

Response to Examiner's Reports of my PhD Thesis, May 2017

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I would like to thank the examiners for their time and input into examining and improving my PhD thesis. Below I have responded to each of their comments and corrections.

Examiner 1

- An additional sentence on the cover page of each published chapter has been included to explain the roles of the candidate in the publication production process.
- Published chapters cover pages have been corrected to indicate that the chapters have been published as they are.
- In response to Examiner 1's concerns about the fire: Unfortunately, given that the first two research chapters are already published I feel it would not aid in furthering the research contained in this thesis to alter the chapters to contain more information on the fire. I feel that the first two research chapters made adequate mention of the fire (particularly chapter 3) and quantified the impacts on the tree and grass growth and phenology. As indicated in Chapter 2, the *Burkea africana* trees which had already flushed at the time of the fire showed no damage to their new leaves most likely due to their large size and the ability to tolerate fires when stem diameters are above 40 cm (Wilson and Witkowski 1993). If the fire had been a planned management fire I could have better planned to alter some of my experiments to better measure the impacts of the fire. However, I feel that given the unexpected nature of the wild fire and the lack of pre-fire data, Chapter 3 managed to highlight some of the after effects of the fire on the phenology of the trees and grasses. It is my opinion that there was little research of novelty to be produced from this particular wild fire and I have included what I felt did show some interesting results. Given that the focus of the PhD study was not on fire I could not invest more time analysing the results any further than I did for this thesis. However I also included a section in the discussion chapter (as per a later request) to further the discussion about the wild fire and the potential impacts it had on the study.
- The definition of the growing season in the glossary has been amended to: "The period of an annual cycle in which plants have green leaves present which enable photosynthesis, nutrient cycling and growth to occur. The start of the growing season is when the first new leaves appear on the canopy irrespective of their functionality."

THESIS ABSTRACT

- Compliments has been changed to complements on pg 2
- A final line has been added to the abstract to highlight the two investigated benefits: This thesis has shown that early-greening broad-leaved savanna trees in South African savannas are more likely to avoid invertebrate herbivory than extend their growing seasons.

CHAPTER ONE

- Pg 20. No action required. Thank you to the examiner for complimenting this section.
- Pg 21. Sentence changed to "Phenological models have been developed to predict green-up events and growth periods for these well studied northern hemisphere systems (Schwartz, 1999)."
- Pg 21. Sentence has been reworded: "The seasonal variation in environmental variables, such as precipitation, coupled with the multifaceted interactions between the different layers of vegetation (trees and grasses) have further confounded modellers abilities to predict how these systems are actually functioning within the context of the broader, global carbon models (Poulter et al. 2014; Ahlström et al. 2015)."
- Pg 21. Removed "an essential"
- Pg 22. A good point but sadly outside the scope of this PhD study.

- Pg 22. Sentenced changed to: “Some species in Asian monsoon forests access subsoil water reserves in the dry season and this enables new foliage to be produced ahead of the first rainfall event (Elliot *et al.*, 2006).”
- Pg 22. Sentenced altered to: “February and Higgins (2016) also found that African savanna trees are able to store and recycle important nutrients, such as nitrogen, between seasons to assist in the rapid deployments of new leaves at the start of the growing season. Therefore, it is clear that rainfall is not necessarily the direct driver of tree phenology in these African savanna systems.”
- Pg 23. Numbers have been included to highlight the four hypotheses in the paragraph.
- This line has been added to the end of section 1.3.4. “This thesis will focus on testing hypotheses (1) and (3) and will return to the rest in the final discussion chapter.”
- Start of Section 1.3.5. changed to: “Comparatively less, in relation to the literature on benefits, is known about the costs of this early-greening strategy employed by savanna trees.”
- Numbers have been added to the Costs section and a concluding sentence stating that the costs were not explicitly tested was added.
- Pg 25. Word annual has been removed.
- Pg 26. Rephrased accordingly.
- Pg 28. Tropical systems now included as well.
- Pg 29. Section 4. ‘Broad’ has been removed from the title.
- Pg 30. ‘changes’ corrected to ‘differences’ in both points.
- Pg 30. Reworded to: “To measure and compare the changes in rates of photosynthesis and transpiration between tree species over the green-up period”
- Pg 31. Section 4.3. changed accordingly.
- Pg 31. Section 4.4. changed to past tense
- Pg 31. Section 4.4. added ‘do’
- Pg 31. “in the occurrence of” deleted
- Pg 32. The definition for the growing season has been altered – see earlier comment.

