Impact of myrtle rust (*Austropuccinia psidii*) infection on community composition and ecological function

Research Impact Report (PBSF038)

May 2023

AUTHOR

Kristy Stevenson, Geoff Pegg, Elizabeth Aitken, John Herbohn, Neal Menzies, Jennifer Firn

Copyright

The material in this publication is licensed under a Creative Commons Attribution 4.0 International license, with the exception of any:

- third party material;
- trade marks; and
- images and photographs.

Persons using all or part of this publication must include the following attribution:

© [University of Queensland] [2021].

Disclaimer

While care has been taken in the preparation of this publication, it is provided for general information only. It may not be accurate, complete or up-to-date. Persons rely upon this publication entirely at their own risk. Australian Plant Biosecurity Science Foundation and its members and stakeholders do not accept and expressly exclude, any liability (including liability for negligence, for any loss (howsoever caused), damage, injury, expense or cost) incurred by any person as a result of accessing, using or relying upon this publication, to the maximum extent permitted by law. No representation or warranty is made or given as to the currency, accuracy, reliability, merchantability, fitness for any purpose or completeness of this publication or any information which may appear on any linked websites, or in other linked information sources, and all such representations and warranties are excluded to the extent permitted by law.

Project Leader contact details

Name: Elizabeth Aitken
Address: The University of Queensland, St Lucia Qld 4072, Australia
P: +61 (0)7 3365 4775
E: e.aitken@uq.edu.au

Australian Plant Biosecurity Science Foundation
3/11 London Circuit, Canberra, ACT 2601

P: +61 (0)419992914
E: info@apbsf.org.au
www.apbsf.org.au

This document should be cited as:

Kristy Stevenson, Geoff Pegg, Elizabeth Aitken, John Herbohn, Neal Menzies, Jennifer Firn, 2022, APBSF Project Final Report, Impact of myrtle rust (*Austropuccinia psidii*) infection on community composition and ecological function

Contents

1. Executive Summary

Austropuccinia psidii, the causal agent of myrtle rust, is an introduced plant pathogen known to have caused significant declines in populations of several Australian native Myrtaceae species. However, limited research has focused on the broader impacts of this pathogen on plant communities after its' establishment. This study was conducted in a wet sclerophyll forest in southeast Queensland that has experienced high levels of tree mortality in the mid- and understorey canopy layers in the last decade due to repeated myrtle rust infection. Because of the advancement of the pathogens affects at this site, we aimed to investigate impacts of myrtle rust tree mortality on key ecosystem functions, notably nutrient cycling, and cross trophic level interactions (i.e., birds and plants). Our results indicate that the mid- and understory of the forest is experiencing a shift in species composition away from several highly susceptible Myrtaceae species toward a more diverse community of tolerant and non-host species, possibly aided by the recent high rainfall years. Several early colonisers from the Myrtaceae family at the site have been significantly reduced in population by repeated myrtle rust infection, creating a space for other native and non-native species that are present to fill this role in the future. The loss of key Myrtaceae species appears to be causing a shift in the dominant seed size and fruit type and has likely caused a general reduction in the availability of fruit, which may affect bird foraging behaviour. We observed no effect of tree mortality on species or functional richness when sampling during a la Nina high rainfall period. Variation in the measured factors associated with nutrient cycling was best explained by variation in topography and soil type. The weak correlation observed between measured factors associated with nutrient cycling and estimators of myrtle rust impact is a positive indicator that this ecosystem function may be relatively resilient to the impacts of the pathogen in this complex forest system at this time.

2. Introduction

Austropuccinia psidii, the causal agent of myrtle rust, poses a unique threat to Australian native forests. The fungal pathogen, native to South America, was first detected in Australia in 2010 (Carnegie et al., 2010). It has since established and spread along much of the east coast (Carnegie and Pegg, 2018). Hosts are in the Myrtaceae family (Glen et al., 2007), with over 480 confirmed host species (Soewarto et al., 2019). Species from this family are dominant in several of Australia's forest communities, including in sclerophyll forests and melaleuca forests (Groves, 1994; Myerscough, 1998). Several highly susceptible species have already experienced rapid population declines, including native guava (*Rhodomyrtus psidioides* (G.Don) Benth.) (Carnegie et al., 2016; Fensham and Radford-Smith, 2021). Research on the impacts of myrtle rust at the community level is limited

(Fernandez-Winzer et al., 2020), with no research so far on impacts of the pathogen on ecosystem functioning. Such research is crucial to develop management strategies for infected forest communities.

Invasive plant pathogens have the potential to modify the diversity of native forests in several ways. The first is direct, reducing, or eliminating populations of susceptible species from the community (Smith et al., 2009). The second is indirect, with pathogen-induced tree mortality causing changes in environmental factors, such as light, that affect the abundance of tolerant species (Mcdougall et al., 2005). Myrtle rust can affect species diversity in both ways (Fernandez-Winzer et al., 2020; Pegg et al., 2017). Changes in species diversity can also reflect changes in the types of 'functions' present in a forest patch such as forage, reproductive behaviour and growth patterns, otherwise known as functional diversity (Díaz and Cabido, 2001). For example, Davis *et al.* (2014) reported a reduction in flowering period and flower abundance in areas subject to cinnamon fungus (*Phytophthora cinnamomi*), with flow-on effects on the diversity and abundance of local nectarivore species. Possible impacts of myrtle rust on functional diversity have not been investigated but are essential to managing potential changes.

Invasive plant pathogens can influence the dynamics of nutrient cycling in native forests. Pathogen-induced tree mortality can modify local environmental conditions, such as canopy cover, a proxy for light availability (Diaz-Torres, 2020), that influence aspects of nutrient cycling, including rates of soil respiration, decomposition, and nitrification (Avila et al., 2016; Ávila et al., 2021; Preston et al., 2016). The duration of a pathogen outbreak affects which aspect of nutrient cycling is affected and to what extent (Borer et al., 2020; Hicke et al., 2012). The majority of field studies have been conducted decades after the establishment of the pathogen (Cobb et al., 2013; Lovett et al., 2010; Shearer et al., 2009), leaving uncertainty around the possible impacts in the early years (± ten years). Notable impacts of myrtle rust were first recorded in the native environment in the Tallebudgera Valley in 2014 (Carnegie et al., 2016). Sites in this region provide a unique opportunity to investigate how invasive pathogens influence the cycling of nutrients in forests during the early stages of an outbreak.

3. Aims

1) To investigate if myrtle rust tree mortality is affecting species and functional richness in seedling communities in a wet sclerophyll forest.
2) To investigate if myrtle rust tree mortality is affecting key processes that influence the cycling of nitrogen and carbon in a wet sclerophyll forest.

4. Methods/Process

The study was conducted on a private property in the Tallebudgera Valley in southeast Queensland. The forest at this site is secondary forest, established after past land clearing in the late 1800's (Cook, D. 2023, Personal communication). The site supports wet sclerophyll forest, an overstory of Eucalypt sp. and brush box (*Lophostemon confertus* (R.Br.) Peter G.Wilson & J.T.Waterh.) with a diverse of rainforest mid- and understorey. Significant impacts of myrtle rust on plant health were first noted in the region in 2014 (Carnegie et al., 2016), shortly after long-term monitoring plots were established (Pegg et al., 2017). The results of this monitoring highlighted significant population decline in four Myrtaceae species that were dominant in the mid- and understory (scaly myrtle (*Gossia hillii* (Benth.) N.Snow & Guymer), silky myrtle (*Decaspermum humile* (G.Don) A.J.Scott), smooth scrub turpentine (*Rhodamnia maideniana* C.T.White) and rose myrtle (*Archirhodomyrtus beckleri* (F.Muell.) A.J.Scott)) due to repeated myrtle rust infection (Pegg et al., 2017). It is after this peak period of tree mortality that this study occurs. This study was conducted over several high rainfall years that followed some of the driest years on record , a significant La Nina event (Bureau of Meteorology, 2021). For example, just 772 mm of rain was recorded in the region in 2019, while 2304 mm of rain was recorded in 2020, the year this study began (Bureau of Meteorology, 2021).

