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Sustainable pasture systems under climate extremes

Abstract

Predicted increases in the frequency and intensity of climate extremes pose a new challenge for pasture-dependent industries. Knowledge of how key pasture grasses and legumes perform under seasonal drought and elevated temperatures, and understanding of the traits and mechanisms driving species differences, can inform future planting decisions and breeding programmes. We applied a winter + spring severe drought treatment to nine field-grown grasses and legumes, and three sets of two species mixtures, over a two-year period; a subset of these were also exposed to a +3°C warming treatment, with the study taking place in the warm temperate climate of Richmond, NSW. There was considerable variation in magnitude of response to drought, with tropical and temperate species exhibiting strong reductions in cool season and annual productivity, along with both improvements and declines in nutritional quality. Most perennial species recovered rapidly once drought was alleviated, with annual productivity in droughted plots being highest for tropical grasses and Lucerne. Warming generally reduced productivity (even during winter, when local mean daily temperatures of 11.5^{o}C were raised by 3°C) and increased greenhouse gas emissions, but had little effect on nutritional quality. We found evidence that plant rooting traits and water use strategies can help explain species differences in climate sensitivity.

Executive summary

Pastures provide much of the feed base that underpins our meat, livestock and dairy industries, the combined value of which is estimated at $22B annually. Further, pastures contribute significantly to soil health through weed and pest control, input of nitrogen that is symbiotically fixed in legume roots, and reduction of soil erosion. Pastures take time to establish and the cost of restoring degraded pastures is significant. There has, therefore, been considerable effort to identify the most suitable species and cultivars, and best management practices, that will ensure sustainable pasture production across diverse growing regions in Australia. Pasture-dependent industries are, however, now facing a substantial, long-term challenge: the need to develop new knowledge and tools to combat an increasingly unpredictable and extreme climate.

A great deal of research has assessed the impacts of higher temperatures or drought on pasture productivity and forage quality, but far less is known regarding the impacts of climate extremes (exemplified in this project by repeated, severe winter and spring drought, in combination with elevated temperatures and heatwaves). Given predicted increases in the frequency and intensity of climate extremes (CSIRO 2015, Climate Council 2019), management of climate risk requires new knowledge of species' performance under future climates and understanding of the traits and mechanisms associated with climate vulnerability.

This project - **PA**stures and **C**limate **E**xtremes (PACE) - evaluated responses of a range of traditional and new pasture species to the more extreme rainfall regimes and temperatures that are predicted to become a consistent feature of climates across south eastern Australia in the coming decades. By quantifying species' responses under common growing conditions (background climate, soils), we have been able to evaluate their relative sensitivity to severe drought and their capacity to recover when drought is alleviated; for a sub-set of species we have also quantified the impacts of elevated temperatures on plant performance, both singly and in combination with drought.

This project has two overarching aims:

1. To establish a knowledge base that can help inform a robust adaptation framework for pasture management under future, more extreme, climates
2. To identify opportunities to increase climate resilience and to minimise future industry exposure to climate-related risk

To achieve these aims, the project included both field and glasshouse experiments. The PACE field experiment was established in Richmond, NSW, which experiences a mean annual temperature of 17.2°C (January mean daily maximum 29.4°C; July mean daily minimum 3.2°C) and annual rainfall of 800 mm (distributed fairly evenly across seasons). Twelve different pasture types (including tropical and temperate grasses and legumes, as single species and mixtures) were sown across 192 plots where we manipulated rainfall and, for a sub-set of plots, temperature. Based on climate predictions (CSIRO 2015) and historical records of extreme winter/spring rainfall for pasture areas across southern and eastern Australia (e.g. Tanganbalamba, Colac, Goulburn, Armidale; BOM, 2017), we instituted a 60% reduction in winter and spring rainfall (henceforth "drought treatment"); a sub-set of plots (two single species, two species mixtures) were also exposed to a year-round elevated temperature (+3°C) treatment, in factorial combination with drought. We monitored a range of environmental, plant and soil parameters including plant-available soil water, leaf-level photosynthesis, productivity and nutritional quality of the swards,

as well as the rooting dynamics and belowground plant-microbe interactions in these plots. We also established two glasshouse experiments to gain mechanistic understanding of the productivity and plant nutritional responses in the field, as well as to understand how manipulation of different plant or soil parameters might help minimize future climate risk for the industry. These experiments involved 1) temperature and water treatments, along with a soil microbial treatment and 2) elevated/ambient atmospheric carbon dioxide (CO_2) treatments, for tropical grasses and legumes growing singly and in mixtures.

New knowledge to inform an industry climate adaptation framework and opportunities to minimise climate risk

An effective climate adaptation framework requires knowledge of pasture species' responses to future, more extreme climate conditions, along with understanding of the mechanisms underpinning those responses. Together, these address key knowledge gaps and help inform best adaptation practice to minimise future climate risk. Here, we summarise major findings from this project as they relate to growers and the meat, livestock and dairy industries. We also identify further areas for research that have emerged from the project that will address targeted knowledge gaps relating to adaptation options to manage the impacts of climate extremes. The 3°C warming treatment we applied to plants growing under ambient temperatures in Richmond (NSW) resulted in seasonal mean temperatures that are similar to those currently experienced in Tara or Gatton (Queensland); experimental warming in this study is thus equivalent to moving our field site ~850 km northwards, under current day conditions. The winter/spring drought treatment represents a (-60%) reduction in rainfall that is not only in line with the upper end of _mean_ rainfall predictions for southeastern Australia, but is well within the range of extreme seasonal rainfall experienced historically in dairy and livestock areas across the South East. For example, Tangambalamba (2000) and Goulburn (2011) both had years with 0 mm rainfall across the 6-month winter/spring period, representing a 100% reduction on long term seasonal means.

Key research findings for growers

1. Species differed considerably in their magnitude of productivity decline in response to extreme winter/spring drought. Averaged across the full two years of the experiment, the smallest overall yield reductions were for Rhodes (-10%) and Digit (-17%), while the biggest overall reductions were seen for Fescue (-46%) and Biserrula (-43%). Biomass produced during the 6-month winter/spring drought period showed strong treatment effects, with drought-related declines in cool season productivity as high as 57% (Biserrula), 58% (Kangaroo) and even 71% (Digit), in 2019.

2. _Most species recovered quickly following the end of the winter/spring drough_t, with relatively few persistent effects carrying over to summer and autumn productivity. Rhodes, Digit, Wallaby and Phalaris had the strongest recovery (i.e. highest drought resilience).

3. _Tropical grasses were, on average, more productive under prolonged winter/spring drought than temperate grasses_, even during the cool season. Whilst tropical species were generally of lower nutritional quality than their temperate counterparts, their crude protein concentrations and digestibility were significantly higher under severe drought conditions and there was evidence of improved nutrition when grown alongside legumes.

4. *Based on productivity responses* across the full two years of this study, species can be categorised according to their drought resistance[#], as follows:

    [#]**Drought Resistance:**   **High**: Rhodes, Digit, Wallaby
                                           Medium: Ryegrass, Phalaris, Lucerne, Kangaroo
                                         Low: Fescue, Biserrula

[#]*Resistance categories correspond to overall yield reductions (all harvests across the 24-month period) in droughted plots, compared to controls: High <20% yield reduction, Medium 20-40% reduction, Low >40% reduction.*

The relatively high drought-resistance of Rhodes, Digit and Wallaby indicates that these species are able to maintain fairly high overall (across both cool- and warm seasons) yields under future, more extreme rainfall regimes. Ryegrass was found to be moderately sensitive to cool season drought, with spring productivity particularly affected; this species also did not tolerate the high temperatures experienced during late spring/early summer at the study location. Digit experienced big reductions in spring productivity when exposed to winter/spring drought but recovered very strongly, producing large amounts of biomass throughout the summer months. As a tropical grass, Digit had lower crude protein and digestible dry matter concentrations than its temperate counterparts, although both were increased when this species was grown with a companion legume and also when droughted. With Digit demonstrating rapid drought recovery, high summer productivity and at least modest nutritional quality, this species appears to be well suited for filling summer feed gaps.

5. *Warming resulted in yield losses* for each of the four species examined, at different times of the year. Lucerne and Phalaris experienced fairly sustained reductions in growth across the first year under elevated, compared to ambient, temperature, while Kangaroo grass was only affected (significant yield reduction) in summer 2020. The combination of warming *and* drought resulted in the biggest declines in annual productivity, compared to control plots, for Phalaris and Fescue. Our findings clearly indicate that sustained increases in temperature, and the associated increase in frequency of summer heatwave events and warmer winter temperatures, are unlikely to have beneficial impacts on cool season productivity, and are quite likely to have negative impacts on summer/autumn and annual production. This is, of course, in the context of ambient temperatures experienced at the study site, where the +3°C warming treatment resulted in an additional 29/10 days when canopy temperatures exceeded 35°C (in summer 2018/19 and 2019/20, respectively) and a reduction in the number of days with winter minimum temperatures below 5°C (43 fewer days in 2018/19 and 32 fewer in 2019/20).

6. *Plant nutritional quality was altered by climate extremes, with species-dependent gains and losses.* Drought resulted in significantly decreased crude protein concentrations for Biserrula, Lucerne and Phalaris, but significantly increased concentrations for Digit and Fescue. The concentration of digestible dry matter increased significantly in Digit, Kangaroo and Rhodes under drought. Warming also resulted in higher summer/autumn concentrations of crude protein (Kangaroo, Phalaris) and digestible dry matter (Kangaroo, Lucerne), partially offsetting yield reductions. Treatment effects on digestibility appear to reflect a decrease in the fibre content and slower growth rates (and thus higher leaf to stem ratios) in droughted plots.

7. *Elevated CO_2 (eCO_2) concentrations increased productivity of tropical legumes.* Productivity was significantly greater under eCO_2 for Desmodium (+38%) and there was a non-significant increase of 45% for Burgundy bean. Tropical grasses were more productive and had higher tissue nitrogen concentrations when growing alongside a companion legume but did not experience a significant

growth benefit from eCO_2 either in monoculture or in a grass-legume mix. Tissue nitrogen concentrations were uniformly lower in eCO_2 compared to ambient conditions, suggesting that productivity gains may be offset by declining protein contents as atmospheric CO_2 continues to rise.

Key research findings for industry

8. *Emissions of nitrous oxide (N_2O) – one of the most potent greenhouse gases - increased under drought and warming.* Fescue switched from being a net sink to a net source of N_2O under drought, while in Lucerne emissions of N_2O were near zero under control conditions but increased substantially under both drought and warming. The challenge of reducing the unintended environmental consequences of pasture production is likely to increase under climate change, and there is a clear need to consider management strategies aimed at reducing greenhouse gas emissions.

9. *Plant-microbe interactions are altered by drought and warming.* Under drought conditions, legume nodule activity and associated transfer of biologically-fixed nitrogen to Lucerne were reduced. The resulting decrease in tissue nitrogen concentrations suggests that legume fertiliser requirements may be increased under drought. Mycorrhizal colonization was generally higher in temperate grasses than tropical grasses, although the latter increased significantly in response to drought. This suggests that tropical species' capacity to increase investment in mycorrhizal symbionts is an important part of their drought-resistance strategy and, therefore, that soil biology plays an important role in plants' climate responses.