CHAPTER 2

- All comments have been noted for this chapter, however, because it has already been published in the South African Journal of Botany I am hesitant to make large alterations to the text contained in this thesis and would prefer to leave this chapter as it is.

CHAPTER 3

- Comment noted.

Abstract

- A line added to abstract to indicate that a fire occurred: “During September 2013 a severe fire burnt through the study site.”

Main Body

- Please see earlier comment on how the fire was handled for this thesis. Given that this chapter has already been published I would prefer not to change too much of it at this stage.
- P78 – 5cm depth added.
- P80 – noted.
- P80 – it is already indicated that the data are available from 1980-2013.
- Pg 81 – corrected to on average
- Pg 82 – noted.
- Fig 2. This was unfortunate.
- Fig 3. Apologies for this.
- Fig 4. Noted.
- Section 4.3. – noted.

- Section 4.4. – AIC modelling: this is a good suggestion and it is a pity that we did not manage to do this prior to the publication of this paper. I will remember this for future studies.
- Section 5.1 noted
- Pg 94. – noted
- Pg 95. – noted
- Pg 96. Corrected to 'homogeneous'

CHAPTER 4

- Section 4.2.2.3 has been rewritten to be less “statistically driven” and more focused on the biology.
- Sentence that was at the bottom of pg 110 has been altered to read: “If they are not photosynthesising then this strategy may only extend the growing season by providing the advantage of faster leaf expansion after rainfall onset, which may extend the growing season of early-greening savanna trees over that of late-greeners.”
- The decision to combine the years was taken due to the patchy data collection which occurred over these two seasons as a result of the licor temporarily malfunctioning. The soil moisture modelling was conducted in order to account for the changes in precipitation over the two seasons. I have included a figure in the appendix of chapter 4 to indicate the two years photosynthesis results for each species as requested by the examiner.

Abstract

- Pg 107 line 5 corrected as stated by Examiner
- Second last sentence clarified to: “It is unclear whether the new leaves of early-greening trees are functional prior to the onset of seasonal rainfall in the NNR, given the low photosynthetic rates of new leaves post rainfall onset.”

Main Body

- Second paragraph has been streamlined and condensed as per Examiner’s request.
- Pg 110 Sentence changed to: “No photosynthesis is observed in new leaves across many northern hemisphere species and it is likely that this is observed across a wider array of species globally as well. However, as the new leaves begin to expand, transpiration commences, their photosynthetic apparatus matures and eventually carbon gain is observed”.
- Pg 110. Sentences have been combined as requested
- Pg 110. Last sentence has been changed to now read: “If they are not photosynthesising then this strategy may only extend the growing season of early-greening trees through providing the advantage of faster leaf expansion after rainfall onset.”
- Pg 111. Aim (2) changed to: “how photosynthetic rates and transpiration are linked to the onset of rainfall through an increase in soil moisture?”
- P114: Our aim was to compare the temporal trends of the chlorophyll within the different leaf age classes. It is unfortunate that a calibration curve was not created for either species, however, the use of SPAD units for comparing chlorophyll has been published (Uddling et al. 2007; Marengo et al. 2009; Cowie et al. 2016) and I therefore feel comfortable sticking with these units for this thesis, however, I will ensure to collect information for calibration curves in future.
- P115: Have now included this sentence to clarify: “Soil moisture was recorded at a depth of 5cm below the surface.”
- Pg 120: Paragraph 4.2.2.2. altered to: “Photosynthetic rates only became positive after the first ~14 days in *B. africana* and ~20 days after the start of leaf flush in *T. sericea* (Figure 3). New leaves of both *B. africana* ($-2.13 \pm 1.0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and *T. sericea* ($-0.95 \pm 1.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) had a negative carbon balance, indicating that respiration exceeded photosynthesis (Figure 3). Photosynthetic rates increased with time from the start of new leaf growth, but then decreased for fully expanded and mature leaves for both *B. africana* ($\chi^2=12.8$; d.f.=2; $p<0.002$; AIC=1460.4, Table 2) and *T. sericea* ($\chi^2=84.6$; d.f.=2; $p<0.001$; AIC=1592.6, Table 2).”