A brief description of the methods of data collection and analysis for study 1 (Aim 1) is described, but for further details please see Stevenson et al. (2023 in press). Forty-eight circular plots (2 m radius, 12.5 m²), each containing four 1 m² quadrats were used for data collection (Appendix 1). Plot location was similar to those used by Pegg et al. (2017) and was determined using six line transects as a base, and random allocation to determine the direction and distance of the plots from the lines (Appendix 1). The presence or absence of dead trees was recorded in each quadrat as a measure of myrtle rust impact level. In this study trees exhibiting basal sprouting were counted as dead as the canopy no longer contained living or dead leaves. Leaf area index was also measured in the centre of each quadrat, LICOR LAI-2200C Plant Canopy Analyzer, as a second indicator of disease impact level. As a negative relationship between canopy cover and the disease impact level was previously recorded at the study site (Diaz-Torres, 2020). The abundance and identity of tree seedlings ≤ 1 m was recorded in each quadrat. Seedlings were identified to the species level using field guides (Harden et al., 2019; Leiper et al., 2017) and expert advice (McDonald W.J.F. and Wills J. 2021, Personal communication). Key reproductive and dispersal traits associated with cross trophic-level interactions were identified from the literature (seed size, fruit size, fruit type, dispersal mechanism) (Katovai et al., 2012; Mayfield et al., 2005; Wills et al., 2017), and the appropriate trait category for each was determined for each observed species using available literature (e.g., Cooper and Cooper, 2013; Dunphy et al., 2020) and expert advice (Catterall C. 2021, Personal communication). Linear mixed effects models, with plot set as a random effect, were used to determine any correlation between tree mortality or leaf area index and species richness or species diversity. Non-parametric multidimensional scaling (nMDS) and PerMANOVA analyses were run, with tree mortality (yes/ no) was set as the grouping variable, to determine possible correlations between species composition and disease impact.

Several types of data were collected for study 2 (Aim 2). Plots used for this study were thirty-two of the circular plots used in study 1, those that were placed using the four line transects established by Pegg et al. (2017) as a base. Variation in topography and aspect resulted in the plots associated with the four transect lines being recognised as being in one of three microhabitats, south-west facing slope, creek at base of south-west facing slope, and north-east facing slope. Disease impact was estimated in each plot using two approaches. First all trees, dead and alive, were counted and the percentage of trees that were dead calculated (PC). The diameter at breast height (DBH) was assessed for each dead and living tree and inserted into allometric equations (one for Eucalypt species and one for hard wood species (Paul et al., 2016)) to determine tree biomass (kg dry weight per tree). This data was used to calculate the percentage of total biomass in each plot that was dead (PB).

Three litter traps made of PVC pipe and nylon mesh were placed in each plot (Appendix 2). Litterfall samples were collected each month, from June to September of 2021, and July to September of 2022. This period was selected as it covers much of the annual peak of myrtle rust infection in eastern Australia (Pegg et al., 2018). Samples were sorted into leaf, wood, reproductive parts, and debris (Proctor, 1983) and dried at 65 °C for 72 hours (Pérez-Harguindeguy et al., 2013). Leaf matter was then ground to powder using a rocklabs grinder and analysed for total carbon (%) and total nitrogen (%) content using a LECO 928 model combustion analyser. From this data the Carbon to Nitrogen (C:N) ratio was determined for each sample.

Tomst sensors were used to measure soil moisture (%) and soil temperature (°C) in each plot continuously from December of 2021 to September of 2022. The sensors were protected from animals by metal cages (Appendix 3). Soil moisture data was calibrated from raw moisture data to volumetric soil moisture using the soil texture data and an online converter provided by Tomst. The decision was made to use an average of the monthly values for each plot for total litterfall (g) and foliar C:N ratio, and an average daily value for soil moisture (%) and soil temperature (°C). As there was no noted change in the number of dead trees in plots between sampling months, as most had already died in the years proceeding our study (Pegg et al., 2017), and we hoped to test for an effect of disease impact rather than of seasonality.

Soil samples were collected at a single time point. Two soil cores (0-10cm depth) were collected from each plot using a randomised approach and homogenised. Half of each sample was sent for soil texture analysis (% coarse sand, % sand, % silt, % clay (Standards Australia, 2003)) at The Chemistry Centre in the Soil and Catchment Science department of the Queensland government. While the other half of each sample was sent to The University of Queensland Analytical Laboratories for analysis of total nitrogen (%) and total carbon (%), determined using a LECO 928 model combustion analyser, and nitrate (mg/ kg) and ammonium (mg/ kg), determined using a SEAL AQ400 colorimetric analyser (Rayment et al., 2011). The C:N ratio for each soil sample was determined from this data. Plans to measure nitrate and ammonium using ion exchange resin bags, and decomposition using the teabag method faced some issues and as a result the data is not included in this report. The leaf area index readings collected in study 1 were also used in this study, an average of the four readings taken in the quadrats was calculated to create one reading for each plot.

Global models were created for each measured factor (i.e., total litterfall (g), foliar C:N ratio, soil C:N ratio, soil ammonium (mg/ kg) and soil nitrate (mg/ kg)). Factors to be included as independent variables, in addition to PC or PB, were determined by a review of the literature (Appendix 4). Evidence of collinearity between factors was checked prior to running the models using the variance inflation factor. The need for random effects (i.e., transect) was checked using maximum likelihood ratio tests. When the results indicated that random effects were not needed (i.e., models for soil C:N ratio, soil nitrate (mg/ kg), soil ammonium (mg/ kg)) global models were run using linear models, while when the results indicated that random effects improved model fit (i.e., total litterfall (g) and foliar C:N ratio) linear mixed effects models were used. AICc values were used to determine model selection. Models with AICc values of ≤ 6 were highlighted as being equally good estimations of our data, however, the simplest model with the lowest AICc was ultimately selected as the best fit model for our data. Soil ammonium (mg/ kg), soil nitrate (mg/ kg) and soil C:N ratio data were square root transformed to address issues of normality and model fit. Principal component analyses, with scaling applied, were used to explore possible influences of microhabitat on the measured factors. Kruskal Wallis analysis, followed by a Dunn test if indicated, were used to test for differences in soil attributes between microhabitats.

5. Achievements, Impacts, and Outcomes

This research project has resulted in several conference presentations, invited presentations, and a manuscript accepted for publication, which is in press with the journal ‘Plants’. An overview of the project aims was presented online at the Ecological Society of Australia Conference 2021 in the form of a speed talk and poster. The results of study 1 were presented at an invited talk at the Queensland Herbarium in June 2022, and as a long talk at the Ecological Society of Australia Conference in Wollongong in November 2022. An abstract describing the results of study 2 has been submitted for consideration as a talk at the upcoming Australasian Myrtle Rust Conference in Sydney in June 2023. The student has also been invited to give two short talks at local community groups to share some of the results. A short talk was given at the Queensland Field Naturalists club in April 2023, and a similar short talk will be given at a meeting of the Moggill Catchment group in June 2023. The results of study 1 have been written up as a manuscript and has been accepted for publication in the journal ‘Plants’, titled “Impacts of myrtle rust induced tree mortality on species and functional diversity within seedling communities of a wet sclerophyll forest in eastern Australia” (Stevenson et al. 2023). A summary of the results and methods contained in this paper are described in this report.

6. Discussion and Conclusion

The results from this study provide some insights on the impacts of myrtle rust on key ecosystem functions in a wet sclerophyll forest during a high rainfall period. Our results indicate that while populations of several rainforest species (i.e., rose myrtle, silky myrtle, scaly myrtle, and smooth scrub turpentine) have been significantly reduced by repeated myrtle rust infection, the forest community appears to be recovering and retaining much of its functioning, likely aided by the recent high annual rainfall years.

Myrtle rusts selective mortality of some species over others has shifted the composition of the forest. Our seedling surveys indicated that there is a diversity of species that have established in the seedling layer. Most of the species recorded are native and associated with the local regional ecosystem, which is a positive indicator for the recovery trajectory of this community. For example, of the fifteen most commonly observed species, that together represented 87 % of all recorded seedings, fourteen are associated with the local regional ecosystem 12.11.2 (Queensland State Government, 2021). Our results support the results of a long-term monitoring study at the site, that observed a decline in the abundance of four of the five Myrtaceae species (rose myrtle, silky myrtle, smooth scrub turpentine and scaly myrtle) that were dominant in the mid- and understorey of the forest when myrtle rust first established (Pegg et al., 2017). We observed each of these species to be at a very low abundance (≤ 1% of the total seedlings). However, we observed the fifth of these species, lilly pilly (*Acmena smithii* (Poir.) Merr. & L.M.Perry), to be the third most abundant species recorded in the seedling community. This is a positive sign for the ongoing survival of this species in this community. Five non-native tree/ shrub species, lantana (*Lantana camara* L.), easter cassia (*Senna pendula* var. *glabrata* (Vogel) H.S.Irwin & Barneby), camphor laurel (*Cinnamomum camphora* (L.) T.Nees & C.H.Eberm), ochna (Ochna serrulata Walp.) and coral berry (*Ardisia crenata* Sims), were also recorded in the surveys, but each at a very low abundance (≤ 1% of total seedlings).

