The impact of natural and simulated herbivory on compensatory leaf production under different light conditions

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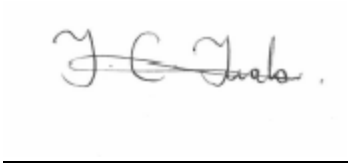
DECLARATION

I declare that this Dissertation is my own work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted by me before for any other degree, diploma or examination at any University or tertiary Institution.



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ABSTRACT

The ability of plants to compensate for disturbances such as herbivory and fire is driven by the type and extent of the disturbance, as well as the availability of resources. In savannas, plants utilise a variety of mechanisms to tolerate disturbances and resource limitations. Compensatory growth, resource reallocation, and photosynthetic and phenological changes have been recognized as mechanisms used by plants to tolerate environmental stress and resource limitation. Herbivory has been extensively researched as one of the main drivers of savanna biomes, but only a few studies have considered the influence of light availability (shading) as a resource affecting plant establishment and recruitment, in particular during the early stages of tree development. There is a gap in our understanding of how juvenile savanna trees (seedlings and saplings) tolerate shade, with many studies considering light as a limiting resource in forests and not in savannas, where light is considered to be a limiting resource to grasses but not woody species. The research that has been conducted on the influence of shading on juvenile trees has neglected how shading affects plant leaf turnover, phenology and leaf traits as well as plant morphology and physiology. Over the past decade only a few seed biology studies have been conducted on *Terminalia sericea.* These studies were aimed at understanding the seed nutrient content and factors influencing seed germination, but there is still limited information about the main drivers of seed germination, predation and physical properties. The main aims of this study were to: (1) investigate the effect of light availability, as well as natural and/or simulated herbivory on the compensatory growth capabilities of *Terminalia sericea*, a deciduous broad-leaved savanna tree species of considerable ecological and economical importance, and (2) determine the effect of seed predation and location on samarae (seed) physical properities, as well as the effect of artificial and natural cues on seed germination. The study took place on the roof top of the Oppenheimer Life Sciences Building at the University of the Witwatersrand in Johannesburg, South Africa, over one growing season (September 2016 to May 2017).

To investigate the effects of simulated herbivory and light availability (shading) on leaf turnover, longevity and phenology, two pairs of adjacent leaf clusters were selected from the canopies of seedlings and saplings in the sun and 80% shade. On each pair, one cluster was exposed to simulated herbivory (removing 50% of the leaf area along the mid-rib vein) while the adjacent

cluster was left untouched. Leaf turnover and longevity were measured at three week intervals and leaf phenology (i.e. timing of leaf age/development classes, e.g. new, fully expanded, mature and senescent) was measured monthly. The herbivory treatments did not affect leaf turnover or phenology. However, simulated herbivory had an effect on leaf longevity in seedlings although not in saplings, with leaf longevity being higher in control leaves compared to herbivory treatment leaves. Shading resulted in lower leaf production and loss, and longer leaf longevity. In addition, there was a higher proportion of new and fully expanded leaf phenophases in full sunlight compared to the shade, with the shade not having an effect on the proportion of mature and senescent leaf phenophases. Although, leaf production was lower in seedlings compared to saplings, the leaves of seedlings quickly matured but delayed leaf senescence, being shed only after the sapling canopy was bare, this is termed phenological avoidance which is an adaptative strategy used to maximise light interception and gain carbon.

The influence of shading on *Terminalia sericea* seedling and sapling architecture, storage reserves (dry mass and allocation patterns), and leaf traits was also tested. This study provided evidence that saplings were larger (taller, higher stem diameter, canopy area and volume as well as biomass), thus indicating that storage reserves increase with an increase in plant stage. Plants grown in the shade allocated more resources to their leaves than sun plants which invested more resources in their roots and shoots. This strategy is used to maximise light capture in the shade through the presence of more leaves (greater leaf size (i.e. length, width, area and thickness)), whereas the high allocation of resources to roots and shoots is an indication that plants were adapted to obtain more water, which is a more important resource in full sunlight, because sunlit plants have higher leaf temperatures and tend to lose more water through transpiration than those in the shade.

On saplings in the sun (only) two pairs of leaf clusters were selected on each plant and chlorophyll content (using the SPAD 502-Plus and CCM-300) and stomatal conductance were measured. *Phalera imitata*, Druce (Notodontidae) larvae were placed on one cluster in each pair with the adjacent cluster left untouched and both clusters were bagged. *Phalera imitata* larvae were removed after 24 hours and chlorophyll content and stomatal conductance were measured 3 days after herbivory. On the same leaves exposed to simulated herbivory (above) leaf chlorophyll content (measured using the SPAD 502-Plus and CCM-300), stomatal conductance and maximum efficiency of photosystem II (F_v/F_m) were measured at 3 week intervals. *Phalera imitata* larvae

herbivory did not have an effect on chlorophyll content and stomatal conductance on herbivory treatment and control leaves. Simulated herbivory did not have an effect on stomatal conductance and F_v/F_m, however, there were variable chlorophyll content results measured with the different devices, where chlorophyll was not influenced by simulated herbivory when using the CCM-300, but control leaves had higher chlorophyll content than herbivory treatment leaves in seedlings only. Chlorophyll content and F_v/F_m were higher in shaded compared to sun leaves, however, stomatal conductance was higher in sun leaves in seedlings. Shading, however, did not have an effect on stomatal conductance in saplings. Overall, seedlings performed better in the shade than in sunlight and saplings were able to grow well in the sun and shade.

I also investigated the influence of seed (samara) predation on seed dimensions from seeds collected from Nylsvley Nature Reserve (Nylsvley) and the Skukuza region of Kruger National Park (Skukuza), as well as the seed germination responses of *T. sericea* under different natural (temperature and photoperiod) and artificial (soaking and scarification) environmental cues. Seed predation was very site specific, with seed predation having an influence on seed dimensions in Nylsvley and not Skukuza. This suggested that seed predation likely occurred during seed development at Nylsvley, whereas it was post-dispersal at Skukuza. From the germination experimental trial, it was observed that soaking seeds in water at ambient temperature (control), nicking the seeds and germinating them at 12/12h photoperiod resulted in higher levels of seed germination, however, overall seed germination was very low due to overall low seed viability.

This dissertation has shown that light should no longer be ignored as a limiting resource in savannas, because it affects many plant responses, particularly during juvenile plant stages. This study has also shown that in order to propagate *T. sericea* from seed, photoperiod (12/12h), seed soaking (control) and nicking are techniques that promote seed germination. My study has also again highlighted the low viability of *T. sericea* seeds.

DEDICATION

"O God, give us the serenity to accept what cannot be changed, the courage to change what can be changed, and the wisdom to know the one from the other" – Reinhold Niebuhr

This dissertation is dedicated to my daughter Phila Lwazi Twala, mother, Johanna Twala, brother, Simphiwe Twala, father, Sithembiso Welcome Twala, and partner, Nceba Sijaji, who have had a very positive influence on my life by keeping me strong in my moments of weakness. Thank you for your emotional, spiritual and financial support.

To make a better life for all of us, I will continue to be by your side the way each and every one of you ha been… I love you!

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LIST OF ABBREVIATIONS

AICc – Akaike information criterion with a correction for finite samples

ANOVA – analysis of variance

CCI – chlorophyll content index

CCM – chlorophyll content meter

CO_2 – carbon dioxide

CV_t – coefficient of variance for the germination time

F_v/F_m – maximum photochemical efficiency of *PSII*

G – germinability

GLM – generalized linear model

LDMC – leaf dry matter content

LLAM – portable laser leaf area meter

LMA – leaf mass area ratio

LME – linear mixed effects model

MR – mean germination rate

MT – mean germination time

Nylsvley – Nylsvley Nature Reserve

OPT – optimal partitioning theory

PPFD – Photosynthetic Photon Flux Density

RMANOVA – repeated measures analysis of variance

SD – standard deviation

SE – standard error

Skukuza – Skukuza region of Kruger National Park

SLA – specific leaf area

U – uncertainty

Z – synchrony

GLOSSARY

Carbon gain: amount of carbon assimilated by a leaf during photosynthesis

Compensatory growth: (1) the production of leaves after exposure to herbivory or other distances and due to leaf loss; (2) An increase in growth after a period of slow growth due to disturbances

Growing season: period of the year where environmental conditions are favourable (temperature and rainfall) for plant growth, this period generally starts in spring and ends in winter for deciduous species

Maximum photochemical efficiency of PS*II*: a chlorophyll fluorescence measure that functions as a plant stress indicator which represents the plant health of a leaf's photosystem II

Phenology: the scientific study of the timing of cyclic biological events which are influenced by climatic conditions

Radicle: the first part to emerge from a seed during germination, it is the embryonic root

Samara(e): a fruit composed of a flattened wing with a hard coat that conseals the seed

Sapling: a young tree younger than 5 years old with a slender trunk

Seedling: a young tree younger than 2 years old

SPAD: the "Single Photon Avalanche Diode" (SPAD) is a hand-held device which is commonly used as an estimate of chlorophyll content or "greenness" in leave

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Chapter 1: General Introduction

1.1. Introduction

Savannas are characterized by a continuous herbaceous layer and a discontinuous woody layer (Scholes and Archer 1997; Sankaran *et al.* 2004). African savannas cover more than 50% of the total surface area of southern Africa and almost one third of South Africa (Huntley and Walker 1982; Scholes and Walker 1993; Scholes and Archer 1997), occurring over a wide range of climatic conditions (Scholes 1987; Higgins *et al.* 2000; Sankaran *et al.* 2005). Savannas support a range of land-use practices such as communal multiple-use systems, livestock farming and nature conservation (Shackleton 1997; Sunderlin *et al.* 2005). Also providing a vast range of ecosystem services for humans such as food, firewood, construction materials (Shackleton *et al.* 2004), and are of aesthetic value. Therefore, it is important to understand the functioning of this biome in all its complexity (Cleland *et al.* 2007).

1.1.1. Determinants of African savannas

In the African savannas, fire, herbivory, water scarcity and nutrient availability are all important drivers in the system (du Toit 1995; Scholes and Archer 1997; Katjiua and Ward 2006; Lehmann *et al.* 2014; Pringle *et al.* 2016; Seymour *et al.* 2016; Case and Staver 2017; Dohn *et al.* 2017; Staver *et al.* 2017; Yadeta *et al.* 2018). These environmental factors influence the structure, composition and productivity of savanna fauna and flora (Scholes and Archer 1997), ultimately influencing species development and survival (Larcher 1995). Plants have developed response mechanisms for these environmental factors which allow them to resist and/or tolerate the stresses associated with each of them (Maurcio 2000). Genetic variability, degree of stress, resource availability (including water, nutrient and light availability) and their interaction play a pivotal role in the ability of plants to invest nutrients in resistance and tolerance (Katjiua and Ward 2006). Tolerance to stresses has been defined as the ability to maintain plant fitness while coping with fitness reducing stress (Pilson 2000; Lehtilä 2003). While, resistance is the ability of plants to reduce the effects of stress either through chemical or mechanical defenses (Cooper and Owen-Smith 1987).

1.1.1.1. Herbivory

Plant development and survival is influenced by a variety of stresses (Larcher 1995). The timing of a disturbance event and the stage of development at the time of defoliation influences the ability of

the plant to cope with defoliation (McNaughton 1983). The frequency and intensity of grazing and browsing is seasonal and is influenced by water availability (Scholes 2009).

Owing to herbivore pervasiveness throughout the savanna, it is almost impossible to escape herbivory (Rosenthal and Kotanen 1994). Therefore, plants utilise two basic mechanisms to cope with herbivory, they either resist herbivory through escape in time (lifecycle) or in space (dispersal) or they tolerate herbivory (Rosenthal and Kotanen 1994; Agrawal *et al.* 2005; Moyo 2013).

Herbivory resistance is the ability of plants to reduce their acceptability to herbivores through defense (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999). Plants may use mechanical or chemical defenses to reduce the negative effects of herbivory (Cooper and Owen-Smith 1987; Bryant *et al.* 1991; Katjiua and Ward 2006; Prado *et al.* 2014). *Terminalia sericea* utilises both chemical (alkaloids) and mechanical (hairs) defenses which reduce the amount of biomass that can be consumed (Cooper and Owen-Smith 1987; Milewski *et al.* 1991; Midgley *et al.* 2001). Mechanical and chemical defenses are expensive to construct forcing plants to balance the trade-offs associated with these strategies (Coley and Aide 1991; Kursar and Coley 2003; Prado *et al.* 2014). Some strategies such as leaf toughness are only effective once the leaf has fully expanded (Coley and Aide 1991; Kursar and Coley 2003; Prado *et al.* 2014). This means that young and expanding leaves must depend on other defense strategies to cope with herbivory; these include trichomes and toxic secondary metabolites (Coley and Aide 1991; Kursar and Coley 2003; Prado *et al.* 2014).

Plant tolerance to herbivory is the ability of plants to maintain fitness (grow and reproduce) after experiencing fitness reducing damage (McNaughton 1983; Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Pilson 2000; Katjiua and Ward 2006). There are three main views on the influence of herbivory on fitness these are that: (1) herbivory is detrimental, (2) plants are able to compensate for low levels of herbivory and (3) moderate levels of herbivory cause overcompensation due to intrinsic and extrinsic herbivory factors (McNaughton 1983). In order to tolerate herbivory plants have developed numerous complex and interlinked tolerance mechanisms, these include: increases in photosynthetic and relative growth rates, resource reallocation, compensatory growth and changes in phenology (McNaughton 1983; Marquis 1992; Tiffin 2000; Schultz *et al.* 2013; Whitecross 2017).

The degree to which plants are able to tolerate herbivory is termed compensation (Strauss and Agrawal 1999). Compensatory growth is the ability of plants to increase growth due to a loss of plant tissue to herbivory (Belsky 1986; Moyo 2013). Various plant tissues have been used to measure compensatory growth including shoots, leaves, inflorescences and seeds (Belsky 1986). Plants respond to defoliation by either achieving partial-, complete- or overcompensation (Fornara and du Toit 2007). Which are the replacement of some, all or more than the defoliated tissue, respectively (Belsky 1986). The ability of plants to compensate for herbivory is influenced by a variety of environmental factors, including water, nutrients and light availability and competition (Rosenthal and Kotanen 1994; Rosenheim *et al.* 1997; Moyo 2013; Whitecross 2017).

Many studies use simulated herbivory, such as herbivore exclusions, clipping and leaf removal to simulate herbivory, due to the difficulty in controlling natural herbivory (Tiffin and Inouye 2000). It is advantageous to measure herbivory tolerance using natural herbivory because natural herbivory produces damage that plants have developed tolerance and/or resistance mechanisms for (Tiffin and Inouye 2000). In some instances replicating natural herbivory can be difficult resulting in different responses from natural and simulated herbivory (Tiffin and Inouye 2000).

Invertebrate and vertebrate herbivory are functionally distinct in the type of damage they inflict; therefore there is a difference in how plants are able to tolerate both types of herbivory damage (Rosenthal and Kotanen 1994; Kotanen and Rosenthal 2000). Invertebrate and vertebrate herbivory share many common features which include: the removal of plant tissue, structural damage, and decline in plant nutrients due to tissue loss as well as impaired plant growth and reproduction; therefore affecting the plants' competitive ability (Hendrix 1988, Marquis 1992). There are many features that differ between both types of herbivory, but only three will be considered: (1) tissue specificity, (2) diversity of damage types, and (3) indirect effects. These three differences are all influenced by the large difference in body size of vertebrates compared with invertebrates, as well as the differences in their behaviour, ecology and physiology (Kotanen and Rosenthal 2000).

1.1.1.1.1. Tissue specificity

Invertebrate herbivores are more tissue specific than vertebrate herbivores (Crawley 1989). The difference in plant and vertebrate herbivore size reduces the ability of vertebrate herbivores to specialize on plant tissue, even though they specialize at a larger scale, such as forage preference

(Kotanen and Rosenthal 2000). Vertebrates may eat a variety of foliage including seeds, roots and leaves while invertebrates specialize on plant tissue organs such as root cortex, xylem sap and leaf parenchyma (Mattson *et al.* 1988). This specialization can be seen in a single weevil genus, *Apion*, where different species prefer roots, stems, leaf buds, flower buds, inflorescence or fruits and seeds (Bernays and Chapman 1994).

1.1.1.1.2. Damage distribution

The spatial distribution of tissue damage due to herbivory is important because of its influence on the compensatory abilities of plants (Rosenthal and Kotanen 1994). Invertebrate and vertebrate herbivory is very specific with both herbivores consuming the most nutritious tissue of the plant (Crawley 1989). Vertebrate and invertebrate herbivory may occur randomly on plant tissue mainly due to herbivore size (Rosenthal and Kotanen 1994; Kotanen and Rosenthal 2000). The proportion of damage produced by a single vertebrate herbivore bite may be similar to that of many invertebrates; however some invertebrate attacks occur over short intervals due to seasonality (Kotanen and Rosenthal 2000). Although invertebrate feeders such as gall forming and sucking insects cause damage to specific tissues, their effects go beyond that of the affected tissue, resulting in physiological and morphological responses to neighbouring tissue within the same individual (Marquis 1992).

1.1.1.1.3. Indirect effects

Both invertebrate and vertebrate herbivory have an indirect positive effect on plants (Kotanen and Rosenthal 2000). The costs of tissue damage and loss are recovered through compensatory growth (Kotanen and Rosenthal 2000). The concept of compensatory growth is very common in systems exploited by vertebrates, where herbivory removes competitor species, increases light availability, reduces fire frequency and leads to nutrient addition (McNaughton 1979; Hobbs 1996). Such events are uncommon for invertebrates, but they can occur when both the herbivore and host plants are abundant (Kotanen and Rosenthal 2000). For example, invertebrates can cause premature leaf abscission, increase soil fertility through faeces deposits and alter keystone species abundance (Trumble *et al.* 1993).

However, the most important indirect effect of invertebrate herbivory is a negative one, the transmission of diseases (Kotanen and Rosenthal 2000). Invertebrate vectors may transmit diseases

actively during feeding or through opportunistic infections in damaged tissue, or passively by herbivores that carry hitchhiking pathogens (Kotanen and Rosenthal 2000). It is important to understand this indirect effect because it could be an underlying factor that may be important in the mechanisms of plant tolerance to herbivory (Kotanen and Rosenthal 2000).

Invertebrate herbivory, particularly insect herbivory, often has a negative effect on the leaf gas exchange of the host plant, including: stomatal conductance, transpiration and net CO_2 assimilation (Martin *et al.* 2009). Measuring gas exchange after invertebrate herbivory is very crucial because it provides us with a quantitative measure of damage prior to any visible damage that may later manifest (Martin *et al.* 2009). These effects may vary depending on the feeding guild of the insect (Martin *et al.* 2009). Welter (1989) found that arthropods directly feeding on leaf matter increased photosynthesis whiles other feeding guilds (root feeders and stem borers) resulted in a decrease in photosynthesis.

1.1.2. Light availability

Biotic and abiotic stress are interrelated, with either an individual or combination of the subsequent stresses causing molecular, biochemical, physiological and morphological changes that have negative impacts on plant growth, productivity and yield (Xu *et al.* 2009). Light, heat, salinity and water stress are the major abiotic stresses that can cause severe cellular damage to plants. Light is a highly heterogeneous resource influencing plant performance and fitness, as well as productivity, survival and competitive ability in plant communities (Valladares and Niinemets 2008). In natural systems, shading is mainly caused by tree canopies, therefore the composition and structure of plant communities is the major cause of light heterogeneity (Valladares and Niinemets 2008). All plants experience at least some degree of shading during their lifetime; this is due to shading between and within plant canopies, creating a contrast of light gradients (Valladares and Niinemets 2008). In order for photosynthesis to occur, light is required to excite electrons, but both low and high levels of sunlight can have a negative impact on plant performance (Valladares and Niinemets 2008). In low levels of light, light availability can reduce plant growth and survival, while high levels of light can cause heat, desiccation, UV radiation and irradiance stress requiring high investment in protection (Valladares and Niinemets 2008), such as plasticity (Abrams and Kubiske 1990). A prime example of morphological plasticity in plants can be observed in the structural differences of sun

and shade leaves (Abrams and Kubiske 1990). Sun and shade leaves have been found to differ in stomatal size and density, leaf thickness, area and specific leaf mass (Abrams and Kubiske 1990).

1.1.2.1. Shade tolerance

Shade tolerance is defined as the ability of a plant to tolerate low levels of light (Valladares and Niinemets 2008). An inverse relationship has been observed between shade tolerance and other limiting factors such as tolerance to flooding and drought (Niinemets and Valladares 2006). The length of the growing season plays a major role in the extent to which plants are able to tolerate environmental stresses in low light (Valladares and Niinemets 2008).

Plants have developed various shade tolerance mechanisms to cope with limitations of light, these include: reduced net photosynthetic rates, stomatal conductance, high concentrations of chlorophyll content, large leaf size and leaf area ratios (Valladares and Niinemets 2008). In order to increase light capture shaded plants are able to re-allocate their resources from roots into shoots and leaves, this is at the expense of water capture (Sack 2004). These traits improve light capture compensating for the low levels of light (Valladares and Niinemets 2008).

1.1.3. Herbivory and light availability interaction

Herbivory affects plant performance and fitness (Marquis 1992), which ultimately influences plant distribution along different light gradients (Louda and Rodman 1996). Numerous studies on different plant species have shown that insect herbivory is higher in shaded habitats (Dudt and Shure 1994; Yamasaki and Kikuzawa 2003; Niesenbaum and Kluger 2006; Muth *et al.* 2008; Cowie *et al.* 2016). Several studies have also shown higher levels of insect herbivory in sun or open habitats (Lincoln and Mooney 1984; Louda and Rodman 1996) or no difference in insect herbivory between sun and shade plants (Coley 1983).

Several studies on herbivory in sun versus shade environments have addressed plant resistance (Salgado-Luarte and Gianoli 2010) and to a less extent plant tolerance strategies. It is important to understand the consequences of herbivory on plant abundance, distribution, performance and fitness (Strauss and Agrawal 1999). Herbivory damage has been found to result in greater fitness loss in shaded plants (Wise and Abrahamson 2007). This difference in herbivory tolerance is due to the fact that carbon gain and compensatory growth are reduced in shaded habitats due to resource availability and time constraints (Salgado-Luarte and Gianoli 2010). Like herbivory, light availability

reduces plant performance and fitness and defoliation by herbivores can affect light exploitation by removing leaf area or by affecting phenotypic responses of plants to shading.

1.1.3. Savanna tree phenology

Phenology is the study of the timing of biological events and their seasonal changes (Seghieri *et al.* 2009; Moyo 2013; Whitecross *et al.* 2016; Whitecross 2017; Whitecross *et al.* 2017a; 2017b). Knowledge of phenology is important in building our understanding of community structure, regeneration and functioning, as well as predicting the effects of climate change on phenological events (Childes 1989; Williams *et al.* 1999; Menzel 2002; Seghieri *et al.* 2009). It is also important to study plant phenology because plants respond to favourable or stressful conditions by changing their phenology (Williams *et al.* 1999; Sekhwela and Yates 2007; Moyo 2013; Whitecross *et al.* 2016). For example, Moyo (2013) and Whitecross *et al.* (2016) showed that nutrient and water availability, as well as fire influence leaf green-up rates and reproductive phenology in *Terminalia sericea* adults in the field.

Phenology can contribute towards understanding and predicting the influence of major biotic and abiotic factors on different plant developmental stages (Shackleton 1997). Phenology is driven by temperature, light, water and nutrient availability, with herbivory and fire acting as selective forces causing leaves to flush before the onset of rain (before the growing season) and shedding them at the end of the growing season, this strategy allows plants to avoid the negative effects of insect herbivory and fire (Scholes and Archer 1997; Fenner 1998; Whitecross *et al.* 2016). A difference in phenological patterns promotes diversity by reducing competition for resources, pollinators and seed dispersers (Cleland *et al.* 2007).

The timing and duration of the presence of different leaf age classes' affects carbon assimilation, energy exchange, water and forage availability (Schwartz 1999; Archibald and Scholes 2007). However, predicting phenological patterns is very complicated, due to seasonal variations (Archibald and Scholes 2007; Whitecross *et al.* 2016), its complexity and sometimes over-simplification of the techniques used to quantify phenology. However over the years there has been an increase in phenological studies utilising different monitoring techniques and phenological attributes to monitor the impacts of climate change and predicting long-term responses (Tucker *et*

al. 1985; Childes 1989; Schwartz 1999; Jolly *et al.* 2005; Cleland *et al.* 2007; Polgar and Primack 2011; Moyo 2013; February and Higgins 2016; Whitecross *et al.* 2016).

1.2. Rationale

1.2.1. Herbivory

Numerous studies have been conducted to understand the ability of plants to tolerate herbivory (Crawley 1983; Belsky 1986; Aarssen 1995; Rooke and Bergström 2007; Moyo 2013; Whitecross 2017). These studies observed compensatory growth in various plant tissues: leaves (Ruiz-R *et al.* 2008; Whitecross 2017), shoots (Hjàlten *et al.* 1993; Gadd *et al.* 2001), inflorescences (Hendrix 1979) and seeds (Hendrix 1979; Islam and Crawley 1983). Although numerous studies have been conducted on the ability of crops (Dyer 1975; Welter and Steggall 1993) and herbaceous species (Maschinski and Whitham 1989; Mauricio *et al.* 1997) to compensate for herbivory, far fewer have studied woody plant species (Boege 2005; Rooke and Bergström 2007; Moyo 2013; Whitecross 2017). With this relative gap in the investigation of compensation in woody species, there is also a gap in the comparison of natural and simulated herbivory in woody species.

It is very important to understand herbivory tolerance in systems where mammalian herbivory is prominent (McNaughton 1979; Detling and Painter 1983) and in natural and agricultural systems where insect herbivory is a common occurrence (Painter 1958; Beck 1965; Trumble *et al.* 1993). This will enable us to understand how foliage availability, through leaf turnover, and other ecosystem services provided by *Terminalia sericea* changes after natural and simulated defoliation. This knowledge can be used by communities who utilize this plant species for medicinal purposes, cattle grazing and fuelwood (Coates-Palgrave 1957).

1.2.2. Light availability

Shade tolerance has been extensively studied in the forest biome, but has been neglected in the savanna due to the sparse distribution of trees in this biome. However, light is also a limiting factor in savannas due to neighbour-to-neighbour associations and bush encroachment. Therefore, it is important to understand the impacts of light limitation in savannas because of shading caused by larger tree species at the landscape scale. This type of shading limits solar radiation reaching herbaceous species and medium and smaller sized trees. An example of this is the shading of *Terminalia sericea* by *Burkea africana* in *Burkea africana-Terminalia sericea* savannas (T. Twala *pers.*

obs.). Where overstory leaves tend to shade understory leaves affecting light capture at the plant level. These challenges make it important to understand how light limitations affect plant growth and foliage availability at the cluster and plant level.

Numerous studies have observed reduced plant tolerance to herbivory in shaded habitats (Pierson *et al.* 1990; Rogers and Siemann 2002); some studies have found similar levels of tolerance in different light conditions (Rogers and Siemann 2002). It is surprising that a large majority of studies on the interaction of herbivory and light availability have focused on plant resistance (defensive traits) and rarely on plant tolerance. In addition to this there has been limited research aimed at understanding the effects of light availability of savanna tree species (Vadigi and Ward 2013), with most studies aimed at understanding the effects of herbivory, temperature, rainfall, and water and nutrient availability (Shackleton 1999; Chidumayo 2001; Jolly and Running 2004; Moyo 2013; Whitecross *et al.* 2016).

1.3. Aims and objectives

The main aims of this study were to investigate the effects of light availability, natural and/or simulated herbivory and their interaction on compensatory growth in *Terminalia sericea,* and the effects of seed predation and collection site on seed physical properties as well as determine the effects of natural and artificial environmental cues on seed germination.

The objectives of this study were to:

1. Determine how simulated herbivory, light availability and their interaction affect leaf production, loss, longevity and phenology of *T. sericea* seedlings and saplings.

2. Investigate the effects of light availability on plant architecture, allocation patterns and leaf traits of *T. sericea* seedlings and saplings.

3. Determine the influence of natural and/or simulated herbivory, light availability and their interaction on the photosynthetic responses of *T. sericea* seedlings and saplings.

4. Investigate the influence of seed predation and collection site on the seed physical properties of *T. sericea*.

5. Investigate the influence of photoperiod, temperature, soaking and scarification on germination in *T. sericea*.

1.4. Study approach and thesis structure

Chapter 1: *Introduction* - Chapter one consists of a general literature review into savannas and disturbances in these systems as well as a general overview of the importance of determining the effects of herbivory and light availability on *T. sericea* growth.

Chapter 2: *Leaf turnover and phenology* - This chapter aimed to determine the effects of simulated herbivory and light availability on leaf compensatory growth (turnover) and phenology of *T. sericea*.

Chapter 3: *Growth patterns and leaf traits* - In this chapter the influence of light availability on *T. sericea* seedling and sapling growth and leaf traits was assessed.

Chapter 4: *Ecophysiological responses* - Chapter four is on ecophysiology, where chlorophyll content, stomatal conductance and photochemical efficiency of photosystem *II* were measured to assess how *T. sericea* seedlings and saplings in full sunlight and shade conditions cope with exposure to natural and/or simulated herbivory. This chapter also compares how well two hand-held chlorophyll content meters measure chlorophyll content.

Chapter 5: *Germination* - This chapter focused on *T. sericea* seed germination under different environmental conditions and compared the influence of seed predation on the seed dimensions of seeds collected from two semi-arid savannas.

Chapter 6: *Synthesis* - This final chapter is a general discussion of the research findings with reference to how *T. sericea* responds to different stresses, it also provides recommendations for future research in regards to understanding the ecology of *T. sericea* and its characteristics, as well as methods and techniques to germinating this unique savanna tree species.

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Chapter 2: The effects of herbivory and light availability on compensatory leaf production and phenology in *Terminalia sericea* (Combretaceae)

Abstract

Leaf turnover, longevity and phenology are important characteristics in determining plant carbon gain and nutrient conservation. These characteristics are largely influenced by light, water and nutrient availability, herbivory and climate. Thus, the aim of this study was to determine the influence of light availability and simulated herbivory on leaf turnover and leaf phenology in *Terminalia sericea* seedlings and saplings. Seedlings and saplings were placed in full sunlight and under 80% shade cloth. On each plant two pairs of leaf clusters were selected. On one cluster from each pair 50% of leaf area (but not including the midrib vein) was removed (herbivory treatment) and the adjacent cluster was left untouched (control). The presence of new leaves and leaf loss were recorded. The time from leaf production to shedding provided leaf longevity. The herbivory treatment did not have an effect on leaf production and loss in either seedlings or saplings. High light availability resulted in higher leaf production in seedlings, with the opposite trend in saplings. In seedlings, control leaves were longer-lived (106 ± 2 days) than herbivory treatment (89 ± 2 days) leaves, with herbivory not having an effect on leaf longevity in saplings (control = 75 ± 2 days, herbivory treatment = 77 ± 2 days). Leaves quickly expanded and matured in seedlings, which may be an adaptive strategy to maintain their leaves and reduce damage from environmental stresses such as herbivory. Seedling leaves senesced and shed later in the growing season than sapling leaves. This suggests that seedlings are changing their leaf phenology in order to avoid and/or reduce herbivory damage, as well as increase carbon gain, by keeping their leaves later into the growing season when shading from taller plants would be reduced. Hence, it is apparent that *T. sericea* seedlings and saplings are shade tolerant and can compensate for 50% herbivory damage at the leaf cluster-level.

Keywords: Compensatory growth, herbivory, leaf longevity, leaf turnover, phenology, shading

2.1. Introduction

Savannas are characterized by a co-dominance of trees and grasses, which comprise of a continuous herbaceous layer and a discontinuous woody layer (Frost *et al.* 1986; Scholes and Archer 1997; Sankaran *et al.* 2004). Trees alter the composition and demographics of savanna ecosystems through light interception, soil nutrient mediation and water availability (Vetaas 1992; Shaw *et al.* 2002; Ludwig *et al.* 2003; Vadigi and Ward 2012). Seedling and/or sapling establishment are important and vulnerable plant stages of savanna trees, which are long lived (Scholes and Archer 1997; Wilson and Witkowski 1998; Witkowski and Garner 2000; Wilson and Witkowski 2003; Chidumayo 2008; Helm *et al.* 2011). If environmental conditions are not favourable, sapling establishment can be a major issue influencing tree recruitment and environmental demographics (Midgley and Bond 2001; Helm and Witkowski 2012). Therefore, the factors that influence the successful recruitment of saplings into savannas have a large effect on savanna functioning (Shaw *et al.* 2002). Disturbances such as fire and herbivory often have intense limitations on savanna sapling establishment (Midgley *et al.* 2010; Helm *et al.* 2011). Apart from disturbances, recruitment is often limited by resource availability (light, nutrient and water) (O'Connor 1995; Kraaij and Ward 2006; Sankaran *et al.* 2008; Riginos 2009; Ward and Esler 2011).

2.1.1. Herbivory

The ability of plants to respond to herbivory is influenced by many intrinsic and extrinsic factors. Plants may develop herbivory resistance traits such as chemical and mechanical defences, causing the plant to return to its juvenile growth form (Bryant 1981), or even affect its leaf turnover and phenology (Williams and Whitham 1986; Whitecross 2017). However the simplest way to respond to herbivory is often through tolerance by rapidly replacing the tissue lost to herbivory. Rapid growth may not be sufficient to prevent mortality to herbivory especially during the juvenile stages of plant development (Bryant *et al.* 1983). Compensatory growth has been well reported in African savannas for various woody species such as *Combretum apiculatum* (Bergström *et al.* 2000), *Schotia brachypetala* (Vadigi 2012), *Acacia nigrescens, A. tortilis* (du Toit *et al.* 1990), *A. erubescens* (Dangerfield and Modukanele 1996) and *Terminalia sericea* (Moyo 2014; Whitecross 2017). The impacts of herbivores on woody seedlings and saplings are also well known (Shaw *et al.* 2002; Riginos and Young 2007; Fornara and du Toit 2008; Vadigi 2012). Understanding the role and impact

of different types of herbivores and herbivory at these crucial growth stages is important to understand in terms of plant survival and establishing of these growth stages.

2.1.2. Shade

Light is a heterogeneous environmental factor that influences plant survival, growth and competitive interactions in the ecosystem (Smith and Shackleton 1988; Ludwig *et al.* 2001; Valladares 2003). Shading in communities is primarily from shading of plant canopies (Kelly and Canham 1992; Randle *et al.* 2018), where contrasting light gradients occur both within and between plant canopies. Light penetration to the soil surface is becoming a scarce resource in African savannas due to bush encroachment (reviewed by Archer *et al.* 1995; Randle *et al.* 2018) and canopy shading, where seedlings, saplings and smaller trees are shaded by taller grasses and adult trees. These plant canopies have both positive and negative effects because they create microsites which facilitate recruitment and survival of woody saplings by intercepting light thereby reducing irradiance, alleviating grass competition by shading them out, enhancing nutrients and increasing soil moisture (Hoffmann 1996; Salazar *et al.* 2012; Vadigi 2012; Abdallah *et al.* 2016; Whitecross *et al.* 2017; Ward *et al.* 2018), and may affect seedling establishment in the understorey especially for shade intolerant species. Plants respond to a change in light availability by altering their physiology, biochemistry, anatomy, morphology, phenology and biomass allocation patterns (Boardman 1977; Harper 1989; Stitt and Schulze 1994; Auguspurger and Bartlett 2003; Catoni *et al.* 2015; Gignoux *et al.* 2016; Valladares *et al.* 2016; Falster *et al.* 2018).

Phenology is often overlooked in plant ecology, particularity in different plant stages and shading conditions, with most data referring to undamaged and/or damaged adult trees (Chidumayo 2001; Archibald and Scholes 2007; Whitecross 2017). The aim of phenology is to monitor and record periodically occurring growth stages and to observe and understand how these different growth stages depend on environmental, physiological and structural factors. Changes in the environment highly affect the timing of phenological events in trees, which are vital for regrowth and survival (Pinto *et al.* 2011; Moyo 2014; Whitecross *et al.* 2016). Extensive research has been conducted on the phenology of savanna trees (Chidumayo 2001; Higgins et al. 2011; Moyo 2014; Whitecross *et al.* 2016, 2017), but there is insufficient understanding on how plant stage, simulated herbivory (such as reducing leaf surface area by cutting) and shading affect leaf phenology in savanna shrubs and trees, in particular indigenous tree species.

Terminalia sericea is an indigenous tree species which commonly occurs in savanna and/or woodlands in association with taller tree species (e.g. *Burkea africana*), thus light availability may play an important role in the productivity, survival, establishment and distribution of this species. Changes in leaf turnover and phenological changes have been identified as key aspects in *T. sericea* biology that change in response to herbivory and/or light availability in adult trees (Moyo *et al.* 2015a, 2015b; Whitecross *et al.* 2016, 2017). Therefore, this study aimed to determine in *T. sericea* seedlings and saplings over a growing season: (1) the effects of simulated herbivory and light availability on leaf turnover and (2) the effects of light availability on leaf phenology.

2.2. Materials and methods

2.2.1. Study site

The study was conducted on the rooftop of the Oppenheimer Life Sciences Building at the University of the Witwatersrand, Johannesburg, South Africa (26° 11' 30" S; 28° 01' 58" E). Johannesburg has a subtropical highland climate characterised by dry sunny days and cold nights during the winter months (May to September), and hot days with afternoon thunderstorms during the day followed by cool nights during the summer months (October to April). The mean annual temperature during the day is 26.2°C in January, dropping to ~16.6°C in June. In the winter months temperatures can drop to freezing at night causing frost. Johannesburg has a mean annual rainfall of 604mm/year most of which falls in summer, receiving its lowest rainfall in July and its highest in January.