- P123: I have taken note of the advice to remove the section on transpiration from the results but would prefer to include it in the thesis. I shall make sure to only include the section on conductance when we submit the paper for publication.
- Figs 2,3,4: The units on these Figures have been corrected as requested.
- Corrected the two Figure 4's to Figures 4 and 5.
- Fig 4 – remove transpiration panels: For the purpose of this thesis I have decided to leave the transpiration panels in Figure 4. However, I will take note of the advice given by the Examiner and remove the transpiration data prior to submitting the publication to the journal.

Modelling

- I have taken note of this suggestion and shall be sure to include further analyses of these models at a leaf age level when I prepare the chapter for publication.
- Sentence in section 4.2.2.3 changed to: "New leaves showed little response to soil moisture, and appear to require a certain amount of time to become functional (Figure 3, Table 2). However, fully expanded and mature leaf photosynthetic rates responded positively to increased soil moisture. (Table 3 (Model 1))."
- The comments regarding model 2 are noted and improvements will be made accordingly when this chapter is submitted for publication
- P128 – changed 'leaf functionality' to 'gas exchange'
- Pg130 – This study supports moved to the start of a new paragraph.
- Pg131 – Sentenced changed to: "Unfortunately, this study was unable to directly test whether new leaves became functional prior to the onset of seasonal rainfall as new leaves produced prior to the onset of rainfall were too small to accurately measure during early-greening seasons (Whitecross et al., 2016 - see Chapter 2)."

CHAPTER 5

- Pg143 line 8 – timing of flush in relation to rainfall added.
- Pg 143 – percentages have been included now
- Pg 148 line 5 – added requested sentence
- As stated earlier in the Chapter 4 comments. Several publications have made use of the SPAD Units and compared trends. I therefore feel comfortable leaving these as is.
- The scales on Figures 4 and 6 have been corrected to show the temporal scale more clearly.
- P162 middle – sentenced changed to: "It is also important to note that the decrease in leaf chlorophyll may impact on the tree's ability gain carbon at the middle and late stages of the growing season (Cornelissen *et al.* 2003; Nabity *et al.* 2009)."
- Pg 163 line 6: changed to: "tolerate invertebrate herbivory"
- Pg164 line 4: changed to: ". Early-greening is risky because it requires the plants to use stored water, carbohydrate and nutrient reserves to produce leaves that will be lost if there is a drought"
- Pg164 – changed to: "Unfortunately the phenology or identification of the invertebrates responsible for this damage was not determined"

CHAPTER 6

- Comment noted. Will be sure to keep this in mind during future work.

CHAPTER 7

- The growing season definition was addressed and the glossary term redefined.
- Section 2.1.2: rephrased to "supporting"
- Table 1 Units have been corrected. Corrected to Figures 4 and 5 as well. If this chapter is prepared for publication, alteration of the models to leaf mass rather than leaf area will be performed.
- P211: Added a paragraph to the end of this section: "As discussed in Chapter 1, little is known about the risks of early-greening for savanna trees, however, the impacts of the 2013 fire on the

phenology of the irrigated *T. sericea* illustrated the potential impacts of fire on early-greening trees. It would be beneficial to explore the impacts of fire on early-greeners in a more controlled experiment in the future to quantify these impacts in a less descriptive manner. “

- P212: This paragraph has been added to the end of this section: “Another area highlighted by this study is the poor understanding behind the potential cues for the commencement of leaf flush in early-greening broad-leaved savanna trees. In Chapter 6, some of the potential cues were explored, however, no conclusive evidence for one specific cue was determined. There is some indication that early-greening in *B. africana* may be linked to temperature in the absence of an increase in soil moisture post-rainfall, however, Do et al. (2005) indicate that changes in atmospheric vapour pressure deficit could also act as a cue.”
- Pg 215 line 4: Sentence streamlined as per request of Examiner.

Examiner 2

GENERAL:

1. Start of sentence in abstract has been changed from ‘I’ to ‘There is likely no other ecosystem...’ as requested. The first person narrative has also been removed from the remainder of the thesis abstract.
2. The words ‘we’ and ‘our’ have been removed and sentences altered throughout the thesis to remove the majority of the first person narrative.
3. The referencing format has been checked and corrected to the same format throughout the thesis.
4. The page numbers in the table of contents have now been corrected.

