leaf N:P mass ratios varied from 20 to 48 for mature leaves and from 78 to 492 in senesced leaves. This expands to tremendous variation in N:P in senesced leaves, most notably in those of *A. dealbata*. As expected, mature leaf N:P ratios differed between species ($P < 0.01$, Man-Whitney U Test, Table 4.2), with higher N:P ratios in *A. dealbata*. Senesced leaves of *A. ataxacantha* had significantly higher N:P ratios than mature green leaves ($P < 0.05$, Wilcoxon Matched Pairs Test), while N:P ratios in senesced and green leaves of *A. dealbata* did not differ at the 5% level ($P = 0.06$, Wilcoxon Matched Pairs Test). There were no observed species interactions in senesced leaf N:P ratios.

4.4.5. Nutrient resorption efficiencies and proficiencies

Both *Acacia* species were remarkably more efficient at P resorption than N resorption ($P < 0.05$ for both, Wilcox Matched Pairs Test; Table 4.2). Nitrogen resorption efficiency, calculated on a mass basis, from senescing leaves is 26% and 44% for *A. dealbata* and *A. ataxacantha*, respectively. Thus, on average, *A. ataxacantha* translocates about half of the N contained in leaves for re-use after senescence, while *A. dealbata* translocates only about a quarter of leaf N during senescence. There was however, considerable within-species variation in N resorption efficiencies (Table 4.2). P resorption was much higher than N resorption, ranging between 63 and 92% (mean 74.2%) in *A. dealbata*, and between 84 and 91% (mean 88.0%) for *A. ataxacantha*. No differences in P resorption efficiency were found between species. P resorption was more than twice as efficient as N resorption in both acacias. No relationship between P resorption efficiency and N resorption efficiency was observed, irrespective of whether data were pooled by species or not (Table 4.3),

suggesting nutrient resorption mechanisms operate independently within these two acacia species.

Resorption proficiencies quantify the levels to which plants are able to reduce nutrient concentrations in their senesced foliage and conserve nutrient resources. Considered separately, the deciduous and evergreen *Acacia* species produced senesced leaves that held remarkably similar N concentrations ($\sim 18 \text{ mg.g}^{-1}$), and P concentrations which were non-significantly lower in *A. dealbata* (0.13 mg.g^{-1}) than in *A. ataxacantha* (0.17 mg.g^{-1} , Table 4.2). In contrast to resorption efficiencies, N and P resorption proficiencies were strongly negatively related within *A. dealbata* individuals, while there was no relation between N and P resorption proficiencies within *A. ataxacantha* (Table 4.3).

Table 4.2 Mean N and P concentrations, N:P ratios of green and senesced leaves and N and P resorption efficiencies. Values for green leaves sampled during mid wet season sampling season, senesced leaves values (resorption proficiency) taken as the bottom 10% quantile if multiple values for senesced leaves available for per individual. Values are presented as mean $\pm$ SE; asterisks indicate significant differences between species ($P < 0.05$)

	<i>A. dealbata</i>	<i>A. ataxacantha</i>
Green leaf concentration (mg.g^{-1})		
Nitrogen	25.60 ± 2.10	33.34 ± 1.65 *
Phosphorus	0.62 ± 0.09	1.43 ± 0.07 *
N:P ratio	42.30 ± 2.71	23.4 ± 1.5 *
Resorption proficiency (senesced leaf concentration, mg.g^{-1})		
Nitrogen (NRP)	18.43 ± 1.26	18.64 ± 1.89
Phosphorus (PRP)	0.13 ± 0.03	0.17 ± 0.02
N:P ratio	200.9 ± 97.6	113.3 ± 16.7
Resorption efficiencies (%)		
Nitrogen (NRE)	27.1 ± 5.1	44.5 ± 3.8 *
Phosphorus (PRE)	74.24 ± 6.73	88.0 ± 1.1

Table 4.3 Correlation coefficients (r) with significance values (P) for N and P concentration in fully expanded green leaves (collected Dec 2011), between N and P resorption proficiencies, and between N and P resorption efficiencies from those leaves.

Interaction	r	N	P
Ngreen vs. Pgreen			
All data	0.85	10	0.002
<i>A. ataxacantha</i>	0.31	5	0.612
<i>A. dealbata</i>	0.93	5	0.021
Resorption proficiency (NRP vs. PRP)			
All data	0.01	9	0.999
<i>A. ataxacantha</i>	0.45	5	0.446
<i>A. dealbata</i>	-0.99	4	0.008
Resorption efficiency (NRE vs. PRE)			
All data	0.20	9	0.607
<i>A. ataxacantha</i>	0.42	5	0.481
<i>A. dealbata</i>	-0.83	4	0.172

4.4.6. Controls over nutrient resorption

We found it difficult to draw any conclusions about the relation between green leaf nutrient status (concentration) and resorption efficiencies between the two *Acacia*, primarily because the number of available observations was low. Nevertheless, our data suggest that N resorption, in terms of both efficiency and proficiency was not responsive to green leaf nutrient status, while green leaf P status was related with both P resorption efficiency and proficiency (Table 4.4). The interaction between resorption efficiency and proficiency of N was positive in *A. ataxacantha*, but unrelated in *A. dealbata*. No significant general interactions were observed (Table 4.4).

Table 4.4 Correlation coefficients (r) relating green leaf nutrient concentrations (N_{green} , P_{green}) and N and P resorption efficiencies (NRE, PRE) and proficiencies (NRP, PRP), and between resorption proficiencies and efficiencies. Asterisks (*) indicate significant relationships ($P < 0.05$)

Interaction	N	r	P
Resorption efficiency vs. nutrient status ^a			
Nitrogen			
All	10	0.55	0.103
<i>A. dealbata</i>	5	0.60	0.289
<i>A. ataxacantha</i>	5	-0.64	0.249
Phosphorus			
All	9	0.64	0.061
<i>A. dealbata</i>	4	0.99	0.003*
<i>A. ataxacantha</i>	5	-0.89	0.043*
Resorption proficiency vs. nutrient status ^a			
Nitrogen			
All	10	0.51	0.133
<i>A. dealbata</i>	5	0.60	0.289
<i>A. ataxacantha</i>	5	0.85	0.070
Phosphorus			
All	9	0.64	0.061
<i>A. dealbata</i>	4	-0.99	0.001*
<i>A. ataxacantha</i>	5	0.95	0.015*
Resorption efficiency vs. resorption proficiency			
Nitrogen			
All	10	-0.44	0.209
<i>A. dealbata</i>	5	-0.38	0.526
<i>A. ataxacantha</i>	5	0.95	0.014*
Phosphorus			
All	9	0.35	0.359
<i>A. dealbata</i>	4	-0.99	0.001*
<i>A. ataxacantha</i>	5	-0.99	0.002*

4.5. Discussion

The nutrient allocation and conservation strategies of two competing acacias (one native and one an alien invasive) in a savanna-grassland riparian zone clearly differ, growing in what our data suggests to be a P-limited ecosystem. Seasonal dynamics of N and P, the most important growth-limiting nutrients in many terrestrial

ecosystems, reflect different strategies and may help explain some of the widespread success of invasive acacias. Furthermore, we considered aspects of N and P resorption, an important nutrient conservation process in perennials, and explored potential controls on these processes. Overall, our results indicated that (1) *A. ataxacantha* was much more efficient at N resorption than invasive *A. dealbata*, while P resorption efficiency did not differ between species, (2) both acacias were more efficient at resorption of P than N from their leaves, (3) there appears to be a convergence of N and P resorption proficiencies between species, (4) there was no clear nutritional controls over N, though P resorption showed equivocal relationships, and (5) the native deciduous *A. ataxacantha* tends to have higher tissue-N and -P concentrations in aboveground components, with stronger seasonal fluctuations, than the exotic evergreen *A. dealbata*.

4.5.1. Allocation of N and P among aboveground tissues

Mean annual nutrient concentrations were consistently higher in *A. ataxacantha* for both N and P (Figure 4.2, Table 4.1) despite high rates of N₂-fixation in *A. dealbata* (see Chapter 3). In agreement with previous findings on nutrient content in aboveground tissues (e.g. Caldeira *et al.*, 2002), high concentrations of N and P were found in the leaves and inflorescences. The greater variability observed in P concentrations relative to N concentrations reflected stronger seasonal translocation of P than N between components or possibly more luxury uptake/storage of P. Variability in nutrient concentrations also tended to be more pronounced in *A. ataxacantha* (Table 4.1) which suggested stronger seasonal fluctuations. These findings were consistent with our expectations of lower tissue N and P concentrations in an evergreen species which tend to have weaker seasonal fluctuations in comparison to a co-occurring (and closely related) deciduous species (Killingbeck, 1996; Aerts and Chapin, 1999). However, because the invasive acacia is an effective N₂-fixer, it was intriguing as to whether or not this commonly observed trend would hold.

