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Climate change in the tropics: Drought effects on the phenology and eco-physiology of rainforest tree species

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James Cook University, 2021

Supervisors: A/Prof. Lucas A. Cernusak
Prof. Michael Bird
Dr. Christopher Wurster
A/Prof. Michael J. Liddell

Dedication

To my parents, Lúcia and Eguimar, with love.

"Those who contemplate the beauty of the earth find reserves of strength that will endure as long as life lasts. There is something infinitely healing in the repeated refrains of nature — the assurance that dawn comes after night, and spring after winter."

(Rachel Carson)

Statement of Sources

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Nara Vogado Date: 19-10-2021

Statement of Contribution of Others

This PhD project was made possible through the assistance of a number of funding sources. I am grateful to have been awarded a James Cook University Postgraduate Research Scholarship, which allowed me to live and study in the Cairns.

I also received funding from the following external grants:

- Skyrail: AU$ 5,000

- Wet Tropics Management Authority: AU$ 3,800

- Holsworth Wildlife Research Endowment:

Round 1 (2019-2020): AU$ 6,375
Round 2 (2020-2021): AU$ 6,065
Total: AU$ 12,440.

This PhD thesis contains several papers (as chapters) that were published during the course of the project; all those who contributed to these articles have been included either as co-authors or mentioned in the acknowledgements. For each of these publications, I conceived, designed and planned the study, carried out the experimental work and undertook the data analysis with the help of others as detailed in the Table below. All co-authors contributed to the interpretation of the data, revisions of the manuscripts and approved the final drafts. Editorial assistance for this thesis was provided primarily by my supervisors. Below I have detailed the contribution of others to these publications in tabular form.

Chapter No	Details of publication(s) on which chapter is based	Nature and extent of the intellectual input of each author, including the candidate
2	Vogado N.O., Bird M.I., Liddell M.J., Engert J.E., Wurster C.M., Cernusak L.A. Climate change and phenology research in Southeast Asia-Oceania: knowledge gaps and future possibilities. *Under review*. 2021.	Nara Vogado was responsible for data collection, analyses, and writing of the manuscript. Michael Bird and Lucas Cernusak contributed to conceptual background and direction for the manuscript. Jayden Engert provided assistance for data collection and analysis. All co-authors contributed to manuscript writing and conceptual direction for the manuscript.
3	Vogado N.O., Liddell M.J., Laurance S.G.W., Campbell M.J., Cheesman A.W., Engert J.E., Palma A.C., Ishida F.Y., Cernusak L.A. The effects of an experimental drought on the ecophysiology and fruiting phenology of a tropical rainforest palm. (2020). *Journal of Plant Ecology*, 13(6): 744–753.	The data was collected by multiple people over a decade. Data collection was maintained by Michael Liddell and the Daintree Rainforest Observatory (DRO). In particular, Michele Schiffer and Andrew Thompson (DRO), helped to manage and undertook data collection activities for some of the phenological data. The drought experiment was led by Susan Laurance, with help from Yoko Ishida and other collaborators. Nara Vogado, Michael Liddell and Lucas Cernusak designed the manuscript. Nara Vogado collected the phenology data from 2018 to 2020, and collected physiological data. Nara Vogado prepared samples for isotopic analyses. Jayden Engert provided assistance for data collection. Ana Palma and Alexander Cheesman provided assistance for physiological data collection. Isotopic analyses were conducted at the Advanced Analytical Centre in Cairns (AAC), with support and help from Jen Whan. Nara Vogado wrote the manuscript and conducted the statistical analyses. Sourav Das provided advice on statistical analysis. All co-authors provided editorial input to the paper.
4	Vogado N.O., Laurance S.G., Liddell M.J., Engert J.E., Wurster C.M., Schiffer M., Thompson A. and Cernusak L.A. Assessing the effects of a through-fall exclusion experiment on the reproductive phenology and ecophysiology of a wet tropical rainforest community. *Under Review*. 2021.	The data were collected by multiple people over a decade. Data collection and curation were carried out by Michael Liddell and the Daintree Rainforest Observatory (DRO). In particular DRO staff Michele Schiffer and Andrew Thompson (DRO) supported data curation, and Andrew Thompson also contributed to phenological data collection. The drought experiment was led by Susan Laurance, with help from Yoko Ishida and other collaborators. Nara Vogado, Michael Liddell and Lucas Cernusak designed the manuscript. Nara Vogado collected the phenology data from 2018 to 2020, collected and prepared samples for isotopic and elemental analyses. Jayden Engert provided assistance for data collection. Isotopic analyses were conducted at the Advanced Analytical Centre in Cairns (AAC), with support and help from Jen Whan. Nara Vogado wrote the manuscript and conducted the statistical analyses. Murray Logan, William Edwards and Sourav Das provided advice on statistical analysis. All co-authors provided editorial input to the paper.
5	Vogado N.O., Winter K., Ubierna N., Farquhar G.D., Cernusak L.A. Directional change in leaf dry matter δ13C during leaf development is widespread in C3 plants. 2020. Annals of Botany, 126(6): 981–990	This paper contains a literature review and data analyses of historical, but previously unpublished, data. These data were collected by Lucas Cernusak between 2009 and 2012, in collaboration with Klaus Winter, Nerea Ubierna and Graham Farquhar (ANU). Nara Vogado conducted the literature review and statistical analyses of the data. Nara Vogado and Lucas Cernusak wrote the manuscript, with inputs from all co-authors.

6	Vogado N.O., Cheesman A.W., Cernusak L.A. Delayed greening during leaf expansion under ambient and elevated CO2 in tropical tree seedlings. Under review. 2021.	Nara Vogado established the glasshouse experiment, with assistance from Jayden Engert. Nara Vogado conducted all measurements. Jayden Engert assisted with some measurements. Alexander Cheesman and Lucas Cernusak provided assistance for physiological measurements. Nara Vogado prepared all samples for stable isotope analysis. Isotopic analyses were conducted at the Advanced Analytical Centre in Cairns (AAC), with support and help from Jen Whan. Seedlings were supplied by Leah Nugent (Cairns Regional Council Nursery). Nara Vogado conducted statistical analyses, with input from Rhondda Jones. Nara Vogado wrote the manuscript. All co-authors provided editorial inputs to the manuscript.
7	Effects of elevated CO_2 and defoliation on wet tropical rainforest tree species in relation to leaf phenology. *In preparation*.	Nara Vogado established the glasshouse experiment, with assistance from Jayden Engert. Nara Vogado conducted all measurements. Nara Vogado designed the method for the measurement of stable isotopes from wood samples with assistance from Christopher Wurster. Nara Vogado prepared all samples for stable isotope analysis. Nara Vogado conducted anatomical work with assistance from Deborah Apgaua, who was responsible for vessel measurements. High-resolution photographs were taken at JCU Townsville, with help from Laboratory and Technical Support staff of the Marine and Terrestrial Division of Tropical Environments, in particular Lexie Edwards. Seedlings were supplied by Leah Nugent (Cairns Regional Council Nursery). Isotopic analyses were conducted at the Advanced Analytical Centre in Cairns (AAC), with support and help from Jen Whan. Nara Vogado conducted statistical analyses and wrote the manuscript.

Acknowledgements

This work would not have been possible if it were not for all the help and support I have received throughout my PhD. For that reason, I am very grateful to many special people.

Firstly, I would like to thank my supervisor A/Prof. Lucas Cernusak for accepting me as his student and believing in my potential, and for giving me all the support, care, and friendship. I am also deeply grateful to my co-supervisors A/Prof. Michael Liddell, Prof. Michael Bird and Dr. Christopher Wurster for all the support, understanding, help and guidance throughout this journey. I am beyond grateful for having such an amazing advisory board.

This research was possible due to the James Cook University Postgraduate Scholarship I was awarded and the grants I have received during my PhD. Hence, I am grateful to JCU and all JCU staff, and to Holsworth Wildlife Research Endowment, Skyrail Rainforest Foundation and Wet Topics Management Authority for the funding received.

Much of my research was supported and enriched by my collaborators who are also friends, in particular Prof. Susan Laurance, Dr. Alex Cheesman, Dr. Ana Palma, Dr. Jen Whan, Dr. Mason Campbell, and Dr. Deborah Apgaua. I extend my deepest gratitude to the staff from the Daintree Rainforest Observatory, especially Dr. Michele Schiffer and Andrew Thompson, and all researchers who were involved in both the phenological data collections and drought experiment. I also thank Leah Nugent (Cairns Regional Council Nursery) for helping me with one of my chapters.

In 2017, I had the opportunity to help Dr. Oliver Binks with his research at the DRO. This was a big scientific adventure, with lots of night shifts in the lab, but also lots of conversations and laughter, and this experience gave me a friend. I am always happy to catch up with you, Steph and the kids, and I am grateful for our friendship.

In 2018, I had the opportunity to develop a research project at the Safe Project in Malaysian Borneo, which was an amazing experience. This project was in collaboration with Dr. Alexander Cheesman, Prof. Patrick Meir, Prof. Yadvinder Malhi's lab, and Dr. Nathan English. This project was also in collaboration with Prof. Noreen Majalap, who was essential, as was help from Roland Robert. I am also grateful for the help, support and friendship of Salwana Jaafar and Fifyliana Karim, as well as for all the lovely people I met at the Safe project.

I extend my gratitude for my friends and colleagues from the Cernusak Lab and the Postgraduate Centre at JCU, for all the important conversations, support, laughter and coffee chats. In particular Arun, Stephanie, Kali, Rachael, and Alex. I am also grateful to Fernanda and Lalita, with whom I shared daily life in the beginning of my life in Australia.

I am deeply grateful to my parents, Lúcia and Eguimar and my Australian family, Diane, Eric, Tamika and Ethan, for all their support and love. I am grateful for the support of my relatives as well, and here I would like to pay homage to my uncle Wilson who always believed in me (*in memorian*).

I am very grateful to my friends that were there for me in the good and bad times. Simone Scalione, Giovanna Ijanc, Caian Gerolamo, Flavia Lemos, Luiza Sellera, Maria Rivera, Arun

and Apoorva Singh, Grace Kerr, Linda Duran, Bruno Ceccato, Laila Ubbi, Deborah Apgaua, Micaela Boarotto, Asta Sillah, your friendship was essential during this journey! Special thanks to Bruno Ceccato, Milene Eingenheer and Luiza Sellera who gave help and support with my process of moving to Australia.

In the second half of my PhD, I faced very challenging health issues that needed special adaptation and surgeries, I was only able to manage with help from multiple health practitioners and therapists. I am especially deeply grateful to Dr. Irene, Dr. David, Dr. Phoebe, and Dr. Tanya. Thank you for helping me to make it through and to be able to finish my PhD. I am deeply grateful for all the support and care from Gislaine Carvalho, and to Amy Hopper who have been helping me with lots of kindness and true care! Also, thank you Ana Palma and Arun Singh for the lovely support during the recovery of my first surgery, and Grace Kerr for spoiling me at the hospital in my second surgery (and all your support in general).

Last but not least, I would not have made it without his partnership, all his help and support. Jayden Engert, thank you for everything, for being such an amazing partner in both life and science, for all your care and wonderful love!

General abstract

Tropical forests are perhaps the most important ecosystem on the planet in terms of biodiversity values as well as for the provision of ecosystem services such as carbon storage and climate regulation. Tropical forests act as carbon sinks on global scales, and are crucial for water cycles extending beyond their boundaries. Despite their vital importance to Earth's climate regulation, tropical forests are still poorly understood, especially when it comes to phenology of wet tropical forests and evergreen tree species. Understanding phenological patterns in tropical rainforests, in association with their underlying processes, is of extreme importance to better predict the effects of climate change in the tropics. In this thesis I explored the reproductive phenological patterns of northeastern Australia rainforest and influence of plant physiological processes on these patterns.

In Chapter 2, I reviewed the published phenological literature across Southeast Asia and Oceania in order to assess common patterns and knowledge gaps. I identified 66 published studies containing phenological observations from natural forest systems. The geographic distribution of these studies is limited, with large regions of exceptional biodiversity unrepresented. Sampling effort in phenological studies was generally low, with 61% of studies lasting five years or less, and only four studies that presented > 10 years of phenological observations. The most frequently identified environmental drivers of phenological variation across all studies were temperature and rainfall, while biotic drivers were found to be rare (1%). In addition, I identified a divergence between seasonality in flowering and fruiting among Oceania, mainland Southeast Asia and insular Southeast Asia. Overall, this review indicates that significant knowledge gaps limit our ability to identify general phenological patterns, and their relation to physiological processes and climate drivers for the tropical forests in the region. These knowledge gaps diminish our capacity to predict climate change effects on forest cycles, productivity and structure.

In Chapter 3, submitted to *Journal of Ecology,* I analysed the influence of an experimental drought (TFE) and climatic drivers on the community patterns of flowering and fruiting (357 trees of 33 species) in Daintree rainforest of North Queensland, Australia. The community presented defined seasonality, with flowering occurring in the wet season and fruiting in the dry season. Flowering was mainly driven by increase in temperature and solar radiation, while fruiting increased with decrease in photoperiod. The experimental imposition of drought led to an increase in fruiting activity, but selected species analysed at population level showed divergent responses to drought. I combined the population level phenology of the three selected species with elemental and stable isotope values from leaves and wood, to assess potential changes in water use efficiency and water source and or transpiration rates under drought conditions. *Syzigium graveolens*, the only species that increased both flowering and fruiting under TFE, presented higher δ^{15}N values, in all seasons and increased δ^{13}C values in the transition season. *Myristica globosa* did not present significant changes in phenological activity but increased δ^{18}O values, in response to drought, while *Cleistanthus myrianthus* decreased fruiting activity, associated with decreased δ^{13}C in the transition season, which indicates a decreased water use efficiency. I concluded that while the whole community presented changes in the fruiting pattern, species showed specific responses, associated with different adaptations to drought.

In Chapter 4, published in *Journal of Plant Ecology* (2020), I investigated the effect of an experimental drought on the phenological pattern of an Australian endemic palm (*Normanbya normanbyi*) in the Daintree rainforest of North Queensland, Australia. To better understand the effects of drought on the population, I combined observations of fruiting phenology, assessing their climate drivers and seasonality, with physiological processes, including leaf gas exchange and stable isotopes from the leaves. I then test how drought affects photosynthesis and water use efficiency, and how these changes influence the reproductive output of the species. The species presents a clear seasonality, fruiting in the dry season. It shows a decline in fruiting after the imposition of drought, with an increase again in 2018, when the rainfall was well above average. During the drought period, the species showed a decline in photosynthetic capacity and increased water use efficiency, with more significant differences in the dry season. After assessing reproductive phenological cycles and their relation to physiological processes, I move on to assess leafing phenology and some of the important physiological processes related to it in the next chapters.

Assessing long-term phenologial observations allowed me to explore how the tree community is influenced by climate variables both seasonally and through time (including an experimental drought). Additionally, it allowed me to explore how species may respond differently to climate and how this difference is mirrored in their physiological processes. These results provided important insights on the forest resources availability and potential changes in its structure.

In Chapter 5, published on *Annals of Botany* (2020), I conducted a literature review of the differences in δ^{13}C value between young and mature leaves across different regions (tropical *vs*. temperate) and leafing patterns (evergreen *vs*. deciduous). The δ^{13}C value is valuable for studying leaf phenology as it allows for the determination of carbon source for the leaf production in trees. Stored carbon has an enriched δ^{13}C value due to translocation from leaves to storage in the roots or wood, and therefore it is possible to test the young leaves to determine whether the main source of carbon was stored carbon or the current photosynthates in the leaves. I found that, while no significant difference was found between tropical and temperate regions, evergreen and deciduous species were significantly different. Deciduous species presented less negative δ^{13}C values, explained by the use of stored carbon after leaf fall, while evergreen species relied on mature leaves as a source of carbon, and had more negative δ^{13}C values. In addition, I showed that less negative δ^{13}C values in young leaves do not represent a higher Water Use Efficiency (WUE), as previously hypothesised. On the contrary, the high δ^{13}C values represent a lower WUE, explained by high rates of daytime respiration in combination with low photosynthetic capacity. Although the literature review provided great insights into this processes, empirical evidence from experiments would be highly desirable to properly test the explanation for these differences.

Given the general context of carbon use and vegetative phenology, in Chapter 6 I tested the source of carbon used by deciduous and evergreen tree species using 160 rainforest tree seedlings grown in a glasshouse under different CO_2 concentrations. Half of the seedlings in each treatment were defoliated, which allowed me to label the newly synthesised carbon, as well as identify the use of stored carbon. Evergreen and deciduous species showed a different use of carbon, with evergreen species using more of the current photosynthate, while deciduous species relied more on stored carbon. Next, I assessed how the two groups responded to elevated CO_2. Deciduous species presented higher photosynthetic capacity under elevated CO_2, and a faster recovery from defoliation, while evergreen species invested more in leaf structure. I conclude that both groups benefited from elevated CO_2 in different ways, and differ, overall, in their physiological response. The difference in use of stored carbon that I identify needs further attention, as more events that lead to leaf loss may exacerbate the use of reserved carbon, resulting in potential tradeoffs between phenology and growth.

Finally, in Chapter 7, submitted to *Austral Ecology*, I explored the differences between species with and without delayed greening and how they respond to elevated CO_2. Delayed greening occurs in about 30% of species in tropical forest trees, but the phenomenon is extremely understudied. I tested the physiological differences between green and red/purple/pink leaves and whether the two leaf types present significant differences in their nutritional contents. Young leaves with delayed greening presented higher N concentration

than the green ones. And lastly, I investigated if they benefit differently from elevated CO_2. I conduct chlorophyll measurements, leaf gas exchange measurements, nutrient and stable isotopes analyses and measurements of leaf expansion in three species, two with delayed greening and one without. The two species with delayed greening had different leaf life spans (one deciduous and one evergreen) to account for any possible variation due to deciduousness. Net photosynthesis rates were significantly lower in recently flushed leaves of species with delayed greening than in the species without. Intrinsic water-use efficiency increased at a steeper rate during leaf development under elevated CO_2 in all three species, irrespective of greening strategy. Although elevated CO_2 did not boost the performance of the delayed greening species more than that of the species without, I found higher nitrogen concentrations in their young leaves, which possibly suggests that delayed greening may have evolved in these species to protect young leaves that are especially rich in nitrogen.

List of Publications

This thesis contains a number of chapters which have been published or submitted.

Below is a list of publications contained within this thesis, and other publications and conference abstracts accepted during the candidature.

Thesis publication details

1. Vogado N.O., Bird M.I., Liddell M.J., Engert J.E., Wurster C.M., Cernusak L.A. Climate change and phenology research in Southeast Asia-Oceania: knowledge gaps and future possibilities. *In preparation*.

2. Vogado N.O., Liddell M.J., Laurance S.G.W., Campbell M.J., Cheesman A.W., Engert J.E., Palma A.C., Ishida F.Y., Cernusak L.A. The effects of an experimental drought on the ecophysiology and fruiting phenology of a tropical rainforest palm. (2020). *Journal of Plant Ecology*, 13(6): 744–753. https://doi.org/10.1093/jpe/rtaa069.

3. Vogado N.O., Laurance S.G., Liddell M.J., Engert J.E., Wurster C.M., Schiffer M., Thompson A. and Cernusak L.A. Assessing the effects of a through-fall exclusion experiment on the reproductive phenology and ecophysiology of a wet tropical rainforest community. *Under Review in Journal of Ecology*. 2021.

4. Vogado N.O., Winter K., Ubierna N., Farquhar G.D., Cernusak L.A. Directional change in leaf dry matter $\delta^{13}C$ during leaf development is widespread in C_3 plants. 2020. *Annals of Botany*, 126(6): 981–990. https://doi.org/10.1093/aob/mcaa114.

5. Vogado N.O., Cheesman A.W., Cernusak L.A. Delayed greening during leaf expansion under ambient and elevated CO_2 in tropical tree seedlings. *Under review in Austral Ecology.* 2021.

Other publications during candidature

1. Apgaua D.M.G., Tng D.Y.P., Forbes S.J., Ishida Y.F., Vogado N.O., Cernusak L.A., Laurance S.G.W. (2019). Elevated temperature and CO_2 cause differential growth stimulation and drought survival responses in eucalypt species from contrasting habitats. *Tree Physiology*, 39(11): 1806–1820. https://doi.org/10.1093/treephys/tpz095.

2. J.E. Engert, N.O. Vogado, K. Freebody, B. Byrne, J. Murphy, G. Sheather, P. Snodgrass, L. Nugent, D. Lloyd, S.G.W. (2020). Laurance. Functional trait representation differs between restoration plantings and mature tropical rainforest, *For. Ecol. Manag.,* 473: 118304. 10.1016/j.foreco.2020.118304.

3. Aguiar BI, Sebbenn AM, Tarazi R, Vogado NO, Morellato LPC, Tambarussi EV, Moreno MA, Pereira LCSM, Montibeller C, Ferraz EM, Gandara FB, Kageyama PY. (2020). Phenology, seed germination, and genetics explains the reproductive strategies of Diospyros lasiocalyx (Mart.) B. wall. *Trop Plant Biol.* 13:23–35. https://doi.org/10.1007/s12042-019-09243-1.

4. Vogado N.O., Engert J.E., Linde T.L., Campbell M.J., Laurance W.F., and Liddell M.J. Climate change increases reproduction in tropical lianas. *Under review in Frontiers in Forests and Global Change.* 2021.

5. Diah and the orangutan – 2021. Children's book published for environmental education and raise money for Southeast Asian institutions that work with forest and orang-utan conservation.

Conference abstracts during candidature

1. ESA2021. Effects of an experimental drought on the phenology and ecophysiology of tropical rainforest tree species. Accepted for oral presentation.

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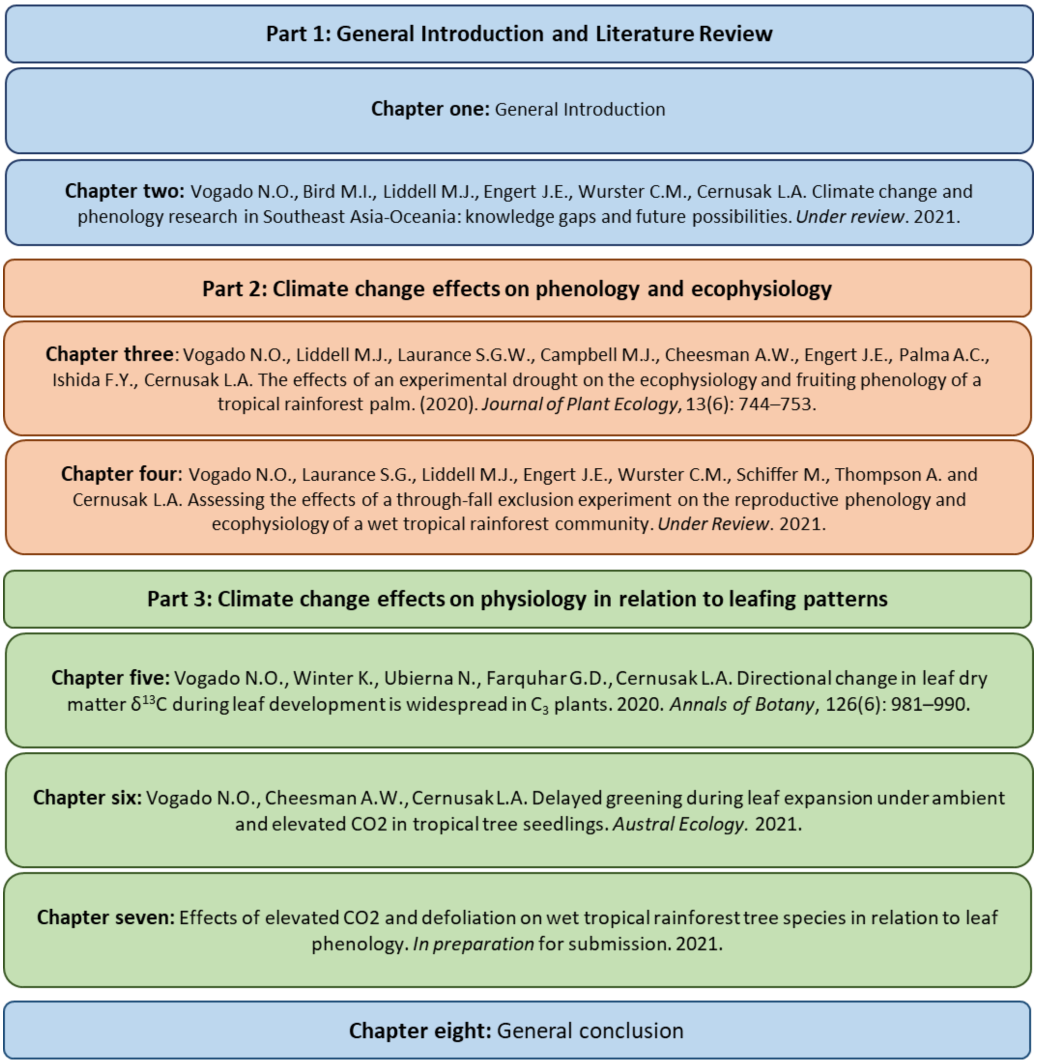
Part 1: Introduction and Literature Review

Part 2: Climate change effects on phenology and ecophysiology

Part 3: Climate change effects on physiology in relation to leafing patterns

Chapter 1 - General introduction

Specifically, this PhD thesis aimed to assess the long-term phenological patterns (>8 years), how they respond to climate drivers and experimental drought, testing how changes in environmental conditions (drought) affected physiological processes, analyzing how different resilience of species related to changes in phenological responses. Additionally, it aimed to provide more insights into the relation between leafing patterns and physiological process, exploring explore how different vegetative phenology depend on physiological processes, and how leaf phenology impacts recovery from defoliation as well as tree species responses to elevated CO_2 (Fig. 1.1.1).



Part 1: General Introduction and Literature Review

Chapter one: General Introduction

Chapter two: Vogado N.O., Bird M.I., Liddell M.J., Engert J.E., Wurster C.M., Cernusak L.A. Climate change and phenology research in Southeast Asia-Oceania: knowledge gaps and future possibilities. *Under review*. 2021.

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Chapter three: Vogado N.O., Liddell M.J., Laurance S.G.W., Campbell M.J., Cheesman A.W., Engert J.E., Palma A.C., Ishida F.Y., Cernusak L.A. The effects of an experimental drought on the ecophysiology and fruiting phenology of a tropical rainforest palm. (2020). *Journal of Plant Ecology*, 13(6): 744–753.

Chapter four: Vogado N.O., Laurance S.G., Liddell M.J., Engert J.E., Wurster C.M., Schiffer M., Thompson A. and Cernusak L.A. Assessing the effects of a through-fall exclusion experiment on the reproductive phenology and ecophysiology of a wet tropical rainforest community. *Under Review*. 2021.

Part 3: Climate change effects on physiology in relation to leafing patterns

Chapter five: Vogado N.O., Winter K., Ubierna N., Farquhar G.D., Cernusak L.A. Directional change in leaf dry matter $\delta^{13}C$ during leaf development is widespread in C_3 plants. 2020. *Annals of Botany*, 126(6): 981–990.

Chapter six: Vogado N.O., Cheesman A.W., Cernusak L.A. Delayed greening during leaf expansion under ambient and elevated CO2 in tropical tree seedlings. *Austral Ecology.* 2021.

Chapter seven: Effects of elevated CO2 and defoliation on wet tropical rainforest tree species in relation to leaf phenology. *In preparation* for submission. 2021.

Chapter eight: General conclusion

Fig. 1.1.1 Thesis structure.

1.1 Phenology, tropical forests and environmental change

Tropical forests store 25% of global terrestrial carbon while covering just 7% of the Earth's land surface (Zuidema *et al.* 2013). Accounting for about 60% of the total terrestrial land surface metabolism, tropical forests have been recognised as a major influence on global biogeochemical cycles and atmospheric function, through carbon uptake of about 41 PgC year-^{1} or 34% of the global total gross primary production (GPP) (Malhi 2012). As these forests process large quantities of carbon yearly, climate-induced changes in their composition and structure may result in the release of substantial quantities of carbon into the atmosphere (Malhi & Grace 2000). Therefore, tropical forests play an essential role in the global carbon cycle, and one that may be affected by global climate change.

Amongst the world's rainforests, much attention has been focused on the Amazon, the largest contiguous tropical rainforest in the world. However, despite receiving significantly less attention, the tropical rainforests of Southeast Asia and Oceania maintain exceptional levels of biodiversity and species endemism, and are also crucial for climate regulation (Sodhi *et al*. 2004, Williams *et al*. 2011). It is essential to take account of more regional responses of forests to climate change in order to better understand the different effects that a drying environment might produce on ecosystems dynamics and equilibria. The effects of climate change, as increased temperature and droughts, can cause substantial changes not only on primary forests but also on secondary forests, which compose more than half of the world's tropical forests (Uriarte *et al*. 2016a, 2016b).

Global mean temperatures have been increasing since 1950 and are predicted to continue to increase into the future (Fig. 1). This increase in temperature is also predicted to lead to regional variations in precipitation (IPCC 2021). As a result, droughts are expected to increase in frequency and intensity all around the globe in the coming decades. When studying climate change models, Dai (2012) found that the regions of Southeast Asia and Oceania have already been experiencing a drying effect due to climate change. Among the seasonal effects of climate change, the same regions also experience extreme events such as El Niño/La Niña and tropical cyclones, which have great impacts on forest function and structure (Christensen *et al*. 2013).

The El Niño Southern Oscillation (ENSO) is a natural climatic phenomenon that involves variation in winds/rainfall regimes and sea surface temperatures over the tropical eastern Pacific Ocean, affecting the climate across the entire Earth system (Cane 2005). Rainfall regimes may present some slight seasonality even in perennially humid tropical lowland forests of Southeast Asia and Australia and these climate norms are being altered by anthropogenic climate change. ENSO events are becoming stronger, giving rise to severe droughts in the region and the impact of these droughts on tropical forests remain unknown (Kitayama & Aiba 2002). It is essential, therefore, to better understand the regional effects of ENSO and the responses of tropical forests to the changes in ENSO periodicity and intensity.

In the tropics, shifts of rainfall patterns due to strong ENSO events, such as the 2015-16 El Niño, can increase precipitation in arid regions and decrease it in wet regions (Gomyo & Koichiro 2009). In northern Australia, Indonesia, Philippines (Meehl 1987, Ropelewsky & Halpest 1987), and Malaysia (Harrison 2001, Juneng & Tangang 2005; Tangang & Juneng 2004, Gomyo & Koichiro 2009), El Niño events have been associated with decreased rainfall. When studying the relationship between rainfall, rainfall seasonality and El Niño in Malaysian Borneo, Gomyo & Koichiro (2009) found that El Niño events affected different regions in different ways over the 40-year study period. Despite differences between the 18 study sites all areas presented vulnerability to El Niño induced drought and significant decline in rainfall.

Generally, in tropical forests, tree species composition and diversity can be affected in different ways by droughts. One response can be the disproportionate mortality of some tree species and size classes, affecting species composition and diversity in forests due to the high relative mortality of pioneer trees (Meir *et al*. 2015). Another impact of drought is an increase in light availability in the forest understory, affecting the establishment of, and growth conditions for, seedlings and young trees (Slik 2004). Trees may also respond by changing photosynthetic activity, leaf exchange pattern and phenology, changing the ecological equilibrium of the forest and the availability of resources for frugivores and pollinators (Morellato *et al*. 2016).

1.2 Plant phenology

Phenology is the study repetitive cycles in biology and their relation to physiological and environmental factors (Lieth 1974). It is considered a reliable tool to verify plant reproductive and growth strategies, and their relation to biotic and/or abiotic drivers. When analysing reproductive phenology, it is essential to consider species characteristics, such as dioecy and seed dispersal syndrome, since zoochorous and anemochorous/autochorous species need different environmental conditions to succeed in their reproduction and seed establishment. Population and community phenology of trees may present different patterns, flowering annually, biannually or supra-annually, and showing different levels of synchronicity. In temperate regions, phenological patterns are usually cued to neatly respond to changes in temperature, while in tropical regions phenological patterns may be dictated by a range of different drivers, such as irradiance, seasonal/extreme drought, or increase in rainfall. In the humid tropics, where the temperature is more constant and water is generally abundant throughout the year, phenological cues such as photoperiod and/or biotic variables may present more of an advantage in reproduction success (Pau *et al.* 2011). The seasonality of regions is also important to understand phenological drivers.

Wet tropical biomes, especially monsoon driven systems, often have very short seasonal transition periods, when species rapidly begin to grow and reproduce. Biotic forces may be the phenological drivers in case of these biomes with low interannual variability of climate variables.

The manner in which rainforest species will respond to rapidly changing cues independent from photoperiod, and how these changes in climate might lead to cascade effects, remains uncertain (Pau *et al*. 2011, Butt *et al.* 2015). However, understanding these relationships will be essential to better understand and predict the effects of climate change on these forests.

Phenological activity is expected to respond to a drying and warming environment by advancing the timing, and/or diminishing the quantity, of flowers and fruits produced (Cleland *et al.* 2007, Menzel *et al.* 2006, Richardson *et al.* 2013, Tang *et al.* 2016). In wet tropical rainforests, phenological activity has been found to be influenced by the annual peak of solar irradiance (Wright & van Schaik 1994, Chapman *et al*. 2018), changes in rainfall (Dunham *et al*. 2018), CO_2 concentration (Pau *et al*. 2018), and temperature (Potts *et al*. 2019). In the Amazon forest, however, different ecoregions have been found to be driven by irradiance or rainfall depending on the region and the availability of resources (Jones *et al*. 2014). The availability of necessary resources, such as water and light, can change primary production in the forest (such as number of fruits available) (Ripoll *et al*. 2014, Meir *et al*. 2018). Within the rainforest community, there are many interdependent relationships that maintain the ecological functioning of the ecosystem. When these relationships are disrupted, phenological mismatches may occur, for example when pollinators become active after flowering events occurred (Gérard *et al*. 2020). These disruptions may lead to the need of adaptation or to the risk of species extinction (Morellato *et al*. 2016).