10. *Arbuscular mycorrhizae were able to reduce the extent of temperature-driven increases in soil nutrient leaching and to offset increased N_2O production* from Lucerne (but not Fescue) soils. The lack of such benefit in Fescue exposed to elevated temperatures reflects the negative effect of warming on mycorrhizal colonisation for this species. These findings highlight the need to consider soil health and the attendant beneficial microbes as a core part of pasture management to achieve productivity, nutritional and environmental aims under more extreme future climates.

11. *Below-ground carbon allocation strategies in response to drought and warming varied considerably between species*. Whilst root productivity was broadly reduced by drought, there were considerable differences among species in terms of their relative allocation of carbon to roots (compared to shoots) and in their root morphological responses to winter/spring drought. Tropical grasses tended to increase their relative investment in roots (compared to aboveground plant material) and increased the level of root mycorrhizal colonization in droughted plots, compared to controls. Some species (e.g. Ryegrass, Digit, Rhodes) altered their root traits towards a more (resource) acquisitive morphology in response to drought, implying an increase in their ability to access water and nutrients as soil water availability declines, relative to species whose root traits were unchanged.

12. *Plant carbon fixation and water use strategies provide insights into species differences in sensitivity to climate extremes*. Rhodes and Digit had high rates of photosynthesis but were operating closer to their hydraulic safety margin and thus have a more "risky" strategy, making them potentially more vulnerable to prolonged, or particularly severe drought. Wallaby and Kangaroo had lower maximum rates of photosynthesis but were functioning with a high margin of hydraulic safety and thus can be considered to have conservative strategies in relation to water use, and hence relatively high resistance to hydraulic failure. Other species were intermediate in strategy between these groups. The "risky" strategy adopted by Rhodes does not explain the relatively small impacts of winter/spring drought on

this species' productivity, but would render it increasingly vulnerable to declines in summer rainfall and/or particularly intense spring drought.

13. *Identifying relationships between multiple above- and belowground plant traits (i.e. suites of traits) and climate sensitivity can inform species and cultivar choice for future, more extreme climates*. We showed trait differences between those grasses that were most resistant (Rhodes) and most sensitive (Ryegrass, Fescue) to the first year's winter/spring drought. Across species, denser root tissues were associated with smaller productivity declines under drought; root tissue density may reflect greater carbohydrate storage as a mechanism of resistance to drought, although data would be required to test this vigorously across a range of species and cultivars. Further work to determine which suite of plant traits are associated with resistance and resilience to climate extremes will help inform species and cultivar choice, as well as breeding programmes for new cultivars that can perform well under future climates.

14. *Many of the physiological and morphological traits that are used to parameterise pasture models exhibited distinct shifts in response to prolonged drought and/or warming*. Incorporation of such trait plasticity (e.g. for maximum photosynthetic rate or root:shoot ratios, both of which were altered by climate treatments in this study) into pasture models such as DairyMod and GrassGro would help improve the predictive power of these important industry tools.

15. *Temperate grass species that play an important role in Australia's southern livestock and dairy industries (Ryegrass, Phalaris and Fescue) experienced large productivity declines under drought and warming*. These findings highlight the vulnerability of three widely used pasture grasses to shifts in seasonal rainfall patterns and, for two species, modest increases in mean, maximum and minimum temperatures. Predicted increases in the frequency and intensity of rainfall and temperature extremes may require consideration of a broader suite of alternative, including tropical, forage species to support industry needs under future climates. The combined drought and warming treatments we applied to plants growing under ambient temperatures and seasonally-explicit mean rainfall conditions in Richmond, NSW are similar to those experienced in a low rainfall year in Gatton, Queensland (e.g. in 2019, when winter+spring rainfall was 81% lower than the 30-year mean seasonal rainfall for this location).

Future research needs

16. Mixed perennial pastures, including tropical and temperate grasses and legumes, have the potential to offer "climate insurance" through increased resource use efficiency and facilitation. However, more work is needed to identify which species combinations offer the best opportunity to optimise multi-season production and digestibility, particularly under prolonged and/or repeated periods of climate stress.

17. Given the importance of forage legumes within the meat, livestock and dairy industries, a key question is how will traditionally-used legumes perform under future climates? This is crucial not only for a sustainable feed base, but also for the role legumes can play in reducing the carbon and nitrogen footprints of the industry.

18. Identification of new legumes that can maintain productivity under severe drought and heat *in southern pasture systems* is a key research need. Deep-rooted, climate tolerant perennial legumes (e.g.

Desmanthus) could enhance soil carbon sequestration (via increased belowground productivity and/or shifts in root traits), reduce fertiliser requirements (via sustained biological nitrogen fixation and transfer to companion grasses) and potentially also reduce ruminant methane emissions (via increased digestibility), all of which are key topics for further research.

19. Expanding morphological, chemical and physiological trait data collection to include more species/cultivars will facilitate a deeper understanding of trait-function relationships for pasture species. This project has collected above- and belowground trait data for a sub-set of species and begun to look at trait-function relationships. However, a more comprehensive evaluation, involving additional species and cultivars, has the potential to provide valuable insights to inform industry climate adaptation strategies.

20. Commercially available smart fertilisers and microbial amendments have the potential to optimise pasture performance and reduce nutrient leaching and greenhouse gas emissions. Research is needed to evaluate the efficacy of these smart fertiliser formulations under future, more extreme climates.

21. Given the focus on temperate species for evaluating the effects of warming in this study, and findings of negative impacts on growth, research into tropical species' responses to increased temperatures is needed, especially given that these may become more widely grown in southern pasture systems in the future.

22. High temporal resolution of plant responses to heatwaves can identify tipping points of soil water content or cumulative days of excessive heat beyond which plant death occurs. Detailed examination of phenology camera and plant tiller data from this project, combined with similar information for additional species, offers the possibility of pinpointing climate thresholds beyond which plant recovery is not possible.

In conclusion, this project has generated new knowledge of species' responses to two important aspects of climate change – altered rainfall regimes (specifically severe winter and spring drought) and increased temperatures. These findings, together with new insights into the carbon allocation and water use strategies that the different species employ when exposed to prolonged drought and warming, provide a basis for *more informed species selection to maximise pasture productivity and nutrition under future, increasingly extreme climates*. The project has addressed several important knowledge gaps in relation to climate risk management, and identified key areas for future empirical and modelling research to guide industry adaptation in the context of climate extremes.

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1 Background

Sustainable and profitable management of productive pasture systems is a key priority for the dairy and livestock industries, and understanding the impact of future climates on pasture performance is central to this endeavour. Global climate change potentially threatens pasture production through changing temperature and rainfall patterns and increasing frequency of extreme weather events. Much is already known, or can be predicted, about how pastures will respond to modest, incremental changes in temperature, altered rainfall and/or elevated CO_2, based on information from single-factor experiments, meta-analyses and models. For example, warmer temperatures are expected to increase pasture production in cool temperate regions (Cullen et al., 2009), while an increase in temperature of 3^oC is predicted to decrease forage production in northern Australian grasslands (McKeon et al 2009). Drought, in turn, plays a particularly important role in pasture dynamics and can lead to long-term degradation of pasture lands (Briske *et al.*, 2005) and a shift towards allocation of biomass belowground at the expense of aboveground forage (Eziz et al. 2017). Plant nutrient uptake and thus pasture quality can also be expected to change in response to climate change (Dijkstra et al., 2015). Drought most commonly increases protein content and improves digestibility of pastures (Dumont et al. 2015) while warming has been found to alter forage quality, with both increases and decreases reported.

A consistent feature of climate model predictions is for more extreme climates, including a greater frequency and severity of droughts, heatwaves and flooding in the future (IPCC, 2013). However, to date, very little research has focused on climate extremes, and even fewer studies have involved combinations of more than one climate driver. In fact, extreme climatic conditions are predicted to become more frequent and more intense in the future, such that what we currently think of as "extreme" is likely to be considered part of normal background climate fluctuations in the coming decades (Bahn *et al.,* 2014). It is also clear that responses to different climate factors are not necessarily independent. For example, effects of elevated temperature depend strongly on background temperatures (Drake *et al*., 2015) and are contingent on water availability (Pendall *et al.,* 2013). Understanding of how future changes in temperature and rainfall regimes affect the productivity, phenology, nutritional quality and resilience of pasture systems is a key knowledge gap that must be addressed in order to develop robust climate adaptation strategies that will underpin sustainable, profitable livestock and dairy industries for the future.

Currently, sowing and management recommendations for pastures across Australia are based on performance data from field trials under contemporary climates, and long-term records of yield in relation to prevailing climate conditions and soil type. It is crucial, however, to establish how traditional and emerging pasture species – both singly and in mixtures - perform under more extreme climates, to inform optimal species selection for the future. Knowledge of how plant-microbial interactions – particularly for N-fixing bacteria and mycorrhizae - are influenced by more extreme temperatures and prolonged or severe drought is also important for managing climate risk.

In light of the knowledge gaps surrounding pasture species' responses to climate extremes we established the **PA**stures and **C**limate **E**xtremes (PACE) experimental facility in 2017, to help inform climate risk management strategies for the meat, livestock and dairy industries. The PACE project exposes nine grass and legume species, and three two-species mixtures, to a simulated severe winter+spring drought, in combination with a year-round 3^oC increase in temperature (collectively

referred to as "climate extremes"), under field conditions. We focused on quantifying productivity and nutritional responses, along with evaluation of the traits and processes associated with climate sensitivity. New knowledge of species performance under more extreme climates, together with improved understanding of the mechanisms underpinning responses, can be used to inform industry adaptation strategies aimed at minimizing the negative impacts of future climates on pasture systems.

The field project was complemented by glasshouse experiments evaluating the role of mycorrhizal fungi in plant response to warming and drought, and the impacts of elevated CO_2 on grass-legume interactions for tropical pasture species.

2   Project objectives

- Provide the knowledge base to inform a robust adaptation framework for pasture management under future, more extreme climates
- Identify opportunities to increase climate resilience and to minimise future industry exposure to climate-related risk

3   Methodology

This project combines a world-first Pastures and Climate Extremes (PACE) field facility investigating pasture responses to future climate scenarios, with glasshouse experiments aimed at understanding some of the mechanisms driving field responses as well as testing conditions that are more difficult to implement in the field (elevated CO_2). All experiments were carried out at the Hawkesbury Campus of Western Sydney University, in Richmond, NSW. Detailed descriptions of facilities and experimental design are located in previous Milestone reports and in associated scientific publications (Churchill et al., in progress).

3.1   Field methods

The Pastures and Climate Extremes (PACE) field facility comprises six, replicate open polytunnel shelters, each with eight treatment plots (**Fig 1**). Treatment plots are subdivided in to four subplots containing either a single pasture species or a two-species mixture (total 192 subplots). Plant species encompass a range of functional groups (temperate-C3/tropical-C4 grasses, legumes) and origins (native and exotic). All pastures were initially established during 2017/18; annual species, as well as those species that were strongly affected by the 2018 drought treatment, were re-seeded in autumn 2019.