The loss of key Myrtaceae species that act as early colonisers may affect the capacity of this community to respond to future disturbance events. Several of the Myrtaceae species that have experienced significant population declines due to repeated myrtle rust infection at the study site, such as rose myrtle, scrub turpentine (*Rhodamnia rubescens* (Benth.) Miq.) and native guava (Pegg et al., 2017), are early coloniser species (Kariuki, 2004; Kooyman, 1996). These species appeared to have played a key role in the re-capturing of the site by forest after past land clearance. Seedlings and saplings from several native early coloniser species that are not hosts of myrtle rust, including cheese tree (*Glochidion ferdinandi* (Müll.Arg.) F.M.Bailey), red kamala (*Mallotus philippensis* (Lam.) Müll.Arg.) and southern salwood (*Acacia disparrima* M.W.McDonald & Maslin) (Kooyman, 1996), were observed in our seedling surveys. However, the presence of non-native species that thrive in disturbed areas, such as lantana and easter cassia, as well indicates the need for vigilance after future disturbance events, to determine which species will fill this early colonising role following inevitable future disturbance and to apply weed management strategies if needed.

The loss of key Myrtaceae species is likely to cause a shift in the dominant fruit type and seed size category in this forest community, which may affect bird foraging patterns in the future. The dominant seed size in the seedling community was category 3 (4-8 mm * 4-8 mm) (Figure 1D), only one of the five key Myrtaceae species (scaly myrtle) produces seeds that fall into this seed size category, while three species (rose myrtle, silky myrtle, and smooth scrub turpentine) contain seeds allocated to lower seed size categories (2 and 1). This shift in seed size may reflect a shift in fruit type. The dominant fruit type recorded in the seedling community was drupe (Figure 1C), while the five key Myrtaceae species (silky myrtle, rose myrtle, scaly myrtle, smooth scrub turpentine and lilly pilly) produce berries. Drupes tend to contain one or a couple of larger seeds, while berries tend to contain many smaller seeds (Godínez-Alvarez et al., 2020; Lambert and Garber, 1998). A shift in dominance from berries to drupes could favour bird species that are true frugivores. There is some evidence that plants that produce drupes tend to favour a strategy of investing in pulp with a higher nutritional value to reward species for dispersing the single seed contained in the fruit (Edwards, 2006; French, 1991; Snow, 1981). Species from the Lauraceae family tend to favour this strategy, producing drupes with a high lipid content (Snow, 1981). Two of the most common species recorded in the surveys, hairy-leaved bolly gum (*Neolitsea dealbata* (R.Br.) Merr.) and jackwood (*Cryptocarya glaucescens* R.Br.), belong to this plant family and together comprised over 40% of total seedlings in this study. An increase in the abundance of fruit from these species in the future may benefit some true frugivores that consume their fruit, such as the topknot pigeon (*Lopholaimus antarcticus*) (Floyd, 2008). In contrast, the predicted decline in the abundance of berry producing trees could reduce the availability of this type of fruit favoured by some generalist bird species. This shift could be mitigated by identifying and protecting any mature berry producing trees in the community that are tolerant to myrtle rust, and potentially planting tolerant species native to the local regional ecosystem that exhibit this trait if all recorded trees are likely to be severely affected by myrtle rust.

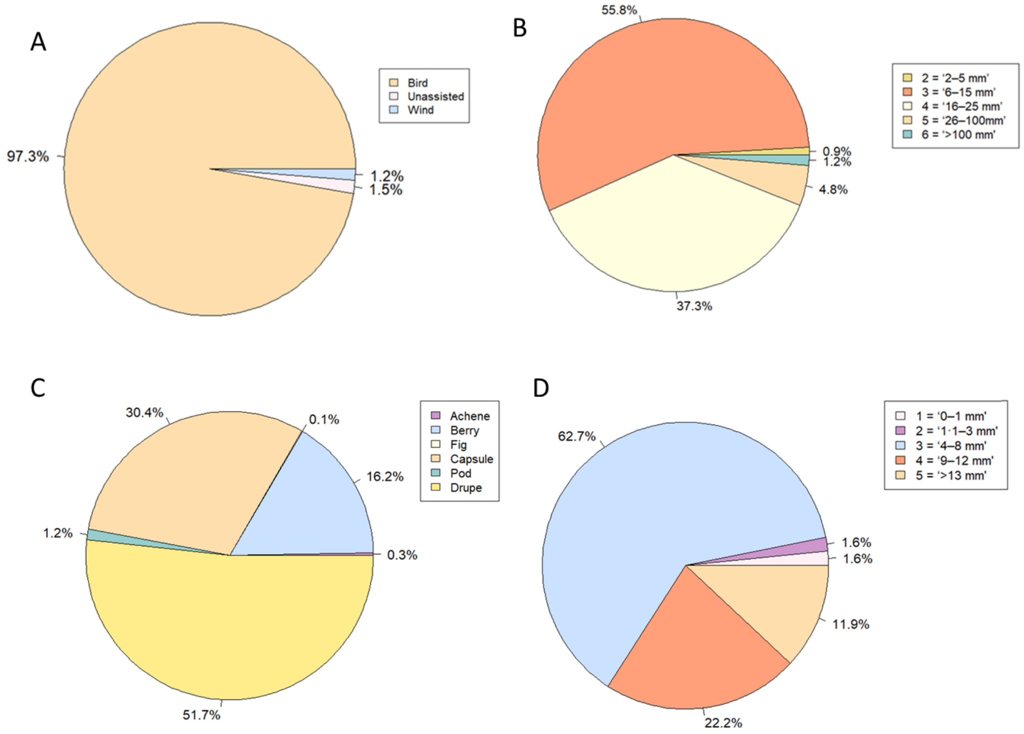


Figure 1. Representation of seedlings (% of total population) for each trait state category in each plant trait. A. Dispersal mechanism, B. Fruit size, C. Fruit type, D. Seed size.

No shift in the dominant dispersal mechanism or fruit size category is expected in this community in response to myrtle rust. Bird dispersal was the most common dispersal mechanism recorded in the seedling layer (Figure 1A); similarly, the key Myrtaceae species all have seeds that are dispersed by birds. While the most common fruit size category recorded in the seedling community was size category 3 '6-15mm x 6-15mm' (Figure 1B). Four of the five key Myrtaceae species (rose myrtle, silky myrtle, scaly myrtle, and smooth scrub turpentine) produce fruit falling into this same seed size category. These results indicate that the eventual replacement of some key Myrtaceae species with species from the seedling layer is unlikely to cause a shift in these traits, nor in browsing behaviour based on these traits.

However, a reduction in the current availability of fruit is expected and could affect browsing behaviour. A significant number of mature trees that produced fruit prior to 2014 at the study site have now been killed by myrtle rust. It will take several years for many of the seedlings surveyed in this study to reach maturity, and until this time there is likely a significant reduction in fruit availability, which may affect the foraging behaviour of local birds. For example, the rose-crowned fruit-dove (*Ptilinopus regina*) is known to consume fruits of rose myrtle, silky myrtle and scrub turpentine (Church, 1997; Gosper and Gosper, 2008; Hawkins, 2014) and has been observed at the study site. The loss of mature fruiting Myrtaceae trees will likely increase the importance of remaining healthy fruit producing trees to local bird species. Increased visitation may be observed at these locations, and care should be taken to protect these trees from damage or removal.