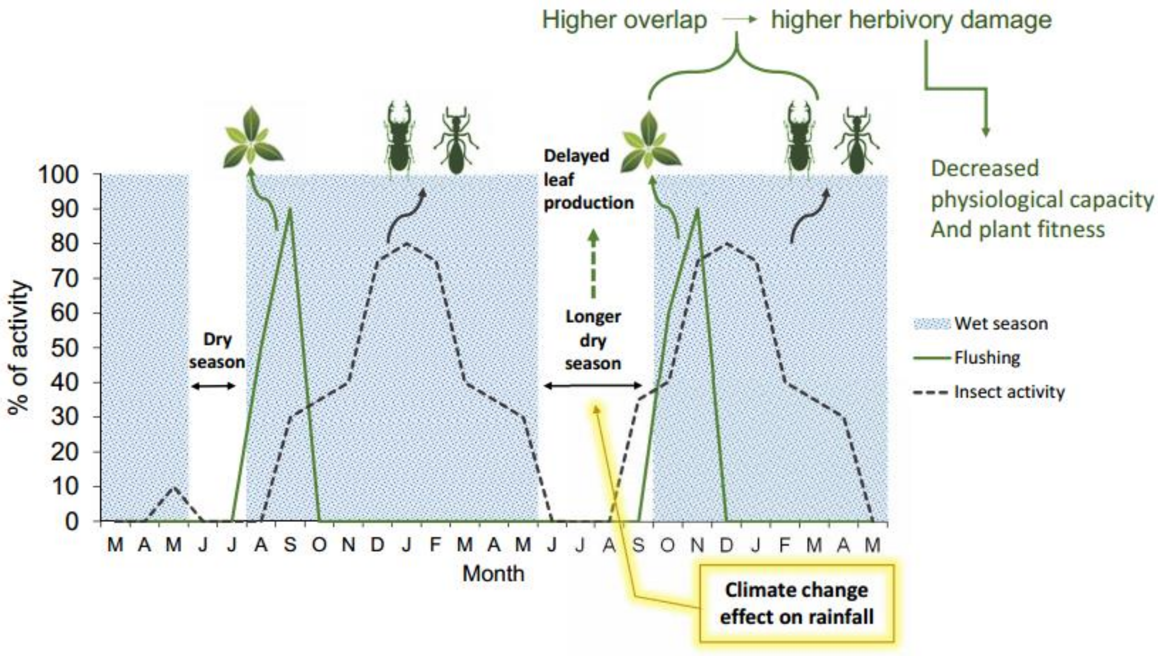

Fig. 1.2.1. Scheme of hypothetical example and possible outcome of human-induced shifts in rainfall pattern and consequence for the relationship between leafing and herbivores activity, if environmental driver of leafing is the beginning of wet season. Adapted from Morellato *et al*. (2016).

Vegetative phenology may also be vulnerable to phenological mismatches as well as to growth rates, which determine carbon balance and reproductive allocation. Phenological mismatches include relationships among biota, such as leaf exchange and herbivory. Delayed leafing activity may lead to a peak of young leaves when herbivores are most active, causing an increased loss of leaves (Fig. 1.2.1). Since leafing activity is essential for carbon assimilation through photosynthesis, changes in vegetative patterns may lead to disruption in growth and carbon storage. At the same time, the production of new leaves often requires the use of stored carbon and, therefore, has implications for carbon reserves and plant growth.

Tropical trees are generally more sensitive to irradiance and water availability rather than mean temperature and need to avoid herbivory during leaf production as young leaves often lack defence mechanisms (Lowman 1992). Deciduous and evergreen trees use different strategies to cope with, and manage, herbivory. While deciduous species in tropical forests usually drop their leaves in the dry season and produce new leaves at the end of the dry season/beginning of wet season, avoiding herbivores that are active in the wet season and optimising photosynthetic gain (Rivera *et al.* 2002), evergreen trees can use various strategies to defend/survive herbivory. In between, there are other levels of deciduousness namely “brevi-deciduous” species, which may drop their leaves for short periods of time, and not exchange the entire canopy. One strategy of evergreen species is exchanging leaves throughout the year, providing trees with a mechanism to survive herbivore attack while keeping mature leaves in the canopy to maintain photosynthetic activity. Another strategy is mass leafing, which satiates insects thereby increasing the survival potential of young leaves (Aide 1992, van Schaik *et al.* 1993), or delayed greening which makes it more difficult for some insects to see the young leaves. These strategies are important since increased herbivory reduces a plant's photosynthetic activity, further decreasing its potential to produce new growth. Although being extremely important to the deep understanding of forest ecology, leafing activity is a poorly understood process in wet tropical rainforests (Borchert *et al.* 2002).

Long-term studies (more than five years) assessing phenological patterns in tropical forests are predominantly found in the Neotropics, but also to a lesser extent in Africa. For example, in Madagascar, Dunham *et al*. (2018) studied how changes in rainfall are likely to affect tree phenology, with decreased fruiting activity in driers periods. Wright and Calderon (2005) found that both flower and seed production both increase during El Niño events in a tropical forest in Panama, suggesting the influence of increased solar radiation. Wright and Calderón (2018) presented a 30-year phenology study in Panama, and found that flowering activity was mainly driven by solar irradiance. Chapman *et al*. (2018) analysed 15 years of phenological data in Uganda and found that the main environmental cues for fruiting are the variability in irradiance and the occurrence of El Niño, which could indicate an increase in fruiting due to more frequent El

Niño events and increased solar radiation due to decreased rainfall. Babweteera *et al*. (2018) in Uganda found, after analysing 24 years of phenological and tree growth patterns, that trees with faster growth rates and higher canopy exposure to light are increasing their flowering and fruiting frequencies, while fruit production broadly declined over time. Potts et al. (2020) analysed 19 years of phenological data in Uganda and found that average temperature was the main driver of monthly fruit production. Although many important studies have been published with long-term phenological observations, as the ones mentioned above, the combined study of phenological activity in relation to physiological processes is still rare. Long-term phenological studies on the ground, with direct observation of flowering and fruiting in tree canopies, do not exist for Australia.

1.3 Physiological processes behind plant phenology

An understanding of the physiological mechanisms driving phenology is essential to the modelling of climate effects on phenology in both species and ecosystem level (Tang *et al*. 2016) and is one of the major, persistent knowledge gaps in the understanding of the effect of climate change on tropical forests. The majority of phenological and physiological processes depend on water and carbon cycles, being tightly related to photosynthetic capacity and plant carbon and water use (Fig. 1.3.1).

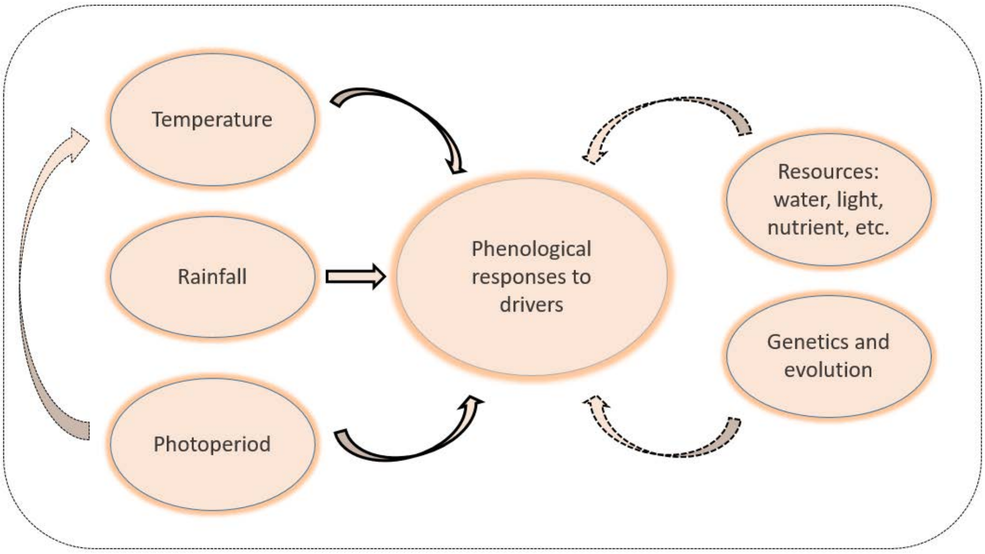

Fig. 1.3.1. Scheme of direct and indirect effects of climate that affect phenological activities. Adapted from Tang *et al*. (2016).

Changes in climate, such as severe drought, will affect the carbon fixation capacity of plants. Current dynamic models of vegetation simulate varying responses of trees to drought, temperature and radiation (Galbraith *et al*. 2010). However, these models remain simplistic due to the lack of physiological and phenological data of *in-situ* tree responses to climate. A significant source of uncertainty in our understanding of causes of variation in the carbon cycle amongst forests (Booth *et al*. 2012) exists due to gaps in our mechanistic understanding of tree mortality due to severe drought or repeated drought occurrence over years or decades (Meir *et al*. 2015).

As plants use atmospheric CO_2 for photosynthesis, increased CO_2 concentration can cause direct and indirect effects on plants, such as an increase in Ribulose-1,5-bisphosphate carboxylase-oxygenase (hereafter just RUBISCO) activity, reduction in stomatal conductance, changes in water use efficiency - the relation between the amounts of CO_2 absorbed in photosynthesis and the amount of water lost from the leaves by evaporation, and changes in carbon uptake and allocation (Cernusak 2020). While studies on the influence of drought on mortality exist (Phillips *et al*. 2010, Meir *et al*. 2015), little is known about the influence of climate variability on growth and physiological responses of trees. During leaf development, plant species have different physiological needs. Mature leaves store excess carbohydrates, which in many cases are redistributed and used by young leaves to meet the energy needs for growth and leaf development (Kerbauy 2004, Cernusak 2020). Most of the carbon fixed during photosynthesis is used in the formation of new carbohydrates that provide energy for respiration (Kerbauy 2004). When there is an excess of is new carbohydrates the plant has a positive amount of carbon that can be used for growth, reserves, and increasing dry matter (Larcher 2000).

In the context of climate change, increases in the atmospheric CO_2 concentration, rising temperatures and variation in rainfall will lead to changes in plant physiology (Cernusak *et al.* 2013). At the same time, plant carbon metabolism that is impacted by changes in climate, such as rising CO_2 concentrations and increased temperature, also feeds back onto the climate system, influencing the intensity of climate change (Dusenge *et al.* 2018). As the balance between CO_2 uptake and emission has been affecting the rate of accumulation of atmospheric CO_2, high rates of dark respiration leading to increased carbon loss are also important consequences of climate change (Clark *et al.* 2003, Clark *et al.* 2021). Decreased tree growth and increased CO_2 emissions, for example, have been suggested to be a result of decreased net primary production in tropical forests as a consequence of a warming climate (Clark *et al.* 2003). At the same time, higher atmospheric CO_2 concentrations may reduce the magnitude of the decrease in photosynthesis during dry periods increase intrinsic water use efficiency and enhance tree growth (Cernusak *et al.* 2013). However, studies presenting long- term growth and gas-exchange responses of trees in tropical forests to the combined effects of rising atmospheric CO_2 concentrations and other global changes, such as warming, are still rare (Nock *et al.* 2011, Linares & Camarero 2012).

1.4 Plant physiology and stable isotopes

Tree physiological processes and their relationship to the climate can be studied through the isotope composition of leaves and growth rings (McCarroll & Loader, 2004). Carbon and oxygen exist as several isotopes, which are atoms with the same number of protons but different numbers of neutrons (Cernusak 2020). This difference in mass causes them to be differentially incorporated during natural processes.

Carbon: The process of photosynthesis is dependent on carbon dioxide as well as water and light availability (Lambers *et al*. 2008). After CO_2 uptake by the stomata, CO_2 is selected by the enzyme RUBISCO which has a preference for the lighter carbon isotope (^{12}C). Carbon stable isotope ratios ($^{13}C/^{12}C$; reported as a $\delta^{13}C$ value), therefore, vary according not only to environmental conditions, but also to physiological processes related to stomata diffusion, RUBISCO discrimination and plant storage. The stable carbon isotope composition of leaf dry matter is generally a reliable recorder of intrinsic water-use efficiency (Cernusak 2020). Water-use efficiency is positively correlated with the $\delta^{13}C$ value, which decreases with high photosynthesis rates and isotopic discrimination.

Oxygen: The ratio of oxygen stable isotopes (ratio of $^{18}O/^{16}O$, reported as a $\delta^{18}O$ value) is related to the source of water used by the plants and transpiration rate (Barbour *et al*. 2002, Cernusak *et al*. 2016). The lighter isotope is favoured in the process of evaporation, increasing the $\delta^{18}O$ value (Farquhar *et al*. 2007). Higher $\delta^{18}O$ values, therefore, can be associated with higher temperature and lower soil moisture. Water molecules in shallower soil layers present a higher $\delta^{18}O$ value as well, due to evaporation (Barbour *et al.* 2007, van der Sleen *et al*. 2017). At the leaf level, the ^{18}O content can be enriched above source water due to the fractionation through stomata and at the leaf boundary layer, the proportional depression of water vapour pressure by the heavier molecule, the water vapour oxygen isotope composition in relation to source water and scaled by the ratio between ambient and intercellular water vapour mole fraction (Barbour 2007). In the wood, oxygen isotopes record variation in oxygen isotope ratios of the source water used by trees, which can be modified by environmental temperature and leaf transpiration, the latter controlled dominantly by vapour pressure deficit (McCarroll & Loader 2004).

Thus, both carbon and oxygen stable isotopes are a reliable tool for understanding water use efficiency and tree responses to physiological processes, as well as changes in climate conditions. δ^{13}C values can also be used to provide more insights into plant phenology. It is known that deciduous trees use stored carbon in the production of leaves and tissues (Borchert *et al.* 2002, Helle & Schleser 2004; Gulbranson *et al*. 2012, Worbes *et al*. 2013), but the source of carbon used to produce new leaves and the leaf-flushing drivers are still uncertain in evergreen species. In general, studies analysing the δ^{13}C value of leaves focus on mature leaves, providing a poor understanding of how isotope composition changes during leaf development and responds to abiotic factors (Damesin *et al*. 1998).

Stable isotopes have been used as a reliable tool to understand time series in geology and paleoclimatology (Bird & Chivas 1989, Bird & Gröcke 1997) and lately in palaeobotany and dendrochronological studies (Brienen *et al*. 2013, Evans & Schrag 2004, Gulbranson *et al*. 2012, Gulbranson & Ryberg 2013). Information about the effect of climate on plant physiology and leafing strategies can be assessed through stable isotopes from the tree growth rings. Through a combination of high-resolution dendrochronology and stable isotope analysis (e.g. δ^{13}C and δ^{18}O) (e.g Poussart et al. 2004) time series information relating to water use and ecophysiology of trees can be derived.

High-resolution stable isotope time series thus can help provide a detailed picture of plant physiological responses to short-term variations in climate, and/or an understanding of recurring seasonal patterns of leaf fall and flushes and use of starch. Helle & Schleser (2004) and Damesin & Lelarge (2003) found the highest values of δ^{13}C value at the beginning of the wet season correlated to leaf flush, explained by the use of carbon reserves to produce new leaves. The observed decline of δ^{13}C value across an individual tree ring (after the maximum value) is suggested to be the result of the gradual incorporation of photosynthates into recently formed cell wall material, once trees use both stored and currently produced carbohydrates for growth and maintenance, often at the same time (Helle & Schleser 2004, Cernusak 2020). Therefore, the use of stable isotopes to enhance the understanding of physiological processes, and their relation to phenological patterns may be of high value to enhance the knowledge in climate change effects on wet tropical forests.

1.5 The Wet Tropics of Queensland

In Australia, the Wet Tropics and adjacent tropical forest tracts along the Queensland coast host 1,200 endemic plants in less than 11,000 km^2, presenting a high species/area ratio and providing a hotspot of biodiversity (Williams *et al*. 2011). This high level of species richness and endemism is explained by genetic differentiation and long-term persistence of species in isolation (Sodhi *et al*. 2004). The tropical rainforests of North Queensland are considered highly sensitive to the climatic changes expected over the next century (Hughes 2003). In addition, forested regions are likely to transition to other vegetation types as the climate becomes less favourable for existing ecosystems. This will lead to a change in vegetation structure, adaptation and potential extinction of plant species, and consequently, extinction of the fauna that relies on them (Hilbert *et al*. 2001, Hughes 2003).

The Australian Wet Tropics rainforest experiences a seasonal climate with a relatively drier period from April to November, and a hot and wet summer from December to March, with heavy rains that are associated with the west Pacific monsoon. Rainfall patterns have been changing in several regions of Australia, with the Wet Tropics experiencing a drying pattern that is consistent with more intense drought predicted in the future (IPCC 2021). Variations in the characteristics of the monsoon influence the intensity of the rainfall variability in this region. Although many climate drivers influence conditions in Northern Australia, El Niño and La Niña events may exert the highest influence on interannual rainfall variability, and precipitation in the wet tropics is usually below average during the early part of the wet season for El Niño years. Analysis of the frequency, duration and intensity of drought in the Pacific islands and northeast Australia show that all these factors associated with drought were greater during the period 1981–2010 than 1951–80, driven by interdecadal Pacific oscillation and ENSO (Mcgree *et al.* 2016). Although there are still uncertainties in predictions of rainfall variability, temperature has been increasing throughout the whole region (IPCC 2021). The responses of tropical tree species to these potential scenarios, however, are still unknown.

In the Wet Tropics of Queensland, few phenological studies have been conducted to date. Boulter *et al*. (2006) developed a long-term analysis of plant phenology through herbaria records in the flowering phenology of the Wet Tropics bioregion of far northern Queensland, Australia, based upon over 36 thousand records from two Queensland-based herbaria. They found a flowering seasonality that correlated with rainfall and temperature and increased with increasing latitude and altitude. Inkrot *et al*. (2007) studied, for two years, the reproductive phenology of the black palm (*Normanbya normambyi*) at the individual and population level. At the population level flowering peaked in the dry season, whereas fruiting was confined to the wet season. Edwards *et al*. (2018) conducted indirect phenological observations using litterfall. They found that peaks in leaf litter coincided with peaks in temperature in December-January of each year, while presence of flower and fruits were strongly positively correlated with wind and negatively correlated with temperature and rainfall.

Time series phenology combined with physiology and site level microclimate may be used to explore the environmental variables controlling tree cycles and to understand the biotic factors driving the observed changes. To date, no studies combining long-term phenology and physiology to understand rainforest tree responses to climate change have been reported in the Wet Tropics, showing a critical knowledge gap in plant ecology, especially regarding the predicted changes in climate for this region. It is also clear that despite some variety of studies on phenology for Southeast Asia and tropical Northern Australia, there remain many knowledge gaps (chapter two). More generally then phenological patterns and the physiological processes behind them are still understudied across Southeast Asia and Oceania, and more effort is needed to improve our mechanistic understanding of the flora from these regions. With the thesis I have aimed to enhance our understanding of the reproductive patterns of rainforest trees the Wet Tropics, underpin this with detailed ecophysiology to explore the drivers and to provide insights in the use of carbon in trees with different leafing strategies.

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Chapter Two: Climate change and phenology research in Southeast Asia-Oceania: knowledge gaps and future possibilities

Climate change and tropical rainforest phenology research in Southeast Asia-Oceania: knowledge gaps and future possibilities

Abstract

Phenological changes remain poorly understood throughout tropical regions, despite being widely used as a reliable indicator of climate change in temperate systems. Here we review published studies on reproductive and vegetative phenology conducted at the species, population and community level across Southeast Asian and Oceanian tropical rainforests, with the aim of providing a comprehensive overview of the current status of phenological research throughout these ecoregions. We identified 66 published studies containing phenological observations from natural forest systems. Our review shows that the geographic distribution of these studies is limited, with large regions of exceptional biodiversity unrepresented. Sampling effort in phenological studies was generally low, with 64% of studies lasting ≤5 years, and only five studies that presented >10 years of phenological observations. In addition, 40% of the reviewed studies analysed climate drivers of phenological patterns. The most frequently suggested environmental drivers of phenological variation across all studies were temperature and rainfall, while biotic drivers were found to be rarely inferred. The methods used to assess the relationships between phenology and drivers were mostly descriptive or correlative statistics, with only 5% of studies reporting modelling of phenological time series. Our review indicates that significant knowledge gaps hinder the understanding of general phenological patterns, and their relation to physiological processes and climate drivers for the tropical forests in the region, limiting our capacity to predict climate change effects on forest cycles, productivity and structure. We therefore highlight the need for research in understudied areas, the setting up of long-term phenological studies with modelling of climate variables, as well as physiological and growth processes. Considering that climatic models predict large changes in the climatic conditions for the tropics, despite variability in the direction of mean rainfall change, it is urgent that we determine the magnitude and direction of potential changes in plant phenology.

Keywords: tropical rainforest, flowering, fruiting, leafing, ecological interactions, Asia-Pacific.

Introduction

Tropical forests are some of the most important ecosystems on the planet in terms of biodiversity, carbon and water cycling, and numerous other ecosystem services (Corlett & Primack 2011, Andresen *et al*. 2018). However, they are often understudied when compared to their temperate counterparts, and their likely responses to climate change are not well understood (Cernusak *et al*. 2013). This disparity in study effort is especially apparent when it comes to phenology of evergreen species in wet tropical forests (Borchert *et al.* 2002). Climate change is expected to increase temperatures globally and increase the intensity and occurrence of drought (IPCC 2021), affecting the phenology and physiology of tree species in different ways. Long-term phenological studies are of critical importance to better understand how trees respond to ongoing and anticipated changes in climate, and how ecological relationships may suffer as a result of these changes (Butt *et al*. 2015).

Southeast Asia and Oceania accounted for around 18.3% of the world's forests in 2010 (FAO 2010), with high levels of deforestation (Estoque *et al*. 2019), and is predicted to suffer some of the most severe impacts of climate change. This region is expected to warm faster than the global average (Christensen et al. 2007), and predictions of future rainfall patterns are highly variable (IPCC 2021). Due to the diversity of biomes, topography and local weather within the region, as well as the influence of large-scale phenomena such as the El Niño Southern Oscillation (ENSO) (Christensen *et al*. 2013), changes in climate vary substantially amongst the different regions within the Asia-Pacific (Wang et al. 2016). For this region, variations in the seasonality of the climate system are expected, including increases in the frequency and intensity of cyclones, heat waves, and shifts in timing of the onset of rainy seasons (Knutson *et al*. 2010, Christensen *et al*. 2013, Ahmed & Suphachalasai 2014), all depending on specific location. Changes in the climate system will have strong effects on forest ecology, increasing floods in wet seasons, landslides, drought events, soil erosion, and occurrence and intensity of forest fires, affecting the economy and the vulnerable biodiversity of the region (IPCC 2021, Wang *et al*. 2016). Occurrence of fires in tropical rainforest occur in low frequency, with high intensity when they occur (Bowman *et al*. 2011). Rainforest fires, however, have been increasing in the recent decades, due to increased deforestation and climate change (Juárez-Orozco *et al*. 2017, IPCC 2021).

The rainforests in Southeast Asia include six of the world's 36 biodiversity hotspots (Sodhi et al. 2004). This region hosts high regional biodiversity and endemism, supports more threatened species than any other comparable continental area (Gray *et al*. 2018), and is one of the world's most threatened regions. The island of New Guinea may be the most floristically diverse island on Earth, being home to over 13,600 described species, amongst which over 9,000 are endemic (Camara-Laret *et al*. 2020). The Philippines, a geologically unique area, is considered the second most biodiverse hotspot in the world (Myers *et al.* 2000), with 5,832 endemic plant species, and only 3% of original vegetation remaining. Following the Philippines is the region of Sundaland, composed by the Malay Peninsula on the Asian mainland, as well as the large islands of Borneo, Java, and Sumatra, and their surrounding small islands, which host around 15,000 species of endemic plants despite only 7.8% of the original vegetation remaining (Myers *et al.* 2000). The region of Indo-Burma is also an important hostpot, with 7,000 endemic plant species and only 4.9% of original vegetation remaining (Myers *et al.* 2000). In Australia, the Wet Tropics and adjacent tropical forest tracts along the Queensland coast are home to 1,200 endemic plants in less than 11,000 km^2, presenting a high species/area ratio and being a hotspot of biodiversity (Williams *et al*. 2011). The tropical rainforests of North Queensland are considered highly sensitive to the climatic changes expected over the next century (Hughes 2003). This will lead to a change in vegetation structure, adaptation and extinction of plant species, and consequently, extinction of the fauna that rely on them (Hilbert *et al*. 2001, Hughes 2003).

All the above-mentioned regions have been threatened not only by land cover change, but also by ongoing changes in climate. Climate variables have been shown to be essential drivers of phenological patterns in forests throughout the neotropics (Mendoza *et al*. 2018). Phenological activity responds to climate cues in different ways. Water availability for example, influences leaf and fleshy fruit production and hence changes in timing or quantity of water availability may cause significant changes in timing and abundance of new leaves and mature fruits. Water availability and temperature also have important effects on tree growth and growth seasonality, which in turn may significantly affect phenological activity due to decreased carbon sources required for fruiting and flowering. Alternatively, temperature may influence phenological patterns by signalling onset of flowering, ripening of fruits and peak of leaf fall. Hence increasing mean temperatures may influence the timing and activity of these phenophases (Scranton & Amarasekare, 2017). As phenological patterns are controled by numerous and varied drivers, understanding what these drivers are and how they may change in the future will be necessary to predict future changes to tropical forest function (Morellato *et al*. 2016, Butt *et al*. 2015). Additionally, identifying species that are more vulnerable in the face of climate change is a basic prerequisite to effectively targeting and tailoring conservation actions in these areas.

In regions with such a high diversity of plant species, the general patterns of flowering, fruiting and leafing activity are essential for understanding the ecological condition of the forests (Tang *et al*. 2016, Butt *et al*. 2015). Community level analyses of changes in timing/activity of tree species reproductive and vegetative cycles, driven by environmental factors, provide us with an appropriate framework within which to manage tree species diversity and conservation in the face of climate change (Corlett & LaFrankie, 1998). Differential responses of species to climate drivers, and hence changes in reproductive success, can have important impacts on forest composition and structure. Additionally, changes in flowering and fruiting activity, for example, may lead to calorie deficits in the fauna that rely on the fruits for food (Wong et al. 2005 in

Malaysia, Bush *et al*. 2020 in Africa) with striking impacts throughout the trophic web (Butt *et al*. 2015). Alternatively, increased leaf fall may alter carbohydrate storage patterns in trees, along with transpiration rates that in turn alter water availability in the forest (Wu *et al*. 2017). Despite the importance of leafing in the carbon and water cycles, the activity of leafing is a poorly understood process in wet tropical rainforests (Borchert *et al*., 2002, but see Jones *et al*. 2014, Moore *et al*. 2016, Wu *et al*. 2017).

Here we review the literature on tropical phenology in the rainforests of Southeast Asia and Oceania. We examine the methods employed in phenological studies, including location, length of monitoring period, analysis of climate drivers, and species studied. Additionally, we assess the results of phenological studies published in the region to date, including in relation to the seasonality of reproductive phenology, comprehensiveness of phenological research in the region, expected changes to climatic conditions, and the impact of climate change on the processes underlying phenological patterns. Finally, we propose some directions for future research to improve the understanding of phenological patterns and their related ecological and conservation implications.

Materials and methods

We undertook a literature search for all published research papers on phenology where observations were conducted at study locations within tropical rainforests of Southeast Asia and Oceania, using ISI Web of Science and Google Scholar. Our specific search terms included “phenology”, “fruiting”, ”flowering”, “leafing” and “Southeast Asia”, “Oceania”, “Australia”, “Micronesia”, as well as the names of specific individual countries and islands in the region. For our study we considered Oceania to comprise New Guinea, Australia, and Micronesia in order to account for similarities among climate and vegetation. We included all papers published prior to the end of the year 2020. We included only papers employing 'on the ground' phenological surveys based on native tree species. No remote sensing studies were included as they employ a different methodology and therefore have a different capacity to assess varied phenological activity across different levels. Taxonomic only papers were also avoided. Some studies were not included where the papers were not accessible.

For each paper identified, we recorded study location (geographic coordinates), number of species observed, observed phenological trait(s), duration of observation, observation method, assessment and any analyses related to environmental drivers. As phenological series were often found in papers not explicitly focused on phenology, we made note of the research topics presented that included studies of phenology. We established the following broad topics that were addressed in the papers: taxonomy, phylogeny, genetics, ecophysiology (including structure, chemistry and physiology of leaf and canopy, leaf decomposability, and spectral reflectance), plant-animal interactions, plant growth and plant reproduction (including fecundity, production, sexual specialisation). To compare seasonality in relation to other regions, we assessed the month of the year where there was a peak of phenological activity, using only studies on communities that were conducted for more than one year. We then used results presented and also assessed the data plots to select the season where higher activity was found. This allowed us to compare the suggested seasonality of the studied region with other patterns found in the tropics.

To assess the geographic coverage of the reported phenological studies, we mapped the locations of the studies using Arcmap. We used the geographic information to assess predicted changes in climate (mean temperature, mean annual rainfall, rainfall standard deviation) for the study sites between the date of study publication and the year 2050. Climate data were obtained from CHELSAcruts for study date, and CHELSA futures (CMIP5) for expected climate in 2050 (Karger *et al*., 2017). Future climate was estimated by averaging five CHELSA climatologies: CESM1-BGC, MPI-ESM-MR, ACCESS1-3, MIROC-5, and CMCC-CM. For phenological studies published between 2016 and 2019, we used CHELSAcruts data for the year 2016 as an estimate of the relevant climate values. We then assessed variation in temperature and rainfall (mean and standard deviation) and how these related to the climatic drivers found for phenological patterns in each location. This allowed us to provide a more complete picture of significant climate drivers of phenology and how they are predicted to change in the future.

Results

In total, 66 papers were identified that reported research on aspects of tropical rainforest plant phenology in the Southeast Asia and Oceania region (Table S2.1). Many studies do not cite phenology in their title (44%) or in the presentation of the work due to other aims of the research, which included foci on seed dispersal, restoration, pollination and frugivore activity, but provide direct or indirect phenological data and time series of observations. Very few studies on phenology from Southeast Asia and Oceania species have been conducted so far that relate phenological patterns to climate variables.

Of the 66 studies we identified, 9 countries and 20 ecoregions within Southeast Asia and Oceania were represented (Fig. 2.1). 65% of the studies were concentrated in Malaysia and Indonesia. No studies of native forest systems were found in either Cambodia or Myanmar despite these countries lying within the Indo-Burma biodiversity hotspot (Myers *et al.* 2000). We also could not identify studies from many ecoregions of Southeast Asia and Oceania, including significant regions such as the lowland rainforests of Sulawesi (Fig. 2.1). Despite being a region of exceptional plant diversity, only two studies have been conducted for the entire island of New Guinea.

Of the 66 studies identified, 27 analysed the relationship between phenological activity and climate drivers. Of the climate drivers indicated, the majority of studies pointed to rainfall patterns (including precipitation, drought, or ENSO), with fewer studies discussing the influence of temperature or other factors (Fig. 2.2). The majority of studies did not statistically analyze the relationship between phenology and climate variables (71.2%). Of the ones that did employ statistical tests, the majority focused on the influence of rainfall (7.6% of total studies) or rainfall and temperature (10.6% of total studies). Studies assessing multiple climate variables ($n > 3$) using statistics were scarce (6% of total studies) (Table S2.1). Predicted future rainfall patterns in Southeast Asia and Oceania are highly variable, with different models predicting higher or lower rainfall than present conditions (+96mm to -137mm) for different ecoregions (Fig. 2.3). However, all ecoregions are expected to become warmer, although the degree of warming is variable (+0.4℃ to +2.1℃) (Fig. 2.3).

The most common statistical methods employed were correlation analyses (10 studies), generalised linear models and mixed models (3 studies), and linear regressions (2 studies). Only 3 studies used more advanced modelling techniques that are able to account for non-linearity, and auto-correlation structures that are often found in phenological time series, such as generalised additive models (GAMs). Few studies tested for correlations between phenological activity and climate variables to determine drivers of intra-annual variation, and reported time-series analyses to de-trend changes in climate from typical patterns of phenological seasonality. To date, two studies with experimental drought plots (Moser *et al*. 2014, Vogado *et al*. 2020), have been conducted within this region, limiting our ability to develop causal relationships between phenology and climate impacts.

We assessed when the peak of flowering and fruiting occurred for the community studies in all sites, classifying them into wet season, dry season, transition (dry-wet or wet-dry), and other (such as aseasonal patterns or bimodal distributions). In total, there were 38 studies assessing community patterns. We compared the peak of phenological activity in seasons between Oceania, Mainland Southeast Asia and Islands Southeast Asia. Flowering and fruiting activity was concentrated in different seasons across the region. While most studies showed a higher levels of flowering in the wet season for Oceania, it was mostly aseasonal or in dry season for both regions in Southeast Asia. Neither the mainland nor the islands of Southeast Asia presented any flowering activity in the wet season. Fruiting activity, on the other hand, occurred mainly in the dry season for Oceania with some aseasonal patterns, but in the wet or transition season for mainland Southeast Asia. Across the islands of Southeast Asia, fruiting occurred in all seasons (Fig. 2.4).