2.2.2. Study species

Terminalia sericea Burch ex. DC is the chosen study species and its common name is the silver cluster leaf. It is commonly found in South Africa, Botswana, Namibia, Zimbabwe, Angola and northwards to the Democratic Republic of Congo and Tanzania (Coates-Palgrave 1957). *Terminalia sericea* is a medium sized deciduous tree, with a height range between 4 and 12 m but some individuals have been known to grow up to 20 m (Coates-Palgrave 1957). It can be easily distinguished by its silver leaves which are borne in clusters and its dark and deeply fissured bark (Grant *et al.* 2005). Sprouting and new leaves occur in the centre of the leaf clusters. *Terminalia sericea* has leaves throughout the growing season dropping them in autumn, while flowers are present in spring which are then replaced by fruit in mid-summer that drop in winter when most of

the tree canopies are bare (Grant 1984; Kikuzawa 1995). It is commonly used for charcoal, timber, firewood and for medicine (Carr 1994). *Terminalia sericea* is a slow growing tree which copes well with fire and cutting, forming dense thickets of coppice growth (Coates-Palgrave 1957; Moyo 2014), indicating its compensatory growth qualities due to tissue loss caused by environmental stresses. *Terminalia sericea* leaves and shoots may be browsed by invertebrates such as *Euproctis fasciata* Walker, 1855 (tussock moth) and *Arcyophora carniola* Hampson, 1912 (moth) (Gandar 1982) and vertebrates such as elephants and impala (Coates-Palgrave 1957). It also constitutes a large part of the diets of many livestock species (Katjiua and Ward 2006). *Terminalia sericea* was chosen as the study species due to its high leaf turnover rates, which suggested to Whitecross *et al.* (2016) that compensatory growth mechanisms may be utilised by this species to tolerate invertebrate herbivory over multiple seasons.

2.2.3. Plant specifics and maintenance

Terminalia sericea seedlings were purchased from The Aloe Farm situated in Hartebeespoort dam, North West, South Africa (25° 43' 15" S; 27° 47' 9"E). Saplings were purchased from Random Harvest Indigenous Plant Nursery situated in Muldersdrift, Gauteng, South Africa (26° 01' 49" S; 27° 53' 38" E). Both seedlings and saplings were sown from samarae (seeds) in greenhouse conditions by their respective nurseries. The seedlings were ± 1.5 years old and the saplings were ± 4 years old from the date of purchase (August 2016). Seedlings were sown in 10 liter bags and saplings were sown in 20 liter bags. Seedlings were planted in sandy soil while saplings were planted in potting soil covered with wood chip mulch. For this study, seedlings were defined as *T. sericea* plants with a height < 1 m and saplings were defined as *T. sericea* plants with a height > 1 m (Table 1).

Table 1. Mean ± S.E. plant height and basal stem diameter of *Terminalia sericea* seedlings and saplings in the sun and shade at the start of the experimental trial.

Light Condition	Plant Stage	Height (m)	Stem Diameter (cm)
Sun	*Seedling*	0.57 ± 0.04	0.98 ± 0.08
	Sapling	1.07 ± 0.09	1.44 ± 0.03
Shade	*Seedling*	0.54 ± 0.06	0.94 ± 0.07
	Sapling	1.05 ± 0.05	1.23 ± 0.05

Plants were left in direct sunlight from August 2016 until 15 October 2016 when 9 seedlings and 10 saplings were moved to the shade (80%) after plants started producing new leaves. Plants were left to acclimate for 2 weeks (16 days) before the experiment started. Sun plants were left in direct

sunlight (photosynthetic photon flux density (PPFD): 1800 µmol m^{-2} s^{-1}) while shade plants were placed under 80% shade cloth (PPFD: 400 µmol m^{-2} s^{-1}) for the duration of the experiment. Both sun and shade plants were watered daily by sprinklers from 08h00 to 08h10 and then again from 16h00 to 16h10.

2.2.4. Experimental design and protocol

2.2.4.1. Leaf turnover

This study used 19 seedlings and 20 saplings for the experiment equating to 39 plants. Nine seedlings and 10 saplings were randomly selected and placed under 80% shade cloth, and the remaining 10 seedlings and 10 saplings were left in full natural sunlight on the roof top of the Oppenheimer Building at the University of the Witwatersrand, Johannesburg, South Africa. On each plant, two pairs of leaf clusters with less than 20% leaf damage were selected (Figure 1).

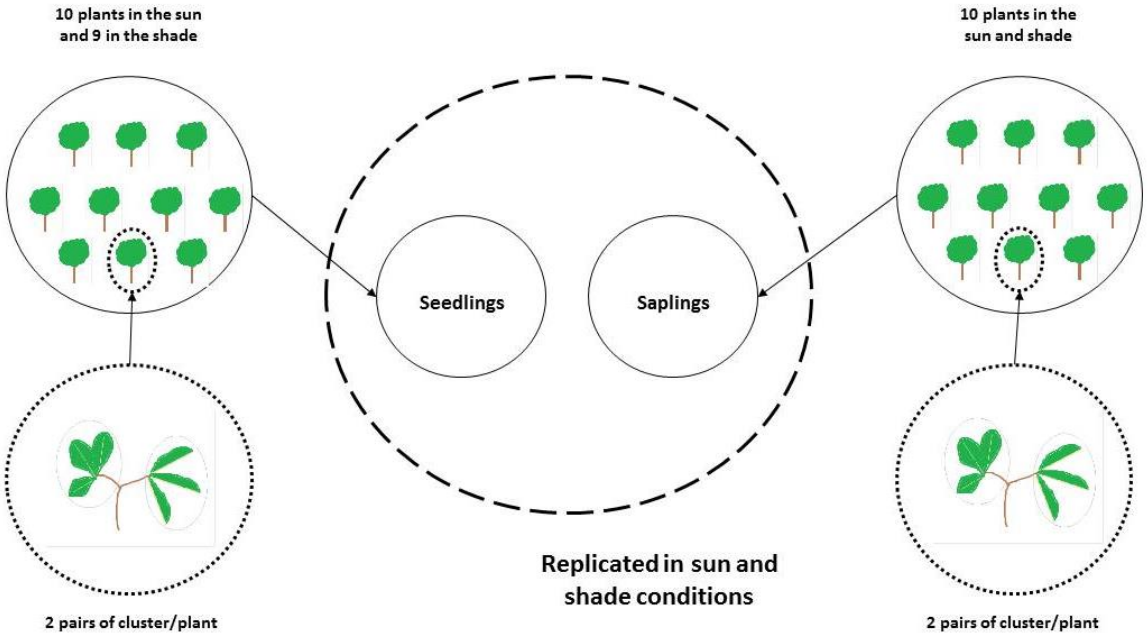

Figure 1. Study design and sample sizes for assessing the influence of herbivory, light availability and/or plant stage on *Terminalia sericea*.

For the simulation of herbivory two pairs of scissors were cleaned with 95% ethanol prior to performing the herbivory treatment and after each use, in order to prevent possible microbial contamination which can affect photosynthetic rates (Venter *et al.* 2013). Each leaf base was

numbered on the left side of the midrib with a permanent marker, numbering from the outside (old leaves) of the leaf cluster inwards (new leaves). Scissors were used to remove all the leaf area on the right hand side of the midrib equating to 50% herbivory. All control and herbivory treatment clusters were covered with organza bags to prevent further herbivory. Different coloured zip ties were used to mark control and herbivory treatment clusters. Each pair had one control and one herbivory treatment cluster adjacent to each other; this method (having the control and herbivory treatments on the same plant) is a well-used experimental design in entomology and biological control using insects (e.g. Cowie *et al.* 2018). Leaves were monitored on day 3, 51, 70, 92, 112, 133, 153 and 174 after herbivory (samples were recorded on a 3 week interval because of the high initial turnover rate). Leaf presence (remaining) and absence (shed) was recorded and new leaves were numbered accordingly. Leaf production and loss were determined by counting the number of leaves produced and lost and leaf longevity was determined by counting the number of days each leaf was present on the cluster. Compensatory leaf production was determined as the difference in leaf production between control and herbivory treatment leaf clusters. Leaves were defoliated in December 2016 (summer) when both seedlings and saplings had ~50% leaf canopy cover.

2.2.4.2. Phenology

Leaf phenology was assessed as the percentage of the plant canopy bearing each of four leaf age classes: new (<50% in size), fully expanded (>50% in size), mature (sun drenched) and senescent (chlorotic) leaves and the percentage of natural herbivory experience by the individual leaves, (Williams *et al.* 1997; Whitecross *et al.* 2016) which were estimated using the Walker aerial cover scale (1976) (Table 2). Leaf phenology was assessed monthly for a full growing season, from September 2016 until May 2017.

Table 2. Seven point percentage Walker (1976) aerial cover scale used to score phenological traits on the canopy.

Score	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Range (%)	0	1 – 10	10 – 25	25 – 50	50 – 75	75 – 90	90 – 99	100

2.2.4.3. Data analysis

Owing to different growing conditions, the data for seedlings and saplings were analysed separately. The R package *ggplot2* (Wickham 2009) was used to produce graphical representations of the data in the statistical program R (version 3.4.2, R Core Team 2017). R was used to test the data for normality by using the Shapiro-Wilks normality test. Linear mixed effects models fit by maximum likelihood were used to determine the effect of light and herbivory on leaf production and loss across the study period using the R package *nlme* (Pinheiro *et al.* 2017); the best model was selected using Akaike's information criteria (AIC), where the difference between the AIC value of the best model was < 2 the model was considered to be well supported. Kaplan-Meier survival curves and log-rank tests were used to determine the effect of herbivory on leaf longevity in seedlings and saplings in the sun and shade. Relationships between leaf longevity and flush date (production date) in sun and shade seedlings and saplings were determined with linear regression analyses. Linear mixed effects models fit by maximum likelihood were also used to determine the effects of light and month on leaf phenology in seedlings and saplings.

2.3. Results

2.3.1. Leaf turnover

A linear mixed effects model fit (LME) by maximum likelihood showed that simulated herbivory at the cluster level did not have a significant effect on leaf production in seedlings and saplings (Table 3). Seedlings in the sun produced more new leaves than seedlings in the shade (Figure 2a,b), while saplings in the shade produced more leaves than saplings in the sun (Figure 2c,d). There were two leaf flushes in herbivory treatment leaves in seedlings and saplings in the sun and shade on week4 (W4), W10 and W13. By the end of the season there were no new leaves produced by seedlings and saplings. In seedlings, leaf production seized 92 days (3 months) post herbivory and 133 days (~4.5 months) post herbivory in saplings, which were also influenced by reductions in day-length and temperature towards the end of the growing season.

In both seedlings and saplings, herbivory had no overall effect on leaf loss and was thus removed from the models (Table 3). Leaf loss fluctuated across the growing season in seedlings, peaking in W7, W13, W19 and W22, however shading did not have an effect on leaf loss (Figure 3a,b). In saplings in the sun leaf loss peaked in W10, and showed two prominent peaks (W13 and W22) in

saplings in the shade (Figure 3c,d). Shading had a significant effect on leaf loss in saplings, with saplings in the sun losing more leaves faster than saplings in the shade.

Leaves not exposed to simulated herbivory had a longer leaf longevity than herbivory treatment leaf clusters in seedlings in the sun (log-rank test: $\chi^2 = 8$, df = 1, p = 0.0048) and shade (log-rank test: $\chi^2 = 4$, df = 1, p = 0.0042) (control sun: 100 ± 3 days; herbivory treatment sun: 84 ± 3 days; control shade: 112 ± 4 days; herbivory treatment shade: 94 ± 4 days) (Figure 4a,b). Herbivory did not have a significant effect on leaf longevity in saplings in the sun (log-rank test: $\chi^2 = 0.4$, df = 1, p = 0.534) and shade (log-rank test: $\chi^2 = 0$, df = 1, p = 0.919) (control sun: 66 ± 2 days; herbivory treatment sun: 67 ± 2 days; control shade: 83 ± 3 days; herbivory treatment shade: 85 ± 3 days) (Figure 4c,d).

Table 3. The outputs of linear mixed effects models for leaf production and loss in *Terminalia sericea* (a) seedlings and (b) saplings. Factor variables describe "Time" as the week number of each observation, "Herbivory" as leaf clusters left untouched and leaf clusters exposed to simulated herbivory and "Shade" as plants in full sunlight and 80% shade.

Plant stage	Turnover Trait	Factor Variable	t-value	SE	p-value	Significance
Seedling	Leaf production	Time	-11.645	0.022	<0.001	***
		Shade	2.908	0.335	0.0038	**
		Herbivory	-	-	>0.05	NS
		Time:Shade	-2.444	0.022	0.0148	*
	Leaf Loss	Time	6.537	0.150	<0.001	***
		Shade	1.747	1.719	0.0812	NS
		Herbivory	-	-	>0.05	NS
Sapling	Leaf production	Time	-8.327	0.031	<0.001	***
		Shade	-2.573	0.488	0.0103	*
		Herbivory	-	-	>0.05	NS
		Time:Shade	2.379	0.031	0.0196	*
	Leaf Loss	Time	6.022	0.172	<0.001	***
		Shade	1.995	1.279	0.0465	*
		Herbivory	-	-	>0.05	NS

- Indicate that the factor variable was excluded from the model because p > 0.05.
NS, not significant; *, p < 0.05; **, p < 0.01;***, p < 0.001.

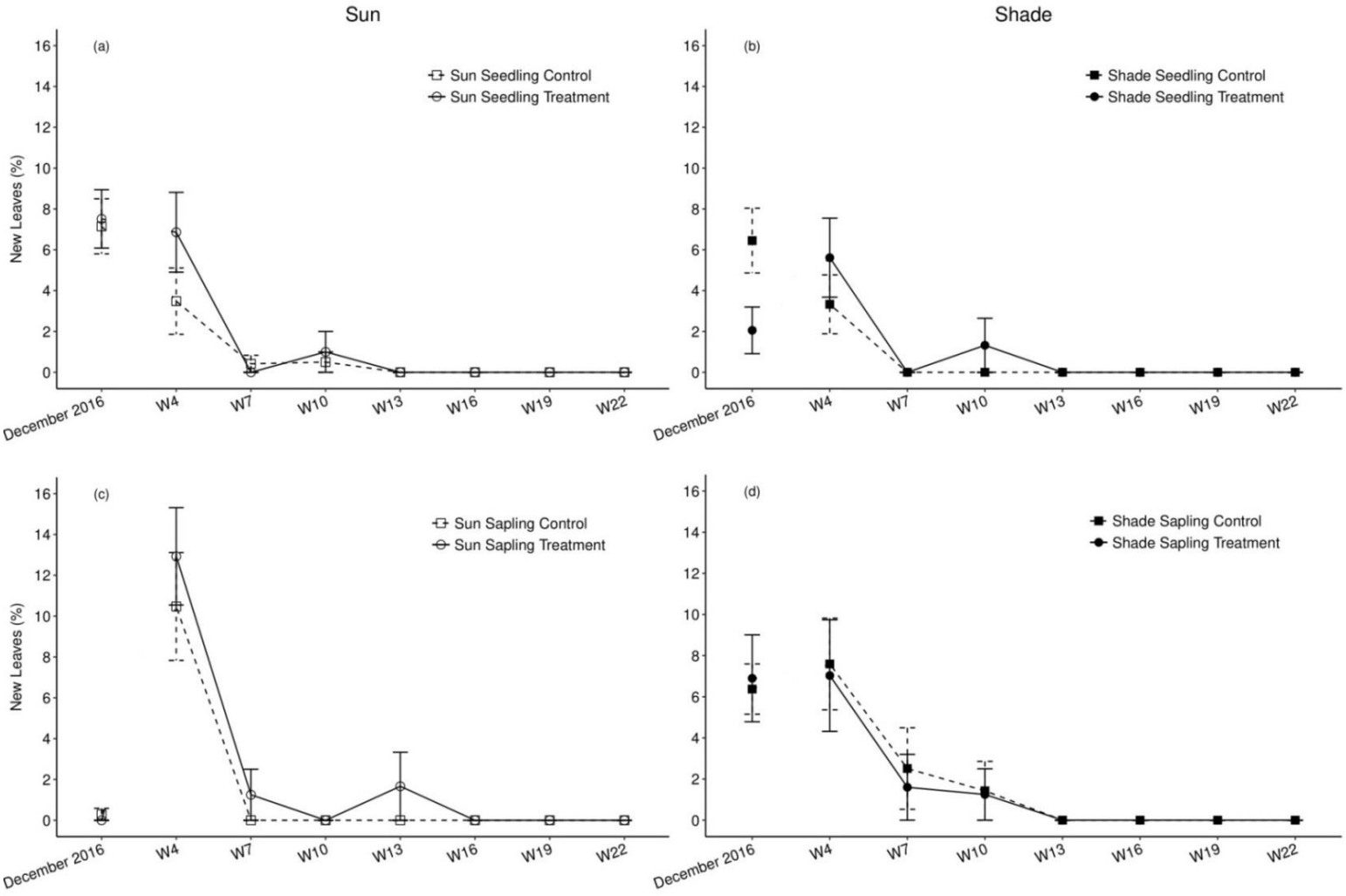

Figure 2. Percentages of new leaves produced (means ± SE) in *Terminalia sericea* control and herbivory treatment leaf clusters in seedlings in the sun (a) and shade (b) as well as saplings in the sun (c) and shade (d) over time from 9th December 2016 (start date) and then at three week intervals (January 2017-May 2017). On the x-axis is the time written in week number.

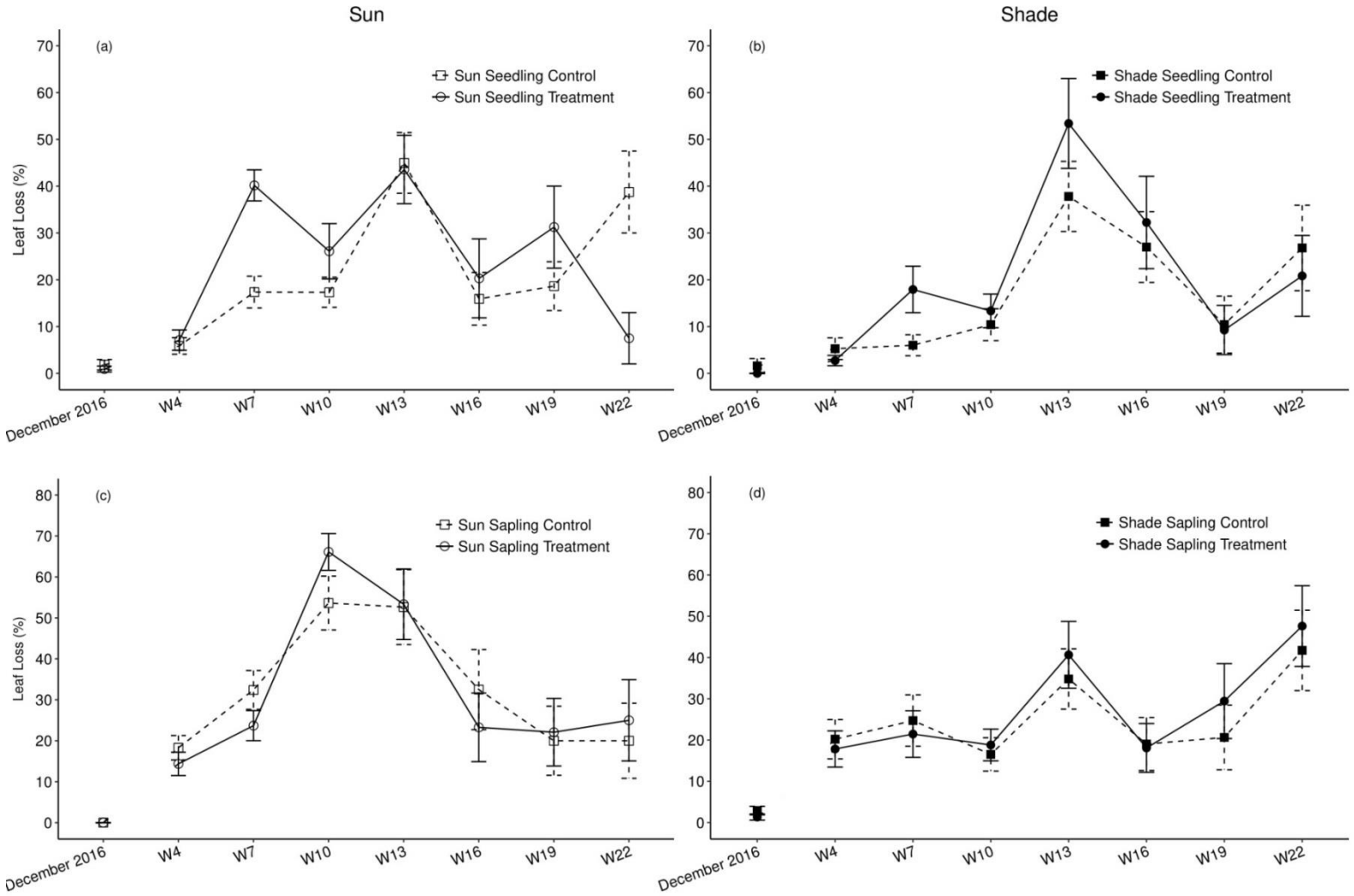

Figure 3. Percentages of leaves lost (means ± SE) in *Terminalia sericea* seedling and sapling control and herbivory treatment clusters in the sun and shade over time from 9th December 2016 (start date) and at three week intervals (January 2017-May 2017). On the x-axis is the time written in week number.

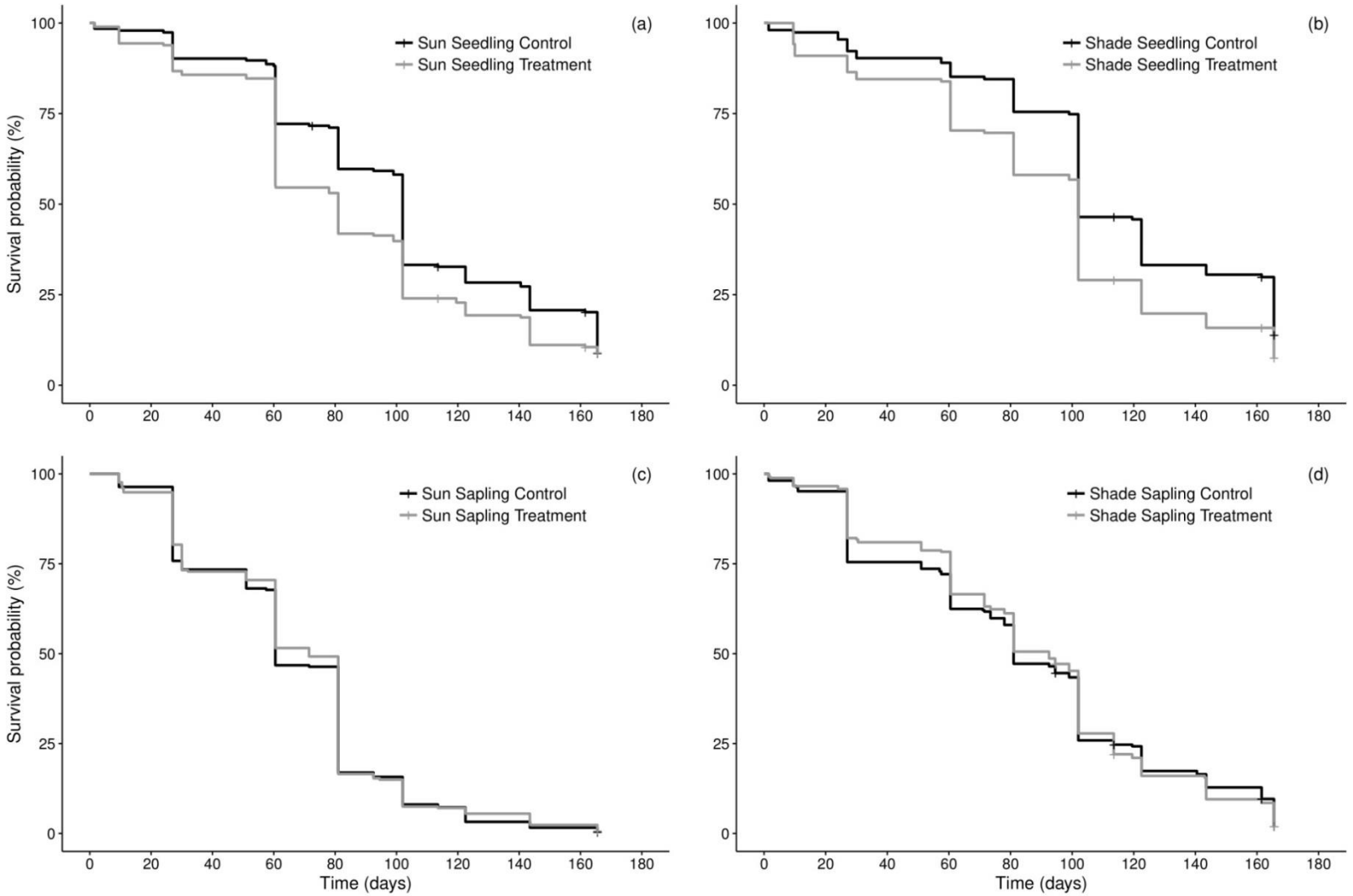

Figure 4. Kaplan-Meier estimates of the survival curve for control (—+—) and herbivory treatment leaves (—+—) in (a) sun seedlings, (b) shade seedlings, (c) sun saplings and (d) shade saplings. + Symbols indicate censored individuals (leaves whose total survival time cannot be accurately determined).

In seedlings and sun saplings, leaves produced earlier in the growing season had a longer leaf lifespan than leaves produced later in the growing season (Table 4) (Figure 5), indicating higher carbon gain for leaves produced earlier in the season than those produced later in the growing season. The relationship between leaf lifespan and flush date was not significant (relatively flat) in saplings in the shade (Table 4) (Figure 5b), suggesting that leaf lifespan was not influenced by the emergence date.

Table 4. Statistical outputs of linear regression analyses between leaf lifespan and emergence date in *Terminalia sericea* seedlings and saplings in the sun and shade.

Light Condition	Plant Stage	t-value	S.E.	Slope	r^2	p-value	significance
Sun	*Seedling*	-3.143	0.160	-0.504	0.025	0.002	**
	Sapling	-8.216	0.064	-0.523	0.121	<0.001	**
Shade	*Seedling*	-8.666	0.140	-1.216	0.197	<0.001	***
	Sapling	-0.502	0.077	-0.038	0.001	0.616	NS

NS, not significant; **, $p < 0.01$; ***, $p < 0.001$.

There was a decrease in leaf lifespan in all treatments with later leaf emergence. However this relationship was not significant in control leaves in seedlings in the sun and in saplings in the shade (Table 5).

Table 5. Statistical outputs of linear regression analyses between leaf lifespan and emergence date in *Terminalia sericea* seedlings and sapling in control and herbivory treatment leaf clusters in the sun and shade. Slope, coefficient of determination (r^2) and significance level (NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) shown.

Light Conditions	Plant Stage	Herbivory	t-value	S.E	Slope	r^2	Significance
Sun	*Seedling*	Control	-1.602	0.256	-0.282	0.0060	NS
		Treatment	-3.003	0.198	-0.596	0.0450	**
	Sapling	Control	-4.154	0.111	-0.459	0.0660	***
		Treatment	0.180	0.078	-0.571	0.1800	***
Shade	*Seedling*	Control	-3.013	0.334	-1.008	0.0560	**
		Treatment	-8.027	0.147	-1.177	0.3010	***
	Sapling	Control	-0.127	0.110	-0.014	0.0001	NS
		Treatment	-0.675	0.106	-0.071	0.0020	NS

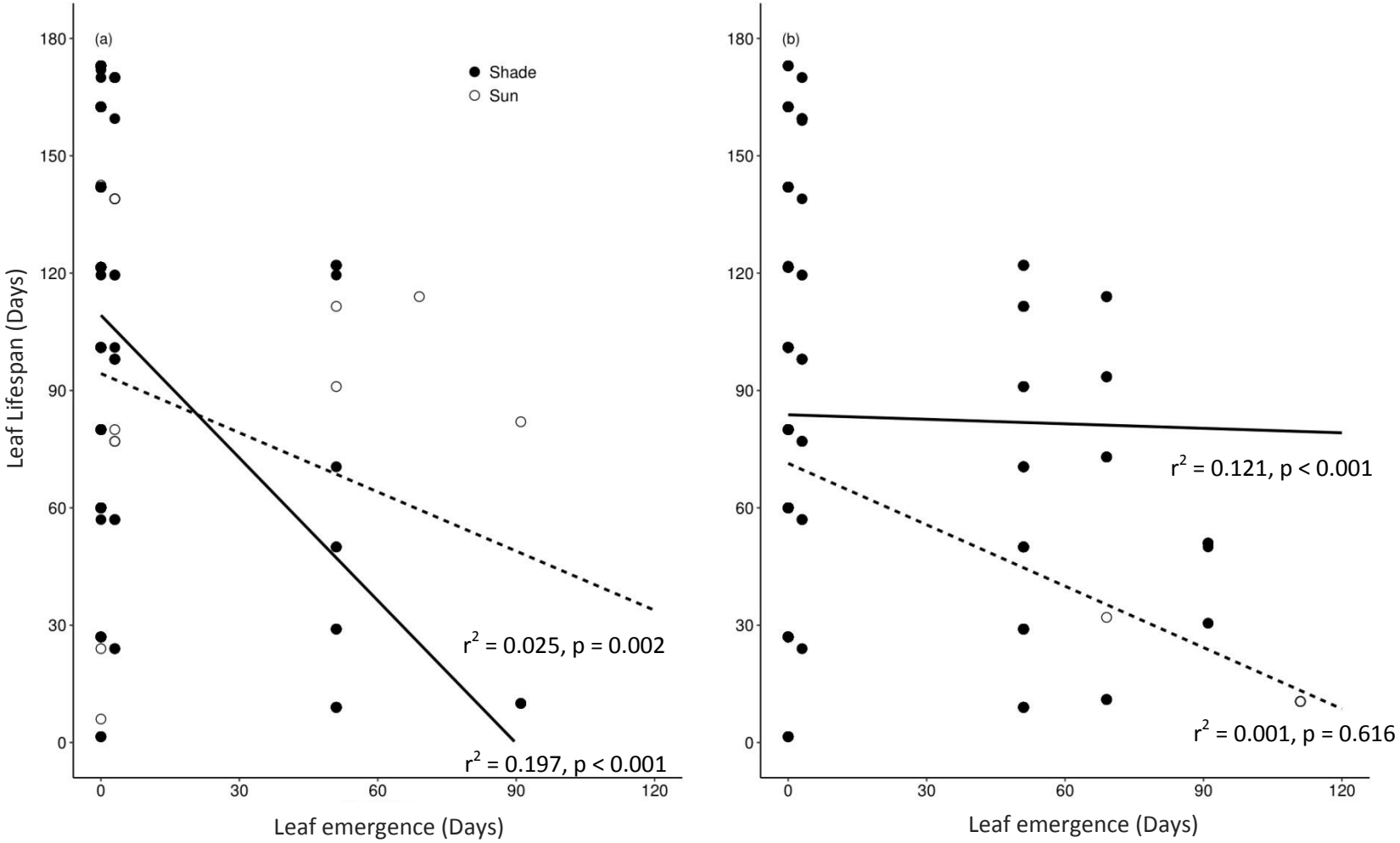

Figure 5. Relationships between leaf lifespan and leaf emergence date of *Terminalia sericea* seedlings and saplings in the sun (dashed line) and shade (solid line). Leaf emergence date starts from 3 December 2016 (day 0) to 28 March 2017 (day 120).

2.3.2. Phenology

Linear mixed effects models showed that leaves were aging as the growing season progressed (Figure 6a-i). New leaves were produced in the beginning of the season, which fully expanded, matured and senesced at the end of the season (Table 5) (Figure 6a-d, f-i). In seedlings, leaves quickly matured and senesced later in the season compared to saplings. There were two leaf flushes in saplings; these were observed in mid-spring (October) and early-autumn (March) (Figure 6f). Shading had a significant effect on the presence of new, fully expanded, mature and senescent leaf cover in seedlings (Table 5). In saplings, shading did not have a significant effect on the presence of new, mature and senescent leaf canopy cover (Table 5). The significant interaction of shading with sampling time indicates that light availability plays a significant role in leaf aging. Natural herbivory on the insectary rooftop was significantly lower in seedlings in the shade compared to seedlings in the sun (Figure 6e). Natural herbivory did not differ between saplings in the sun and shade (Figure 6j).

2.4. Discussion

2.4.1. Leaf turnover and longevity

There is variation in the pattern of leaf production among species (Hikosaka 2005). Deciduous shrubs and trees simultaneously produce their leaves at the beginning of the growing season with little to no new leaf production later (Kikuzawa 1983, 2003; Whitecross *et al.* 2016). Leaf production is divided into three categories; flush, succeeding and intermediate types (Kikuzawa 1983). Successive leaf flushing (i.e. multiple leaf flushes) was observed in both *T. sericea* seedlings and saplings as well as across both light treatments in herbivory treatment leaves with the first flush occurring at the beginning of the growing season and second flush in the middle (week 10-13 = March). The first flush lasted longer (> 5 weeks) than the second flush in seedlings and saplings across both light conditions, indicating that the first flush was a better carbon investment than the second flush (Schulze *et al.* 1977; Chabot and Hicks 1982; Falster *et al.* 2011). This flush of leaves in the herbivory treatment clusters compensates for leaf area lost to herbivory (McNaughton 1983).

Table 5. The outputs of linear mixed effects models for leaf phenology and canopy herbivory in *Terminalia sericea* seedlings and saplings. Factor variables describe "Time" as the week number of each observation and "Shade" as plants in full sunlight and 80% shade.

Plant stage	Phenophase	Factor variable	t-value	SE	p-value	Significance
Seedling	New	Time	-8.932	0.245	<0.001	***
		Shade	-7.706	1.485	<0.001	***
		Time:Shade	6.855	0.245	<0.001	***
	Fully expanded	Time	-3.396	0.243	<0.001	***
		Shade	-5.321	1.474	<0.001	***
		Time:Shade	5.031	0.243	<0.001	***
	Mature	Time	1.482	0.720	0.1406	NS
		Shade	-6.229	4.368	<0.001	***
		Time:Shade	6.883	0.720	<0.001	***
	Senescent	Time	12.976	0.539	<0.001	***
		Shade	5.687	3.284	<0.001	***
		Time:Shade	-6.931	0.539	<0.001	***
	Herbivory	Time	-	-	>0.05	NS
		Shade	5.877	0.736	<0.001	***
		Time:Shade	-	-	>0.05	NS
Sapling	New	Time	-4.974	0.563	<0.001	***
		Shade	-	-	>0.05	NS
		Time:Shade	-	-	>0.05	NS
	Fully expanded	Time	-2.732	0.329	0.0070	**
		Shade	-6.467	1.987	<0.001	***
		Time:Shade	7.642	0.329	<0.001	***
	Mature	Time	9.808	0.518	<0.001	***
		Shade	1.958	3.128	0.0521	NS
		Time:Shade	-2.499	0.518	0.0135	*
	Senescent	Time	5.979	0.440	<0.001	***
		Shade	1.571	2.663	0.1182	NS
		Time:Shade	-2.552	0.440	0.0117	*
	Herbivory	Time	1.187	0.422	0.2370	NS
		Shade	-	-	>0.05	NS
		Time:Shade	-	-	>0.05	NS

- Indicate that the factor variable was excluded from the model because $p > 0.05$.

NS, not significant; *, $p < 0.05$; **, $p < 0.01$;***, $p < 0.001$

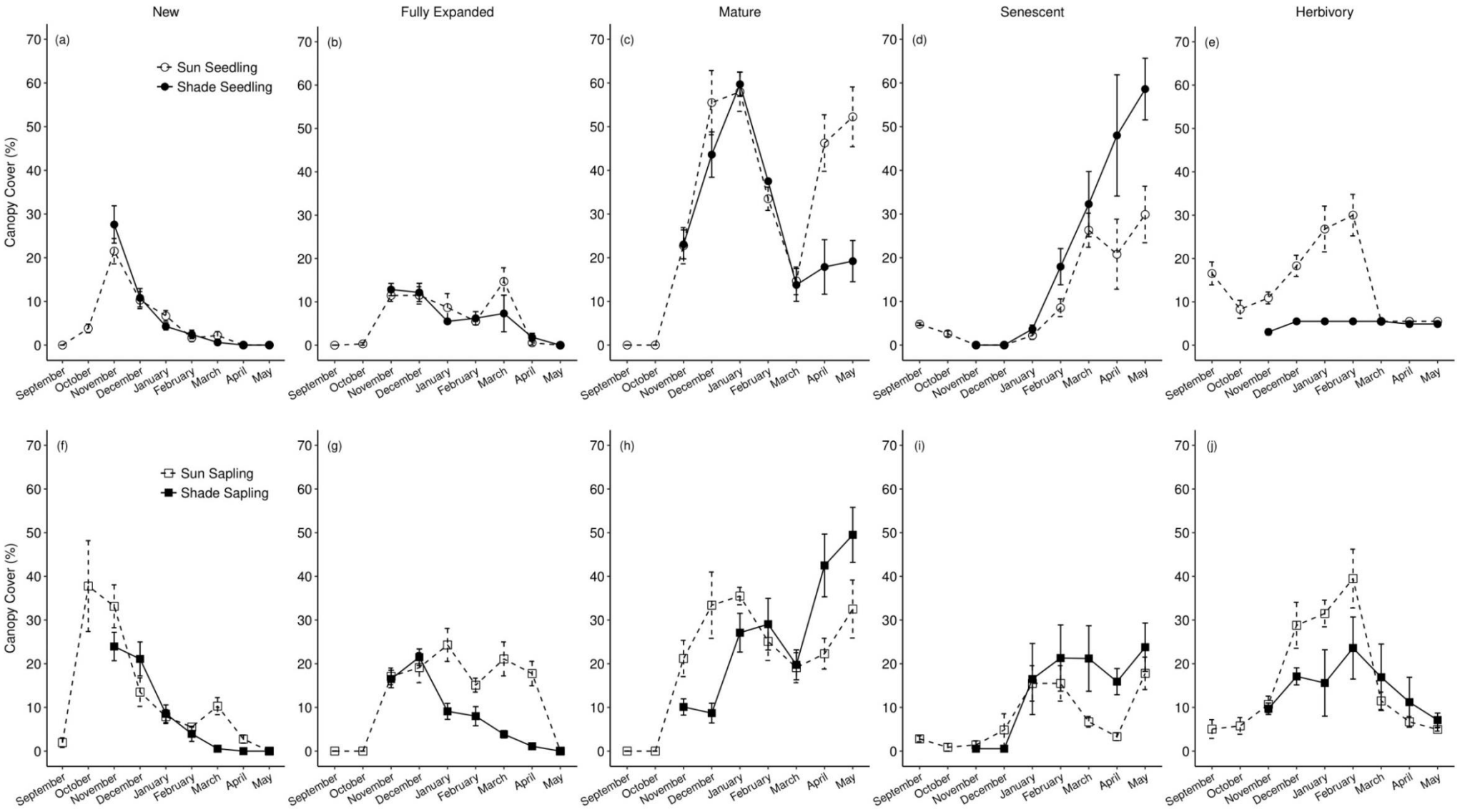

Figure 6. Leaf phenology (mean ± SE) of *Terminalia sericea* seedlings (⊖,●) and saplings (□,■), in the sun and shade, of new, fully expanded, mature and senescent leaves, and percentage of natural herbivory damage on the leaves in the canopy. Time is in months on the x-axis.