Experimental treatments include an extreme drought, comprising a 60% reduction in winter and spring rainfall. This reduction is in line with the upper end of climate predictions for southern and eastern Australia, as well as being within the 1-in-100-year frequency for past climate records in many southern and southeastern pasture regions. In essence, this project therefore evaluates the impacts of severe winter/spring drought, such as has already occurred periodically in the past but that is ***predicted to become a more consistent feature of future climates*** across SE Australia by the end of the century. Importantly, we initiated the winter/spring 2018 drought treatment after individual swards for each pasture were considered to be fully established, resulting in staggered starting times and durations of drought in the first year; this meant that some species received a full 6-month drought (Fescue, Digit, Kangaroo, Rhodes), while others had a 3-month (Lucerne, Ryegrass, Biserrula) or 6-week (Phalaris,

Wallaby) drought in 2018. All species received a full six-month drought in 2019. Rainfall regimes in control (non-droughted) plots were based on long-term, seasonally-explicit averages for both the timing and size of rain events in the local area. Four pasture species/mixtures (Fescue, Lucerne, Phalaris/sub-clover and Kangaroo/Wallaby) were exposed to year-round elevated temperature (+3°C) treatments achieved using infra-red heating lamps, in combination with the winter/spring drought. We monitored soil moisture to track the effects of treatments on available water, as well as measuring soil and canopy temperature, air temperature, relative humidity and photosynthetically active radiation.

Fig 1. *Interior of a shelter at the PACE facility showing different pasture subplots. The IR heating array (with ceramic lamps - red arrow) for the + 3 °C treatment is visible in the centre of the image.*

Table 1. *Details for pasture species and species combinations within the PACE experiment.*

Common name*	Species Name	Cultivar	Growth Form	Photosynthetic pathway	Monoculture	Mixed Sward
Biserrula	*Biserrula pelecinus*	Casbah	Legume	C3	Y	Y- Digit
Digit	*Digitaria eriantha*	Premier	Grass	C4	Y	Y- Biserrula
Tall Fescue	*Festuca arundinacea*	Quantum II MaxP	Grass	C3	Y	N
Kangaroo	*Themeda triandra*	Badgerys Creek, NSW	Grass	C4	Y	Y-Wallaby
Lucerne	*Medicago sativa*	SARDI 7 series2	Legume	C3	Y	N
Phalaris	*Phalaris aquatica*	Holdfast GT	Grass	C3	Y	Y- Sub clover
Rhodes	*Chloris gayana*	Katambora	Grass	C4	Y	N
Perennial Ryegrass	*Lolium perenne*	Kidman	Grass	C3	Y	N
Sub clover	*Trifolium subterraneum*	Campeda	Legume	C3	N	Y- Phalaris
Wallaby	*Rytidosperma caespitosum*	Evans	Grass	C3	Y	Y- Kangaroo

All species are referred to by their common name throughout the text, with Tall Fescue shortened to Fescue and Perennial Ryegrass shortened to Ryegrass.

Data collected during the first two years of the project, and associated methodologies, have been reported in previous Milestone reports. This Final Report focuses on above- and below-ground biomass responses, treatment effects on plant nutritional quality and a mechanistic evaluation of physiological and carbon allocation strategies, for both the first and second years of the project.

For the aboveground biomass we assessed macro- and micro- nutrient concentrations and elemental stoichiometry, along with metrics of nutritional quality including concentrations of protein, cellulose, carbohydrates and plant dry matter digestibility. Digestible dry matter (DDM) was calculated using the formula: DMD = 88.9- (0.779*Acid detergent fibre percentage of dry matter); Linn and Martin, 1989). We also report new data on soil nutrient availability and nitrous oxide fluxes, methods for which are outlined in brief, below.

Ion exchange resins were used to gain an integrated measure of NO_3, NH_4 and PO_4 availability over time (Giblin et al., 1994). Resins bags were deployed (10 cm depth) at monthly intervals from July 2019 to March 2020. Data presented here represent the cumulative N and P availability for the winter-spring 2019 drought period. Responses of nitrous oxide (N_2O) fluxes and soil nutrient availability to warming and drought were assessed in Lucerne and Fescue plots from July 2018 to June 2019. Fluxes were measured via a standard, static chamber technique (Kim et al., 2019) on a monthly basis. The N_2O concentrations were analysed with gas chromatography (Agilent 7890A; Agilent Corp., USA) equipped with electron capture detector (ECD) and headspace auto sampler (Teledyne Tekmar, USA).

Physiology measurements were carried out between September 2018 and February 2020. Gas exchange and hydraulic measurements were carried out on individuals within each plot (of the species studied) to analyse treatment effects and also on individuals transplanted into pots (from the control plots in the field) to analyse the physiological response of each species to a continuous dry-down. Hydraulic vulnerability measurements were conducted in June 2019 (for cool season species) and February 2020 (for warm season species) where individuals were uprooted from control plots and dehydrated under laboratory conditions.

Throughout this report there are a number of common calculations and statistical analyses used to present findings. These include reporting estimates of the "effect size" of change associated with drought or warming treatments and "sensitivity" of productivity to drought. All references to effect size have been calculated using equation 1, below:

$$Equation\ 1. \quad Effect\ size = \frac{(Drought\ value - Control\ value)}{Control\ value} * 100\ \%$$

For pastures also exposed to warming or drought and warming, we present effect size as the difference from the control-unwarmed pasture (Con-aT) for pairwise comparisons. Negative values therefore indicate a reduction from control levels in response to drought for that particular pair of droughted and control plots (i.e. either warmed or ambient pairs).

All statistical analyses associated with the field experiment use linear mixed effects models to examine whether drought and/or warming resulted in a significant treatment effect, relative to control plots (Pinheiro et al., 2014). For these analyses drought application and/or warming was included as a fixed effect, and shelter identity - associated with the location of sample collection - was included as a random effect to account for any spatial patterns across the site; all analyses were conducted using the R package lme4 (Bates et al., 2015). Individual measurements were checked for assumptions of normality and appropriate transformations were applied as necessary. For example, all aboveground

biomass measurements were natural log-transformed for statistical analyses to account for unequal variance between control and drought plots.

Over the course of the field experiment we measured a wide range of above- and belowground traits that are associated with plant morphology, chemistry, and strategies relating to carbon fixation and allocation, and water acquisition and use. We have used these data in a multi-trait analysis – based on 2018 control plot trait values - to examine whether there are particular traits or trait combinations that are associated with relative sensitivity/resistance to drought/warming amongst the species examined. Variation among species in these multiple traits was summarized using principal components analysis (PCA) on species-level trait values. Then we test a central question of this project— whether interspecific variation in these traits drives species differences drought sensitivity. Such trait-sensitivity relationships may be helpful in identifying species and/or cultivars - beyond those directly examined in the field and glasshouse experiments - that are more vulnerable to predicted changes in climate. Future work will extend these metrics to include 2019 trait data.

3.2   Glasshouse experiment 1: Warming, drought and the role of mycorrhizal fungi

The first PACE glasshouse experiment focused on warming and drought treatments alongside experimental manipulation of the soil community. The experiment was conducted in glasshouse chambers at Western Sydney University's Hawkesbury campus, New South Wales and involved two species grown at the PACE field facility - Fescue and Lucerne. Temperature treatments for this study included an ambient regime (aT; 26/18 °C day/night 15:9 light/dark cycle) and an elevated temperature regime of + 4°C (eT; 30/22 °C day/night, also with a 15:9 light/dark cycle). The ambient regime represents the average daily maximum temperature for Richmond, NSW over the last 30 years, and the elevated regime is consistent with the predicted end-of-century increase in maximum temperature of 4 °C for this region (CSIRO, 2018). Drought treatments were applied after plant recovery from a mid-experiment aboveground harvest conducted in June 2018 (see previous Milestone reports for details). The drought regime exposed plants to a gradual decline from well-watered (field capacity) to ~40 % of field capacity, which was maintained for one week before re-wetting to well-watered conditions. This was followed by a short period of re-watering (to field capacity, for one week), followed by a second drought that lasted 12 days with the VWC maintained at 40% of field capacity.

In addition to drought and warming treatments, this experiment also included manipulations of the soil microbial community, full details of which can be found in the June 2018 Milestone Report. In brief, all soils were sterilized to eliminate indigenous arbuscular mycorrhizal fungi (AMF) and other soil microoganisms, as well as adding mycorrhizal inoculum to half of the pots in a factorial design. This resulted in two soil community treatments; sterile soil with mycorrhizae added (M), sterilized soil with no additional mycorrhizae (nM). In addition, rhizobial bacteria (*Easyrhiz*, strain AL) were added to all Lucerne pots to promote legume nodulation, as is standard procedure for this species in the field. Sample collection associated with this experiment ran April-August 2018 and included aboveground and belowground biomass, greenhouse gas fluxes, and soil nutrient leaching.

3.3 Glasshouse experiment 2: eCO$_2$ and tropical grass/legume mixtures

The second glasshouse experiment, also conducted at Western Sydney University, focused on the effects of elevated CO$_2$ on tropical grass-legume nutrient facilitation. We used six glasshouse chambers – three at ambient CO$_2$ conditions (420 ppm; aCO$_2$) and three with an elevated CO$_2$ regime (630 - 650 ppm; eCO$_2$, consistent with predicted end-of-century concentrations – Szulejko et al., 2017). Glasshouse chambers were maintained at a consistent temperature equating to a daily average of 27°C (00-06 hours: 22°C, 06-09 hrs: 27°C, 09-18 hrs: 32°C, 18-21 hrs: 27°C, 21-00 hrs: 22°C) and all pots were well watered. Tropical species included two grass-legume pairs: Rhodes-Burgundy Bean (*Chloris gayana* and *Macroptilium bracteatum*) and Panic-Desmodium (*Panicum maximum* var. *trichoglume* and *Desmodium intortum*); both legumes' seeds came pre-inoculated for appropriate rhizobia (Heritage Seeds, Australia). Two individuals were transplanted into each pot - either two of the same species or one each of the associated species in a mixture. The experimental design included two CO$_2$ treatments (ambient and elevated), six types of plant combinations in pots (single grass$_1$, single legume$_1$, mixed$_1$, single grass$_2$, single legume$_2$, mixed$_2$), and twelve replicates per treatment (four replicates per chamber).

Sample collection for this experiment occurred from Sept-Dec. 2018. Measurements included above and belowground productivity, specific leaf area and greenness (presented in the July 2019 report). A sub-set of leaves were used to measure leaf area and then weighed to derive specific leaf area (SLA). We also measured plant nutritional quality and stable isotope signatures (δ^{15}N) on aboveground material for each species, in monoculture and mixture.