Tree mortality did not correlate with measures of species richness during a period of high rainfall at the study site. Tree mortality (presence or absence) was not observed to correlate with leaf area index (fixed effect= -0.02, standard error= 0.03, t= -0.46; Figure 2A), Shannon's diversity Index (fixed effect= -0.01, standard error= 0.08, t= -0.15; Figure 2B) or species richness (fixed effect= -0.04, standard error= 0.09, t= -0.47; Figure 2C). Nor did it appear to affect species composition (F= 1.60, p= 0.14; Figure 3). While no correlation between these factors was observed in this study that occurred during a high rainfall time period, previous studies at the same site during drought conditions observed a decrease in species composition where myrtle rust was more prevalent (Diaz-Torres, 2020). The continuous monitoring of the forest community at this site, facilitated by APBSF funding over two projects over the last five years, allowed authors to visually observe the flush of growth at the site with the arrival of higher rainfall years. These changes from an El Nino event (low rainfall) to a La Nina event highlights the importance of long-term monitoring to detect the effects of varying climate in complex ecosystems like sub-tropical forests.

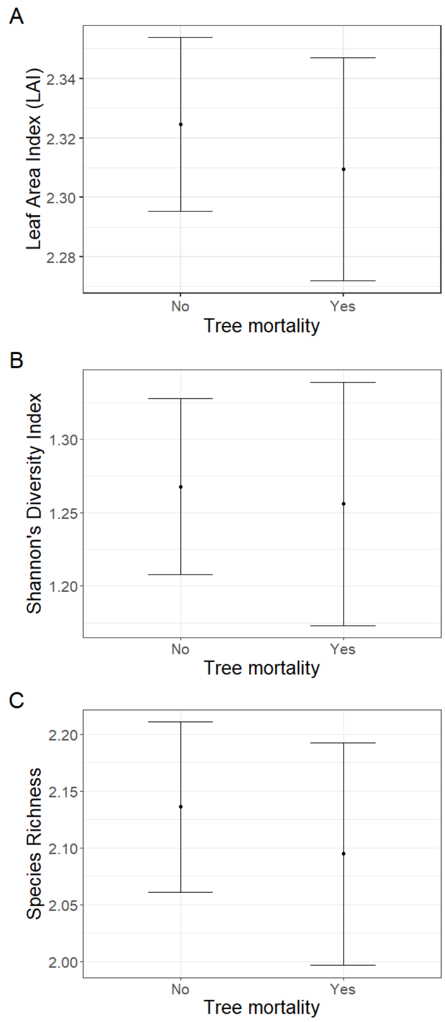


Figure 2. There were negligible effects of tree mortality on (A) Leaf Area Index (LAI), (B) Shannon's diversity index, or (C) species richness, when comparing the mean fixed effects and standard error estimates.

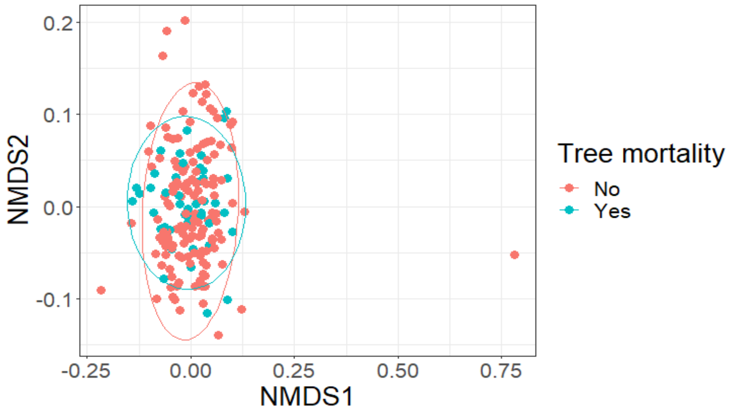


Figure 3. Tree mortality present in the plots (yes/ no) was not observed to markedly affect the grouping of species composition data among the plots using an nMDS analysis (k=2, stress= 0.22).

Disease impact level had minimal impacts on the richness of plant traits. There was a very weak effect of tree mortality on the richness of fruit size categories (F= 4.22, p <0.01 & F= 4.95, p <0.01; Appendix 5), however, it was not observed to affect the richness of any other traits. The absence of an effect of myrtle rust on the richness of most traits could indicate that the disturbance brought about by myrtle rust was not sufficient to filter for species with markedly different traits in affected plots. However, it may also be that the pathogen has not been established in the community for long enough to see definitive change. Significant impacts of the pathogen were only detected in the region relatively recently in 2014 (Carnegie et al., 2016). In contrast, Lovett et al. (2010) investigated impacts of beech bark disease on a different ecosystem function, nutrient cycling, and observed the strongest correlations in plots that had been exposed to the pathogen for the longest period, with infection recorded in their region of study over 50 years before the study took place.

In study 2, several key results were observed, the first being that correlations between estimators of myrtle rust impact (PC and PB) and the measured factors were minimal, however, notable given the complexity of these forest systems and the relatively short period (~10 years) that the pathogen has been established in the region. While neither PC nor PB, was included in any of the best fitting models for the data, each was retained in four or more of the equivalent acceptable models for each measured factor. However, the fixed effects estimates, or slope estimates, were consistently low (≤ 0.05) indicating only a minimal correlation. This was also supported by PCA analyses where both PC and PB represented between 1 and 3 % of variation in the first two principal components (Figure 4). This result is a positive indicator that this ecosystem function may be relatively resilient to the impacts of myrtle rust in this forest community during a high rainfall period.

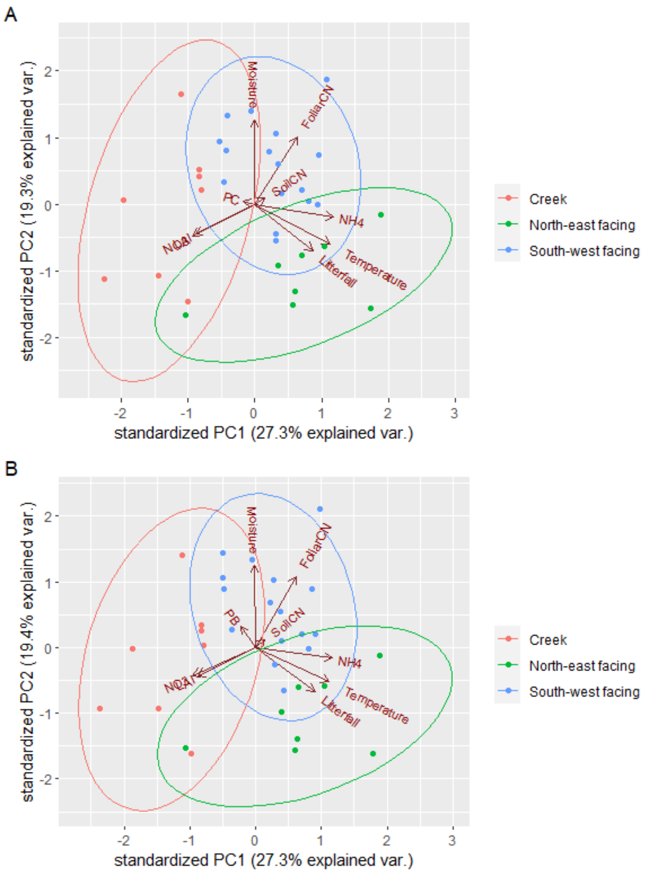


Figure 4. PCA analysis containing PC (A) and PB (B) indicated high similarity of plots in the three microhabitats. Ellipses represent 95% confidence intervals.

Variation in the measured factors associated with nutrient cycling were best explained by variation in topography and soil type. Variation in total litterfall was best explained by soil temperature, with a strong positive correlation observed between these factors (Fixed effects= 5.99, Standard error= 2.83, t= 2.12; Figure 5). The microhabitats within which our plots were located likely influenced the strength of soil temperature as a driver in this study. PCA analyses indicated that both soil temperature and total litterfall were strongly associated with the microhabitat located on the north-east facing slope (Figure 4). North facing aspects in the southern hemisphere receive greater direct sun exposure in winter, the period when this study took place, compared to south facing aspects (Oke, 1987). The higher interception of direct sunlight on this slope may represent a warmer microhabitat distinct from the other microhabitats that were located on south-west facing slopes or on creek banks at the base of a south-west facing slope.

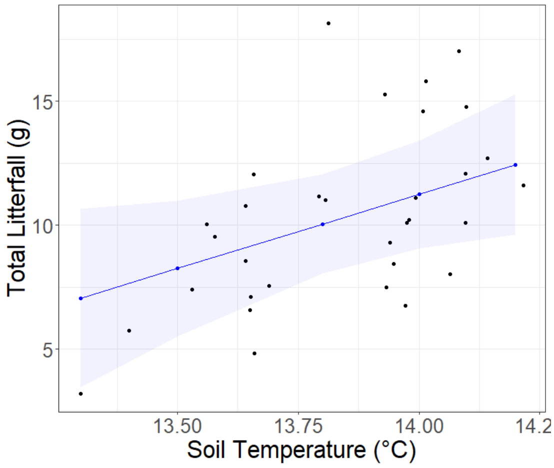


Figure 5. Fixed effects and standard error estimates indicate a marked positive correlation between total litterfall (g) and soil temperature (°C).