Both seedlings and saplings were continuously shedding their leaves, although this was less frequent in saplings. This strategy allows *T. sericea* to discard older damaged leaves and maintain a functional canopy. In addition, this strategy also minimises self-shading (Whitecross 2017), maximises carbon gain during the growing season and avoids the stressful conditions of the cold and dry season (Kikuzawa 1995; Sharma *et al.* 2012). The successive leaf flushing strategy may enable *T. sericea* to compensate for herbivory and utilise sunlight more efficiently.

Simulated herbivory did not have an effect on leaf production and loss. Therefore, indicating that *T. sericea* is able to completely compensate for simulated herbivory damage at the leaf cluster level in both plant stages (McNaughton 1983). The lack of effect of the herbivory treatment in both plant stages may be due to the study design, because control and herbivory treatment clusters were adjacent to each other. Both plant stages must have shunted resources between control and herbivory treatment leaves in order to compensate for herbivory damage. In addition to this, it is suggested that the removal of 50% of the leaf area, but not including the midrib vein, did not significantly affect leaf function of the remaining half of the leaf. Studies have shown that control and non-midrib vein insect and simulated herbivory defoliated leaves had a higher photosynthetic rate than leaves where the midrib vein had been damaged (Oleksyn *et al.* 1998; Aldea *et al.* 2005; Delaney and Higley 2006). Thus, it may be suggested that gaseous exchange and transpiration may have not been significantly altered between control and herbivory treatment leaves, therefore not significantly affecting leaf functionality, and in turn not affecting leaf production, loss and longevity in these leaf clusters.

The leaf production response of seedlings and saplings to shading differed, seedlings produced more new leaves in the sun and saplings produced new more leaves in the shade. This difference in leaf production response to light conditions may not be entirely due to irradiance limitation but may also be due to differences in soil types that the seedlings and saplings were grown in. Owing to saplings being grown in nutrient-rich soil and seedlings in nutrient-poor soil, this likely created a more conducive environment (i.e. high nutrient and water availability) for saplings to be able to construct new leaves to compensate for low irradiance in comparison to seedlings. This may suggest that both plant stages are able to compensate for shading, however nutrient and/or water availability plays an important role in the ability of *T. sericea* to respond to shading. Moyo *et al.* (2015a) conducted a study on *T. sericea* in the field and reported that both nutrient and water availability play an important role in *T. sericea* growth.

Leaf longevity was higher in control than herbivory leaves in seedlings, while herbivory did not have an effect on leaf longevity in saplings. Long leaf longevity is a strategy that allows plants to conserve nutrients, increase nutrient-use efficiency and balance carbon (Waring and Franklin 1979; Chapin 1980; Chabot and Hicks 1982; Mooney and Gulmon 1982; Harper 1989; Kikuzawa 1991; Reich *et al.* 1991; Witkowski *et al.* 1992; Ackerly 1999; Wright *et al.* 2004; Kikukzawa *et al.* 2013; Li *et al.* 2016). Longer leaf longevity is also an adaptive strategy utilized by plants in the understory (shaded) to gain access to light when the overstory leaves have fallen off (Harrington 1989). Short leaf longevity is a strategy for rapid carbon fixation (Chabot and Hicks 1982; Reich *et al.* 1991) and growth (Coley 1988; Reich *et al.* 1991; Ryser and Urbas 2000). These differences in leaf longevity strategies are very important because (1) they allow seedlings to maintain their leaves for longer while gaining carbon in the understory where photosynthetic income is low, this carbon can be utilised for leaf construction, stems and roots (Coley 1983) and for carbohydrate storage for the next growing season (Myers and Kitajima 2007), and (2) they allow saplings to rapidly grow so they can compete for light and other resources against taller more developed adult trees.

In addition, differences in leaf-longevity between seedlings and saplings can be attributed to two theories: (1) the optimal strategy in resource rich-environments which is associated with a rapid turnover of productive leaves which are short-lived as seen in the short leaf longevity of sapling and sun leaves, and the (2) cost-benefit theory which states that leaves should have a longer leaf longevity in more resource poor environments (Chabot and Hicks 1982; Williams *et al.* 1989; Kikuzawa 1991; Ackerly and Bazzaz 1995), as seen in the difference in soil types between seedlings (sandy soil) and saplings (potting soil covered with wood chip mulch), with the sapling soil type being more nutrient-rich and is associated with a higher water retention than the seedling soil type as well as in the shade environment (low light availability). This adaptation is used as a means to obtain more carbon from each individual leaf (Chabot and Hicks 1982; Kikuzawa 1995; Givinish 2002; Hikosaka 2005). In addition, leaves with longer leaf longevities use resources more efficiently than leaves with shorter leaf longevities (Chabot and Hicks 1982; Escudero and Mediavilla 2003). Therefore, indicating that the differences in leaf longevity between seedlings and saplings was not necessarily due to plant age, but was rather driven by nutrient availability.

2.4.3. Phenology

The presence of the different leaf phenophases differed as the growing season progressed, with new leaves being present at the beginning of the growing season, followed by fully expanded, mature and senescent leaves at the end of the season. This suggests that leaves were aging as the season progressed, which is a common characteristic of deciduous trees with new leaves being present at the beginning of the growing season and shed at the end of the season when conditions are no longer favourable (Kikuzawa 1983; Muthuri *et al.* 2009; Whitecross 2017).

Saplings produced more new leaves than seedlings throughout the growing season, with saplings producing leaves earlier than seedlings under full sunlight and seedlings producing more leaves than saplings in the shade. The ability of plants to produce new leaves has been strongly linked to plant stage, with resource acquisition and reserve storage being larger in older plant stages than younger plant stages (Strauss and Agrawal 1999; Kelly and Hanley 2005; Hanley and Fegan 2007), thus adult plants have more stored resources to produce more new leaves than their younger counterparts as seen in the high leaf production in saplings compared to seedlings. This trend was consistent in fully expanded leaves, with saplings having more fully expanded leaves than seedlings under full sunlight and in the shade. These two phenophases were lower in the shade compared to in the sun, indicating higher productivity in sun conditions compared to shaded habitats (Seiwa 1999). However, differences in phenophases cannot exclusively be linked to plant stage, as seedlings and saplings were planted in different soil types which were in different pot sizes. Therefore, differences between seedlings and saplings could also be due to differences in nutrient and moisture availability.

The early phenophases quickly matured in seedlings compared to saplings. Seedling canopies possessed a higher percentage canopy cover of mature leaves compared to saplings in both light conditions. This may be a strategy to maintain the leaves that were already produced in order to delay leaf loss and increase carbon gain, especially in this resource-poor environment where leaf construction may be too expensive. Seedlings tend to be more vulnerable to disturbances than their older counterparts because they have less stored reserves to allocate to defence and have softer leaves than mature plants, where losing one leaf can have more of an effect than in older plant stages (Eichhorn *et al.* 2010); this is more apparent in low-light environments. Mature leaves tend to have more chlorophyll content (Kamble *et al.* 2015; Whitecross 2017), thus they have a higher photosynthetic capacity making them an important phenophase for plant growth. In addition, mature leaves are also tougher (more lignin, thicker cuticle, more fibre and dry

mass) making them less susceptible to stresses such as herbivory and light stress (Coley and Aide 1991; Kursar and Coley 2003; Loney *et al.* 2006; Prado *et al.* 2014). As the leaves senesced seedlings retained most of their leaves later into the growing season than saplings, which may be a strategy to avoid shading from saplings and taller plants. Phenological avoidance of shade conditions by seedlings may be a means of increasing their carbon gain (Uemura 1994; Cleland *et al.* 2007). This strategy is commonly used by juvenile trees in order to maximize light capture enabling them to persist in shaded habitats such as tree understories during the growing season (Gill *et al.* 1998; Seiwa 1998; 1999). This is a useful strategy for *T. sericea* juveniles which can establish under the canopies of other (deciduous) woody species, and is particularly noted in the higher canopy cover *Burkea africana* savanna woodlands at Nylsvley, which are also dominated by *T. sericea* (Wilson and Witkowski 2003; Whitecross *et al.* 2016).

Natural herbivory was higher in seedlings in the sun compared to seedlings in the shade. These findings are consistent with those of numerous other studies (Lincoln and Mooney 1984; Salgado-Luarte and Gianoli 2010). Increased levels of herbivory in high irradiance environments may be due to light induced differences in defence traits, differences in herbivore abundances between the two environments (Collinge and Louda 1988; Salgado-Luarte and Gianoli 2010) or due to differences in leaf traits (Guerra *et al.* 2010). In addition to this, herbivory may have been higher in seedlings in the sun as they were left exposed, while seedlings in the shade where under the shade-netting mesh, that may have excluded some invertebrates approaching from above. This suggests that the mesh may have reduced herbivory access to seedlings in the shade. However, percentage herbivory damage on saplings was not significantly different between saplings in the sun and shade. As previously mentioned, *T. sericea* is able to compensate for herbivory by shedding old damaged leaves and replacing them with new foliage.

2.5. Conclusions

Terminalia sericea seedlings and saplings continuously turnover leaves during the growing season, with leaf flushes at the beginning and middle of the growing season, regardless of the light treatment. In seedlings and saplings, leaves aged as the growing season progressed, with leaves being produced, expanding, maturing and senescing at the end of the growing season which is characteristic of deciduous trees. Owing to the more shaded conditions where seedlings usually establish, seedlings utilised a phenological avoidance strategy to maximise light capture, by retaining their leaves well after leaf fall in the saplings. A change in phenology

is a compensatory mechanism that may be used by plants not only to avoid and/or reduce the negative effects of herbivory but also to tolerate stress factors such as light and water limitations as well as herbivory. Both plant stages showed complete-compensatory growth after herbivory damage as seen in the similar leaf production rates between herbivory treatment and control leaves. However, this may be in part due to the experimental design because control and herbivory treatment leaf clusters were adjacent to each other. Future studies should: (1) plant seedlings and saplings in the same size pots and growth medium, (2) place herbivory treatments and controls on separate plants in order to observe whole plant effects and (3) a higher defoliation percentage should be used where at least 50% of the whole plant is defoliated (and/or to have a range of percentage herbivory treatments) in a similar manner as in this study in order to be able to track leaf longevity.

2.6. References

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Chapter 3: Effects of shading on allocation patterns and leaf traits of *Terminalia sericea* seedlings and saplings.

3.1. Abstract

This study analysed the growth response of *Terminalia sericea* seedlings and saplings to different light conditions, by measuring plant architecture, allocation patterns and leaf traits. Seedlings and saplings were placed in either full sunlight or under 80% shade treatments. From a previous experiment two leaf clusters on each plant were selected, where one cluster in each pair was exposed to cutting (removing 50% of the leaf area) and the adjacent cluster left untouched. Plant height and stem diameter were continuously measured over 7 months during the growing season (December 2016 – June 2017). Canopy area and volume were measured at the beginning and end of the study. At the end of the study, 50% of the seedlings and saplings in the sun and shade were cut, oven-dried and weighed to determine their dry mass and biomass allocation patterns. Leaves were stripped from the remaining plants and their leaf traits measured in order to determine leaf length, width, area, specific leaf area (SLA), leaf dry matter content and leaf thickness. Plant stage had a significant effect on plant architecture, biomass and leaf traits in sun and shade conditions with saplings being larger than seedlings. Plant architecture, stem dry mass and biomass, as well as shoot biomass were not influenced by shading. Seedlings and saplings in the sun and shade allocated most of their resources to roots, followed by shoots, stems and leaves. Shade leaves were larger, higher in leaf length, width, area and SLA and thinner than sun leaves. However, leaf dry matter content was not influenced by shading or plant stage. The strongest leaf trait correlations were between leaf length, width and area across all shading treatments and plant stages. Shading influenced the sign of the correlation between leaf traits with different shading treatments having opposite signs. Overall, these results emphasise the importance of plant stage (size) in the interrelationships between plant morphology, allocation patterns, and leaf traits, with light and shade tolerance.

Keywords: Biomass allocation, leaf traits, light, plant architecture, plant stage

3.2. Introduction

In African savannas fire, herbivory as well as nutrient and water availability are considered the main drivers of the biome (Scholes and Walker 1993; Scholes and Archer 1997), influencing plant distribution, establishment and development. Savanna tree species present adaptations to these drives such as: (1) fire: high sprouting ability, a thick bark, more carbohydrate reserves and large root:shoot ratio, (2) browsing: cage morphology, more chemical substances, greater occurrence of spines and thorns (Rooke *et al.* 2004). However, savanna tree species are also faced with light limitations, although not to the same extent as forest species, which create very shaded microclimates below their canopies. Thus, making it apparent that light competition in savannas is a complex matter. Owing to some degree of overlap of canopies between savanna trees, light is mostly a limiting factor to smaller plants such as grasses, forbs, juvenile trees and shrubs (Randle *et al.* 2018). Savanna plant species are impacted by light availability in different ways, for example some species are able to grow isolated while others grow associated with other species (Gignoux *et al.* 2016). Younger or juvenile plant stages are the most sensitive to resource limitations and environmental stresses (*reviewed by* Bond 2008; van Langevelde *et al.* 2011). Thus, strategies evolved in response to plant stressors such as light limitation should be apparent in the early stages of plant development.

Phenotypic plasticity is the ability of plants to alter their phenotype in response to a change in environmental conditions (Gratani 2014). Phenotypic plasticity is a sufficient way for sessile organisms, such as plants, to cope with environmental heterogeneity (Bradshaw 1965; Sultan 2000). Studies have shown that plants are plastic for numerous ecological traits, such as morphology, anatomy, physiology, breeding system patterns, reproductive and developmental timing, and offspring developmental patterns (Sultan 2000). The magnitude of plasticity may depend on plant organ or age (Watson *et al.* 1995). Plasticity can be a complex mechanism affecting leaves, meristems, stems and roots rather than the whole plant (Kroon *et al.* 2005). Phenotypic plasticity can vary depending on the type of environmental stress (e.g. herbivory) or the availability of a resource (light, nutrient and water availability; e.g. Witkowski *et al.* 1990).

Two adaptive strategies are used by plants when exposed to shading; they can either avoid or tolerate the stress (Gommers *et al.* 2013; Gong *et al.* 2015). Plants can avoid shading by maximising light capture, repositioning their leaves, moving them out of the shade using photoreceptor signaling networks, increasing stem length to escape shading (Vandenbussche *et al.* 2005; Franklin 2008; Casal 2012; 2013), or tolerate shading by optimising light capture and

utilisation efficiency, this is done by decreasing leaf thickness as well as increasing specific leaf area and chlorophyll content which contribute to carbon gain (Valladares and Niinemets 2008). The most common strategy used by savanna trees is tolerance because shading usually has a larger effect on juvenile plants while both shade resistance and tolerance are strategies used by forest species (Wu *et al.* 2017).

When competing for light savanna trees tend to have thick leaves (leaf mass:area ratio) (Cornelissen *et al.* 2003), increase assimilation rate to maximize growth rate (Taiz and Zeiger 2006), allocate their biomass to stems and leaves to increase height and compete for light, thus shading their neighbours (Gignoux *et al.* 2016). In addition, trunk shape is usually cylindrical to increase height at a low biomass cost. Plant growth rates are influenced by resource availability (water, nutrients and light), resources captured and efficiency of resource use. The amount of light captured and the efficiency of its use in trees is determined by leaf traits and crown characteristics (Poorter and Bonger 2006). The tree crown is composed of photosynthetic and structural organs which are vital in determining the amount of light captured, and the ability to shade neighbouring trees. Therefore, crown attributes such as architecture are commonly used to model tree growth. Tree crowns are also responsive to the spatial arrangements and sizes of neighbouring trees.

Canopy structure and biomass allocation have been postulated to be important plant traits when predicting plant growth. The partitioning of resources to seeds, roots, stems and leaves is of adaptive significance (Tilman 1988; Witkowski and Lamont 1996), because plants rotate between above- and below-ground resource acquisition (Bloom *et al.* 1985), as a means of attaining the most limiting resources (Tilman 1988; Chapin *et al.* 1993). Plants have a holistic response to stress with certain traits being correlated to each other.

Leaves are considered the most plastic plant organ (Dickison 2000). They are important plant organs for productivity due to their role in carbon assimilation (Wright *et al.* 2004; Xu *et al.* 2009), gaseous exchange and water loss through transpiration during photosynthesis, therefore playing an important role in carbon gain, particularly under shade conditions (Valladares and Niinemets 2008; Rodriguez *et al.* 2016). Leaf physiology and morphology usually reflect resource uptake and use efficiency strategies, thus these traits have an influence on plant growth rates (Reich *et al.* 1999). Environmental conditions affect the plasticity of leaf traits (Sisó *et al.* 2001; Pandey and Nagar 2002; Kessler and Sinha 2004; Barkoulas *et al.* 2007; Rodriquez and Kumari 2016), therefore leaf traits can provide a link between environmental conditions and leaf

function (Givinish 1987; Roche *et al.* 2004). However, variation in leaf trait patterns are influenced by intra- and interspecific variation, water, nutrient and light availability and wind velocity (Aguiar *et al.* 2002).

Terminalia sericea is an indigenous tree species which grows in woodland savannas across sub-Saharan Africa and has been found to grow in areas where it is shaded by larger neighbouring tree species such as *Burkea africana* (T. Twala *pers. obs*.). In this chapter the plant architecture, biomass allocation patterns and leaf traits in different light environments are explored, to simulate savannas with low or high tree densities. Linking these measurable parameters will allow us to understand tree growth and structure strategies used by juveniles of this savanna tree species to respond to variable light conditions below the understory of other savanna tree species or when covered by large herbaceous species. The aim of the study was to investigate the effects of shading on plant architecture, allocation patterns and leaf traits in seedlings and saplings of *T. sericea*. The two objectives were to (1) determine the effects of shading on plant architecture and allocation patterns and (2) to compare leaf traits under different light conditions.

3.2. Materials and methods

3.2.1. Study site

The experiment was conducted between November 2016 and May 2017 on the rooftop of the Oppenheimer Life Sciences Building, at the University of the Witwatersrand, Johannesburg, South Africa (26 ° 11’ 30’’ S; 28° 01’ 58’’ E). The site is subject to a subtropical highland climate with cool summer nights and hot summer days with occasional afternoon thundershowers, as well as experiencing dry winter days and cold winter nights.

3.2.2. Study species

The selected study species was *Terminalia sericea* Burch ex DC (Combretaceae), a medium sized deciduous tree species with a height range of between 4 to 12 m, but some individuals have been known to grow up to 20 m (Coates-Palgrave 1957). The silver leaves (due to trichomes) are borne in clusters (Grant 2005), where new leaves are borne in the centre of the leaf cluster (Grant 1984; Kikuzawa 1995). The leaves are present most of the year dropping in autumn, while flowers are present in spring and replaced by fruit mid-summer (Grant 1984; Kikuzawa 1995). *Terminalia sericea* is browsed by moth species such as *Euproctis fasciata* and *Arcyophora carniola* (Gandar 1982), as well as vertebrates such as impala and elephants (Coates-Palgrave

1957). It plays a vital role in the diet of livestock during the winter months (Katjiua and Ward 2006).

3.2.3. Plant specifics and maintenance

Nineteen seedlings were purchased from The Aloe Farm in Hartebeesport dam, North West, South Africa (25° 43' 15" S; 27° 47' 9" E), while twenty saplings were purchased from Random Harvest Indigenous Plant Nursery in Muldersdrift, Gauteng, South Africa (26° 01' 49'' S; 27° 53' 38'E'). All the seedlings and saplings were sown from samarae (seed) under greenhouse conditions by their respective nursery. Upon purchase in August 2016, seedlings were $\geq$ 1.5 years old and saplings were $\geq$ 4 years old. All plants were left in full sunlight (photosynthetic photon flux density (PPFD): 1800 µmol m^{-2} s^{-1}) from 30 August 2016 until 15 October 2016, where upon 9 seedlings and 10 saplings were moved under 80% shade cloth (PPFD: 400 µmol m^{-2} s^{-1}), and the remaining 20 plants were left in full sunlight. For this study, seedlings were defined as *T. sericea* plants with a height < 1 m and saplings were defined as *T. sericea* plants with a height > 1 m. All the plants were left to acclimate for two weeks prior to conducting the herbivory treatment experiments. All the plants were watered twice a day for 10 minutes, at 8h00 and at 16h00.

3.2.4. Experimental design and protocol

Ten seedling and saplings were placed in full sunlight and ten saplings and nine seedlings were placed under 80% shade (factorial design). Plant architecture, allocation patterns and leaf traits were measured on all the plants.

3.2.4.1. Plant architecture

In November 2016 and then monthly, a measuring tape was used to measure plant height (H_1) and stem diameter 10 cm and 30 cm above the ground for seedlings and saplings, respectively, this was done until May 2017. In November 2016 and May 2017 canopy height (H_2), trunk height (H_3), maximum canopy diameter (d_1) and canopy diameter at right angles to the maximum (d_2) were measured in order to obtain measures of total sapling growth. Canopy area and volume (Witkowski and Garner 2000) were determined by using the following equations:

Equation 1: Area (m^2) = $\pi \left(\frac{d_1}{2} \times \frac{d_2}{2}\right)$, and

Equation 2: Volume (m^3) = $\frac{4}{3} \pi \left(\frac{d_1}{2}\right)\left(\frac{d_2}{2}\right)\left(\frac{H_2}{2}\right)$.

3.2.4.2. Allocation patterns

In order to understand allocation patterns in *T. sericea,* 10 saplings in the sun and shade were randomly selected on the 31st of May 2017. Each plant was separated into four parts: leaves, stems, shoots (main stem) and roots; leaves, stems and shoots were stripped from the plant using cutters and placed in separate paper bags (Figure 1). The roots were obtained by cutting the black bags, removing the soil by hand until roots were visible and then rinsing them with distilled water before placing them into bags. The paper bags were placed in an oven at 60°C for 7 days to obtain oven-dried mass. Once oven-dried, all the plant parts were weighed on a four point balance to determine their dry weight.

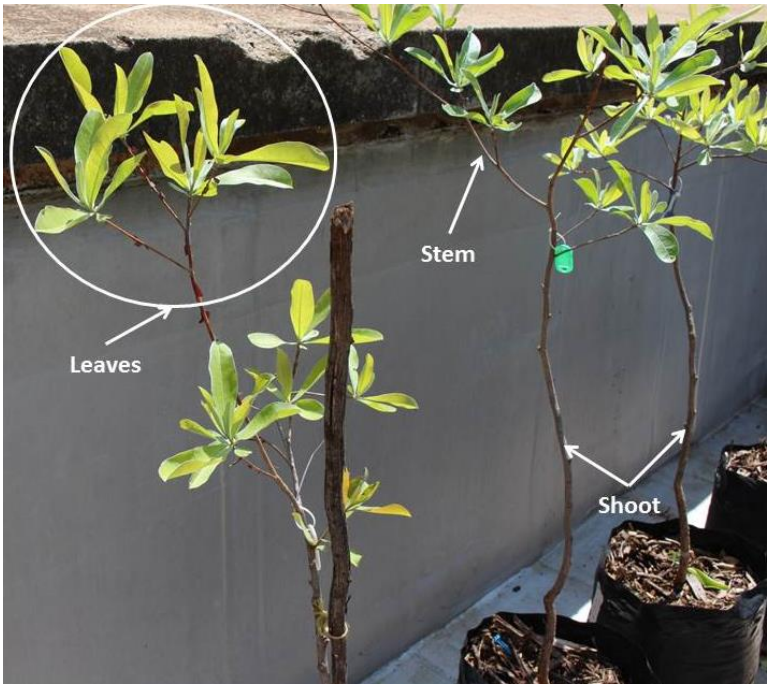

Figure 1. Plant parts measured to determined plant dry mass and allocation patterns.

3.2.4.3. Leaf traits

Ten saplings in both the sun and shade were randomly selected, on the remaining plant canopies (19 saplings) all the leaves were stripped and leaf length, maximum width and area were measured on the 1st of June 2017 after the leaves were on the canopy for ~8 months. Leaf thickness, specific leaf area (SLA) and leaf dry matter content (LDMC) were calculated based on fresh and dry mass of the collected leaves.

3.2.4.3.1. Leaf length, width and area

A permanent marker was used to number each leaf. Leaf length was defined as the distance between the leaf tip and base, and leaf width as the distance between the widest width of the

leaf; which can vary depending on leaf shape. Leaf length, width and area were measured for every leaf using a portable laser leaf area meter (LLAM) (CID Bio-Science, CI-202-ver. 3.06, 2009). This was done by scanning each leaf in the LLAM. Once a leaf was scanned the LLAM produced a measure of leaf length, width and area.

3.2.4.3.2. Specific leaf area, leaf dry matter content and leaf thickness

The same leaves used to determine leaf length, width and area were weighed to determine their fresh leaf mass after harvesting them. Leaf samples were then placed in brown paper bags and oven dried at 60°C for 3 days to obtain their oven-dried mass. The following equations were used to determine SLA, LDMC and leaf thickness which was estimated as a whole-leaf average thickness estimate adopted from Vile *et al.* (2005):

Equation 3: Specific leaf area ($m^2.kg^{-1}$) = $\frac{\text{Fresh Leaf Area } (m^2)}{\text{Dry Leaf Mass (kg)}}$,

Which is area of the fresh leaf per gram of dry mass,

Equation 4: Leaf dry matter content ($mg.g^{-1}$) = $\frac{\text{Dry Leaf Mass (mg)}}{\text{Fresh Leaf Mass (g)}}$,

Which is milligram dry mass per gram of fresh mass, and

Equation 5: Whole-leaf average thickness (µm) = $\frac{1}{\text{SLA } (m^2.kg^{-1}) \times \text{LDMC } (mg.g^{-1})}$,

Where the inverse of SLA and LDMC previously measured were used to determine leaf thickness.

3.2.4.3. Data analysis

Due to the difference in growing conditions, the data for seedlings and saplings were analysed separately. The R package *ggplot2* (Wickham 2009) was used to produce graphical representation of the data and statistical analyses were conducted in the statistical program R (R Core Team 2017, version 3.4.2). Shapiro Wilk tests were conducted to test if the data fit a normal distribution. In order to determine the effects of shading and time on plant height and stem diameter, the R package *nlme* was used to run linear mixed effects models (LME) fit by maximum likelihood (Pinheiro *et al.* 2017); the best model was selected using Akaike's information criteria (AIC). One-way repeated measures analysis of variance (RMANOVA) tests were used to determine the influence of shading on canopy area and volume over time. Differences between the treatments were tested using pairwise-t-tests. One-way ANOVA's were

run to determine the effect of shading on plant dry mass and allocation. The effect of shading on the leaf traits was analysed using generalised linear models (GLM) from the R package *MASS* (Venables and Ripley 2002). Spearman's rank correlation analysis was used to compare leaf traits between treatments.

3.3. Results

3.3.1. Plant architecture

Linear mixed-effects models fit by maximum likelihood showed that plant height was not influenced by shading in seedlings ($t = 0.609$, SE = 0.031, $p = 0.551$) (Table 1) (Figure 2a). However, shading had a significant effect on saplings, with saplings in the sun (1.16 ± 0.02 m) being taller than saplings in the shade (1.08 ± 0.01 m) ($t = 2.209$, SE = 0.018, $p = 0.040$) (Figure 2b). In both seedlings and saplings, sampling time had no overall effect on plant height and thus was excluded from the model ($p > 0.05$), indicating that plant height did not significantly change across the study period. From December 2016 to June 2017 seedling stem diameter decreased by 2.90% ($t = 9.507$, SE = 0.016, $p < 0.001$) and sapling stem diameter increased by 2.61% ($t = 10.789$, SE = 0.013, $p < 0.001$) (Figure 2c,d). Shading did not have a significant effect on seedling stem diameter, therefore it was removed from the model ($p > 0.05$). Saplings in the sun had a larger stem diameter compared to saplings in the shade, with a mean difference of 0.178 cm ($t = 3.301$, SE = 0.028, $p = 0.005$).

Table 1. Comparison between the best linear mixed effects models fit by maximum likelihood to describe plant height and stem diameter in relation to shading (Shade) and sampling dates (Time) in seedlings and saplings. The inclusion of a factor variable in the model is indicated by "+".

Plant Stage	Measurement	Model	Intercept	Shade	Time	Shade:Time	AICc	ΔAICc	Weight
Seedling									
	Height	1	0.552				-348.7	0.00	0.688
		2*	0.551	+			-347.1	1.63	0.305
		3	0.552		+		-338.6	10.05	0.005
	Stem Diameter	1*	1.093		+		-233.9	0.00	0.699
		2	1.091	+	+		-232.2	1.70	0.299
		3	1.091	+	+	+	-222.4	11.58	0.002
Sapling									
	Height	1*	1.116	+			-192.2	0.00	0.465
		2	1.116	+	+		-191.3	0.87	0.302
		3	1.116				-189.8	2.42	0.139
	Stem Diameter	1*	1.450	+	+		-324.4	0.00	0.951
		2	1.450		+		-317.6	6.81	0.032
		3	1.450	+	+	+	-316.4	7.99	0.018

* Model selected

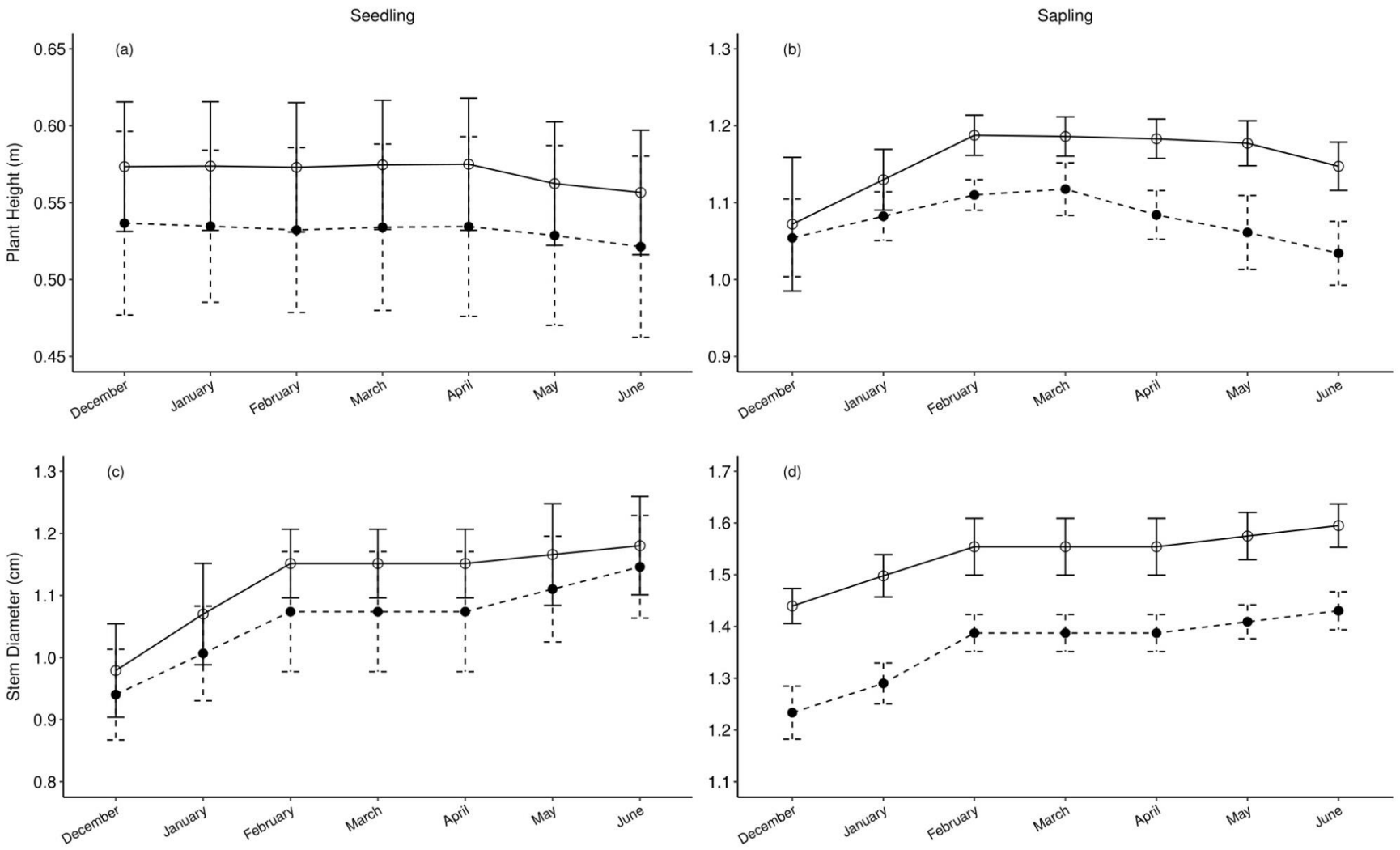

Figure 2. Mean ± SE (a, b) plant height and (c,d) stem diameter of sun (-○-) and shade (-●-) *Terminalia sericea* seedlings and saplings over time (months).

Change in canopy architecture in the growing season was measured by comparing the canopy area and volume of seedlings and saplings over time. There was no significant change in canopy area and volume from December 2016 (summer) to June 2017 (winter) (Table 2) (Figure 3). Shading did not have a significant effect on canopy area and volume in seedlings or saplings.

Table 2. Statistical outputs of one-way repeated measures ANOVAs for canopy area and volume of *Terminalia sericea* seedlings and saplings between December 2016 and June 2017. Factor variables describe "Shade" as plants in full sunlight or 80% shade and "Time" as the month of each observation within one growing season.

Plant Stage	Plant Architecture	Factor variables	F-value	D.F.	p-value	Significance
Seedling	Canopy Area	Shade	0.111	1	0.741	NS
		Time	0.484	1	0.491	NS
		Shade:Time	0.235	1	0.631	NS
	Canopy Volume	Shade	0.007	1	0.935	NS
		Time	0.872	1	0.357	NS
		Shade:Time	0.198	1	0.659	NS
Sapling	Canopy Area	Shade	0.542	1	0.467	NS
		Time	1.839	1	0.184	NS
		Shade:Time	0.273	1	0.605	NS
	Canopy Volume	Shade	0.883	1	0.354	NS
		Time	0.544	1	0.466	NS
		Shade:Time	0.705	1	0.407	NS

NS, not significant

3.3.2. Plant dry mass and allocation patterns

3.3.2.1. Plant dry mass

Leaf, stem, shoot, root and total dry mass were not significantly different between seedlings in the sun and shade (one-way ANOVA: $p > 0.05$), but were significantly different between saplings in the sun and shade (one-way ANOVA: $p < 0.05$) (Table 3). Leaf dry mass was ~6 times higher in saplings in the shade (12.10 ± 1.53 g) than in the sun (1.81 ± 0.49 g) (Figure 4A). Saplings in the sun had a greater stem, shoot, root and total dry mass than saplings in the shade with a percentage difference of 47.44%, 48.88%, 47.77% and 43.20%, respectively (Figure 4B-D).

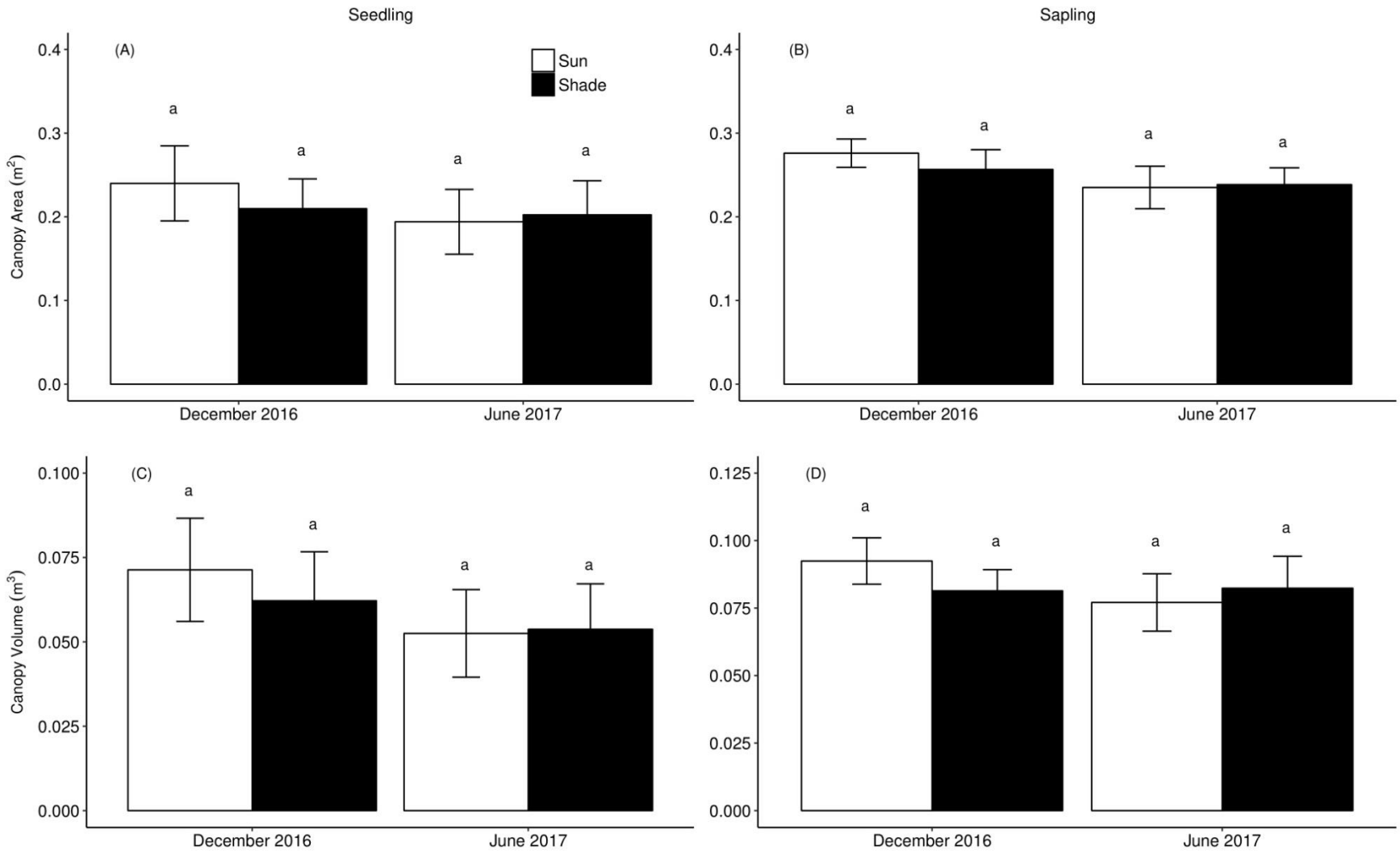

Figure 3. Mean ± SE (A,B) canopy area and (C,D) volume of *Terminalia sericea* seedlings and saplings in the sun and shade. Time is on the x-axis, for the first (December 2016) and final (June 2017) measurements. Lowercase letters indicate significant difference between each time interval within each treatment (one-way ANOVA: $p > 0.05$).