4 Results

4.1 Pastures and Climate Extremes (PACE) field experiment

4.1.1 Environmental conditions

All pastures received the same duration and intensity of drought (six months of -60% rainfall, compared to controls) in the second year and, in general, showed similar patterns of soil moisture throughout the winter/spring period. Soil water contents in warmed plots differed slightly from ambient plots across the six-month drought period. Following precipitation events in early September 2019, the warmed plots for Fescue had lower soil moisture than ambient ones (**Fig 2A**). In contrast, from that point onwards, warming was associated with higher soil moisture for Lucerne, until plants were harvested in mid-November (**Fig 2B**). It is worth noting that for both Fescue and Lucerne, periods when soil moisture contents were higher in warmed, compared to ambient temperature, plots corresponded with reduced aboveground biomass and, therefore, likely reduced transpirational water loss from these plots.
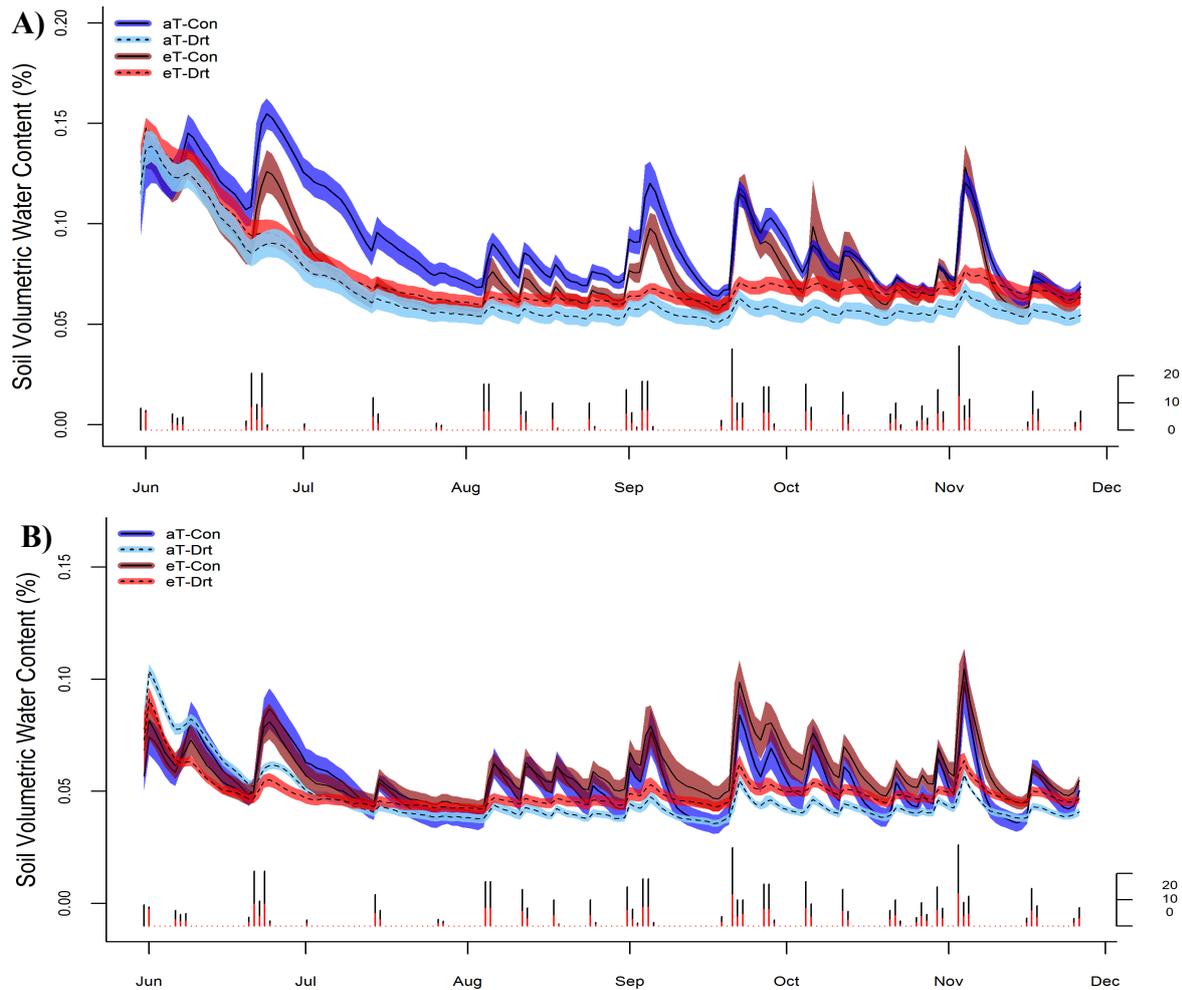
Fig 2. Soil moisture in the upper 0-10 cm during the winter-spring drought period in 2019 in A) Fescue and B) Lucerne pastures experiencing both drought (Con/Drt) and elevated temperature (aT/eT). Values shown are treatment means with a 95% confidence interval. The lower insert in both panels shows individual applied precipitation events for the drought (red bars) and control (black) treatments.

Of further note is that the level of drought imposed in this experiment – a 60% reduction in winter+spring rainfall, compared to the long-term average for the site – whilst considered to be at the more "extreme" end of climate predictions is by no means exceptional and has been recorded on several occasions in the past 100 years. Indeed, in 2002, the local field site received only 27% of the long-term winter+spring rainfall amount (i.e. a reduction of 73%). Furthermore, during the winter of 2019, our droughted plots received amounts of water (51.3 mm) that were very similar to ambient rainfall inputs in the local area (56.7 mm - on-site data). 2019 was the 7th driest winter in the past 30 years in Richmond, while 2018 (50.4 mm) was the 5th driest. This highlights that our drought treatment is very much in line with recent experiences of consecutive years of significantly below average winter rainfall (2018, 2019 - BOM).

The warming treatment achieved a +3°C increase in surface temperature consistently across the experiment (**Fig 3**). Winter daily average surface temperatures were generally lower in 2018 compared with 2019 (**Fig 3**; Appendix **Table A1**). While there were generally more days on which surface temperatures exceeded 35 °C in the 2018-2019 year (aT-Con: 112 days, eT-Drt: 168 days) than in 2019-2020 (aT-Con: 103 days, eT-Drt: 138 days), very hot surface temperatures (>45°C) were reached on

more days during the second year (Appendix **Table A1**). Soil moisture content for Fescue during the winter-spring drought period in 2018 is shown in Appendix **Fig A1**.
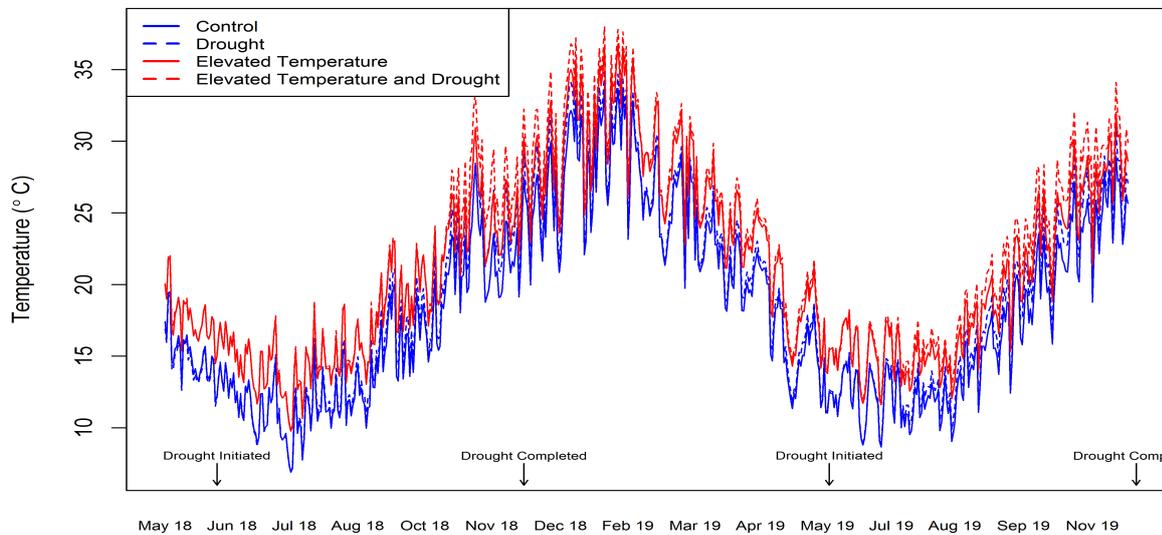
Fig 3. *Average daily surface temperature over the experimental period, in ambient and warmed plots*.

4.1.2   Productivity responses to drought and warming treatments

This section is presented first as individual harvest-level data from June 2018 to November 2019, then as summed responses for the two drought periods (winter/spring 2018 and 2019) and the recovery (summer/autumn 2019 and 2020) periods following consecutive droughts. Collectively, these data highlight both the phenology of drought and warming impacts and the magnitude of responses in terms of cool-season, warm-season and annual production (expressed on a dry weight basis).

4.1.3   Temporal patterns in aboveground biomass for individual harvests

Harvests were conducted when individual species reached pre-specified heights or growth stages, following the Pasture Trials Protocol (Kemp et al., 2011); this resulted in between six and nine harvests per species over the period from June 2018 to January 2020. **Figs 4-6** illustrate temporal patterns in productivity and the magnitude of treatment effects at each harvest, across the 2-year period. Annual species such as Biserrula (**Fig 5A**) and Ryegrass (effectively an annual in Richmond, due to high summer temperatures; **Fig 5B**) each had six harvests, while the Digit-Biserrula mixed pasture was harvested a total of nine times, reflecting growth phenology differences between constituent species (**Fig 4B**).

Annual productivity in the first year was greatest in Kangaroo and Kangaroo-Wallaby mixed species plots (17,384 and 16,045 kg ha^{-1}, respectively). This was followed by Lucerne and Digit (11,201 and 10,507 kg ha^{-1}) and Rhodes and the Digit-Biserrula mixed pasture (9,664 and 9,069 kg ha^{-1}). Temperate grasses produced substantially less biomass overall, with values ranging from 7,790 kg ha^{-1} (Wallaby grass) to 5,074 kg ha^{-1} (Fescue). Productivity in the second year of the experiment was higher for some species (e.g. Biserrula, Ryegrass and Lucerne) but lower for others (e.g. Kangaroo, Phalaris, Wallaby). Clear summer productivity peaks are evident for C4 grasses, although Kangaroo grass also performed well in control plots in early spring 2019 (**Fig 4C**). Peak biomass for temperate species differed slightly between years; for annual species that were re-sown in 2019 (Biserrula and Ryegrass), earlier biomass peaks in winter 2019 reflect earlier sowing dates, compared to the previous year (**Fig 5**). Lucerne exhibited an

increase in productivity over time in control plots and was one of only two temperate species (the other being Fescue) that performed strongly (in control plots) during the recent, very warm summer.