It was difficult to compare our mean annual nutrient concentration to other works simply because the majority of published data reports season specific nutrient concentrations. Mature green leaf N concentrations (our wet season sample) were considerably higher than the global average estimates of 12 – 19 mg.g⁻¹ (Lambers *et*

al., 2010). P concentrations in mature leaves were slightly lower than global average (0.9 – 1.1 mg.g⁻¹; Lambers *et al.*, 2010). This was also reflected green leaf N:P mass ratios which were higher than the average for sub-tropical trees (N:P ~10, Aerts and Chapin, 2000), although legumes associated with N₂-fixing bacteria tend to have higher N:P ratios (Ratnam *et al.*, 2008). Our data lend support this trend, with higher N:P ratios observed in the N₂-fixing *A. dealbata*.

4.5.2. Seasonal trends in nutrient allocation among aboveground components

Seasonal patterns of N and P allocation between our native and invasive acacias were relatively distinct, which was expected considering differences in phenological guilds and N₂-fixation capacity. Deciduous species, such as *A. ataxacantha*, generally allocate a high proportion of nutrients from storage or uptake to leaves early in the growing season, while evergreen species are thought to depend less on reserve storage than deciduous species, as leaf senescence typically coincides with new leaf growth (Chapin and Shaver, 1989). It therefore seems reasonable to expect evergreen trees to be less reliant on long distance seasonal translocation of N and P than deciduous trees. Evergreen species also tend to produce tissues with lower year-round nutrient concentrations and longer lifespans, which may reflect the extra investment in structural tissue (higher C content) in long-lived leaves and/or a trade-off in a nutrient conservation strategy (Killingbeck, 1993; Aerts, 1995; Killingbeck, 1996). Our results are in agreement with these notions, with consistently lower mean annual N and P concentrations in aboveground components of the evergreen invasive than the native deciduous species.

Seasonal allocation patterns of N and P in tree species are likely to depend on both the environmental availability of the nutrient t(s) and interspecific differences in seasonal requirements. Overall, there were no general trends. We found little evidence of co-variation in tissue-N and -P content across seasons. For example, a more than three-fold increase in P concentrations (0.3 – 1.4 mg.g⁻¹) in *A. ataxacantha* leaves was accompanied by a relatively minor decrease (~4mg.g⁻¹) in N concentrations, while a doubling of P concentrations in *A. dealbata* inflorescences was not associated with a notable change in N content (Figure 4.3). Where co-variation was evident, it was not observed across species, suggesting specific nutrient requirements in aboveground

components vary both spatially (within the plant) and temporally (between seasons). The general lack of co-variation in N and P observed here contradict previous work on African and Australian acacias. For example, Tolsma *et al.* (1987) observed co-variation in N and P concentrations in leaves from eight African *Acacia* species growing in arid savanna in Botswana over a one-year period, and Fife (Fife *et al.*, 2008) noted similar patterns on N and P co-variation in the leaves of *A. mearnsii* (black wattle – a close relative of *A. dealbata*) growing in its natural distribution in southern Australia. Both Tolsma *et al.* (1987) and Fife *et al.* (2008) undertook monthly sampling over a 12 month period, allowing for quantification of variability on a finer temporal scale. It is possible that a higher degree of co-variation in N and P content would have been observed in this study had we followed a similar sampling interval. However, a key finding here was the temporal variation of maximum concentrations of the respective nutrients between species. This may be one of the reasons Australian acacias are able to compete with native African acacias.

Maxima in nutrient concentrations were expected during the growing and reproductive season(s), as allocation to productive and reproductive tissue is required. *Acacia dealbata* had maximum P concentrations during mid dry season (August) and mid wet season (December) in several components, corresponding with its flowering and growing season respectively. This species typically flowers from July to October, thus our mid dry season (August) sample was in the peak flowering season for this species, and the elevated P concentrations coincided with the flowing season. Inflorescences were first observed and collected in late wet season (April), but were small and poorly developed. Decreasing P concentrations in aging inflorescences suggested breakdown and translocation of this limiting nutrient from the inflorescences. We suggest that a major portion of this P was incorporated into the development of seed pods. This is supported by the observed decline in aboveground component P content during early seed development. Mid dry season peaks in leaf-P is in agreement with studies on closely related Australian acacias (Fife *et al.*, 2008), however, in our study, peaks in leaf-P concentrations in *A. dealbata* were not accompanied by peaks in leaf-N (as noted by Fife *et al.*, 2008) but instead showed only slight elevation in the late wet season (April) sampling period. The co-variation of green and senesced leaf nutrient contents in *A. dealbata* suggests that there is little

seasonal variation in N and P resorption efficiency in this evergreen species. Also interesting is the peak in P concentrations in bark (phloem) in mid wet season in both species. This indicates phloem loading of P compounds during the growing season, however, this was not evident for N. It is possible that N is more tightly retained in the leaves during the growing season due to its importance in photosynthetic compounds (such as chlorophyll), while P is incorporated into ATP and phospholipids which are transported to the roots for growth and further nutrient acquisition.

Seasonal fluctuations in nutrient content of the deciduous *A. ataxacantha* were very pronounced, with elevated N and P concentrations during the wet season months. *A. ataxacantha* flowers from October – March, thus overlapping with the growing season. N concentrations in leaves declined gradually over the growing season in *A. ataxacantha*, in agreement with previous work on deciduous African acacias (Tolsma *et al.*, 1987) and the majority of woody perennials (Lambers *et al.*, 2008a). There are numerous processes which can drive this decline in N. First, resorption of N from the leaves is a multi-phase process, starting in the early growing season, with the most considerable uptake during leaf senescence (Fife *et al.*, 2008). Second, leaf expansion is generally accompanied by an increase in the relative proportion of structural and defensive compounds (cell wall material, lignins, tannins, etc.), therefore the relative proportion (i.e. concentration) of N may be diluted during leaf expansion (Aerts and Chapin, 1999; Lambers *et al.*, 2008a). Thus, apparent changes in tissue N concentration may not always reflect decreases in the amount of N in the respective tissue. To test this hypothesis, fine scale temporal data on the changes in leaf biomass and nutrient concentrations are required.

We did not repeatedly sample attached dead branch as it was assumed that nutrient changes in nutrient concentrations over a sampling period (if any) would be a result of nutrient leaching rather than translocation processes. Surprisingly, our baseline value for dead branch nutrient concentration in *A. ataxacantha* was not the minimum for woody material. This suggests that translocation of nutrients out of senescing branches is low, although decomposition of dead wood has the potential to elevate nutrient concentrations through respiration. Nevertheless, it may be that low allocation of nutrients to woody material is a bigger nutrient conservation mechanism than resorption itself.

4.5.3. *N and P resorption efficiencies and proficiencies*

The vast majority of woody species, spanning multiple functional types and geographic distributions, conserve P more tightly than N (Aerts, 1996; Killingbeck, 1996; Ratnam *et al.*, 2008). This pattern was also observed in our study, with efficient P resorption and inefficient N resorption. Resorption of N was lowest in *A. dealbata* where only ~ 27% of leaf N was withdrawn from leaves during senescence, compared to ~45% N resorption in *A. ataxacantha*. These average N resorption efficiencies were lower than most values reported for related species growing in South African savannas (58% for fine-leaved trees in Kruger National Park; Ratnam *et al.* 2008), and the global average (56%) for woody plants reported and corrected by Aerts (1996) and Van Heerwaarden (2003), suggesting highly inefficient N resorption in the invasive acacia. On the other hand, P resorption efficiencies (PRE) reported here (74% and 88% for *A. dealbata* and *A. ataxacantha*, respectively) were considerably higher than the global average of 52% (Aerts, 1996), but similar to those previously reported for arid *Acacia* species in north western Australia (up to 80%, He *et al.*, 2011). High P resorption values indicated that delayed senescence was not a primarily cause of inefficient N resorption in this N-fixing species. Preferential resorption of P over N was also reflected in the excessively high N:P ratios in senesced leaves (Table 4.2).