3.3.2.2. Plant allocation patterns

In seedlings, shading did not have a significant effect on leaf, stem and shoot allocation as well as the root:shoot ratio (one-way ANOVA: $p > 0.05$) (Table 3). Root allocation was higher in seedlings in the sun (4.21 ± 0.24 g) than seedlings in the shade (3.68 ± 0.67 g) (Figure 5D). Leaf allocation was higher in saplings in the shade than in the sun with a mean difference of 4.52% (one-way ANOVA: $F_{(1,9)} = 49.130$, $p < 0.001$) (Figure 5A). Stem, shoot and root allocation did not differ between sun and shade saplings (one-way ANOVA: $p > 0.05$) (Figure 5B-D). Sun and shade saplings had a similar root:shoot ratio (one-way ANOVA: $F_{(1,9)} = 0.009$, $p = 0.92600$) (Figure 6).

Table 3. Statistical outputs of one-way ANOVAs for plant dry mass of *Terminalia sericea* seedlings and sapling in the sun and shade.

Plant Stage	Plant Dry Mass	F-value	D.F.	p-value	Significance
Seedlings	Leaf mass	1.546	1	0.2490	NS
	Stem mass	0.387	1	0.5510	NS
	Shoot mass	0.096	1	0.7650	NS
	Root mass	1.975	1	0.1980	NS
	Total mass	0.723	1	0.4200	NS
	Leaf allocation	2.208	1	0.1790	NS
	Stem allocation	0.118	1	0.7400	NS
	Shoot allocation	0.159	1	0.7010	NS
	Root allocation	5.742	1	0.0434	*
	Root:Shoot	0.541	1	0.4830	NS
Saplings	Leaf mass	48.130	1	<0.001	***
	Stem mass	5.175	1	0.0490	*
	Shoot mass	15.320	1	0.0036	**
	Root mass	17.880	1	0.0022	**
	Total mass	18.640	1	0.0019	**
	Leaf allocation	49.130	1	<0.001	***
	Stem allocation	0.090	1	0.7710	NS
	Shoot allocation	0.937	1	0.3580	NS
	Root allocation	3.180	1	0.1080	NS
	Root:Shoot	0.009	1	0.9260	NS

NS, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

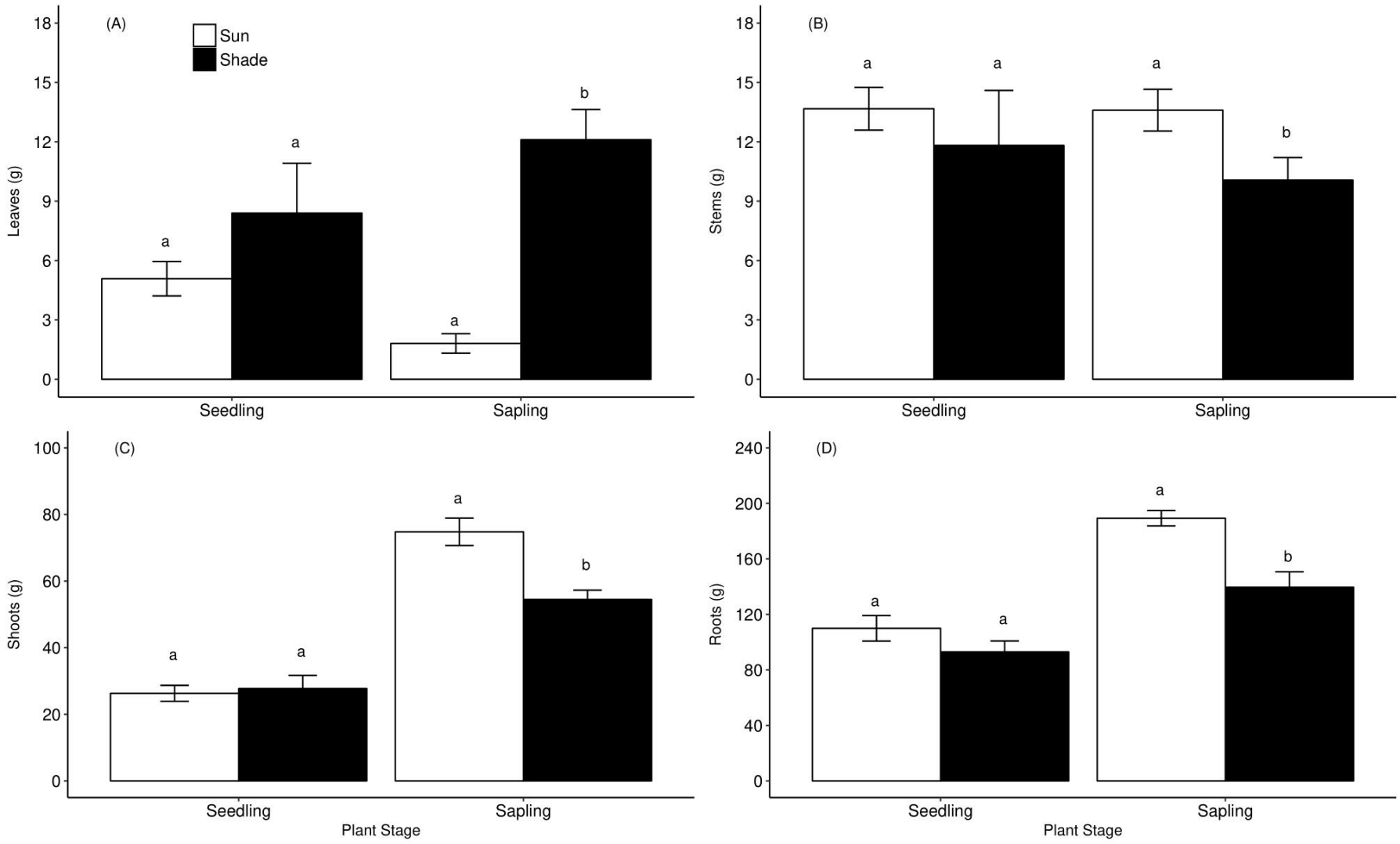

Figure 4. Mean ± SE (A) leaf, (B) stem, (C) shoot, and (D) root (mean ± SE) dry mass of sun and shade *Terminalia sericea* seedlings and saplings. Lowercase letters indicate differences within plant stages (one-way ANOVA: $p < 0.05$).

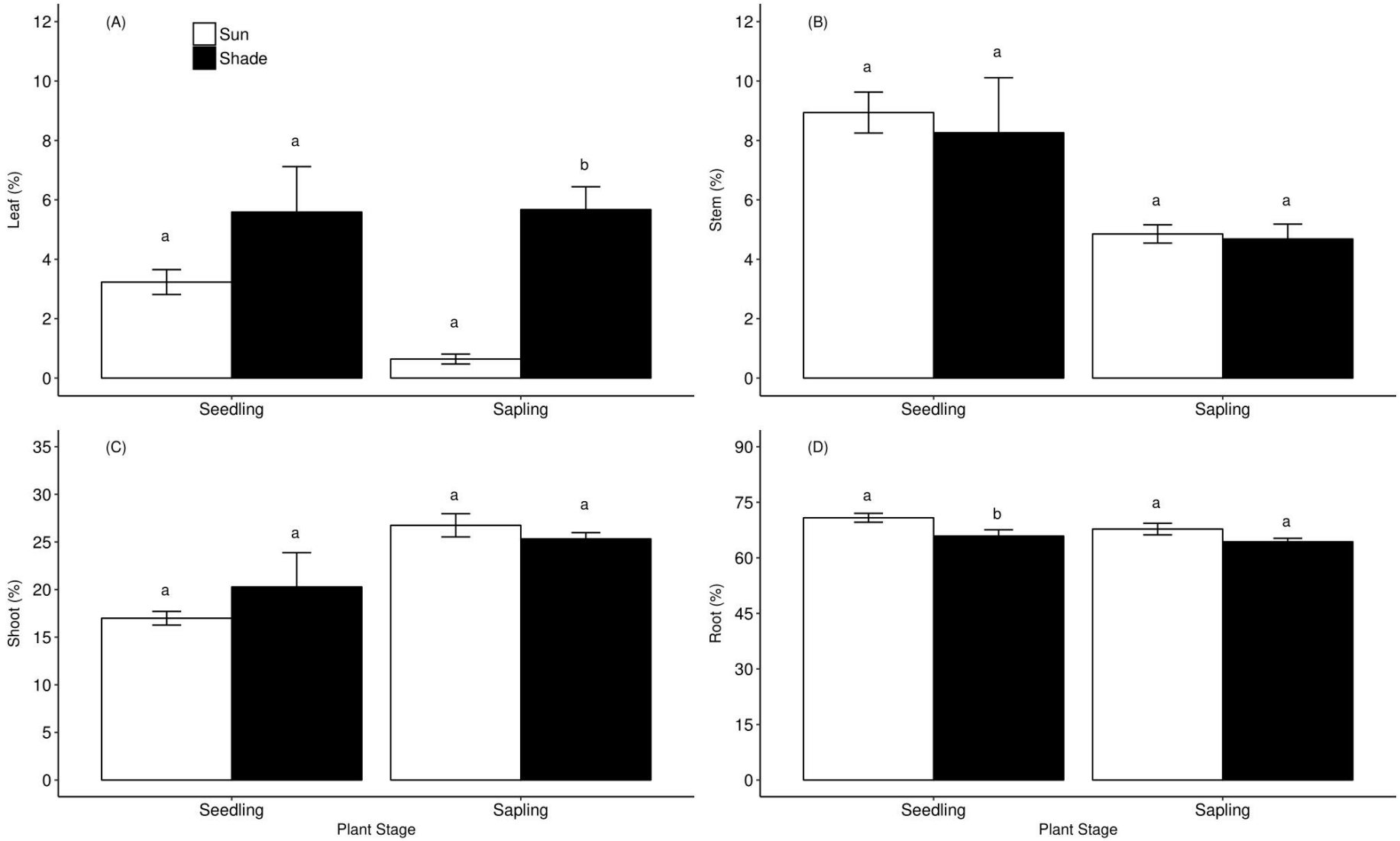

Figure 5. Mean ± SE (A) leaf, (B) stem, (C) shoot, and (D) root (mean ± SE) allocation patterns of sun and shade *Terminalia sericea* seedlings and saplings. Lowercase letters indicate differences within plant stages (one-way ANOVA: $p < 0.05$).

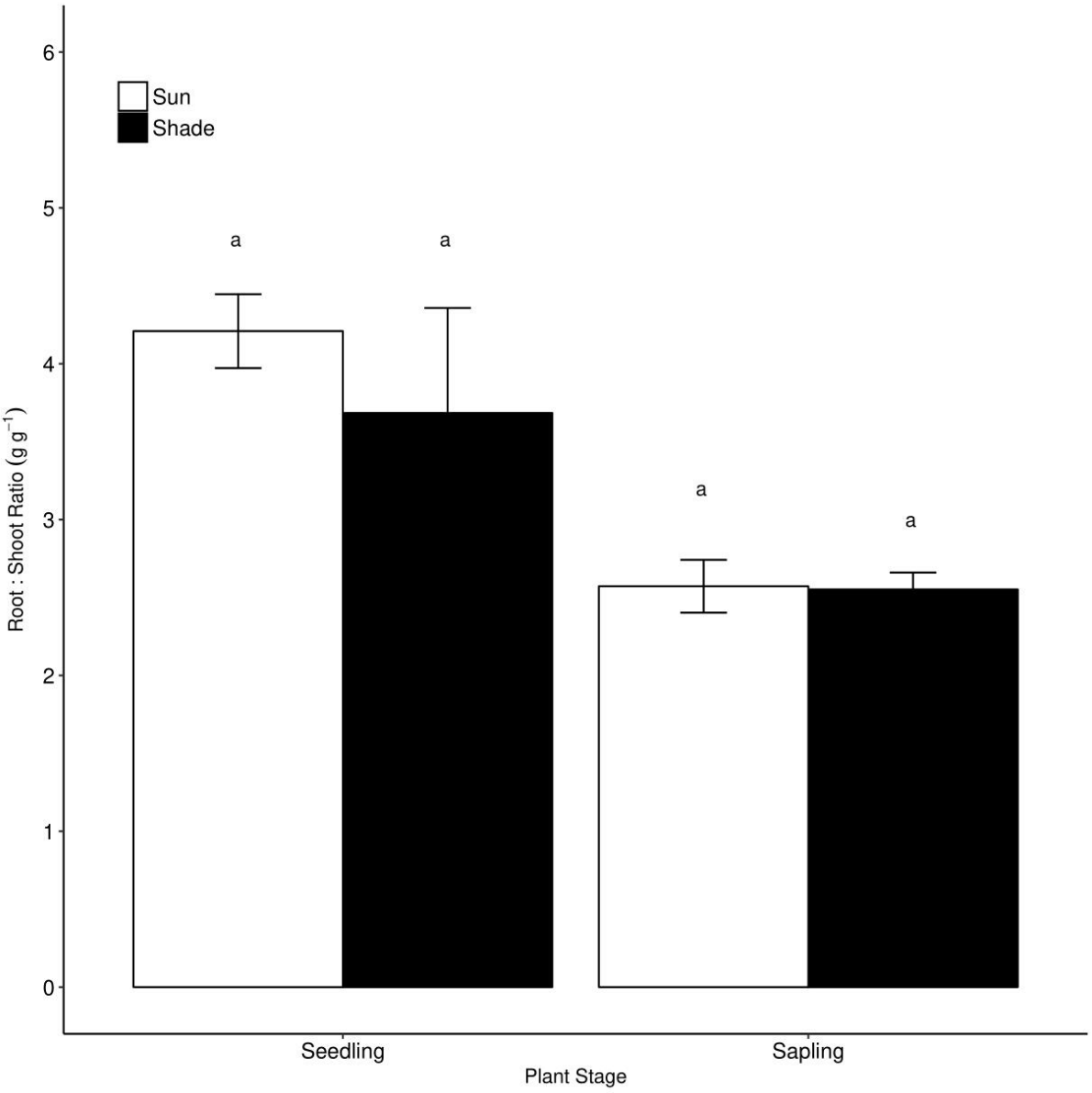

Figure 6. Root:shoot ratio (mean ± SE) of sun and shade seedlings and saplings. Lowercase letters indicate differences within plant stages (one-way ANOVA: $p < 0.05$).

3.3.3. Leaf traits

Seedlings

Leaves from seedlings in the shade were found to be 16.46% longer than from seedlings in the sun ($W = 4173$, $p = 0.0044$) (Figure 7A). Shade leaves (1.49 ± 0.06 cm) were wider than sun leaves (1.25 ± 0.03 cm) ($W = 4386.5$, $p = 0.0005$) (Figure 7B). Seedlings in the shade had a larger (37.76%) leaf area than seedlings in the sun ($W = 4245$, $p = 0.0021$) (Figure 7C). Leaves from seedlings in the sun had 32.36% thicker leaves than from seedlings in the shade ($W = 323$, $p < 0.001$) (Figure 7D). Specific leaf area (SLA) was larger in seedlings in the shade than seedlings

in the sun (mean difference: 3.00 $m^2.kg^{-1}$) (W = 6169, p < 0.001) (Figure 7E). Seedlings in the sun (492.93 ± 3.03 $mg.g^{-1}$) had leaves with a larger LDMC than seedlings in the shade (450.98 ± 4.74 $mg.g^{-1}$) (W = 935, p < 0.001) (Figure 7F).

Saplings

Shade leaves were 41.38% longer, 44.12% wider and had a 78.69% larger leaf area than sun leaves (p < 0.001) (Figure 7A-C). Sun leaves in saplings (0.0004 ± 0.00002 µm) were thicker than shade leaves (0.0003 ± 0.000004 µm) (W = 273, p < 0.001) (Figure 7D). Specific leaf area was higher in saplings in the shade than saplings in the sun with a mean difference of 1.49 $m^2.kg^{-1}$ (W = 1295, p < 0.001) (Figure 7E). Saplings in the sun (513.20 ± 19.13 $mg.g^{-1}$) had a larger LDMC than saplings in the shade (506.13 ± 39.92 $mg.g^{-1}$) (W = 292, p = 0.0025) (Figure 7F).

Overall, there was a consistent leaf trait pattern between seedlings and saplings. In both plant stages leaf length, width, area and SLA was higher in plants in the shade than plants in the sun, and leaf thickness and LDMC was higher in sunlit plants than plants in the shade, indicating that these leaf traits are highly influenced by light availability.

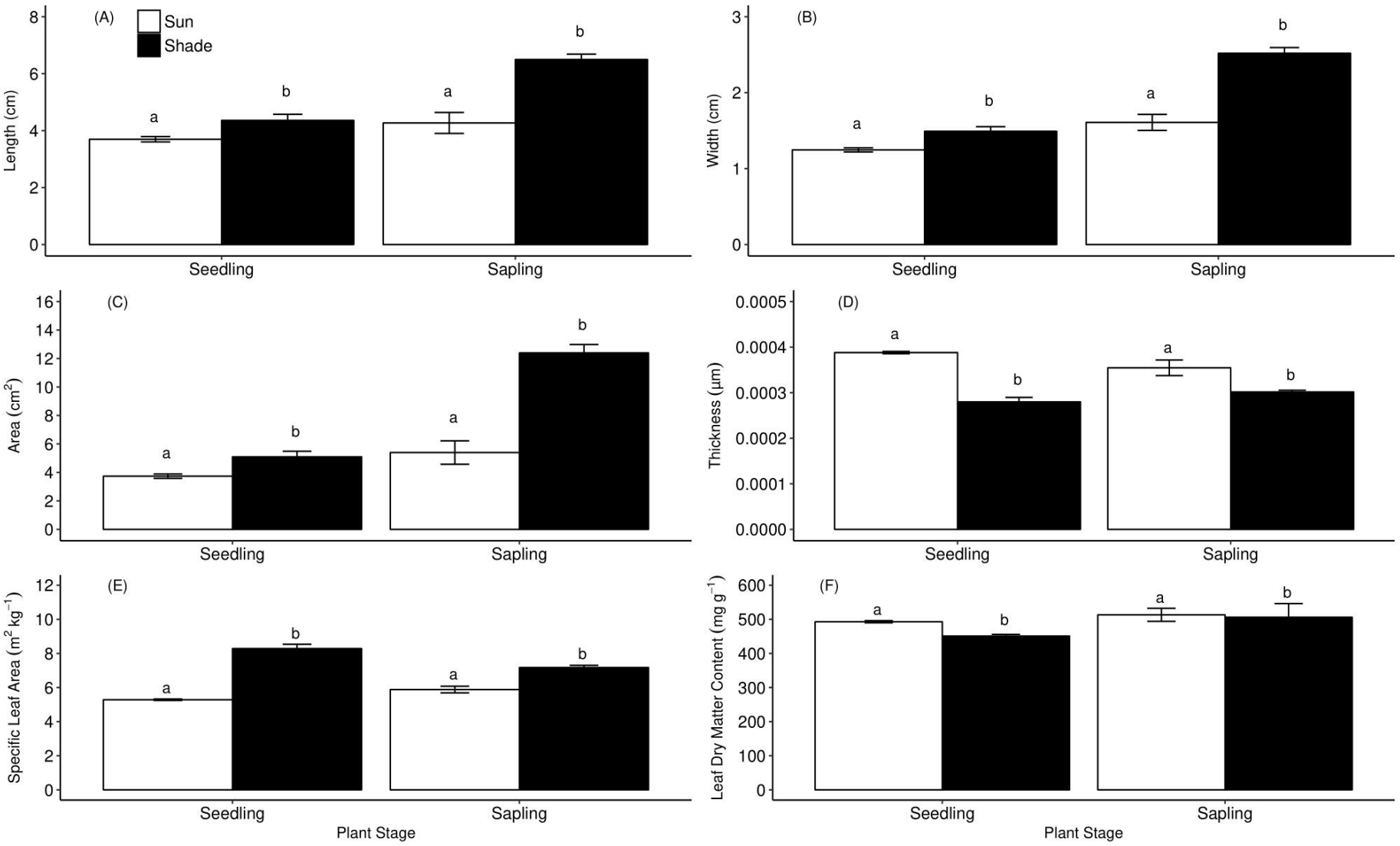

Figure 7. Leaf (A) length, (B) width, (C) area, (D) thickness, (E) specific leaf area, and (F) leaf dry matter content (means ± SE) of sun and shade *Terminalia sericea* seedlings and saplings at the end of the growing season. Lowercase letters indicate differences within plant stages (Wilcoxon sum rank test: $p < 0.05$).

3.3.4. Leaf trait correlations

A strong significant relationship was observed between leaf thickness and SLA in seedlings in the sun and shade, saplings in the sun and shade and for pooled seedling and sapling data (ρ = -0.710, ρ = -0.948, ρ = -0.886, ρ = -0.885, ρ = -0.860, ρ = -0.836, $P < 0.001$, respectively) (Table 4). There was a weak relationship between SLA and leaf length, width and area across all treatments; these relationships were positively correlated in plants in the sun and negatively correlated in plants in the shade. There was a weak correlation between leaf thickness and leaf length across all treatments with ρ values of -0.066, 0.408, 0.380, 0.452, -0.186 and 0.155 in seedlings in the sun and shade, saplings in the sun and shade and for pooled seedling and sapling data, respectively. There was a positive relationship between leaf thickness and area in plants in the shade ($\rho_{seedlings}$ = 0.386; $\rho_{saplings}$ = 0.427) and a negative relationship in plants in the sun ($\rho_{seedlings}$ = -0.980; $\rho_{saplings}$ = -0.104). Leaf length and width was strongly correlated in seedlings in the sun and shade, saplings in the shade and for pooled data (ρ ranged from 0.889 to 0.962), but there was a moderate relationship between these two leaf traits in saplings in the sun (ρ = 0.669, $P < 0.01$). Leaf width and area were significantly correlated (positive) across all treatments (ρ ranged from 0.840 to 0.977). In seedlings in the sun and shade, saplings in the sun and shade and for the pooled seedling and sapling data there was a highly significant relationship between leaf length and area (ρ = 0.992, ρ = 0.988, ρ = 0.932, ρ = 0.980, ρ = 0.989 and ρ = 0.983, $P < 0.001$, respectively) (Table 4); this was the strongest relationship across all leaf traits. The weakest relationships (negative) were observed between LDMC and the other leaf traits (ρ ranged from -0.006 to -0.586). Overall, strong significant correlations were observed between leaf length and width, leaf width and area as well as leaf length and area.

Table 4. Spearman rank correlation coefficient (ρ) between measured leaf traits in seedlings and saplings in the sun and shade and pooled measurements. LDMC, leaf dry matter content. SLA, specific leaf area.

Treatments	Leaf Traits	Variable	LDMC	SLA	Thickness	Width	Length
Seedling Sun	SLA	ρ	-0.407				
		P	***				
	Thickness	ρ	-0.289	-0.710			
		P	***	***			
	Width	ρ	-0.155	0.214	-0.149		
		P	NS	**	NS		
	Length	ρ	-0.212	0.183	-0.066	0.962	
		P	**	*	NS	***	
	Area	ρ	-0.187	0.191	-0.980	0.987	0.992
		P	*	*	NS	***	***
Seedling Shade	SLA	ρ	-0.486				
		P	**				
	Thickness	ρ	0.238	-0.948			
		P	NS	***			
	Width	ρ	-0.058	-0.229	0.277		
		P	NS	NS	NS		
	Length	ρ	0.082	-0.391	0.408	0.927	
		P	NS	**	**	***	
	Area	ρ	0.060	-0.366	0.386	0.949	0.988
		P	NS	*	*	***	***
Pooled Seedling	SLA	ρ	-0.586				
		P	***				
	Thickness	ρ	0.191	-0.860			
		P	**	***			
	Width	ρ	-0.264	0.324	-0.280		
		P	***	***	***		
	Length	ρ	-0.262	0.260	-0.186	0.948	
		P	***	***	**	***	
	Area	ρ	-0.256	0.282	-0.223	0.982	0.989
		P	***	***	**	***	***
Sapling Sun	SLA	ρ	-0.114				
		P	NS				
	Thickness	ρ	-0.254	-0.886			
		P	NS	***			
	Width	ρ	-0.145	-0.064	0.139		
		P	NS	NS	NS		
	Length	ρ	0.118	0.236	0.380	0.669	
		P	NS	NS	**	**	
	Area	ρ	-0.018	0.154	-0.104	0.840	0.932
		P	NS	NS	NS	***	

Table 2. Continued.

Treatments	Leaf Traits	Variable	LDMC	SLA	Thickness	Width	Length
Sapling Shade	SLA	*ρ*	-0.317				
		P	***				
	Thickness	*ρ*	-0.123	-0.885			
		P	NS	***			
	Width	*ρ*	-0.272	-0.271	0.389		
		P	**	**	***		
	Length	*ρ*	-0.209	-0.280	0.452	0.889	
		P	*	**	***	***	
	Area	*ρ*	-0.219	-0.315	0.427	0.940	0.980
		P	*	**	***	***	***
Pooled Sapling	SLA	*ρ*	-0.408				
		P	***				
	Thickness	*ρ*	-0.006	-0.836			
		P	NS	***			
	Width	*ρ*	-0.372	0.027	0.125		
		P	***	NS	NS		
	Length	*ρ*	-0.285	-0.333	0.155	0.909	
		P	**	NS	NS	***	
	Area	*ρ*	-0.314	-0.027	0.166	0.955	0.983
		P	***	NS	NS	***	***

P is the correlation significance (NS, not significant; $P > 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***).

3.4. Discussion

This study compared plant architecture parameters (plant height, stem diameter and canopy area and volume), dry mass and biomass allocation pattern and leaf traits across two light treatments (full sunlight and 80% shade) in *T. sericea* seedlings and saplings. Substantial differences were noted between the two shading treatments for stem diameter, leaf and root dry mass and allocation patterns as well as leaf traits.

3.4.1. Plant architecture

Shading did not have an effect on plant height, canopy area and volume. These patterns suggest that *T. sericea* growth (architecture) is not driven by light availability. Moyo *et al.* (2015) showed that *T. sericea* adult growth in the field was largely driven by nutrient and water availability, where different watering and nutrient addition levels influenced shoot production, diameter and length, as well as tree resprout ratio (the ratio between original stump diameter and resprout shoot diameter). Furthermore, the lack of difference between plant growth could be due to the slow growing nature of *T. sericea*. The higher stem diameter of plants in the sun compared to shaded plants could be a mechanism used by sunlit plants to increase water

acquisition and transportation across the plant. This is advantageous in such an environment where water loss (transpiration) is a commonplace.

3.4.2. Plant biomass

Allocation patterns showed a common trend in all plants; with roots > shoots > stems > leaves (Figure 5A-D). Regardless of light availability, allocation to below-ground mass and biomass was higher than that of leaf, stem and shoot mass. This suggests that under different light conditions *T. sericea* reallocates the majority of its resources to its roots at the end of the growing season. There may be two explanations for this: (1) because destructive sampling occurred at the end of the growing season, plants may have redirected resources from light interception for carbon assimilation that can aid in plant growth, to root storage in preparation for the dry and cold season when frosts, fires and herbivory commonly occur and conditions for growth are not optimal due to the long dry season; this may have been selected for after numerous severe frost events (Whitecross *et al.* 2012; Muller *et al.* 2016), frequent fires in order to protect resources from the reach of fires (Bond and Van Wilgen 1996; Higgins *et al.* 2000) and herbivory pressure when there is a lack of available foliage (Katjiua and Ward 2006), as well as (2) the optimal partitioning theory which suggests that plants allocate biomass that will aid in the acquisition of the most limiting factor at that particular time (Thornley 1972; Bloom *et al.* 1985), suggesting that light was not the only factor influencing plant dry mass and biomass allocation at that time. Therefore, it is suggested that nutrient and possibly water availability could have influenced partitioning to these plant organs. Nutrient and water availability have been considered as important drivers of plant growth in *T. sericea* adults at Wits Rural Facility (in the Lowveld), in a semi-arid savanna (Moyo *et al.* 2015). In addition, shading did not have a significant effect on stem and shoot mass and allocation; this indicates that these parameters are not driven by light availability in *T. sericea* juveniles. It is thus suggested that other environmental factors such as water and nutrient availability, herbivory and fire may be shaping these characteristics.

Shading did not have a significant effect on the root:shoot ratio. A high root:shoot ratio has been associated with nutrient- and/or water-stress, this relationship can be shifted by mild water stress (Khurana and Singh 2000). Hódar *et al.* (2008) reported that the addition of fertiliser had more of an effect on the root:shoot ratio than other biomass parameters (leaves and shoots) in *Pinus sylvestris* subsp. *nevalensis*. Also, according to McConnaughay and Coleman (1999) water-stressed plants can have a high root:shoot ratio investing more resources to

below-ground biomass in order to optimise their forging ability, thus supporting the optimal partitioning theory.

Overall, it is apparent that shading affects water absorption (roots) and loss (leaves), and does not affect water transport systems (stems and shoots). With plants in the sun investing more on roots (water absorption) than leaves in order to reduce water loss and shade plants investing more on leaf biomass than plants in the sun (maximize light interception). This strategy is vital for *T. sericea* which is a co-dominant species when it occurs in the midslope portions of a soil catena or a dominant species when it occurs in seasonally waterlogged soils at the base of the catena (Yeaton 1988), suggesting that water availability is the most important resource for *T. sericea* survival and growth (Moyo *et al.* 2015; Whitecross 2017).

3.4.3. Leaf traits and correlations

Several plant species have shown phenotypic variation to maintain plant performance under stressful environmental conditions. These modifications are common in light dependent species when growing in shaded environments (Markestejin *et al.* 2007). Shaded leaves were significantly larger (high leaf length, width, area and SLA) than sun leaves. This increase in leaf size has been implemented as a means of maximising light interception (Valladares *et al.* 2000; Baesse *et al.* 2014; Venâncio *et al.* 2016). There have been mixed reports on the effect of shading on leaf area, however, it has been reported that light intensity does have a significant effect on leaf area. Leaf area has been proportionally linked with the demand for light capture (Giertych *et al.* 2015), transpiration, photosynthesis, water balance, growth and biomass estimations (Westoby *et al.* 2002; Kucharik *et al.* 1998; Boeger and Gluzezak 2006; Gross *et al.* 2007). Numerous studies have supported my results showing that leaf area decreases with an increase in light availability (Humphries and Wheeler 1963; Giertych *et al.* 2015). My results are also supported by the optimal partitioning theory; with light being a limiting factor in the shade, an increase in leaf area increases the surface area available to intercept light. Leaf area showed highly significant positive correlations with leaf length and width in both light treatments and in the pooled data. These results are also supported by those of Rodriquez *et al.* (2016) who conducted a study in Linares (North eastern Mexico) during winter (June – July) on 34 woody shrub and tree species which showed that leaf area was strongly positively correlated with leaf length and breadth (width). This reveals that leaf area, length and width play a significant role in plant productivity. In summary, smaller (length, width and area) and thinner leaves in the sun

are an adaptation to increase convective heat loss, reduce transpiration thus conserving water (Parkhurst and Loucks 1972; Bongers and Popma 1988; Boeger and Gluzezak 2006).

Sun leaves were thicker than shade leaves (Figure 6D). This is a common trend in many studies which observed that leaf thickness increases with an increase in light intensity (Chabot and Chabot 1977; Sobrado and Medina 1980; Björkman 1981; Witkowski and Lamont 1991). The high leaf thickness in sun leaves can be due to numerous layers of cells in the mesophyll or the elongation of the palisade parenchyma (Zimmermann and Brown 1971; Witkowski and Lamont 1991; Cao 2000; Boeger *et al.* 2004; Boeger *et al.* 2006), this is directly influenced by the balance between water loss and carbon gain (Givnish and Vermeij 1976).

Specific leaf area reflects the area available for light capture per unit photosynthate invested in the leaf (Wright *et al.* 2004). It is positively related to relative growth, hence it has been used to assess plant growth (Pérez-Harguindeguy *et al.* 2013). It is also used as an indicator of plant productivity in high stress environments (Niklas and Christianson 2011). Specific leaf area was lower in sun compared to shade leaves. This is a strategy to reduce the excessive temperature and transpiration it experiences (Soares *et al.* 2012; de Melo Junior and Boeger 2016). dos Santos *et al.* (2006) observed that leaf mass area ratio (LMA) increased in *Cedrela fissilis* to compensate for low light availability. Species with a high SLA are considered to have higher concentrations of organic acids, proteins and minerals (Rodriquez *et al.* 2016) and species with a low SLA possess greater quality of cell wall components such as lignin, also possessing greater LDMC and greater leaf and root longevity (Rodriquez *et al.* 2016). However, there were mixed results in this study with sun leaves having a shorter leaf longevity than shade leaves (*see* Chapter 2). In addition to this, a low SLA has been linked to a slow-growing strategy (Freschet *et al.* 2011).

Shading had contrasting effects on leaf thickness and SLA with sun leaves being thicker than shade leaves and shade leaves having a higher SLA than sun leaves (Figure 6D,E). Specific leaf area was strongly negatively correlated with leaf thickness across both light treatments and in the pooled data (Table 2). As shown in this study, leaves with a smaller SLA tend to be thicker (larger number of mesophyll cell layers), thus increasing CO_2 absorption per unit area, which in turn increases water use efficiency in water stressed environments (Burslem *et al.* 1999). This decreases susceptibility to wilting (Wright and Westoby 1999). Numerous studies have shown a positive relationship between SLA and relative growth rate (Poorter and Remkes 1990; Lambers and Poorter 1992; Poorter and van der Werf 1998; Khurana and Singh 2000), photosynthetic

rates (by increasing their light capturing ability) and plant survival (Reich *et al.* 1998; Evans and Poorter 2001; Garnier *et al.* 2001b; Shipley and Vu 2002). Both these leaf traits have been thought to capture ecological variation among species (Westoby *et al.* 2002). Thick leaves with a low SLA have been associated with a long leaf lifespan, these leaves are expensive to construct and tough making them less susceptible to physical damage, thus less palatable to herbivores (Coley 1983; Diaz *et al.* 2004). Specific leaf area was positively correlated with leaf length, width, and area in plants in the sun and for the pooled data, but was negatively correlated with leaf length, width, thickness and area in plants in the shade. This suggests that for plants in the sun and/or *T. sericea* in general, maximising the amount of photosynthetic compounds per unit area was more important than minimising diffusive resistance (Niinements 1999; Wright *et al.* 2003).

Numerous studies have been conducted to determine the relationship between SLA and LDMC (Garnier *et al.* 1997; Poorter and van de Werf 1998; Cunningham *et al.* 1999; Poorter and de Jong 1999; Wilson *et al.* 1999; Garnier *et al.* 2001a; Garnier *et al.* 2001b; Shipley and Vu 2002; Vendramini *et al.* 2002). A general increase in SLA is associated with a decrease in LDMC (Garnier *et al.* 2001b). Our results are in agreement with these findings, although the correlation between these leaf traits was not significant in plants in the shade. However, the relationship between SLA and LDMC was fairly consistent between shading treatments. Specific leaf area and LDMC have been associated with a trade-off between nutrient conservation and rapid biomass production (Poorter and de Jong 1999). They are considered to be indicator traits of different resource-use strategies thus making it important to evaluate these traits in different environments. In summary, variation in SLA and the lack of variation in LDMC among the shading treatments suggests that LDMC is less sensitive to shading. Thus, it appears that SLA is a better indicator of resource-use strategy in *T. sericea* in different light treatments than LDMC in *T. sericea* juveniles.

3.5. Conclusions

Shading exerted strong control on some allocation patterns and leaf traits, however plant architecture was not influenced by the shading treatment. This means that shading during the early stages of plant development in *T. sericea* is not the main limiting factor in plant growth. Suggesting that other environmental parameters such as nutrient and water availability play an important role in plant growth as these are common drivers of African savannas (Scholes and Walker 1993; Scholes and Archer 1997). *Terminalia sericea* appears to have fully compensated for being in a shaded environment by increasing its leaf size and SLA. Indicating that *T. sericea* is

not a sun-demanding species which is unable to recruit in the shade as previously thought. *Terminalia sericea* is able to recruit under larger tree canopies (e.g. *Burkea africana*) as observed in the field (T. Twala *per. obs.*). Differences in the response of seedlings and saplings to shading may be due to differences in: (1) soil types between the plant stages, (2) pot sizes, (3) plant age or (4) genetics. Therefore, *T. sericea* is not restricted to recruiting in gaps but can also be found in shaded areas. It is suggested that future studies should: (1) conduct a field study on the distribution of *T. sericea* found in gaps and under the canopies of other tree species, (2) compare the growth patterns and leaf traits of *T. sericea* seedlings, saplings and adults in the understorey and canopy gaps in order to determine how different age classes of *T. sericea* cope with shading under field conditions, and (3) identify what other limiting factors apart from light availability influence *T. sericea* recruitment in different plant stages.

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Chapter 4: How shade and herbivory affect leaf-level photosynthetic responses in *Terminalia sericea*

Abstract

The aim of this study was to determine the effects of natural and/or simulated herbivory and light availability on leaf chlorophyll content, stomatal conductance and maximum photochemical efficiency of *PSII* (F_v/F_m) on *Terminalia sericea* seedlings and saplings, and to compare the capability of the SPAD 502-Plus and CCM-300 to measure chlorophyll content. Saplings in the sun were exposed to natural herbivory by the larvae of the insect *Phalera imitata* (Notodontidae). Seedlings and saplings were placed in full sunlight and 80% shade. On each plant two pairs of leaf clusters were randomly selected. One leaf cluster in each pair was exposed to 50% simulated herbivory with the adjacent leaf cluster left untouched. Chlorophyll content, stomatal conductance and F_v/F_m were measured throughout the growing season at three week intervals. Invertebrate herbivory did not significantly affect chlorophyll content and stomatal conductance. Chlorophyll content, stomatal conductance and F_v/F_m parameters decreased as the growing season progressed. Simulated herbivory did not affect photosynthetic response in *T. sericea* seedlings and saplings. This suggests that these plant stages were able to tolerate this level of simulated herbivory, regardless of light stress. Seedlings compensated for reduced light availability in the shade with higher F_v/F_m (0.77 ± 0.01) and lower stomatal conductance (133 ± 7.7 $mol.m^{-2}.s^{-1}$) than those in the sun (F_v/F_m: 0.72 ± 0.01; stomatal conductance: 161 ± 10 $mol.m^{-2}.s^{-1}$). In contrast shading had no effect on photosynthetic responses in the saplings. At the end of the experiment (~8 months) chlorophyll content was measured using the SPAD 502-Plus, CCM-300 and then extracted using ethanol. The SPAD 502-Plus ($r^2_{chlorophyll\ a}$ = 0.88, $r^2_{chlorophyll\ b}$ = 0.47, $r^2_{total\ chlorophyll}$ = 0.83) was better at indicating chlorophyll *a* and total chlorophyll (*a* + *b*) than the CCM-300 ($r^2_{chlorophyll\ a}$ = 0.74, $r^2_{chlorophyll\ b}$ = 0.45, $r^2_{total\ chlorophyll}$ = 73). Overall, these findings suggest that *T. sericea* seedlings and saplings can tolerate these levels of herbivory damage at the cluster scale. Secondly, shading does not influence photosynthetic responses in saplings, but seedlings performed better in shaded conditions than in full sunlight. Therefore, seedlings and saplings are likely to be able to recruit in the understorey and in gaps between canopies.