Drought significantly reduced biomass during the second (2019) winter/spring drought period in nearly all species (**Figs 4-6**). Carry-over effects of the second drought were, however, minimal in summer 2020, indicating rapid recovery from the biomass reductions that were evident at the end of spring. Rhodes actually had significantly greater productivity in formerly droughted plots in summer 2020, indicating compensatory growth once rainfall returned to control levels.
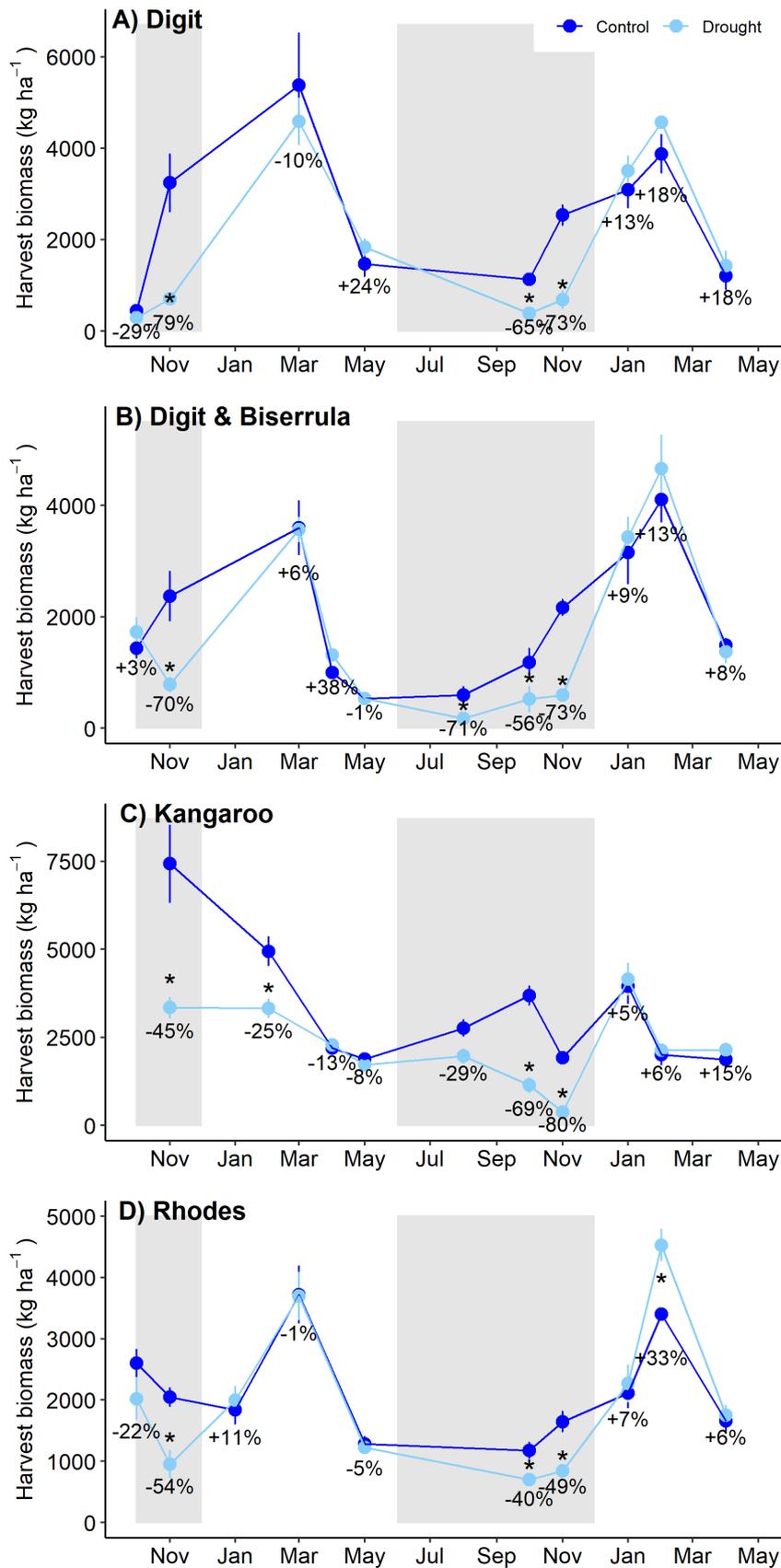
Fig 4. *Aboveground dry biomass associated with individual harvest events June 2018-April 2020 for tropical species, including A) Digit, B) Digit-Biserrula mix, C) Kangaroo grass and D) Rhodes. Values shown are means ± 1 SE, with drought-related effect sizes on yields also indicated for each harvest. Significant (p < 0.05) reductions are indicated with *. The drought period - June 1st - Nov 30th - is indicated by grey shading.*
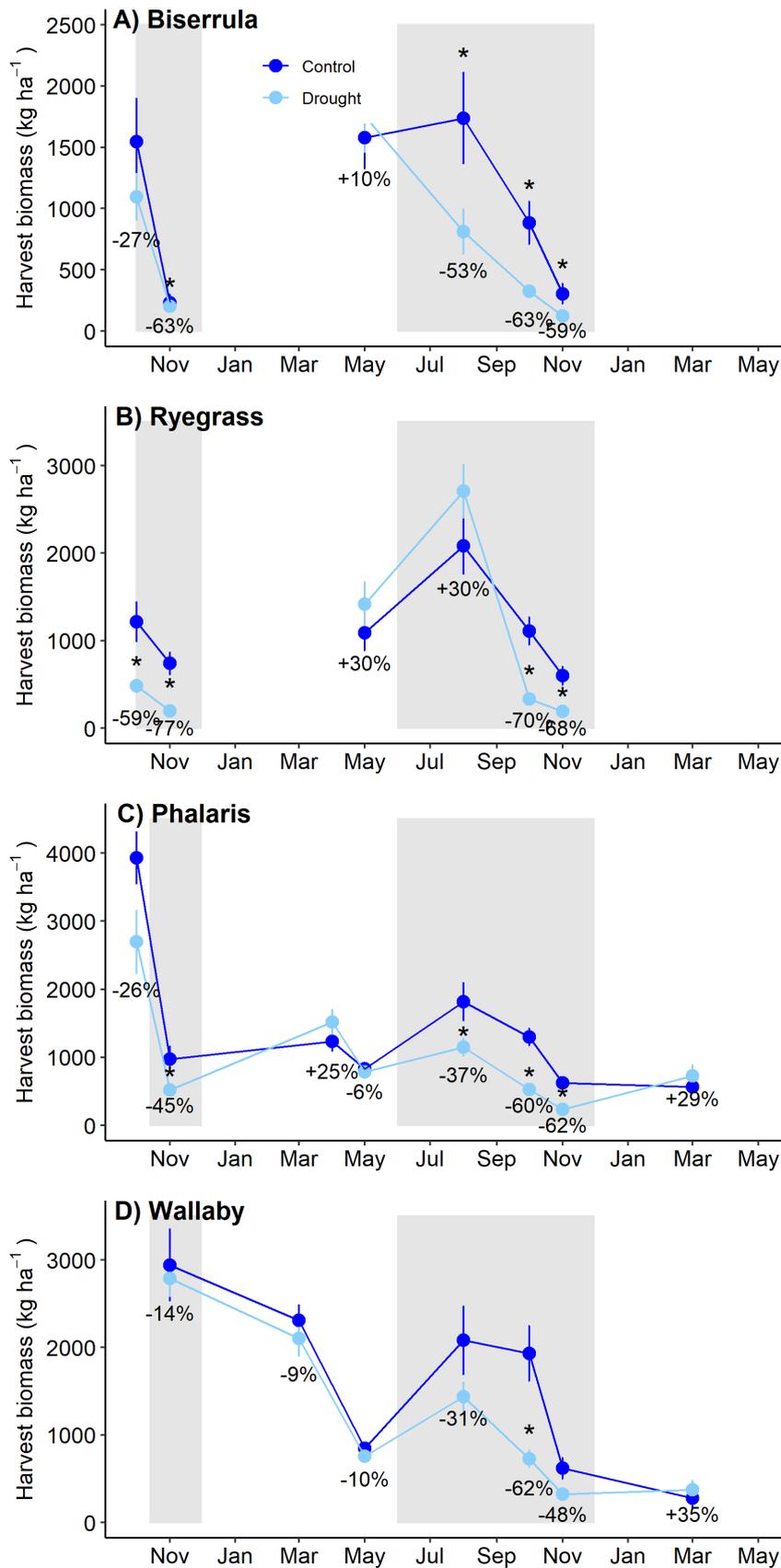
Fig 5.
*Aboveground dry biomass associated with individual harvest events June 2018-March 2020 for temperate species, including A) Biserrula, B) Ryegrass, C) Phalaris, and D) Wallaby grass. Values shown are means ± 1 SE, with drought-related effect sizes on yields also indicated for each harvest. Significant (p < 0.05) reductions are indicated with *. The drought period - June 1st - Nov 30th - is indicated by grey shading.*
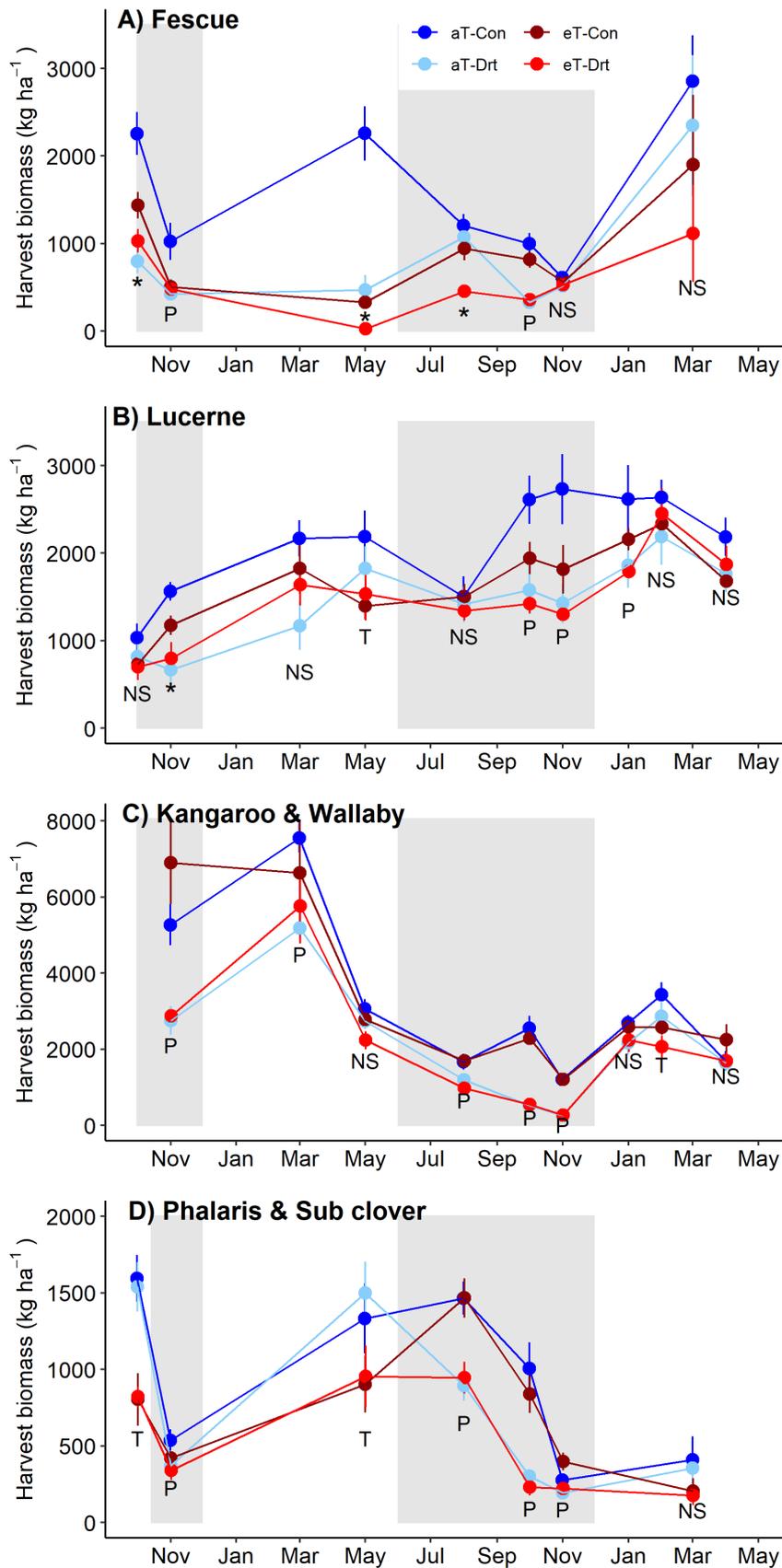
Fig 6.

Aboveground dry biomass associated with individual harvest events June 2018-April 2020 PACE pastures exposed to both drought and warming, including A) Fescue, B) Lucerne, C) Kangaroo and Wallaby grass mixed pasture, and D) Phalaris and Sub clover mixed pasture. Values shown are means ± 1 SE, with drought-related effect sizes on yields also indicated for each harvest. Significance codes are as follows: P= precipitation effect, T = temperature effect, and * = P x T effect. The drought period - June 1st - Nov 30th - is indicated by grey shading.