CHAPTER 1:

- A map has now been added to chapter one to illustrate the location and layout of the study site.
- A few sentences explain the fire history and frequency in Nylsvley have been added to the section mentioned.

CHAPTER 2:

A section has now been included in the introduction chapter (pg19) which states: “The start of the rainfall season in semi-arid southern African savannas is considered to be after the first rainfall event that is >20 mm within a 24 hr period (Hachigonta et al., 2008), however, several other definitions for the onset date of rainfall exist in the literature including Bate et al. (1982) who stated that 15 mm of rainfall is sufficient to commence grass growth in African savannas.”

CHAPTER 5:

An early-greening is a tree species that is capable of producing leaves prior to the onset of seasonal rainfall in savannas. I have added the early-greening term under the early-greening section in the glossary.

CHAPTER 6:

The section on soil influence on pg (181) has been expanded: “This study chose to use NDVI over the enhanced vegetation index (EVI), as an assessment into the relative changes through time and not the absolute values of greenness at each site was the main priority, so differences in soils were deemed not important. This study acknowledges that soil bio-geochemistry can influence water infiltration among other factors (Scholes and Walker 1993), however, all of the sites selected were found on deep, sandy soils with a similar texture and composition throughout.”

A section has been added with data which I had not included showing why I accepted the use of TRMM data over ground-based measurements: “To compare the accuracy of TRMM rainfall estimates with ground-based rainfall collection data (South African Weather Service’s Bela Bela station), a t-test was conducted comparing the estimates of each and found no overall difference

(paired t-test: $t=-0.09$; d.f.=21; $p=0.92$). This study thus accepted that TRMM data were sufficient for analysing the onset of rainfall across the study region.”

Examiner 3

- The sentence on pg 44 (now pg 45 after changes) has been reworded to make it clearer and more logical to reader: “Savanna trees, however, are able to access deep water supplies through an extensive deep-root network and have the ability to store water in both their trunks and roots, and by using these stored reserves prior to rainfall onset, trees are able to produce green leaf material when grasses are brown (Chidumayo, 2001; Archibald and Scholes, 2007; Higgins *et al.*, 2011).”
- The sentence on pg 95 (now pg 96 after changes) has been reworded to make it clearer and more logical to reader: “A delay in the onset of seasonal rainfall provides trees with an opportunity to commence green-up during a period when grasses remain dormant, and through the early onset of growth, trees are able to have faster acquisition of nutrients at the start of seasonal rainfall (Scholes and Walker, 1993).”
- Pg212. The sentence has been reworded to improve its clarity: “Nutrient-poor savannas occur in higher rainfall areas and their structural composition is often maintained through fires rather than driven by mammalian herbivory (Charles-Dominique *et al.* 2016).”
Minor comments:
- Inconsistencies in reference formatting throughout the thesis have been addressed
- Removed S.L. from the in text Childs citation on pg 44
- Inconsistency in reference formatting addressed on pg 72
- PLOS ONE changed to PloS One throughout thesis
- Inconsistent data formatting has been corrected throughout all reference lists
- Missing volume numbers have been corrected.
- Inconsistent formatting has been corrected and ‘pp.’s have been removed

References

- Cowie, B.W., Venturi, G., Witkowski, E.T.F., and Byrne, M.J. 2016. Does climate constrain the spread of *Anthonomus santacruzi*, a biological control agent of *Solanum mauritianum*, in South Africa? *Biological Control* 101: 1-8. <http://dx.doi.org/10.1016/j.biocontrol.2016.06.005>
- Marenco, R., Antezana-Vera, S., and Nascimento, H. 2009. Relationship between specific leaf area, leaf thickness, leaf water content and SPAD-502 readings in six Amazonian tree species. *Photosynthetica* 47: 184. doi:10.1007/s11099-009-0031-6
- Uddling, J., Gelang-Alfredsson, J., Piikki, K., Pleijel, H., 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynthesis Research* 91, 37–46. <http://dx.doi.org/10.1007/s11120-006-9077-5>.
- Wilson, B., Witkowski, E.T.F., 2003. Seed banks, bark thickness and change in age and size structure (1978–1999) of the African savanna tree, *Burkea africana*. *Plant Ecology* 167, 151–162.