Despite the lower than average N resorption values observed in this study, our results followed the same general pattern as previous findings, with higher resorption efficiency in a deciduous species than an evergreen species (Killingbeck, 1996). Arguments put forward to explain these patterns suggest that the generally longer leaf lifespan of evergreen species require a larger proportion of leaf N to be stored in less accessible nutrient pools (i.e. structural compounds) with a higher cost of mobilization (Wright and Westoby, 2003). That is, the resorption-nutrient cost for these N pools is potentially higher than the cost of acquiring N from the soil. Where soil derived nutrients become less expensive to acquire than those from resorption is where we would expect resorption efficiency to be limited (Wright and Westoby, 2003). Following on from this thinking, is the notion that the lower N concentration of evergreen leaves is perhaps a greater nutrient conservation mechanism than resorption

itself (Killingbeck, 1996). The same argument can be used for the non-significantly higher P resorption efficiency in *A. ataxacantha* than in *A. dealbata*.

Confounding these interpretations is N₂-fixation capacity, a trait which is known to influence N resorption in woody plants (Killingbeck, 1993; Killingbeck, 1996). It could be argued that the high N₂-fixation capacity of *A. dealbata* as indicated by the ¹⁵N natural abundance data (Chapter 3) increases N supply from the roots (N availability) and shifts a theoretical crossover point between the cost of resorption and the cost of acquisition toward a lower proportional N-resorption. This argument carries two key assumptions. Firstly, it assumes that there has been an evolutionary trade-off between N₂-fixation and efficient resorption of N in acacias, and that these trade-offs for a continuum between the cost of acquisition and the cost of conservation. This is supported by disparity between N and P resorption efficiencies in the study species, which suggests the ability to fix N does not lead to a physiological inability to resorb nutrients. Secondly, the argument assumes that the cost associated with the symbiosis with N₂-fixing rhizobia (estimates of 30% additional carbon expenditure – Pate and Layzell, 1990) is less than the relative cost of resorption at the molecular level, yet the energetic costs of uptake vs. resorption remain unknown. Such questions related to the selection pressures that have, and have not, influenced the evolution of the resorption process itself are typically better addressed using resorption proficiencies (the terminal nutrient content in senesced leaves).

Resorption proficiency values showed greater convergence across species than resorption efficiencies. This was especially evident with N, which was reduced to ~18 mg g⁻¹ in senesced leaves of both species. Based on Killingbeck's (1996) model that defines resorption proficiency in terms of senesced leaf nutrient content, neither *Acacia* in our study was very proficient in N resorption. The Killingbeck (1996) model states resorption of N is highly proficient (complete resorption) in plants that reduce N concentration in leaves to below 7 mg.g⁻¹, suggesting relatively poor N conservation in these species growing at Komati study site. However, proficiency of N resorption in the acacias in this study were remarkably consistent with previous findings in fine-leaved legumes growing in nearby savannas landscapes (mean of 18mg.N.g⁻¹; Ratnam *et al.*, 2008), and were only slightly higher than some Australian acacias growing in the Great Sandy Desert (13 mg.N.g⁻¹; He *et al.*, 2011), supporting the idea that

phylogeny and environment are strong determinants of potential resorption (Stewart, 1968; Killingbeck, 1996). These N resorption proficiencies were also similar to the global average for N-fixing species (16.1 mg.g^{-1} ; Killingbeck, 1996). In contrast to the inefficient N resorption observed in our study, P resorption proficiency in both *A. ataxacantha* and *A. dealbata* (0.17 and 0.13 mg.g^{-1} respectively) are lower than the widely accepted thresholds for complete resorption in deciduous ($<0.5 \text{ mg.g}^{-1}$) and evergreen ($< 0.4 \text{ mg.g}^{-1}$) species (Killingbeck, 1996). Thus it appears that P conservation is highly developed in both *Acacia* species, despite their divergent biogeographical histories. This is highly plausible as P limitation is a key attribute of many both south eastern Australia and southern African landscape (Lambers *et al.*, 2008b). These species, therefore, have both undergone long-term adaptation to P limitation which could explain their complete (as defined by Killingbeck, 1996) resorption potential. The slightly higher P resorption proficiencies the *A. dealbata* may suggest that evergreens are able to colonize P-deficient sites more effectively than deciduous species, a possible factor contributing the success of Australian *Acacia* in South African landscapes. However, this is largely speculation as we do not have data on the use efficiency of P for the species which would assist in this analysis.

How do we draw conclusions from these conflicting findings based on two ecologically important, but fundamentally different, attributes of the same process? Although the resorption efficiency values agree that P is conserved to a higher degree relative to N, the interspecific divergence in resorption efficiency values support a conclusion opposite to that offered by resorption proficiency, in that they suggest a convergence in resorption ability. Efficiency values are unique in that they encompass both nutrient demand (green-leave nutrient content) and nutrient withdrawal, allowing for comparisons of the relative degree to which individuals, species, or communities can conserve foliar nutrients. On the other hand, proficiency values represent the absolute levels to which nutrients are reduced in senescing leaves, and perhaps provide a more definitive, objective and interpretative measure of selection pressure and resorption success (Killingbeck, 1996). Based on the patterns that emerged from our data on N and P resorption, we suggest that that selection for nutrient conservation strategies has been stronger for P than for N, leading us to conclude that P acquisition is relatively more expensive than that of N at the Komati study site.

When comparing our results of nutrient resorption efficiency with those of other studies, it is important to consider that resorption efficiencies were calculated and are expressed on a mass basis and not leaf mass area (LMA) basis as many authors recommend (Killingbeck, 1996; He *et al.*, 2011). Although there are obvious advantages to the area based calculations (these are less affected by the loss of leaf mass during senescence), the calculation of leaf area for fine leaved species such as *A. dealbata* and *A. ataxacantha* is generally subject to a considerable error as the compound leaf structure and the small area of the individual leaflets complicates leaf area measurements. Moreover, obtaining accurate mass measures of senesced leaves of fine-leaved species is also subject to error with leaflet drop. Therefore, we chose to limit our expression of resorption efficiencies to mass basis (concentrations on mass basis) only and acknowledge that our mass based estimates are likely to overestimate resorption efficiencies (He *et al.*, 2011). Furthermore, since the reduction in leaf mass during senescence is typically less than 10% (Chapin *et al.*, 1990), the errors in these estimates are relatively small.

4.5.4. Controls over nutrient conservation mechanisms

We found no evidence that N resorption efficiency was related to plant nutrient status (as represented by green leaf N concentrations). This was in agreement with some previous findings (Chapin and Moilanen, 1991; Aerts, 1996), but disagreed with others (e.g. Pugnaire and Chapin, 1992; Pugnaire and Chapin, 1993; Kobe *et al.*, 2005; Drake *et al.*, 2008; Ratnam *et al.*, 2008). In studies where N resorption efficiency varied with nutrient availability, hypotheses have pointed to changes in the ratio of soluble/insoluble forms of N (Pugnaire and Chapin, 1993), changes in the source-sink interactions (Chapin and Moilanen, 1991; Pugnaire and Chapin, 1992), and luxury nutrient uptake (Shaver and Melillo, 1984), however, evidence in support of these has been somewhat equivocal. We also found no significant relationship between the N resorption proficiency and plant nutrient status, though N concentrations in senesced leaves of *A. ataxacantha* did tend to be higher in plants with higher green-leaf-N.

Phosphorus resorption efficiency was related to green leaf-P concentration, though the direction of this relationship differed between species (negatively related in *A. ataxacantha*, positively related in *A. dealbata*). A bi-directional relationship

between PRE and green leaf-P content was difficult to interpret. Less efficient P resorption in plants with high leaf-P status has been identified in previous studies (e.g. Chapin and Moilanen, 1991; Ratnam *et al.*, 2008) and has been hypothesised to occur when the costs of nutrient resorption relative to acquisition from the soil increase as nutrients become more readily available (Wright and Westoby, 2003). Alternatively, a positive relationship between leaf-P and P resorption efficiency might arise if the ratio of soluble/insoluble P compounds in leaves increases with elevated leaf-P concentrations (Pugnaire and Chapin, 1993), possibly as a result of luxury P uptake where the “luxury” P is stored as soluble compounds. Although interesting, identification of the mechanisms behind these relationships would require the determination of nutrient fractions and the relative cost of resorption and acquisition, both of which are beyond the scope of this work.