Topography and soil texture appeared to explain the variability in soil nitrate at the study site at this single time point. Measured factors included in the global model for soil nitrate appeared to be poor predictors, with the null model indicated as the best fit model. However, multivariate analysis indicated that soil nitrate was strongly associated with the creek microhabitat (Figure 4). This microhabitat differed from the others in two distinct ways. The first is that soil collected from plots in this location contained a higher percentage of sand and a lower percentage of clay than recorded in the other microhabitats (Appendix 6). Nitrate is easily leached from soils with a high sand content (Beaudoin et al., 2005; Hoffmann and Johnsson, 1999), which increases the risk of leaching into nearby water bodies, such as the creek in this microhabitat. We observed no significant difference in soil nitrate levels detected between the microhabitats in this study (Appendix 6). The second is that several plots at this site were located directly on the banks of the creek, where canopy cover was naturally lower. There is strong evidence that nitrification and soil nitrate can be elevated in canopy gaps (Prescott, 2002). Significantly lower soil ammonium levels and a higher soil nitrate to ammonium ratios recorded in soils collected from this microhabitat appears to support the suggestion of elevated nitrification at this site (Appendix 6). However, soil nitrate levels can vary significantly overtime in response to factors such as rainfall and plant uptake (Breitenbeck, 1990; Kariuki et al., 2010). Soil samples were only collected once in this study, so our results give an indication of impacts at this single time point.

Soil temperature was observed to be the best predictor of soil ammonium in our study, with a strong positive correlation between the factors observed ($F_{1,30}$= 18.69, p<0.01; Figure 6). The process that transforms organic nitrogen into ammonium releasing carbon dioxide is called mineralization (Schlesinger and Bernhardt, 2020). There is strong evidence for a positive relationship between soil temperature and the rate of mineralisation (Bai et al., 2013; Guntiñas et al., 2012; MacDonald et al., 1995), supporting our result of a positive correlation between soil ammonium content and soil temperature at our site.

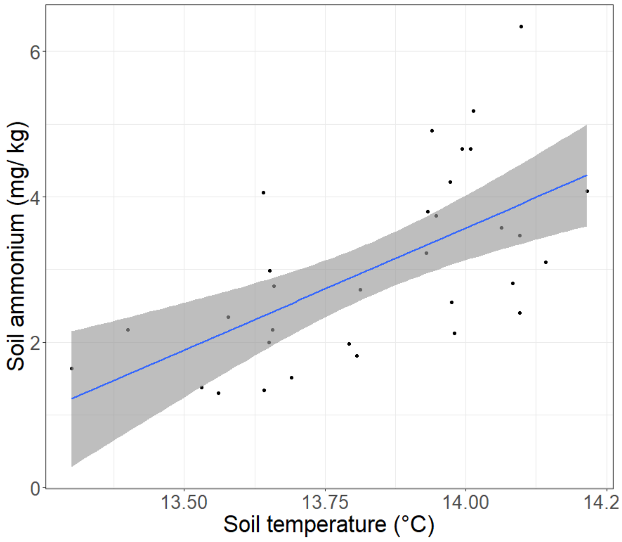


Figure 6. Significant positive relationship between soil ammonium (mg/ kg) and soil temperature (°C).

Our data appeared to poorly explain variation in soil C:N ratio and foliar C:N ratio, with the null models indicated as the best fit models. This suggests that there were factors not measured in our study that were driving the results observed. One such factor may be species composition, for example, regional and local studies have indicated that that the dominant canopy species is a strong driver of soil C:N ratio (Cools et al., 2014; Finzi et al., 1998). The PCA analyses indicated foliar C:N ratio was most strongly associated with the southwest facing microhabitat (Figure 4). While Eucalypt sp. and brush box are dominant in the overstory across the study site, litter from these species was particularly prominent in samples collected from the south-west facing slopes. This contrasted with the other microhabitats where litterfall from rainforest species, including red cedar (*Toona ciliata* M.Roem.) and hard quandong (*Elaeocarpus obovatus* G.Don) were also prominent. There is some evidence to suggest that rainforest species can contribute a disproportionally large level of total nitrogen in litterfall biomass relative to overstory Eucalypts in wet sclerophyll forests (Ashton and Attiwill, 1994; Baker and Attiwill, 1981), which may have contributed to the positive association of foliar C:N ratio with this microhabitat. However, further data collection would be required to confirm this observation as litter was not sorted by species in this study given the high species diversity (> 65 species).

7. Recommendations

The results from this study provide some insights on the impacts of myrtle rust on key ecosystem functions in a wet sclerophyll forest during a high rainfall period, several recommendations can be made based on these results. Our results, support those of past studies at the site, indicating that populations of several early colonising species (i.e., rose myrtle, scrub turpentine and native guava) have been significantly reduced by repeated myrtle rust infection. We recommend close monitoring of plant recruitment after severe disturbance (e.g., fire or flooding) at myrtle rust infected sites so weed control can be applied if it appears non-native species are filling this early colonising role. The predicted decline in the abundance of berry producing trees, and current reduction generally in fruit availability in forest communities affected by myrtle rust, could increase the importance of remaining fruit producing trees. Increased visitation may be observed at these locations, and care should be taken to protect these trees from damage or removal. If it appears all berry producing trees in a community are likely to be severely affected by myrtle rust, planting patches of tolerant or non-host tree species native to the local regional ecosystem that exhibit this trait may mitigate impacts on local birds. Variation in the measured factors associated with nutrient cycling was best explained by variation in topography. The weak association of measured factors associated with nutrient cycling with estimators of myrtle rust impact is a positive indicator that this ecosystem function may be resilient to the impacts of pathogen in this complex forest system at this stage of the outbreak.

9. Appendices, References, Publications

References

Adamek, M., Corre, M.D., Hölscher, D., 2009. Early Effect of Elevated Nitrogen Input on Above-Ground Net Primary Production of a Lower Montane Rain Forest, Panama. Journal of Tropical Ecology 25, 637–647.

Ashton, D.H., Attiwill, P.M., 1994. Major vegetation types: Tall open forests, in: Groves, R.H. (Ed.), Australian Vegetation. Cambridge University Press, Cambridge, pp. 157–197.

Ávila, J.M., Gallardo, A., Ibáñez, B., Gómez-Aparicio, L., 2021. Pathogen-Induced Tree Mortality Modifies Key Components of the C and N Cycles with No Changes on Microbial Functional Diversity. Ecosystems 24, 451–466. https://doi.org/10.1007/s10021-020-00528-1

Avila, J.M., Gallardo, A., Ibáñez, B., Gómez-Aparicio, L., 2016. Quercus suber dieback alters soil respiration and nutrient availability in Mediterranean forests. Journal of Ecology 104, 1441–1452. https://doi.org/10.1111/1365-2745.12618

Bai, E., Li, S., Xu, W., Li, W., Dai, W., Jiang, P., 2013. A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. NEW PHYTOL 199, 441–451. https://doi.org/10.1111/nph.12252

Baker, T.G., Attiwill, P.M., 1981. Nitrogen in Australian eucalypt forests. Presented at the Autralian forest nutrition workshop: Productivity in perpetuity., CSIRO, Melbourne, pp. 159–72.

Beaudoin, N., Saad, J.K., Van Laethem, C., Machet, J.M., Maucorps, J., Mary, B., 2005. Nitrate leaching in intensive agriculture in Northern France: Effect of farming practices, soils and crop rotations. Agriculture, Ecosystems & Environment 111, 292–310. https://doi.org/10.1016/j.agee.2005.06.006

Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems. ECOL MONOGR 75, 139–157. https://doi.org/10.1890/04-0988

Borer, E.T., Asik, L., Everett, R.A., Frenken, T., Gonzalez, A.L., Paseka, R.E., Peace, A., Seabloom, E.W., Strauss, A.T., Waal, D.B.V. de, White, L.A., 2020. Elements of disease in a changing world: modelling feedbacks between infectious disease and ecosystems. Ecology letters. https://doi.org/10.1111/ele.13617

Breitenbeck, G.A., 1990. Nitrogen Nutrition of Cotton: Practical Issues. American Society of Agronomy, Madison, WI, USA, pp. 77–87. https://doi.org/10.2134/1990.nitrogennutritionofcotton.c10

Bureau of Meteorology, 2021. Monthly rainfall: Tallebudgera Guineas Creek Road [WWW Document]. Climate Data Online. URL http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=139&p_display_type=dataFile&p_startYear=&p_c=&p_stn_num=040196 (accessed 1.11.21).