Studies focusing on how these changes may impact phenological activity were not common. We found that while 27 studies assessed phenological responses of rainforest phenological patterns to climate drivers, 12 studies analysed individual species responses at population level. A large portion (58%) of the studies presented community phenological patterns. Two studies presented phenology at both community and population level (Lambert & Marshall 1991, Harrison 2001). Of the 21 studies that analysed phenology at a population level, six assessed species-specific responses for more than five species. Some studies presented an extremely short observation period - such as an 80 day campaign during a mass flowering event (LaFrankie & Chan 1991). 25 publications were from the last ten years, three of which were from Australia. We found that the majority of studies (40 out of 67) carried out observations for less than five years (Fig 2.5b). Five of the studies presented more than 10 years of observations, inclusive, at time of publication.

Several important studies with long-term (>5 years) phenological time series were found in books, mainly exploring frugivory and animal behaviour. From the 66 studies on phenology, 26 additionally assessed plant-animal interactions, three assessed taxonomy/phylogeny, one presented genetics analyses, one assessed plant growth and two assessed plant production (Fig. 2.6). The limited coverage of ecoregions, temporal spans and both community and species coverage places limits on the reliability of broad conclusions about phenological trends across the region, but, when all studies are considered together, this does provide a baseline for current conditions.

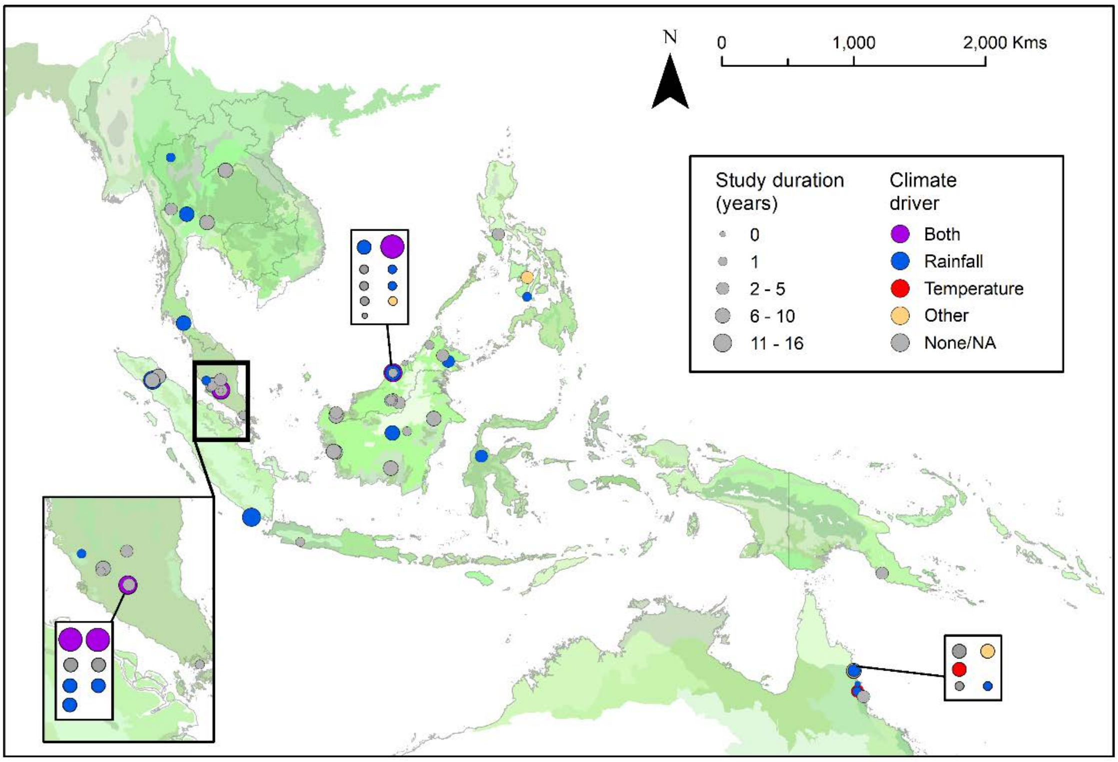

Fig 2.1. Study sites in tropical Southeast Asia and Oceania where phenological studies have been conducted. Markers indicate study site location, while marker size indicates study duration and marker colour indicates which climate drivers were assessed. Different shades of green indicate different ecoregions (WWF).

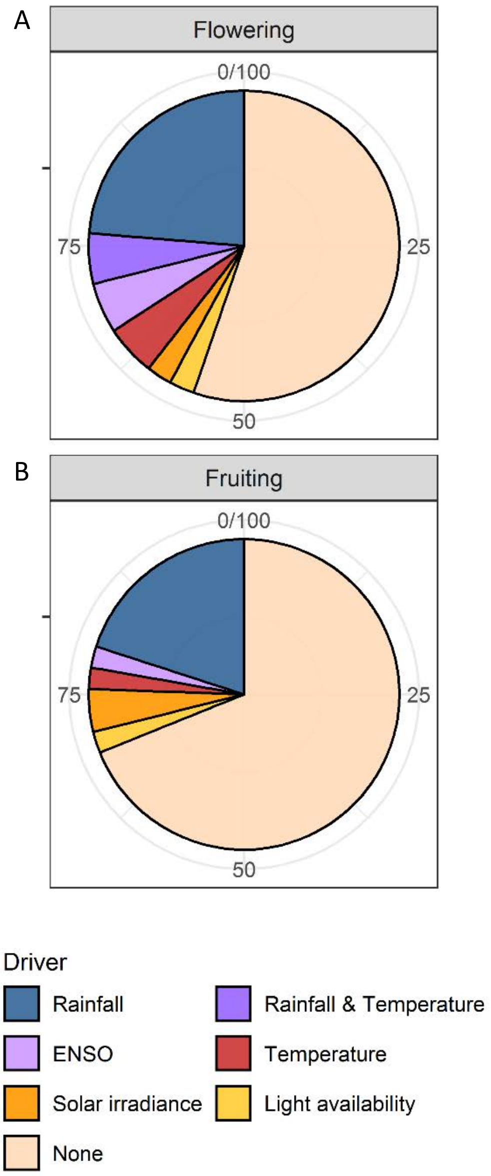

Fig. 2.2. Phenological drivers of A) flowering and B) fruiting, found in the papers and their respective percentage in relation to total number of reviewed papers. Although ENSO can be considered a component of rainfall, we considered it separately. None means no climate driver was found or climate drivers were not tested.

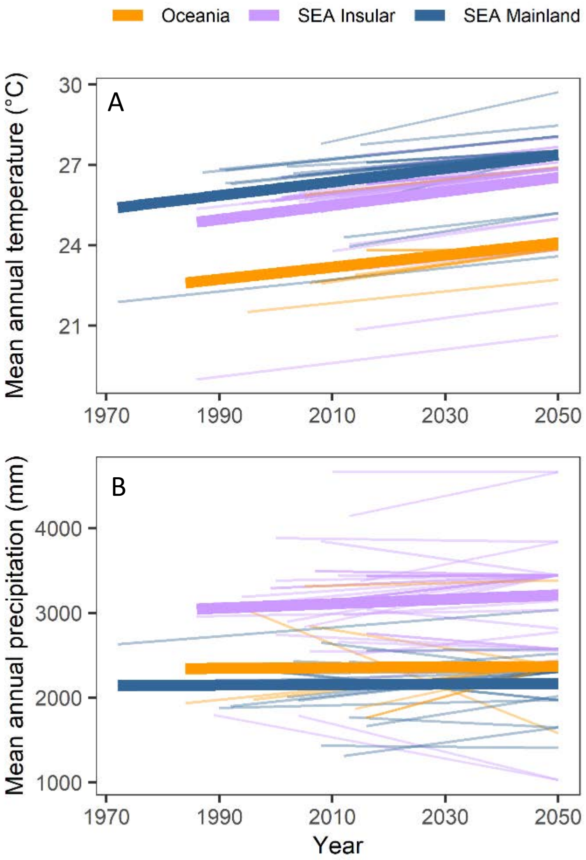

Fig. 2.3. Differences in A) mean annual temperature, B) annual rainfall from the publication date of each study and projection for 2050 for each Eco Region. Thick lines represent the average per region.

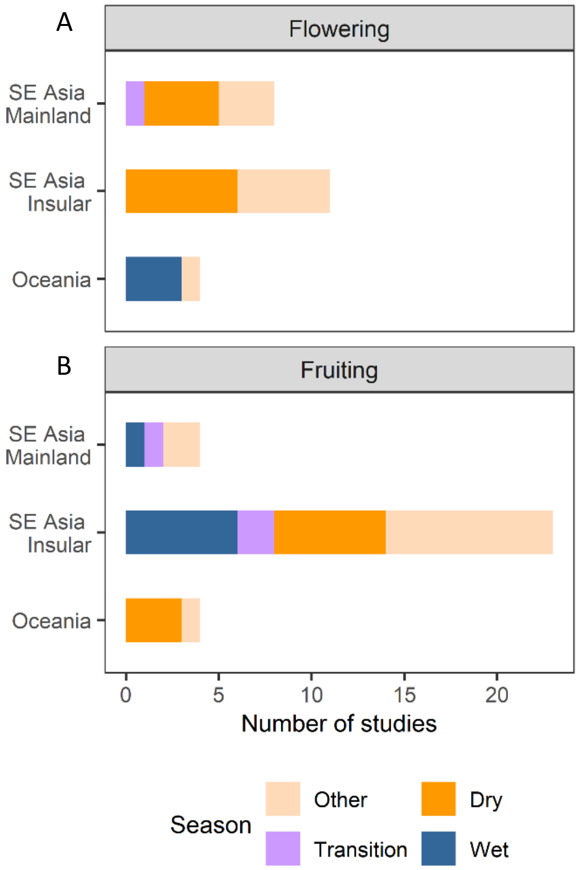

Fig. 2.4. Number of studies per region in each season for A) flowering and B) fruiting. Other represents bimodal or aseasonal distributions.

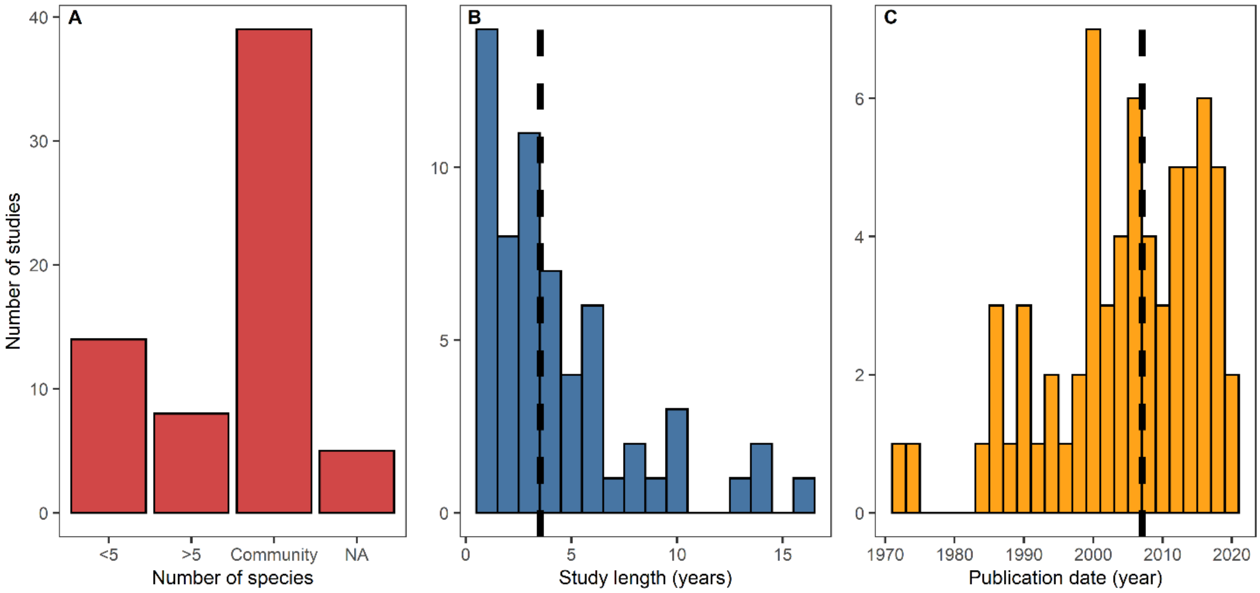

Fig. 2.5. Information summary of tropical rainforest phenological publications from Southeast Asia and Oceania a) number of species assessed in each study, b) duration of studies and c) period of publication. Dashed line indicates 50% of studies.

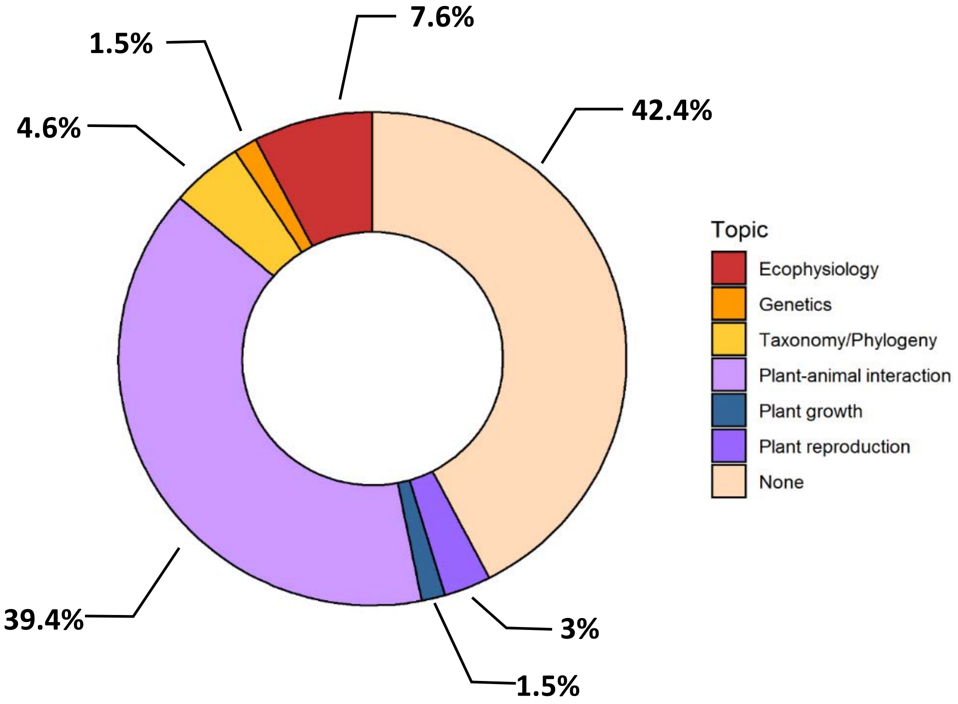

Fig. 2.6. Research topics assessed in the studies besides phenology. 'None' indicates only phenology was studied.

Discussion

Compared to temperate forests, tropical forests in general have received substantially less study effort in plant phenology. What our review has uncovered is that despite being a region of outstanding biological value, including six biodiversity hotspots (Mittemeier *et al*. 2011) and containing the island with the greatest plant diversity on the planet (New Guinea; Camara-Leret *et al*. 2020), very few phenological studies (66) have been published with phenological information from the tropical rainforests of Southeast Asia and Oceania. In comparison, Mendoza *et al*. (2017) has uncovered 97 datasets published with fruiting phenology in tropical rainforests from the Neotropics. One data paper was found presenting an important long-term dataset of litterfall patterns in Malaysia (Niiyama *et al*. 2019). Despite this region's incredible biodiversity values, its importance for carbon storage and cycling, and its provision of critical ecosystem services to over half a billion people (WorldBank 2021), only 20 of its 93 ecoregions have received study effort. Challenges in the requirement for constant observations, logistics, funding, etc. usually increase the difficulty of phenological studies. Knowledge of changes in vegetation resources and their availability for human and wildlife populations will become increasingly valuable as climate change and anthropogenic impacts are expected to modify environmental conditions in Southeast Asia and Oceania in myriad ways in the coming decades. These changes in resource availability are not only driven by extreme events or long-term climate changes, but also highly influenced by climate seasonality.

Seasonality

In Oceania, flowering activity is reported to be mainly concentrated in the wet season, a pattern found in other regions such as the Brazilian Atlantic rainforest (Morellato *et al.* 2010, Talora & Morellato 2000, Cardoso *et al.* 2018). An increase in flowering in the wet season may be due to increased rainfall and temperature and/or increasing the rates of litter decomposition or nutrient cycling (Pau *et al.* 2013). Nevertheless, expected increases in temperature due to climate change are likely to have direct effects on flowering activity. The fact that an increase in temperature also leads to increased evapotranspiration may have significant effects on water availability which can then influence reproductive success following flowering activity (Brando *et al.* 2006, Gallagher & Campbell 2021). However, in Southeast Asia flowering is more often found during the dry season, with mainly bimodal or aseasonal patterns mainly in the case of mast flowering events. Flowering in the dry season has also been reported in the Neotropics (Mendoza *et al.* 2017), Africa (Bush *et al.* 2020, Chapman *et al.* 2018, Adamescu *et al.* 2018) and Hawaii (Pau *et al.* 2018). Fruiting, on the other hand, seems to have varied patterns across the region of Southeast Asia and Oceania (Fig. 2.4). While in Oceania fruiting activity was found to be more concentrated in the dry season, in mainland Southeast Asia it occurred in all seasons except during the dry, while in the Southeast Asian islands the seasonality was highly variable (Fig. 2.4).

Climate

Only 40% of the phenological studies we identified included analyses of the influence of climate on phenological patterns. Though technical constraints may have limited the ability of earlier studies to properly analyse climate drivers, somewhat surprisingly, more than half of the papers that did not look at climate variables were published after the year 2000. Of the climate variables considered, the most common was rainfall (inclusive of drought, typhoon, or ENSO), with studies including one or more of these variables. Rainfall not only varies across ecoregions but also leads to varied responses among tree species. Flowering activity was found to increase with increased rainfall in Australia (Spencer *et al.* 1996), Thailand (Kuaraksa *et al.* 2012) and Phlippines (Heideman, 1989), while it was related to decreased rainfall in Malaysia (Sakai *et al.* 2006) and Indonesia (Brearley *et al.* 2007). The effects of rainfall on fruiting activity also varied in relation to region and study, with the relationship being positive in Australia, Malaysia and Thailand, and negative in Indonesia and Malaysia (Table S2.1). The same varied response was found for leafing phenology (Table S2.1). Future predictions of rainfall quantity and seasonality vary significantly between ecoregions and climate models across the region (Fig. 2.5).

In general, reproductive phenology was found mostly to be positively correlated with rainfall (Heideman 1989, Spencer *et al.* 1996, Eltz *et al.* 2001, Boulter *et al.* 2006, Sakai *et al.* 2006, Brearley *et al.* 2007, Kuaraksa *et al.* 2012, Sulistyawati *et al.* 2012, Edwards *et al.* 2018, Kurten *et al.* 2017, Chen *et al.* 2018) but in some studies was negatively correlated with rainfall (prolonged or extreme drought), such as can occur as a result of El Niño events (Medway 1972, Appanah 1985, Numata *et al.* 2003). The mass phenological masting events of flowering and fruiting in Southeast Asia have been the focus of the majority of studies in Malaysia and Indonesia. In Southeast Asia, most El Niño events were linked to peaks in flowering and fruiting activity, however no comparable analyses have been reported in Oceania that have linked reproductive phenology to El Niño events. In Australia, Edwards *et al.* (2018) conducted indirect phenological observations using litterfall and modelled the patterns in relation to climate drivers. They found that peaks in leaf litter fall coincided with peaks in temperature in December-January of each year, while flowering and fruiting fall were strongly positively correlated with wind and negatively correlated with temperature and rainfall.

Mass flowering or general flowering is the phenomenon mostly associated with the Dipterocarp forests where trees produce a massive amount of seeds in synchroneity after an interval of many years. Mass flowering has been found to be a response to extreme drought, especially El Niño events (research conducted in Malaysia, Table S2.1). Given that "general flowering" in lowland rainforests in Southeast Asia appears to be driven by irregular/prolonged droughts, changing drought frequency or intensity could have substantial impacts on forest dynamics (IPCC 2021) and the resources available for frugivores, as well as the reproductive success of trees. Ashton *et al*. (1988) presented a review of mass flowering of dipterocarp forests, discussing the potential environmental cues identified in a number of studies at that time. They concluded that *Dipterocarpaceae* species may have developed their phenological characteristics under drivers more characteristic of the seasonal tropics, combined with droughts driven by the ENSO cycle.

While the general flowering/mast seeding is a phenomenon generally attributed to Southeast Asia, masting or mast fruiting has been found to also occur in other regions including temperate forests, being defined as seed production that is highly variable and synchronous among individuals within a population and years (Kelly 1994, Kelly & Sork 2002, Koenig *et al*. 2003). The hypothesis raised to explain masting events include reducing long-term seed predation by limiting predator populations (Janzen 1971) or increasing pollination efficiency through synchronized flowering effort (Moreira *et al*. 2014), most common in wind-pollinated species (Kelly *et al*. 2001). Masting has been recently found in other tropical regions in South America (Norden *et al*. 2007), such as the Nouragues rain forest in French Guiana (Mendoza *et al*. 2018). The phenological event of masting has also been suggested to occur in the Wet Tropics of Queensland, in Australia, with a frequency of around seven years, apparently being triggered by weather conditions that are drier than usual (Moore *et al*. 2016).

Besides masting, flowering and fruiting, phenological activity in non-masting years is essential for maintenance of both flora and fauna. From the studies we examined in this review, the majority found the main driver to be rainfall, followed by temperature and the combination of rainfall with temperature (Fig. 2.2). In the Neotropics, fruiting has been found to be mainly driven by rainfall, followed by air temperature and day length (Mendoza *et al*. 2018). In Africa, fruiting activity has been found to respond to increase in rainfall (Dunham *et al*., 2018), solar radiation and ENSO (Chapman *et al*. 2018) and decrease in temperature (Potts *et al*. 2020).

Increases in temperature are expected to occur across all ecoregions of Southeast Asia and Oceania under the projected climate of 2050, with the degree of increase ranging from 0.32 to 2.13 ˚C (Fig. 2.3). Athough there is a general increase in temperature, predicted trends in rainfall showed varied directions (Fig. 2.3). Despite the imperative to understand the impact of a warmer climate on phenology in this region, only 15% of the studies analysed the relationship between temperature and phenology. In general, the relationship between temperature and flowering/fruiting was found to be negative, with the initiation of flowering phenology being driven by a decrease in temperature. This is of concern since temperature is predicted to increase across all regions in 2050 and it is not clear if it is a decrease in temperature or a threshold temperature that is triggering the reproductive phenology. In some studies, masting activity in Southeast Asia has also been related to the combined effect of drought and lower temperatures. For example, there is a tendency to a decrease in the frequency of low temperatures and prolonged droughts in peninsular Malaysia (Numata *et al*., 2013). This means that in the region, species that require lower temperatures to flower, such as *Shorea leprosula*, studied by Chen *et al*. (2018), might suffer a decrease in flowering activity due to the ongoing climate warming.

Long-term trends

While temperature and water availability (usually analysed as rainfall, drought, or ENSO phase) are the main climate variables related to phenological patterns that are expected to change in the future, there are other factors that often influence phenology. Light availability (quantified as crown light availability, photoperiod, or solar irradiance) and mycorrhiza were also found to influence phenological patterns in some studies (Table S2.1). Light availability will likely change under climate change scenarios due to cloud cover change (along with potential impacts of climate change on canopy structure) and hence phenological patterns will be caught between dynamic drivers such as temperature and water, and semi-static drivers such as photoperiod. Long-term trends in phenology are assessed through time series where the seasonal component is excluded. They are of particular value as they provide important information on decreases or increases in phenophase activity, allowing modelling and predictions in relation to climate cues. Besides the environmental effects on phenology, there are also biotic factors that may influence species reproductive and vegetative patterns. Phylogeny, circadian rhythms, physiological responses and ecological relationships might also explain some phenological patterns. In Southeast Asia and Australia, only one study showed this kind of relationship (Curran 1994). The thesis written by Curran (1994) presented a study of 54 species in a long-term (nine year) observation period in Indonesia and found a relationship between fruiting patterns and ectomycorrhizae that allow nutrient uptake and accumulation which in turn facilitates mast seeding (Corrales *et al*. 2018), showing that an ecological relationship might be the phenological driver for some species.

Abiotic drivers such as temperature, rainfall, irradiance, or daylength may vary not only amongst microclimates but also amongst different phenophases. For example, populations of the same species might have their reproductive phenology driven by rainfall but their leafing phenology driven by irradiance. The drivers may also vary between species. Especially in phenology and tree growth, long-term studies are fundamental to determining these ecological drivers and phenological patterns, allowing one to reliably determine whether they present any variation. While reproductive phenology is extremely important to understand species survivorship and adaptation, vegetative phenology provides insights into various ecological processes. Different types of flowering patterns may be found in the forests, such as supra-annual patterns. Some species might respond to changes in the environment by changing their leafing pattern due to the plasticity and adaptation capability of that species. In both cases, important information may be lost in short-term studies. The possible changes in leaf production and fall are extremely important for carbon cycling, water and energy fluxes, resource availability and nutrient cycling (Reich 1995, Polgar & Primack 2011, Richardson *et al*. 2013). Interannual shifts in climate can be more intense during severe dry seasons, driving shifts in the intensity and timing of trees' phenological activity. These changes in seasonality are an indicator of the pattern community deciduousness, as changes in species leafing patterns can cause shifts in leaf turnover at the community level, and can provide essential insights into different levels of ecological responses (Reich 1995, Condit *et al*. 2000, Camargo *et al*. 2018). Across the Southeast Asia and Oceania region the paucity of reported and on-going studies tracking long-term vegetative phenology indicates a clear data gap, one that remote sensing is only able to fill at a relatively coarse spatial resolution compared to ground based studies.

In addition to the climate drivers mentioned above, rising CO_2 may also lead to changes in phenological patterns, and has currently been overlooked in phenology studies. Experimental studies have demonstrated that elevated CO_2 can produce an increase in flower and fruit production (Jablonski et al. 2002). Additionally, field studies in Panama have found a link between increasing CO_2 concentrations and an increase in flowering activity (Pau et al. 2018). The positive effect of CO_2 on flowering could be due to carbon allocation, and an earlier reproductive age, leading to enhanced reproductive output (LaDeau & Clark, 2006). It has similarly been suggested that increased atmospheric CO_2 concentrations may lead to altered leaf turnover (Sigurdsson 2001), though no field studies have yet demonstrated this effect. Therefore, the impacts of climate drivers would occur synergistically with elevated CO_2, and accounting for this will be necessary for future phenological and ecological modelling. To date there is no literature reported from the Southeast Asia and Oceania region looking at the impacts of elevated and rising CO_2 on phenology.

Ecological interactions

Many important phenological studies have been developed in order to better understand the relationship between animals and their food sources. We found 39.4% of studies containing phenological series data were focussed on assessing plant-animal interactions, and often the term "phenology" was not cited in the study title. Many of these studies are found in books or unpublished data, such as PhD theses. Most show the importance of understanding fruiting seasonality and patterns for frugivores, how their behaviour relates to fruiting season and even how their health follows fruit availability. Marshall & Leighton (2006) for example, found that the population density of gibbons is strongly correlated with density of figs in Gulung Palung, Indonesia. In contrast, Wich *et al.* (2006) found no evidence supporting a relationship between large fruit production and orangutan grouping or ranging in Ketambe, Indonesia. Although the main concern in this regard is centred on primate specialists due to the risk of extinction, even generalists may suffer nutritional stress due to limited fruit availability, as fruits have a higher carbohydrate and fat content when compared to leaves. For example, it has been found that sun bears lose weight during low fruiting periods and gain weight again in fruiting seasons (Wong *et al.* 2005). In Papua New Guinea, Wright (2005) found that during periods of high fruit availability, Dwarf Cassowaries (*Casuarius bennetti*) had preference for species that presented seasonal fruiting patterns (annually or biannually) over species that fruited continuously, which were preferred in the lean season.

Knott (1998) found that during periods of highest fruit availability, fruit made up almost 100% of orang-utans' diet. During fruit-poor periods, orangutans relied on leaves, bark, pith and insects as fall-back foods. In Malaysia, Hanya & Bernard (2012) found that the red leaf monkey increased seed and whole fruit consumption in response to the supra-annual increase in fruit availability, however, they depended on young leaves as a fallback food in lean periods. Impacts of climate change on plant phenology, therefore, have important implications for animal conservation (Marshall & Leighton 2006, Marshall & Wich 2016, Marshall & Wich 2013). Additionally, frugivory and seed dispersal are important factors in the recovery of plant diversity and succession of forests on degraded and cleared lands (Corlett 2017). Large fruits and seeded fruits depend on bigger animals for dispersal, which are less abundant and extremely vulnerable to landscape changes and hunting (Corlett 2017).

These ecological interactions not only have fundamental implications for animal-flowering/fruiting relationships, but also for the relationship between herbivores and leafing phenology. Many herbivores selectively feed on young instead of mature leaves, possibly due to the lower fibre and higher protein content that some plant species young leaves have in comparison to mature leaves (Milton 1979). The preference for young leaves by herbivores have led plants to develop different strategies to protect their canopies, amongst which there are mass flushing, flushing in periods when herbivores are less abundant, and flushing in colours that herbivores have more difficulty recognising (such as purple, red and pink). In Malaysia, Yoneyama & Ichie (2019) studied the relationship between leaf phenology and defences against herbivores in five dipterocarp species, finding that species with high leaf flushing frequency had greater chemical defences, while those with lower flushing frequency had tougher leaves with greater photosynthetic capacity. Although of extreme importance, leafing phenology has been understudied, especially in relation to the processes behind it. Ichie et al (2004) and Palakit *et al*. (2018), however, provided important contribution to the understanding of processes that relate to leafing activity, such as changes in rainfall combined with cambial activity, and herbivory.

Floristic composition and phenological mismatches

Species can also present phylogenetic and genetic signals in how they respond to environmental changes, and studies assessing phylogeny/taxonomy, as well as genetics, combined with phenology can provide important information. Our review found several studies focused on the relationship between phenology and taxonomy or phylogeny, and one study analysed genetics of trees in combination with phenology (Fig. 2.6).

Impacts of climate change on plant phenological patterns vary both between different geographical regions and different species (Sakai & Kitajima 2019). Changes in climate cues may lead species to respond differently depending on their adaptive capacity and trait plasticity. Species-specific responses to changing climate conditions may alternatively lead to reductions or increases in phenological activity as well as the advancing or delaying of phenological cycles. Varied changes in phenological activity can lead to changes in recruitment rates for different species and ultimately contribute to a change in plant community composition and structure. Similarly, changes in timing of phenological activity may lead to breakdowns in relationships with pollinators and seed dispersers that can also lead to species-specific changes in recruitment rates (Burkle & Alarcon, 2011).

Additionally, changes in floristic composition, as well as timing of flowering and fruiting, have impacts that flow through to higher trophic levels by altering resource availability for pollinators and frugivore species. In extreme cases these changes may start cascade effects that impact entire ecological communities (Butt et al. 2015, Morellato et al. 2016). Therefore, studying species-specific phenological patterns and responses to climatic drivers will be vital to detect future changes in plant community composition and the flow on impacts.

Beyond what we can see

Physiological processes play an important role in shaping phenological patterns, however, these two aspects of plant performance are rarely studied in concert. Understanding how phenological and physiological processes inter-relate will be important in order to predict responses of trees to environmental and climatic changes. For example, leafing phenology has been found to be highly influenced by water availability and water potential in some species (Borchert *et al.* 1994, Morisette *et al.* 2009, Polgar & Primack 2011). However, production of fruits and flowers, and hence reproductive capacity is also dependent on availability of water and stored resources, hence trade-offs will occur between growth and reproduction in drying environments. While some species may invest in reconstructing the canopy in face of extreme events, others may invest in flowering or fruiting. Changes in the triggering of these activities may alter forest processes. For example, it has been suggested that a trade-off in use of carbon reserves might occur in mast fruiting events due to demands from growth and reproductive activity (Miyazaki 2015, Ichie *et al.* 2005). Hence, links between carbon reserves and phenological patterns provide important insights into the processes that modulate shifts in reproductive or vegetative outputs. However, current dynamic models of vegetation remain simplistic due to the lack of physiological and phenological data that describes in situ tree responses to climate.

To better understand species responses to changes in climate, an experimental approach is very useful. Although difficult to study the effects of increased temperature on mature trees in the forest, throughfall exclusion and FACE (Free Air CO_2 Enrichment) experiments provide important information on the impact of drought and elevated CO_2 effects, respectively (Ainsworth & Long 2004, Norby & Zack 2011). Increasing atmospheric CO_2 concentration has been found to increase flowering activity (Pau *et al*. 2018) and increased drought has been found to cause a range of responses in different species (Ogaya & Penuelas 2004, Brando *et al*. 2006). In addition to experiments, assessing the effects of extreme events on trees' physio-phenological processes may provide important information about how vulnerable or resilient species are, such as the research conducted by Nunes et al. (2018), that investigated how rainforest trees responded to an El Niño event at a site in northern Borneo combining remote sensing with functional trait approaches.

Dendrochronology and stable isotopes represent valuable tools with which to reconstruct tree physiology and phenology (growth and leafing) to better understand the long-term effects of climate change and can also provide a better understanding of trade-offs between phenology and growth. Our review found only one study assessing a combination between phenology and tree growth, and two studies focused on plant reproduction, but none of these used dendrochronology for long-term studies, all in Southeast Asia. Through a combination of high-resolution dendrochronology and stable isotope analysis (Poussart *et al*., 2004), time series information on tree water use and ecophysiology can be derived. In addition, the intrinsic water use efficiency (WUEi) and use of stored carbon to produce new tissues may be tightly related to phenological patterns (Cernusak 2020). Therefore, stable isotope analysis may provide important information not only on temporal change in climate variables but also for tree physiological and phenological cycles, allowing us to better understand the changes in water use efficiency, photosynthetic activity, and use of carbon (Loader *et al*. 2011). For example, Palakit *et al*. (2018) aimed to relate leafing phenology to dendrochronology, using *Melia azedarach* in Thailand. Although they did not use stable isotopes, they tracked leafing phenology and growth using cambial marks and found that the relationship between monthly climate, leaf phenology and wood increments indicated significant correlations between soil moisture and the abundance of mature dark green leaves.