Identification of controls over resorption metrics in these species was limited by our small resorption dataset, nevertheless, the conflicting nature of our results were in agreement with previous reviews on the topic (Aerts, 1996; Kobe *et al.*, 2005). Additional putative controls on nutrient resorption which were not tested in our study include soil moisture availability (Pugnaire and Chapin, 1992), source-sink interactions (Chapin and Moilanen, 1991; Pugnaire and Chapin, 1992), and duration of leaf abscission period (del Arco *et al.*, 1991; Escudero *et al.*, 1992). Because controls on resorption are unlikely to act independently, variations in nutrient resorption efficiency are likely to be the net results of the interplay between these, and other, controls. In recognition of the complex interplay of controlling factors, some authors have even suggested that resorption efficiency is not a meaningful measure of interspecific variation (Fife *et al.*, 2008). However, we argue that measures of the proportional resorption of nutrients from senescing structures provide valuable insights into nutrient availability, as plants would only tend to have efficient retranslocation when other benefits can be derived from it and when no extra costs are incurred. Although often inconclusive, investigations around the ecological controls over resorption continue to shed insight into different plant life history strategies in nutrient limited environments.

4.5.5. *Concluding comments and future directions*

We conclude that co-occurring native and alien invasive *Acacia* growing in the Komati grassland-riparian environment differ in respect to their N and P allocation to aboveground parts, seasonal N and P dynamics and controls over nutrient resorption. Seasonal fluctuations in N and P concentrations were more pronounced in the native acacia (*A. ataxacantha*, a deciduous non-fixer), which also had almost consistently higher seasonal nutrient concentrations than the alien acacia (*A. dealbata*, an evergreen N₂-fixer). As expected, reproductive and productive tissues had the highest nutrient concentrations among aboveground components in both species, and maxima in N and P concentrations in these parts coincide with the flowering and growing seasons of the respective species. Nutrient resorption, a key nutrient translocation process, was shown to be an important nutrient conservation mechanism, with up to 44% N and 88% P contained in the mature leaves resorbed during senescence. We found strong evidence of species divergence in terms of resorption efficiency, with proportionally lower N and P resorption in the invasive relative to the native. Inefficient N resorption in the invasive acacia (~27%) was attributed to phenological guild and N₂-fixing capacity. Conversely, our measures of resorption proficiency (level to which nutrients were reduced in senesced leaves) indicated species convergence, suggesting phylogenetic or environmental controls over resorption. This was supported by the agreement of our senesced leaf N and P concentrations with published works on closely related species, pointing to genotypic limit to resorption. There was no relationship between green-leaf N concentrations and NRE or NRP, but P resorption was responsive to green-leaf P concentrations.

Overall, these results indicate that nutrient resorption mechanisms in this riparian grassland ecosystem result in P being more tightly conserved than N, suggesting P limitation in the Komati study site. Inferring nutrient limitation in based on nutrient resorption is questionable, although it does provide an interesting alternative to the use foliar N:P ratios which are often confounded by species adaptations to variable nutrient supply (Craine *et al.*, 2008). However, they are also vulnerable to a skewed interpretation of availability if N₂-fixing species are used (for which NRE is more sensitive than NRP). Differentiation in N and P conservation and allocation strategies in dominant species can lead to changes in nutrient cycling within

the invaded ecosystem, and may well be a major contributing factor to the success of an invading species in riparian systems.

An interesting question would be: are there seasonal differences in resorption efficiency in evergreen N₂-fixing perennials which experience leaf turnover throughout the year? As N₂-fixation is inhibited by low soil moisture, N acquisition via the symbiotic N₂-fixation may be limited during the dry season. This might lead plants evergreen N₂-fixers to conserve more N by increasing N resorption from leaves undergoing senescence during the dry season(s). This would need to be explored this in a non-riparian habitat, with strong seasonal changes in soil moisture.

4.6. References

- Aerts, R. (1995) The advantages of being evergreen. *Trends in Ecology & Evolution* 10(10):402-407.
- Aerts, R. (1996) Nutrient Resorption from Senescing Leaves of Perennials: Are there General Patterns? *Journal of Ecology* 84(4):597-608.
- Aerts, R. and Chapin, F. S. (1999) The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. Pp 1-67 in A.H. Fitter and D.G. Raffaelli, editors. *Advances in Ecological Research*. Academic Press.
- Boerner, R. E. J. (1986) Seasonal nutrient dynamics, nutrient resorption, and mycorrhizal infection intensity of two perennial forest herbs. *American Journal of Botany* 73(9):1249-1257.
- Caldeira, M. V. W., Schumacher, M. V., and Spathelf, P. (2002) Quantification of nutrient content in above-ground biomass of young *Acacia mearnsii* De Wild., provenance Bodalla. *Ann. For. Sci.* 59(8):833-838.
- Chapin, F. S. and Kedrowski, R. A. (1983) Seasonal Changes in Nitrogen and Phosphorus Fractions and Autumn Retranslocation in Evergreen and Deciduous Taiga Trees. *Ecology* 64(2):376-391.
- Chapin, F. S., Matson, P. A., and Mooney, H. A. (2002) *Principles of terrestrial ecosystem ecology*. Springer, New York, USA.
- Chapin, F. S. and Moilanen, L. (1991) Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. *Ecology* 72(2):709-715.
- Chapin, F. S., Schulze, E., and Mooney, H. A. (1990) The Ecology and Economics of Storage in Plants. *Annual review of ecology and systematics* 21(1):423-447.
- Chapin, F. S. and Shaver, G. R. (1989) Differences in growth and nutrient use among arctic plant growth forms. *Functional Ecology* 3(1):73-80.
- Craine, J. M. (2009) *Resource strategies of wild plants*. Princeton University Press, Princeton, USA.
- Craine, J. M., Morrow, C., and Stock, W. D. (2008) Nutrient concentration ratios and co-limitation in South African grasslands. *New Phytologist* 179(3):829-836.
- del Arco, J. M., Escudero, A., and Garrido, M. V. (1991) Effects of site characteristics on nitrogen retranslocation from senescing leaves. *Ecology* 72(2):701-708.

- Drake, D. C., Naiman, R. J., and Bechtold, J. S. (2006) Fate of nitrogen in riparian forest soils and trees: an ^{15}N tracer study simulating salmon decay. *Ecology* 87(5):1256-1266.
- Drake, D. C., Peterson, B. J., Deegan, L. A., Harris, L. A., Miller, E. E., and Warren, R. S. (2008) Plant nitrogen dynamics in fertilized and natural New England salt marshes: a paired ^{15}N tracer study. *Marine Ecology Progress Series* 354:35-46.
- Ehrenfeld, J. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6(6):503-523.
- Ernst, W. (1975) Variation in the Mineral Contents of Leaves of Trees in Miombo Woodland in South Central Africa. *Journal of Ecology* 63(3):801-807.
- Escudero, A., del Arco, J. M., Sanz, I. C., and Ayala, J. (1992) Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. *Oecologia* 90(1):80-87.
- Fife, D. N., Nambiar, E. K. S., and Saur, E. (2008) Retranslocation of foliar nutrients in evergreen tree species planted in a Mediterranean environment. *Tree physiology* 28(2):187-196.
- Forrester, D. I., Schortemeyer, M., Stock, W. D., Bauhus, J., Khanna, P. K., and Cowie, A. L. (2007) Assessing nitrogen fixation in mixed-and single-species plantations of *Eucalyptus globulus* and *Acacia mearnsii*. *Tree physiology* 27(9):1319-1328.
- Gill, H. S., Abrol, I. P., and Samra, J. S. (1987) Nutrient recycling through litter production in young plantations of acacia nilotica and eucalyptus tereticornis in a highly alkaline soil. *Forest Ecology and Management* 22(1-2):57-69.
- He, H., Bleby, T. M., Veneklaas, E. J., and Lambers, H. (2011) Dinitrogen-fixing Acacia species from phosphorus-impooverished soils resorb leaf phosphorus efficiently. *Plant, Cell & Environment* 34(12):2060-2070.
- Hooper, D. U. and Vitousek, P. M. (1998) Effects of plant composition and diversity on nutrient cycling. *Ecological Monographs* 68(1):121-149.
- Killingbeck, K. T. (1993) Inefficient nitrogen resorption in genets of the actinorhizal nitrogen fixing shrub *Comptonia peregrina*: physiological ineptitude or evolutionary tradeoff? *Oecologia* 94(4):542-549.
- Killingbeck, K. T. (1996) Nutrients in Senesced Leaves: Keys to the Search for Potential Resorption and Resorption Proficiency. *Ecology* 77(6):1716-1727.
- Kobe, R. K., Lepczyk, C. A., and Iyer, M. (2005) Resorption efficiency decreases with increasing green leaf nutrients in a global dataset. *Ecology* 86(10):2780-2792.
- Lajtha, K. and Klein, M. (1988) The effect of varying nitrogen and phosphorus availability on nutrient use by *Larrea tridentata*, a desert evergreen shrub. *Oecologia* 75(3):348-353.
- Lambers, H., Brundrett, M. C., Raven, J. A., and Hopper, S. D. (2010) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil* 334(1-2):11-31.
- Lambers, H., Chapin, F. S., and Pons, L. P. (2008a) *Plant physiological ecology: Second edition*. Springer Science, New York.
- Lambers, H., Raven, J. A., Shaver, G. R., and Smith, S. E. (2008b) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution* 23(2):95-103.