4.1.3.1 Aboveground biomass responses under drought and warming and associated post-drought "recovery"

Year 2 responses

Aboveground biomass responses to the second year of winter/spring drought and continuous warming, were diverse across species/mixtures (**Fig 7**). All except Ryegrass experienced a significant reduction in the total amount of biomass produced during the 6-month drought period, with decreases ranging from -71% (Digit) to -35% (Fescue) (**Table 2**). Productivity in droughted Ryegrass plots was 15% lower than control plots across the May-November period in 2019, **Fig 7,** (compared to 48% lower in 2018); this reflects strong winter growth that partly offset the substantial drought-associated reductions (of up to -70% for individual harvests) seen during spring (**Fig 5**).

There was no evidence of any species/mixture responding positively to the 3°C warming across the second winter/spring period. Warming resulted in a significant (-18%) reduction in Fescue's 2019 cool-season biomass production (**Fig 7A**, **Table 2**), as well as an 19% reduction in summer/autumn 2020 productivity (**Fig 6**). A non-significant (-23%) reduction in winter/spring biomass was also observed for Lucerne exposed to elevated temperatures; this species also had lower (-29%) summer/autumn productivity in 2020, although this reduction was also not statistically significant (**Fig 7B, Table 2**). Overall, species responses to a second year of warming treatment were consistent with findings in 2018 and provide strong evidence that warmer winter/spring temperatures will not be associated with increased cool-season growth, but that some species will be detrimentally affected by this change, particularly during the warmer months.
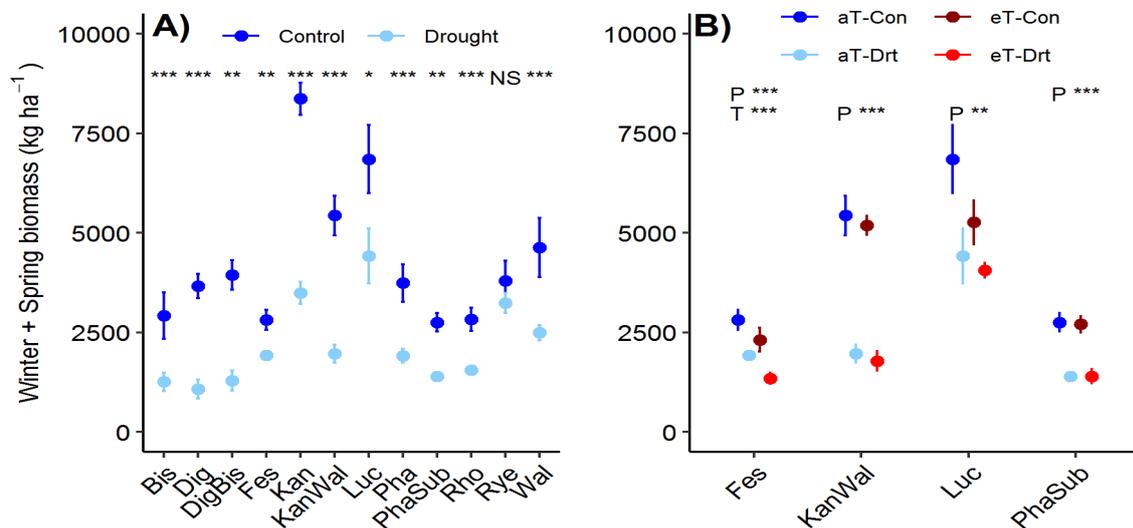
Fig 7. Aboveground dry biomass of droughted and control plots, summed over the second six-month period of drought treatment (June-Nov 2019, year 2) for A) all species/mixtures exposed to drought and B) species/mixtures exposed to both drought (Con/Drt) and warming (aT/eT) treatments. */**/*** represents significant drought treatment effects at the p<0.05/0.01/<0.001 level of significance while NS = not significant. P/T indicate significant precipitation/temperature effects, respectively. Species abbreviations are as follows: Biserrula (Bis), Digit (Dig), Digit & Biserrula mixture (DigBis), Fescue (Fes), Kangaroo (Kan) , Kangaroo & Wallaby mixture (KanWal), Lucerne (Luc), Phalaris (Pha), Phalaris & Sub clover mixture (PhaSub), Rhodes (Rho), Ryegrass (Rye) and Wallaby (Wal).

Comparison of drought responses between years

Three species experienced similar levels of drought-associated biomass reduction in winter/spring productivity between years (**Table 2**), namely Digit (strongly affected both years), Kangaroo (moderately affected both years) and Lucerne (moderately affected both years, despite the difference in duration of applied drought). Those species/mixtures that had greater reductions in productivity during the second year's drought were generally those that had been exposed to only a short drought period during the first year. With the exception of Wallaby, these were plots that included annual legume species, suggesting that annual legumes may be particularly sensitive to the duration of drought, compared to perennial species.

Two species experienced substantially smaller proportional responses to drought during the second year of treatment - Fescue and Ryegrass (**Table 2**). For Ryegrass, this may reflect earlier seeding in 2019 than in 2018 (n.b. perennial ryegrass functions as an annual in Richmond NSW, due to high summer temperatures), such that a greater proportion of this species' growth occurred during the cooler part of the year, when soil moisture was high and VPD was low. It is also noteworthy that droughted Ryegrass plots actually had higher productivity than control plots in the early part of the winter 2019 (see detailed phenology in **Figure 5B**, and discussion in **Section 4.1.2.2**, below), which offset strong drought-associated declines in aboveground biomass production in spring 2019. Smaller drought impacts on Fescue productivity in 2019, compared to 2018, may reflect this species' adaptation following repeated drought exposure, as well as, potentially, the opportunity to develop a deeper rooting system over time.

Recovery from drought ("Resilience") and the role of warming

The effects of drought did not generally persist beyond the end of spring (**Table 2**), with most species showing rapid recovery of aboveground production once drought treatments ended (i.e. high resilience); indeed, Rhodes had significantly greater (+18%) summer/autumn productivity in previously droughted plots in 2020. For Fescue, while there were large, significant legacy effects of drought on 2019 warm-season productivity, which were exacerbated by warming, there were no significant carry-over effects of either treatment in 2020.

Although the biggest reductions in summer/autumn biomass were generally seen in warmed and previously droughted plots, treatment interactions were only significant for Fescue in 2019 (where the combined treatment resulted in a less-than-additive reduction in biomass).

Taken together, findings from the two years of drought and warming treatments suggest that most perennial species in this study were resilient in the face of prolonged, severe drought, such that plots generally returned to control levels of productivity over a period of 2-6 months once the drought treatment ended. This may in part have been facilitated by higher soil N content in droughted plots at the end of the drought period being available to support growth once the drought was alleviated, as was observed during the 2019 winter-spring drought period (**Fig A5**). Our findings also indicate that +3°C warming did not generally affect drought recovery (i.e. resilience) for those species/mixtures exposed to the combined treatments. The main exception was Fescue in the first year of the experiment, where there were very few live plants (low tiller density and associated very low biomass) after the first drought to support recovery in early 2019. However, this species did not have a significant carry over effect of the second winter/spring drought into summer 2020, indicating that it was better able to recover in the second year of this study.

Table 2. *Aboveground productivity in control plots and yield reductions under drought, during the two years of drought treatments (June-Nov 2018, June-Nov 2019), two subsequent 6-month "recovery" periods (Dec 2018-May 2019, Dec 2019-April 2019), and averaged for the full 1st year (June 2018-Many 2019) and 2nd year (June 2019-April 2020) of the experiment.*

Pasture	Treat-ment	1st Drought Jun-Nov 18 (kg ha⁻¹)	Effect size 1st drought	1st Recovery Dec18-May19 (kg ha⁻¹)	Effect size 1st recovery	2nd Drought (Jun-Nov19) (kg ha⁻¹)	Effect size 2nd drought	2nd Recovery Dec18-May19 (kg ha⁻¹)	Effect size 2nd recovery	Annual production Year 1 (kg ha⁻¹ y⁻¹)	Effect size (annual) Year 1	Annual production Year 2 (kg ha⁻¹ y⁻¹)	Effect size (annual) Year 2	Overall effect size##
Biserrula†	Drt	1776 ± 367	-11.9%	NA	NA	2924 ± 585	**-56.9%**	NA	NA	1776 ± 367	-11.9%	2924 ± 585	**-56.9%**	-42.9%
Digit	Drt	3683 ± 684	**-70.1%**	6844 ± 1374	4.3%	3662.9 ± 307.6	**-70.8%**	7524 ± 579	16.1%	10507 ± 1638	**-23.2%**	11187 ± 968	-11.3%	-17.0%
Digit/Biserrula†	Drt	3806 ± 540	**-30.5%**	5129 ± 642	13.4%	3940 ± 371	**-67.3%**	7818 ± 1249	8.2%	9069 ± 750	**-6.9%**	11758 ± 1492	-14.6%	-13.1%
Kangaroo	Drt	7433 ± 1110	**-52.6%**	9018 ± 334	**-18.1%**	8366 ± 404	**-58.3%**	7177 ± 1044	7.4%	17384 ± 485	**-34.3%**	15543 ± 1336	**-27.8%**	-31.3%
Phalaris‡	Drt	4907 ± 438	**-32.7%**	2064 ± 245	25.1%	3737 ± 469	**-48.9%**	562 ± 87	29.1%	5801 ± 680	**-18.1%**	4205 ± 510	**-38.2%**	-26.4%
Rhodes	Drt	4645 ± 371	**-36.8%**	6842 ± 747	2.9%	2822 ± 293	**-45.2%**	6328 ± 957	18.5%	9664 ± 980	**-14.9%**	9151 ± 1009	-4.7%	-9.5%
Ryegrass	Drt	2348 ± 327	**-48.5%**	NA	NA	3794 ± 510	**-14.8%**	NA	NA	2348 ± 327	**-48.5%**	3794 ± 510	**-14.8%**	-22.3%
Wallaby‡	Drt	2942 ± 417	14%	3158 ± 205	-9.4%	4631 ± 746	**-46.3%**	275 ± 130	35.1%	7790 ± 912	**-3.1%**	4860 ± 777	**-38.0%**	-19.8%
Fescue	Drt	3279 ± 418	<u>-59.2%</u>	2257 ± 310	<u>-81.1%</u>	2817 ± 251	**-31.9%**	2853 ± 523	-17.8%	5074 ± 508	**-67.8%**	5195 ± 835	-20.5%	-45.9%
	Wrm		<u>-38.4%</u>		<u>-83.4%</u>		**-17.8%**		-33.3%		-57.0%		-18.7%	-38.7%
	Drt*Wrm		<u>-52.3%</u>		<u>-98.2%</u>		**-52.3%**		-61.0%		**-71.1%**		-48.9%	-61.3%
Kangaroo / Wallaby	Drt	5270 ± 537	**-46.6%**	10612 ± 551	-24.5%	5433 ± 495	**-63.8%**	6956 ± 976	-15.0%	16045 ± 739	**-32.3%**	12389 ± 1013	**-34.6%**	-33.5%
	Wrm		32.2%		-10.3%		-4.5%		-5.1%		-2.7%		-3.8%	-1.3%
	Drt*Wrm		**-42.3%**		-25.5%		**-67.2%**		-22.7%		**-31.0%**		**-40.5%**	-35.3%
Lucerne†	Drt	3393 ± 400	**-25.7%**	4356 ± 416	-27.9%	6846 ± 854	**-35.4%**	6638 ± 1172	-22.4%	11201 ± 1225	**-27.0%**	13484 ± 1961	**-27.6%**	-27.7%
	Wrm		-9.1%		-23.8%		-23.1%		-17.1%		-17.4%		-15.1%	-16.6%
	Drt*Wrm		**-17.5%**		-23.4%		**-40.6%**		-18.7%		**-21.0%**		**-25.4%**	-23.3%
Phalaris/ Sub clover‡	Drt	3005 ± 121	-4.1%	1333 ± 230	25.0%	2747 ± 2312	**-49.4%**	409 ± 153	-13.3%	4080 ± 426	1.3%	3088 ± 345	**-43.0%**	-17.6%
	Wrm		*-27.3%*		*-29.1%*		-1.6%		-49.6%		-29.5%		-0.0%	-18.7%
	Drt*Wrm		*-34.7%*		*-19.0%*		**-49.1%**		-56.7%		**-32.4%**		**-50.2%**	-39.7%

NA refers to species that died back at the end of spring and therefore had no summer/autumn biomass; these species were re-sown in autumn 2019. Values in bold indicate significant effects of the drought treatment, italicised values indicate significant effects of warming treatments and underlined values indicate significant drought x warming treatment interactions. †Species/mixtures that received a 3-month drought during spring 2018; ‡Species that received 1 month of drought in spring 2018. ##The overall effect size is calculated as the overall % yield reduction across all harvests for the 2-year period June 2018 to May 2020.