Conclusions and future directions

Phenological observations are a valuable tool for quantifying the response of plant species and vegetation communities to environmental conditions. Therefore, changes in phenological patterns allow us to understand impacts of climate change and how the response of individual species may affect community composition and structure, and flow to other trophic levels in an ecosystem. Following this, we can use phenological studies to assess our ability to predict future impacts of environmental changes on Southeast Asia and Oceania’s tropical forests. Due to the large variation in phenological patterns in relation to environmental drivers and the lack of long-term observations, especially in combination with physiological measurements, reproductive and vegetative patterns for Asian and Oceanian tropical rainforests remain poorly understood. Since plants depend on photosynthesis to maintain their growth rate and the physiological processes responsible for their reproductive cycles, studies on plant physiology combined with phenology are critical to deepening our understanding of tree biological cycle responses to climate change.

Our review found that numerous ecoregions of exceptional value in terms of biodiversity, carbon storage, and ecosystem services are chronically understudied in Southeast Asia and Oceania. The lowland forests in Sumatra and Sulawesi, for example, do not have published phenological records, despite these regions containing exceptional levels of floral and faunal diversity. Additionally, mega-diverse regions such as the Philippines and New Guinea are represented by only a few studies. Myanmar, whose floral diversity can only be estimated due to lack of study effort was not represented by a single published record of native plant phenology. Without adequate knowledge of plant phenological patterns, and how they respond to environmental cues, we are less able to predict responses of these communities to changing climatic conditions. This alarming lack of understanding of plant phenology presents problems not only for tree species, but also for the wildlife species that depend on fruit, flower, or leaf resources as exemplified by leaf monkeys in Malaysia, sun bears in Indonesia, sun bears and black bears in Thailand, and others. The lack of phenological data even impedes our ability to model global processes such as carbon cycling, as leafing phenology is tied to the carbon balance of forests. We emphasise the need for greater attention to the under-studied ecoregions in Southeast Asia and Oceania. We understand, the increase in budget and staffing that would be required to enhance the sampling effort across the multitude of countries, including many developing nations that are part of this region. We propose that the establishment of a phenological network across the region could be a first step in getting this underway, something consistent with the overarching goals of the Asia-Pacific Biodiversity Observation Network (Takeuchi *et al.* 2020).

Numerous studies outlining the impacts of climate change and land-cover change on forest phenology and composition have demonstrated the importance of long-term monitoring. We show that few long-term phenological studies have been carried out in Southeast Asia and Oceania, limiting our capacity to understand historic patterns and whether they have been impacted by global changes. As it is not possible to retroactively establish baseline monitoring programs, long-term monitoring plots must be supplemented with alternative methods of reconstructing historic patterns. Dendrochronology and stable isotope analyses, for example, have been used to assess growth and leafing phenology, and should be further developed.

Finally, we provide some specific recommendations to advance the understanding of phenological patterns within Southeast Asia, and improve our ability to predict future changes for this region:

1. Develop long-term monitoring plots for reproductive and vegetative phenology in high-priority, understudied regions such as New Guinea, Myanmar, and Vietnam. This is important not only in mature forests, but also in logged and regrowth forests.
2. Improve the assessment of climate drivers of phenological patterns in the region, through the use of long-term monitoring plots, and where possible, comparing published historical records to historical climate data.
3. Quantify phenological patterns at the level of species as well as plant communities in order to identify species-specific responses to environmental change, and predict future impacts on community composition and function.
4. Assess effects of land change and microclimate in phenological patterns.
5. Combine phenological studies with studies of the underlying physiological functions required to facilitate phenological activity (for example, water use efficiency and photosynthetic activity).

6. Use genetic and molecular studies (hormones, transcriptomes) to understand environmental effects on phenology, deciphering processes such as the regulation of growth cessation, bud set, dormancy and bud break.
7. Explore potential techniques for the reconstruction of historic phenological patterns in order to identify longer-term shifts due to environmental changes.

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Competing Interests

The authors declare no competing interests.

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Supplementary material

Table S2.1. Phenology papers used in the literature review with corresponding information.

Year	Authors	Reference	Country	N. species	Flowering	Fruiting	Leafing	Time (years)	Method
1975	Crome	Crome 1975	Australia	Community		x		<5	Direct – On the ground
1984	Spain	Spain 1984	Australia	NA			X	<5	Indirect - Litterfall
1996	Spencer *et al*.	Spencer et al. 1996	Australia	<5	x	x	X	<5	Direct - On the ground
2006	Boulter *et al*.	Boulter et al. 2006	Australia	Community	x			_	Indirect - Herbaria
2007	Inkrot *et al*.	Inkrot et al. 2007	Australia	<5	x	x		<5	Direct - On the ground
2008	Webber *et al.*	Webber et al. 2008	Australia	<5	x			5-10	Direct - On the ground
2014	Parsons *et al.*	Parsons et al. 2014	Australia	Community			X	<5	Indirect - Litterfall
2017	Edwards *et al.*	Edwards et al. 2017	Australia	<5	x	x	X	5-10	Indirect - Litterfall
2020	Vogado *et al*.	Vogado et al. 2020	Australia	<5		x		5-10	Direct - On the ground
1994	Kitamura *et al.*	Kitamura et al. 1994	Brunei	<5	x			_	Direct - On the ground
1986	van Schaik	van Schaik 1986	Indonesia	Community	x	x	X	<5	Both - On the ground and litterfall
2000	Curran & Leighton	Curran & Leighton 2000	Indonesia	Community		x		5-10	Direct -On the ground
2000	Wich & van Shaik	Wich & van Shaik 2000	Indonesia	Community	x	x		>10	Direct -On the ground
2002	McConkey *et al*.	McConkey et al. 2002	Indonesia	Community		x		<5	Direct -On the ground
2006	Fredriksson *et al.*	Fredriksson et al. 2006	Indonesia	Community		x		5-10	Direct -On the ground
2007	Brearley *et al.*	Brearley et al. 2007	Indonesia	Community	x	x		5-10	Direct -On the ground
2007	Cannon *et al.*	Cannon et al. 2007	Indonesia	Community		x		5-10	Direct -On the ground
2008	Vogel *et al.*	Vogel et al. 2008	Indonesia	Community		x		<5	Direct -On the ground
2010	Harrison *et al.*	Harrison et al. 2010	Indonesia	NA		x		<5	Direct -On the ground
2011	Wich *et al.*	Wich et al. 2011	Indonesia	NA		x		5-10	Direct -On the ground
2011	Poesie *et al.*	Poesie et al. 2011	Indonesia	>10		x		<5	Direct -On the ground
2012	Sulistyawati *et al.*	Sulistyawati et al. 2012	Indonesia	Community	x	x		<5	Direct -On the ground
2013	Smith *et al.*	Smith et al. 2013	Indonesia	Community	x	x		<5	Direct -On the ground
2014	Moser *et al.*	Moser et al. 2014	Indonesia	Community			X	<5	Indirect - Litterfall
2015	Dillis *et al.*	Dillis et al. 2015	Indonesia	Community		x		5-10	Direct -On the ground
2008	Kato *et al.*	Kato et al. 2008	Laos	Community	x			5-10	Direct -On the ground
1972	Medway	Medway 1972	Malaysia	Community	x	x	X	5-10	Direct -On the ground
1986	Kaul et al.	Kaul et al. 1986	Malaysia	>10	x		X	<5	Direct -On the ground
1991	Lambert & Marshall	Lambert & Marshall 1991	Malaysia	>10		x		<5	Direct -On the ground

1992	Toy et al.	Toy et al. 1992	Malaysia	>10		x		<5	Direct -On the ground
1999	Davies & Ashton	Davies & Ashton 1999	Malaysia	>10	x	x		<5	Direct -On the ground
1999	Sakai *et al.*	Sakai et al. 1999	Malaysia	Community	x	x		<5	Direct -On the ground
2000	Harrison *et al.*	Harrison et al. 2000	Malaysia	<5	x	x	x		Direct -On the ground
2001	Eltz *et al.*	Eltz et al. 2001	Malaysia	<5	x			<5	Direct -On the ground
2001	Harrison	Harrison 2001	Malaysia	>10		x	x	<5	Direct -On the ground
2001	Kimura *et al.*	Kimura et al. 2001	Malaysia	Community		x		<5	Direct -On the ground
2002	Moog et al.	Moog et al. 2002	Malaysia	<5	x			<5	Direct -On the ground
2003	Numata et al.	Numata et al. 2003	Malaysia	NA	x			<5	Direct -On the ground
2004	Ichie *et al.*	Ichie et al. 2004	Malaysia	Community			x	<5	Direct -On the ground
2004	Itioka *et al.*	Itioka et al. 2004	Malaysia	Community			x	<5	Direct -On the ground
2005	Wong *et al.*	Wong et al. 2005	Malaysia	Community		x		<5	Direct -On the ground
2006	Sakai *et al.*	Sakai et al. 2006	Malaysia	Community	x	x		5-10	Direct -On the ground
2013	Numata et al.	Numata et al. 2013	Malaysia	Community	x			<5	Direct -On the ground
2015	Ong et al.	Ong et al. 2015	Malaysia	<5			x	<5	Indirect - Litterfall
2016	Harrison *et al.*	Harrison et al. 2016	Malaysia	Community		x		5-10	Direct -On the ground
2016	Winarni et al.	Winarni et al. 2016	Malaysia	>10	x	x		>10	Direct -On the ground
2017	Iku *et al.*	Iku et al. 2017	Malaysia	Community	x	x		<5	Direct -On the ground
2019	Nakagawa *et al.*	Nakagawa et al. 2019	Malaysia	NA			x	>10	Indirect - Litterfall
2018	Aoyagi et al.	Aoyagi et al. 2018	Malaysia	Community	x	x			Indirect - Litterfall
2018	Chen *et al.*	Chen et al. 2018	Malaysia	5-10	x			>10	Indirect - Litterfall
2019	Satake *et al.*	Satake et al. 2019	Malaysia	5-10	x			>10	Direct -On the ground
2019	Yoneyama & Inchie	Yoneyama & Inchie 2019	Malaysia	5-10			x	<5	Direct -On the ground
2020	Roth *et al.*	Roth et al. 2020	Malaysia	Community		x		5-10	Direct -On the ground
2001	Intachat *et al.*	Intachat et al. 2001	Malaysia	Community	x	x	x	<5	Direct -On the ground
1995	Brown & Hopkins	Brown & Hopkins 1995	Papua New Guinea	Community	x	x		<5	Direct -On the ground
1989	Heideman	Heideman 1989	Philippines	Community	x	x		<5	Direct -On the ground
2004	Hamann	Hamann 2004	Philippines	Community	x	x		<5	Direct -On the ground
2015	Caringal	Caringal 2015	Philippines	<5	x	x	x	<5	Direct -On the ground
1987	Corllet	Corllet 1987	Singapore	<5	x	x		<5	Direct -On the ground
1990	Corllet	Corllet 1990	Singapore	Community	x	x		<5	Direct -On the ground

1991	Lucas & Corllet	Lucas & Corllet 1991	Singapore	Community	x	x		<5	Direct -On the ground
2008	Williams *et al.*	Williams et al. 2008	Thailand	Community			x	5-10	Direct -On the ground
2012	Kuaraksa *et al.*	Kuaraksa et al. 2012	Thailand	<5	X	x	x	<5	Direct -On the ground
2013	Steinmetz *et al.*	Steinmetz et al. 2013	Thailand	Community		x		<5	Direct -On the ground
2017	Kurten *et al.*	Kurten et al. 2017	Thailand	Community	X	x		5-10	Direct -On the ground
2017	Suwanvecho *et al.*	Suwanvecho et al. 2017	Thailand	Community	X	x	x	5-10	Direct -On the ground

Chapter Three: The effects of an experimental drought on the ecophysiology and fruiting phenology of a tropical rainforest palm

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The effects of an experimental drought on the ecophysiology and fruiting phenology of a tropical rainforest palm

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Abstract

Aims Anthropogenic climate change is predicted to increase mean temperatures and rainfall seasonality. How tropical rainforest species will respond to this climate change remains uncertain. Here, we analysed the effects of a 4-year experimental throughfall exclusion (TFE) on an Australian endemic palm (*Normambya normanbyi*) in the Daintree rainforest of North Queensland, Australia. We aimed to understand the impact of a simulated reduction in rainfall on the species' physiological processes and fruiting phenology.

Methods We examined the fruiting phenology and ecophysiology of this locally abundant palm to determine the ecological responses of the species to drought. Soil water availability was reduced overall by ~30% under a TFE experiment, established in May 2015. We monitored monthly fruiting activity for 8 years in total (2009–2018), including 4 years prior to the onset of the TFE. In the most recent year of the study, we measured physiological parameters including photosynthetic rate, stomatal conductance and carbon stable isotopes (δ^{13}C, an integrated measure of water use efficiency) from young and mature leaves in both the dry and wet seasons.

Important Findings We determined that the monthly fruiting activity of all palms was primarily driven by photoperiod, mean solar radiation and mean temperature. However, individuals exposed to lower soil moisture in the TFE decreased significantly in fruiting activity, photosynthetic rate and stomatal conductance. We found that these measures of physiological performance were affected by the TFE, season and the interaction of the two. Recovery of fruiting activity in the TFE palms was observed in 2018, when there was an increase in shallow soil moisture compared with previous years in the treatment. Our findings suggest that palms, such as the *N. normanbyi*, will be sensitive to future climate change with long-term monitoring recommended to determine population-scale impacts.

Keywords: climate change, water use efficiency, stable isotopes, seasonality, reproductive phenology

摘要：据预测，人为气候变化将季节性地增加平均温度和降雨。热带雨林物种将如何应对这种气候变化仍不确定。本研究分析了对澳大利亚昆士兰北部丹特里雨林的一种澳大利亚特有棕榈(*Normambya normanbyi*)进行4年降雨实验的影响，目的是了解模拟降雨减少对物种生理过程和果实物候的影响。我们考察了这种本地丰富的棕榈的果实物候和生理生态学特性，以确定该物种对干旱的生态响应。2015年5月，通过排涝实验，降低了约30%的土壤水分有效性。我们总共监测了8年(2009–2018年)的月度果实活性，包括排涝实验开始之前的4年。在最近几年的研究中，我们测量了干、湿两季幼嫩和成熟叶片的光合速率、气孔导度和碳稳定同位素等生理参数。研究结果表明，所有棕榈树的月度果实活性主要受光周期、平均太阳辐射和平均温度的驱动。然而，暴露于较低土壤水分的植株，其果实活性、光合速率和气孔导度均显著下降。我们还发现这些生理表现受到排涝实验、季节以及两者的相互作用的影响。2018年观察到排涝实验的棕榈的果实活力有所恢

复，土壤浅层水分也有所增加(与前几年相比)。我们的研究结果表明，像*N. normanbyi* 这样的棕榈树对未来的气候变化非常敏感，建议对其进行长期监测，以确定其对种群规模的影响。

关键词：气候变化，水分利用效率，稳定同位素，季节性，生殖物候

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Chapter Four: Assessing the effects of a through-fall exclusion experiment on the reproductive phenology and ecophysiology of a wet tropical rainforest community

Assessing the effects of a through-fall exclusion experiment on the reproductive phenology and ecophysiology of a wet tropical rainforest community

Abstract

Climate change is expected to increase the intensity and occurrence of drought, thereby affecting the phenology and physiology of tree species. Phenological activity may respond to a drying and warming environment by advancing in timing, and/or diminishing the production of flowers and fruits. These changes have the potential to disrupt important ecological processes, with ensuing and potentially wide-ranging effects on tropical forest function. Here, we analysed tree community monthly flowering and fruiting (357 individuals from 33 species) over eight years in a lowland tropical rainforest in north-eastern Australia and the effect of an experimental reduction in effective moisture as a result of throughfall exclusion (TFE) on part of this community. Solar radiation (daily) and mean temperature (daily) were the main drivers of flowering activity (positive correlation) while photoperiod was the main driver of fruiting phenology (negative correlation). Counter to expectations, the TFE drove a significant increase in flowering and fruiting activity in the community with varied effects on the three selected dominant species. Additionally, we examined nutrient (C:N) and leaf stable isotope composition, δ^{13}C value as a proxy for water use efficiency and δ^{15}N value as a proxy for nitrogen source. The selected dominant species presented varied responses to the TFE, with *Syzygium graveolens* significantly increasing flowering and fruiting activity, with an increase in δ^{13}C and δ^{15}N that indicates higher water use efficiency and access to water in deeper soil layers. In contrast, *Cleistanthus myrianthus* presented a significant decrease in flowering and fruiting activity combined with decreased δ^{13}C vakue, while *Myristica globosa* did not show any significant change in phenology but an increase in cellulose δ^{18}O values indicating a decrease in transpiration rates. Our results suggest that changes in frequency and intensity of climate events are likely to lead to species-specific responses in the timing and intensity of flowering and fruiting. Understanding species-specific responses to climate events may provide insights into future changes in tropical forest structure and function.

Introduction

Phenological time series for evergreen tropical rainforests are required to understand the physiological processes that sustain the reproductive patterns of forest communities and populations, enabling us to better predict both tree level and community level responses to a drying environment (Butt *et al*. 2015). The importance of temperature and water availability in the context of global change biology is well understood, but specific effects are rarely studied in tropical rainforests, with most experimental studies carried out in temperate systems (Ogaya & Peñuelas, 2007). Studies analysing the effect of drought on the phenology of tropical rainforest tree species are rare (but see Brando *et al*. 2016). Studies combining physiological proxies with assessments of phenological responses to environmental changes, in an attempt to better understand species resilience, are even rarer (Vogado *et al*. 2020). As a result, there is a significant gap in our understanding of how tropical rainforests are responding to changes in climate and how they will respond in the future.

Phenology and physiology of tree species are affected by climate and may suffer changes due to increases in the intensity and frequency predicted for future droughts. In the tropics, flowering and fruiting activity has been found to be mainly influenced by climate variables, such as rainfall (Dunham *et al*. 2018) and temperature (Pau *et al*. 2013). Photoperiod and solar irradiation (van Schaik *et al*. 1993, Chapman *et al*. 2018, Wright & Calderon 2018) may also be important triggers of variation in tropical plant phenology, although they are significantly understudied. In humid tropical forests, photoperiod has been suggested to be important for the timing and synchronicity of plant phenology as equatorial regions experience lower seasonality and more constant water availability (van Schaik *et al*. 1993, Borchert *et al*. 2005, Calle *et al*. 2010, Morellato *et al*. 2000, Borchert *et al*. 2015). Additionally, temperature and water availability are important for numerous physiological processes in tropical tree species and, through changes in resource availability, may have an important influence on phenological processes even when they are not considered direct climate drivers. This combined effect of mean temperature and water availability on phenological patterns and the physiological capabilities of tropical tree species has important consequences for the structure and function of tropical forests, the persistence of tropical tree species, and potentially the persistence of rainforests more broadly.

Trees are expected to change their reproductive activities as a result of physiological adjustments in response to drought, for example, by increasing the diffusive limitation on photosynthesis by stomatal closure with impacts on non-structural carbohydrate synthesis (Brando *et al*. 2006, Nepstad *et al*. 2002). Species that are more capable of acclimating to drought might exhibit a higher water use efficiency, allowing them to maintain uptake of carbon for photosynthesis while reducing water loss through transpiration and avoiding embolisms (McDowell *et al*. 2008). Effective use of water implies maximal soil moisture capture for transpiration, which also involves reduced non-stomatal transpiration and minimal water loss by soil evaporation. Alternatively, species with less capacity for tight stomatal control to increase water use efficiency might struggle to maintain photosynthetic rates during drought as transpiration becomes limiting (McDowell *et al*. 2008). A lower capacity to increase water use efficiency or make more effective use of water through rooting adjustments might cause a reduction in flower and fruit production as the plants are placed under both water and carbohydrate stress, as a result of a trade-off between vegetative and reproductive needs. These potential trade-offs are poorly understood for most tropical tree species, despite their importance in understanding climate change effects on phenology and reproductive success.

The use of stable isotopes, as an integrative measure of physiological processes, provides a reliable tool to understand how trees are responding to climate change, in particular through the relationship between intrinsic water use efficiency (expressed as WUEi) and δ^{13}C value (Cernusak *et al*., 2013). WUEi can be assessed using the stable carbon composition of plant tissue (represented by δ^{13}C value) (Cernusak *et al*. 2020), since the process of photosynthesis is impacted by stomatal activity that is balancing transpiration to minimise water loss. Additionally, the δ^{18}O value of plant tissue may be used as a reliable tool to understand water relations, especially transpiration rates and water sources (Barbour 2007). Stable nitrogen isotope composition (δ^{15}N value) provides information on plant nutrient use under applied environmental conditions and, when combined with the carbon to nitrogen ratio (C:N), these variables provide a more detailed understanding of physiological responses of plant species to a drier environment (Craine *et al*. 2015, Nel *et al*. 2018, Lorenz *et al*. 2020).

Here we analyse the phenological patterns of a tropical rainforest community (357 trees from 33 species) at the Daintree Rainforest Observatory in the wet tropics of north-eastern Australia. The observation of flowering and fruiting patterns and their responses to climate in general, as well the response of part of the community to a drought brought about through an experimental reduction in through-fall, was carried out to provide insights into possible future mismatches between plant performance and future climate. Reproductive phenology when combined with concurrent ecophysiological measurements enhances our understanding of how tropical tree species may respond to a drier or more seasonal future environment. We specifically aimed to characterise: 1) general tree species reproductive patterns in the lowland rainforest of the Australian Wet Tropics; 2) phenological patterns in relation to seasonality and climate variables; 3) the impact of the through-fall reduction experiment on flowering and fruiting activity of the tree community;

4) how reduced soil moisture affected the stable isotope composition and nutrient use in the dominant species across seasons; 5) relationships between phenology, water use efficiency and nutrient cycling at the species level as a result of the through-fall exclusion.

Materials and methods

Study site

The phenological observations and physiological measurements for this study were conducted in lowland tropical rainforest at the James Cook University administered Daintree Rainforest Observatory (DRO; 16°06’020″S, 145°26’040″E), in northeastern Queensland, Australia (Fig. 1). The forest at the study site is classified as Complex Mesophyll Vine Forest (Tracey 1982, Goosem *et al*. 1999), mainly formed by mature coastal lowland tropical rainforest. The Wet Tropics region experiences a seasonal moist tropical climate, with a distinct wet season from November to May and a dry season from June to October (Fig. 1). The region is affected by El Niño Southern Oscillation (ENSO) and tropical cyclones (TC). During our phenological study, the area has been affected by TC Ita in 2014. Other cyclones occurred before the start of our phenological observations. The most recent El Niño, observed through Southern Oscillation Index (SOI) events occurred in 2002-03 (SOI: weak), 2006-07 (SOI: weak), 2009-10 (SOI: weak to moderate) and 2015-16 (SOI: weak) (Fig. 1c). Detailed phenological observations in the 1ha plot have been carried out since 2009, with canopy access facilitated by a canopy crane (Liddell *et al*. 2007). In 2015, to simulate drought conditions, an experimental throughfall reduction plot was established within 0.4 ha of the existing plot (details in Tng *et al*., 2018) – this is referred to as the through-fall reduction experiment (TFE) plot. The remaining 0.6 ha with no treatment provides a baseline reference and as such is referred to as the reference plot.

The design of the overall rainfall reduction experiment is a before-after-control-impact (BACI) design (Green 1979) and throughout we will use ‘before’ (the experiment) to refer to data collected in the reference plot and TFE plots in the years prior to the experiment (period 2009 - 2014). We will use ‘after’ to refer to data collected in the reference plot and TFE plots in the years after the experiment began (period 2015 - 2018).

To assess the effects of the TFE at species level, we selected three tree species: two subcanopy (*Cleistanthus myrianthus* (Hassk.) Kurz and *Myristica globosa subsp. muelleri* (Warb.) W.J.de Wilde) and one canopy (*Syzygium graveolens* (F.M.Bailey) Craven & Biffin). Each species was represented by more than 5 individuals in both the reference-plot and TFE, *Cleistanthus myrianthus* n=28 in the reference-plot and n=25 in TFE, *Myrtistica globosa* n=23 in the reference-plot and n=15 in TFE, and *Syzygium graveolens* n=7 in the reference-plot and n=6 in TFE. We then tested the effect of the TFE on each species flowering and fruiting activity, as well as on the carbon and nitrogen ratio, leaf δ^{15}N and δ^{13}C values, and wood δ^{13}C and δ^{18}O values.

Phenological observations

Using the canopy crane, flowering and fruiting activity observations were conducted on a monthly basis. For analyses, we selected species that had at least 5 individuals in the whole 1 ha plot. We also selected individual trees that had activity observed at least once and did not die during the whole study period, totalling 357 individual trees (225 in the reference plot and 132 in the TFE) of 33 species (30 in the reference plot and 29 in the TFE). Presence or absence of flowers and fruit was recorded monthly from 2009 to 2018, scanning the trees canopy using the canopy crane. Because it was a long-term study and depended on multiple observers, training and cross-validation checks have been done between observers. We considered the presence of flower buds and open flowers as flowering as well as the presence of unripe and ripe fruits as fruiting. Reproductive phenology for four years prior to the implementation of the TFE experiment and four years after the implementation of the TFE experiment were utilized for subsequent analyses. Data collected during the years 2013-15 were excluded, as research station construction did not permit proper sampling. During the transition from the dry to the wet season in 2018, we sampled, at monthly intervals, the intensity of leaf flush and leaf fall using the Fournier index – the percentage of the canopy presenting the phenological activity (from 0 to 4, with 25% interval) (Fournier 1974) for the selected trees used in the isotopic analysis.

Meteorological data

Site climate data were collected using a permanently mounted automatic weather station (AWS) and included measurements of rainfall, solar radiation (incoming shortwave) and air temperature. In the study site, the peak mean daily temperature and photoperiod (hours of sunlight per day) occurs in the wet season (from November to May), while peaks in average solar radiation occur towards the end of the dry season (from June to October) (Fig. 1). Across the period January 2009–April 2014 the AWS used was a Weathermaster 2000 (Environdata, Warwick, QLD, Australia). This unit was replaced in May 2014 with a custom-built weather station, consisting of a data-logger (CR1000, Campbell Scientific, Logan, UT, USA) an integrated meteorological sensor (WXT520, Vaisala, Helsinki, Finland), for measurement of wind speed and direction, precipitation, barometric pressure, temperature and relative humidity; a pyranometer (SP-110, Apogee, Logan, UT, USA), for measurement of total incoming shortwave radiation, and a tipping bucket rain gauge (RIM8000, Campbell scientific, Logan, UT, USA).

Soil moisture at the site was measured using time domain reflectometry probes (CS616, Campbell Scientific, UK), these were installed at 150, 100, 50 and 10 cm depths in eight soil pits, four pits in each of the reference plot and TFE plot. Eight separate data-loggers were used to collect soil moisture readings at 15-min intervals.

Stable isotope composition and nutrient analysis

To analyse stable carbon isotope composition (δ^{13}C value), total carbon abundance (%), total nitrogen abundance (%), and intrinsic water use efficiency (WUEi) from the selected species (*Cleistanthus myrianthus*, *Myristica globosa* and *Syzygium graveolens*), we sampled young (5-10 leaves per tree) and mature leaves (5-10 leaves per tree) from three trees per species in the reference-plot, and three trees per species in the TFE ($n = 18$; 9 in drought and 9 in the reference plot). Young leaves were visually identified through size, softness and colour. Sampling was conducted in September and November 2018 and February 2019. Sun exposed leaves were accessed using the canopy crane at the DRO. The leaves were subsequently dried at 60°C for seven days and then homogenized using a rock mill grinder (Rocklabs, Auckland, New Zealand).

We also analysed δ^{13}C value, total carbon (%), and total nitrogen (%) from wood micro-cores sampled at breast height using a TREPHOR. Isotope measurements were conducted on five trees per species that showed flowering and fruiting during the study period. Micro-cores were subsequently dried at 60°C for seven days and had the most recent 2 mm of wood sampled, after the bark and phloem were cut out. We then conducted cellulose extraction following Brendel method for small samples (Brendel *et al*. 2000). All carbon (both from bulk and α-cellulose) and nitrogen isotope composition and elemental abundances were determined using an elemental analyzer (ECS 4010 CHNSO Analyzer;

Costech Analytical Technologies INC, Valencia, CA, USA) fitted with a Costech Zero Blank Autosampler coupled via a ConFloIV to a Thermo Scientific Delta V^{PLUS} using Continuous-Flow Isotope Ratio Mass Spectrometry (EA-IRMS) at the Advanced Analytical Centre, James Cook University, Cairns. δ^{13}C and δ^{15}N values are reported as per mil (‰) deviations from the VPDB and AIR reference scales, respectively. The extracted α-cellulose was additionally analysed for δ^{18}O values using a with a Thermo-Fisher TCEA (Temperature Conversion Elemental Analyzer) fitted with a Costech Zero-Blank autosampler and coupled to a Thermo Scientific Delta V^{Plus} isotope ratio mass spectrometer. All cellulose samples were lyophilized just prior to analysis to remove water. δ^{13}C, δ^{15}N, and d18O values are reported as per mil (‰) deviations and anchored to the VPDB, AIR and VSMOW reference scales by normalization using at least two reference materials. All elemental and isotopic analyses were conducted at the Advanced Analytical Centre, James Cook University in Cairns, Australia.

Statistical analyses

Temporal and seasonal trends

For visualising seasonal patterns and modelling temporal trends, we utilised Generalised Additive Models (GAMs) as they are able to capture nonlinear relationships across multiple covariates as well as the periodic properties of phenological time series (Yang *et al*. 2012, Polansky & Robins 2013, Gaira *et al*. 2011, Potts *et al*. 2018, Pau *et al*. 2020). First, to visualise the seasonal patterns of the whole community, we fitted GAMs with month as the predictors for both flowering and fruiting of the entire community, the fleshy-fruit species only (biotic – animal dispersed), the dry-fruit species only (abiotic –

wind/gravity dispersed), and the dominant species (based on highest number of individuals per species). This allowed us to understand the seasonality of the groups and how overall patterns were driven by the dominant species. For seasonal pattern assessment, we used only reference plots from the period of 2009 to 2012. Because we first aimed to assess the seasonal patterns of the whole community, we opted for this period since it allowed us to have the maximum number of observations, as prior to TFE implementation both plots were under ambient conditions.

To assess long-term phenological patterns and potential seasonal variations in the tree community due to TFE implementation we developed GAMs for flowering and fruiting. We aggregated time series of monthly phenology (the proportion of trees in flowering/fruiting activity) as the response variables and used date, month, and treatment as the explanatory variables. GAMs were conducted using a Beta error distribution with a log link function. Because phenological patterns are cyclical, we ran the model with a cyclical smoother. Changes in the detrended, long-term flowering and fruiting activity patterns were assessed through the first derivatives that indicate significant changes in the non-linear slopes (Clement & Thas, 2009; Simpson 2018). Prior to analysis, any data gaps were filled using Kalman smoothing imputation in the “ImputeTS” package (Moritz & Bartz-Beielstein 2017). Residuals were assessed and analysed using the “DHARMa” package.

Following the assessment of seasonal patterns and long-term trends, we assessed the potential climate drivers of the community phenological patterns. We used Generalised Additive Model for Location, Scale and Shape (GAMLSS) to model relationships between climatic drivers and flower and fruit production as data exploration revealed non-linear relationships between phenological patterns and covariates (Hudson *et al*. 2010). The main benefit of GAMLSS over GAM for modelling climate effects on phenology is that they accommodate unconventional distributions, and the auto-correlated nature of the phenological series. In addition, they can identify the main drivers of the response variable, allowing for non-linear effects of the explanatory variables as well as statistically detecting thresholds (Hudson *et al*. 2010).

We developed the models using a cubic spline smoothing function. Each model was run using a Beta distribution, as phenological data was reported as proportions. We included an auto-regressive AR(1) correlation structure for the response (term of 1 month). Collinearity was checked with concurvity, which is a generalisation of collinearity to the GAM setting. For model construction, we used proportion of trees in flowering and fruiting activity as the response variables, and monthly rainfall (mm), monthly mean temperature (°C), photoperiod (h/day) and monthly mean solar radiation (W/m^2) as the predictors. Optimal models were selected using the Aikaike Information Criteria (AIC).

Effects of the TFE

To assess year to year variation in both reference-plot and TFE, we developed Generalised Linear Mixed Models (GLMM, family=Beta(link=”log”)) with proportion of individuals flowering and fruiting as the response variables and year as the grouping factors for each treatment. To account for pseudo-replication, we added month as the random effect. We then conducted pairwise comparisons using the marginal means from the fitted models.

To assess changes in phenophase seasonality we used circular statistics. First, we transformed each month into an angular value of 30° (January 0° to December 330°), and calculated the mean angle α (month with highest number of individuals/species in activity) and the magnitude of the r vector (degree of dispersion which acts as a proxy for seasonality) (Zar, 1999). To assess if the concentration of flowering activity in wet season changed among years, we tested if the activity was concentrated in the wet season in all years using a Rayleigh test with 0° as the mean angle for both reference-plot and the TFE. To assess if the concentration of fruiting activity in the dry season changed between the years, we tested if fruiting activity was concentrated in the dry season in all years using Rayleigh test with 180° as the mean angle for both reference-plot and the TFE.