Keywords: Chlorophyll content, chlorophyll fluorescence**,** photochemical efficiency of PS*II*, stomatal conductance, *Terminalia sericea*

4.1. Introduction

Terrestrial plants are exposed to a variety of stresses such as natural (e.g. light, heat, nutrients and water) and anthropogenic-related stresses (e.g. pollution, acid rain and cutting) (Lichtenthaler and Burkart 1999). All environmental drivers and stresses either directly or indirectly affect photosynthetic apparatus structure or function (Lichtenthaler and Burkart 1999). Light is one of the main factors affecting phytochemical concentration and photosynthetic apparatus in plants (Correia *et al.* 1990; Kopsell and Kopsell 2008). During photosynthesis, photosynthetic pigments absorb solar radiation where the resulting energy is channeled into reaction centres, which in turn releases electrons that set the photochemical process into motion (Schreiber 2003). Chlorophyll *a* and *b* are the most important of these pigments playing a vital role in energy transformation (Richardson *et al.* 2001; Richardson *et al.* 2002; Sims and Gamon 2002). How leaf chlorophyll content varies between and within species is an important physiological process to understand, however, leaf pigmentation is also important to ecophysiologists and landscape managers from an application perspective. The amount of solar radiation absorbed by a leaf is largely influenced by the concentration of these photosynthetic pigments in the leaf, therefore low concentrations of these pigments in chlorophyll directly limits the photosynthetic potential of the plant, thus affecting its primary production (Curran *et al.* 1990; Witkowski *et al.* 1992; Field *et al.* 1995; Filella *et al.* 1995).

Many stresses, including light stress, manifest themselves in decreased foliar chlorophyll content or changes in pigment ratios (Peñuelas and Filella 1998; Pinkard *et al.* 2006; Demmig-Adams *et al.* 2012) and are usually visibility detectable. Traditional methods for measuring chlorophyll require the extraction of chlorophyll in a solvent analysed using spectrophotometry in order to determine the absorbance of the chlorophyll in the solution (Lichtenhaler 1987; Parry *et al.* 2014), which is then converted from absorbance to chlorophyll concentration using equations such as those of Arnon (1949) and modifications thereof such as Lichtenthaler and Buschmann (2001). This is the most accurate method of photosynthetic pigment determination, however, it is time consuming, labour intensive and requires destructive sampling which affects leaf development and may not be feasible in some studies, such as those where changes in chlorophyll content over time on a single leaf are monitored (Yamamoto *et al.* 2002; Pinkard *et al.* 2006).

When very precise methods are not feasible, non-destructive methods may be sufficient, especially in the field (Richardson *et al.* 2002; Pinkard *et al.* 2006; Parry *et al.* 2014). In the past 25 years

chlorophyll content meters (CCM) have been developed to solve these problems (Biber 2007). Manufactures of these devices include Optical-Sciences (2002) and Konica Minolta single photon avalanche diode (SPAD) who manufacture the chlorophyll content meter 300 (CCM-300) and SPAD 502-Plus, respectively. Most chlorophyll content meters use light emitting diodes and receptors in order to calculate a chlorophyll content index (CCI) (Chang and Robison 2003; Biber 2007; Goodall *et al.* 2012; Kalaji *et al.* 2014; Zivcak *et al.* 2014); which is an expression of relative chlorophyll content and not chlorophyll concentration per gram of leaf tissue, or absolute chlorophyll content per unit leaf area (Richardson *et al.* 2001). The CCI is defined as the ratio of the percentage of light transmission at 931 nm (near-infrared) to that at 653 nm (red) transmitted through a leaf sample (Richardson *et* al. 2002). The red light is absorbed by chlorophyll and the infrared light is reflected adjusting for differences in leaf structure producing chlorophyll content readings (Richardson *et al.* 2001; Chang and Robison 2003; Parry *et al.* 2014). However, many chlorophyll content meter readings are dimensionless and require calibration equations with tradition chlorophyll extraction methods to associate optical chlorophyll content meter readings to chlorophyll concentrations (Markwell *et al.* 1995; Richardson *et al.* 2002)

The use of non-destructive optical chlorophyll content meters has been highly favoured as they provide rapid, simple and accurate measurements of leaf chlorophyll in intact leaves, without destructive chlorophyll assays and have been successively used to measure chlorophyll content in many studies (Markwell *et al.* 1995; Gamon and Surfus 1999; Loh *et al.* 2002; Richardson *et al.* 2002; Abdelhamid *et al.* 2003; Pinkard *et al.* 2006; Biber 2007; Marenco *et al.* 2009; Goodall *et al.* 2012; Cowie *et al.* 2016; Kalaji *et al.* 2017; Whitecross 2017). However, there are several constraints to the use of chlorophyll content meters, some authors have found that leaf age (Silla *et al.* 2010), morphological traits (Yamamoto *et al.* 2002; Marenco *et al.* 2009), leaf water content (Martinez and Guimet 2004) or the distribution of chloroplasts (Nauš *et al.* 2010) influence the relationship between optical chlorophyll content and absolute chlorophyll content.

Chlorophyll fluorescence measurements have been shown to be a non-destructive, quantitative and rapid way of determining the effects of various environmental stresses and changes on the properties of the photosynthetic apparatus (Baker and Rosenqvist 2004). Chlorophyll fluorescence is composed of photons of far-red and red light which are emitted by chlorophyll *a* after light absorption (Porcar-Castell *et al.* 2014). During chlorophyll fluorescence light energy is absorbed by

chlorophyll molecules which get excited, and de-excitation of this energy is attained through non-radiative thermal energy dissipation (NPQ), radiative loss of photons (chlorophyll fluorescence) and photosynthesis (Cendrero-Mateo *et al.* 2016). As these three processes compete for energy a change in one process results in a change in the other two processes (Cendrero-Mateo *et al.* 2016). Hence, by measuring chlorophyll fluorescence we can gain information on light regulated and non-regulated heat dissipation, heat dissipation through non-photochemical quenching, energy balance between yields of photosystems and electron transport rate (Maxwell and Johnson 2000; Kramer *et al.* 2004; Porcar-Castell *et al.* 2014). Chlorophyll fluorescence has been used as an indicator of plant health and vitality by measuring the efficiency of photosynthesis (Witkowski *et al.* 1992; Flexas *et al.* 2002; Schächtl *et al*. 2005; Corp *et al.* 2009; Zarco-Tejada *et al.* 2013; Kancheva *et al.* 2008).

In addition to chlorophyll content and fluorescence, stomatal regulation also plays an important role in the photosynthetic status of a plant. Stomata respond to a variety of signals and the opening and closing of stomata is influenced by many factors such as CO_2 concentration, light intensity and air humidity (McDermitt 1990; Baroli *et al.* 2008). The photosynthetic apparatus is affected by blue and red light which is absorbed by chlorophyll *a* and *b* (Sæbø *et al.* 1995; Son and Oh 2015), with blue light initiating stomatal opening (Shimazaki *et al.* 2007).

The effect of light is a complex matter when considering differences in light availability experienced by seedlings, saplings and adult plants. While established adults are usually exposed to high irradiance, younger plants are exposed to a variety of light intensities ranging from abundant in canopy gaps or clearings to severe light limitations in understories, or when covered by herbaceous biomass. Thus, plasticity in response to light and shade tolerance may play an important role in the development of juvenile savanna tree species. Tolerance (the ability to maintain plant fitness after experiencing fitness reducing stress) has rarely been studied within an ontogenetic context. This is important because seedlings and juvenile (saplings) plants are associated with few lateral buds and limited stored sources which can be utilised in response to damage. These young plant stages are quite constrained in the acquisition and utilisation of stored reserves. Younger plant stages are more plastic than their older counterparts (Pigliucci 1998). In this chapter, tolerance to natural and simulated defoliation was investigated in *Terminalia sericea* seedlings and saplings under two light treatments, with the objectives being to: (1) determine the effect of natural herbivory on chlorophyll content and stomatal conductance, (2) determine the long term effects of simulated

herbivory and shading on chlorophyll content and photosynthetic responses, and (3) compare the performance of two commercially used hand-held chlorophyll absorbance meters.

4.2. Materials and methods

4.2.1. Study site

The study ran from the beginning of December 2016 to the end of May 2017 and was conducted on the roof top of the Oppenheimer Life Sciences Building, Johannesburg, South Africa (26° 11' 30'' S; 28° 01' 58'' E) which has a subtropical highland climate. Johannesburg experiences dry sun days and cold nights during the winter months (May – September), and hot days with afternoon thundershowers and cool nights during the summer months (October – April). The highest mean annual temperature is experienced in January (26.2°C) with the lowest being July (~16.6°C), where temperatures can drop to freezing at night. The mean annual rainfall is 604 mm/year in the summer months. The lowest rainfall occurs in July while the highest is in January.

4.2.2. Study species

Terminalia sericea Burch. ex DC (common name: silver cluster leaf) is a medium sized deciduous tree, with a height range of between 4 – 12 m but some individuals have been known to grow up to 20 m. The silver leaves are borne in clusters (Grant 2005), with new leaves sprouting in the center of the cluster (Grant 1984; Kikuzawa 1995). The leaves are present all year dropping in autumn, while the cream flowers are present in spring followed by the fruits in mid-summer (Grant 1984; Kikuzawa 1995). *Terminalia sericea* is browsed by invertebrates such as *Euproctis fasciata*, Walker, 1855 and *Arcyophora carniola*, Hampson, 1912, which are lepidopteran species, as well as vertebrates such as elephants and giraffe (Coaste-Palgrave 1957). It is foliage forms a vital part of livestock foliage during the dry months (Katjiua and Ward 2006).

4.2.3. Plant specifics and maintenance

Seedlings were defined as *T. sericea* plants with a height < 1 m and saplings were defined as *T. sericea* plants with a height > 1 m. Nineteen seedlings were purchased from The Aloe Farm, Hartebeespoort dam, North West, South Africa (25° 43' 15" S; 27° 47' 9" E), and 20 saplings were purchased from Random Harvest Indigenous Plant Nursery situated in Muldersdrift, Gauteng, South Africa (26° 01' 49'' S; 27° 53' 38'' E). All plants were grown from samarae under greenhouse conditions. Upon purchasing the plants on August 2016, seedlings were ± 1.5 years old and saplings

were ± 4 years old. The seedlings were planted in 10 litre plastic bags with sandy soil, while saplings were planted in 20 litre plastic bags with potting soil covered with wood chip mulch. Plants were placed in full sunlight from August 2016 to 15 October 2016, when 9 seedlings and 10 saplings were moved under 80% shade cloth and left to acclimate for 14 days. Photosynthetic photon flux density in full sunlight was 1800 µmol m^{-2} s^{-1}, compared with 400 µmol m^{-1} s^{-1} in the shaded treatment. Sprinklers were used to water all plants at 08h00 to 08h10 and 16h00 to 16h10 daily.

4.2.4. Experimental design and protocol

Ten seedlings and saplings were placed in full sunlight and ten saplings and nine seedlings were placed under 80% shade cloth. On each plant two pairs of leaf clusters were randomly selected. On each pair one cluster was exposed to simulated herbivory where a pair of scissors was used to remove the leaf area on the right hand side of the mid-rib vein and leaving the adjacent cluster untouched (*see* Chapter 2). On each cluster one leaf was selected and chlorophyll content (measured using the SPAD 502-Plus and CCM-300), stomatal conductance and maximum photochemical efficiency of *PSII* (F_v/F_m) were measured before and after exposure to simulated herbivory. This type of replication technique was used to determine the effects of herbivory at the leaf-level and to identify where the herbivory treatments had a leaf-level effect on the aforementioned photosynthetic responses. This is a well-used experimental design in entomology and biological control for determining the responses of plants to herbivory damage (Cowie *et al.* 2018).

4.2.4.1. Natural herbivory

Two pairs of leaf clusters were randomly selected on 3 saplings in the sun (only) on the 24th of November 2016. Chlorophyll content and stomatal conductance were measured on each leaf in the selected clusters using a SPAD 502-Plus chlorophyll meter (Minolta, Osaka 542, Japan) and a chlorophyll content meter 300 (CCM-300, Optical-Science Inc. NH 03051, USA), as well as a SC-1 leaf porometer (Decagon Devices Inc., Pullman, Washington, USA), respectively. From each pair two *Phalera imitata*, Druce, 1896, (Notodontidae) larvae that were starved for 2 days were placed onto one leaf cluster and bagged using an organza bag and the other cluster was left untouched and also bagged. The larvae were left in the bags for 24 hours and then removed (Figure 1). Three days post herbivory chlorophyll content and stomatal conductance were again measured.

Figure 1. *Terminalia* sericea leaves after 24 hours exposure to *Phalera imitata* larvae.

4.2.4.2. Simulated herbivory

One leaf per cluster from both the control and herbivory treatment clusters on the seedlings and saplings in the sun and shade was randomly selected (*see* Chapter 2) on the 5th of December 2016. A pair of scissors was used to remove the right side of all the leaves in the herbivory treatment leaf clusters; the scissors was dipped in 95% ethanol after cutting each leaf to reduce contamination. Chlorophyll content, stomatal conductance and chlorophyll fluorescence were measured on the left side of each leaf before and two weeks post herbivory treatment, and then again at three week intervals over 15 weeks post simulated herbivory. The same leaves were measured over the growing season without replacement.

4.2.4.3. Relationships between chlorophyll meters readings and measured chlorophyll content

Five leaves were collected from 5 seedlings in the sun and shade, as well as 5 saplings in the sun and shade (n = 20). In each leaf a single-hole punch was used to extract ~2cm^2 of leaf sample from each leaf; making sure to clean the punch with 95% ethanol after punching. Chlorophyll content was measured using a CCM-300 (accurate to ± 1.0 mg.m^{-2}) and SPAD 502-Plus for each leaf (accurate to ± 1.0 SPAD units). Once chlorophyll content was measured each leaf was weighed on a 5 point balance scale. Each sample was cut into small pieces using a pair of scissors and placed into separate labelled test tubes; the scissors were rinsed in 95% ethanol and wiped with a paper towel after each use to reduce cross contamination. In order to extract chlorophyll *a* and *b,* 10ml of 95% ethanol was poured into each test tube, sealed using foil and left at room temperature for 24 hours. A total of 6ml was transferred into cuvettes using a dual-beam spectrophotometer at $A_{664.2}$, $A_{648.6}$ and A_{470}. Recordings were also done at A_{750} to correct for contaminating coloured compounds and

turbidity, this wavelength was appropriate because chlorophyll *a* and *b* do not absorb at this wavelength. If turbidity was found for any of the samples the test tubes were dark centrifuged at 1 120 g force for 10 minutes before reanalysis (Lichtenthaler and Buschmann 2001). The concentrations of the extracts were converted into actual leaf chlorophyll content ($l.m^{-2}$ DW) using equations (1-3) described by Lichtenthaler (1987). Ethanol (95%) was poured into a cuvette, placed into the spectrophotometer and calibrated to zero absorbance.

Equation 1: chlorophyll "*a*" = $(13.36\ A_{664.2}) - (5.19\ A_{648.6})$

Equation 2: chlorophyll "*b*" = $(27.43\ A_{648.6}) - (8.12\ A_{664.2})$

Equation 3: total chlorophyll = chlorophyll (*a* + *b*)

4.2.4.4. Data analysis

Owing to different growing conditions, the data for seedlings and saplings were analysed separately. The R package *ggplot2* (Wickham 2009) was used to produce graphical representations. Statistical analyses were done in the statistical programme R (version 3.4.2, R Core Team 2017). Shapiro-Wilks normality tests were used to test the data for normality in R. To determine the influence of natural herbivory on chlorophyll content and stomatal conductance one-way repeated measures analysis of variances (RMANOVA) were conducted across the whole study period and ANOVAs at each time interval. The R package *nlme* was used to run linear mixed effects models (LME) (Pinheiro *et al.* 2017), which were conducted to determine the effects of shading and simulated herbivory on chlorophyll content measured using the SPAD 502-Plus and CCM-300, stomatal conductance and chlorophyll fluorescence. The effects of these factors were determined from week 5 (W5) to W13 in seedlings and from W5 to W10 in saplings. Because the same leaves were measured without replacement, the sample size decreased as the season progressed, and therefore these data were statistically analysed until these stop dates, which are based on the mean leaf longevities of *T. sericea* seedlings and saplings (*see* Chapter 2). Kruskal-Wallis tests were conducted to determine the effects of these factors on the photosynthetic parameters on W5, W7, W10 and W13 in seedlings and W5, W7 and W10 in saplings, with Kruskal Multiple Comparisons (kruskalmc) post-hoc analysis from the *pgirmess* package in R (Giraudox 2014) conducted to identify the underlying differences. To determine the effect of shading on SPAD values, chlorophyll content meter index (CCM), chlorophyll *a* and *b* and total chlorophyll, one-way ANOVAs were conducted.

The relationship between SPAD values and CCM against the measured chlorophyll content concentrations was determined using simple linear regressions.

4.3. Results

4.3.1. Natural herbivory

Phalera imitata larvae did not have an effect on the chlorophyll content of the remaining undamaged half-leaf tissue (one-way RMANOVA: $p > 0.05$) (Figure 2). Before the introduction of the herbivory treatment chlorophyll content was not significantly different between herbivory treatment and control leaves (one-way ANOVA: SPAD 502-Plus: $F_{(1,37)} = 2.65$, $p = 0.112$; CCM-300: $F_{(1,37)} = 3.451$, $p = 0.0712$). Three days post herbivory, chlorophyll content was still not significantly different between herbivory treatment and control leaves (one-way ANOVA: SPAD 502-Plus: $F_{(1,33)} = 0.124$, $p = 0.727$; CCM-300: $F_{(1,33)} = 0.57$, $p = 0.456$). There was no significant change in stomatal conductance across the study period or between herbivory treatments (one-way RMANOVA: herbivory treatments: $F_{(1,55)} = 0.069$, $p = 0.7942$; time: $F_{(1,55)} = 2.961$, $p = 0.0904$; interaction: $F_{(1,55)} = 0.382$, $p = 0.5389$) (Figure 2c). Prior to defoliation there was no significant difference in stomatal conductance between control (171.5 ± 15.3 $mol.m^{-2}.s^{-1}$) and herbivory treatment (185.0 ± 16.1 $mol.m^{-2}.s^{-1}$) leaves (one-way ANOVA: $F_{(1,27)} = 0.038$, $p = 0.847$). Three days post herbivory there was still no significant difference in stomatal conductance between herbivory treatment (213.1 ± 16.5 $mol.m^{-2}.s^{-1}$) and control leaves (199. 6 ± 15.7 $mol.m^{-2}.s^{-1}$) (one-way ANOVA: $F_{(1,27)} = 0.456$, $p = 0.505$). These findings suggest that herbivory by *P. imitata* larvae did not have an immediate or short-term effect on chlorophyll content and stomatal conductance in *T. sericea* under the experiment conditions imposed.

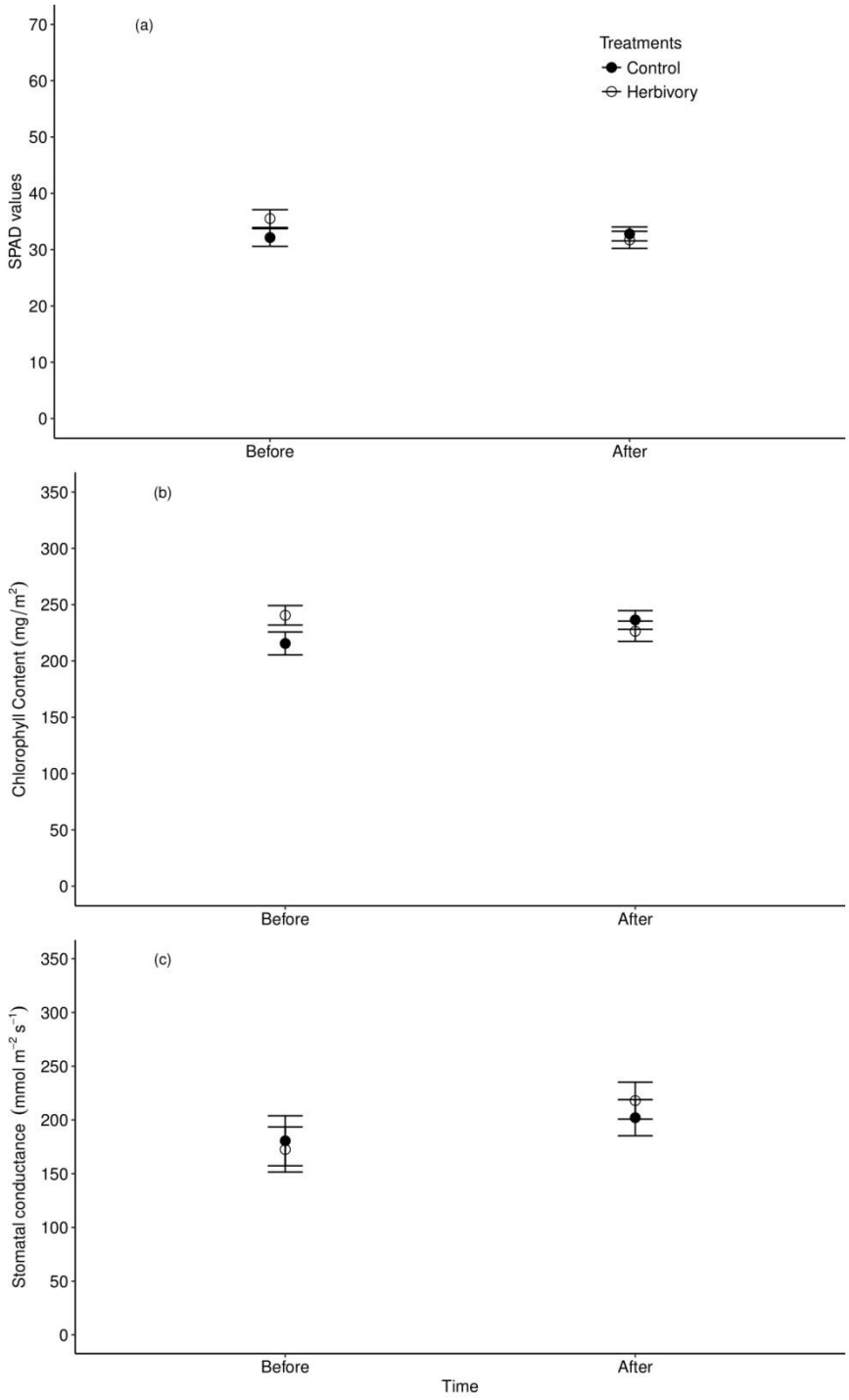

Figure 2. Mean ± SE of chlorophyll content measured using the SPAD 502-Plus (a) and CCM-300 (b) and stomatal conductance (c) before and three days after exposure to herbivory by *Phalera imitata* larvae.

4.3.2. Simulated herbivory

4.3.2.1. Chlorophyll content measured using the SPAD 502-Plus

Seedlings

A linear mixed effect model fit by maximum likelihood (LME) (Table 1) showed that chlorophyll content measured using the SPAD 502-Plus remained higher in seedlings that were in the shade (41.33 ± 0.7 SPAD units) than those in the sun (31.02 ± 0.73 SPAD units) (Figure 3a,b). Between W5 and W13, significant decreases in chlorophyll content were observed (LME: $p < 0.001$) (Figure 3), however, this decrease was not influenced by the herbivory treatment (LME: $p > 0.05$) (Table 1).There was a significant difference in chlorophyll content, using the SPAD 502-Plus, between the control and herbivory treatment as well as sun and shade leaves between W5 and W13 (Kruskal-Wallis: $p < 0.01$). In seedlings in the shade, chlorophyll content in control leaves was higher than in herbivory treatment leaves in W5 and W7 and lower in W10 and W13 (Kruskalmc: $p < 0.05$), however herbivory did not have an effect on chlorophyll content in seedlings in the sun (Kruskalmc: $p > 0.05$).

Saplings

Shading did not have a significant effect on chlorophyll content measured using the SPAD 502-Plus, although chlorophyll content tended to be higher in the saplings in the shade (33.55 ± 0.73 SPAD units) than those in the sun (29.35 ± 0.62 SPAD units) (Table 1) (Figure 3c,d). There was no significant change in chlorophyll content across the study period and the herbivory treatment did not have a significant effect on chlorophyll content, hence they were removed from the model (LME: $p > 0.05$). On W5 and W7 chlorophyll content was significantly different between herbivory treatment and control leaves in the sun and shade (Kruskal-Wallis: $p < 0.05$), however there was no significant different between the treatments in W10 (Kruskal-Wallis: $p > 0.05$). These differences were between herbivory treatment leaves in the shade (35.42 ± 1.70 SPAD units) and control leaves in the sun (31.06 ± 1.33 SPAD units) in W5, and between sun (28.02 ± 1.15 SPAD units) and shade herbivory treatment leaves (35.02 ± 2.86 SPAD units) in W7.

Overall, the herbivory treatment did not have a significant effect on chlorophyll content in either of the plant stages (Table 1). Seedlings were able to compensate for shading by producing more chlorophyll than those in the sun; however shading did not have an effect on chlorophyll content in the saplings.

Table 1. Statistical outputs of linear mixed effects models fit by maximum likelihood for the different photosynthetic measurements of seedlings and saplings over one growing season. Factor variables describe "Shade" as leaves in either full sunlight or under 80% shade, "Herbivory" as leaves exposed to either 0% herbivory or 50% herbivory, and "Time" as the week number of each observation within the growing season. Factor variables that were excluded from the model ($p > 0.05$) are represented by the "-" symbol.

Photosynthetic measurements	Plant stage	Factor variables	t-value	S.E.	p-value	Significance
SPAD 502-Plus	*Seedling*	Shade	-8.449	1.157	<0.001	***
		Herbivory	-	-	>0.05	NS
		Time	-9.993	0.890	<0.001	***
	Sapling	Shade	-1.692	1.990	0.0921	NS
		Herbivory	-	-	>0.05	NS
		Time	-	-	>0.05	NS
CCM 300	*Seedling*	Shade	-	-	>0.05	NS
		Herbivory	-	-	>0.05	NS
		Time	-6.059	19.587	<0.001	***
	Sapling	Shade	0.242	15.472	0.9013	NS
		Herbivory	-	-	>0.05	NS
		Time	-1.765	7.749	0.0791	NS
		Shade:Time	2.714	13.110	0.0038	**
Stomatal Conductance	*Seedling*	Shade	2.254	17.557	0.0377	*
		Herbivory	-2.666	9.493	0.0085	**
		Time	1.704	11.834	0.0904	NS
		Shade:Time	3.958	17.110	<0.001	***
	Sapling	Shade	-	-	>0.05	NS
		Herbivory	-1.834	11.406	0.0697	NS
		Time	-	-	>0.05	NS
Chlorophyll Fluorescence	*Seedling*	Shade	-3.866	0.014	0.0012	**
		Herbivory	-	-	>0.05	NS
		Time	-1.463	0.011	0.1450	NS
	Sapling	Shade	-3.981	0.012	0.0009	***
		Herbivory	-1.733	0.012	0.0862	NS
		Time	0.029	0.012	0.9766	NS
		Herbivory:Time	-2.092	0.168	0.0391	*

NS, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

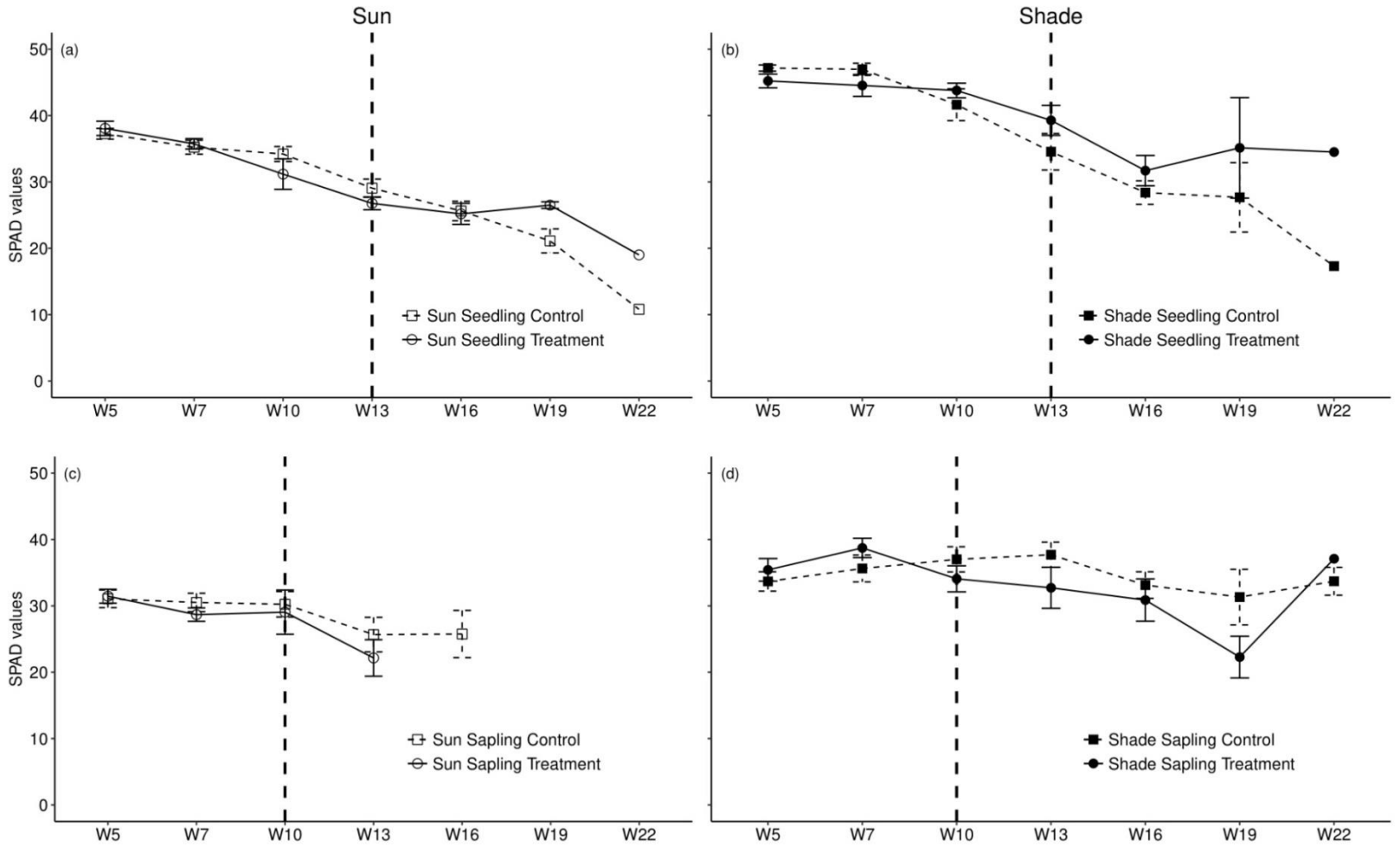

Figure 3. Change in chlorophyll content (mean ± SE) (SPAD 502-Plus) over time for *Terminalia sericea* (a, b) seedlings and (c, d) saplings in the sun and shade, with and without simulated herbivory (cutting). On the x-axis is time in week number from 31 January 2017 to 28 May 2017. The vertical dashed line indicates the mean leaf longevities (*see* Chapter 2) where statistical analysis was conducted between the start date and the mean leaf longevity. This was done because the same leaves were measured without replacement, and hence sample sizes decreased across the study period.

4.3.2.2. Chlorophyll content measured using the CCM-300

Seedlings

A linear mixed effects model fit by maximum likelihood showed that chlorophyll content decreased by 48% in sun and 53% in shade leaves between W5 and W13 (Table 1) (Figure 4a,b). Shading and herbivory did not have a significant effect on chlorophyll content, hence they were removed from the model (LME: $p > 0.05$). In W5, W7 and W13 there were no significant differences in chlorophyll content between control and herbivory treatment leaves in the sun and shade (Kruskal-Wallis: $p > 0.05$). Chlorophyll content differed between control and herbivory treatment leaves in W10 (Kruskal-Wallis: $H = 8.149$, $df = 3$, $p = 0.0430$), however there were no overall differences between the treatments (Kruskalmc: $p > 0.05$).

Saplings

There was no significant change in chlorophyll content across the study period in saplings (Table 1) (Figure 4c,d). Shading did not have a significant effect on chlorophyll content (Table 1). Between W5 and W10, sapling leaves in the shade retained their chlorophyll content better than those in the sun (Table 1). Herbivory did not have an effect on chlorophyll content, hence it was removed from the model (LME: $p > 0.05$). In W5 to W10 chlorophyll content did not differ between control and herbivory treatment leaves in the sun and shade (Kruskal-Wallis: $p > 0.05$).

4.3.2.3. Stomatal conductance

Seedlings

A linear mixed effects model fit by maximum likelihood showed that stomatal conductance did not significantly change across the study period (Table 1) (Figure 5a,b). Stomatal conductance was higher in control than herbivory treatment leaves (mean difference: 29.4 $mol.m^{-2}.s^{-1}$). Seedling leaves in the sun had a higher stomatal conductance (160.5 ± 10.0 $mol.m^{-2}.s^{-1}$) than those in the shade (133.3 ± 7.7 $mol.m^{-2}.s^{-1}$). Across the study period, stomatal conductance was higher in sun than shade leaves (Table 1). In W7 and W10 stomatal conductance was not significantly different between control and herbivory treatment leaves in the sun and shade (Kruskal-Wallis: $p > 0.05$). However, in W13 leaves exposed to full sun (control) tended to have higher stomatal conductance than those in the shade (mean difference = 95.9 $mol.m^{-2}.s^{-1}$) (Kruskalmc: $p > 0.05$).

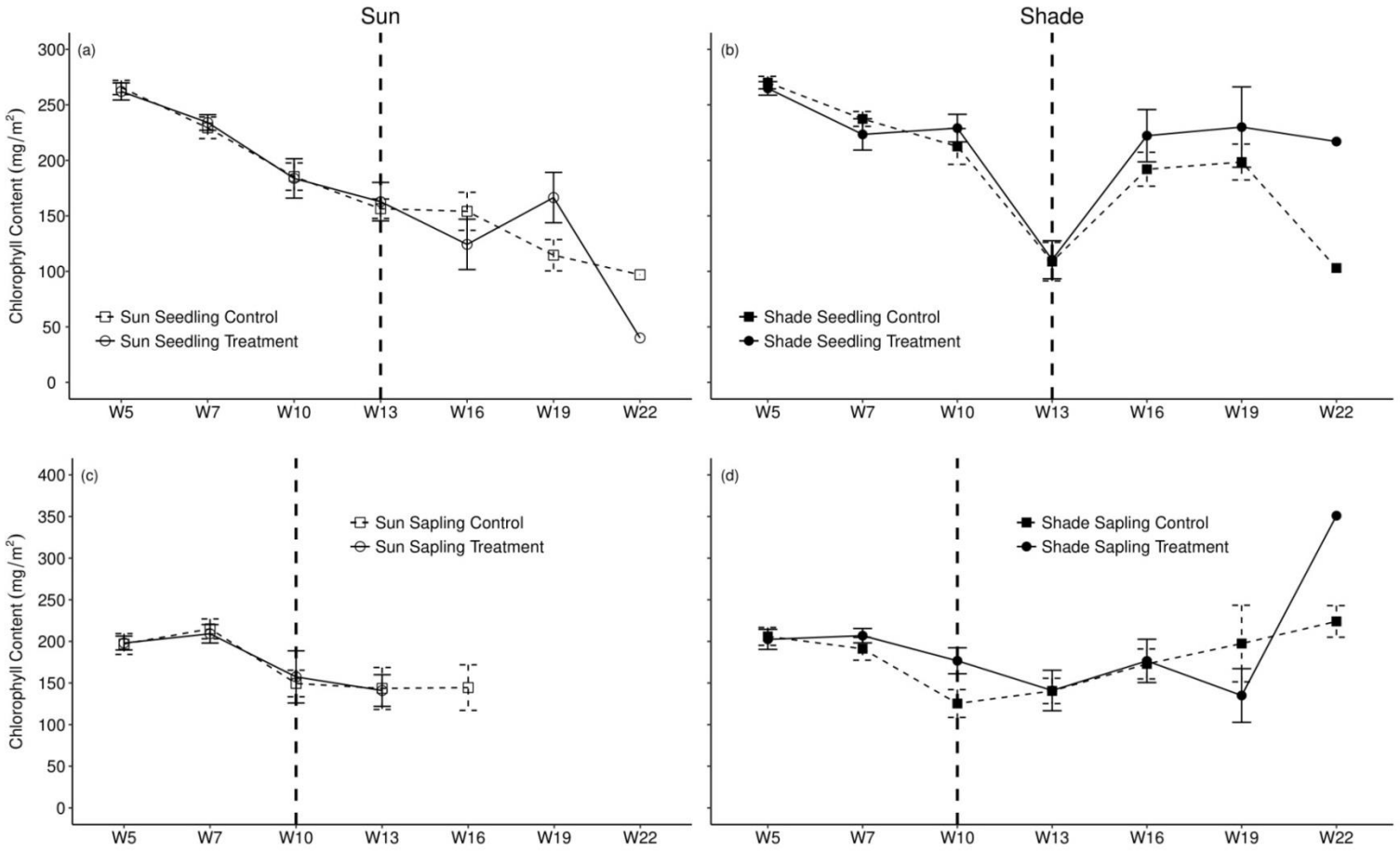

Figure 4. Change in leaf chlorophyll content (mean ± SE) (CCM-300) over time fin *Terminalia sericea* (a, b) seedlings and (c, d) saplings, in the sun and shade, with and without simulated herbivory (cutting). On the x-axis is time in week number from 26 January 2017 to 30 May 2017. The vertical dashed line indicates the mean leaf longevities where statistical analysis was conducted between the start date and the mean leaf longevity. This was done because the same leaves were measured without replacement, and hence sample sizes decreased across the study period.