4.1.4 Nutritional quality of pastures under drought and warming

4.1.4.1 Treatment effects on nutritional quality

The nutritional analyses presented here were performed on total aboveground harvested material (leaves, stems and flowers, where present), including a mix of both live and dead material. During the winter-spring drought period in 2018, crude protein concentrations (CP) were generally more strongly affected by climate treatments than the other nutritional traits **(Fig. 8A-B)**. Crude protein concentrations were significantly lower in drought-treated plots of Biserrula and Phalaris when grown in mixtures, and in Lucerne. In contrast, CP concentrations were significantly greater in Digit (in mixture), Fescue and Ryegrass **(Fig. 8A)**. Warming and warming + drought treatments resulted in a non-significant increase in CP concentrations of at least 10% (relative to controls) for Fescue and Kangaroo (mixed) and a non-significant decrease of at least 10% for Lucerne and Phalaris (mixed) **(Fig. 8B)**. As expected, legumes and temperate grass species generally had higher CP concentrations than tropical grasses; Digit and Phalaris both had higher CP concentrations when grown with a companion legume, compared to monoculture (**Fig 9A-B**). Warming increased CP concentrations of Phalaris and Kangaroo during the summer-autumn of 2019 (Appendix-**Table A3**), relative to ambient temperature plots.

The proportion of digestible dry matter (DDM) was not affected by drought for Biserrula, Fescue, Lucerne, Phalaris, Ryegrass or Wallaby **(Fig. 8C)**. Neither did warming affect any of the species exposed to this treatment **(Fig. 8D)**. However, there was a general trend for DDM of tropical grasses (Digit, Kangaroo and Rhodes) to increase under drought during winter/spring, reflecting a decreased fibre content for these species (Appendix- **Table A3**). Indeed, Kangaroo DDM increased by more than 9% under drought and drought + warming. Temperate species, including Ryegrass, Fescue, Phalaris and Lucerne, all maintained digestible dry matter values above 63% (Appendix-**Table A3),** regardless of climate treatment, while Biserrula and Kangaroo were notable for their low digestibility when grown in mixtures. The nutritional quality (DDM and CP) of Biserrula, Kangaroo and Phalaris was greater when they were grown alone (under control conditions), compared to mixtures, although the opposite was true for Digit. As a general guide, 70-80% digestibility is required for high livestock production; 60-70% digestibility is required for moderate production; 55-60% digestibility is required to maintain stock weight, while, at digestibility values below 55%, stock will lose weight (DPI, 2020; **Fig 8, Fig 9**).
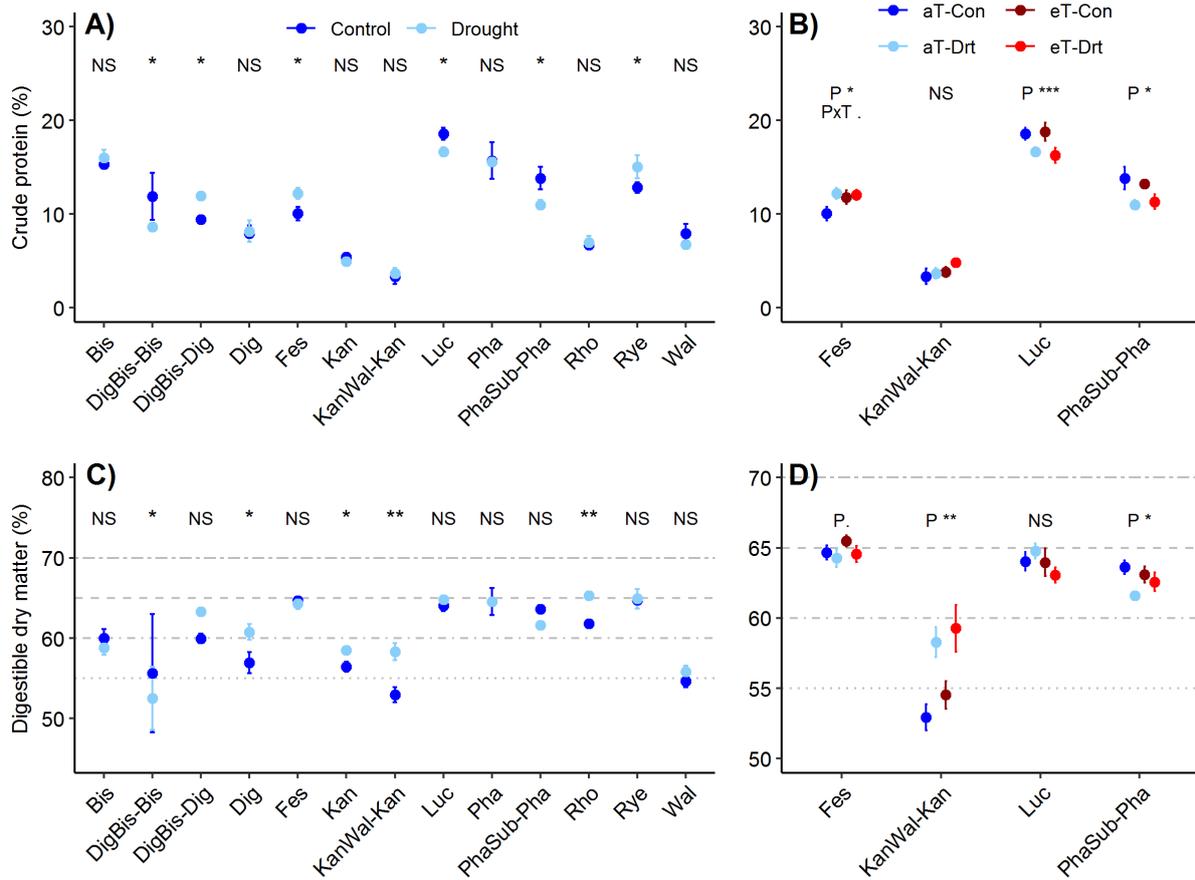
Fig 8. *Nutritional quality of pastures under control and drought/warming treatments during the first winter-spring drought period (material harvested Nov. 2018). A) Crude protein (CP) concentrations (% dry weight) under control and drought treatments; B) CP for pastures under drought (Con/Drt) and warming (aT/ET); C) Digestible dry matter (DDM; % dry weight) under control and drought conditions; and D) DDM for pastures under drought (Con/Drt) and warming (aT/ET). Digestible dry matter (DDM) was calculated using the formula: DDM = 88.9- (0.779*Acid detergent fibre percentage of dry matter) (Linn & Martin, 1989). Horizontal lines on panels C and D reflect thresholds where stock can lose weight (dotted line), maintain weight (dot-dashed line) or have moderate weight gain (dashed line) (NSW DPI).*

There was a near-universal trend seen for previously-droughted plants to have greater CP concentrations than non-droughted ones, during recovery from drought, although differences were mostly non-significant. Crude protein values were lower for all treatments during the summer/autumn (post-drought recovery period) than during the winter/spring (drought treatment period) for Digit, Lucerne and Phalaris in monoculture **(Fig. 9A-B)**, most likely reflecting plant phenology. Similarly, DDM values were greater for all treatments during the summer/autumn than during the winter/spring (drought treatment) period for Fescue, Kangaroo, Phalaris and Wallaby **(Fig. 9C-D)**.
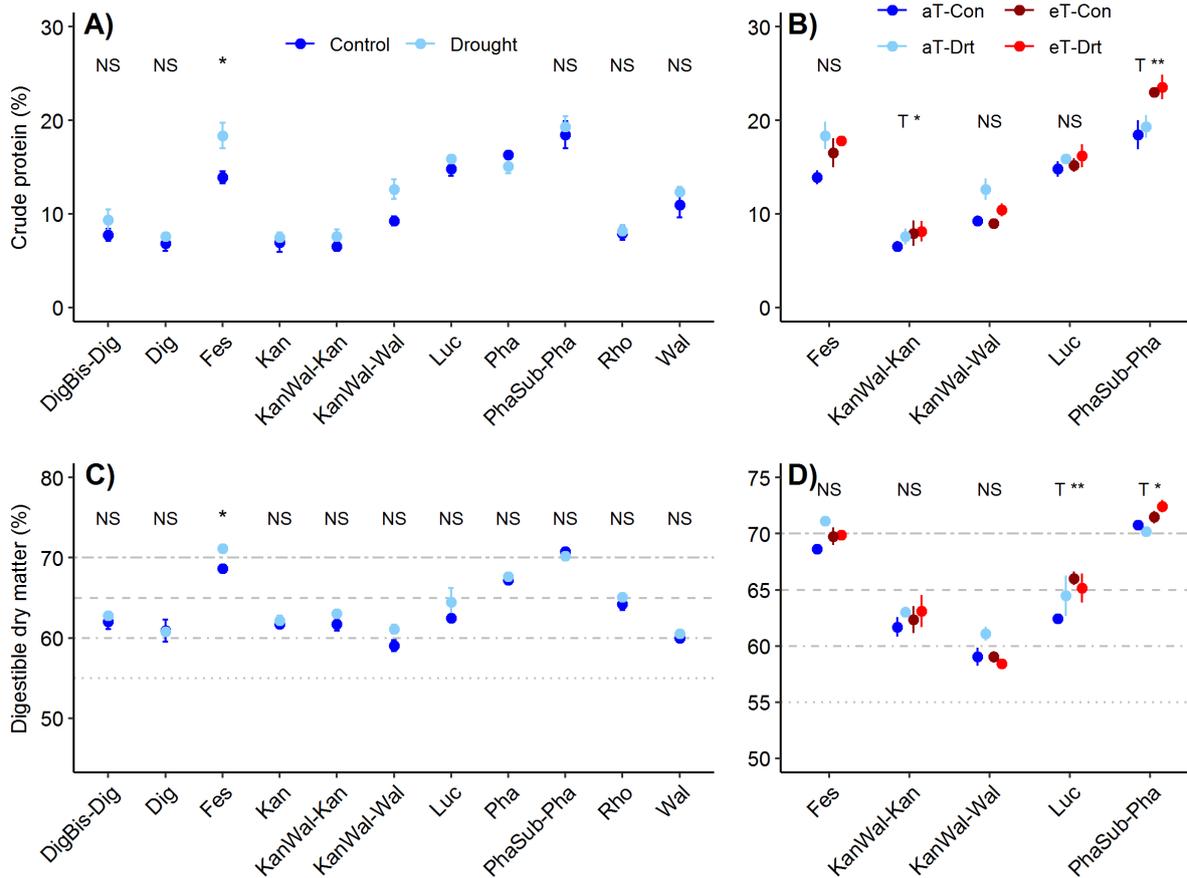
Fig 9. *Nutritional quality of pastures under control and treatment conditions during the first post-drought recovery phase (summer-autumn 2019). A) Crude protein (CP) concentrations (% DM) under control and drought conditions; B) CP for species under both drought (Con/Drt) and warming (aT/ET); C) Digestible dry matter (DDM; % DM) under control and drought; and D) DDM for pastures under both drought (Con/Drt) and warming (aT/ET). Horizontal lines on panels C and D reflect thresholds where stock will lose weight (dotted line), maintain weight (dot-dashed line) or have moderate weight gain (dashed line) (NSW DPI).*