- Miller, J. T., Murphy, D. J., Brown, G. K., Richardson, D. M., and González-Orozco, C. E. (2011) The evolution and phylogenetic placement of invasive Australian *Acacia* species. *Diversity and Distributions* 17(5):848-860.
- Mucina, L. and Rutherford, M. C. (2006) *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute Pretoria, Pretoria, South Africa.
- Pate, J. S. and Layzell, D. B. (1990) Energetics and biological costs of nitrogen. Pp 1-42 in P.K. Stumpf, E.E. Conn, B.J. Mifflin, and P.J. Lea, editors. *The Biochemistry of Plants, a Comprehensive Treatise: Intermediary Nitrogen Metabolism Assimilation*. Academic Press, San Diego, USA.
- Pereira, J. C., Caldeira, M. V. W., Schumacher, M. V., Hoppe, J. M., and Santos, E. M. (2000) Determinação do conteúdo de nutrientes em um povoamento de *Acacia mearnsii* De Wild. no Rio Grande do Sul. *Revista Árvore*. Viçosa 24:193-199.
- Pugnaire, F. I. and Chapin, F. S. (1992) Environmental and physiological factors governing nutrient resorption efficiency in Barley. *Oecologia* 90(1):120-126.
- Pugnaire, F. I. and Chapin, F. S. (1993) Controls over nutrient resorption from leaves of evergreen mediterranean species. *Ecology* 74(1):124-129.
- Ratnam, J., Sankaran, M., Hanan, N., Grant, R., and Zambatis, N. (2008) Nutrient resorption patterns of plant functional groups in a tropical savanna: variation and functional significance. *Oecologia* 157(1):141-151.
- Scholes, M. C., Scholes, R. J., Otter, L. B., and Woghiren, A. J. (2003) Biogeochemistry: The cycling of Elements. Pp 130-148 in J.T.D. Toit, K.H. Rogers, and H.C. Biggs, editors. *The Kruger Experience: Ecology and Management of Savanna Heterogeneity*. Island Press, Washington, D.C., USA.
- Shaver, G. R. and Melillo, J. M. (1984) Nutrient budgets of marsh plants: efficiency concepts and relation to availability. *Ecology* 65(5):1491-1510.
- StatSoft (2004) STATISTICA (data analysis software system) version 6. StatSoft Inc., Tulsa, USA.
- Sterner, R. W. and Elser, J. J. (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton, USA.
- Stewart, M. M. (1968) Notes on Reptiles from Northern Malawi, Africa. *Journal of Herpetology* 2(3/4):163-164.
- Tolsma, D. J., Ernst, W. H. O., Verweij, R. A., and Vooijs, R. (1987) Seasonal Variation of Nutrient Concentrations in a Semi-Arid Savanna Ecosystem in Botswana. *Journal of Ecology* 75(3):755-770.
- Van Heerwaarden, L. M., Toet, S., and Aerts, R. (2003) Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. *Oikos* 101(3):664-669.
- Vitousek, P. (1982) Nutrient cycling and nutrient use efficiency. *The American Naturalist* 119(4):553-572.
- Vitousek, P. M. (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57(1):7-13.
- Vitousek, P. M. and Howarth, R. W. (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13(2):87-115.
- Vitousek, P. M., Porder, S., Houlton, B. Z., and Chadwick, O. A. (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications* 20(1):5-15.

Wright, I. J. and Westoby, M. (2003) Nutrient concentration, resorption and lifespan: Leaf traits of Australian sclerophyll species. *Functional Ecology* 17(1):10-19.

CHAPTER 5: ESTIMATION OF N AND P STANDING STOCKS AND STUDY SYNTHESIS

Implications and patterns around Australian acacia invasions are among the best researched and understood of any invasive trees in southern Africa (e.g. Musil, 1993; Le Maitre *et al.*, 1996; Mehta, 2000; Dye and Jarman, 2004; Richardson and Van Wilgen, 2004; Yelenik *et al.*, 2004; Yelenik *et al.*, 2007; Jovanovic *et al.*, 2009; Le Maitre *et al.*, 2011; Morris *et al.*, 2011a; Richardson and Rejmánek, 2011). In fact, in their global review of invasive trees and shrubs, Richardson and Rejmánek (2011) noted that “few groups of woody plants have provided us with as many opportunities for gaining insights into the complex interplay of factors” determining species introductions, establishments and naturalisations than invasive Australian acacias. Furthermore, many of the lessons learned around human perceptions of introduced species and the impacts they have in different environments have emerged from this group of species. Yet despite an research focus on invasive Australian acacias, our understanding of their effects on nutrient cycling in non-native regions is limited, with the majority of studies based in the Cape Floristic Region (e.g. Witkowski, 1991; Stock *et al.*, 1995; Yelenik *et al.*, 2004) and none to my knowledge is based in the savanna or grasslands biomes or southern Africa.

In this study, I quantified aspects of nutrient (specifically N and P) uptake, allocation and conservation in two species of *Acacia*, the alien invasive *A. dealbata* native to south-eastern Australia, and the native *A. ataxacantha*, an abundant African species with similar growth habit and habitat as *A. dealbata*. These aspects were considered in conjunction with key traits in the nutrient-use strategy (such as leaf lifespan and N₂-fixation) of species. Furthermore, I assessed biomass allocation strategies and evaluated the use of easy-to-measure predictor variables (diameter and height) in biomass prediction models. Together, the results indicated that the invasive evergreen acacia tends to have high N₂-fixation rates (mean of 70% leaf N derived through symbiotic N₂-fixation), high biomass (~20% AGB) and nutrient allocation (~47% and 55% of aboveground N and P, respectively) to leaves, weak resorption of N (27%) and relatively efficient resorption of P (74%) from senescing leaves, and strong P allocation to reproductive tissue. On the other hand, the native deciduous acacia,

which did not fix N, had more efficient N resorption (~44% of the N stored in leaves) and highly efficient P resorption (>85%) from leaves, low biomass allocation to leaf tissue (~5% AGB), stronger seasonal variation in component nutrient concentrations and a shorter leaf lifespan. Nutrient concentrations between species differed in green leaves, with higher concentrations of both N and P in the native acacia for all aboveground components. However, the nutrient concentration in senesced leaves (resorption proficiency) demonstrated convergence between acacias. These findings suggest that N₂-fixation and phenological guild (deciduous vs. evergreen) play key roles in nutrient allocation and conservation strategies in acacias. Ecosystem-level changes brought about by invasion hinge on these differences. High rates of N₂-fixation in the invasive acacia are likely to facilitate the species high biomass allocation to leaf tissue, aiding high growth rates which contribute to its competitive advantage in many non-native environments. However, to better understand the potential impact of invasion of *A. dealbata* in systems with native African acacias, these results need to be synthesized for prediction and comparison of nutrient standing stocks, in conjunction with pool turnover rates and nutrient source.

5.1. Estimating N and P Stocks in Major Aboveground Components

Overall, my allometric equations derived for predicting total aboveground biomass in the two acacias were remarkably similar, but at the component level there were several species differences (see Chapter 2). For example, leaf biomass was proportionally higher (typically >4-fold, e.g. 21% vs. 5%) and in *A. dealbata*, while a high proportion (~30%) of aboveground biomass (dry weight) in *A. ataxacantha* was contained in senesced branches. But how do these disparities in biomass partitioning influence N and P stocks?