Saplings

Herbivory did not have a significant effect on stomatal conductance in sapling leaves (Table 1) (Figure 5c,d). Shading and sampling time did not have an effect on stomatal conductance, hence they were removed from the model (Table 1).In W7 and W10 stomatal conductance did not differ between control and herbivory treatment leaves in saplings in the sun and shade (Kruskal-Wallis: $p > 0.05$).

4.3.2.4. Maximum photochemical efficiency of PS*II*

Seedlings

There was no significant change in F_v/F_m from W4 to W13, and the herbivory treatment had no overall effect on F_v/F_m and was thus excluded from the model (Table 1) (Figure 6a,b). Maximum photochemical efficiency of *PSII* was higher in seedlings in the shade (0.77 ± 0.01) compared to those in full sunlight (0.72 ± 0.01), suggesting that seedlings in full sunlight were more stressed than shaded seedlings. The interaction of shading and the herbivory treatment in seedlings had a significant effect on F_v/F_m in W7, W10 and W13 (Kruskal-Wallis: $p < 0.05$).

Saplings

The LME likelihood showed that there was no significant change in F_v/F_m from W4 to W10 (Table 1) (Figure 6c,d). Maximum photochemical efficiency of *PSII* was higher in saplings in the shade (0.77 ± 0.01) than those in full sunlight (0.70 ± 0.01), these are similar findings to those in seedlings (Table 1). Herbivory did not have a significant effect on F_v/F_m (Table 1). However, from W4 to W10 F_v/F_m was higher in herbivory treatment than control leaves (Table 1), suggesting that the herbivory treatment had long term effects on F_v/F_m in saplings. Maximum photochemical efficiency of *PSII* differed significantly between control and herbivory treatment leaves in the sun and shade in W7 and W10 (Kruskal-Wallis: $p < 0.05$).

Overall, F_v/F_m did not differ between seedlings and saplings with overall efficiency values of 0.73 ± 0.01 in seedlings and 0.73 ± 0.01 in saplings. Both seedlings and saplings had higher F_v/F_m values in shade than sun leaves. The herbivory treatment did not influence F_v/F_m in either plant stages.

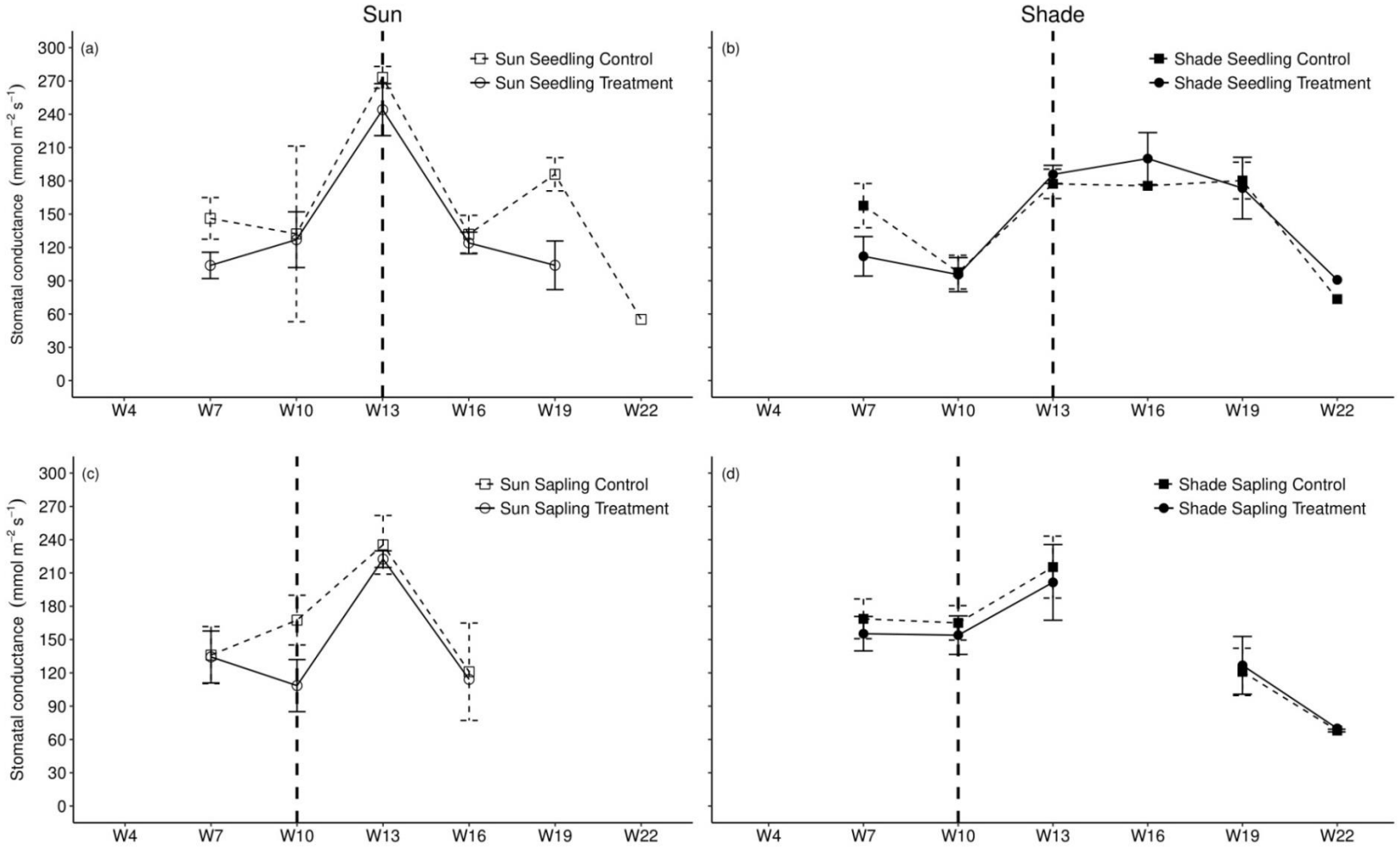

Figure 5. Changes in stomatal conductance (mean ± SE) over time in *Terminalia sericea* (a, b) seedlings and (c, d) saplings, in the sun or shade, and with or without simulated herbivory (cutting). On the x-axis is time in week number from 26 January 2017 to 30 May 2017. The vertical dashed line indicates the mean leaf longevities where statistical analysis was conducted between the start date and the mean leaf longevity (*see* Chapter 2). This was done because the same leaves were measured without replacement, and hence sample sizes decreased across the study period.

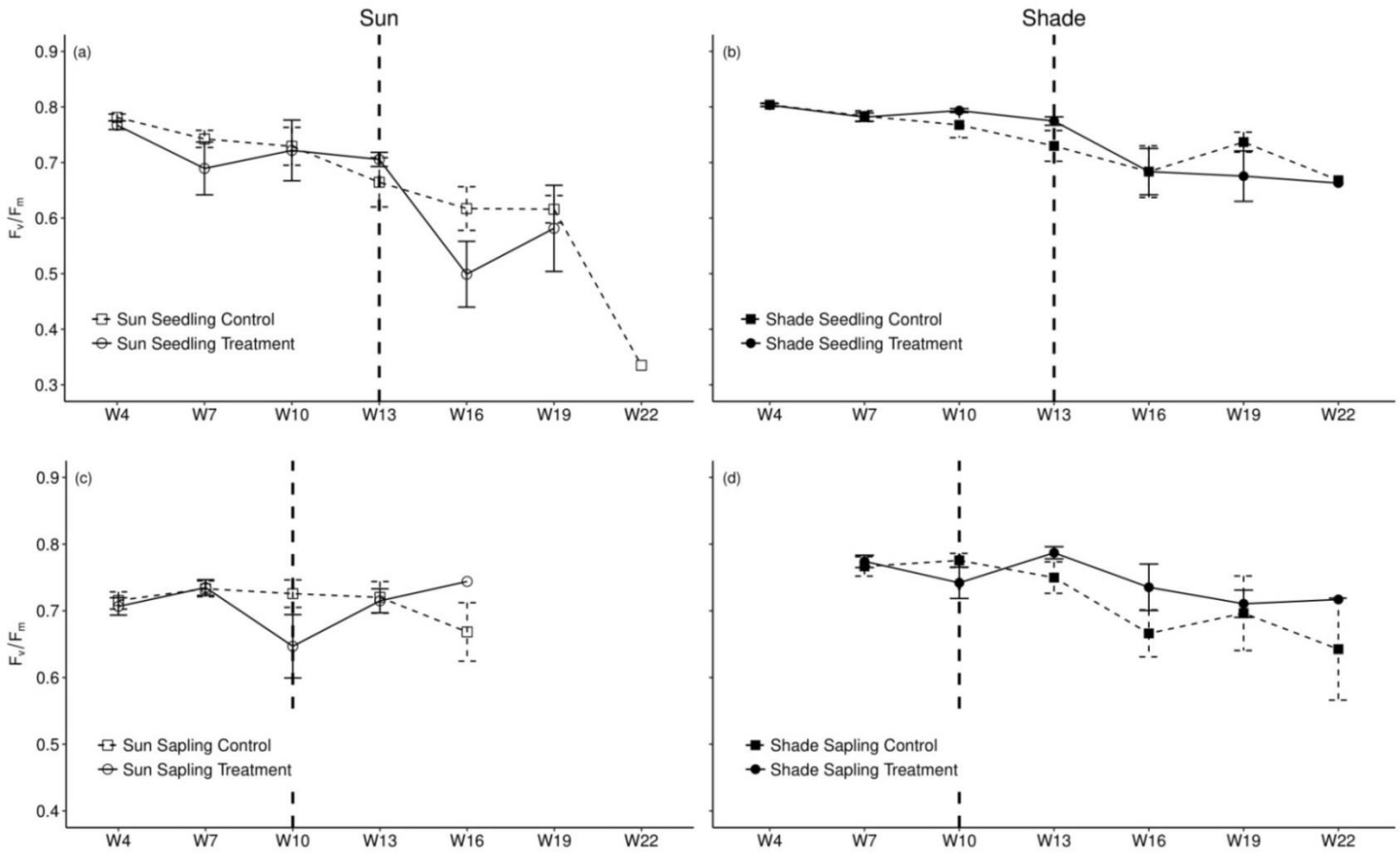

Figure 6. Changes in maximum *PSII* quantum efficiency (F_v/F_m) (mean ± SE) over time in *Terminalia sericea* (a, b) seedlings and (c, d) saplings, in the sun or shade, and with or without simulated herbivory (cutting). On the x-axis is time in week number from 26 January 2017 to 30 May 2017. The vertical dashed line indicates the mean leaf longevities where statistical analysis was conducted between the start date and the mean leaf longevity (*see* Chapter 2).This was done because the same leaves were measured without replacement, and hence sample sizes decreased across the study period.

4.3.3. Relationships between chlorophyll indices and measured chlorophyll content

When using the SPAD 502-Plus, chlorophyll content did not differ significantly between sun and shade leaves (one-way ANOVA: $F_{(1, 17)} = 3.30$, $p = 0.0871$), although chlorophyll content tended to be higher in shade leaves (Table 2). Chlorophyll content measured using the CCM-300 was higher in shade than sun leaves, with a mean difference of 213.92 $mg.m^{-2}$ ($F_{(1,17)} = 11.78$, $p = 0.0032$). Shading had a significant effect on the concentrations of chlorophyll *a*, *b* and total chlorophyll in *Terminalia sericea,* with shade leaves having higher chlorophyll concentrations then sun leaves (chlorophyll *a*: $F_{(1,17)} = 4.84$, $p = 0.0419$, chlorophyll *b*: $F_{(1,17)} = 9.90$, $p = 0.0059$, total chlorophyll: $F_{(1,17)} = 9.60$, $p = 0.0065$).

Table 2. Mean ± SE of SPAD 502-Plus and chlorophyll content meter (CCM-300) values as well as directly measured concentrations of chlorophylls (Chl) ($l.m^{-2}$) in leaves of *Terminalia sericea* in the sun and shade.

Light Availability	SPAD	CCM-300	Chl *a*	Chl *b*	Total Chl
Sun	$19.30 \pm 2.20_a$	$125.30 \pm 22.82_a$	$1.46e^{-03} \pm 2.29e^{-04}{}_a$	$6.98e^{-04} \pm 1.29e^{-04}{}_a$	$2.16e^{-03} \pm 3.45e^{-04}{}_a$
Shade	$28.01 \pm 4.44_a$	$339.22 \pm 60.78_b$	$3.84e^{-03} \pm 8.66e^{-04}{}_b$	$1.75e^{-03} \pm 3.48e^{-04}{}_b$	$5.58e^{-03} \pm 1.07e^{-03}{}_b$

Different lowercase letters indicate significant differences between sun and shade leaves.

The relationship between the SPAD 502-Plus, CCM-300 and chlorophyll concentrations was positive and linear (Figure 7). The SPAD 502-Plus was a better indicator of chlorophyll *a*, chlorophyll *b* and total chlorophyll than the CCM-300, the calibration equations for the SPAD 502-Plus had coefficient of determinations of $r^2 = 0.88$, $r^2 = 0.47$ and $r^2 = 0.83$ for chlorophyll content *a*, *b* and total chlorophyll, respectively (Figure 7 a,c,e). These values were somewhat higher than those measured with the CCM-300, with coefficient of determinations of $r^2 = 0.74$, $r^2 = 0.45$ and $r^2 = 0.73$ for chlorophyll content *a*, *b* and total chlorophyll, respectively (Figure 7 b,d,f).

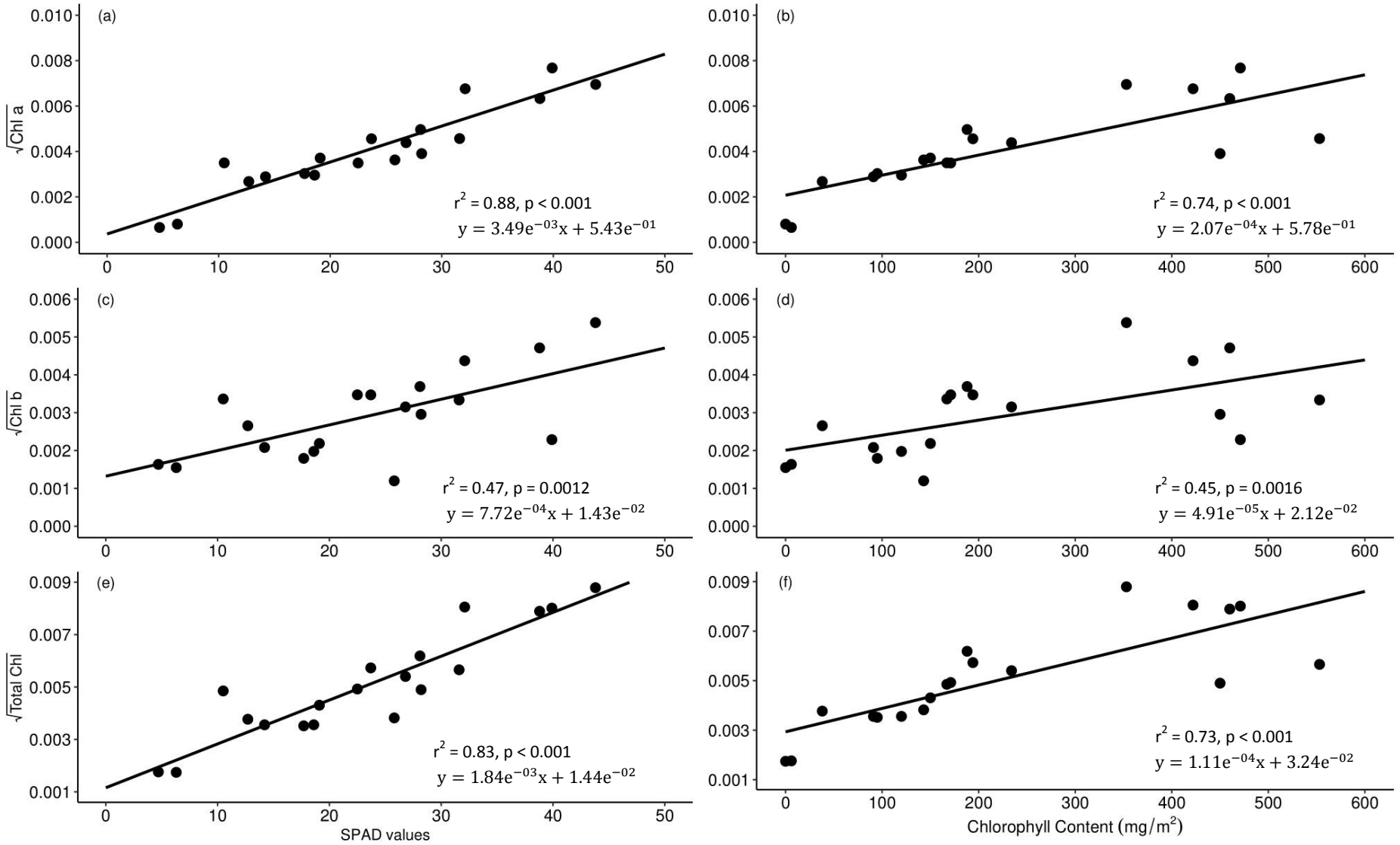

Figure 7. Calibration equations to convert the SPAD and CCM chlorophyll indices to actual chlorophyll content for *Terminalia sericea* seedlings and saplings. The calibration equation converts from SPAD and CCM index values to chlorophyll (Chl *a*, Chl *b* and Total Chl) content.

4.4. Discussion

This study has demonstrated that both seedlings and saplings of *T. sericea* were able to completely compensate for 50% leaf herbivory damage at the leaf cluster-level. Photosynthetic responses of *T. sericea* to shading differed with plant stage. This was observed in chlorophyll content, measured using the SPAD-502 Plus, and stomatal conductance results, where shading had a significant effect on these photosynthetic responses in the seedlings but not in the saplings. Saplings were more shade tolerant than seedlings, because shading did not have a significant effect on chlorophyll content (measured using the SPAD-502 and CCM-300) and stomatal conductance, and saplings in the shade were less stressed (high F_v/F_m) than those in the sun (Minotta and Pinzauti 1996; Boege and Marquis 2005; Barton and Koricheva 2010; Boege *et al.*2011; Koricheva and Barton 2012; Barton 2013). The ability of the different plant stages to compensate for herbivory damage may be influenced by three other factors: (1) the distribution of the herbivory damage, where most of the herbivory was on one half of the leaf blade and the other half left untouched, as well as the un-punctured midrib vein, therefore not having a large influence on leaf functionality, (2) possible genetic differences between the two plant stages as they were purchased from different nurseries, and (3) soil type (and pot size) which influences water and nutrient availability to the different plant stages that could compensate differently for herbivory damage. However, possible genetic and soil type differences do not negate the effect that removing half the leaf blade has on leaf functionality, as the un-punctured midrib vein may play a significant role in leaf functionality by possibly reducing water and nutrient loss compared to when the midrib vein is punctured.

4.4.1. Natural herbivory

Saplings in full sunlight were able to compensate for herbivory by *P. imitata* larvae, expressed as similar chlorophyll content and stomatal conductance in the herbivory treatment and control leaf clusters over time. However, there was limited plasticity in these physiological traits, with none of these traits showing a significant change in response to herbivory across the study period. Only chlorophyll content measured with the SPAD 502-Plus and CCM-300 varied in response, although not significantly, leading to a tendency for chlorophyll content to increase in control leaves and decrease in herbivory treatment leaves three days after herbivory. Stomatal conductance has also been used as a proxy for photosynthetic rate; an increase in stomatal conductance results in an increase in photosynthetic rate in general (Heichel and Turner 1983; Chapin *et al.* 1987; Fay 1993) and more specifically in *T. sericea* adult plants in the field (Whitecross 2017). Therefore, the increase in stomatal conductance of herbivory treatment

leaves compared to control leaves, although not significant, is an indication of compensatory adjustment in photosynthetic rate (Heichel and Turner 1983; Senock *et al.* 1991; Moyo 2014). Compensatory increases in photosynthesis have been shown as a mechanism for alleviating the negative effects of herbivory in many common savanna tree species, such as *Acacia nigrescens*, *Combretum apiculatum* (du Toit 1990; Rooke and Bergström 2007) and *T. sericea* (Moyo 2014).

4.4.2. Simulated herbivory

Chlorophyll content, stomatal conductance and F_v/F_m were not significantly different between herbivory treatment and control leaf clusters in either seedlings or saplings. This indicates that both plant stages were able to fully compensate for 50% simulated herbivory at the leaf cluster-level within 2 weeks of defoliation. It is suggested that the lack of difference in photosynthetic responses between herbivory treatment and control leaves may be due to resources being shunted from the adjacent control leaf into the herbivory treatment leaf in order to compensate for leaf area loss, and the overall low herbivory treatment applied. The shunting of resources from source to sink areas is a common mechanism used by plants in order to compensate for herbivory damage (*reviewed by* Koricheva *et al.* 1998; Throop 2005; Katjiua and Ward 2006; Yoshizuka and Roach 2011; Erwin *et al.* 2014).

All photosynthetic parameters were high at the beginning of the season but decreased towards the end of the season, which is common in many deciduous plant species, and is used as a means of reallocating resources, known as reabsorption, from the leaves to other plant organs (e.g. stems, shoots and roots) before leaf senescence at the onset of the dry cold season (Kershaw and Webber 1986; Demarez 1999; Mishra *et al.* 2013; Devmalkar *et al.* 2014; Sheik *et al.* 2017; Whitecross 2017). This enables plants growing in a nutrient limited system (such as broad-leaved savannas) to conserve resources for the next growing season (Huntley and Morris 1982). Chlorophyll pigment concentrations tend to be higher in summer and lowest in winter, this is a common trend in many plant species across different functional types (Lewandowska and Jarvis 1977; Sheik *et al.* 2017). This has also been shown and in *T. sericea* adults in the field (Whitecross 2017), suggesting that chlorophyll content is influenced by seasonality which is observed with a change in temperature, rainfall and day length (Sheik *et al.* 2017). The high chlorophyll content at the beginning of the season increases the photosynthetic capacity of the plant and thus increases carbon gain, which can be used for growth. Oh and Koh (2014) reported that F_v/F_m and stomatal conductance of tea plants (*Camellia sinensis* L.) were highest during summer and decreased as the season progressed, reaching their lowest level in the

winter months, they also attributed these findings to differences in precipitation, atmospheric vapour pressure and temperature. Junker and Ensminger (2016) reported that leaf chlorophyll content and F_v/F_m of *Acer saccharum* leaves decreased from summer to autumn, indicating a decrease in photosynthetic efficiency.

4.4.2.1. Chlorophyll content response to shading

Shading had a significant effect on chlorophyll content in seedlings but not in saplings when measured with the SPAD-502 Plus, however, the effect of shading was not significant in seedlings and saplings when measured with the CCM-300. The difference in chlorophyll content results between the SPAD-502 Plus and CCM-300 suggests that the SPAD readings may be more affected by environmental light conditions. Nauš *et al.* (2010) reported that SPAD readings are affected by environmental light conditions due to chloroplast movement. Xiong *et al.* (2015) showed that SPAD readings are also affected by nutrient availability and leaf characteristics, when studying rice and soybean. Chlorophyll content, measured using the SPAD-502 Plus, in seedlings was higher in the shade than in the sun, these are characteristic of shade acclimated plants (Jarvis 1964; Ziegenhagen and Kausch 1995; Gross *et al.* 1996; Pons *et al.* 2001). This suggests that juvenile stages of *Terminalia sericea* are able to tolerate shading by altering the leaf chlorophyll content.

4.4.2.2. Stomatal conductance response to shading

The shading treatment had a significant effect on stomatal conductance in seedlings, however this was not the case in saplings. Mediavilla and Escudero (2003) reported that stomatal conductance decreased with advancing plant stage. They observed that stomatal conductance was higher in *Quercus ilex* seedlings than adults, both in favourable and unfavourable conditions. *Terminalia sericea* seedlings had a higher stomatal conductance in sun compared to shade leaves (Table 1) (Figure 5). Sellin *et al.* (2008) also reported that in silver birch (*Betula pendula*), a high light intensity resulted in an increase in stomatal conductance. The watering regime in this study must have made growing conditions favourable, allowing for plants to keep their stomata open more frequently in the sun with water not being as scarce a resource as in the field in semi-arid savannas, where rainfall is highly seasonal. Therefore, water was not a limiting resource in the greenhouse environment because seedlings were able to maintain stomatal conductance, suggesting that there was no trade-off to conserve water in full sunlight, where the vapor pressure deficit could be high due to heat from the sun (Favaretto *et al.* 2011; Zhang *et al.* 2015).

4.4.2.3. Maximum photochemical efficiency of PS*II* in response to shading

Maximum photochemical efficiency of *PSII* measures the efficiency of photosystem II under normal conditions. Björkman and Demmig (1987) reported that most healthy plant species have an optimal F_v/F_m value of 0.83. A F_v/F_m value lower than this tends to indicate different levels of plant stress and in some instances demonstrating photoinhibition (a decrease or loss in photosynthetic functioning of PS*II*) which can be seen as a decline in leaf F_v/F_m at low light intensities (Osmond 1994) in stressful conditions (Eastman and Camm 1995; Maxwell and Johnson 2000; Baker and Rosenqvist 2004). *Terminalia sericea* shade seedlings and saplings had higher F_v/F_m compared to sun seedlings and saplings, however, the F_v/F_m values were lower than the maximum value of 0.83 (Björkman and Demmig 1987); suggesting that in essence all the plants were under a degree of stress (Björkman and Demmig 1987; Gross *et al.* 1996; Thiele *et al.* 1998; Cerrillo *et al.* 2004; Oizounis *et al.* 2015). These low F_v/F_m values between sun and shade leaves from the beginning to the end of the growing season may either indicate protective measures (Demmig and Björkman 1987; Gonçalves *et al.* 2001) and/or reveal photodamage to PS*II* (Powles 1984; Baker 2008) in sun leaves, thus resulting in photoinhibition or non-photochemical quenching processes (Murchie and Lawson 2013; Cowie *et al.* 2016). However, Epron *et al.* (1992) and Gross *et al.* (1996) showed that this process is reversible and is used as a means to protect PS*II* from permanent damage.

4.4.3. Relationships between chlorophyll indices and measured chlorophyll content

Leaf chlorophyll content has been associated with dry matter production (Ghosh *et al.* 2004) and photosynthetic rate (Mao *et al.* 2007). Chlorophyll content measured using the SPAD-502 Plus was not significantly different between sun and shade leaves, however, chlorophyll content measured using the CCM-300 was higher in shade than sun leaves. Differences in the results between the two hand-held devices could be due to differences in the (1) ratio vegetation index (near infrared/red radiances) where the SPAD-502 Plus has a larger range than the CCM-300, (2) leaf area measured where the CCM-300 measured a larger area (0.28 cm^2) than the SPAD-502 Plus (0.06 cm^2) and (3) that readings were taken on detached leaves. These results suggest that SPAD-502 Plus readings are not only affected by light conditions, but rather that long term monitoring of chlorophyll content in different light conditions have an effect on SPAD-502 Plus readings. Studies have shown that time of measurement, irradiance, and leaf water status may affect the accuracy of SPAD measurements (Hoel and Solhaug 1998; Martínez and Guimet 2004). Chlorophyll *a*, *b* and total chlorophyll were significantly higher in shade compared to sun leaves. Low concentrations of chlorophyll in sun leaves may reflect pigment damage (Shao *et al.*

2014). This suggests that high light levels may impair photosystem function (Masarovico and Minorcic 1984; Masarovico and Stefanick 1990; Minotta and Pinzauti 1996; Hawkins *et al.* 2009; Favaretto *et al.* 2011; Shao *et al.* 2014). Under irradiance chlorophyll is synthesized and degraded (photo-oxidation), however at high irradiance degradation occurs more rapidly, resulting in lower chlorophyll concentrations as observed (Broadman 1977; Gonçalves *et al.* 2001). In addition, the differences in chlorophyll content between sun and shade leaves may be a strategy for sun or shade acclimation which includes other mechanisms such as a smaller leaf size in shade leaves and thinner and larger SLA in sun leaves (*see* Chapter 3) (Favaretto *et al.* 2011). These results are characteristic of shade-tolerant plants (Boardman 1977; Reich *et al.* 1998).

As far as we are aware, this is the first study to compare the performance of the latest versions of these two chlorophyll content meters, with a study by Richardson *et al.* (2002) having compared the older versions (SPAD-502 and CCM-200). The relationships between chlorophyll content meter readings and chlorophyll concentrations were all linear, positive and significant. These results are in line with many studies (Cate and Perkins 2003; Wang *et al.* 2004; Kalaji *et al.* 2017), however some studies have reported a non-linear relationship between relative chlorophyll content and actual chlorophyll content (Markwell *et al.* 1995; Richardson *et al.* 2002; Abdelhamid *et al.* 2003; Uddling *et al.* 2007; Hawkins *et al.* 2009; Coste *et al.* 2010; Cerovic *et al.* 2012). The linear trend suggests that the accuracy of the SPAD-502 Plus and CCM-300 is not affected by the change in chlorophyll content (Steele *et al.* 2008). These differences (linear versus non-linear responses) have been attributed to differences in leaf anatomy and the heterogeneity of the distribution of chlorophyll in the leaf (Marquard and Tipton 1987; Fukshansky *et al.* 1993; Sims and Gamon 2002; Hunt and Daughtry 2014; Parry *et al.* 2014), which affects the reflective and absorbance properties of the leaf thus having an impact on the determination of chlorophyll content.

It would be expected that a device that samples a larger area would be more accurate at measuring chlorophyll than a smaller leaf area. However, this study has shown that the SPAD-502 Plus is better at indicating chlorophyll *a* and total chlorophyll, despite it measuring a smaller leaf area, than the CCM-300, as observed in the better and more significant relationships between the SPAD-502 Plus and the direct chlorophyll measurements. These finding are consistent with those of Richardson *et al.* (2002), Pinkard *et al.* (2006), Coste *et al.* (2010), Djumaeva *et al.* (2012) and Kalaji *et al.* (2017), who also showed that the SPAD-502 was

effective at determining foliar chlorophyll content; showing a good relationship between chlorophyll content meter values and actual directly measured chlorophyll content.

Our results also suggest that specific conditions, such as light conditions, may change leaf properties influencing the chlorophyll content values obtained by the different chlorophyll content meters. These findings are in line with those of Sibley *et al.* (1996) who reported that the SPAD-502 is a reliable tool to estimate chlorophyll content in *Acer rubrum* in similar environmental conditions, however, they caution the use of calibration equations on plants grown under different environmental conditions that were not tested. In addition to this, Kalaji *et al.* (2017) reported that nutrient deficiency also had a significant effect on the accuracy of the SPAD-502 and CCM-200 in crop species.

4.5. Conclusions

Terminalia sericea seedlings and saplings were able to compensate for 50% herbivory at the leaf cluster-level. This may be due to the samples not being completely independent of each other, so the control leaf clusters (source area) were able to shunt resources to herbivory treatment leaf clusters (sink area). Therefore, to understand the whole plant effects of herbivory (whether directly by invertebrates, or simulated) the control and herbivory treatments would need to be on separate plants. Under natural field conditions, Yeaton (1988) and Pohjonen (1992) stated that *T. sericea* adult plants performed better in good light conditions. However, my study has shown that *T. sericea* seedlings and saplings are able to tolerate shading by altering the photosynthetic responses (chlorophyll content, stomatal conductance and maximum photochemical efficiency), in order to tolerate shade conditions. This suggests that seedlings and saplings are able to recruit in sun (canopy gaps) and shade (understorey) environments (*see* Chapter 3). This will give them an advantage in order to establish in the highly competitive savanna landscape. The SPAD-502 Plus was more highly significantly correlated to foliar chlorophyll *a* and total chlorophyll (chlorophyll content), which suggests that the ~20% cheaper SPAD-502 Plus is the better instrument to determine chlorophyll content in *T. sericea*.

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Chapter 5: Dimensions, predation and germination of *Terminalia sericea* (Combretaceae) samarae.

Abstract

Terminalia sericea is a multipurpose savanna tree species whose propagation is affected by its extremely low seed viability/germination rate. Knowledge of the physical properties and germinability of *T. sericea* samarae (seeds) is important in attempting to promote and improve its propagation. The aim of the study was to determine the effects of: (1) seed predation on seed dimensions in seeds collected from the Skukuza region of Kruger National Park (Lowveld) and Nylsvley Nature Reserve (Highveld), as well as (2) photoperiod, scarification, soaking and temperature on seed germination. Seeds were collected from the Skukuza region of KNP and Nylsvley Nature Reserve, and seed length, width, thickness and mass were measured as well as geometric mean diameter and surface area calculated. Non-predated seeds from Skukuza were germinated at different photoperiods (8/16 and12/12 light/dark hours), scarification (nicked and not nicked), soaking (control, hot and cold) and temperature (25 and 30°C) treatments. Seeds from the Highveld were longer, wider, heavier and had a larger geometric mean diameter and surface area than seeds from the Lowveld; however, Lowveld seeds were thicker than Highveld seeds. Non-predated seeds had a larger seed length, width, mass, geometric mean diameter and surface area than predated seed. Predated seeds were relatively smaller than non-predated seeds, suggesting that predation occurs during seed development, resulting in smaller seeds. Overall, percentage seed germination was very low, irrespective of treatment. Nicking, soaking in water at room temperature or cold water, incubating at 25°C at a 12/12h (light/dark) photoperiod gave the highest mean germination percentage (5%). Overall, temperature did not have an effect on seed germination, however the seeds soaked at ambient temperature (1%) had a higher germination than seeds soaked in cold (0.875%) and hot (0.375%) water. The highest germination of 1.25% was obtained at a photoperiod of 12/12h compared to 0.25% at a photoperiod of 8/16h. Scarification had a significant effect on germination with a mean ± SE germination of 0.25 ± 0.15% and 1.25 ± 0.32% in not nicked and nicked seeds, respectively. In order to improve *T. sericea* germination, an equal (12/12h) photoperiod, 25°C/18°C germination temperature, nicking, soaking in water at room temperature or cold water are suggested as pre-treatments for germinating *T. sericea*. It is common

knowledge that germination percentage varies in seed crops collected between years in *T. sericea*, thus it is apparent that germination percentage was very low (0 – 5%) in this year's seed crop. Therefore it would be valuable to compare a seed crop from a year of high germination percentage with this seed crop (2016) in order to better understand the germination ecology of this species.

Keywords: Germination, photoperiod, physical properties, soaking, scarification, temperature

5.1. Introduction

In different parts of Southern and Eastern Africa *Terminalia sericea* is used as a source of fuel (firewood and charcoal), to produce fence posts, carvings, construct hand tools and in construction (Coates-Palgrave 1988). In sub-Saharan African it contributes to the browse of many domestic animals and wildlife, particularly during the dry season (Katjiua and Ward 2006). *Terminalia sericea* is also used as an ethnomedicine where the leaf extract is used to manage stomach disorders, and the extracts of the root bark are used to treat bilharzia, colic, diarrhea and pneumonia (Coates-Palgrave 1988); this is mainly due to its antifungal and antibacterial properties (Fyhrquist *et al.* 2004). It provides several ecosystem services such as soil drainage, enriching nutrient poor soils, reducing soil erosion and shading out weeds (Eckman and Deborah 1993). The seeds are used to treat asthenia, candidosis, dermatitis, diabetes, eczema, gonorrhea, leprosy, malaria and scurfy affection (Fyhrquist *et al.* 2004).

Seed physical properties, in particular seed size, have been linked to seedling emergence in *Terminalia bellerica* (Kuniyal *et al.* 2013). Seed size is a contributor to genetic diversity and is a visible evolutionary trait (Aniszewski *et al.* 2001), as well as a fitness trait contributing to successful seedling establishment (Zhang 1998). On the other hand, small seed size is considered to be advantageous because it reduces the visibility of the seed thus making it less vulnerable to predators, which ultimately increases the chances of survival (Moles *et al.* 2003). Seed predators affect plant health and recruitment in many species (Fenner and Thompson 2005). Insect seed predation has been linked to >90% of seed loss before dispersal (Fenner and Thompson 2005). However, the role of seed predation has not been adequately assessed. Ernst *et al.* (1989) found high seed predation rates in African *Acacia* species and low predation rates in *Brachystegia spiciformis*, where *B. spiciformis* were more abundant than *Acacia* species in a miombo woodland.

The determination of seed physical properties is important as it aids in the designing of machines for processing, handling and storage of the seed, which requires some understanding of seed physical properties in order to convert these materials into feed, food, medicine and for germination. Knowledge of seed surface area can be useful in heating, cooling and drying seeds either for germination or storage. Understanding seed physical properties, including the effects of the removal of the seed coat, may be useful to develop a better understanding of its germination, as well as survival and growth of *T. sericea* seedlings in agroforestry systems. Furthermore, these

traits may play an important role in understanding the conservation and propagation of *T. sericea* (Khurana and Singh 2001).

Knowledge on the seed biology is required for the artificial germination and domestication of *T. sericea* (Maghembe *et al*. 1994). Owing to its low germination rate under natural conditions (IITO 2003), there is a need for its adequate propagation and domestication. The low germination rate of *T. sericea* seeds has been attributed to seed dormancy (Hilhorst 1995; Baskin and Baskin 1998), seed predation and predation during germination (Chidumayo 1991, 1992a, 1992b). Several studies on seed dormancy have been conducted over the years aimed at breaking seed dormancy and improving propagation, which have investigated the use of different pre-treatments (Likoswe *et al.* 2008; Amri 2010; Bodede *et al.* 2015).

Few experimental studies have been conducted on the effects of soaking treatments, photoperiod, temperature regime and scarification on seed germination of *T. sericea* (Likoswe *et al*. 2008; Amri 2010). Research that has been done on *T. sericea* has mostly focused on its raw materials for industrial purposes, ethnomedicinal uses, the potential to use the seed as a food ingredient, feed for livestock and as a source of essential oils (Chivandi *et al.* 2008; Chivandi and Erlwanger 2011; Chivandi *et al.* 2011a, 2011b). Very little is known about the effects of seed predation and location on seed physical properties and the interaction of environmental conditions on seed germination in many species including *T. sericea*. Furthermore, owing to the importance of *T. sericea* in providing many uses and ecosystem services, more efforts need to be aimed at propagating this indigenous savanna tree species. Thus, this study aimed to determine: (a) if a relationship between predation and seed dimensions exists, (b) to compare the influence of predation on *T. sericea* seeds in Lowveld and Highveld populations where *T. sericea* commonly occurs, and (c) the germination requirements of *T. sericea* seeds in a range of treatments simulating natural and artificial environmental cues; including varying photoperiods, temperatures, scarification and pre-conditioning treatments.