To assess if the similarity between the reference-plot and TFE were different between the periods, we subtracted the monthly proportion of the reference-plot from the TFE and used a Wilcoxon rank test to identify significant differences. We then compared Reference *vs.* TFE for both flowering and fruiting in the community and each selected species.

Species level

To test the correlation between changes in percentage of flowering and fruiting and changes in soil moisture for each focused species, we conducted Spearman correlation analyses between the annual phenological activity and annual soil moisture. Because we wanted to test whether wetter/drier years led to an increase/decrease in phenological activity, we correlated all years from both treatments using the averaged proportion of phenological activity for the years of 2009-2018 in the reference-plot and 2016-2018 in the TFE altogether.

To better understand the effects of the TFE on the isotope composition and nutrient concentration of the selected species, and how this is related to possible acclimation to a drier environment, we tested for differences in leaf δ^{13}C value, δ^{15}N value and C:N in each season (dry, transition and wet), and wood δ^{13}C and δ^{18}O values. Differences were tested using ANOVA. All analyses were conducted in R (R Core Team 2019), using the packages “circular” (Agostinelli & Lund 2017), “timeSeries” (Wuertz 2020), “glmmTMB” (Brooks *et al*. 2017), “emmeans” (Lenth 2020), and “mgcv” (Wood 2017).

Results

Fruiting activity at the Daintree Rainforest Observatory was mainly driven by fleshy fruit species, which contributed an 84% share of the tree species activity at the site, with dry fruit species representing 16% of the tree species activity. Flowering activity of the tree community (proportion of individuals) peaked in the wet season, while the peak fruiting occurred in the dry season (Fig. 4.2, Fig. 4.3). When comparing only fleshy fruit species, flowering peaked in the period from December to February, while fruiting peaked in July (Fig. 4.2). Wind-dispersed (abiotic) species presented an overall low level of flowering and fruiting activity, with no outstanding seasonality detected (Fig. 4.2). The highest overall activity occurred for flowering in March of 2010 (21.3%) and March of 2011 (25.5%) and for fruiting in April/May of 2010 (28.5%) (Fig. 4.3).

The GAMLSSs produced models with high explanatory power for flowering (R^2=0.66) and intermediate explanatory power for fruiting (R^2=0.32) (Table 4.1). Flowering activity was positively correlated with monthly mean temperature and solar radiation (Table 1). Flowering activity started when mean daily temperature was around 25°C and average daily solar radiation around 100 W/m^2. Fruiting activity was negatively correlated with photoperiod, presenting a linear relationship, peaking when the photoperiod was at around 11h/day.

TFE experiment

After implementation of the through-fall reduction in the TFE plot, soil moisture decreased in both seasons (Fig. 4.4) and by 2017 the difference in soil moisture between wet and dry seasons was smaller in the TFE plot when compared with the reference-plot.

Differences in soil moisture between seasons were less pronounced for the years of 2012, 2016 and 2018 in the reference-plot (Fig. 4.4).

Flowering activity in both the reference-plot and TFE-plot occurred mainly in the wet season for all years (Fig. 4.3, Fig. 4.5, Fig. 4.6). Generally, the peak in fruiting occurred in the dry season in both reference-plot and TFE. Fruiting activity in both plots was concentrated in the dry season but there was activity in both seasons (Fig. 4.3, Fig. 4.6). Prior to the experiment implementation (2009-2012), peak fruiting occurred in June for the reference-plot and in August for the TFE; during the experiment (2016-2018) peak fruiting occurred in June for reference-plot but in September for the TFE, both one month later than in the previous period (Fig. 4.2, Fig. 4.6). The analysis of first derivatives from the time series showed no significant change in flowering long-term trend, but a significant increase in the fruiting activity during 2010 in both reference and TFE. In the reference-plot, however, there was a significant decrease after the 2010 peak, which was not observed in the TFE time series (Fig S4.1).

Seasonality in flowering activity was significant for all years with the exception of 2009 in the reference-plot. Seasonality in fruiting activity was significant for all years with exception of 2017 and 2018 in the reference-plot, and 2012 and 2016 in the TFE plot (noting that 2012 was prior to the experiment) (Table 4.2). The concentration of flowering activity in the wet season was significant for all years except for the year of 2009 in both the reference-plot and TFE-plot. Fruiting activity was significantly concentrated in the dry season in 2009 and 2010 in both plots. In 2012 and 2016, only the reference-plot fruiting activity was significantly concentrated in the dry season. In 2017 and 2018, only the TFE-plot fruiting activity was significantly concentrated in the dry season (Table 4.2). Both fruiting and flowering activity were higher in the TFE plot after the establishment of the

TFE experiment (Table 4.3, Fig. 4.3). Overall flowering and fruiting activity in the both reference-plot and TFE-plot varied considerably over the years (Table S4.1).

Species level phenology

Seasonal phenological patterns were found to differ among each selected species and between each species and the community pattern (Fig. 4.7). When comparing the three common species, we found differences related to both species and the effect of TFE upon the commencing the experiment. A Wilcox test to assess possible significant changes in the relationship between the reference-plot and TFE-plot between periods (before/after TFE) showed that only *S. graveolens* experienced significant changes in flowering activity. For fruiting activity, with the exception of *M. globosa*, all species (and the community) experienced significant changes in activity (Table 4.3, Fig. 4.8). While *M. globosa* did not seem to be affected by the TFE, *C. myrianthus* presented a decline in fruiting activity and *S. graveolens* showed an increase in fruiting activity between before and after TFE. The three species showed, therefore, very different responses to the TFE (Table 4.3, Fig. 4.8).

We analysed the general relationship between the annual proportion of trees flowering or fruiting and soil moisture, across all years in both the reference-plot and TFE-plot for the community, *C. myrianthus*, *M. globosa* and *S. graveolen*s. *C. myrianthus* showed a positive relationship between both flowering and soil moisture ($R^2=0.72$, $P<0.01$) and fruiting and soil moisture ($R^2=0.53$, $P<0.05$). *M. globosa* did not present a significant relationship between flowering and soil moisture ($R^2=0.03$, $P=0.68$), but fruiting was positively correlated with soil moisture, although the correlation was not significant ($R^2=0.45$, $P=0.056$). *S. graveolens* flowering activity was not correlated with soil moisture ($R^2=0.06$, $P=0.52$), but fruiting was negatively correlated ($R^2=-0.85$, $P<0.001$). The community showed a negative correlation between flowering and soil moisture, although this was not significant (R^2=-0.38, *P*=0.086), and there was no correlation between soil moisture and fruiting activity (R^2=0.01, *P*=0.79).

Stable isotopes

We assessed the $\delta^{13}C$ and $\delta^{15}N$ values as well as C:N from the leaves and the $\delta^{13}C$ and $\delta^{18}O$ values from the wood for the three selected species in both the TFE-plot and the reference-plot. For leaves, we assessed the differences in each season (dry, transition and wet). Significant differences in $\delta^{13}C_{leaf}$ between the TFE-plot and the reference-plot were found for *Cleistanthus* and *Syzygium* in the transition season. *Syzygium* presented significant differences in $\delta^{15}N_{leaf}$ between the reference-plot and TFE-plot in all seasons (Fig. 4.9).

Values of $\delta^{13}C_{wood}$ were significantly affected by species only (F=3.57, *P*<0.05), with no significant difference between the TFE-plot and the reference-plot (F=1.50, *P*=0.231) or the interaction between species and the TFE experiment (F=2.66, *P*=0.087). Post hoc comparisons indicated that in the TFE-plot, the only significant difference occurred between *Cleistanthus* and *Syzygium* (t=-3.44, *P*<0.05). The assessed $\delta^{18}O_{wood}$ were significantly affected by the TFE experiment (F=6.22, *P*<0.05) only, with no significant effect due to species (F=2.14, *P*=0.137) or the interaction between species and TFE (F=3.21, *P*=0.055). Post hoc pairwise comparisons indicated that the only significant difference occurred in *Myristica* between the reference-plot and the TFE-plot (t=-3.40, *P*<0.05). Despite the variability in response, it is possible to see that under the TFE, *Cleistanthus* presented decreased $\delta^{13}C_{wood}$ values while *Syzygium* increased and *Myristica* maintained similar values. When analysing $\delta^{18}O_{wood}$, although the differences are not significant, *Cleitstanthus* presented an increase under TFE, while *Syzygium* showed no difference between the reference-plot and the TFE-plot.

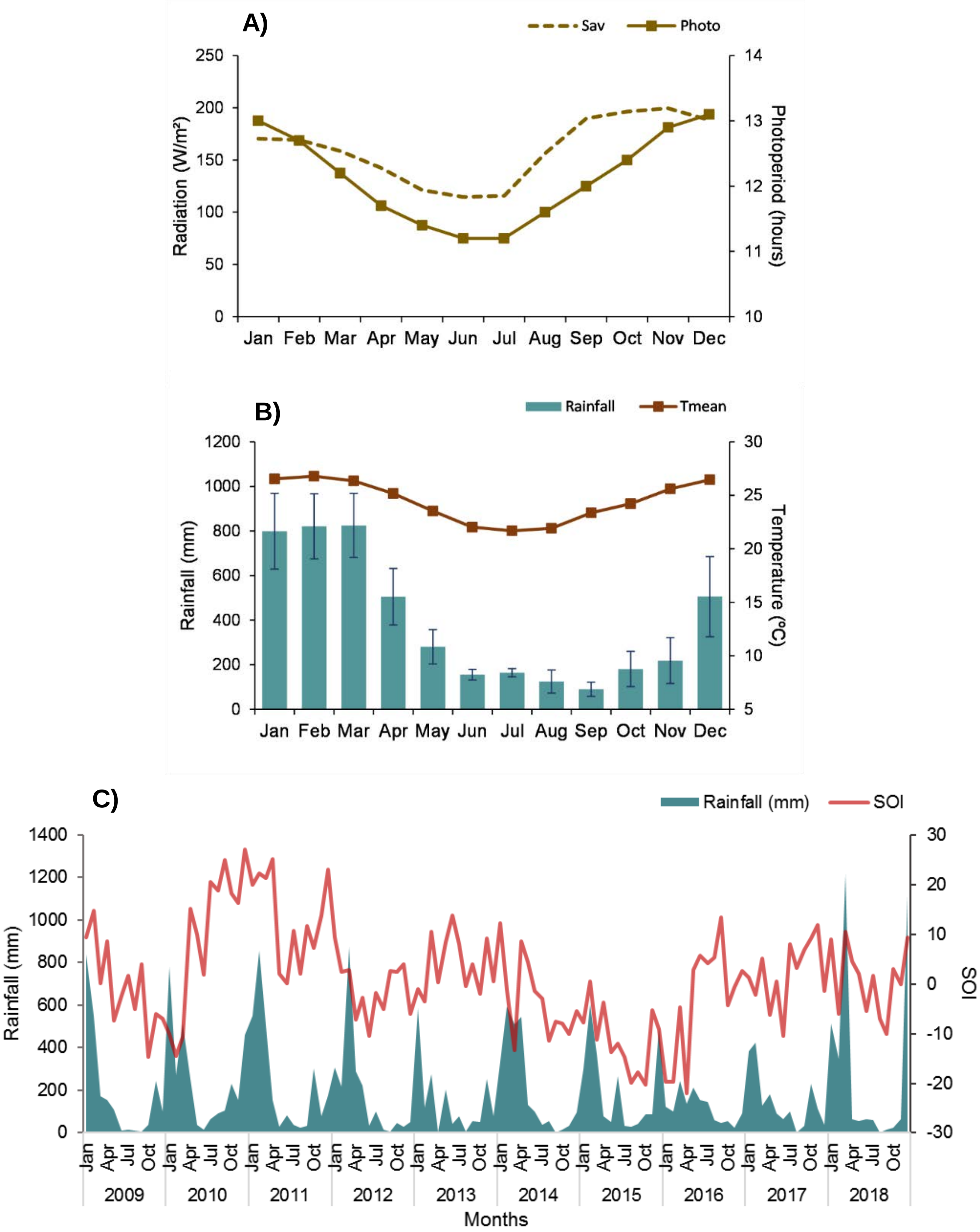

Fig 4.1. A) Monthly meteorological patterns of average daily solar radiation (Sav, W/m²) and photoperiod (photo, h/day), B) average mean monthly temperature – mean ± SD (Tmean, °C) and rainfall (mm/month), and C) and SOI (Southern Oscillation Index) over the studied period at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia.

Community level

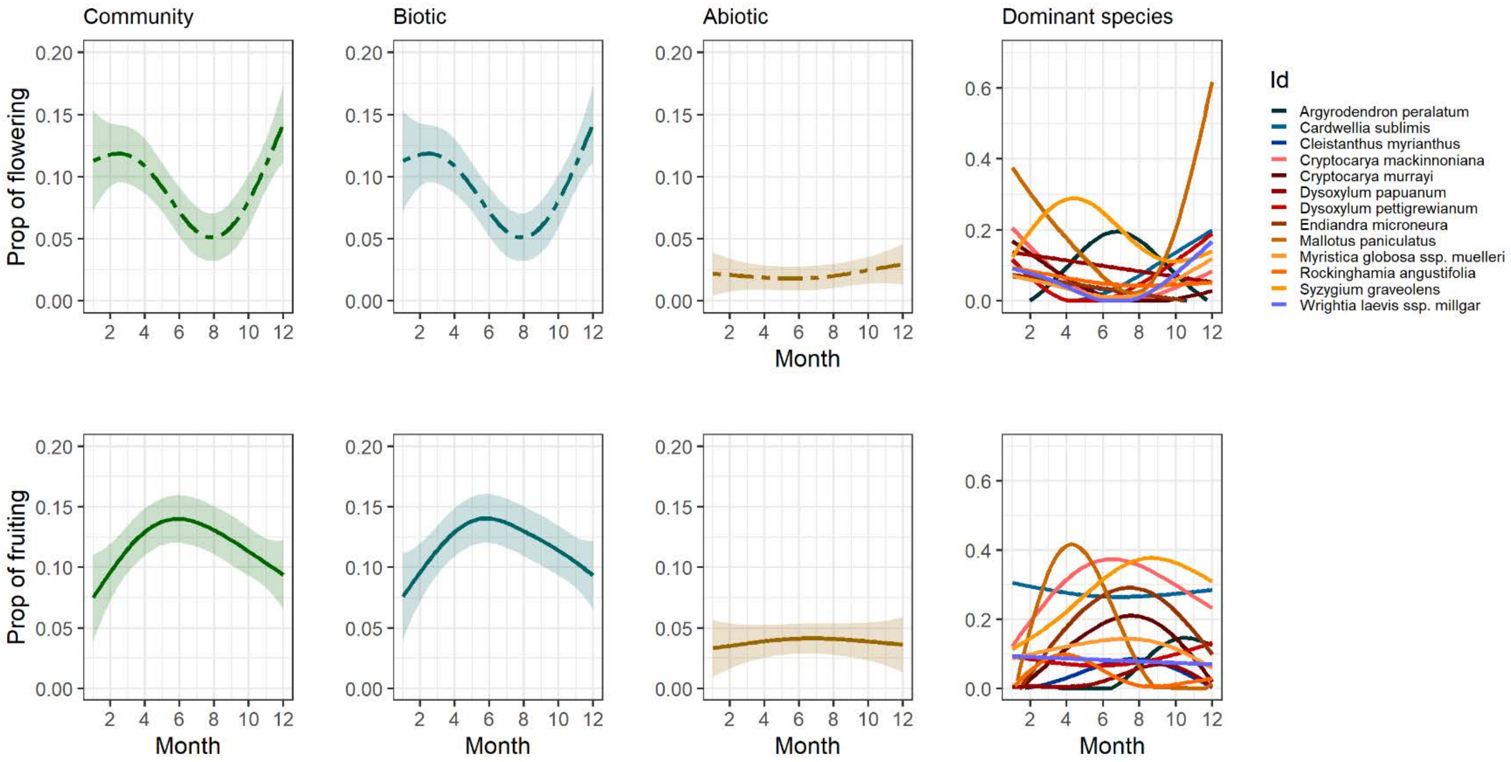

Fig. 4.2. Fitted generalized additive models for the proportion of individuals flowering and fruiting for the whole community, biotic species, abiotic species and dominant species (biotic in shade of red and abiotic in shades of blue) in relation to months over the first half of studied period (2009-2012) in the reference-plot at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia.

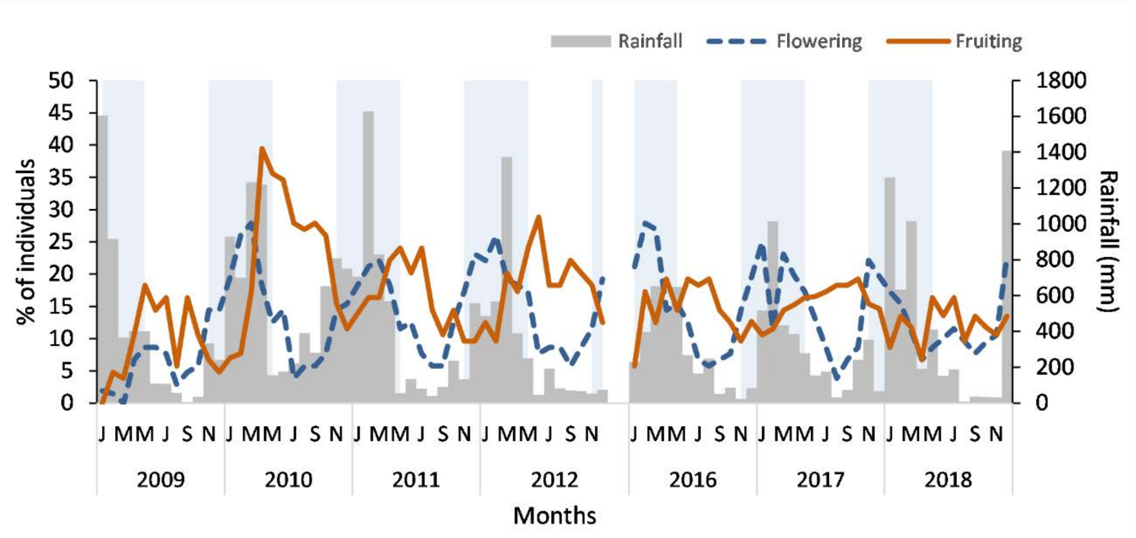

Fig. 4.3. Time series of percentage of individuals displaying flowering and fruiting activity throughout the studied period (2009-2012 and 2016-2018) in the reference-plot at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia. The blue shaded area indicates the wet season and grey bars the mean monthly rainfall (mm).

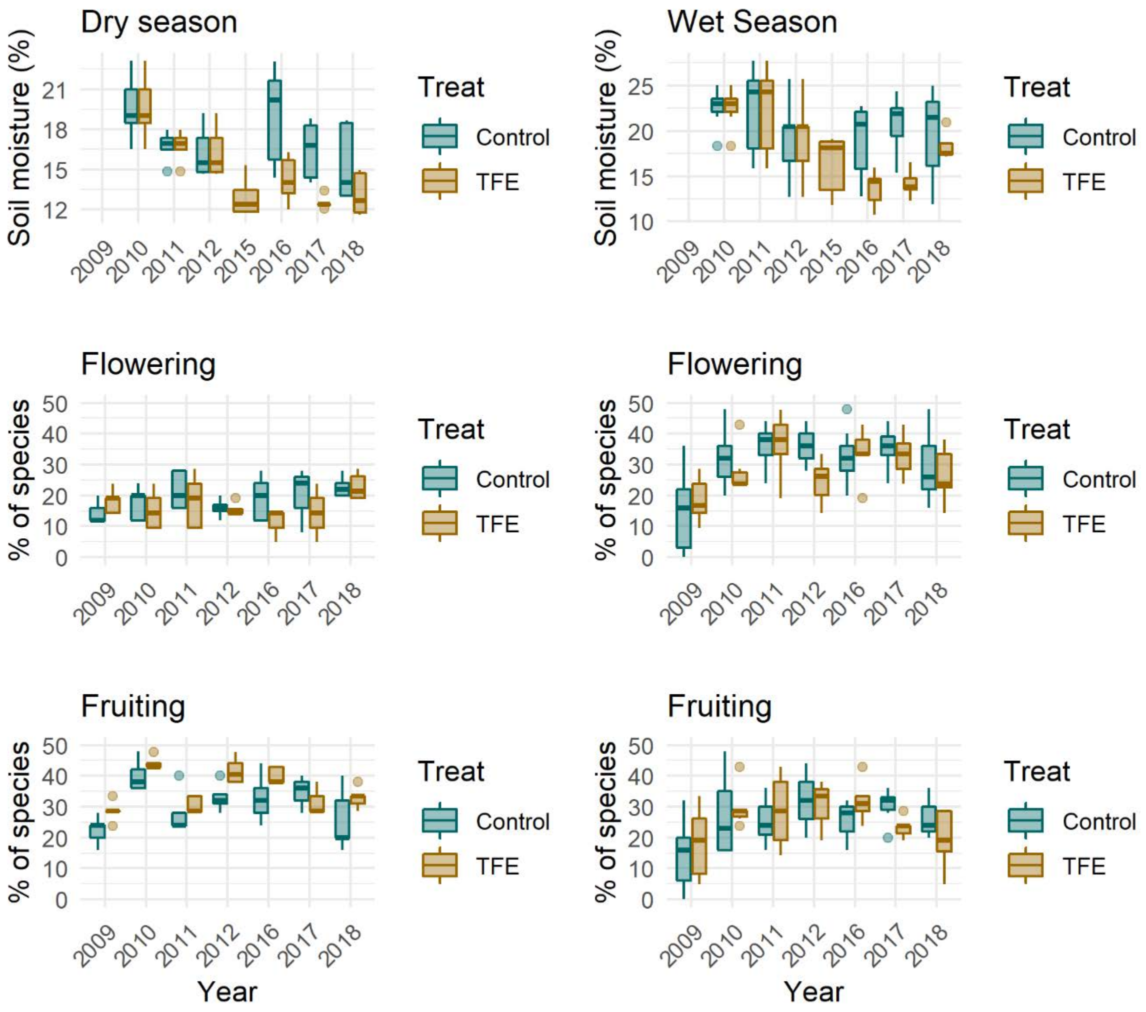

Fig 4.4. Boxplots of soil moisture (volumetric water content %), flowering (% of species in activity) and fruiting (% of species in activity) in both reference-plot (left) and TFE-plot (right) per season, throughout the studied period at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia.

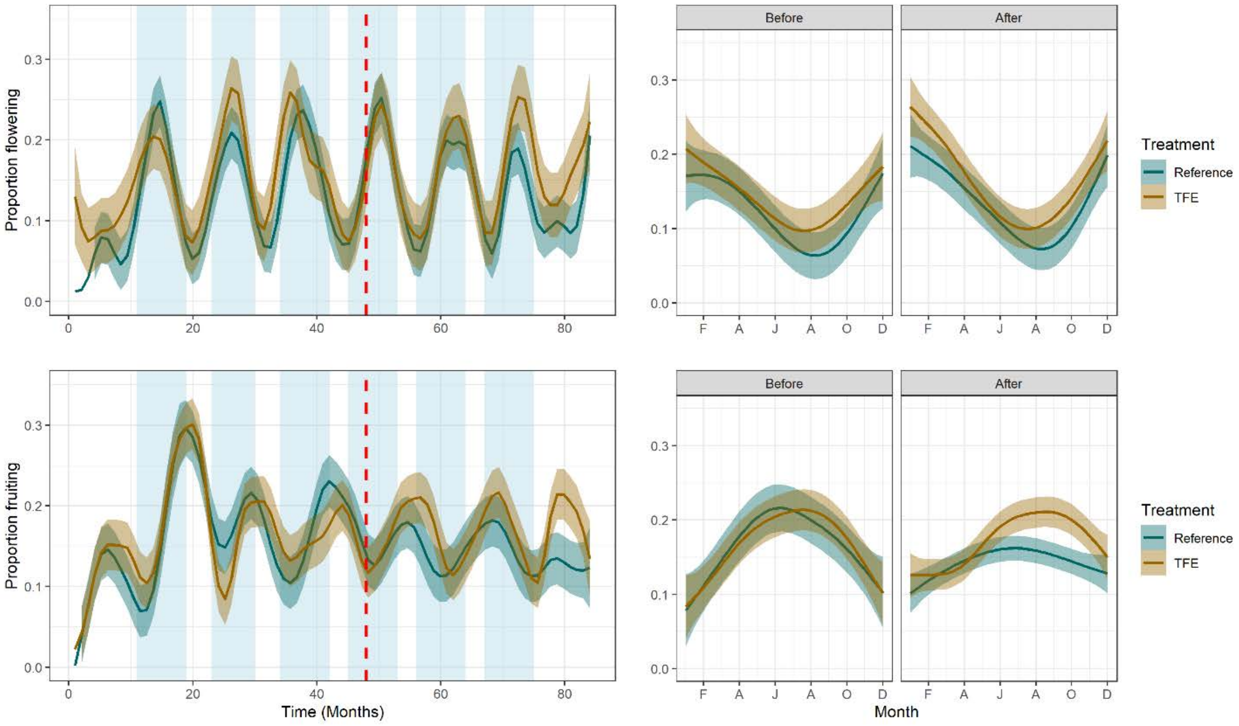

Fig 4.5. Fitted generalized additive models of the time series (left) and seasonal proportion (right) of individuals flowering in reference-plot and TFE plot. Seasonal plots show before TFE and after TFE began in relation to month for the studied period at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia. Red dotted line shows the beginning of TFE and blue vertical bars indicate the wet seasons.

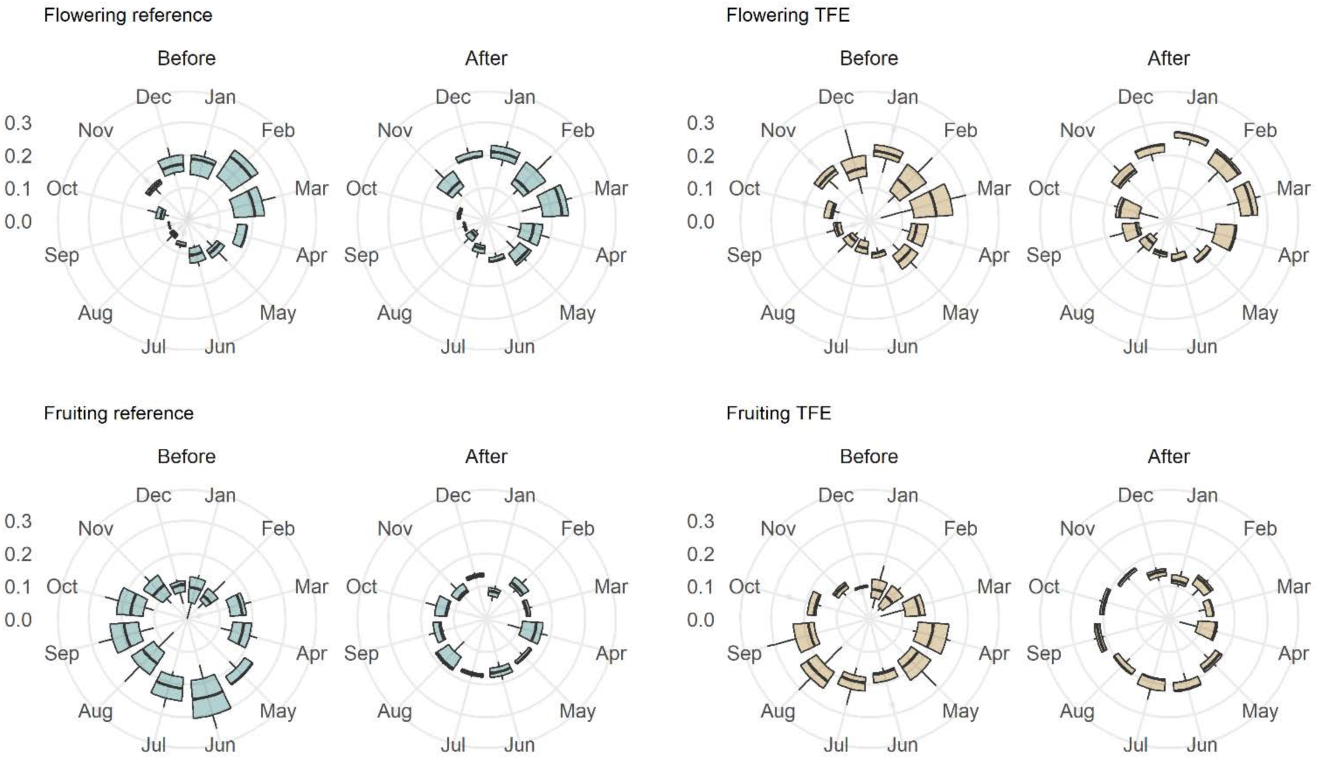

Fig. 4.6. Circular boxplots showing the mean (bold horizontal lines), interquartile range (shaded grey boxes) and 95th centile range (horizontal black lines) of proportion of individuals flowering and fruiting, from the community in the reference-plot and TFE both before and after-periods, and each calendar month amongst years, at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia.

Species level

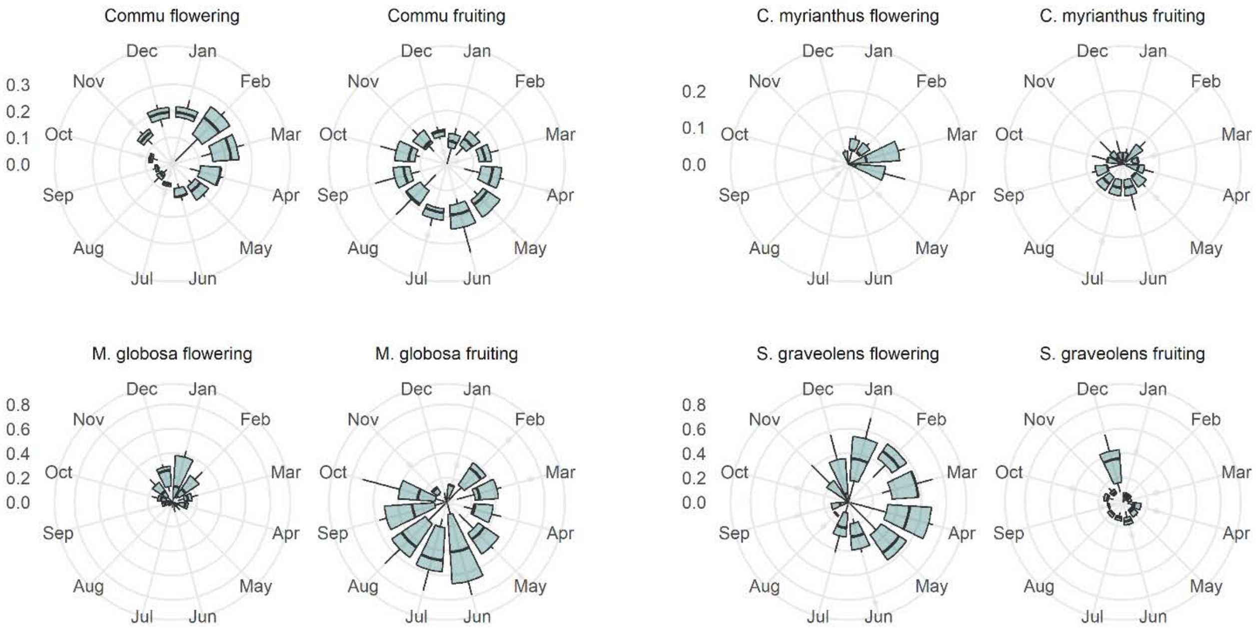

Fig. 4.7. Circular boxplots showing the mean (bold horizontal lines), interquartile range (shaded grey boxes) and 95th centile range (horizontal black lines) of proportion of individuals flowering and fruiting, from the community (Commu) and each selected species, each calendar month amongst years, at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia.

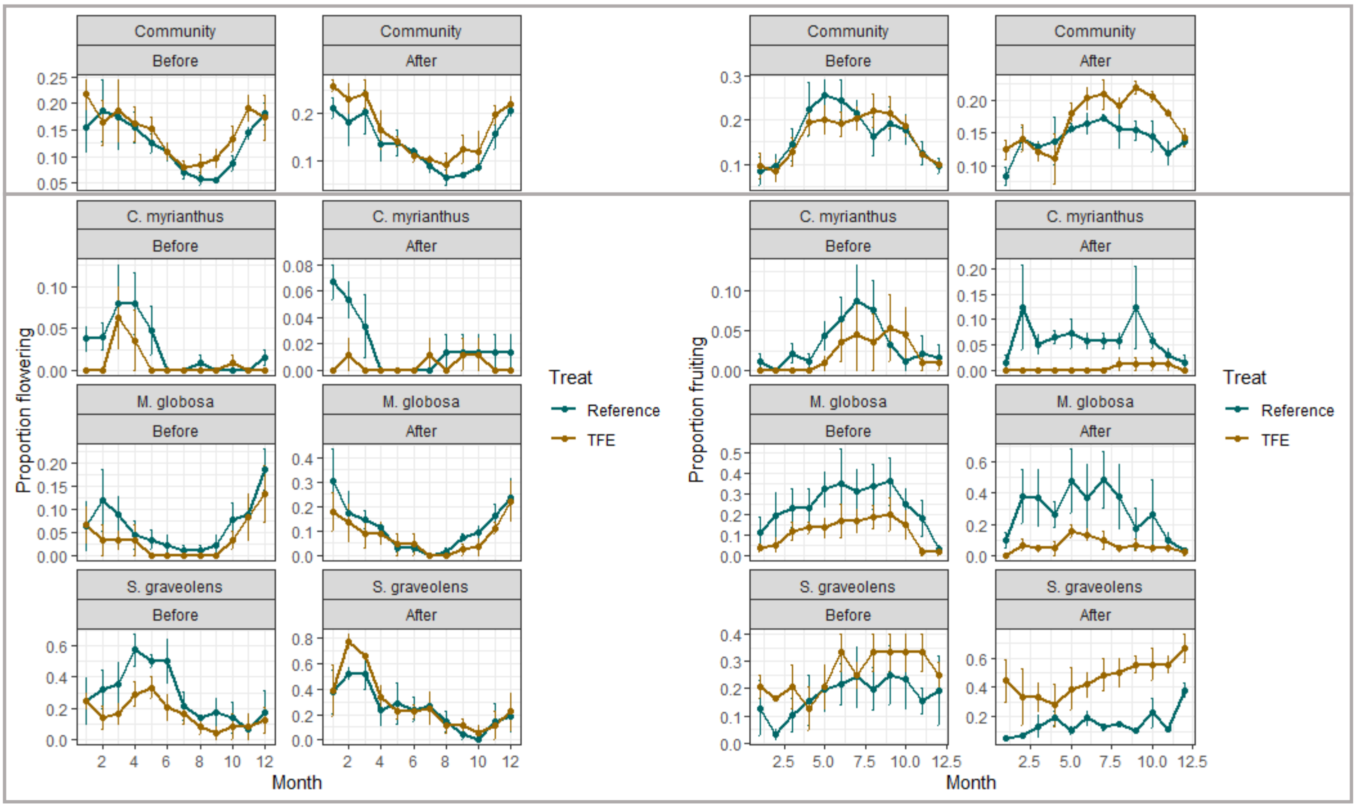

Fig. 4.8. Seasonal patterns of the proportion of individuals flowering and fruiting for the whole study period (before and after start of the experiment) for three studied dominant species in both the reference-plot and TFE plots at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia.