Carnegie, A.J., Kathuria, A., Pegg, G.S., Entwistle, P., Nagel, M., Giblin, F.R., 2016. Impact of the invasive rust Puccinia psidii (myrtle rust) on native Myrtaceae in natural ecosystems in Australia. Biological Invasions 18, 127–144. https://doi.org/10.1007/s10530-015-0996-y

Carnegie, A.J., Lidbetter, J.R., Walker, J., Horwood, M.A., Tesoriero, L., Glen, M., Priest, M.J., 2010. Uredo rangelii, a taxon in the guava rust complex, newly recorded on Myrtaceae in Australia. Australasian Plant Pathology 39, 463–466. https://doi.org/10.1071/AP10102

Carnegie, A.J., Pegg, G.S., 2018. Lessons from the Incursion of Myrtle Rust in Australia 56, 457–478. https://doi.org/10.1146/annurev-phyto-080516-035256

Church, R., 1997. Avian frugivory in a subtropical rainforest: eleven years of observations in Lamington National Park. The Sunbird journal of the Queensland Ornithological Society 27, 85–97.

Cobb, R.C., Eviner, V.T., Rizzo, D.M., 2013. Mortality and community changes drive sudden oak death impacts on litterfall and soil nitrogen cycling. The New Phytologist 200, 422–431.

Cools, N., Vesterdal, L., De Vos, B., Vanguelova, E., Hansen, K., 2014. Tree species is the major factor explaining C:N ratios in European forest soils. FOREST ECOL MANAG 311, 3–16. https://doi.org/10.1016/j.foreco.2013.06.047

Cooper, W., Cooper, W.T. (William T., 2013. Australian rainforest fruits : a field guide. CSIRO Pub., Collingwood, Vic.

Davis, R.A., Valentine, L.E., Craig, M.D., Wilson, B., Bancroft, W.J., Mallie, M., 2014. Impact of Phytophthora-dieback on birds in Banksia woodlands in south west Western Australia. Biological Conservation 171, 136–144. https://doi.org/10.1016/j.biocon.2014.01.027

Diaz-Torres, S., 2020. Ecological Impact of Myrtle Rust (Austropuccinia psidii) in a Wet Sclerophyll Forest (PhD). Queensland University of Technology, Brisbane, Australia.

Díaz, S., Cabido, M., 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology & Evolution 16, 646–655. https://doi.org/10.1016/S0169-5347(01)02283-2

Dunphy, M., McAlpin, S., Nelson, P.N., Chapman, M., Nicholson, H. (Hugh R.W., 2020. Australian rainforest seeds a guide to collecting, processing and propagation. CSIRO Publishing, Clayton, Vic.

Edwards, W., 2006. Plants reward seed dispersers in proportion to their effort: The relationship between pulp mass and seed mass in vertebrate dispersed plants. Evolutionary Ecology 20, 365–376. https://doi.org/10.1007/s10682-006-0006-z

Fensham, R.J., Radford-Smith, J., 2021. Unprecedented extinction of tree species by fungal disease. Biological Conservation 261, 109276. https://doi.org/10.1016/j.biocon.2021.109276

Fernandez-Winzer, L., Berthon, K., Entwistle, P., Manea, A., Pegg, G., Carnegie, A., Leishman, M., 2020. Direct and indirect community effects of the invasive plant pathogen Austropuccinia psidii (myrtle rust) in eastern Australian rainforests. Biological Invasions 22, 2357–2369. https://doi.org/10.1007/s10530-020-02260-2

Finzi, A.C., Van Breemen, N., Canham, C.D., 1998. Canopy Tree-Soil Interactions within Temperate Forests: Species Effects on Soil Carbon and Nitrogen. Ecological Applications 8, 440–446. https://doi.org/10.2307/2641083

Floyd, A.G. (Alexander G., 2008. Rainforest trees of mainland south-eastern Australia, [Revised ed.]. ed. Terania Rainforest Publishing, Lismore.

French, K., 1991. Characteristics and abundance of vertebrate-dispersed fruits in temperate wet sclerophyll forest in southeastern Australia. Australian Journal of Ecology 16, 1–13. https://doi.org/10.1111/j.1442-9993.1991.tb01476.x

Glen, M., Alfenas, A.C., Zauza, E.A.V., Wingfield, M.J., Mohammed, C., 2007. Puccinia psidii: a threat to the Australian environment and economy —a review. Australasian Plant Pathology 36, 1–16. https://doi.org/10.1071/AP06088

Godínez-Alvarez, H., Ríos-Casanova, L., Peco, B., 2020. Are large frugivorous birds better seed dispersers than medium- and small-sized ones? Effect of body mass on seed dispersal effectiveness. ECOL EVOL 10, 6136–6143. https://doi.org/10.1002/ece3.6285

Gosper, C.R., Gosper, D.G., 2008. Foods of Pigeons and Doves in Fragmented Landscapes of Subtropical Eastern Australia. Australian field ornithology 25, 76–86. https://doi.org/10.3316/informit.308760687742663

Groves, R.H. (Richard H., 1994. Australian vegetation, 2nd ed. ed. Cambridge University Press, Cambridge.

Guntiñas, M.E., Leirós, M.C., Trasar-Cepeda, C., Gil-Sotres, F., 2012. Effects of moisture and temperature on net soil nitrogen mineralization: A laboratory study. European Journal of Soil Biology 48, 73–80. https://doi.org/10.1016/j.ejsobi.2011.07.015

Harden, G., Nicholson, H., McDonald, B., Nicholson, N., Tame, T., Williams, J., 2019. Rainforest plants of Australia- Rockhampton to Victoria.

Hardwick, S.R., Toumi, R., Pfeifer, M., Turner, E.C., Nilus, R., Ewers, R.M., 2015. The relationship between leaf area index and microclimate in tropical forest and oil palm plantation: Forest disturbance drives changes in microclimate. AGR FOREST METEOROL 201, 187–195. https://doi.org/10.1016/j.agrformet.2014.11.010

Hawkins, B.A., 2014. Birds, fruit and nectar: spatio-temporal patterns of regional bird abundance and food availability in subtropical eastern Australia (PhD). Monash University, Melbourne.

Hicke, J.A., Allen, C.D., Desai, A.R., Dietze, M.C., Hall, R.J., Hogg, E.H.T., Kashian, D.M., Moore, D., Raffa, K.F., Sturrock, R.N., Vogelmann, J., 2012. Effects of biotic disturbances on forest carbon cycling in the United States and Canada. Global Change Biology 18, 7–34. https://doi.org/10.1111/j.1365-2486.2011.02543.x

Hoffmann, M., Johnsson, H., 1999. A method for assessing generalised nitrogen leaching estimates for agricultural land. Environmental modeling & assessment 4, 35–44. https://doi.org/10.1023/A:1019087511708

Jiang, L., He, Z., Liu, J., Xing, C., Gu, X., Wei, C., Zhu, J., Wang, X., 2019. Elevation Gradient Altered Soil C, N, and P Stoichiometry of Pinus taiwanensis Forest on Daiyun Mountain. Forests 10. https://doi.org/10.3390/f10121089

Kariuki, M., 2004. Modelling dynamics including recruitment, growth and mortality for sustainable management in uneven-aged mixed-species rainforests. Southern Cross University.

Kariuki, S.K., Schroder, J.L., Zhang, H., Hanks, T., McGrath, J.M., Payton, M.E., 2010. Temporal Variability of Soil Property Dynamics in a Grazed Pasture. Communications in Soil Science and Plant Analysis 41, 2744–2754. https://doi.org/10.1080/00103624.2010.518265

Katovai, E., Burley, A.L., Mayfield, M.M., 2012. Understory plant species and functional diversity in the degraded wet tropical forests of Kolombangara Island, Solomon Islands. Biological Conservation 145, 214–224. https://doi.org/10.1016/j.biocon.2011.11.008

Kooyman, R.M., 1996. Growing rainforest : rainforest restoration and regeneration : recommendations for the humid sub-tropical region of northern New South Wales and south east Queensland. Greening Australia, Brisbane, Australia.