5.2. Materials and Methods

5.2.1. Study Site

5.2.1.1. Skukuza (Kruger National Park), Mpumalanga

Seeds were collected from the Skukuza region (Lowveld) of Kruger National Park in the hot Lowveld savanna with an altitude of ~300 m above sea level (m.a.s.l.), Mpumalanga, South Africa (S 24° 59' 4''; E 31° 35' 34''). The region is hot semi-arid with a mean annual rainfall of approximately 500 mm with most of the rainfall being concentrated in the mid-summer months (December – February), receiving the lowest and highest rainfall in June and December, respectively. Average maximum temperatures are 31.7°C in January and 24.5°C in July (Kruger *et al.* 2002). The vegetation is characterised by tree species from the Combretaceae (e.g. *Terminalia sericea* and *Combretum apiculatum*) and Mimosaceae (e.g. *Acacia nigrescens*) families which are characteristic of the Mixed Bushveld and Thornveld vegetation types (Acocks 1988).

5.2.1.2. Nylsvley Nature Reserve, Limpopo Province

Seeds were also collected from Nylsvley Nature Reserve (Highveld) located in the warm wet sandy bushveld savanna area (1080m-1155 m.a.s.l.), Limpopo, South Africa (24° 39' 50''S ; 28° 39' 54'' E). The region is warm semi-arid, with a mean annual rainfall of approximately 648 mm which is in the summer months (October – March) and the least rainfall is concentrated in the winter months (Huntley and Morris 1982). The average maximum temperatures range from 22°C in July to 31°C in January (Huntley and Morris 1982). The common soil types in this region are infertile loamy and sandy soils which are derived from Waterberg sandstone (Scholes and Walker 1993). The vegetation is dominated by deciduous trees including *Terminalia sericea* and *Burkea africana* which are characteristic of the Central Sandy Bushveld vegetation type (Rutherford *et al.* 2006).

5.2.2. Study Species

Terminalia sericea Burch ex DC (Combretaceae), also known as the Silver cluster tree, has a wide tropical distribution throughout sub-Saharan Africa; where it is commonly found in miombo woodlands in south, east and central Africa occurring either as a dominant or co-dominant species or scattered in open woodlands (Hitchings *et al.* 1996). It usually grows in close association with *Colophospermum* species, *Acacia* species, *Brachystegia* species and other *Combretum* species (Drummond 1981). *Terminalia sericea* is considered to be drought and saline tolerate, and can

tolerate some degree of frost (Palmer and Pitman 1972; Yeaton 1988; Pohjonen 1992). It thrives in soil types with good drainage but it commonly occurs in deep sandy soil (Coates-Palgrave 1988). *Terminalia sericea* occurs as a tree that grows on average to a height of 6 to 9 m, but some individuals have been known to reach a height of 23 m (Coates-Palgrave 1988). The leaves have silvery hairs and have an obovate-elliptic shape and are borne in clusters at the end of the branch (Drummond 1981). The creamy-white flowers are borne in auxiliary buds. *Terminalia sericea* samarae (seeds) are single-winged and pinkish in colour, becoming darker upon maturity and contain one seed (Likoswe *et al.* 2008; Chivandi *et al.* 2013). Its seeds are considered to be dense in macro-nutrients which make them possible alternative sources of nutrients for humans and animals (Chivandi *et al.* 2008; Chivandi *et al.* 2013).

5.2.3. Experimental Design and Protocol

5.2.3.1. Physical Properties

On the 1st week of October 2016, *T. sericea* samarae (seeds) were randomly collected on the ground under different tree canopies (>5 individuals) in the Skukuza region of Kruger National Park (Lowveld) and air dried. Once air dried the seeds were placed in a box and brought to the University of the Witwatersrand, Johannesburg (October 2016). The seeds were than separated into predated (n = 150) and non-predated (n = 150) seeds (predated: 28%; non-predated: 72%; n = 5167), where predated seeds were classified as having a hole in the seed coat and not intact (indicating that the seed inside may have been affected by an invertebrate) and non-predated seeds were classified as being intact (Figure 1a). On the 10th of February 2017, seeds were also randomly selected from the canopy of 10 *T. sericea* trees in Nylsvley Nature Reserve and placed in brown paper bags, where after they were brought to the University of the Witwatersrand and left to air dry. These seeds were also separated into predated (n = 200) and non-predated (n = 200) seeds (predated: 30%; non-predated: 70%; n = 2226). All the seeds were than numbered using a permanent marker (Figure 1 c - f). The following methods were used to determine some of the physical properties of *T. sericea* seeds.

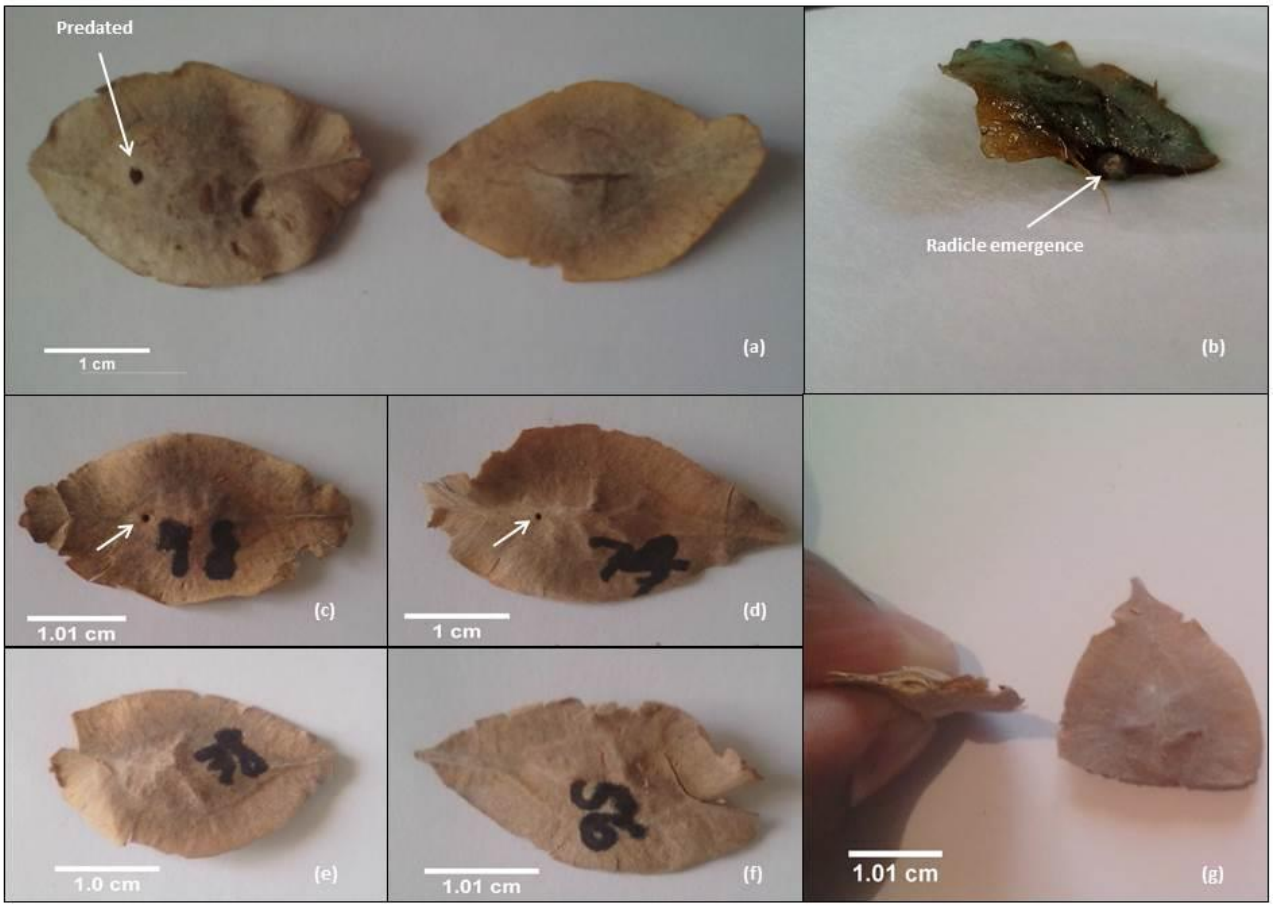

Figure 1. *Terminalia sericea*: (a) Seeds defined as being predated (left) and non-predated (right), (b) emergence of the radicle (germination), (c - d) examples of images used to examine predated seed physical properties, (e - f) examples of images analysed to determine non-predated seed physical properties , and (g) nicking by using a nail clipper.

A 5 cm line was drawn on clear white paper and a photograph of each seed was taken at a 90° angle from the paper (Figure 1 c - f). The computer program Image J was used to measure seed length, width and area. Seed thickness at the thickest point in the center of the seed was measured using Vernier calipers.

The geometric mean diameter (mm) was determined by using the following formula (Mohsenin 1986; Joshi *et al.* 1993):

Equation 1: $D_g = (LWT)^{1/3}$,

where: *L*, the length is the dimension along the longest axis in mm; *W*, the width is the dimension along the longest axis perpendicular to *L* in mm and *T*, the thickness, is the dimension along the longest axis perpendicular to both *L* and *W* in mm. The surface area was determined by using:

Equation 2: $S = \pi D_g^2$,

where D_g is geometric mean diameter. In order to determine seed mass, *M*, seeds were weighed to the nearest 0.001g. Both geometric mean diameter and surface area incorporate seed length, width and thickness, thus acting as parameters to measure seed shape (Gupta and Das 1997; Garnayak *et al.* 2008).

5.2.3.2. Germination Experiment

5.2.3.2.1. Seed Collection and Storage

From the same *T. sericea* seed crop as the seeds collected in Skukuza (only), intact seeds were separated (apart from the ones used to determine seed physical properties) from predated seeds (predated: 28%; intact: 72%; n = 5167). The seeds were left to air dry in a dark place for two weeks under ambient laboratory conditions from mid-October 2016 to end of October 2016.

The experiment was conducted at the University of the Witwatersrand, Oppenheimer Life Sciences Building, Johannesburg, Gauteng, South Africa (S 26° 11' 30"; E 28° 01' 58"). The seeds were exposed to: (1) three soaking treatments: control (water at ambient temperature), cold and hot (2) two photoperiods: 12/12h and 8/16h (light/dark), (3) two scarification treatments: not nicked and nicked, and (4) two thermoperiods: 25°C and 30°C and for both treatments dark temperatures were at ambient temperatures (~18°C) during the study period, equating to 24 sub-treatments. Alternating light and dark (day/night) temperatures are associated with improved germination and seedling growth (ISTA 2006; Muhl 2009). The photosynthetic photon flux density in all the incubators was 102 µmol $m^{-2}s^{-1}$ during the light photoperiod. Four incubators were used for this experiment which were set to: (1) 12/12h at 25/18°C, (2) 12/12h at 30/18°C, (3) 8/16h at 25/18°C, and (4) 8/16h at 30/18°C (Figure 2).

5.2.3.2.2. Seed Soaking

All seeds were surface sterilized using 3% bleach (Jik) and left to soak for 30 minutes and then well rinsed with distilled water. Ice and water were added to a bucket and a glass beaker with 800 seeds

was placed inside the bucket until the water reached ~5°C. The water temperature in the glass beaker was maintained at ~5°C by adding ice into the bucket and the seeds were left to soak for 12 hours. Distilled water was boiled to ~100°C and then removed from the heat. The water (1l) was placed in a beaker together with 800 seeds. Water temperature was monitored using a thermometer, once the temperature of the water temperature reached 25°C the beaker with seeds was placed in an incubator and the temperature of the water maintained at 25°C for the remainder of the 12-hour period. Distilled water at ambient temperature (~25°C) was poured into a glass beaker with 800 seeds. The seeds were soaked at ambient temperature for 12 hours.

5.2.3.2.3. Seed Scarification

Half the seeds from each soaking treatment (n = 1200) were nicked at the micropyle (Figure 1g) using a nail clipper which was sterilized in hot water and then ethanol for each seed; so as to expose the seed from the seed coat (samara). The remainder of the seeds (n = 1200) were left untouched.

5.2.3.2.4. Germination

Ten seeds were placed in 90 cm diameter petri dishes lined with 2 layers of Whatman filter paper at the bottom and top. The seeds were watered with 10 ml of distilled water with Efekto Virikop fungicide and bactericide every morning for the first 20 days and every other day for the last 20 days in order to maintain adequate moisture content and prevent contamination. During watering, germination was observed over the 40 day period from 8 November 2016 to 20 December 2016. Seeds were placed in four incubators set at 25/18°C: 12/12h, 25/18°C: 8/16h, 30/18°C: 12/12h and 30/18°C: 8/16h (light/dark) simulated environmental conditions (Figure 2). Germination was defined as the emergence of the radicle from the seed coat (Figure 1b) (Likoswe *et al.* 2008).

5.2.3.2.4.1. Germination Parameters

Germination parameters were calculated as per Ranal *et al.* (2009). Germinability, is the percentage germination: $G = \sum_{i=1}^{k} n_i/n \times 100$, where n_i: number of seeds germinated at the i^{th}: observation (days), k: last time of germination and n: number of seeds sowed. Germinability is defined as the percentage of seeds germinating (producing a seedling) from the seed population. Mean germination time was calculated using the following expression: $MT = \sum_{i=1}^{k} n_i\, t_i / \sum_{i=1}^{k} n_i$, where t_i: time from the beginning of the experiment. Mean germination time is the average time it

takes for each seed to germinate. The coefficient of variation of the germination time was calculated by using the following expression: $CV_t = \frac{S_t}{MT} \times 100$, where S_t is the standard deviation of the germination time calculated using the following equation: $\sqrt{\sum_{i=1}^{k}(t_i - MT)^2 / \sum_{i=1}^{k} n_i - 1}$. Mean germination rate was calculated as the reciprocal of the mean germination time ($\frac{1}{MT}$). The uncertainty of the germination process was calculated using the following expression: $U = -\sum_{i=1}^{k} f_i \log_2 f_i$, where $f_i = n_i / \sum_{i=1}^{k} n_i$. Synchrony of the germination process was calculated by using the following expression: $Z = \sum_{i=1}^{k} C_{n_i,2} / C_{\sum n_i,2}$, whereby $C_{n_i,2} = n_i(n_i - 1)/2$ and $C_{n_i,2}$: is the combination of seeds germinated at the i^{th} time.

In each sub-treatment 10 seeds were placed in one petri dish with 10 petri dishes for each sub-treatment, and hence a 100 seeds for each of the 24 sub-treatments. Each germination measurement was conducted for each petri dish and the mean, median, standard deviation (SD) and standard error of the mean (SE) of all ten petri dishes for all the sub-treatments was calculated.

5.2.3.3. Data analysis

The R package *ggplot2* (Wickham 2009) was used to produce graphical representations of the data. Statistical analyses were done in the statistical program R ver. 3.4.2 (R Core Team 2017). All data was tested for normality using the Shapiro-Wilk test (Giraudoux 2017), and all data were found to be non-parametric therefore non-parametric tests were used. General linear model (GLMs) with a Tukey post hoc analysis from the *emmeans* package in R (Lenth 2017) was used to compare differences between and within predation treatments and sites. The influence of the soaking treatments (control, cold and hot) on germination were analysed using a Kruskal-Wallis test. Wilcoxon sign rank tests were run to determine the effects of photoperiod, temperature and scarification on percentage seed germination. Linear mixed effects models were conducted on pre- and incubator treatments to determine the influence of these treatments on percentage germination.

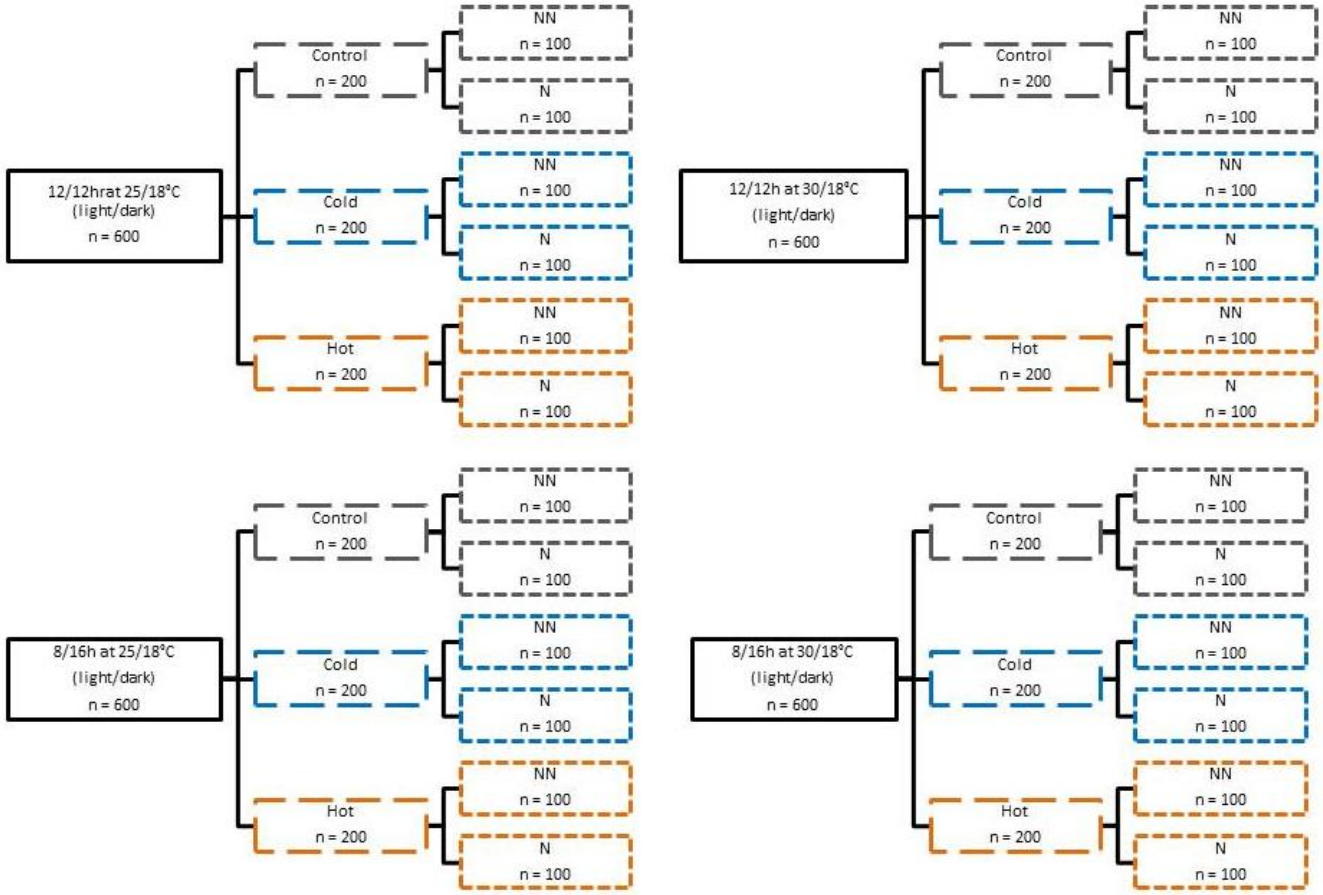

Figure 2. Factorial design of how seeds were germinated at two photoperiods (12/12h and 8/16h (light/dark)), two temperatures (25 and 30°C), three soaking treatments (control (ambient temperature), cold and hot water soaksoakinging) and two scarification treatments (not nicked and nicked). The grey boxes indicate seeds soaked in water at ambient temperature, the blue indicate seeds soaked in cold water and the orange boxed indicate seeds soaked in boiling water whose temperature reduced over time. “NN” and “N” indicate seeds that were not nicked and nicked, respectively. All seeds were soaked for 12 hours.

5.3. Results

5.3.1. Physical Properties

Seeds from Nylsvley were 24% longer and 8% wider than seeds from Skukuza (Table 1) (Figure 3a,b). Geometric mean diameter and surface area did not differ between seeds collected from Nylsvley and Skukuza (Table 1) (Figure 3d,e). However, seeds from Skukuza were 24% thicker and 32% heavier than seeds from Nylsvley (Table 1) (Figure 3c,f). Overall, non-predated seeds were 2% longer, 4.6% wider, 0.9% thicker, had a 2.7% larger geometric mean diameter, 5.1% larger surface area and were 3.9% heavier than predated seeds (Table 1). There were significant interactions between site by predation on seed length, width, thickness, geometric mean diameter, surface area and mass (Table 1).

Seed length, width, thickness, geometric mean diameter, surface area and mass were not significantly different between non-predated and predated seeds from Skukuza ($p > 0.05$). Non-predated seeds were significantly longer, wider, thicker, had a larger geometric mean diameter and surface area and were heavier than predated seeds from Nylsvley, with a mean difference of 1.82 mm, 1.69 mm, 0.16 mm, 0.87 mm, 0.72 cm^2 and 0.01 g, respectively ($p < 0.05$). This suggests that seed predation occurs later in seed development in Skukuza compared to Nylsvley.

Table 1. The statistical outputs of general linear models for physical properties of *Terminalia sericea* seeds. Factor variables describe "Predation" as predated or unpredated seeds and "Site" as seeds collected either at Skukuza or Nylsvley.

Physical Properties	Factor Variables	t-value	S.E.	p-value	Significance
Length	Predation	3.647	0.013	< 0.001	***
	Site	-15.072	0.014	< 0.001	***
	Predation x Site	-3.658	0.019	< 0.001	***
Width	Predation	7.198	0.011	< 0.001	***
	Site	-2.759	0.012	0.006	**
	Predation x Site	-5.025	0.017	< 0.001	***
Thickness	Predation	3.412	0.014	< 0.001	***
	Site	18.922	0.015	< 0.001	***
	Predation x Site	-3.697	0.021	< 0.001	***
Geometric mean diameter	Predation	6.298	0.010	< 0.001	***
	Site	1.900	0.011	0.058	NS
	Predation x Site	-5.483	0.015	< 0.001	***
Surface area	Predation	6.086	0.020	< 0.001	***
	Site	1.625	0.021	0.105	NS
	Predation x Site	-5.398	0.030	< 0.001	***
Mass	Predation	3.926	0.023	< 0.001	***
	Site	15.097	0.025	< 0.001	***
	Predation x Site	-2.896	0.035	0.004	**

NS, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

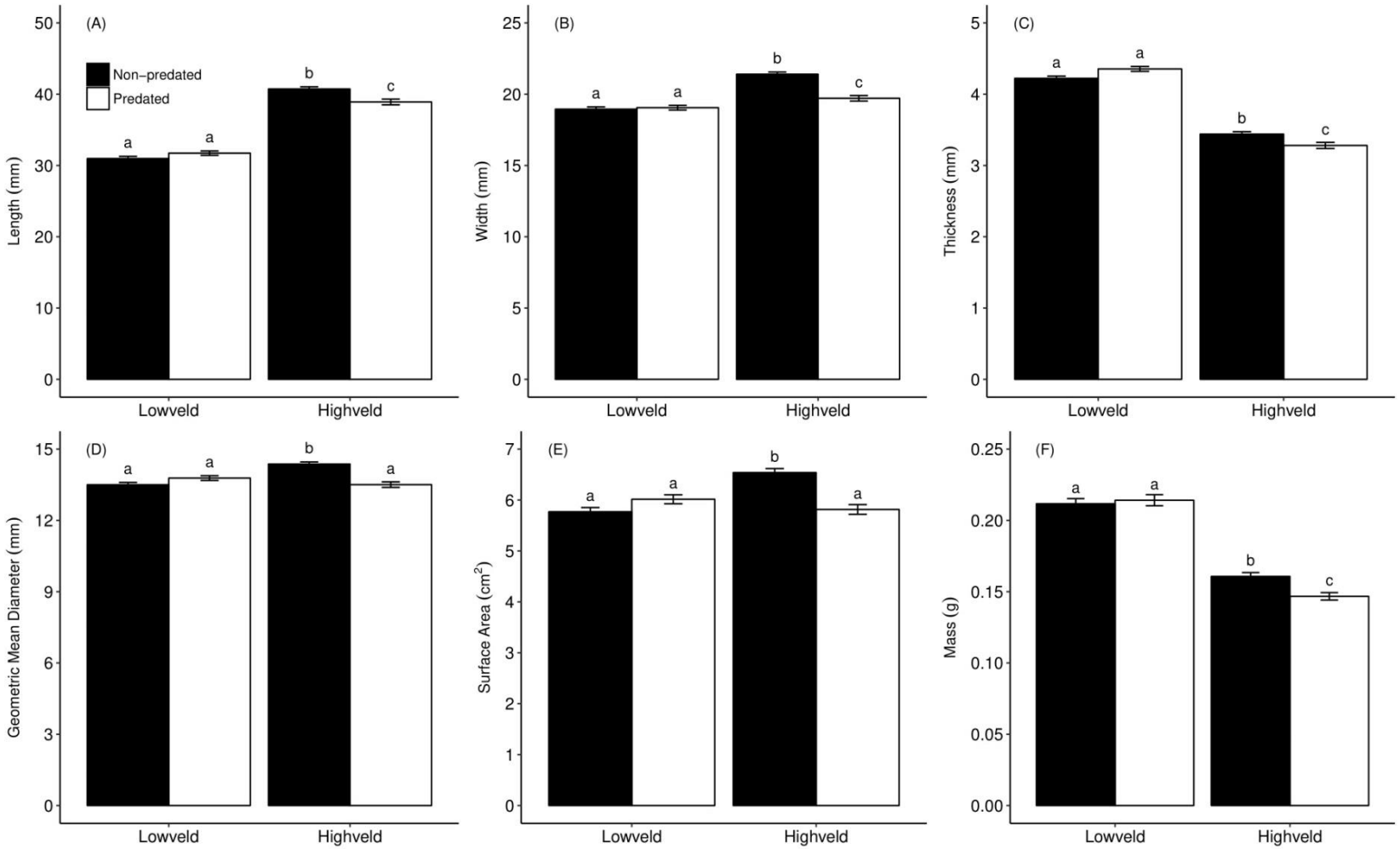

Figure 3. Mean ± SE of seed (A) length, (B) width, (C) thickness, (D) geometric mean diameter, (E) surface area and (F) mass of non-predated and predated *Terminalia sericea* seeds from Skukuza (Lowveld) and Nylsvley Nature Reserve (Highveld). Different lowercases indicate significant differences between the treatments (Tukey HSD: $p < 0.05$).

5.3.2. Germination

Of the 24 sub-treatments, 11 of these sub-treatments resulted in at least one seed germinating (Table 1). Nicking, soaking in water at ambient temperature (control) and germinating at 25°C with a 12/12h photoperiod yielded the highest germinability (mean ± SE: 5 ± 2.69 %). The least germinability was obtained for seeds soaked in the hot water soaking treatment, not nicked, and/or germinated at a short day length (8/16h) or at 30°C.

Seeds soaked in cold water, not nicked and germinated at 30°C with a 12/12h photoperiod had the lowest mean germination time (*MT*) (mean: 6 days) of the seeds that germinated, with germination occurring faster than in the other sub-treatments. Seeds soaked in hot water, nicked and germinated at 30°C with a 12/12h photoperiod took the longest amount of time to germinate (mean: 26.5 days); the coefficient of variance for the germination time (CV_t) was 29.3%. This was followed by seeds soaked in cold water, nicked and germinated at 30°C with a 12/12h photoperiod with a *MT* of 21 days (mean). The pooled sub-treatments had a *MT* of 1.31 ± 0.31 days (mean ± SE) for treatments where germination actually did occur, the CV_t for germination time was 9.78 ± 1.12% (mean ± SE).

Of the seeds that germinated, seeds soaked in hot water, nicked and germinated at 30°C with a 12/12h photoperiod had the lowest mean germination rate (*MR*) (mean: 0.04 $days^{-1}$). The highest *MR* was for seeds soaked in cold water, not nicked and germinated at 30°C with a 12/12h photoperiod (mean: 0.17 $days^{-1}$). The combined sub-treatments had a *MR* of 0.12 ± 0.0 $days^{-1}$ (mean ± SE).

Seeds soaked in hot water, nicked and germinated at 30°C with a photoperiod of 12/12h had the lowest uncertainty (*U*) indicating that germination was more concentrated in time (mean: -1 bit). Synchrony could only be determined in two sub-treatments: (1) with seeds soaked in hot water, nicked and germinated at 30°C with a photoperiod of 12/12h indicating no overlap in germination (mean: 0) and (2) seeds soaked in water at ambient temperature, nicked and germinated at 25°C with a photoperiod of 12/12h indicating some degree of overlap in germination (mean ± SE: 1 ± 0).

Although germination did occur in 11 of the sub-treatments the median germinability for all the sub-treatments and the pooled sub-treatments was 0% (Table 2), while all the other parameters were similar to the mean (Table 1). Germinability, mean germination time, coefficient of variation of the germination time, mean germination rate, uncertainty and synchrony was; 0 ±

0.31%, 0 ± 4.64 days, 0 ± 16.95%, 0.14 ± 0.05 days^{-1}, 0 ± 0.24 bit and 1 ± 0.58 (median ± SD), respectively for the pooled sub-treatments.

For pooled treatments, temperature did not have an effect on seed germination with 10 and 8 seeds germinating at 25°C and 30°C, respectively (Wilcoxon Test: W = 718800, p = 0.6363) (Figure 4A). The duration of the photoperiod had a significant effect on seed germination with 1.25% and 0.25% of the seeds germinating at 12/12h and 8/16h, respectively, when comparing germination across all treatments (Wilcoxon Test: W = 727200, p = 0.0045) (Figure 4B). Overall, the soaking treatments did not have an effect on germination of *T. sericea* seeds (Kruskal-Wallis: H = 2.35, df = 2, p = 0.3088), with 1%, 0.875% and 0.375% of the seeds germinating after the control, cold and hot soaking treatments, respectively (Figure 4C). Germination was higher for nicked seeds (mean ± SE: 1.25 ± 0.32%) than seeds that were not nicked (mean ± SE: 0.25 ± 0.15%) across all treatments (Wilcoxon Test: W = 727200, p = 0.0045) (Figure 4D).

Linear mixed effects models fit by maximum likelihood showed that the preconditioning and scarification treatments as well as the temperature and photoperiod did not have a significant effect on percentage germination (Table 3). In both the pre- and incubator treatments the percentage of seeds germinated decreased over the 40 day germination period, with the first seed germinating after 6 days and the last after 21 days. These results are contradictory to those found using either the Kruskal Wallis test or Wilcoxon Sign Ranked tests, indicating that germination time has a significant effect on the ability of pre- and incubator treatments to affect *T. sericea* seed germination.

Table 3. Statistical output of linear mixed effects models fit by maximum likelihood for the seeds pre-treatments and incubator treatments.

Treatments	Factor Variables	t-value	S.E.	p-value	Significance
Pre-treatments	Preconditioning	-	-	> 0.05	NS
	Scarification	-1.88789	0.00057	0.06030	NS
	Germination Time	-2.54001	0.00005	0.01110	*
Incubator Treatments	Temperature	-	-	> 0.05	NS
	Photoperiod	-1.88789	0.00114	0.0603	NS
	Germination Time	-2.54001	0.00005	0.01110	*

NS, not significant; *, p < 0.05.

Table 1. Germination measurements (mean ± SE) of *Terminalia sericea* samarae collected at Skukuza, 2016. (*G*: germinability, MT: mean germination time, CV_t coefficient of variation of the germination time, MR: mean germination rate, *U*: uncertainty, and *Z*: synchrony).

Treatments	*G* (%)	MT (day)	CV_t (%)	MR ($\mathbf{day^{-1}}$)	*U* (bit)	Z
Control Not Nicked 12/12 25°C	0 ± 0	0 ± 0				
Control Nicked 12/12 25°C	5 ± 2.7	7 ± 0	0 ± 0	0.1 ± 0.0	-0.2 ± 0.1	1 ± 0
Cold Not Nicked 12/12 25°C	0 ± 0	0 ± 0				
Cold Nicked 12/12 25°C	3 ± 1.5	15 ± 4.7		0.1 ± 0.0		
Hot Not Nicked 12/12 25°C	0 ± 0	0 ± 0				
Hot Nicked 12/12 25°C	1 ± 1	20		0.1	0	
Control Not Nicked 12/12 30°C	1 ± 1	7		0.1	0	
Control Nicked 12/12 30°C	1 ± 1	8		0.1	0	
Cold Not Nicked 12/12 30°C	1 ± 1	6		0.2	0	
Cold Nicked 12/12 30°C	1 ± 1	21		0.1	0	
Hot Not Nicked 12/12 30°C	0 ± 0	0 ± 0				
Hot Nicked 12/12 30°C	2 ± 2	26.5	29.4	0.0	-1	0
Control Not Nicked 8/16 25°C	0 ± 0	0 ± 0				
Control Nicked 8/16 25°C	0 ± 0	0 ± 0				
Cold Not Nicked 8/16 25°C	1 ± 1	7		0.1	0	
Cold Nicked 8/16 25°C	0 ± 0	0 ± 0				
Hot Not Nicked 8/16 25°C	0 ± 0	0 ± 0				
Hot Nicked 8/16 25°C	0 ± 0	0 ± 0				
Control Not Nicked 8/16 30°C	0 ± 0	0 ± 0				
Control Nicked 8/16 30°C	1 ± 1	8		0.1	0	
Cold Not Nicked 8/16 30°C	0 ± 0	0 ± 0				
Cold Nicked 8/16 30°C	1 ± 1	7		0.1		
Hot Not Nicked 8/16 30°C	0 ± 0	0 ± 0				
Hot Nicked 8/16 30°C	0 ± 0	0 ± 0				
Pooled sub-treatments	0.78 ± 0.21	1.31 ± 0.31	9.78 ± 1.12	0.12 ± 0.0	-0.07 ± 0.02	0.67 ± 0.04

Table 2. Germination measurements (median ± SD) of *Terminalia sericea* samarae collected at Skukuza, 2016. (*G*: germinability, MT: mean germination time, CV_t coefficient of variation of the germination time, MR: mean germination rate, *U*: uncertainty, and *Z*: synchrony).

Treatments	***G* (%)**	**MT (day)**	CV_t (%)	**MR ($\mathbf{day^{-1}}$)**	***U* (bit)**	**Z**
Control Not Nicked 12/12 25°C	0 ± 0	0 ± 0				
Control Nicked 12/12 25°C	0 ± 8.5	7 ± 0	0 ± 0	0.1 ± 0	-0.2 ± 0.3	1 ± 0
Cold Not Nicked 12/12 25°C	0 ± 0	0 ± 0				
Cold Nicked 12/12 25°C	0 ± 4.8	7 ± 14.7		0.1 ± 0.1		
Hot Not Nicked 12/12 25°C	0 ± 0	0 ± 0				
Hot Nicked 12/12 25°C	0 ± 0	20		0.05	0	
Control Not Nicked 12/12 30°C	0 ± 3.2	7		0.1	0	
Control Nicked 12/12 30°C	0 ± 3.2	8		0.1	0	
Cold Not Nicked 12/12 30°C	0 ± 3.2	6		0.2	0	
Cold Nicked 12/12 30°C	0 ± 3.2	21		0.1	0	
Hot Not Nicked 12/12 30°C	0 ± 0	0 ± 0				
Hot Nicked 12/12 30°C	0 ± 6.3	26.5	29.4	0.0	-1	0
Control Not Nicked 8/16 25°C	0 ± 0	0 ± 0				
Control Nicked 8/16 25°C	0 ± 0	0 ± 0				
Cold Not Nicked 8/16 25°C	0 ± 3.2	7		0.1	0	
Cold Nicked 8/16 25°C	0 ± 0	0 ± 0				
Hot Not Nicked 8/16 25°C	0 ± 0	0 ± 0				
Hot Nicked 8/16 25°C	0 ± 0	0 ± 0				
Control Not Nicked 8/16 30°C	0 ± 0	0 ± 0				
Control Nicked 8/16 30°C	0 ± 3.16	8		0.13	0	
Cold Not Nicked 8/16 30°C	0 ± 0	0 ± 0				
Cold Nicked 8/16 30°C	0 ± 3.16	7		0.14		
Hot Not Nicked 8/16 30°C	0 ± 0	0 ± 0				
Hot Nicked 8/16 30°C	0 ± 0	0 ± 0				
Pooled sub-treatments	0 ± 3.14	0 ± 4.64	0 ± 16.95	0.14 ± 0.05	0 ± 0.24	1 ± 0.58

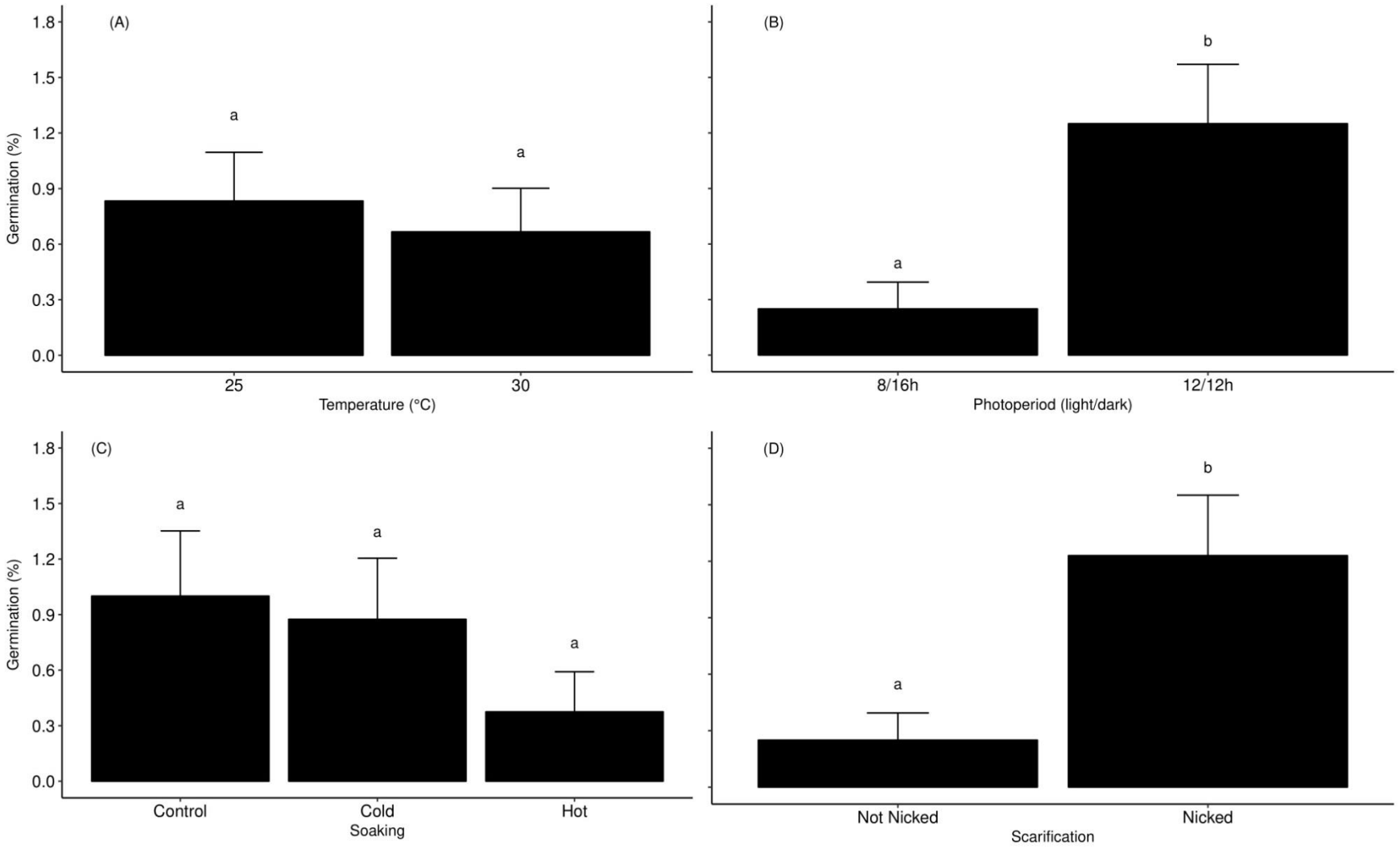

Figure 4. The effect of (A) temperature, (B) photoperiod, (C) soaking and (D) scarification on overall germination (means ± SE) of *Terminalia sericea*. Different lowercase letters indicate significant differences between the germination percentage and the independent variable (Kruskal Wallis: $p < 0.05$).