Comparing aboveground biomass and associated nutrient stocks between invasive and native species from different phenological guilds requires seasonal considerations as leaves can comprise or be a major component of aboveground biomass. Here, I first consider biomass and nutrient (N and P) standing stocks using mean annual nutrient concentrations, providing a time-integrated perspective on within plant standing nutrient stocks, Next, annual changes in aboveground pools are modelled using season-specific component N and P concentrations. Reproductive

components were not incorporated into this model. Despite this minor limitation, all major aboveground components have been included for both species allowing for a comparative analysis of aboveground stocks.

Total aboveground N and P stocks increase exponentially with stem diameter (Figure 5.1). Nitrogen stocks, estimated using mean annual tissue concentrations, are higher in *A. dealbata* than *A. ataxacantha* for any given stem diameter, and this difference increases with increasing stem diameter. For P, these curves are more similar between species, although again *A. ataxacantha* tends to have higher stocks at larger stem diameters. These species models of *total* aboveground stocks in relation to plant size provide potentially important inferences around species effects on nutrient cycling. Examination of component-level standing stocks, however, allow for more specific insights into potential alterations.

The relative nutrient allocation to leaves increases much more dramatically in *A. dealbata* with stem diameter (Figure 5.2). In the invasive acacia, >50% of total aboveground N and P stocks were in the leaves, while <6 % of total aboveground N and P was stored in leaf component of the native acacia (on average across the sampled range of stem diameters). Instead, the majority of aboveground N in *A. ataxacantha* is stored in woody components (live bole and branches, dead branches and bark, Figure 5.2). Phosphorus, as noted in Chapter 4, has allocation patterns similar to that of N, and thus we observed similar differences in the partitioning of aboveground P stocks between acacias. The proportion of P stored in the leaves of *A. ataxacantha* decreases with stem diameter, while the opposite was observed in *A. dealbata* (Figure 5.2).

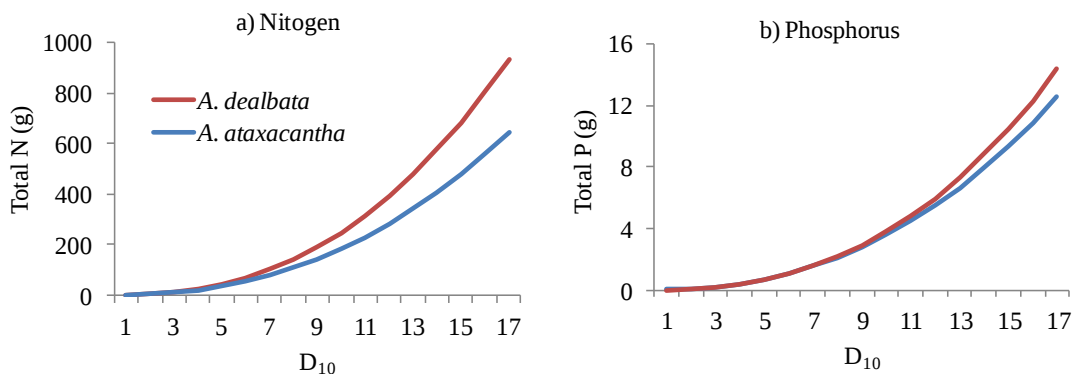


Figure 5.1 Total aboveground standing stocks of (a) nitrogen and (b) phosphorus as a function of stem diameter (D_{10}) for the native *A. ataxacantha* and the invasive *A. dealbata*. Mean annual tissue nutrient concentrations used for standing stock estimation.

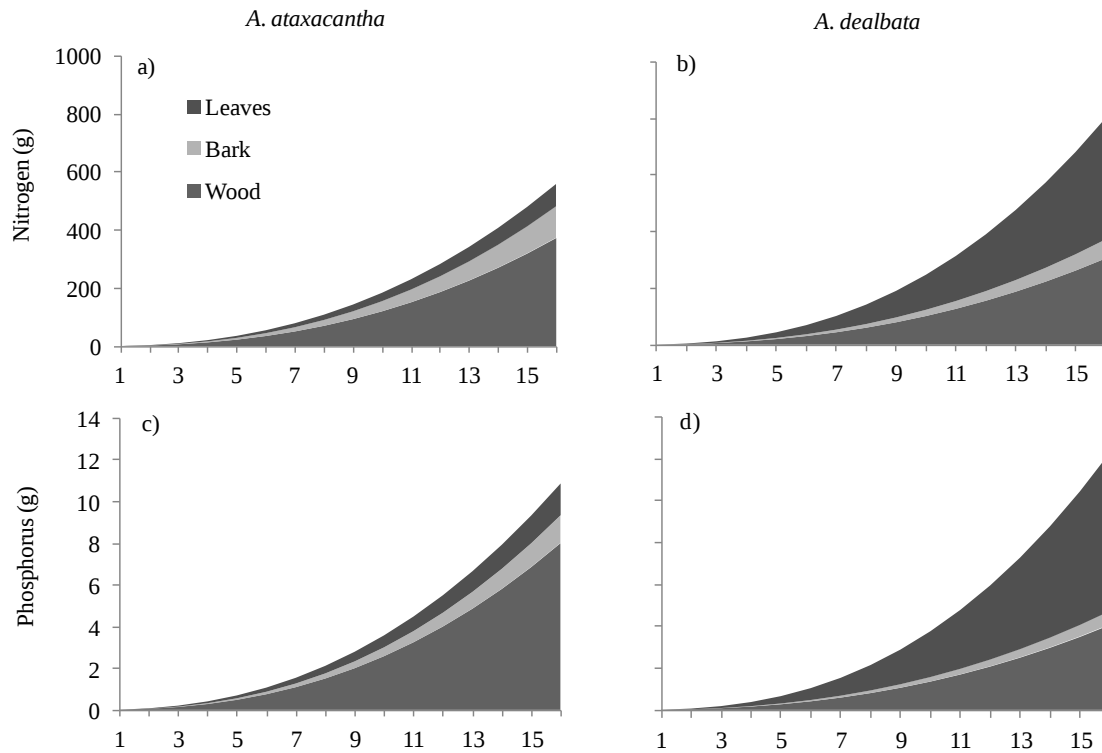


Figure 5.2 Component N and P stocks as a function of stem diameter (D_{10}). Nutrient standing stock modelled using allometric equations derived for relative components and mean annual component N and P concentrations.

Seasonal variation in nutrient stocks were far stronger in the deciduous *A. ataxacantha* and total P was more variable (3- to 6-fold variation) than total N (< 3-fold variation), and appear to be strongly linked to deciduousness in *A. ataxacantha* and investment in reproductive tissues in both species. Estimation of season-specific N and P stocks for a tree of basal stem diameter (D_{10}) of 12 cm shows considerable temporal variability in aboveground standing stocks of acacia trees (Figure 5.3), though relative differences in component N and P pools vary with stem diameter (Figure 5.2). For discussion on seasonal changes in component nutrient concentrations, refer to Chapter 4.

High N and P resorption efficiency requires nutrient transport (phloem loading), but this is likely to be most pronounced in the branch tips (Chapin *et al.*, 2002). This may explain the relatively subtle fluctuations in bark nutrient stocks observed in these models. Even relatively immobile forms of leaf P are likely to be resorbed considering 88% of all green leaf P is resorbed (Chapter 4). The high P

concentrations observed in inflorescences suggest that reproductive tissue constitutes a large seasonal P sink, especially for *A. ataxacantha*, where flowering coincides with a dramatic drop in leaf/wood/bark P stocks (October, Figure 5.3). Decreases in P stocks in *A. dealbata* do not coincide with flowering but do correspond to the early development of seed pods (first collected in the late dry season sample, October; Figure 5.3, also see Chapter 4). Again, decreases in wood/bark/leaf N pools coincide with development of reproductive tissue (*A. ataxacantha* – inflorescences, *A. dealbata* – seeds). These are most notable in the wood component.