Lambert, J.E., Garber, P.A., 1998. Evolutionary and ecological implications of primate seed dispersal. American journal of primatology 45, 9–28. https://doi.org/10.1002/(sici)1098-2345(1998)45:1<9::aid-ajp3>3.0.co

Leiper, G., Glazebrook, J., Cox, D., Rathie, K.A., 2017. Mangroves to mountains : a field guide to the native plants of south-east Queensland, Second edition. ed. Society for Growing Australian Plants Queensland Region Inc., Logan River Branch, Logan Village, Qld.

Li, J., Guo, Q., Ma, H., Zheng, W., 2021. Effects of slope aspect on altitudinal pattern of soil C:N:P stoichiometry in alpine forest of Tibet. E3S Web of Conferences 269, 1012. https://doi.org/10.1051/e3sconf/202126901012

Li, X., Zheng, X., Han, S., Zheng, J., Li, T., 2010. Effects of nitrogen additions on nitrogen resorption and use efficiencies and foliar litterfall of six tree species in a mixed birch and poplar forest, northeastern China. CAN J FOREST RES 40, 2256–2261. https://doi.org/10.1139/X10-167

Li, Y., Zhou, G., Huang, W., Liu, J., Fang, X., 2016. Potential effects of warming on soil respiration and carbon sequestration in a subtropical forest. Plant Soil 409, 247–257. https://doi.org/10.1007/s11104-016-2966-2

Lovett, G.M., Arthur, M.A., Weathers, K.C., Griffin, J.M., 2010. Long-term Changes in Forest Carbon and Nitrogen Cycling Caused by an Introduced Pest/Pathogen Complex. Ecosystems 13, 1188–1200. https://doi.org/10.1007/s10021-010-9381-y

MacDonald, N.W. (Grand V.S.U., Allendale, MI.)., Zak, D.R., Pregitzer, K.S., 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. SOIL SCI SOC AM J 59, 233–240. https://doi.org/10.2136/sssaj1995.03615995005900010036x

MacDuff, J.H., White, R.E., 1985. Net mineralization and nitrification rates in a clay soil measured and predicated in permanent grassland from soil temperature and moisture content. Plant and soil 86, 151–172.

Mayfield, M.M., Boni, M.F., Daily, G.C., Ackerly, D., 2005. Species and Functional Diversity of Native and Human-Dominated Plant Communities. Ecology 86, 2365–2372. https://doi.org/10.1890/05-0141

Mcdougall, K., Hobbs, R., St J Hardy, G., 2005. Distribution of understorey species in forest affected by Phytophthora cinnamomi in south-western Western Australia. Australian Journal of Botany 53, 813–819. https://doi.org/10.1071/BT04203

Myerscough, P., 1998. Ecology of Myrtaceae with special reference to the Sydney region. Cunninghamia 5, 787–808.

Myrold, D.D. (Oregon S.Univ., Corvallis, OR (USA). Dept. of Soil Science), Matson, P.A., Peterson, D.L., 1989. Relationships between soil microbial properties and aboveground stand characteristics of conifer forests in Oregon. Biogeochemistry 8, 265–281. https://doi.org/10.1007/BF00002892

Ngao, J., Epron, D., Delpierre, N., Bréda, N., Granier, A., Longdoz, B., 2012. Spatial variability of soil CO2 efflux linked to soil parameters and ecosystem characteristics in a temperate beech forest. Agricultural and Forest Meteorology 154–155, 136–146. https://doi.org/10.1016/j.agrformet.2011.11.003

Oke, T.R., 1987. Boundary Layer Climates. Routledge, Abingdon, Oxon. https://doi.org/10.4324/9780203407219

Paul, K.I., Roxburgh, S.H., Chave, J., England, J.R., Zerihun, A., Specht, A., Lewis, T., Bennett, L.T., Baker, T.G., Adams, M.A., Huxtable, D., Montagu, K.D., Falster, D.S., Feller, M., Sochacki, S., Ritson, P., Bastin, G., Bartle, J., Wildy, D., Hobbs, T., Larmour, J., Waterworth, R., Stewart, H.T.L., Jonson, J., Forrester, D.I., Applegate, G., Mendham, D., Bradford, M., O'Grady, A., Green, D., Sudmeyer, R., Rance, S.J., Turner, J., Barton, C., Wenk, E.H., Grove, T., Attiwill, P.M., Pinkard, E., Butler, D., Brooksbank, K., Spencer, B., Snowdon, P., O'Brien, N., Battaglia, M., Cameron, D.M., Hamilton, S., McAuthur, G., Sinclair, J., 2016. Testing the generality of above-ground biomass allometry across plant functional types at the continent scale. GLOBAL CHANGE BIOL 22, 2106–2124. https://doi.org/10.1111/gcb.13201

Pegg, G., Carnegie, A., Giblin, F., Perry, S., 2018. Final Report Managing myrtle rust in Australia CRC2063. Plant Biosecurity Cooperative Research Centre, Brisbane, Australia.

Pegg, G., Taylor, T., Entwistle, P., Guymer, G., Giblin, F., Carnegie, A., 2017. Impact of Austropuccinia psidii (myrtle rust) on Myrtaceae-rich wet sclerophyll forests in south east Queensland. PloS one 12, e0188058. https://doi.org/10.1371/journal.pone.0188058

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, S., Cornelissen, J.H.C., 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian journal of botany 61, 167–234. https://doi.org/10.1071/bt12225

Prescott, C.E., 2002. The influence of the forest canopy on nutrient cycling. Tree Physiology 22, 1193–1200. https://doi.org/10.1093/treephys/22.15-16.1193

Preston, D.L., Mischler, J.A., Townsend, A.R., Johnson, P.T.J., 2016. Disease Ecology Meets Ecosystem Science. Ecosystems 19, 737–748. https://doi.org/10.1007/s10021-016-9965-2

Proctor, J., 1983. Tropical forest litterfall. I. Problems of data comparison, in: Tropical Rain Forest : Ecology and Management. Blackwell Scientific Publications, Oxford, pp. 267–273.

Queensland State Government, 2021. Regional ecosystem details for 12.11.2 [WWW Document]. Regional ecosystem descriptions. URL https://apps.des.qld.gov.au/regional-ecosystems/details/?re=12.11.2 (accessed 2.10.22).

Rayment, G.E., Lyons, David J, Lyons, D. J, 2011. Soil chemical methods : Australasia. CSIRO, Collingwood, Vic.

Schlesinger, W.H., Bernhardt, E.S., 2020. Chapter 6 - Biogeochemical Cycling on Land, in: Schlesinger, W.H., Bernhardt, E.S. (Eds.), Biogeochemistry (Fourth Edition). Academic Press, pp. 183–248. https://doi.org/10.1016/B978-0-12-814608-8.00006-2

Shearer, B.L., Crane, C.E., Fairman, R.G., Dunne, C.P., 2009. Ecosystem dynamics altered by pathogen-mediated changes following invasion of Banksia woodland and Eucalyptus marginata forest biomes of south-western Australia by Phytophthora cinnamomi. Australasian Plant Pathology 38, 417–436. https://doi.org/10.1071/AP09018

Smith, K.F., Behrens, M.D., Sax, D.F., 2009. Local Scale Effects of Disease on Biodiversity. EcoHealth 6, 287–295. https://doi.org/10.1007/s10393-009-0254-9

Snow, D.W., 1981. Tropical Frugivorous Birds and Their Food Plants: A World Survey. Biotropica 13, 1–14. https://doi.org/10.2307/2387865

Soewarto, J., Giblin, F., Carnegie, A.J., 2019. Austropuccinia psidii (myrtle rust) global host list. Version 2. Australian Network for Plant Conservation, Canberra, ACT.

Standards Australia, 2003. Method 3.6.3: Soil classification tests — Determination of the particle size distribution of a soil — Standard method of fine analysis using a hydrometer.