5.4. Discussion

5.4.1. Physical properties

The influence of site on seed physical properties in *T. sericea* was apparent. Seeds from Nylsvley were 24% longer and 8% wider than Skukuza, however geometric mean diameter and surface area were not significantly different between the two sites (Figure 3a,b,d,e). Seeds from Skukuza were 24% thicker and 32% heavier than in Nylsvley (Figure 3c,f). These results suggest that: (1) *T. sericea* seed shape is not influenced by seed collection site, and (2) although seeds from Nylsvley are longer and wider, the embryo of seeds collected in Skukuza maybe larger than that of Nylsvley seeds, this maybe of ecological value as Skukuza is relatively drier than Nyslvley. Studies have reported that larger seeds have a tendency to be found in drier habitats (Schimpf 1977, Sorenson and Miles 1978, Stromberg and Pattern 1990). In addition, larger seeds are have been associated with greater amounts of carbohydrates in their cotyledons or endosperms than smaller seeds and have more nutrients and mobilisable reserves which provide an advantage for initial growth until the seedling becomes autotropic; also enabling the plant to grow faster (Hewitt 1998; Mwase and Mvula 2011), this may be particularly vital in resource poor environments. However, the factors contributing to differences in seed traits between the two sites cannot be clearly stated as these sites are ecologically different; owing to the difference in site altitude, longitude, soil types, fauna, and flora as well as invertebrate species diversity and climate.

Overall, non-predated seeds had significantly higher physical property values than predated seeds. Non-predated seeds were 2% longer, 5% wider, 0.9% thicker and 3.9% heavier, and had a 2.7% and 5.1% larger geometric mean diameter and surface area, respectively. In the field (Nylsvley Nature Reserve) *T. sericea* seeds were observed to be predated on the plant rather than on the ground causing the seeds to become contoured (T. Twala *pers. obs.).* This is a common phenomenon in many seed predators, thus delayed seed abscission or collection may affect seed mortality in turn affecting seed viability (Hossain *et al.* 2005). However, in Skukuza which is relatively hotter than Nylsvley there were no differences in seed physical properties between predated and non-predated seeds. This suggests that: (1) seed predation occurs earlier in seed development in Nylsvley compared to Skukuza, (2) there are different species of seed predators (generalists and/or specialists) predating on seeds at the different sites, and (3) other biological, physiological,

environmental, geographical and topographical factors or genetic differences may affect seed predation in these sites.

5.4.2. Germination

5.4.2.1. Interaction of pre-treatments, temperature and photoperiod

The results in this study showed the importance of temperature, photoperiod length, and pre-treatment of seeds in the form of soaking and scarification on their ability to influence seed germination. Germination varied among the different sub-treatments with the vast majority (99% of 2400) of the seeds not germinating. The highest germination was 5% in the seeds soaked in water at room temperature (control), nicked and germinated at 25°C at a photoperiod of 12/12h, making it the best sub-treatment. This combination of pre-treatments and conditions are supported by findings by Likoswe *et al.* (2008) and Amri (2010). Likoswe *et al.* (2008) reported that *T. sericea* seeds soaked in water at ambient temperature germinated better than seeds soaked in hot water; they also showed that nicking improved germination than not nicking. Amri (2010) found that *T. sericea* seeds germinated at 25°C had the highest germination (35%) suggesting that the optimum temperature to germinate *T. sericea* was 25°C and that seeds germinated at a photoperiod of 12/12h had the highest germination percentage (33%) compared to photoperiods of 4/20h, 8/16h, 16/8h and 0/24h (light/dark).

5.4.2.2. Temperature

The high overall germination percentage of seeds incubated at 25/18°C (0.833 ± 0.263%) compared to 30/18°C (0.667 ± 0.235%) (Figure 4A) , although not significant, is supported by those of Muhl (2009) who found that *Moringa oleifera* seeds germinated at 25/15°C and 20/10°C germinated faster than those germinated at a high temperature (30/20°C). This suggests that moderate temperatures (20-25°C) are sufficient for germinating woody species, including *Typha latifolia, M. oleofera* (Lombardi *et al.* 1997; Muhl 2009) and *T. sericea*. These findings are not surprising because according to Washitani and Saeki (1986) temperature is one of the most important environmental factors affecting germination rate. The optimum temperature to germinate *T. sericea* is the same in many tropical species (Everham *et al.* 1996; Valio and Scarpa 2001). Where, sensitivity to environmental conditions such as temperature increases the chances of seed germination and seedling survival (Ramirez-Padila and Valverde 2005). Amri (2010) also reported that a temperature

above the optimum temperature reduced and in some instances prevented seed germination in *T. sericea*. High temperatures have been known to prevent the elongation of the radicle and shoot by inhibiting nucleic acid and protein synthesis (Sivaramakrishnan *et al.* 1990).

5.4.2.3. Photoperiod

Photoperiod had a significant effect on *T. sericea* seed germination with 1.25 ± 0.32% and 0.25 ± 0.14% of the seeds germinating at a photoperiod of 12/12h and 8/16h, respectively (Figure 4B). Photoperiod affects seed germination due to a difference in light quality, fluency, direction and duration. This is a common trait in plants due to their ability to monitor these traits in order to adjust their reproduction and development to daily and seasonal changes. A photoperiod of 8 to 12 hours in the light is sufficient for germination and growth (Amri 2010), this corresponds to the findings of this study that showed that seeds were able to germinate at a 8/16h and 12/12h photoperiod, more so at a 12/12h photoperiod; similar findings were found in *Hypericum perforatum*, *Melastoma malabatricum* and *T. latifolia* (Lombardi *et al.* 1997; Cirack *et al.* 2004; Faravani and Bakar 2007).

5.4.2.4. Scarification

Overall, nicking resulted in a higher germination (1.5 ± 0.32%) than not nicking (0.25 ± 0.15%). The results of this study are also supported by those of Nainar *et al.* (1999) and Likoswe *et al.* (2008) who showed that scarification results in higher germination in *Terminalia chebula* (60%) and *Terminalia sericea* (51%), respectively. Many savanna woodland species have hard seed coats that can withstand unfavourable conditions such as mechanical damage, severe drought and heat caused by sunlight (Doran *et al.* 1983; Baskin and Baskin 1998). If sown untreated many *Terminalia* species are difficult to germinate (Likoswe *et al.* 2008). Physical seed dormancy has been known to be broken by nicking in plants with hard seed coats which inhibit gas exchange and water uptake as observed in *Terminalia* and *Acacia* species (Hossain *et al*. 2005); where nicking allows for the entrance of air and water into the seed (Likoswe *et al.* 2008; Mwase and Mvula 2011). The hard seed coat acts as a barrier that inhibits water from entering the seed coat, which is necessary for germination (Mwase and Mvula 2001), this allows for the seed to remain dormant for long periods of time until conditions become favourable. The exposure of the seed to water and air after scarification results in enzymatic hydrolysis thus initiating germination, with the embryo transforming into a seedling (Mwase and Mvula 2011). Mwase and Mvula (2011) found that

seedlings whose seeds were nicked germinated faster and had a faster height growth rate than seeds that were not nicked in *Bauhinia thonningii*. Although nicking is vital to enable the entrance of water and for gaseous exchange, extreme care needs to be taken in particular when nicking is done at the micropyle in order to prevent damage to the embryo. However, nicking is time consuming and slow, making it only feasible in small sample sizes. To solve this problem, a more efficient mechanism need to be designed that can scarify large quantities of seeds rather than nicking one seed at a time.

5.4.2.5. Soaking

Regardless of the scarification treatment, temperature and photoperiod, the lowest germination rate was observed in seeds soaked in hot water where the highest and lowest germination percentages were 2 ± 2% and 0 ± 0%, respectively. This was also the case in the pooled data where control, cold and hot water soaking had a seed germination percentage of 1 ± 0.352%, 0.875 ± 0.330% and 0.375 ±0.216%, respectively. However, Likoswe *et al.* (2008) reported that soaking seeds in hot water for 12 h after nicking resulted in a higher germination rate and growth in *T. sericea*, and Mwase and Mvula (2011) showed that soaking seeds in hot water had the second highest germination percentage (53%) in *B. thonningii*. Although soaking in hot water softens the seed coat allowing for chemical inhibitors to leach out, soaking in boiled water (like in this study) makes the seed coat permeable to water but also makes the seed imbibe as it cools (Baskin and Baskin 1998; Mwase and Mvula 2011). This treatment has yielded beneficial results in numerous legume seeds. The high initial water temperature has large effects on the germination rate than the periods when the seed is cooling (Willan 1985). The hot water treatment works better in seeds with little resistance to germination. However, the use of the hot water treatment should be applied carefully in attempts not to kill the seed with high heating (Phartyal *et al.* 2005).

The low germination rate can also be attributed to the seed collection time which as Likoswe *et al.* (2008) has shown to have an effect on seed viability and longevity or could be due to the nicking in the instance of nicked seeds in *T. sericea*. Seed germination has been found to vary in various *Terminalia* species with species such as *T. ivorensis*, *T. superba*, and *T. prunioides* having a germination percent of 10-93%, 60-80% and 15-35%, respectively (Lemmens *et al.* 1995). However, the germination percentage of *T. sericea* has been found to vary from 1-3% (Carr 1994) to up to 60% (Likoswe *et al.* 2008). Also, germination could have been influenced by the substrate choice for

germination, as filter paper was used as the substrate for this germination study. Valencia-Díaz and Montaña (2003) germinated *Flouensia cernua* (shrub Asteraceae) on agar and filter paper; they reported that germination was higher on the agar substrate than on a substrate of filter paper with cotton attributing these findings to the constrast in moisture conditions between the substrates. The influence of substrate choice cannot be accurately determined, however the filter paper did have its own advantages as we were constantly aware of how much moisture surrounded the seeds and it made it easy to observe whether seeds had germinated or not.

5.5. Conclusions

There were differences in seed physical properties between seeds collected from Nylsvley Nature Reserve and Skukuza (KNP). There was a significant interaction between seed collection site seed predation for all the seed physical properties in across the two sites. Non-predated seeds were significantly longer, wider, thicker, heavier and had a larger geometric mean diameter and surface area since seed predation occurs on the plant in *T. sericea* in Nlysvley, thus seed predation could have significantly reduced seed size. Therefore, it is important to know when and where (on the tree or ground) to collect the seeds, however this can be tricky because the seed needs enough time to mature on the plant while not remaining attached long enough to get attacked by seed predators (Hossain *et al.* 2005). A more comprehensive study needs to be conducted to identify the underlying factors affecting seed size and seed selection by predators across different temporal scales, altitudes, latitudes and longitudes in more savanna species.

Many studies have established that *T. sericea* seeds germinate better at an equal photoperiod (12/12h), at a 25°C temperature, after soaking and nicking the seeds as shown in this study (Likoswe *et al.* 2008; Amri 2010). Kuniyal *et al.* (2013) suggested that *T. bellerica* be germinated from large seeds under nursery conditions in order to introduce *T. bellerica* into other habitats, this could possibly be the solution to solving the low germination and propagation rate of *T. sericea*. While different methods demonstrated here may break the seed coat and seed dormancy enabling gaseous exchange and allowing water to infiltrate, thus enhancing seed germination; there is a need to recommend to *T. sericea* farmers the adoption of the best treatment method. These methods need to be simple, cost effective and quick because at the moment purchasing *T. sericea* is difficult due to it being so hard to propagate (T. Twala *pers. obs.,* The Aloe Farm, Michelle Hofmeyer (Skukuza), Random Harvest Nursery).

From these findings it is suggested that in order to optimise *T. sericea* seed germination, seeds should be germinated at a 12/12h photoperiod, 25°C temperature, soaked in water at ambient temperature and nicked prior to germination. Future studies on the germination and survival rate of seedlings established from seeds of different sizes and from different sites should be informative. A smoking treatment should be tested because fire is a common disturbance in savannas (Scholes and Archer 2007). It is also suggested that nicking should be done prior to soaking the seeds in cold or control soaking treatments and after soaking in the hot treatment. Seed germination percentage varies between the years in *T. sericea*, thus it is apparent that germination percentage was very low (0 – 5%) in this years seed crop therefore it would be valuable to compare a seed crop from a year of high germination percentage with this seed crop (2016) in order to better understand the germination ecology of this species.

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Chapter 6: Synthesis and conclusions

6.1. Introduction

Savannas are complex and dynamic systems which are associated with strong climatic seasonality that influences germination, establishment, physiology, morphology and phenology of deciduous savanna trees (Huntley and Walker 1982; Williams *et al.* 1997; Wilson and Witkowski 1998; Chidumayo 2001; Moyo 2013; Sales *et al.* 2013; Whitecross *et al.* 2016; Whitecross *et al.* 2017). They are characterised by the co-existence of trees, shrubs and herbaceous species (Scholes and Walker 1993; Scholes and Archer 1997; Sankaran *et al.* 2004). Trees alter the savanna landscape through light interception and the utilisation of nutrients and water (Vetaas 1992; Ludwig *et al.* 2003). Seed germination, seedling establishment and sapling recruitment are critical life-history stages of savanna trees which are associated with long lifespans (Scholes and Walker 1993; Scholes and Archer 1997; Wilson and Witkowski 1998; Chidumayo 2008; Venter and Witkowski 2010; Helm and Witkowski 2012). If environmental conditions are not favourable, seed germination, seedling establishment and sapling recruitment can be of concern for tree recruitment (Witkowski and Garner 2000; Midgley and Bond 2001).

Disturbances (fire and herbivory) and resource availability (nutrients, water and light) strongly limit plant dynamics (O'Connor 1995; Gashaw and Michelsen 2002; Riginos 2009; Midgley *et al.* 2010; Ward and Elser 2011; Vadigi 2013). However, one major plant resource has been greatly ignored in savannas, light availability. This is mainly because light availability, which is considered as a limiting resource in forests due to their complex, multi-layered canopies that shade newly established and smaller plants, is considered unlimited in the savannas of southern Africa (Gignoux *et al.* 2016). Although, savanna trees are scattered across the landscape, some seeds manage to germinate and establish under the canopy of other trees, which ameliorate stressful conditions.

These canopy microsites facilitate tree recruitment and enhance woody plant survival by intercepting light, thus reducing high irradiance and temperature, enhancing nutrients due to the decomposition of leaf litter, and increasing soil moisture (Hoffmann 1996). However, seedling response to shading is species-specific (Hoffmann 1996). Although shade is considered to promote seedling establishment, some savanna species exhibit shade intolerance such as *Colophospermum*

mopane (Smith and Shackleton 1988; Belsky 1994). In addition to this, *Terminalia sericea* is also considered to be shade intolerant (Yeaton 1988; Pohjonen 1992). However, the magnitude of this intolerance has yet to be tested in the different plant stages and under the stress of herbivory. Seedlings and saplings are the most vulnerable plant stages in plant development due to their slow growth (Harper 1977; Bond 2008; Chidumayo 2008). Therefore, this study represents a means to understand how this co-dominant/dominant savanna tree species copes with shading and herbivory at the seedling and sapling stages and to understand the effects of seed predation on seed physical properties and its seed germination response.

6.2. Conceptual framework

The framework developed in this study considers the influence of light availability (shading) and natural and simulated herbivory on *T. sericea* seedling and sapling leaf dynamics, phenology and traits, plant morphology and physiology, as well as factors influencing *T. sericea* seed predation and germination (Figure 1). When considering the effect of shading and herbivory the framework implies that shading plays a greater role in influencing plant growth and physiology than herbivory in seedlings and saplings. Seed predation is site specific, influencing seed physical properties in the Nylsvley but not the Skukuza trees. Seed germination was influenced by photoperiod and scarification. Table 1 provides a summary of the difference in leaf dynamics, phenology and traits, plant architecture, dry mass, biomass and physiology within different light treatments and plant stages.

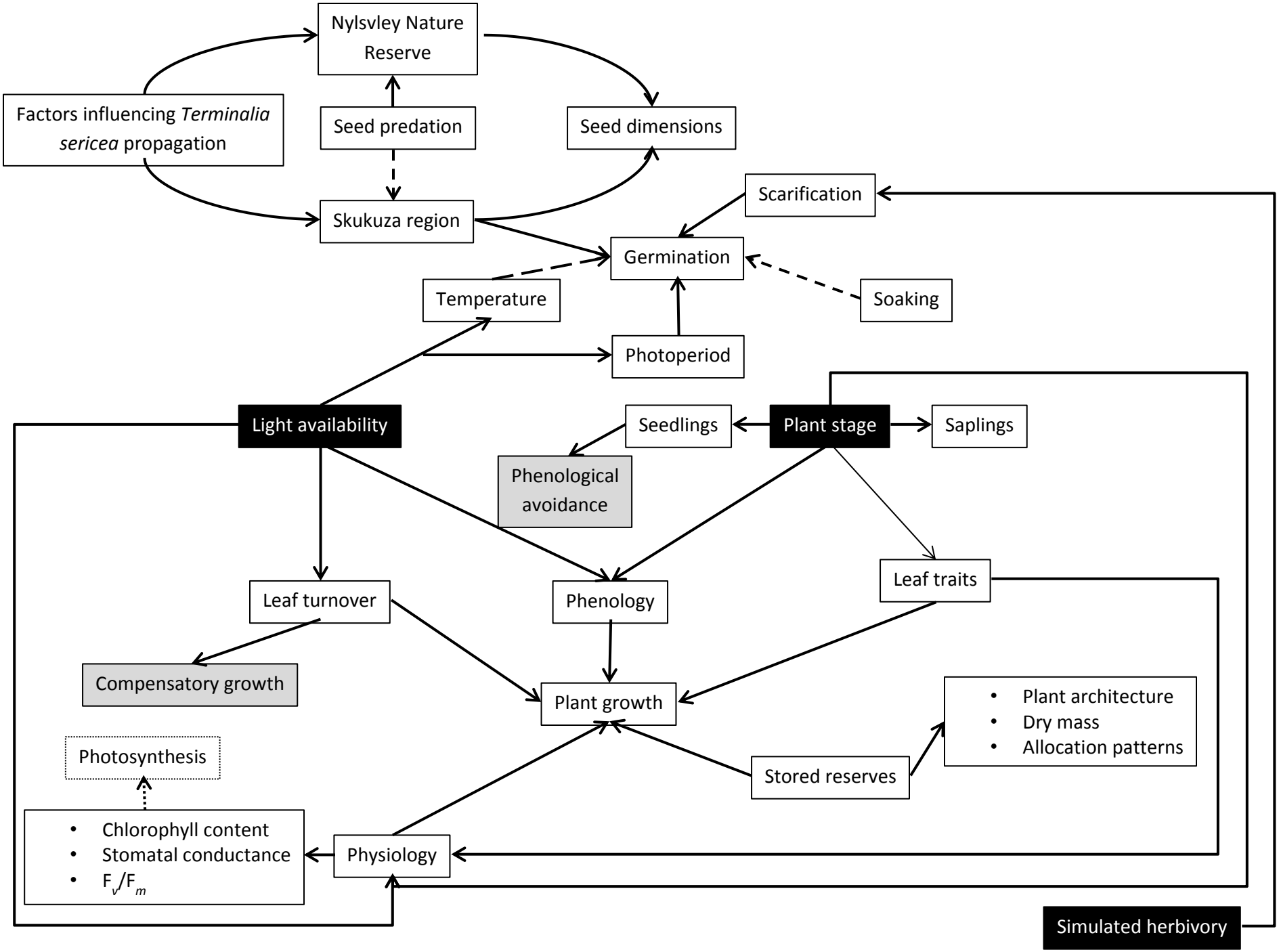

Figure 1. The overall conceptual framework of the factors influencing the compensatory growth, phenology, leaf traits, physiology, and propagation of *Terminalia sericea* seedlings and saplings.

Table 1. Characteristic differences observed between sun and shade plants, as well as between seedlings and saplings, of *Terminalia sericea*.

	Light Treatment		**Plant Stage**	
Variables	Sun Plants	Shade Plants	Seedlings	Saplings
Plant Level			Less dry mass and biomass	More dry mass and biomass
			Short	Tall
			Small stem diameter	Large stem diameter
Canopy Level	High leaf production	Low leaf production	Low leaf production	High leaf production
	Early and high leaf shedding	Late and low leaf shedding	Late leaf shedding	Early leaf shedding
	Short leaf longevity	Long leaf longevity	Long leaf longevity	Short leaf longevity
Morphology	Small leaves	Large leaves	Small leaves	Large leaves
	Thick leaves	Thin leaves	Thick leaves	Thin leaves
Anatomy	Small SLA	Large SLA	Small SLA	Large SLA
Biochemistry	Less chlorophyll	More chlorophyll	More chlorophyll	Less chlorophyll
	High stomatal conductance	Low stomatal conductance	High stomatal conductance	Low stomatal conductance
	Low F_v/F_m	High F_v/F_m	High F_v/F_m	High F_v/F_m

6.3. Research summary and recommendations

6.3.1. The influence of herbivory on leaf dynamics and physiology in *Terminalia sericea* seedlings and saplings

This study aimed to monitor the leaf cluster effects of natural and/or simulated herbivory on leaf dynamics and physiology. The study demonstrated that natural and/or simulated herbivory did not have a significant effect on leaf dynamics and physiology in *T. sericea* seedlings and saplings at the leaf cluster level. Suggesting that *T. sericea* seedlings and saplings are able to compensate for herbivory at the cluster level. This may be due to the adjacent control (untouched) leaf clusters shunting resources into the herbivory treatment leaves in order to compensate for leaf area loss. This type of herbivory may not significantly influence physiological responses because most of the undamaged half of the leaf remains functional (Marquis 1992). These results are not surprising as plants have been known to be able to re-allocate resources between source and sink areas in response to herbivory and resource availability (Ayres 1993; Folgarait and Davidson 1994; Koricheva *et al.* 1998; Katjiua and Ward 2006; Prik and Farji-Brener 2013; Johnson and Ebersole 2017). However, the herbivory treatment had a significant effect on leaf longevity in seedlings but not in saplings indicating that seedlings have the ability to tolerate herbivory, but are more vulnerable to herbivory than saplings due to their smaller sizes and hence lower levels of stored reserves.

6.3.2. What are the effects of shading on *Terminalia sericea* seedlings and saplings?

The ability of *T. sericea* seedlings and saplings to compensate for shading was investigated by determining whether these two plant stages were able to perform better in shaded (80% shade) compared to full sunlight conditions by monitoring their leaf dynamics, phenology, plant morphology, leaf traits and physiology – thereby confirming if *T. sericea* is shade intolerant as suggested by Yeaton (1988) and Pohjonen (1992).

6.3.2.1. Leaf dynamics and phenology

In **Chapter 2** the leaf dynamics and phenology of *T. sericea* seedlings and saplings was monitored over one growing season. This study has shown that seedlings produced more leaves in the sun while saplings produced more leaves in the shade. This may be due to differences in stored resources between the two plant stages, where younger plant stages are associated with less stored resources than older plant stages which can rapidly produce leaves by mobilising stored

carbohydrate reserves. Although, leaf production was lower in seedlings relative to saplings, they were able to accelerate leaf expansion and maturation while delaying leaf senescence and shedding, therefore enabling seedlings to phenologically avoid shading under field conditions, thus allowing seedlings to maximise light interception and carbon gain (Uemura 1994) long after the canopy of saplings was bare.

Based on these results, future studies should consider investigating the effects of shading by adult *T. sericea* and other species associated with *T. sericea* (e.g. *Burkea africana*, *Ochna pulchra, Grewia flavescens*) on *T. sericea* seedlings and saplings, as well as investigating the effects of natural herbivory (invertebrate and vertebrate) on plant establishment, fitness, tolerance and phenology. Shading, herbivory and other drivers influence tree establishment and dynamics across different plant stages and in different biomes (Vesk 2006; Riginos 2009; Moutakas and Evans 2015). However, understanding the intensity of these drivers will help determine the ability of plants to resist, tolerate and compensate for these drivers. This will enable us to properly categorise plant stages according to their tolerance to shading and herbivory, which will enable us to better predict the distribution of *T.* sericea across the landscape and how they may affect vegetation structure and composition.

6.3.2.2. Plant morphology and leaf traits

Chapter 3 investigated the influence of light availability on plant growth (plant architecture), stored reserves (dry mass and allocation patterns) and leaf traits. This study found that plant architecture and allocation patterns were not dependent on shading. These findings could be attributed to: (1) acclimation time under the different light treatments before the study commenced (Givinish 1988), (2) a short study period, while other studies have shown that one growing season may not be sufficient to build a thorough understanding of the ecology of a plant species (e.g. Whitecross *et al.* 2016), and/or (3) that these parameters are not greatly influenced by shading in these plant stages, suggesting that both plant stages were able to tolerate shading in regards to these parameters. Moyo (2013) and Whitecross *et al.* (2017) showed that *T. sericea* adult growth was also largely influenced by nutrient and water availability. Although shading did not significantly influence plant architecture and biomass, leaf traits were significantly affected by shading. These results suggest that some plant traits are more sensitive to certain resource limitations and disturbances than

others. Therefore, indicating that leaves are the most plastic plant organs as stated by Dickison (2000).

It is suggested that future studies should continue to assess leaf traits and plant dry mass allocation patterns when conducting short-term studies, but when seeking a more robust understanding of the growth patterns and strategies of a plant, long-term studies with continuous sampling should be conducted. It is also suggested that future studies should conduct a similar experimental trial (considering shading under tree species that *T. sericea* is associated with and measuring plant architecture, dry mass, biomass and leaf traits) on seedlings, saplings and adult individuals.

6.3.2.3. Plant physiology

Chapter 4 tested the effects of shading and herbivory on the physiology of *T. sericea.* The decrease in leaf functionality (chlorophyll content, stomatal conductance and F_v/F_m) towards the end of the growing season is a common resource conservation strategy utilised by deciduous tree species, by reabsorbing resources towards the end of the growing season and storing them in sink organs (e.g. stems, shoots and roots) for use in the next growing season (Demarez 1999; February and Higgins 2016; Whitecross 2017). Some of the processes involved in this strategy have been illustrated in **Chapters 2**-**4,** where leaves reabsorbed resources as seen in changes in chlorophyll content (**Chapter 4**), reallocating resources into stems, shoots and/or roots (**Chapter 3**), and then shedding their leaves at the end of the growing season (**Chapter 2)**. This is used as a compensatory response to tolerate herbivory damage and to reduce the effects of the cold and dry season (winter) (McNaughton 1983; Marquis 1992; Tiffin 2000; Whitecross 2017). In addition to this the results in **Chapter 4** indicate that plant stage plays an important role in plant functioning and stress tolerance, reiterating the importance of considering different plant stages in many aspects of plant ecology (**Chapter 2** and **5**), morphology (**Chapter 3**) and physiology.

All the physiological parameters measured were influenced by shading in seedlings, with chlorophyll content and F_v/F_m being higher in the shade and stomatal conductance being higher in the sun, however shading did not have an effect on the corresponding physiological parameters in saplings, indicating an adaptive light or shade response. Stomatal conductance and net photosynthesis have been shown to be highly positively correlated (Epron and Dreyer 1993). The high stomatal conductance in the sun compared to the shade in seedlings, and the insignificant difference in

stomatal conductance between saplings in the sun and shade, suggests that shading plays in important role in the photosynthetic ability of different plant stages, with seedlings performing better in the sun and saplings being able to maintain photosynthetic capacity in the sun and shade. Seedlings compensate for this shading effect on photosynthesis by utilising phenological avoidance (**Chapter 2**) to increase carbon assimilation and storage of carbon during the end of the growing season period when the canopy of older plant stages is bare. Saplings, may perform well in both environments because: (1) shaded environments are associated with favourable conditions such as lower irradiance and heat (Jagtap 1995), which may affect photosystem functionality, and (2) the higher storage reserves (**Chapter 3**) in saplings may give them an added advantage to invest more nutrients within their leaves, which may improve photosynthesis efficiency . The low chlorophyll content and F_v/F_m values in full sun compared to shaded plants is an adaptive strategy that enables the plants to reduce the damaging effects of high light energy on the chlorophyll and photosystems.

Further research should be conducted to understand the effects of prolonged shading on plant physiology by allowing the leaves to grow under one light treatment and then at some point in the growing season moving the plants from that light treatment to the other and measuring the subsequent changes in plant physiology after the shift. This study will give us an understanding of how plants perform after a disturbance such as fire or tree felling by large herbivores, environmental hazards (e.g. lightening) or people, where neighbouring plants may be removed, thus increasing light availability or a neighbouring plant may shade the plant therefore, outcompeting it for light.

6.3.3. *Terminalia sericea* seed predation and propagation

Chapter 5 compared the effect of seed predation on seed physical properties between two semi-arid savannas. The influence of seed predation on seed physical properties differed between the two savanna regions with seed physical properties from the Skukuza region (Lowveld, hot semi-arid savanna) not being influenced by seed predation. Seed physical properties from Nylsvley Nature Reserve (Highveld, warm semi-arid savanna), however, differed between non-predated and predated seeds with non-predated seeds being significantly larger than predated seeds. These results suggest that the different environmental conditions at the different sites (such as rainfall, temperature, altitude, longitude and/or genetics) may affect seed physical properties which in turn have an influence on seed predator dynamics.

The seeds collected from Skukuza (only) were also germinated under different photoperiods (light/dark: 12/12h, 8/16h) and temperature conditions (25/18°C, 30/18°C), as well as different pre-conditionint water temperatures (ambient, cold, hot) and scarification (not nicked, nicked) treatments. The equal photoperiod (12/12h), soaking in water at ambient temperature and nicking the seeds resulted in higher germination percentages, with temperature not having an effect on seed germination. Across all treatment combinations, only 18 seeds (0.75%) germinated. These results indicate that even in favourable germination conditions, *T. sericea* seeds have a low germinability, probably as a result of seed crops with overall low seed viability. This suggests that additional factors not tested in this study may further influence the germinability of *T. sericea* or that the species has a naturally low germination rate owing to its presence in a largely nutrient poor ecosystem (Scholes and Walker 1993; Scholes *et al.* 2002). In general, *T. sericea* germination varies between seasons (Carr 1994) with seed collection time playing an important role in seed germinability (Likoswe *et al.* 2008). In addition, the low *T. sericea* germination rate may be due to seed abortion, resulting in low seed viability. The seed abortion strategy has been used to reduce the effects of stress (Sun *et al.* 2004; Meyer *et al.* 2014), seed predation (Marquis 1992; Ghazoul and Satake 2009), to save resources invested in reproduction (Meyer *et al.* 2014), and selective abortion of lower quality offspring due to unfavourable genotypes (Kärkkäinen *et al.* 1999; Melser and Klinkhamer 2001), which is used as a strategy to increase progeny fitness (Vaughton and Carthew 1993). In **Chapter 5** I showed that seed predation in Skukuza may be at a greater level post-dispersal, while it might be more important pre-dispersal at Nylsvley. Pre-dispersal seed predation may play a vital role in seed abortion, thus influencing seed viability and vigour. In addition to this, the seed abortion strategy used by *T. sericea* may act as a means to reduce the negative effects of seed predation by increasing seed abortion (low seed germinability), therefore decreasing seed predation rates on viable seeds. This may also suggest that low seed production acts as a bottleneck in the reproductive process of *T. sericea*.

Future studies should consider comparing the germinability of seeds with different seed dimensions and measuring how, once germinated, seed dimensions influence plant establishment and survival. Seed abortion, predation and germination studies on different populations across different savannas should be conducted in order to build ecological understanding into the influence of seed abortion and predation on plant reproduction and persistence (Ehrlén 1996; Pizo 1997; Crawley

2000; Steffan-Dewenter 2001; Marino *et al.* 2005). In addition to this, methodologies to test *T. sericea* seed vigour and viability need to be developed in order to test the seed abortion theory and to determine the relative importance of pre- versus post-dispersal predation on these traits.

6.4. Are *Terminalia sericea* seedlings and saplings shade tolerant?

Terminalia sericea seeds need enough light in order to germinate (*see* **Chapter 5**), this means that it has to germinate in the canopy gaps. Once established, *T. sericea* is able to recruit in the shade by altering leaf turnover, phenology (*see* **Chapter 2**), leaf traits and morphology (*see* **Chapter 3**), as well as its physiology (*see* **Chapter 4**) in order to acclimate to these low irradiance conditions, thus suggesting that *T. sericea* seedlings and saplings are shade tolerant. This has large implications on the demographics of *T. sericea* in semi-arid savannas.

Once the seeds have germinated in the canopy gaps, one would expect to find *T. sericea* seedlings and saplings growing in the understories of *Burkea africana*, *T. sericea* itself and under canopies of other woody species. These findings are aligned with those of Yeaton (1988), who reported that *T. sericea* can occur under the canopy of *B. africana* and itself in larger size classes, suggesting that *T. sericea* has to establish earlier and in the canopy gaps of *T. sericea* adults and *B. africana* prior to being over-topped by the adult trees. It is also light tolerant (Stranger 1974), as shown in this study, which also suggests that *T. sericea* seedlings and saplings can be found in the edges of woodlands. This suggests that *T. sericea* is largely limited by light availability due to its germination requirements, and nutrient and moisture availability (Moyo 2014; Whitecross 2017), once they have established. However, the size-class of *T. sericea* found within a landscape will be largely influenced by the degree of shading as plant growth is reduced by extreme shading (**Chapter 3**).

6.5. Further Suggestions

Owing to the seedlings and saplings being in different pot sizes and growth mediums, they could not be statistically compared as one could not disentangle whether the differences in responses between seedlings and saplings were due to plant stage, pot size or growth mediums. Therefore, it is suggested that future studies pot seedlings and saplings in the same pot sizes and growth medium; preferably collecting soil under the canopies of *T. sericea* in the field (e.g. at Nylsvley Nature Reserve or Skukuza Nature Reserve) and planting them in this soil.

This study was mainly conducted at the leaf cluster level and this meant that the effects of the herbivory treatment were tested on adjacent leaf clusters within each plant replicate, and therefore the response of the whole plant to the herbivory treatment could not be tested as the adjacent control leaf cluster shunted resources into the herbivory treatment cluster to compensate for herbivory. This meant that the actual effects of natural and simulated herbivory could not be tested at the whole plant level, which would have been a more ecologically meaningful level of study. Therefore it is suggested that future studies apply the herbivory treatment on individual plants (and not leaf clusters) and that if 50% herbivory is to be applied to each canopy this must be calculated according to the canopy size of each plant stage as the canopies of seedlings, saplings and adults are not the same.

Whitecross (2017) has shown the importance of monitoring plants over a long time period due to large differences in seasonality and variability in disturbances between years. Hence it is suggested that a more long term study be conducted to measure how well *T. sericea* is able to compensate for shading and compare these responses to field studies, but this would most likely be a PhD study, not the time limited MSc level.

6.6. Conclusions

The main aim of this study was to investigate the effect of light availability, natural and/or simulated herbivory on the compensatory growth abilities of *Terminalia sericea* seedlings and saplings and to investigate the seed germination response of *T. sericea*. This study has shown that *T. sericea* utilises tolerance mechanisms to cope with herbivory and shading. These include increases in photosynthetic rates (stomatal conductance), resource reallocation, compensatory growth and phenological changes (McNaughton 1983; Marquis 1992; Tiffin 2000). However, the extent of this tolerance is largely influenced by plant stage. This study has shown that *T. sericea* seedlings and saplings are not affected by natural and/or simulated herbivory (50% leaf area loss) at the leaf cluster level, with control and herbivory treatment leaf clusters maintaining leaf turnover and physiological functioning. This study has also highlighted the influence of shading on leaf turnover and longevity, phenology, leaf traits, plant morphology and physiology, and how these plant characteristics can differ between plant stages. Furthermore, it has also shown that seedlings are using phenological avoidance as a strategy to tolerate shading and herbivory by accelerating leaf expansion and maturation, as well as delaying leaf senescence and shedding their leaves well after

the saplings, therefore gaining physiological advantages, while reducing the negative effects of herbivory on new leaves and maximising light interception after the sapling canopy is bare. Seedlings and saplings utilise different light or shade adaptive strategies to survive in high and low irradiance environments through leaf trait plasticity. These results suggest that light availability (shade) is one of the main resources influencing plant morphology and physiology, with shading influencing light quality, therefore having a photomorphogenetic effect on the plants (Gross *et al.* 1996). One of the major findings of this research is highlighting the importance of considering plant stage in phenological, morphological and physiological research on savanna trees in building our understanding of tolerance to disturbances and environmental stresses. In addition to this, I showed the importance of doing germination studies in order to understand the ecology of savanna trees by highlighting techniques that can be used to germinate *T. sericea* and I showed how different factors influence the germinability of a co-dominant/dominant savanna tree species which can influence overall tree population dynamics. One of the major outcomes of this study is highlighting the importance of measuring different plant characteristics when considering plant compensatory capabilities to resource limitations and disturbances, especially when conducting short-term studies, and considering measurement scales (cluster-, canopy- to plant-level) when conducting these studies as they give us different insights into plant tolerance.

6.7. References

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