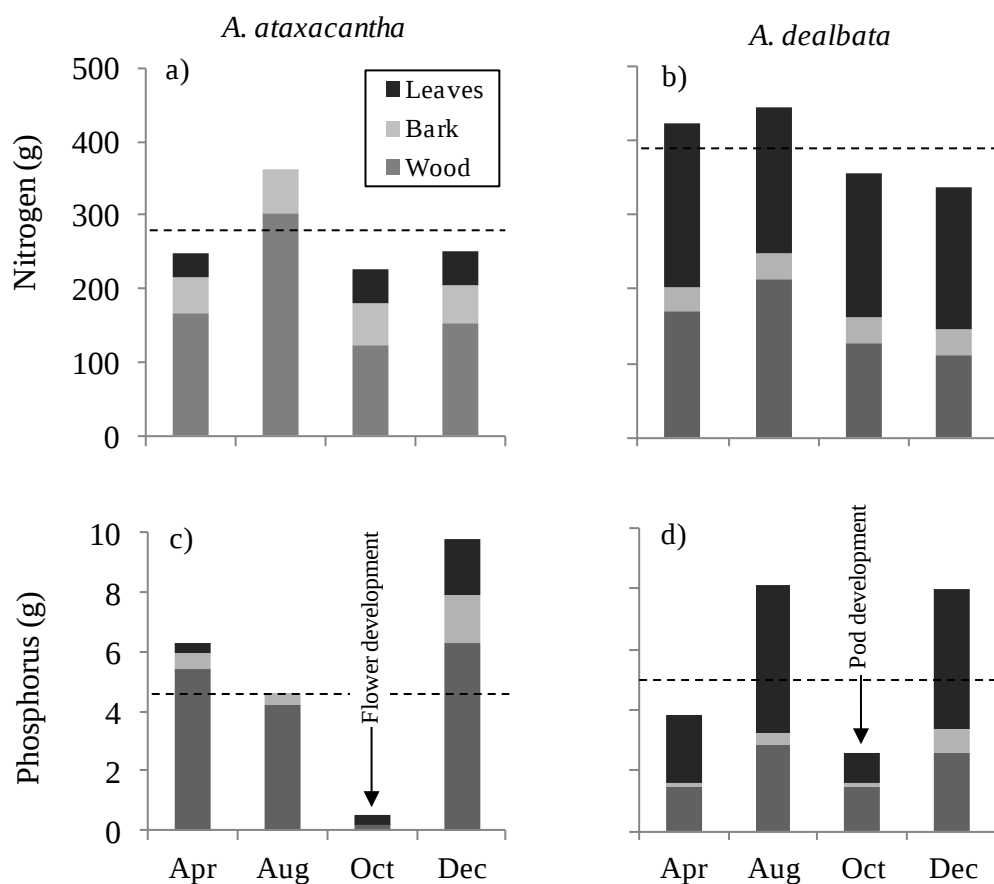


Figure 5.3 Seasonal changes in total aboveground and component N (a and b) and P (c and d) stocks in *A. ataxacantha* (right) and *A. dealbata* (left) based on average seasonal nutrient concentrations. Pool sizes are estimated for a stem diameter of 12 cm. Dashed line indicates total aboveground stock estimated using mean annual nutrient concentration (see Figure 5.2)

5.1.1. Limitations to standing stock models

Models of nutrient standing stocks in relation to stem diameter should provide a useful tool for evaluating site-level nutrient budgets (e.g. carbon budgets and carbon stocks, Montagu *et al.* 2005, Kaonga and Bayless-Smith, 2010) as well as identifying potential changes in the cycling and availability of growth-limiting nutrients in relation to turnover rates. The models provided here (Figure 5.1 and Figure 5.2) were developed using species-component specific allometric equations in conjunction with their respective N and P concentrations. It is important to note that these models provide best estimates for the relevant standing stocks. Error associated with allometric equations and component nutrient concentrations used to develop this model have been reported and discussed in Chapters 2 and 4, respectively. Once errors are combined, the resultant uncertainty associated with estimation might be substantial and should be considered where applying these models for empirical estimates of site-level standing stocks. Nevertheless, best-estimate scenarios still provide a useful standpoint for comparative analyses of the nutrient allocation strategies between species.

5.2. How Australian *Acacias* Impacts N and P Cycling in Southern Africa

In South Africa, information on functional impacts of invasive acacias has mostly been confined to the fynbos biome. The common finding of these studies has been the alteration in the spatial distribution of nutrient pools (N and P in particular). For example, Witkowski (1991) compared N and P return and leaf litter decomposition beneath the canopy of the invasive *Acacia saligna* and *Acacia cyclops* (both native to Australia) with that of the indigenous sclerophyllous vegetation. Beneath the canopy of invasive acacias, soil N increased, while no consistent pattern in soil P status was noted. Similar patterns of N enrichment in surface soils of fynbos ecosystems invaded by woody N₂-fixing *Acacia* species was observed by Stock *et al.* (1995) and Yelenik *et al.* (2004). Higher soil N availability in invasive acacia soils led Yelenik *et al.* (2004) to predict that the impacts of *A. saligna* would be long-lasting considering the residence times of organic N in soils. In all of these studies, invasive acacia species produced greater amounts of litter with higher N concentrations than the resident fynbos/strandveld species. However, it was unclear whether similar impacts would

occur in biomes where N and P limitation is less severe, such as grassland and savanna systems.

Mechanisms driving N enhancement appear to manifold. In the fynbos studies, higher litterfall rates couple with higher tissue N concentrations in invasive acacias result in an overall higher soil N contribution in invaded areas. Elevated leaf N concentrations of the invasive acacias were hypothesised to be mediated by symbiotic N₂-fixation and/or more efficient N uptake from the soil (Witkowski, 1991). This was supported by Stock *et al.* (1995) who used the relatively depleted ¹⁵N abundance of acacia litter as an indication of N₂-fixation. It is well known that N₂-fixing alien invasive plant species can alter the nutrient cycling of a community, especially if they differ from the dominant native species in their nutrient use strategies (Vitousek and Walker, 1989; Ehrenfeld, 2003). The capacity for N₂-fixation is also thought to contribute to the competitive success of Australian acacias as invaders (Sprent, 2009). Inputs of novel N from N₂-fixing aliens can increase primary production, increase rates of nutrient cycling, and facilitate N-limited competitors (Ehrenfeld, 2003).

Studies on invasive plant impacts often fail to identify the pathways through which the predicted or observed impacts alter ecosystem structure and/or functioning (Levine 2003). Apart from increased N inputs which may be expected from N₂-fixing plants, there are a number of other pathways through which Australian acacias, and invaders in general, may alter nutrient cycling. For example, mineralization rates are highly dependent on the C:N ratios and the lignin:N ratios of leaf litter. If these differ considerably between the invader and the resident species we would expect changes in nutrient cycling through changes in N and P cycling rates. Increased production of material which is easily decomposable (i.e. low C:N ratios, low lignin:N ratios) will increase nutrient cycling rates, while litter with higher C:N ratios than native species may result in decreased N mineralisation rates. Another major, albeit indirect, pathway through which invaders can alter nutrient cycling in non-native environments is through changes to the fire regime. Increase fire return frequency may increase N losses and reduce soil P status as P will be concentrated in ash. Change in litter inputs through differences in biomass partitioning and/or leaf lifespan will influence N and P cycling.

5.2.1. The potential consequences of *A. dealbata* invasion in the Komati on N and P dynamics

The invasion of *A. dealbata* in the upper Komati catchment carries a number of potential changes, with sometimes conflicting impacts on N and P dynamics. Displacement of *A. ataxacantha* by *A. dealbata* will result in elevated N inputs through N₂-fixation and higher N and P standing stocks in leaf material. Because leaf material has higher turnover rates than woody material, nutrient contributions through litterfall and decomposition under the canopy of the invasive acacia should also increase. Leaf lifespan in *A. dealbata* (~ 22 months for closely related *A. mearnsii*, Fife *et al.* 2008) is more than double that of *A. ataxacantha* (between 7 and 9 months), however, leaf biomass was >8-fold higher in *A. dealbata*. At senescence, terminal N and P concentrations in senesced leaves (resorption proficiencies) are similar between species. Assuming that in *A. ataxacantha*, the entire mid wet season leaf stock turns over annually, while in *A. dealbata* approximately half the leaf biomass turns over (complete turnover every 2nd year), litterfall rates (mass per year) should theoretically be >4-fold higher under the invasive acacia. This may be a conservative estimate in that it does not include leaf loss to herbivory (*Acacia ataxacantha* is likely more palatable with lower leaf C:N ratios; Hattingh, 2012).