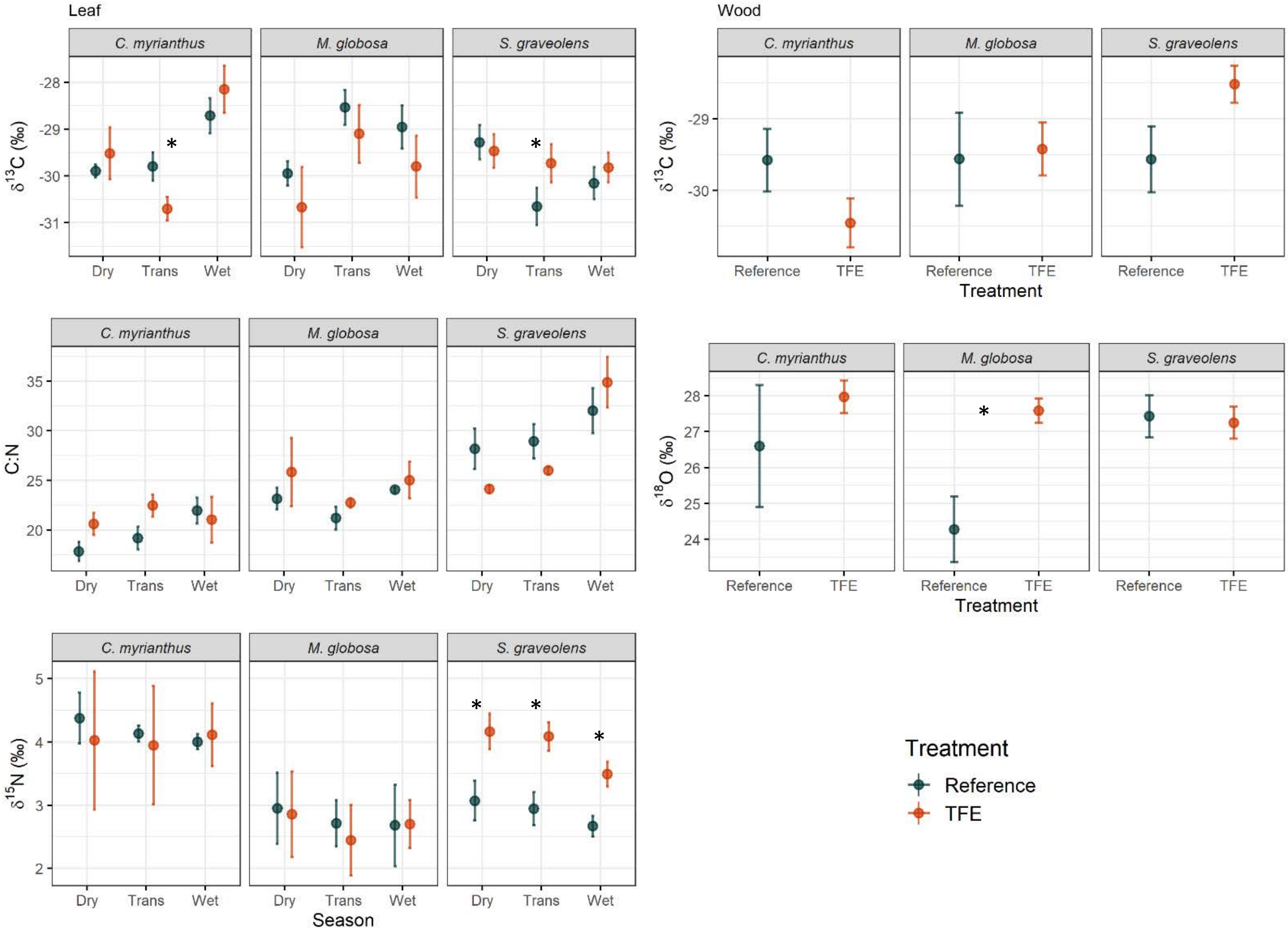

Fig. 4.9. Intra-seasonal variation in δ^{13}C, δ^{15}N and C:N in mature leaves (left panel) from dry season (D), transition dry-to-wet (T) and wet season (W) and δ^{13}C and δ^{18}O from wood microcores (right panel) at the Daintree Rainforest Observatory (DRO), Cape Tribulation, Australia sampled in 2018 and 2019. Asterisks (*) indicate significant differences (ANOVA at *P*<0.05).

Table 4.1. Parameter estimates and goodness of fit statistics for the GAMLSS models. GD, AIC and SBC denotes scaled deviance statistic, Akaike information criterion and Schwarz Bayesian Criterion goodness of fit statistics, respectively. Pseudo R^2 (Cox Snell) are presented as well.

Phenophase	Predictors	β estimate	Std error	T value	P value	GD	AIC	SBC	R^2
Flowering	Intercept	-6.53	0.45	-14.56	< 0.0001	-272.65	-252.65	-229.88	0.66
	Mean Temperature	0.20	0.02	10.14	< 0.0001				
	Mean Solar Radiation	0.00	0.00	-3.15	< 0.01				
Fruiting	Intercept	1.95	0.67	2.94	< 0.01	-225.47	-213.47	-199.81	0.32
	Photoperiod	-0.31	0.06	-5.59	< 0.0001				

Table 4.2. Results of circular statistics analyses testing for the occurrence of seasonality in flowering and fruiting activity in both the reference and TFE plots at the DRO, Cape Tribulation, Australia. A Rayleigh test was performed for significance of the mean angle (a) or mean date (given inside parentheses when significant).

Flowering														
Year	2009		2010		2011		2012		2016		2017		2018	
Treatment	Reference	TFE	Reference	TFE	Reference	TFE	Reference	TFE	Reference	TFE	Reference	TFE	Reference	TF
Number of Observations	78	134	172	170	177	231	174	162	180	188	177	204	142	21
Mean date	Sep 13th	Nov 1st	Feb 9th	Jan 25th	Jan 21st	Jan 9th	Feb 3rd	Feb 24th	Feb 3rd	Jan 27th	Jan 24th	Jan 14th	Dec 17th	De
Mean Vector (μ)	253.05°	300.22°	39.52°	25.48°	21.996°	9.104°	33.78°	54.39°	33.096°	27.568°	24.86°	14.856°	347.464°	34
Length of Mean Vector (r)	0.151	0.191	0.348	0.234	0.26	0.268	0.288	0.208	0.326	0.322	0.272	0.274	0.168	0.
Concentration	0.306	0.389	0.743	0.481	0.538	0.557	0.603	0.425	0.69	0.679	0.566	0.57	0.342	0.
Circular Variance	0.849	0.809	0.652	0.766	0.74	0.732	0.712	0.792	0.674	0.678	0.728	0.726	0.832	0.
Circular SD	111.35°	104.27°	83.22°	97.70°	94.073°	92.94°	90.34°	101.53°	85.77°	86.31°	92.43°	92.22°	108.13°	10
Rayleigh Test (Z)	1.786	4.885	20.865	9.283	11.946	16.632	14.481	7.012	19.141	19.44	13.117	15.298	4.031	7.
Rayleigh Test (p)	0.168	0.008	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.018	<
Rayleigh Test 0° (Z)	-0.044	0.095	0.2656	0.2086	0.2381	0.2619	0.2371	0.1198	0.2701	0.2818	0.2442	0.2617	0.1626	0.
Rayleigh Test 0° (p)	0.7	0.06	0	<0.001	0	0	0	0.0155	0	0	0	0	0.003	<

Fruiting														
Year	2009		2010		2011		2012		2016		2017		2018	
Treatment	Reference	TFE	Reference	TFE	Reference	TFE	Reference	TFE	Reference	TFE	Reference	TFE	Reference	TF
Number of Observations	114	80	287	150	203	112	231	115	179	126	193	116	152	11
Mean date	Jun 27th	Jul 20th	Jun 12th	Jul 2nd	May 5th	Jun 17th	Jun 22th	Jul 9th	Jun 3rd	Jul 20th	Jul 18th	Aug 15th	Jun 28th	Au
Mean Vector (μ)	177.07°	200.67°	162.26°	182.02°	125.48°	167.82°	172.18°	189.49°	153.177°	200.809°	198.878°	225°	178.904°	21
Length of Mean Vector (r)	0.37	0.263	0.285	0.261	0.2	0.212	0.144	0.1	0.17	0.112	0.115	0.167	0.081	0.
Concentration	0.796	0.544	0.595	0.541	0.408	0.435	0.29	0.2	0.346	0.226	0.231	0.339	0.162	0.
Circular Variance	0.63	0.737	0.715	0.739	0.8	0.788	0.856	0.9	0.83	0.888	0.885	0.833	0.919	0.

Circular SD	80.82°	93.70°	90.74°	93.91°	102.80°	100.85°	112.86°	123.08°	107.81°	119.85°	119.17°	108.36°	128.53°	10
Rayleigh Test (Z)	15.591	5.515	23.363	10.221	8.12	5.054	4.771	1.139	5.191	1.586	2.551	3.245	0.991	3.
Rayleigh Test (p)	<0.0001	0.004	<0.0001	<0.0001	<0.0001	0.006	0.008	0.32	0.006	0.205	0.078	0.039	0.371	0.
Rayleigh Test (Z) 180°	0.2035	0.2187	0.0919	0.1616	-0.0603	0.0873	0.0711	0.0711	0.029	0.0936	0.0938	0.1636	0.0466	0.
Rayleigh Test (p) 180°	0.001	0.0027	0.0138	0.0025	0.88	0.0957	0.1408	0.1408	0.2915	0.0687	0.0326	0.0063	0.2087	0.

Table 4.3. Results from Wilcox rank test for the differences between TFE and reference-plot (TFE − reference) comparing between the periods before and after for community and each selected species.

		Flowering		Fruiting	
		W	*P*-value	W	*P*-value
Community	Before *vs*. After	886	0.846	**1195**	**<0.001**
C. myrianthus	Before *vs*. After	843	0.832	**427.5**	**<0.0001**
M. globosa	Before *vs*. After	845	0.864	745.5	0.285
S. graveolens	Before *vs*. After	**1316.5**	**<0.0001**	**1348.5**	**<0.0001**

Discussion

Our study represents the first integrated study of the physiology and phenology of a tropical rainforest community in Southeast Asia and Oceania. We found that the tree community in the ancient Daintree rainforest exhibits seasonality in reproductive activity, which may be influenced by El Niño and drought events. We highlight the climate drivers of flowering and fruiting and show that responses to through-fall reduction are species-specific and influenced by physiological processes.

Community reproductive phenology and seasonality

The community wide phenological patterns of the study area exhibited seasonality that was driven mainly by the fleshy-fruited species. The majority of flowering occurred in the wet season and fruiting occurred in the beginning of the dry season. As fleshy fruits are expected to be more vulnerable to changes in water availability, changes in their phenological patterns induced by climate change will likely shape patterns in the overall community (Mendoza *et al*. 2017, Gray & Ewers 2021. The peak of flowering and fruiting activity in the wet and dry season, respectively, contrasts with conclusions of the majority of studies in tropical evergreen rainforests in the Neotropics (Mendoza *et al*. 2017), Africa (Bush *et al*. 2020, Chapman *et al*. 2018, Adamescu *et al*. 2018) and Southeast Asia (Medway 1972, Kurten *et al*. 2017), but follows flowering patterns in the evergreen regions of the Brazilian Atlantic rainforest (Morellato *et al*. 2000; Talora & Morellato 2000, Cardoso *et al*. 2018). Fruiting activity was mainly concentrated in the dry season, similar to patterns found in some areas of the Brazilian Atlantic rainforest (Morellato *et al*. 2000; Talora & Morellato 2000; Staggemeier & Morellato 2011) and in five sites studied by Adamescu *et al*. (2018) in Africa, but contrasting with patterns found in the ever-wet equatorial Amazon rainforest. The similarities between the phenological seasonality of the Daintree and Atlantic rainforests may be due to similarities in floristic structure due to common latitudinal range and the influence of coastal processes in both regions (Wagner *et al*. 2020). Fruiting presented varying degrees of seasonality across the years, but fleshy fruit species fruiting in the dry season suggests that trees either have access to water even in drier periods or have high water storage capacity.

Our results showed that flowering activity was correlated with increases in monthly mean temperature and solar radiation. The relationship between flowering and temperature and solar radiation has also been found in other regions (Pau *et al*. 2013). Wright & Calderón (2018) demonstrated that, in a seasonal dry tropical forest in Panama, periods of sustained high irradiance are the proximate cue for flowering in some species. An increase in flowering due to an increase in temperature may be due to both the effects on photosynthesis and rates of litter decomposition or nutrient cycling (Pau *et al*. 2013) that increase the carbon availability and gross primary productivity (GPP) (Pau *et al*. 2018). Nevertheless, expected increases in temperature due to climate change are likely to exert direct effects on flowering activity. The fact that an increase in temperature also leads to increased evapotranspiration may have significant effects on water availability which can influence reproductive success following flowering activity.

Fruiting activity in the study area was related to a reduction in photoperiod, similar to patterns observed in the Brazilian Atlantic rainforest (Morellato, 2000). This is indirectly supported by other results from the Daintree rainforest where Edwards *et al*. (2018) found that flowering/fruiting inputs in litterfall over 7 years were negatively correlated with temperature. From Fig. 1 we can see that the minimum in photoperiod is very close to the coolest time of year (N.B. litter analyses are gravimetric analyses where flowering/fruiting is dominated by fruiting). The results also align with other studies that have found correlations between fruiting and a decrease in temperature (Potts *et al*. 2019), but contrast with studies from other regions that typically show fruiting activity responding to increases in rainfall (Dunham *et al*., 2018). From 218 studies in the Neotropics assessed by Mendoza *et al*. (2017) 73.4% indicated rainfall as the main climate driver for fruiting, followed by air temperature (19.3%), then day length (9.17%) with other minor drivers including flooding, irradiance, ENSO and humidity. Other studies have shown positive correlations between solar radiance (Chapman *et al*. 2018) and fruiting which is quite different to what we observe in the Daintree. Climate drivers for rainforest tree species may be dependent on the interplay between local environmental drivers for the plant community, since signalling for phenology, combined with limited resources, may lead to changes in what drives the onset of phenological activity.

The notable increase in fruiting that occurred in 2010 may be explained by the moderate El Niño followed by an intense La Niña which occurred in that year. Effects of extreme events on fruiting activity of tropical rainforests have also been found by Chapman *et al*. (2018), who studied the phenological record from Kibale National Park, Uganda, for 15 years finding that solar irradiance and the occurrence of El Niño are the strongest positive predictors of fruiting. The timing of La Niña and El Niño events have been found to drive masting events in Southeast Asia, in dipterocarp forests (Appanah 1993) and this has recently been found in the rainforests of South America (Norden *et al*. 2007, Mendoza *et al*. 2018). ENSO climate anomalies such as El Niño, in addition to reducing rainfall and soil moisture availability, increase solar radiation, air temperature and vapour pressure deficit (Meir *et al*. 2018). The relationship between reproductive phenology and El Niño episodes has been described for Panama (Wright *et al*., 1999), in the Brazilian Atlantic rainforest (Engel & Martins 2005, Menezes *et al*. 2018), in Hawaii (Pau *et al*. 2010), Uganda (Chapman *et al*. 2018), as well as in Malaysia (Appanah 1993, Numata *et al*. 2003, Sakai 2006, Satake *et al*. 2019).

The trigger in reproductive phenology driven by El Niño events might be either a response to drought or to increased solar radiation that accompanies periods with less rain and, therefore, less clouds (Nepstad *et al*. 2002). El Niño events have also been found to be usually shortly preceded by increased leaf fall, and followed by an increase in seed production, which mimics the phenological response of species during the dry season (Detto *et al*. 2018). Our results showing a peak in fruiting activity as a response to a combined El Niño / La Niña event, the change in community activity being large enough to suggest a possible mast fruiting event. This possibility is currently being confirmed through additional longer-term phenological studies as they will enable the observation of recurrence interval of ENSO events, increasing the number of events and their effects. As extreme events are expected to increase due to ongoing changes in climate, understanding supra-annual cycles is essential for assessing the vulnerability of community phenology patterns, particularly in relation to resource availability. Plants may shift resource allocation from leafing to fruiting as a response to higher light availability during a warming phase of ENSO, while coping with higher atmospheric water demand and lower water availability (Detto *et al*. 2018). Masting activity has also been suggested to have trade-offs with growth, as trees use their stored carbohydrates in the mast production of flowers and fruits (Nakagawa *et al*. 2012).

TFE effects on the forest community phenology

Although drought has been previously found to lead to a decrease in flowering (Meir *et al*. 2018) and fruiting (Dunham *et al*. 2018), our results showed no change in flowering and an increase in fruiting activity due to the TFE. Our results contrast with other studies. Brando *et al*. (2006) studied one tree species (*Coussarea racemose*) in the Amazon and found that through-fall exclusion had no effect on the peak of reproductive activity but produced a delay in both flowering and fruiting. Also in the Amazon, a long-term TFE reduced the reproductive output of trees for the first five years of the experiment, followed by a recovery of both flowering and fruiting after 10 years of the experimental drought (Meir *et al*. 2018).

An increase in fruit production in dry years has been suggested to occur due to higher levels of photosynthetically active radiation (PAR) that leads to high photosynthetic activity (Nepstad *et al*. 2002). When developing an experiment and increasing PAR artificially, Graham *et al*. (2003) found an increase in fruit production for two Panamanian tree species. The increase in fruit production as a response to increased PAR suggested that fruiting is light limited. Phenological responses to drought, therefore, may vary between regions, species and vegetation types.

Along with droughts and El Ninos, other extreme events such as cyclones may also modulate the phenological activity of forest plant communities. When examining phenological activity between periods (before-TFE vs. after-TFE) in the reference-plot, there was a decrease in reproductive activity in the after-period. On the other hand, the comparison between before- and after- in the TFE-plot showed a similar level of fruiting activity. The difference in activity in the reference-plot between before- and after- may be related to a trade-off between canopy reconstruction and reproductive outputs due to TC cyclone Ita which impacted the plot in 2014. Cyclones often cause substantial damage to leaf demography, altering photosynthetic capacity and forcing trees to reconstruct their crowns which in turn may lead them to make increased use of carbon reserves (Nakagawa *et al*. 2012). The results suggests that species in the TFE-plot may have invested more in fruiting than in reconstructing the canopy after cyclone, while trees in the reference-plot invested more in leafing.

TFE effects on species phenology, stable isotopes and nutrients

Phenological and physiological responses to TFE were variable between species. One species, *S. graveolens,* presented an increase in both flowering and fruiting activity during the TFE, while *C. myrianthus* and *M. globosa* exhibited reduced flowering and fruiting activity. Apgaua *et al.* (2015) previously found that *S. graveolens* had traits conducive to drought resilience, such as high sap velocity and rate of sap flow, moderate WUE_i, contributing to an extremely low vulnerability index (based on wood vessel packing per unit area), while *M. globosa* had a higher drought vulnerability. The studied tree species altogether exhibited a higher intensity of leaf flushing in the reference-plot than in the TFE-plot, and a lower intensity of leaf fall (Fig. S2).

Physiological responses to TFE were also species dependent. *C. myrianthus* and *S. graveolens* presented higher intrinsic water-use efficiency (based on $\delta^{13}C_{leaf}$) in the transition dry-to-wet season. Drought may affect $\delta^{13}C_{leaf}$ and WUE_i by changing the c_i/c_a relationship, as decreases in stomatal conductance generally leads to decreased intercellular CO_2 concentration. When the ambient air becomes drier, photosynthetic activity can be reduced by the increased diffusion resistance caused by less open stomata. Interestingly, the significant effect of TFE on $\delta^{13}C_{leaf}$ value and therefore integrated water-use efficiency was found only in the transition between the dry and wet seasons, which may be due to the end of the dry season having the lowest soil water availability and highest VPD. *S. graveolens* exhibited higher $\delta^{15}N_{leaf}$ values under TFE conditions in all seasons. This indicates that the species has a divergent capacity to respond and acclimate to drought. Despite difficulty in pinpointing the mechanism causing the variation in $\delta^{15}N$ values, our results suggest a change in the use of nitrogen and possibly access to deeper layers of soil water by *S. graveolens,* consistent with a shift in rooting depth in response to TFE, which was not found in the other two species (Craine *et al*. 2015, Nel *et al*. 2018, Lorenz *et al*. 2020). The isotopic signals from $\delta^{13}C_{wood}$ and $\delta^{18}O_{wood}$ showed no significant effect of the TFE, except for the $\delta^{18}O_{wood}$ values of *M. globosa* which were substantially higher under experimental drought. The $\delta^{18}O_{wood}$ values in *M. globosa* signals that, under TFE, there is an increased enrichment of leaf water as a result of lower stomatal conductance and/or higher leaf temperature (van der Sleen et al. 2017).

Conclusion

Both phenological and physiological data suggested a species-specific response to drought, as found previously for other temperate species (Ogaya & Peñuelas 2007). This suggests that responses to climate change are heterogeneous and a drying climate will affect not only tree species but also forest floristic composition and structure. The species level changes in phenology were influenced by physiological processes which in turn are dependent on functional traits. These results are important because in highly diverse tropical rainforests, species may respond very differently in the face of climate change, making predictive modelling of community responses particularly challenging. More detailed studies relating changes in phenology to changes in physiological processes and species-specific traits are clearly necessary to better analyse and understand how species and communities will respond to predicted and ongoing changes in climate. In the Wet Tropics, we can already see phenological responses to extreme events that are expected to increase in the next years. This will not only alter the structure of the forest, but also the resource availability for the endemic fauna of the globally unique biodiversity found in the Daintree lowland rainforests.

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Supplementary information

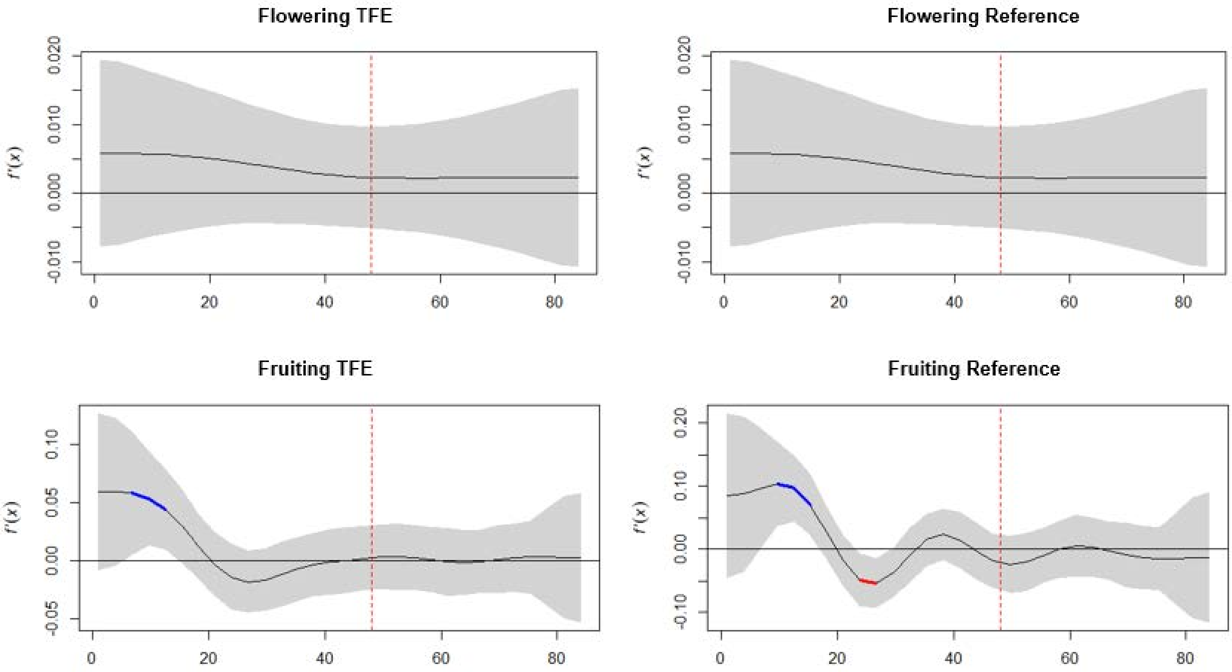

Fig. S4.1. First derivatives from the detrended time series of flowering and fruiting in both TFE and reference-plot. Lines in blue indicate significant increase while red indicate significant decrease. Dotted red lines indicate the start of the TFE.

Table S4.1. Pairwise comparison of marginal means from the GLMMs of reproductive activity in relation to month for all years in the reference plot and TFE.

		Reference plot								TFE						
		2009	2010	2011	2012	2016	2017	2018		2009	2010	2011	2012	2016	2017	2018
Flowering	2009								2009							
	2010	**-5.561**							2010	-2.222						
	2011	**-5.847**	-0.348						2011	**-4.339**	-2.220					
	2012	**-5.760**	-0.217	0.131					2012	-1.605	0.629	2.819				
	2016	**-5.825**	-0.368	-0.02	-0.151				2016	-2.575	-0.37	1.853	-0.997			
	2017	**-5.825**	-0.318	0.029	-0.101	0.049			2017	**-3.443**	-1.272	0.956	-1.889	-0.902		
	2018	**-5.029**	0.898	1.244	1.115	1.263	1.213		2018	**-3.562**	-1.385	0.829	-2.000	-1.017	-0.12	
		2009	2010	2011	2012	2016	2017	2018		2009	2010	2011	2012	2016	2017	2018
Fruiting	2009								2009							
	2010	**-7.040**							2010	**-5.897**						
	2011	**-4.616**	2.731						2011	-2.967	3.016					
	2012	**-5.539**	1.686	-1.059					2012	**-3.320**	2.633	-0.375				
	2016	**-4.094**	**3.360**	0.642	1.697				2016	**-4.325**	1.615	-1.398	-1.023			
	2017	**-4.487**	2.875	0.153	1.211	-0.488			2017	**-3.571**	2.396	-0.619	-0.243	0.782		
	2018	-2.912	**4.556**	1.915	2.954	1.280	1.763		2018	-3.009	2.943	-0.056	0.318	1.341	0.562	

Transition from Part II to Part III:

In the first part of this thesis, I assessed the climate influence on the reproductive phenology of trees, through an *in-situ* drought experiment, evaluating responses at community, population and individual level.

The second part of this thesis focuses on the physiological processes that may be related to leaf phenology. Using stable isotopes and physiological measurements, both in literature review and glasshouse experiments, I aimed to answer some important questions on how patterns related to leaves are influenced, and influence, physiological processes such as photosynthesis, water use and carbon translocation.

The ambient changes influence on physiological processes are found to have significant impacts on both reproductive and vegetative phenological patterns.

Chapter Five: Directional change in leaf dry matter $\delta^{13}C$ during leaf development is widespread in C_3 plants

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RESEARCH IN CONTEXT

Directional change in leaf dry matter δ^{13}C during leaf development is widespread in C_3 plants

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- **Background and aims** The stable carbon isotope ratio of leaf dry matter ($\delta^{13}C_p$) is generally a reliable recorder of intrinsic water-use efficiency in C_3 plants. Here, we investigated a previously reported pattern of developmental change in leaf $\delta^{13}C_p$ during leaf expansion, whereby emerging leaves are initially ^{13}C-enriched compared to mature leaves on the same plant, with their $\delta^{13}C_p$ decreasing during leaf expansion until they eventually take on the $\delta^{13}C_p$ of other mature leaves.
- **Methods** We compiled data to test whether the difference between mature and young leaf $\delta^{13}C_p$ differs between temperate and tropical species, or between deciduous and evergreen species. We also tested whether the developmental change in $\delta^{13}C_p$ is indicative of a concomitant change in intrinsic water-use efficiency. To gain further insight, we made online measurements of ^{13}C discrimination (Δ^{13}C) in young and mature leaves.
- **Key Results** We found that the $\delta^{13}C_p$ difference between mature and young leaves was significantly larger for deciduous than for evergreen species (−2.1 ‰ vs. −1.4 ‰, respectively). Counter to expectation based on the change in $\delta^{13}C_p$, intrinsic water-use efficiency did not decrease between young and mature leaves; rather, it did the opposite. The ratio of intercellular to ambient CO_2 concentrations (c_i/c_a) was significantly higher in young than in mature leaves (0.86 vs. 0.72, respectively), corresponding to lower intrinsic water-use efficiency. Accordingly, instantaneous Δ^{13}C was also higher in young than in mature leaves. Elevated c_i/c_a and Δ^{13}C in young leaves resulted from a combination of low photosynthetic capacity and high day respiration rates.
- **Conclusion** The decline in leaf $\delta^{13}C_p$ during leaf expansion appears to reflect the addition of the expanding leaf's own ^{13}C-depleted photosynthetic carbon to that imported from outside the leaf as the leaf develops. This mixing of carbon sources results in an unusual case of isotopic deception: less negative $\delta^{13}C_p$ in young leaves belies their low intrinsic water-use efficiency.

Key words: Carbon isotope ratio, intercellular CO_2 concentration, leaf development, water-use efficiency.

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Chapter Six: Delayed greening during leaf expansion under ambient and elevated CO_2 in tropical tree seedlings

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Delayed greening during leaf expansion under ambient and elevated CO_2 in tropical tree seedlings

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Abstract Tropical tree species employ varying strategies in young leaves to minimise losses to herbivory. The young leaves of species with delayed greening are thought to be less visible to herbivores, but likely incur a cost to leaf-level carbon gain *via* lower photosynthetic rates during leaf development. Increasing atmospheric CO_2 concentrations may reduce the cost of delayed greening, and/or modify leaf expansion rates, altering the ecological interaction of species. In this study, we evaluated the effects of elevated CO_2 concentrations on physiological responses of three Australian rainforest tree species, two with delayed greening in young leaves. Net photosynthesis rates were significantly lower in recently flushed leaves of species with delayed greening than in the species with normal greening. Yet, surprisingly, total nitrogen concentrations were higher in the former than in the latter. Intrinsic water-use efficiency increased at a steeper rate during leaf development under elevated CO_2 in all three species, irrespective of greening strategy. Leaf growth rate, in terms of area expansion, did not increase under elevated CO_2 in any of the species. Although elevated CO_2 did not boost the performance of the delayed greening species more than that of the normal greening species, we found higher nitrogen concentrations in their young leaves. This supports the intriguing possibility that delayed greening may have evolved in these species to protect young leaves that are especially rich in nitrogen.

Key words: anti-herbivory, climate change, leaf colour, leaf flush, wet tropics.

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Chapter Seven: Effects of elevated CO$_2$ and defoliation on wet tropical rainforest tree species in relation to leaf phenology

Chapter Seven: Effects of elevated CO_2 and defoliation on wet tropical rainforest seedlings in relation to leaf phenology

Abstract

The phenology and physiology of evergreen tropical tree species has important implications for the carbon balance of tropical forests. Understanding how leaf exchange patterns influence recovery from defoliation may enhance predictions of tropical rainforest carbon budgets, and responses of this ecosystem to environmental changes. Producing new leaves represents a substantial cost to a tree as it involves the use of the tree's stored carbon and, among other things, emerging leaves are not immediately photosynthetically competent. However, elevated CO_2 may allow new leaves to do more photosynthesis, and this benefit may differ depending species' functional traits, such as leafing pattern. In this study, we aimed to gain insights into the physiological drivers of leaf production in relation to environmental and endogenous conditions by analysing the source of carbon used to produce new leaves. Using seedlings of Australian rainforest species, we assessed the impacts of defoliation and elevated CO_2 on $\delta^{13}C$ composition of leaves and wood, and growth rates. We found that deciduous species relied more on stored carbon than evergreen species, which primarily incorporated $\delta^{13}C$ from current photosynthates. Upon defoliation, we found that evergreen species used stored carbon to produce the new leaves. Elevated CO_2 increased growth rates in plants recovering from defoliation, and increased vessel density and LMA only in evergreen species. Under elevated CO_2, deciduous individuals which were defoliated were able to flush new leaves faster. In general, our findings show that the studied deciduous and evergreen species relied on different strategies to recover from defoliation, with deciduous species investing in growth while evergreen species invested more in improving leaf resistence traits, such as leaf mass per area.