Stevenson, K., Pegg, G., Wills, J., Herbohn, J., Firn, J., 2023. Impacts of Myrtle Rust Induced Tree Mortality on Species and Functional Richness within Seedling Communities of a Wet Sclerophyll Forest in Eastern Australia. Plants 12. https://doi.org/10.3390/plants12101970

Suo, Y., Yuan, Z., Lin, F., Wang, X., Ye, J., Bai, E., Hao, Z., 2016. Local-scale determinants of elemental stoichiometry of soil in an old-growth temperate forest. Plant Soil 408, 401–414. https://doi.org/10.1007/s11104-016-2939-5

Uriarte, M., Turner, B.L., Thompson, J., Zimmerman, J.K., 2015. Linking spatial patterns of leaf litterfall and soil nutrients in a tropical forest: a neighborhood approach. ECOL APPL 25, 2022–2034. https://doi.org/10.1890/15-0112.1

Vitousek, P., 1982. Nutrient Cycling and Nutrient Use Efficiency. The American Naturalist 119, 553–572.

Wills, J., Herbohn, J., Moreno, M.O.M., Avela, M.S., Firn, J., 2017. Next-generation tropical forests: reforestation type affects recruitment of species and functional diversity in a human-dominated landscape. Journal of Applied Ecology 54, 772–783. https://doi.org/10.1111/1365-2664.12770

Xu, S., Liu, L.L., Sayer, E.J., 2013. Variability of above-ground litter inputs alters soil physicochemical and biological processes: A meta-analysis of litterfall-manipulation experiments. BIOGEOSCIENCES 10, 7423–7433. https://doi.org/10.5194/bg-10-7423-2013

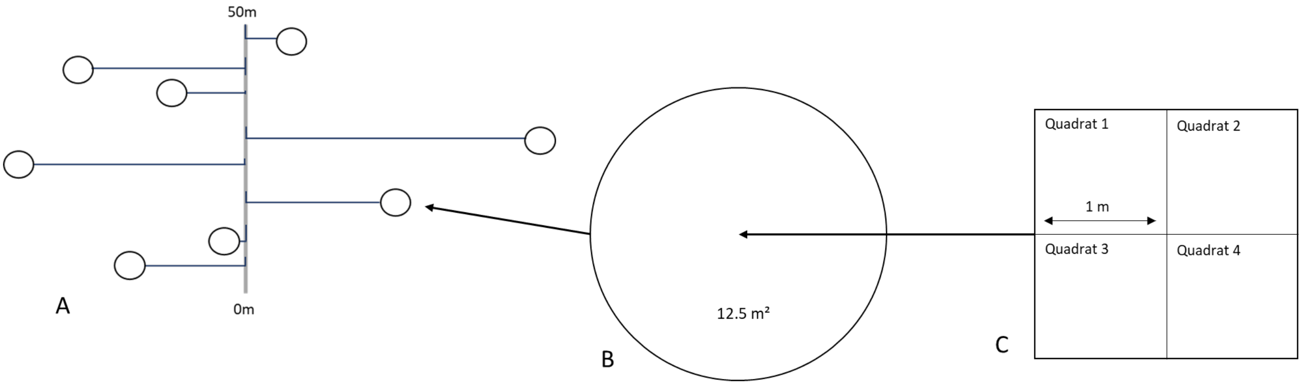
Yan, W., Farooq, T.H., Chen, Y., Wang, W., Shabbir, R., Kumar, U., Riaz, M.U., Alotaibi, S.S., Peng, Y., Chen, X., 2022. Soil Nitrogen Transformation Process Influenced by Litterfall Manipulation in Two Subtropical Forest Types. Frontiers in plant science 13, 923410–923410. https://doi.org/10.3389/fpls.2022.923410

Yang, J., Wu, F., Wei, X., Zhang, X., Wu, Q., Yue, K., Ni, X., 2022. Global Positive Effects of Litter Inputs on Soil Nitrogen Pools and Fluxes. Ecosystems. https://doi.org/10.1007/s10021-022-00800-6

Yang, W.H., Ryals, R.A., Cusack, D.F., Silver, W.L., 2017. Cross-biome assessment of gross soil nitrogen cycling in California ecosystems. Soil Biology and Biochemistry 107, 144–155. https://doi.org/10.1016/j.soilbio.2017.01.004

Zheng, L., Chen, H.Y.H., Yan, E., Gilliam, F., 2019. Tree species diversity promotes litterfall productivity through crown complementarity in subtropical forests. J ECOL 107, 1852–1861. https://doi.org/10.1111/1365-2745.13142

Appendices



Appendix 1. Diagram highlighting an example of random placement of plots with reference to a line transect (A) and circular plots (2 m radius, 12.5 m²) (B) that contained the four 1 m² quadrats (C).

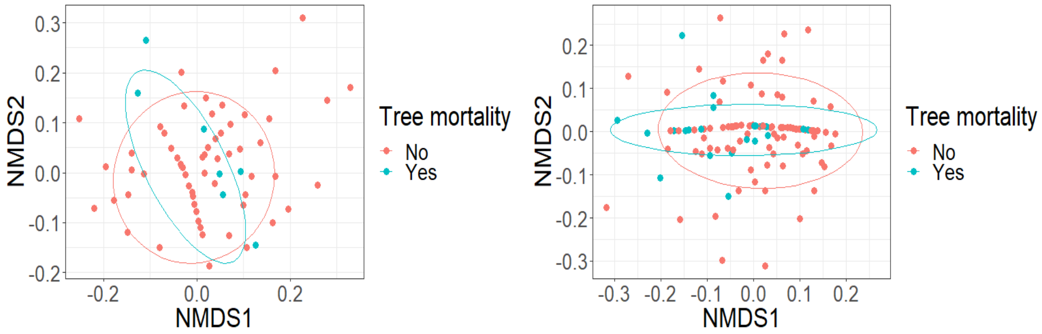
Appendix 2. Litter trap design created using PVC pipe, nylon mesh and nylon thread. The surface area of the traps was 0.25 m² (0.5 m x 0.5 m), mesh size of 1 mm, and trap height of 0.8 m.

Appendix 3. Tomst sensor under metal protective cage.

Appendix 4. Independent variables included in the global models for the measured factors/ dependent variables (i.e., total litterfall (g), foliar C:N ratio, soil C:N ratio, soil nitrate (mg/ kg) and soil ammonium (mg/ kg)).

Dependent variables	Independent variables	Supporting citations
Total litterfall (g)	Soil moisture (%), soil temperature (°C), soil nitrate (mg/ kg), soil ammonium (mg/ kg) and PC or PB	(Adamek et al., 2009; Li et al., 2010, 2016; Zheng et al., 2019)
Foliar C:N ratio	Soil C:N ratio, soil nitrate (mg/ kg), soil ammonium (mg/ kg) and PC or PB	(Li et al., 2010; Vitousek, 1982)

Soil C:N ratio	Foliar C:N ratio, soil moisture (%), soil temperature (°C), total litterfall (g), leaf area index and PC or PB	(Jiang et al., 2019; Li et al., 2021; Ngao et al., 2012; Suo et al., 2016; Uriarte et al., 2015; Xu et al., 2013; Yang et al., 2022)
Soil nitrate (mg/ kg) or soil ammonium (mg/ kg)	Soil C:N ratio, soil moisture (%), soil temperature (°C), total litterfall (g), leaf area index and PC or PB	(Booth et al., 2005; Hardwick et al., 2015; MacDuff and White, 1985; Myrold et al., 1989; Yan et al., 2022; Yang et al., 2022, 2017).



Appendix 5. Some grouping of plots based on (A) fruit size trait state richness (k= 2, stress= 0.11) and (B) fruit size trait state richness adjusted for seedling abundance (k=2, stress= 0.10) was observed in response to tree mortality using nMDS plots.

Appendix 6. Results of Kruskal- Wallis analyses and Dunn tests, investigating a correlation between microhabitat and soil attributes. Microhabitat 1: south-west facing slope, microhabitat 2: creek banks at base of south-west facing slope, microhabitat 3: north-east facing slope.

Variable	Chi squared value	Degrees of freedom	P value	Difference detected between microhabitats using Dunn test
Clay content (%)	19.15	2	<0.01	M2 < M1 = M3
Sand content (%)	19.23	2	<0.01	M2 > M1 = M3
Soil nitrate (mg/ kg)	2.69	2	0.26	NA
Soil ammonium (mg/ kg)	14.02	2	< 0.01	M2 < M1 = M3
Soil nitrate: ammonium ratio	10.76	2	< 0.01	M2 > M1 = M3
Soil C:N ratio	1.29	2	0.52	NA