Nutrient inputs through litterfall constitute a major flux in both N and P cycles, and represent the primary pathway through which impacts to N and P dynamics are expected to occur. Decomposition rates of fallen leaves, however, may moderate the N and P contribution rates through this pathway. Decomposition is primarily influenced by local climatic factors (temperature and humidity) and litter quality, for which C:N ratios are often used as an indicator (Scholes *et al.*, 2003). Green-leaf C:N ratios in common species of *Acacia* in the Komati study site (Hattingh, 2012) indicate that *A. dealbata* has significantly higher C:N ratios than *A. ataxacantha* (mean = 22.3 and 19.7, respectively). This difference was expected to increase in senesced leaves, with proportionally less N withdrawn during senescence, and suggested that decomposition rates of *A. dealbata* litter would be slower than that of *A. ataxacantha* litter.

The contribution of novel bioactive N by invasive species is particularly important in terms of impacts to ecosystem functioning. Nitrogen isotope analyses suggested that approximately 70% ($\pm$ 7% SE) of the N in leaves of *A. dealbata* (of

mixed representative ages) was fixed, while *A. ataxacantha* did not fix N. If estimated N contributions through litterfall are >4-fold higher in *A. dealbata* (with this differences increasing with stem diameter), the additional N contributions through litterfall must be novel inputs. Furthermore, novel N contributions scale with leaf biomass, which in turn scales exponentially with stem diameter. Novel N contributions through litterfall were thus estimated to range between 1.5 to 1.7 g tree⁻¹ for stems ranging between 10 and 17 cm diameter.

5.3. Concluding Remarks

Theoretical considerations and the data available have suggested that phosphorus is the limiting nutrient at the Komati study site, which may partially explain the competitive advantage of an ECM-species (*A. dealbata*) over an AM species (*A. ataxacantha*). The ability of *A. dealbata* to form symbiotic relationships with N₂-fixing bacteria has obvious benefits for N acquisition (~70% N derived from atmosphere), but may further facilitate P uptake in this species (Houlton *et al.*, 2008). In agreement with previous experimental works on African acacias, *A. ataxacantha* (a member of non-nodulating group) did not fix N. This small group of acacias (section *Monacantha*) appears to have lost their capacity for N₂-fixation symbiosis, which may be a trade-off in low light forest edge environments where the cost of nodule development is too high. It is likely that elevated N and P acquisition in *A. dealbata* support high biomass allocation to leaf tissue in this species. The primary discrepancy observed in predictive models of component biomass in aboveground components was the higher allocation to leaf biomass in *A. dealbata*. High leaf biomass facilitates high growth rates which are typical of many invasive Australian acacias in South Africa (Morris *et al.*, 2011a). Although there were species differences in resorption efficiency (for which leaf life-span and N₂-fixation ability appear to be trade-offs), there was a convergence in resorption proficiencies, which may be more relevant in an evolutionary context. Models of total aboveground biomass indicated similar total biomass for a given diameter, yet N and P standing stocks in aboveground components were higher in *A. dealbata* trees. Estimated rates of N inputs were >4-fold higher under the invasive acacia relative to *A. ataxacantha*, suggesting increased rates of N and P cycling. The high contributions of N₂-fixation to aboveground leaf N in the invasive

acacia indicate that novel N inputs alone (through litterfall and decomposition) are higher than total N litterfall inputs through litterfall in *A. ataxacantha*.

Overall, this study has also provided considerable evidence that invasions by the alien invasion *A. dealbata* have the potential to alter N cycling considerably, and P cycling to a lesser extent. Although the riparian zone of the upper Komati is unlikely to be severely N-limited, increased N inputs through dense stands of *A. dealbata* might facilitate the establishment of N-hungry exotics. In conclusion, it is clear that invasive Australian acacias successfully compete for resources in non-native environments, allowing them to proliferate at remarkable rates under variety of resource limitations.

5.4. References

- Chapin, F. S., Matson, P. A. & Mooney, H. A. (2002) *Principles of terrestrial ecosystem ecology*. Springer, New York, USA.
- Dye, P. & Jarmain, C. (2004) Water use by black wattle (*Acacia mearnsii*): implications for the link between removal of invading trees and catchment streamflow response. *South African Journal of Science*, **100**(1): 40-44.
- Ehrenfeld, J. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems*, **6**(6): 503-523.
- 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.
- Hattingh, W. (2012) *Are there differences in the leaf functional traits of nitrogen and non-nitrogen fixing woody plant species?* Honours thesis, University of the Witwatersrand, Johannesburg, South Africa.
- Houlton, B. Z., Wang, Y.-P., Vitousek, P. M. & Field, C. B. (2008) A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature*, **454**(7202): 327-330.
- Jovanovic, N. Z., Israel, S., Tredoux, G., Soltau, L., Maitre, D. L., Rusinga, F., Rozanov, A. & Van der Merwe, N. (2009) Nitrogen dynamics in land cleared of alien vegetation (*Acacia saligna*) and impacts on groundwater at Riverlands Nature Reserve (Western Cape, South Africa). *Water SA*, **35**: 37-44.
- Kaonga, M. L. & Bayliss-Smith, T. P. (2010) Allometric models for estimation of aboveground carbon stocks in improved fallows in eastern Zambia. *Agroforestry Systems*, **78**(3): 217-232.
- Killingbeck, K. T. (1996) Nutrients in Senesced Leaves: Keys to the Search for Potential Resorption and Resorption Proficiency. *Ecology*, **77**(6): 1716-1727.
- Le Maitre, D. C., Gaertner, M., Marchante, E., Ens, E. J., Holmes, P. M., Pauchard, A., O'Farrell, P. J., Rogers, A. M., Blanchard, R. & Bignaut, J. (2011) Impacts of invasive Australian acacias: implications for management and restoration. *Diversity and Distributions*, **17**(5): 1015-1029.
- Le Maitre, D. C., Van Wilgen, B. W., Chapman, R. A. & McKelly, D. H. (1996) Invasive plants and water resources in the Western Cape Province, South

- Africa: modelling the consequences of a lack of management. *Journal of Applied Ecology*, **33**: 161-172.
- Mehta, S. (2000) The Invasion of South African fynbos by an Australian immigrant: The story of *Acacia saligna*. *Restoration and Reclamation Review*, **6**(5).
- Montagu, K. D., Duttmer, K., Barton, C. V. M. & Cowie, A. L. (2005) Developing general allometric relationships for regional estimates of carbon sequestration: an example using *Eucalyptus pilularis* from seven contrasting sites. *Forest Ecology and Management*, **204**: 113-127.
- Morris, T. L., Esler, K. J., Barger, N. N., Jacobs, S. M. & Cramer, M. D. (2011) Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions*, **17**: 898-910.
- Musil, C. F. (1993) Effect of invasive Australian acacias on the regeneration, growth and nutrient chemistry of South African lowland fynbos. *Journal of Applied Ecology*, **30**(2): 361-372.
- Richardson, D. M. & Rejmánek, M. (2011) Trees and shrubs as invasive alien species – a global review. *Diversity and Distributions*, **17**(5): 788-809.
- Richardson, D. M. & Van Wilgen, B. W. (2004) Invasive alien plants in South Africa: how well do we understand the ecological impacts? *South African Journal of Science*, **100**: 45-52.
- Scholes, M. C., Scholes, R. J., Otter, L. B. & Woghiren, A. J. (2003) Biogeochemistry: The cycling of Elements. *The Kruger Experience: Ecology and Management of Savanna Heterogeneity* (eds J. T. D. Toit, K. H. Rogers & H. C. Biggs), pp. 130-148. Island Press, Washington, D.C., USA.
- Sprent, J. I. (2009) *Legume nodulation: a global perspective*. Wiley-Blackwell, Chichester, UK.
- Stock, W. D., Wienand, K. T. & Baker, A. C. (1995) Impacts of invading N₂-fixing *Acacia* species on patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies and ¹⁵N natural abundance values. *Oecologia*, **101**(3): 375-382.
- Vitousek, P. M. & Walker, L. R. (1989) Biological invasion by *Myrica Faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. *Ecological Monographs*, **59**(3): 247-265.
- Witkowski, E. T. F. (1991) Effects of invasive alien acacias on nutrient cycling in the coastal lowlands of the cape fynbos. *Journal of Applied Ecology*, **28**(1): 1-15.
- Yelenik, S. G., Stock, W. D. & Richardson, D. M. (2004) Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. *Restoration Ecology*, **12**(1): 44-51.
- Yelenik, S. G., Stock, W. D. & Richardson, D. M. (2007) Functional group identity does not predict invader impacts: differential effects of nitrogen-fixing exotic plants on ecosystem function. *Biological Invasions*, **9**(2): 117-125.