Introduction

Climate change due to human activity is causing shifts in meteorological patterns, seasonality and how trees respond to those changes. For example, some tropical rainforests are facing increasing dry seasons which may affect leaf demography due to unexpected leaf shedding. In predicted scenarios, trees will likely suffer changes in their canopy leaf area due to changes in soil moisture, water availability, herbivory and fire, having important implications for canopy gas exchange and carbon allocation (Trumble *et al*., 1993; Wu *et al*., 2017; Palacio *et al*., 2018). The need to reconstruct the canopy after disturbance changes the number of leaves in each stage of development and, because photosynthetic capacity depends on leaf development, physiological processes are also expected to be disrupted, having significant impacts on water and CO_2 exchange (Cernusak, 2020). Thus, tropical rainforest species could experience changes in leaf production and leaf exchange seasonality, affecting photosynthetic activity, carbon and water use, as well as growth rates.

Ecophysiology and phenology studies aim to understand interactions between tropical trees and climate, an area that is becoming increasingly important due to changing climatic conditions and the adverse effects of them on tropical rainforests. Increased seasonality of, and reductions in, water availability as a result of climate change is expected to impact leafing phenology in three important ways: (1) changing the timing of and intensity of leaf exchange patterns, (2) increasing leaf mortality rates due to drought, and (3) increasing defoliation events through climatic extremes including fires. Producing new leaves represents a substantial cost to a tree as it involves the use of the tree's stored carbon and, among other things, emerging leaves are not immediately photosynthetically competent but obtain carbohydrates from mature leaves or storage, and start to become more photosynthetically capable throughout expansion. During expansion, young leaves gradually increase their photosynthetic capacity, switching from higher respiration levels to higher photosynthetic rates (Choinski *et al*., 2003; Cernusak *et al*., 2006, 2009). Deciduous and evergreen species co-exist in tropical forests and employ different ecophysiological strategies in order to maximise photosynthetic capacity or resilience to unfavourable conditions.

Differences in the responses of deciduous and evergreen species to increased leaf loss result in changes in the carbon balances of tropical forests. Additionally, as deciduous and evergreen species co-exist in tropical forests, differences in their responses to increasing leaf loss events may influence floristic composition and structure of tropical forests under future climate change conditions (van Ommen Kloeke et al. 2012). Therefore, it becomes essential to understand their different ecophysiologies to provide a better understanding on how these species may respond to changes in their canopy area and leaf production as a result of changing environmental conditions. Since leaf-herbivore relationships also depend on abiotic factors, understanding how these strategies differ and how they affect trees' physiological processes may allow us to improve predictions of how climate change will affect the floristic structure and carbon balance of tropical rainforests. Studies presenting the combination of physiology and leafing patterns in evergreen tropical rainforests are required to understand the physiological processes that sustain the exchange of leaves throughout the year (Wu *et al*., 2017). One way of studying the physiological drivers of leaf production in relation to environmental and endogenous conditions is to analyse the source of carbon used in the process. The source of carbon and changes in water use efficiency may be studied through the use of carbon stable isotopes (δ^{13}C) (Helle & Schleser, 2004; Gulbranson *et al*., 2012; Worbes *et al*., 2013; Cernusak, 2020).

Carbon stable isotope ratios (δ^{13}C) are useful in determining the source of carbon used to produce new leaves in evergreen species. The δ^{13}C of carbon in C_3 plants reflects the selection of the lighter isotope in the photosynthetic process, as well as environmental conditions at the time of photosynthesis. However, it is not understood if the carbon used to build new leaves originates from a trees' stored carbon, and therefore carries a δ^{13}C signal from a previous time, or from carbon exported by mature leaves carrying a contemporaneous δ^{13}C signal. Besides that, stable isotope ratios (δ^{13}C) of leaves are a valuable tool to assess changes in water-use efficiency in C_3 plants (Farquhar *et al.* 1982). Despite still not being totally understood, the process of storing and translocating carbohydrates makes δ^{13}C less negative, causing the δ^{13}C used in leaf production to be usually enriched, making recently flushed leaves present less negative values of δ^{13}C (for further discussion see Cernusak *et al.*, 2009). This enrichment due to translocation provides information on the source of carbon used in the production of new leaves. Studies analysing the δ^{13}C that focus on mature leaves generally provide a poor explanation of how isotope composition changes in leaf production and leaf expansion, and how these processes respond to environmental factors (Damesin *et al.*, 1998). Therefore, it is yet to be determined if the carbon used by young leaves of tropical evergreen species in their synthesis originates from stored carbon, carrying the δ^{13}C signal from the period in which the carbon was taken up, or from carbon exported from mature leaves contemporaneously, carrying the δ^{13}C signal of the current atmospheric conditions. This deeper understanding will also enhance interpretation of wood isotopic time series and their relation to physiological processes occurring in the leaves.

Changes in leaf demography effects on physiological process may depend on the leafing strategy of species. For example, deciduous species have been found to have higher photosynthetic rates and hydraulic conductivity (Chabot & Hicks, 1982; Reich *et* *al*., 1992, 1999; Choat *et al*., 2005), while evergreens would spend more of their energy production in longevity and defense mechanisms (Reich *et al*., 1992, 2004). Atmospheric carbon is also increasing, which may benefit trees through carbon fertilization and reduce the impacts of leaf loss events. Since evergreen species have higher water use efficiency they will likely benefit more from carbon fertilization. Differences in how species respond to the competing impacts of leaf loss and CO_2 fertilization may influence floristic composition, and hence forest structure and dynamics. While changes in water cycles are expected to negatively affect tropical trees, the increase of CO_2 in the atmosphere would be expected to provide some benefits in photosynthetic water-use efficiency and growth (Dusenge *et al*., 2018). Therefore, it is essential to understand the effects of changes in leaf performance in relation to leaf phenology, and test how they would respond to increased CO_2.

Here, we assessed the carbon source used by evergreen and deciduous species in the production of new leaves using carbon stable isotopes under ambient and elevated CO_2. We tested the hypotheses that: 1) Deciduous and evergreen species would present differences in nutritional contents and water use efficiency and that young leaves would differ from mature leaves dependent upon leafing strategies; 2) expanding leaves of deciduous species would depend more on their own photosynthesis for leaf expansion than evergreen species; and 3) elevated CO_2 would aid defoliated plants in recovering from defoliation due to higher photosynthesis during leaf expansion. To test these hypotheses, we conducted an experiment with seedlings from three deciduous and three evergreen species from the Australian tropical rainforest, growing them within chambers of controlled environment and different air CO_2 concentration with accompanying difference in $\delta^{13}C$ of CO_2 in the air.

Materials and methods

Experimental design

Experimental procedures were carried out using a glasshouse divided into three chambers, located at James Cook University, Cairns, Australia, in 2019. Chamber 1 was treated with ambient CO_2 concentration (about 400 ppm) to establish a control. Chambers 2 (hereafter short-elevated CO_2) and 3 (hereafter long-elevated CO_2) were treated with elevated CO_2 concentrations of approximately 800 ppm from June 29th onwards and May 29th onwards respectively (Fig. 7.1). The choice of 800ppm was to account for an extreme increase in CO_2. Half of the individuals of each species in each chamber were defoliated on June 29th. The difference in the time under elevated CO_2 allowed the individuals to either store carbon with $\delta^{13}C$ impacted by the elevated CO_2 treatment prior to defoliation (Chamber 3) or to have only the $\delta^{13}C$ signal of the ambient CO_2 treatment prior to defoliation (Chamber 2). The CO_2 cylinders used to elevate the CO_2 concentrations contained CO_2 with $\delta^{13}C$ signal typical of fossil fuels (relatively depleted in ^{13}C). Using a chamber air with different $\delta^{13}C$ of CO_2 allowed us to examine the source of carbon used to produce new leaves after defoliation and the elevated CO_2 treatment itself enabled us to analyse the species capacity to recover from defoliation as a result of higher photosynthetic rates.

To keep track of changes in $\delta^{13}C$ in each treatment, we grew two individuals of sugar cane in each chamber. The sugar cane has a C_4 photosynthetic pathway, and C_4 plants discriminate less against the stable isotope ^{13}C during photosynthetic CO_2 fixation than C_3 plants. Therefore, having a sugar cane in each chamber allowed us to calculate the ambient $\delta^{13}C$. The $\Delta^{13}C$ of the C_4 species was assumed to be 3.8‰ (Meinzer and Saliendra, 1998) and was used to back-calculate δ_a in each chamber:

$$\delta_a = \Delta^{13}C_{C4}(1 + \delta_{pC4}) + \delta_{pC4}$$

where subscript C_4 refers to sugar cane (Dalling *et al*., 2016).

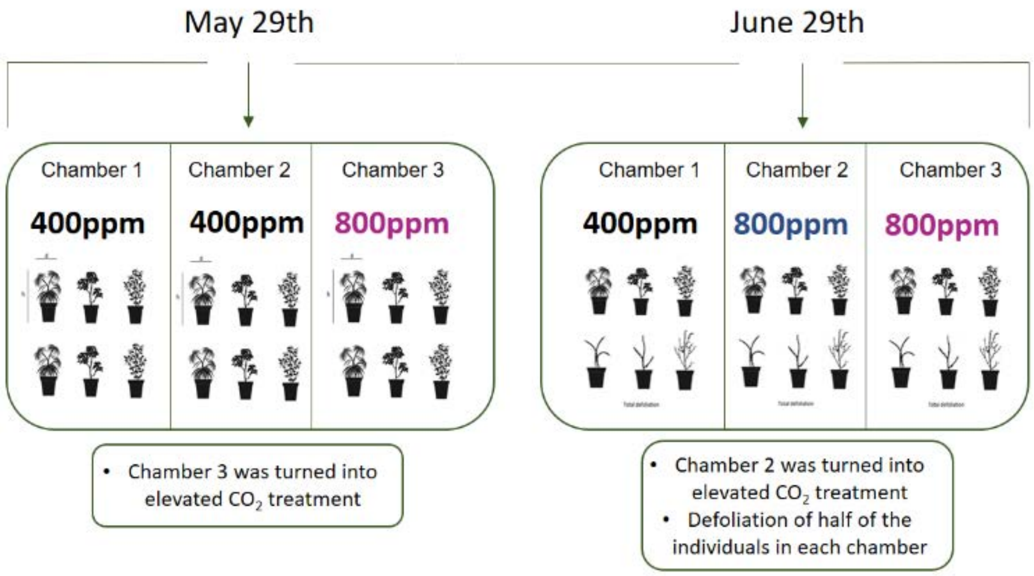

Fig. 7.1. Simple scheme of experimental design with 10 individuals from each species in each chamber, totalling 60 individuals per chamber.

Species

We chose six Australian native rainforest species, with each species represented by 10 individuals in each chamber, totaling 60 individuals per chamber. To compare the influence of leafing phenology on performance post-defoliation, we chose three deciduous species (*Brachychiton acerifolius* (A.Cunn. ex G.Don) F.Muell. (Malvaceae), *Melia azedarach L. (*Meliaceae*)*, *Terminalia catappa* L. (Combretaceae) and three evergreen species (*Phaleria clerodendron* (F.Muell.) Benth. (Thymelaeaceae), *Aglaia sapindina* (F.Muell.) Harms (Meliaceae), and *Cryptocarya laevigata* Blume (Lauraceae) (Table 2). Hereafter, we named chamber 1, chamber 2 and chamber 3 as control, short-elevated CO_2 and long-elevated CO_2, respectively.

Data collection

To compare the source of carbon used to produce new leaves between defoliated and non-defoliated deciduous and evergreen species, we collected the first flushed leaves of both groups as soon as defoliated individuals started to flush again. Sampled flushes were then dried at 60°C for 7 days and then leaf clippings were collected for analysis. Leaf tips of young leaves were collected to maintain consistency. To determine leaf carbon isotope ratio (δ^{13}C) and carbon and nitrogen concentrations, the dry leaf plugs were analysed at the Advanced Analytical Centre, James Cook University in Cairns, Australia. We measured height (cm) and diameter (cm) fortnightly of all individuals in the three chambers from the defoliation date (June 29th) until the end of the experiment. We calculated stem volume (cm^3) of each individual as $V = 0.333(\pi(d/2)^2)h$, where d is the diameter (cm) and h is individual height (cm). Along with the growth measurements, we recorded the date of first flushing for all defoliated individuals to assess effects of phenology and CO_2 treatment on the timing of new leaf production.

To evaluate the effect of defoliation and atmospheric carbon concentration on leaf traits, we measured leaf mass per area (LMA) in leaves of all stages of development in both control and long-elevated CO_2. In order to compare the effect of leaf exchange pattern and carbon concentration on leaf expansion rates of species, we measured leaf expansion through time (at an interval of 2-4 days). This approach allowed us to compare leaf development under different conditions.

Wood Anatomical traits and wood stable isotopes

At the end of the experiment, individuals were harvested and stem samples were collected and naturally dried for posterior anatomic and isotopic analyses. To facilitate microscopic examination, we shaved the ends of stem samples with a GSL1 microtome (Gärtner *et al.*, 2014) until we obtained a smooth xylem surface with visible vessels. Stem wood samples were examined under a light microscope at 60x magnification to detect the presence of growth rings and vessels formed due to the increase in CO_2. Digital photographs of the stem sections were then taken using the camera mounted onto a Leica compound and dissecting camera microscope (Leica EZ4W). We counted the vessels present in an area of $1mm^2$ in the most outer ring using the software ImageJ (National Institutes of Health, Bethesda, MD, USA) and obtained vessel density (VD).

To understand the isotopic variation due to defoliation and elevated CO_2, The biggest stem samples from *Terminalia catappa*, *Brachychiton acerifolius* and *Phaleria clarodendron* from both control (*n*=2 per species) and short-elevated CO_2 (*n*=2-3 per species × defoliation) were selected for high-resolution stable isotope analyses. This allowed us to better understand the isotopic variation in relation to growth, providing some insight on the incorporation of carbon into growth. We then sampled the whole radius of the stem cross section in 0.2mm resolution using a GSL1 microtome (Gärtner, Lucchinetti & Schweingruber 2014). We calculated the proportion of growth for each radius and pinpointed the approximate date in the proportional growth at which defoliation took place, which allowed us to check the possible variation in the stable isotopes due to new leaves production. The wood samples were then analysed for $\delta^{13}C$ using a Costech Elemental Analyser with a zero-blank auto-sampler coupled via a ConFloIV to a ThermoFinnigan Delta V PLUS Isotope Ratio Mass Spectrometry (EA-IRMS) at the Advanced Analytical Centre at James Cook University in Cairns, Australia.

Statistical analyses

Differences in nutrients and isotopes between young and mature leaves regarding phenology

To test for differences in δ^{13}C and δ^{15}N values between young and mature leaves in relation to phenology as well as treatment, we used Kruskal-Wallis test, with phenology factor and/or treatment as the grouping, followed by post hoc Dunn's test for pairwise comparisons. The variation in δ^{13}C and in relation to leaf stage and phenology, treatment and defoliation was assessed through a linear model with δ^{13}C as the response variable and treatment, defoliation, stage and phenology as explanatory variables.

We assessed the high-resolution δ^{13}C variation through time, using the short-elevated CO_2 treatment as a label for the treatment period. We assessed the time 0 of treatment, the day of defoliation, using stem diameter measurements combined with a segmented analysis in a Bayesian framework. The significant change in slope due to change in CO_2 concentration was then considered day 0 of treatment. We then assessed changes in δ^{13}C through proportion of the experiment period with linear and segmented linear regressions. We compared defoliated and non-defoliated individuals of *Brachychiton*, *Phaleria* and *Terminalia*, comparing their regression shapes and slopes, which provided us with information on how fast new CO_2 was incorporated into the wood. To test for significant differences between non-defoliated individuals at species level in elevated CO_2, we used a linear model with δ^{13}C as response variable with time and species as an interaction for the explanatory variable. We conducted one linear model for non-defoliated individuals applying a segmented regression and one linear model for defoliated individuals.

Growth and leaf expansion

To compare the time until the production of first flushes after defoliation, we used chi-square test with proportion of individuals first flushing in each week comparing amongst phenology and treatment.

To analyse the effect of treatment and defoliation on growth in relation to deciduousness, we tested the differences between the final and initial stem diameter measure, comparing between phenology, treatment and defoliation using Kruskal-Wallis test, followed by post hoc Dunn's test. We then built generalized linear models (GLM) with %Volume (percentage of volume difference between the last and first measurement) as the response variable and phenology and treatment as the explanatory factors.

To analyse the effect of deciduousness and treatment on the leaf expansion, our approach consisted in a polynomial linear regression with growth rate as the response variable and time, proportion of expansion, leaf phenology and treatment as the independent variables. The effect of phenology and elevated CO_2 on vessel density was assessed through Kruskal-Wallis test, followed by a Dunn's post hoc test, with vessel density as the response variable and phenology and treatment as the grouping factors.

Results

Experiment conditions

A summary of conditions in each chamber is presented in Fig. 1. The temperature in the chambers was set to track ambient based on a temperature sensor outside the glasshouse. Mean values and standard deviations are shown for day (from 7a.m. to 6p.m.) and night (from 6p.m. to 7a.m.) periods for temperature and relative humidity. The CO_2 concentrations in the three chambers throughout the course of the experiment are shown in Fig. 7.2 and Table 7.1.

Table 7.1. Air $\delta^{13}C$ (δ_a) in each treatment throughout the course of the experiment.

Treatment	Month	δa
Control May	May	-8.80
Control June	June	-8.87
Control October	October	-7.40
Short-elevated CO_2 before	May	-7.10
Short-elevated CO_2 before	June	-6.58
Short-elevated CO_2 after	October	-21.53
Long-elevated CO_2 before	May	-9.30
Long-elevated CO_2 after	June	-17.41
Long-elevated CO_2 after	October	-19.29

Nutritional and isotopic differences between deciduous and evergreen species

When analysing $\delta^{13}C$ from just flushed young leaves in relation to phenology, treatment and defoliation, our results showed a significant effect of the interaction of the three terms in both $\delta^{13}C$ and $\delta^{15}N$ (Kruskal-Wallis, $P<0.05$). Under non-elevated CO_2 conditions, defoliated deciduous and evergreen did not significantly differ, but non-defoliated deciduous had significantly less negative $\delta^{13}C$. Defoliation did not significantly change $\delta^{13}C$ values in deciduous species but had a significant effect on evergreen species, with non-defoliated individuals showing significantly more negative $\delta^{13}C$ values than defoliated individuals. Under short-elevated CO_2, deciduous and evergreen species did not show significant changes within defoliation, but between defoliated and non-defoliated individuals, with both deciduous and evergreen presenting less negative $\delta^{13}C$ values in defoliated individuals than in the non-defoliated individuals (Fig. 7.3, Table 7.2). Under the long-elevated CO_2 treatment, however, defoliation only significantly affected evergreen $\delta^{13}C$ values, with defoliated evergreen individuals having less negative $\delta^{13}C$ than non-defoliated individuals (Fig. 7.3, Table 7.2).

Changes between young and mature leaves

Deciduous and evergreen individuals presented a divergent directional difference when comparing their young and mature leaves, however this difference was not significant. Young leaves of deciduous species showed less negative $\delta^{13}C$ while in evergreen species, young leaves had more negative $\delta^{13}C$ (Fig. 7.2, Table 7.3). Comparisons within each treatment showed that significant differences between young and mature leaves of defoliated individuals were found only for evergreen species under ambient conditions, but for both evergreen and deciduous under short-elevated CO_2 (Table 7.3). Under long-elevated CO_2, only defoliated evergreen species presented a significant difference between young and mature (Table 7.3).

Leaf growth

There was a statistically significant interaction between the effects of CO_2 treatment and phenology on flushing. Deciduous species in elevated CO_2 had all individuals flushed within the first 4 weeks, while evergreen in elevated CO_2 flushed until week 6. When testing effect of treatment, leaf pattern on growth rate in relation to proportion of leaf expansion, significant differences were found in the shape of relation between deciduous and evergreen ($\chi^2=31.11$, $P<0.0001$), the interaction between proportion of expansion and leaf pattern ($\chi^2=34.77$, $P<0.0001$) and the interaction between leaf pattern, treatment and species ($\chi^2=15.83$, $P<0.05$) (Fig. 7.7). Elevated CO_2, on the other hand, was found to increase LMA in evergreen species. We analysed the effect of the CO_2 treatment on LMA in relation to leaf pattern in mature leaves. Elevated CO_2 significantly affected deciduous species, but did not differ amongst deciduous in the control and evergreen species. LMA was significantly higher only in evergreen trees in the elevated CO_2, but not different in the others (Fig. 7.8).

Total growth

The CO_2 treatment did not affect individuals differently in relation to leaf phenology. For non-defoliated individuals, the mean diameter was 0.269 ± 0.057 and 0.392 ± 0.062 in short- and long-elevated CO_2, respectively. For deciduous species, mean diameter was 0.617 ± 0.05 and 0.627 ± 0.051 for short- and long-elevated CO_2, respectively. Significant differences were found only between defoliation and non-defoliated individuals (ANOVA, $P<0.001$), but not in relation to phenology and treatment.

Wood anatomical traits and stable isotopes

Evergreen species presented higher vessel density than deciduous in the control but not under elevate CO_2 (Fig. 7.8). Elevated CO_2 did not affect vessel density in deciduous species, but decreased vessel density in evergreen species (Fig. 7.8).

Using the elevated CO_2 as a label to δ^{13}C variation in the wood throughout the growth it was possible to see that patterns of δ^{13}C throughout experiment time were more similar between *Brachychiton* and *Terminalia* than *Phaleria*. Defoliated individuals showed a linear relationship between δ^{13}C and time, whilst non-defoliated individuals presented a two-segment linear regression (Fig. 7.4, Table 7.4, Table 7.5). *Phaleria* showed a steeper first segment and a later break point than *Brachichyton* and *Terminalia* (Fig. 7.4, Table 7.4, Table 7.5). When testing for differences between species under elevated CO_2, the linear mixed models with polynomial adjustment indicated that *Phaleria* was the significantly different species (t-value= 2.17, $P<0.05$).

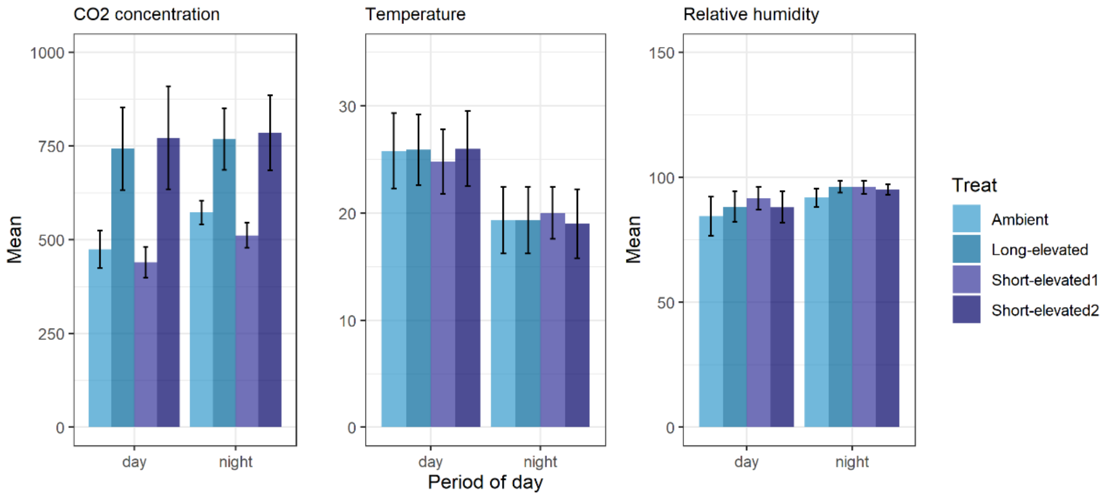

Fig. 7.1. Environmental conditions of the glasshouse during the experiment period: CO_2 concentration (ppm), Temperature (°C), and Relative humidity in the ambient treatment, short-elevated 1 (before set to elevated CO_2), short-elevated 2 (after set to elevated CO_2) and long-elevated treatment.

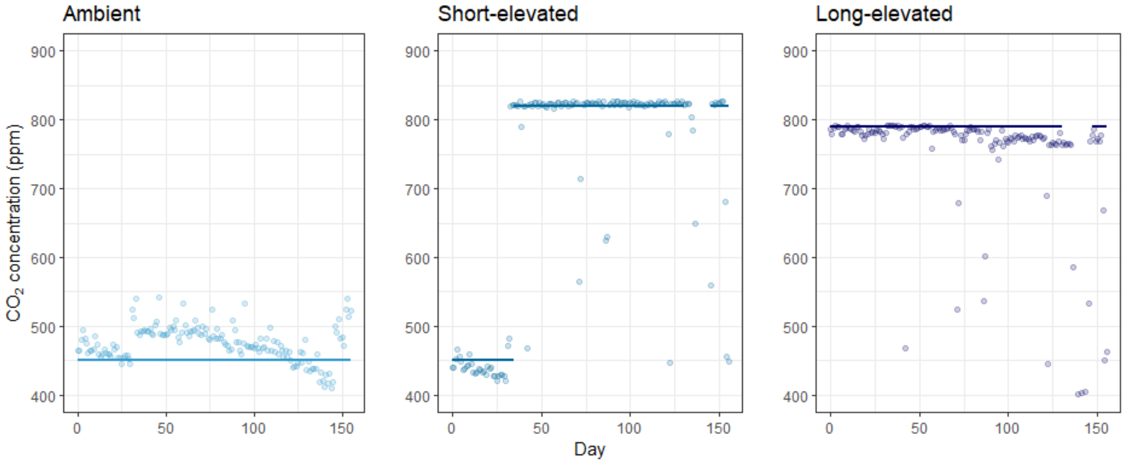

Fig. 7.2. Time series of mean daily CO_2 concentration (ppm) in each treatment (ambient CO_2, short-elevated CO_2 and long-elevated CO_2) for the whole period of the experiment.

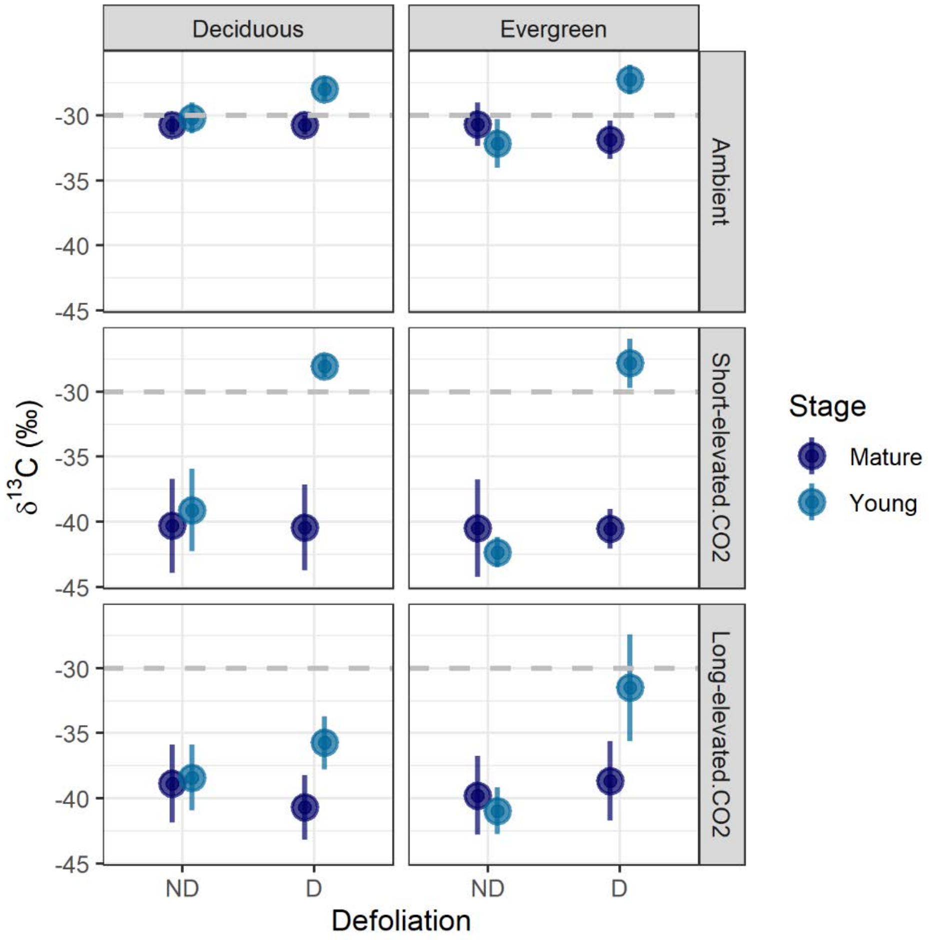

Fig. 7.3. δ^{13}C values from just flushed leaves collected in control, short- elevated CO_2 and long-elevated CO_2, showing the differences between leaf phenology (deciduous *vs*. evergreen) and defoliation status.

Table 7.2. δ^{13}C and δ^{15}N values in young leaves from defoliated and non-defoliated individuals grown at different CO$_2$ concentration. Significant differences between treatments were determined by the Kruskal–Wallis test (chi-square presented), separated by Dunn's test ($P < 0.05$) and indicated by different letters. Asterisks (***) indicate P<0.0001.

	Ambient				Short-elevated CO$_2$				Long-elevated CO$_2$				
	Defoliated		Non-defoliated		Defoliated		Non-defoliated		Defoliated		Non-defoliated		
	Deciduous	Evergreen	Deciduous	Evergreen	Deciduous	Evergreen	Deciduous	Evergreen	Deciduous	Evergreen	Deciduous	Evergreen	χ^2
n	15	13	14	13	15	12	15	12	13	14	14	12	
δ^{13}C	-27.85±(0.93) ^{ab}	-27.15±(1.30)^{a}	-29.47±(1.67)^{abc}	-31.61±(2.12)^{cd}	-28.12±(0.91)^{abc}	-27.46±(1.53)^{a}	-39.38±(3.00)^{e}	-40.21±(4.65)^{e}	-35.23±(2.17)^{de}	-31.38±(4.25)^{bcd}	-38.36±(2.62)^{e}	-41.07±(1.65)^{e}	129.34 (***)

Table 7.3. Outputs of Kruskal-Wallis test followed by post hoc Dunn’s test, assessing effects of treatment, defoliation, stage and phenology on δ^{13}C of young and mature leaves in each treatment.

	Evergreen				Deciduous			
	Defoliated		Non-defoliated		Defoliated		Non-defoliated	
	Young	Mature	Young	Mature	Young	Mature	Young	Mature
Ambient	27.24 ± 1.05^{c}	31.88 ± 1.38^{b}	32.18 ± 1.78^{b}	30.69 ± 1.59^{ab}	27.96 ± 0.83^{ac}	30.74 ± 0.66^{ab}	30.20 ± 1.07^{abc}	30.75 ± 0.63^{ab}
Short-elevated	27.82 ± 1.77^{b}	40.53 ± 1.44^{a}	42.33 ± 1.08^{a}	40.5 ± 3.47^{a}	28.02 ± 0.8^{b}	40.43 ± 3.13^{a}	39.08 ± 3.01^{a}	40.31 ± 3.42^{a}
Long-elevated	31.5 ± 3.93^{b}	38.67 ± 2.9^{a}	40.96 ± 1.7^{a}	39.79 ± 2.86^{a}	35.73 ± 1.67^{ab}	40.71 ± 2.01^{a}	38.41 ± 2.26^{ab}	38.86 ± 2.9^{a}

Fig. 7.4. Fitted segmented regression in defoliated (blue) and linear regression in non-defoliated (red line) individuals of each species showing relationship between high-resolution variation in δ^{13}C and experiment time, from the defoliation day (0) until the harvest day (1).

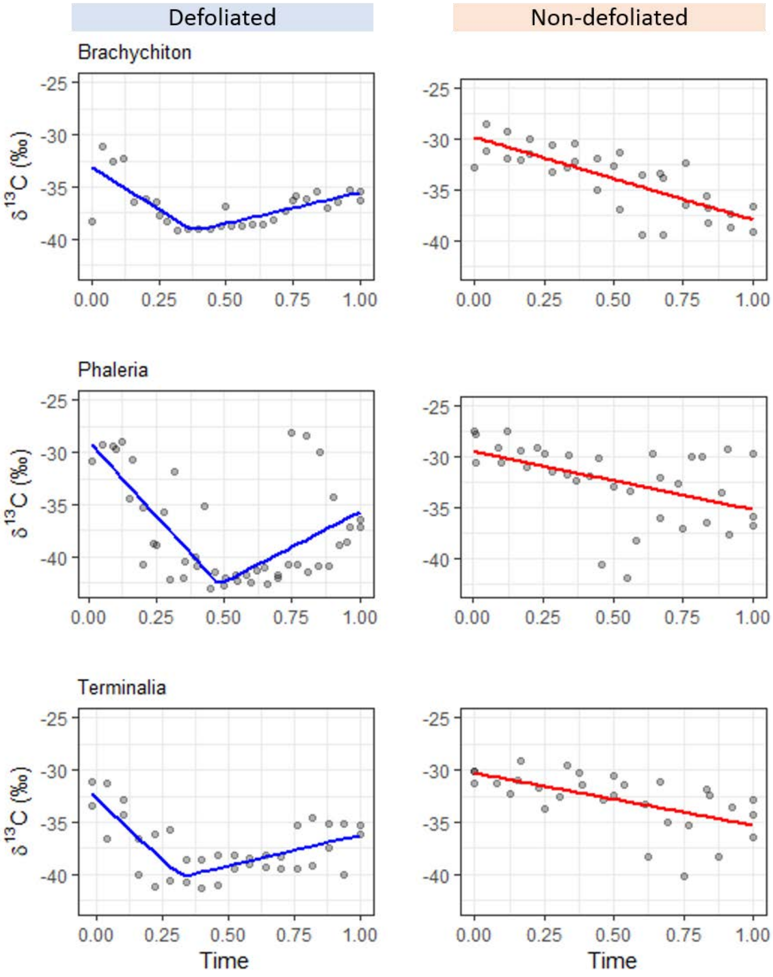

Table 7.4. Outputs from analysis of the two-segment linear regression and linear regression models for each species within defoliated and non-defoliated groups.

Species x Defoliation	Regression[a]	S1δ^{13}C [b] or Sδ^{13}C [b] (‰)	S.E.	C.I.	Y-Intercept[c]	S.E.	δ^{13}C BP[d]	S.E.	C.I.	S2TR [e]	S.E.	R^2
Brachychiton non-defoliated	Segmented	-16.09	4.01	-23.95 _ -8.23	-33.11	0.87	0.33	0.04	0.25-0.41	21.98	4.39	0.55
Phaleria non-defoliated	Segmented	-28.44	5.95	-40.10_ -16.78	-28.97	1.704	0.47	0.05	0.37-0.57	41.93	7.57	0.48
Terminalia non-defoliated	Segmented	-23.17	5.36	-33.68_ -12.66	-32.80	0.887	0.36	0.05	0.26-0.46	29.1	5.68	0.56
Brachychiton defoliated	Linear	-8.15	1.17	-10.44 _ -5.86	-29.85	0.685	_	_	_	_	_	0.62
Phaleria defoliated	Linear	-5.71	1.8	-9.24 _ -2.18	-29.54	1.064	_	_	_	_	_	0.23
Terminalia defoliated	Linear	-4.98	1.26	-7.45 _ -2.51	-30.33	0.747	_	_	_	_	_	0.36

[a] The type of the regression fit for the response.

[b] The first slope of the segmented regression or the slope of the linear response, respectively.

[c] The theoretical δ^{13}C when Time is null.

[d] The Time threshold at which a breakpoint (BP) in the δ^{13}C response to Time is observed.

[e] The second slope of the segmented regression.

Table 7.5. Results of linear models analysing the relationship between δ^{13}C and time × species, with segmented regression fitted for non-defoliated individuals and simple linear regression fitted for defoliated individuals.

	Parameter	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Non-defoliated	Time	1	236	236	45.5814	**<0.0001**
	Species	2	36.32	18.16	3.5073	**<0.05**
	U1.Time	1	556.11	556.11	107.4085	**<0.0001**
	psi1.Time	1	0.94	0.94	0.1808	0.672
	Time:Species	2	96.75	48.38	9.3435	**<0.0001**
	Parameter	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Defoliated	Time	1	350.06	350.06	51.4956	**<0.0001**
	Species	2	50.81	25.41	3.7375	**<0.05**
	Time:Species	2	15.83	7.92	1.1645	0.31667

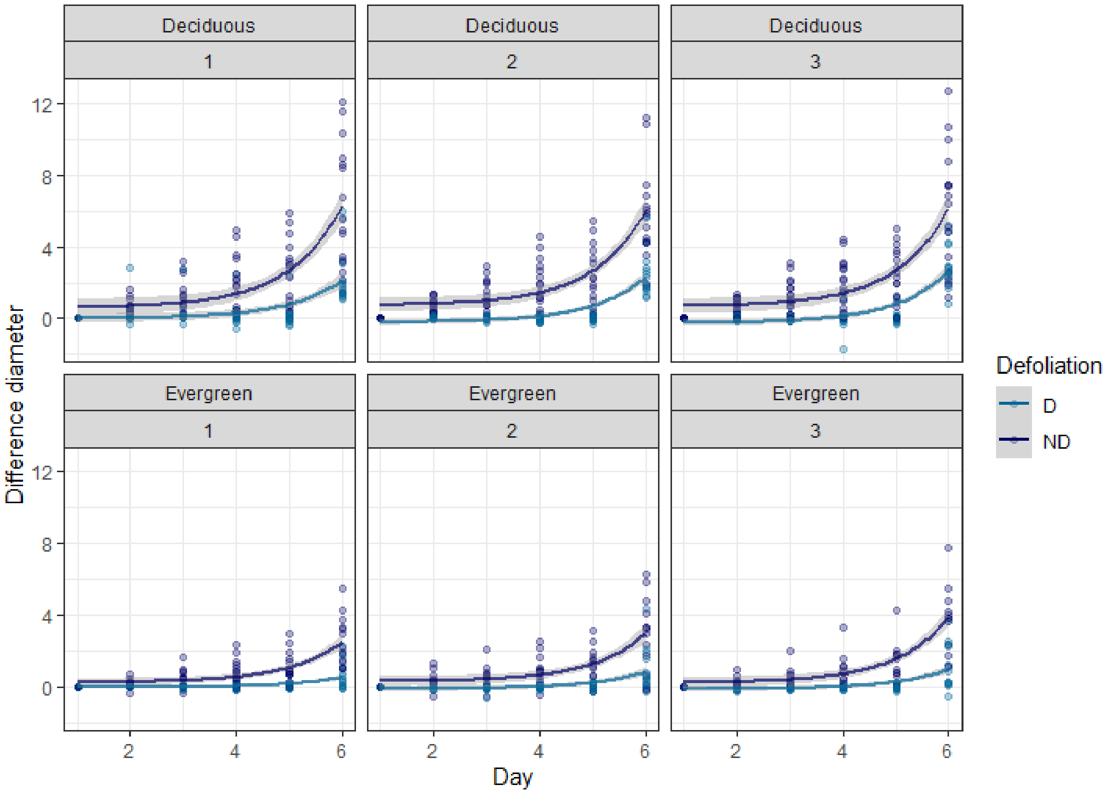

Fig. 7.5. Diameter growth against each measurement week. Measurements have been conducted fortnightly. Plot shows difference between deciduous and evergreen species, defoliated and non-defoliated individuals, in each treatment (ambient, short-elevated CO_2 and long-elevated CO_2). Significant difference was found between defoliated and non-defoliated in all treatments except long-term elevated CO_2 (3) for both evergreen and deciduous (Dunn's test, $P<0.05$).

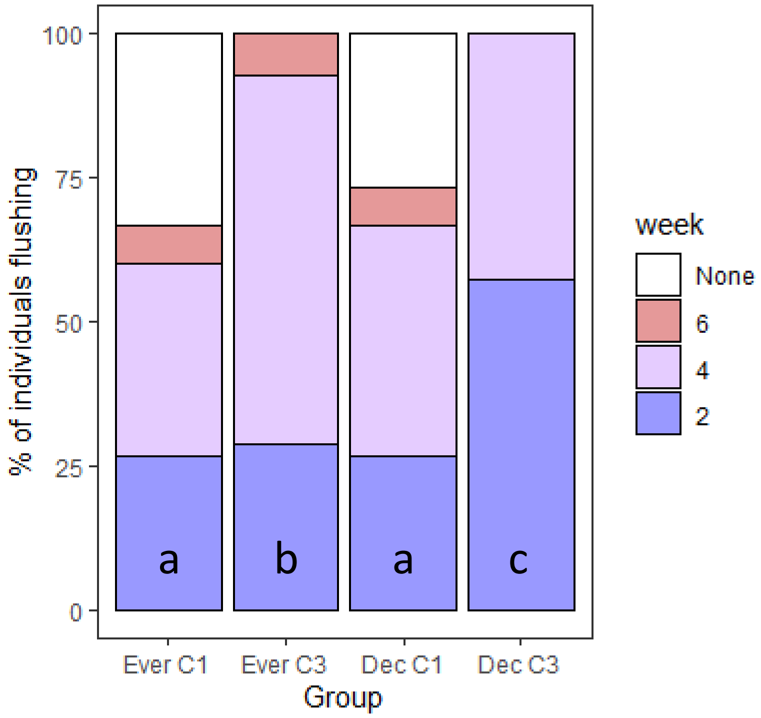

Fig. 7.6. Comparison of first leaf flushing activity in defoliated individuals within evergreen in control (Ever C1), evergreen in elevated CO_2 (Ever C3), deciduous in control (Dec C1) and deciduous in elevated CO_2 (Dec C3). Different letters represent significant differences (Chi-square, $p<0.05$).

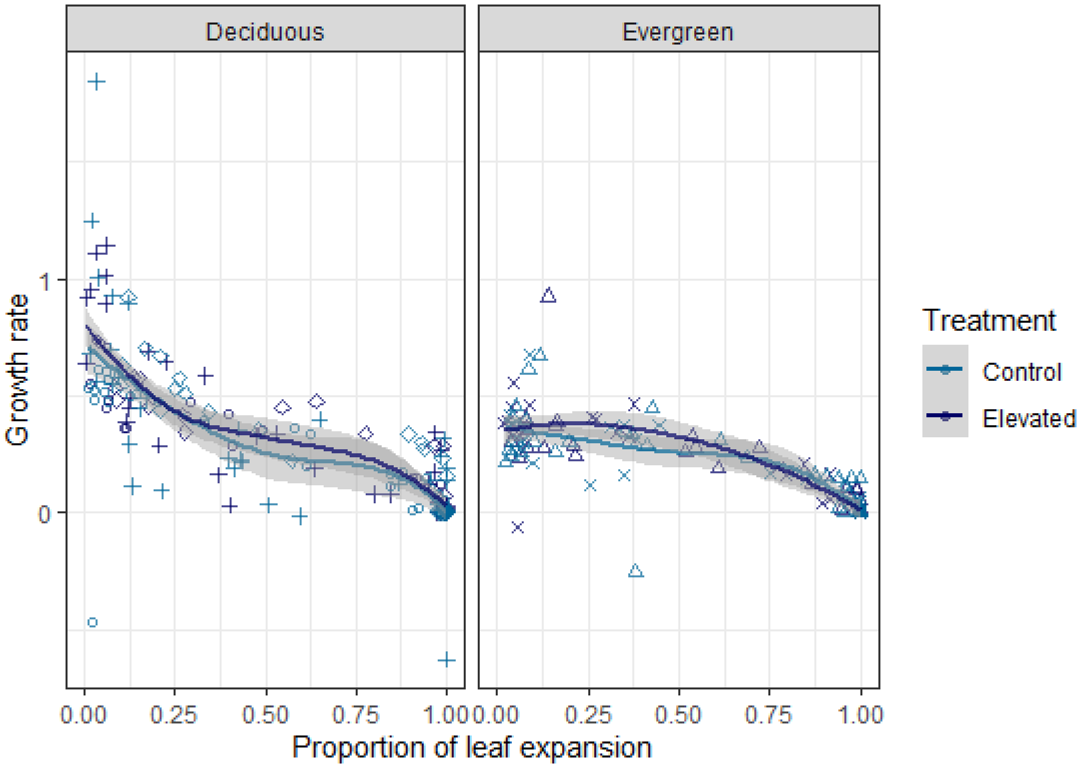

Fig. 7.7. Comparison of proportion of leaf expansion rate according to leaf phenology in control (light blue line) and elevated CO_2 (dark blue line). Significant differences were found in the shape of relation between deciduous and evergreen (χ^2=31.11, *P*<0.0001), the interaction between proportion of expansion and leaf pattern (χ^2=34.77, *P*<0.0001) and the interaction between leaf pattern, treatment and species (χ^2=15.83, *P*<0.05).

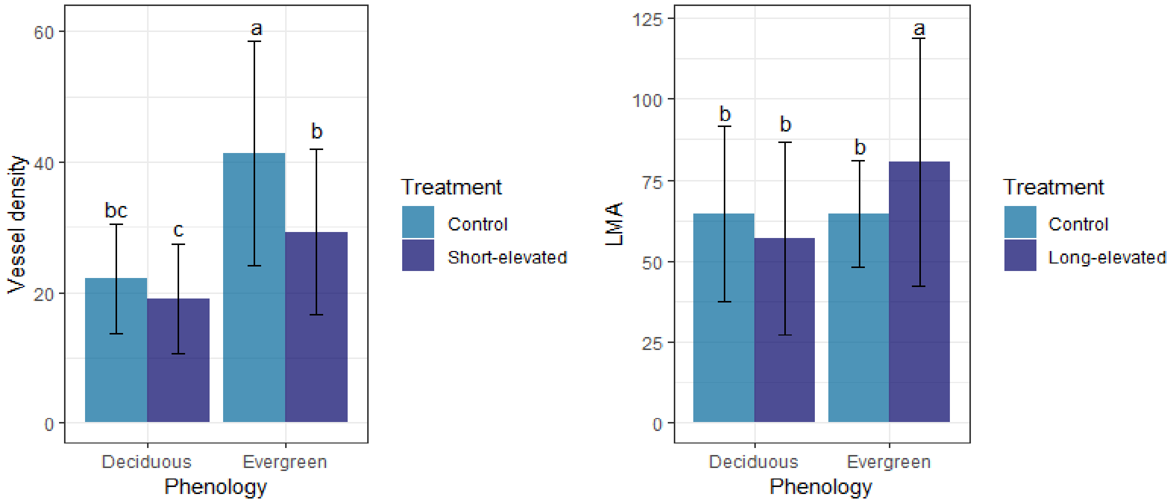

Fig. 7.8. a) Vessel density and b) Leaf mas per area (cm^2/kg^{-1}) in each phenological group and treatment. Different letters indicate significant differences as tested by Dunn`s test (*P*<0.05) and Tukey's test (*P*<0.05).

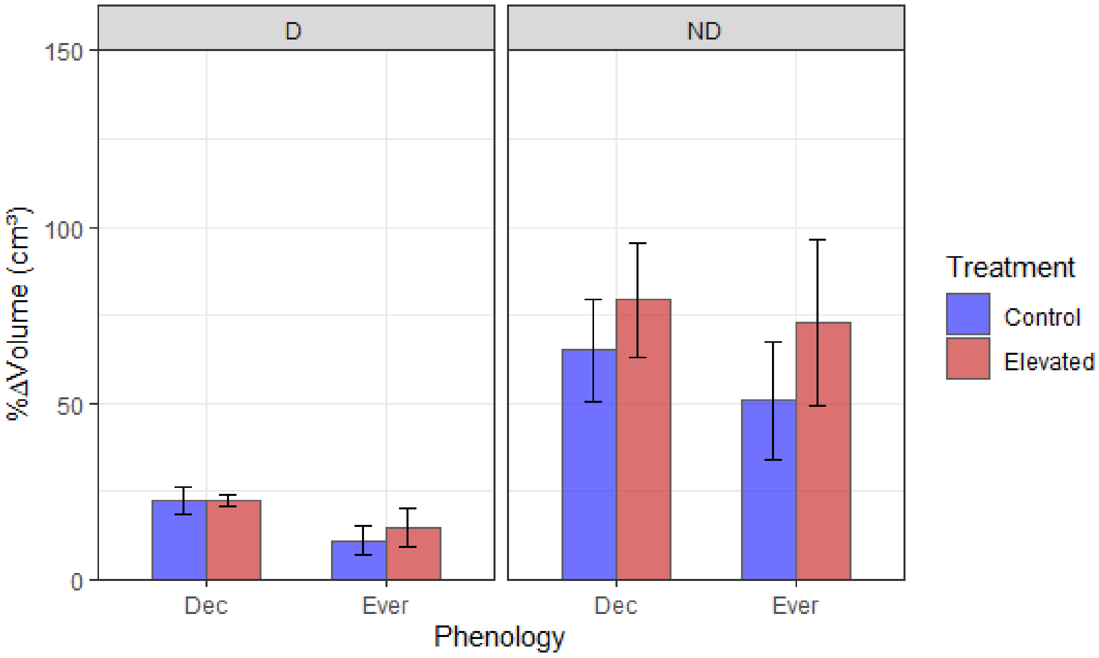

Fig. S7.1. Percentage of volume (cm³) difference of species between first and last measurement in each treatment (chamber 1 – control and chamber 3 – elevated CO_2), in relation to leaf phenology.

Discussion

Deciduous and evergreen presented differences in the δ^{13}C of young leaves, pattern of incorporating ambient CO_2 into the wood and anatomical traits. Under elevated CO_2, both groups were similarly affected, presenting variation in carbon source and increase in growth in the defoliated individuals. Changes in leaf expansion and traits, as well as decrease in vessel production, depended on the leafing pattern.

Leaf stable isotopes

Leafing patterns in tropical forests are influenced by different environmental factors (temperature, relative humidity, soil moisture), as well as intrinsic factors such as species traits. Because tropical trees are sensitive to small environmental changes, shifts in leaf physio-phenology may occur amongst species (Singh & Kushwaha, 2016), and deciduousness may have an important influence on that. These changes affect the use of reserves and may cause trade-offs in species due to their different capacity of adaptation to them. The δ^{13}C of just flushed leaves may indicate the source of carbon used in leaf production, which provides us with some clues on how defoliation may affect carbon balance in the forests. Because leaf phenology is highly influenced by leaf age and physiology (Caldararu *et al*., 2014), it becomes essential to better understand how phenology affects water and carbon cycles, and vice-versa. Evergreen and deciduous species face different challenges related to optimizing physiological processes in relation to leaf production, with deciduous species being more adapted to leaf loss and canopy reconstruction than evergreen species. Because of that, different use of carbon source and shifts in leaf ontogeny may cause varied effects in carbon balance and growth of trees with different leafing patterns. This different use of carbon, however, may not vary as much in seedlings as compared to adult trees, since seedlings often do not show deciduous behaviour. Therefore, our results may show a less intense pattern in comparison to the ones found in mature trees.

In deciduous species, where the carbon source for leaf flushing cannot be from contemporaneous mature leaf photosynthesis, the $\delta^{13}C$ of new leaf flushes has been found to tend toward enrichment, presumably due to fractionation associated with movement into and out of storage and translocation in the stem. Therefore, $\delta^{13}C$ value would be less negative than the carbon received from mature source leaves (discussed in Cernusak *et al.,* 2009). Additionally, deciduous species may add more of the leaf's own photosynthesis during leaf expansion than evergreens, as mature source leaves exporting carbon are absent when new leaves are being built.

By defoliating evergreen and deciduous seedlings under different atmospheric CO_2 concentrations, we confirmed that these groups utilised different carbon sources in the production of new leaves. Deciduous species under ambient CO_2 concentrations showed no difference in $\delta^{13}C$ ratios from the recently flushed young leaves when comparing between defoliated and non-defoliated individuals, indicating that stored carbon was used to build the new leaves. Alternatively, evergreen species showed a more substantial difference in $\delta^{13}C$ values from recently flushed young leaves between defoliated and non-defoliated individuals. This suggests that defoliated plants require stored carbon to produce new leaves, whereas non-defoliated plants preferred contemporaneously assimilated carbon from mature leaves. There is likely a translocation cost to using stored carbon, so it is more efficient to use newly fixed carbon for leaf growth in the case of non-defoliated evergreen species. This result also suggests an innate use of stored carbon for leaf production that is independent of defoliation status in deciduous species.

By defoliating seedlings on the same day as the atmospheric carbon concentration was changed (short-elevated CO_2), we were able to demonstrate that for both deciduous and evergreen species, stored carbon was required to construct the new leaves, and $\delta^{13}C$ reflected only carbon fixed prior to the defoliation event. Conversely, in non-defoliated plants, the carbon used to construct new leaves came from the contemporaneous photosynthesis of other mature leaves, a difference that was accentuated in the evergreen species. New leaves were sampled early, such that there was no contribution to the leaf's carbon from its own photosynthesis and therefore carbon source could be properly assessed. The mature leaves for defoliated plants that have flushed in the elevated CO_2 label reflect entirely, or almost entirely, the CO_2 fixed during its own photosynthesis as it expanded or current photosynthate exported by the other mature leaves on the plant.

Under long-elevated CO_2, emerging leaves on defoliated plants reflected an influence on their storage carbon pool of the experimental elevated CO_2 label, as seen by comparing young leaves in control and short-elevated CO_2. Additionally, the evergreen species turned over their stored carbon pool or added to it less in the one month of elevated CO_2 prior to defoliation than the deciduous species. The results from the long-elevated CO_2 treatment also show that the emerging leaves on undefoliated plants reflect completely current photosynthate from other mature leaves and not stored carbon.

Although not statistically significant, deciduous mature leaves presented a more negative $\delta^{13}C$ while evergreen species presented a less negative, contrasting to previous results on differences between young and mature leaves (Vogado *et al.*, 2020). The results obtained in long-elevated CO_2 showed that stored carbon in deciduous species mostly comprises carbon fixed in the previous month. On the other hand, stored carbon in evergreen species mostly comprises carbon fixed more than a month previously, supporting the previous discussed pattern. These results are supported by the differences between young and mature leaves presented in Table 3. In the non-defoliated individuals, young leaves of evergreen species had a more negative $\delta^{13}C$ than mature leaves, while for deciduous species the young leaves had less negative $\delta^{13}C$ than mature leaves, supporting the idea that deciduous species mostly use stored than current carbon.

Combining the leaf values of $\delta^{13}C$ with high-resolution $\delta^{13}C$ from the wood we can obtain a deeper understanding of how leaf pattern may differ in carbon use. The non-defoliated deciduous species, *Brachychiton* and *Terminalia*, showed a similar flatter regression between them, but different than the one found for *Phaleria*, the evergreen species. The steeper slope in *Phaleria* under elevated CO_2 suggests a higher incorporation of current photosynthates being assimilated, while the deciduous species seem to mix the stored carbon with the current one, presenting a less steep line. For the three species, however, the defoliated individuals presented similar regression lines, showing the common use of stored carbon in all of them due to defoliation.

The difference in the carbon source has important ecological implications when we consider that defoliation events are expected to increase due to climate change (Carnicer *et al*., 2011; Anderegg *et al*., 2013) and consequent more often and more intense droughts, fires and phenological mismatches between leaf production and herbivores activity are expected to occur (Kozlov, 2008; Richardson *et al*., 2013). The fact that deciduous species mostly use stored carbon to produce new leaves show that these species are more adapted to deal with defoliation events, since more used to reconstructing a canopy, whereas the same is not true for evergreen species. Conversely, stored carbon in evergreen species could be used for the production of fruit and flowers instead of vegetative outputs, and therefore tradeoffs may occur with reproduction and/or growth (Miyazaki, 2015; Ichie *et al*., 2005). Previous studies have found that evergreen and deciduous species differ in non-structural carbon (NSC), with evergreen having less NSC in the stem than deciduous but more in branches and leaves

(Fajardo, 2013). One implication is that more defoliation events may disadvantage evergreen species, possibly causing changes to floristic structure in the future and resource availability for frugivores. Another important implication is that while evergreens would be expected to be photosynthesizing, after a defoliation they would mostly respire due to leaf age as young leaves do not have yet fully phosotynthetic capacity (discussed in Vogado *et al.*, 2020), releasing carbon instead of uptaking it.

Leafing and growth activity

Deciduous species were expected to have higher capacity of recovering from defoliation as they are more adapted to leaf shedding and canopy reconstruction. Evergreen species, on the other hand, were expected to suffer more to reconstruct the canopy, having a trade-off between growth and leaf production. After defoliation, deciduous species were the fastest to produce the first flushes in long-elevated CO_2, followed by evergreen individuals in long-elevated CO_2. Although deciduous trees have been found to have an early flushing in different countries such as Thailand (Ishida *et al.*, 2010), India (Singh and Kushwaha, 2005), Java (Rivera *et al.*, 2002), Australia (Williams *et al.*, 1997), Brazil (Rivera *et al.*, 2002) and Costa Rica (Rivera *et al.*, 2002; Gutiérrez-Soto *et al.*, 2008), no significant difference was found in the control between deciduous and evergreen in our study. On the other hand, elevated CO_2 aided deciduous species in a more robust way than evergreen species.

Although the first flushes after defoliation did not change between leaf phenology, evergreen species have been found to take longer to build their leaves, explained by the necessity to build leaves with more defense compounds and more structure to survive longer in the tree (thicker leaves) (Eamus, 1999). On the other hand, deciduous species would be expected to grow faster in order to begin photosynthetic activity earlier. These expectations have been supported by our results, which showed significant differences in the pattern of leaf growth between deciduous and evergreen, but not effect of the treatment on them. Evergreen species showed a more constant growth pattern than deciduous species, which grew leaves faster in the beginning of leaf expansion. The differences in first flushing and leaf expansion were not translated into changes in total plant growth (volume). In addition, differences in growth may have presented species-specific variation due to differences in wood density. For example, faster growing species have been found to be more capable to recover from defoliation, showing the important role that growth strategies play in canopy recovery (Atkinson *et al*., 2014).

The effects of growth suppression and carbon allocation due to defoliation could also be different when trees have reached maturity and need to use their stored carbon to produce both vegetative and reproductive outputs. The capacity of leaf flushing earlier in elevated CO_2 found in deciduous species did not translate into a higher growth in height and diameter. However, deciduous species in elevated CO_2 did present an increase in leaf area per leaf, while evergreen individuals presented similar area between treatments but higher LMA. It seems that deciduous species could use the higher water use efficiency to increase NSC investing in reserves, since growth and leaf expansion were not affected, while evergreen species increase LMA, investing in leaf defences and longevity. Thicker leaves are less vulnerable to herbivores, desiccation, amongst others. The increase in CO_2, therefore, might aid evergreen species in constructing more protected leaves that could be more resistant to drought/herbivory events. Since evergreen trees need a high investment of carbon to build new leaves, a long leaf life-span gives the advantage of trees to be able to quickly start carbon assimilation in more favourable moments instead of having to construct leaves that stay for a while with low photosynthetic capacity (Ishida *et al*., 2010). However, the drought signal also increases the concentration of abscisic acid and although tougher, leaves from evergreen species are still expected to suffer and even be dropped due to drier conditions. The increase in leaf defenses will be more useful for the increase in herbivory protecting more evergreens than deciduous. It is important, however, to additionally consider the concurrent and synergistic effects of elevated CO2 and higher temperatures, lower rainfall and humidity, as they are commonly found and expected in the tropics. These interactions between the environmental variables can intensify or change the effects of climate change on physiological processes. Nevertheless, our study shows that evergreen and deciduous might respond differently to climate change and differences in leaf phenology are important to more correctly understand and predict ecological and metereological changes.

Wood anatomy

The species responded differently to elevated CO_2. Deciduous species had higher vessel density than evergreen species, and were not affected by elevated CO_2, while evergreen had a decrease in vessel density. We were not able to find previous studies that assessed effects of elevated CO_2 on vessel density of tropical tree species. In temperate species, no significant change was found in vessel density under elevated CO_2 for most of the species studied (Watanabe *et al.,* 2010; Domec *et al*., 2017; Rhea, 2010; Kostiainen *et al*., 2014). Atwell *et al*. (2007) studying *Eucalyptus teriticornis,* however, found that vessel number (% of stem occupied by vessels) was generally larger under elevated CO_2 although not significant. Contrasting to our results, Lotfiomran *et al*. (2015) studying European beech (*Fagus sylvatica*) found that elevated CO_2 led to an increase in total vessel lumen area (TVLA) and vessel density (VD). Our results suggest that effects of elevated CO_2 on wood anatomical features from tropical species are different from temperate species and more research is needed to improve our understanding of climate change effects on tropical rainforests.

Overall, our findings suggest that physiological processes are influenced by leaf phenology, and trees might respond differently to climate change depending on their capacity to adapt to increasing disturbance events such as defoliation. For example, we found that the capacity to recover from leaf loss was influenced by leaf phenology, as evergreen and deciduous species seem to have different carbon sources to produce new leaves as well as different leaf costs, but when defoliated, both relied on stored reserves. Additionally, they might benefit differently from elevated CO_2, with evergreen species investing in leaf defence traits while deciduous species may invest more in short-term benefits such as faster growth. Therefore, it is important to understand the impact of leaf phenological patterns on tree eco-physiological processes an vice-versa, to better predict how climate change will affect tropical rainforest in all different levels.

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Chapter Eight: General conclusions

The aim of this PhD thesis was to explore the influence of climate and climate change on phenological processes in tree species from tropical rainforests of Oceania and Southeast Asia through literature review and experiments both *in-situ* and in glasshouse. I assessed the climate drivers, and response to changing climate (drought), of reproductive phenological patterns, as well as their association with physiological processes. Additionally, I assessed how leaf phenology influenced carbon use and recovery from defoliation, and how species with different leafing strategies responded to elevated CO_2. Specifically, this PhD thesis aimed to 1) assess long-term reproductive phenology patterns (>8 years) and how they respond to climate drivers and experimental drought, 2) test how changes in environmental conditions (drought) affected physiological processes, 3) analyse how different resilience of species related to changes in phenological responses, 4) explore how different vegetative phenology depend on physiological processes, 5) test how leaf phenology impacts recovery from defoliation and how leafing patterns influence trees responses to elevated CO_2.

When assessing the phenological patterns of the Wet Tropical species, I found phenological activity was seasonal, influenced by climate variables, and sensitive to drought. Flowering activity of the community studied in the Daintree rainforest was mainly driven by temperature and solar radiation (wet season), while fruiting was mainly influenced by photoperiod, rainfall, and mean temperature (dry season). Both community and individual species responded to drought by changing their phenological pattern, with either a delay in timing or an increase/decrease in activity. Although the community showed an increase in fruiting activity under drought, individual species had divergening responses dependent on their resilience and physiological capacity to adapt to water loss, and their use of carbon.

Species that showed higher acclimation capacity had increased reproductive activity when under drought, while the ones less able to acclimate had decreased reproductive activity. Acclimation strategies such as increased water use efficiency, access to deeper water, and use of stored carbon were some of the physiological changes found to underly phenological processes. Species with increased water use efficiency, and suggested capacity to reach water at deeper soil levels, such as *S. graveolens*, were able to increase flowering and fruiting activity under drought conditions. The species less able to acclimate, *C. myrianthus*, had decreased water use efficiency and phenological activity under the experimental drought.

The CO_2-defoliation experiment revealed an influence of leafing phenology on recovery from defoliation. Deciduous species were able to recover faster from defoliation, by having leaves that flushed earlier and expanded faster, while evergreen species seemed to invest more in long-term traits such as leaf thickness. Evergreen species showed a higher dependence on current photosynthates while deciduous species relied in stored carbon for growth and leaf production following defoliation. Elevated CO_2, however, did not seem to have impacted the groups differently and did not significantly increase capacity of recovery. Attention is needed, however, when interpreting and applying this result, as the experimental studies of defoliation and CO_2 responses were conducted on seedlings and the field-based phenology was conducted on adult trees *in-situ*. These results, however, are important to the understanding of the processes behind phenology.

The importance of these varied responses to climate is that climate variation will affect both reproductive and vegetative phenology of tropical tree species. Defoliation may occur due to extreme drought, massive herbivore attacks and/or fires, and affect carbon balance of trees. This will in turn affect carbon storage and uptake for the production of flowers, fruits, and leaves. Considering how inter-related these processes are, better understanding their responses to changing climate conditions becomes essential to improve the mechanistic understanding of tropical tree species. Inter-species variation in phenological responses, as well as growth, seem to be influenced by different carbon sources and varied physiological capacities to adapt to environmental changes. These capacities have been shown to be influenced by leafing patterns, leaf morphology, type of fruit (fleshy or dry), and other functional traits. The fact that each species respond differently demonstrates the importance of phenological patterns and changes both at community and population level, as well as the combination of phenological studies with assessment of functional traits and plant physiology.

It is expected that the increase in temperature and frequency of droughts will impose great challenges on plant species. The increase of CO_2, however, has been suggested to minimize negative impacts through fertilization and increases in water use efficiency. Therefore, although our understanding of plants responses to changes in environment has been increasing, the effects are mostly synergetic, and little has been tested in terms of varied effects from the combination of climate variables. The results from this PhD identify important responses to climate change at both the community and species level. The capacity of some species to adjust to environmental changes might lead to varied phenological responses, with important consequences for forest ecology and structure and, although elevated CO_2 was previously expected to reduce some of the negative impacts of climate change, the results from the experiment showed that this may not be true for all species.

In general, the results of this study show how physiology and carbon allocation are related to phenology, and how important it is to understand species' capacity for physiological adjustment while assessing long-term phenological processes**.** The varied responses to changes in environmental conditions indicate a possible vulnerability of tropical forests to maintain forest structure and diversity in face of climate change**.** This is tightly related to use of resources, and trade-offs between reproductive needs and growth.

The lack of understanding of leaf processes as well is of concern, as this study has shown how different leafing patterns may influence carbon use and, therefore, growth patterns in the context of defoliation. Defoliation rates in tropical forests may increase in the future, due to different environmental changes such as increased drought, fires and phenological mismatches, all predicted under climate change. As tropical rainforests are composed of different tree species with varied leafing and reproductive phenological patterns, as well as varied functional traits, these results suggest a myriad of responses to climate change that can alter not only resources availability, but also species distribution and forest structure.

The findings of this PhD contribute to an improvement in our understanding of phenological patterns in the Wet Tropics and the physiology processes behind them. However, as phenology has been found to be highly influenced by climate and extreme events, long-term studies assessing cyclone and ENSO events on phenology would be highly beneficial. Events such as El Niño increasing fruiting have been found in other tropical forests, and concomitantly the large demand place on carbon reserves for the mass flower and fruit production has been suggested to decrease resource allocation for growth afterwards. This indicates that an increase in frequency of ENSE events would lead to a tradeoff between reproduction and growth, and possibly negative impact on trees. Having a better understanding of how these events affect the different tropical forests would enhance predictions and mechanistic models of tropical species.

Concurrent studies of time-series physiological processes, growth patterns and phenological observations also would provide a more detailed understanding of phenophysiological responses to environmental changes. However, such studies require high levels of logistic organisation and funding. Finally, improving techniques of phenological reconstruction would provide us with great potential to understand long-term series and patterns in regions that have not been the subject of long-term monitoring. Improving the use of techniques such as dendrochronology and stable isotope analysis, as most species found in regions with low climate seasonality lack annual growth rings, would be highly desirable to enhance our understanding of past events and long-term patterns of responses to environmental conditions in tropical species.