4.1.4.2   Legume biological nitrogen fixation with drought and warming

Lucerne swards under drought had lower nodule biomass (**Fig. 10a**) and lower nodule N-fixing activity (**Fig. 10b**) compared to control plots. Warming was associated with non-significant increases in nodule weight in droughted plants. Nodule analysis can only give a snapshot of how nodule rhizobia have been affected by warming and drought at a given point in time, whereas N isotope and nitrogen concentration data in plant material provide a more integrated evaluation of N-fixing performance over time. Biologically fixed N traditionally has values of $\delta^{15}N$ that are approximately the same as the atmosphere (~0 ‰), with slight variations associated with individual legume species (Lucerne is -0.68 ‰; Unkovich et al., 2008). Soil-derived nitrogen pools are typically enriched in ^{15}N due to loss of lighter forms of N over time through leaching or denitrification, and in pastures may reach values as high as 14 ‰ (Bedard-Haughn et al., 2003). Thus, lower $\delta^{15}N$ values indicate a higher proportion of plant N derived from N fixation. Here we found Lucerne shoot N concentrations, but not $\delta^{15}N$ (a proxy for N-fixation by nodule bacteria), were significantly reduced under drought (**Fig. 10c-d**). These data suggest that drought did not affect the relative contribution of biologically fixed N to the plant N budget but did reduce the absolute amount of nodule-derived N, in line with the

nodule activity data. The effect of warming was small, compared to drought. Together, these findings suggest that drought reduced the benefit of symbiotic N-fixation, resulting in a trend for lower tissue N in Lucerne (**Fig 11**). This means that under drought conditions, legume growth is more dependent on soil N uptake, resulting in greater soil nutrient depletion and/or increased need for external N inputs.
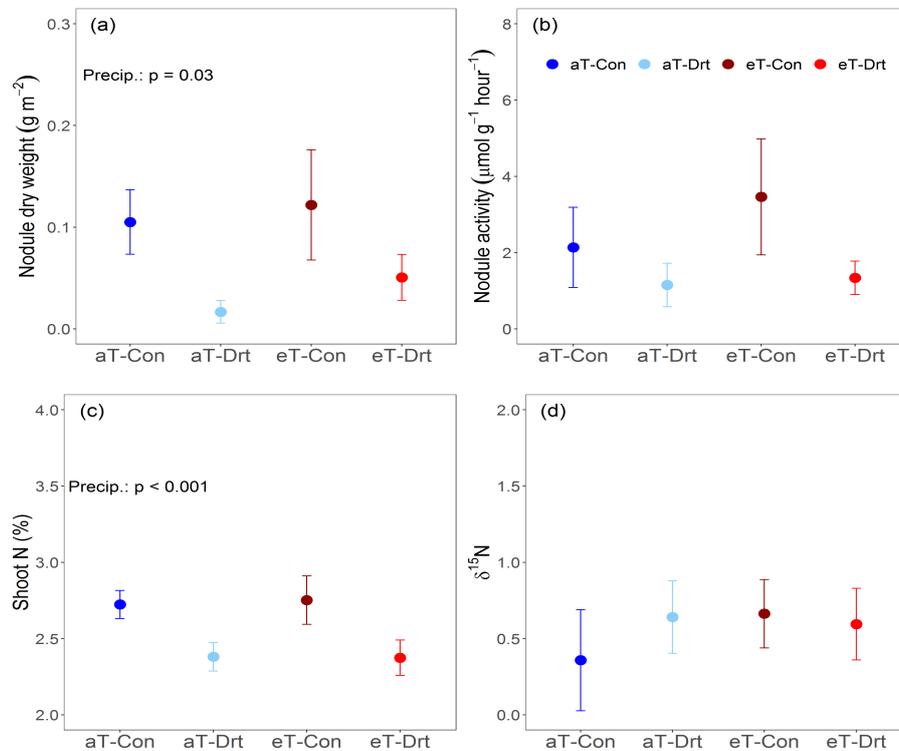
Fig 10. *Lucerne root nodule biomass (a) and activity (b), along with and shoot N concentrations (c) and isotopic ratios (δ^{15}N, d) under drought and warming treatments. # indicates marginal significance (P=0.05-0.1), all other notations match Fig 7. Error bars indicate +/- 1 standard error.*

4.1.5   Species mixtures

Pastures are often planted as multiple species mixtures with the aim of extending the period of available forage or improving forage quality by the inclusion of legumes along with grasses. We assessed the benefits of mixed species cultivation, compared to monoculture, and the effects of winter-spring drought for three sets of species mixtures (Digit/Biserrula, Kangaroo/Wallaby and Phalaris/Sub-clover).

Grass-legume mixtures experienced lower biomass reductions under drought than their respective single species swards during the 2018 drought (**Table 2**). This benefit was not, however, apparent during the 2019 drought (Digit/Biserrula, **Fig 4B**; Phalaris/Sub-clover **Fig 6d**). The difference between years likely reflects the longer drought exposure in 2019 (full 6 months) compared to 2018 (3 months for Biserrula and 1 month for Phalaris/Phalaris-sub clover), implying that the potential for legumes to provide nutritional benefits to companion grasses depends on the duration and severity of drought. The Kangaroo-Wallaby grass mixture was slightly less affected by drought in terms of its first year's cool-season productivity (-47%), compared to its dominant species growing in monoculture (-53% for Kangaroo), although this trend was reversed in the second drought period

(**Table 2**). The difference between years for this species combination seems to be a reflection of the reduced tiller density of Wallaby in the mixed pastures over time and especially under drought (evidence for increased competition between species); with no recruitment of Kangaroo to fill the space left behind by Wallaby decline, overall plant densities were therefore lower in mixed, compared to single species plots. For all three mixtures aboveground production was strongly dominated by one species, and the differences in density of the dominant species explain differences in total biomass production between mixed and monoculture plots.

Nutrient facilitation in mixed pastures

One of the main benefits of planting legumes and grasses together is the potential nutrient transfer from legumes and associated rhizobia to grasses grown in close proximity. Transfer of biologically-fixed nitrogen occurs via three routes: (1) decomposition of legume root tissues and nodules, (2) exudation of soluble N compounds and (3) mycorrhizal-mediated N transfer. While steady facilitation (increased legume-derived nitrogen availability for the neighbouring grass) throughout the legume's life cycle is likely to provide some benefit to neighbouring grasses during the period of legume activity (Rasmussen et al., 2013), dieback of the two annual legumes at the beginning of summer in our study would potentially also provide a pulse of additional nitrogen. The amount of legume-derived nitrogen transferred to companion grasses can be inferred from the isotopic signatures of plant tissue N, and has been found to account for up to 12% of the plant nitrogen budget in Alfalfa-Tall Fescue mixtures and up to 47% in mixtures of Ryegrass and White Clover (Louran et al., 2015).
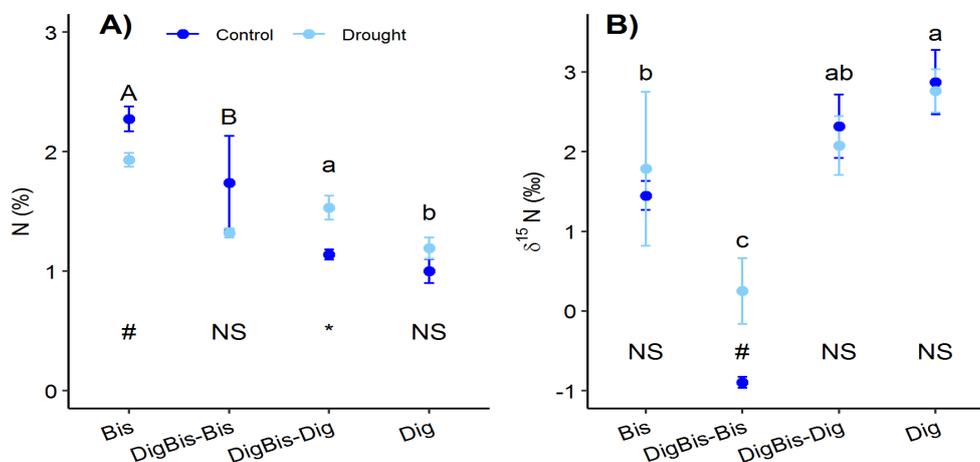
Fig 11. *Comparison of mixed sward component species, and their respective monocultures, for A) δ^{15}N (evidence of nitrogen fixation contributions to plant total nitrogen) and B) plant tissue nitrogen concentration in November 2018. Species abbreviations follow Fig 7 monocultures, while individual species within mixtures are indicated as follows: Biserrula in DigBis (DigBis-Bis), Digit in DigBis (DigBis-Dig). Significance of drought effects are indicated by * (P<0.05) with marginal significance (p < 0.10) indicated by #. NS - not significant. Different letters indicate species level contrasts, for panel B contrasts are among all species/mixtures, and in panel A contrasts are between same species only - indicated by upper and lower case.*

Isotopic signatures of plant material in our study imply that monoculture Biserrula derives 64% of its N from the soil, with the remaining 36% coming from biological nitrogen fixation (**Fig 11B**). However,

when grown with Digit, Biserrula appears to derive more of its nitrogen from its root nodules, as indicated by a substantially lower $\delta^{15}N$ value for this species in mixtures. Under drought, however, the contribution of biologically fixed (relative to soil-derived) N to Biserrula's nitrogen budget was lower, particularly so when it was growing with Digit. For Digit growing in these mixed plots, isotopic signatures indicate that it derived 15% of its total nitrogen via facilitation benefits from Biserrula, a contribution that increased to 27% under drought conditions. The transfer of nitrogen from Biserrula to Digit was reflected in plant tissue concentrations, with higher nitrogen concentrations in Digit growing in mixture compared to monoculture, and also greater concentrations under drought (**Fig 11A**).

4.1.6   Belowground allocation under drought and warming

On any given sampling occasion, belowground biomass comprises both recently produced roots and those that have accumulated over time. Belowground net primary productivity (BNPP, expressed on a dry mass basis) is a measure of new root growth over a discrete time interval. This value, together with a measure of total root biomass (also referred to as root standing crop) can be used to estimate root turnover rate, i. e. the number of times root biomass is replaced each year - the inverse of root longevity - an important factor influencing rates of soil carbon accumulation.
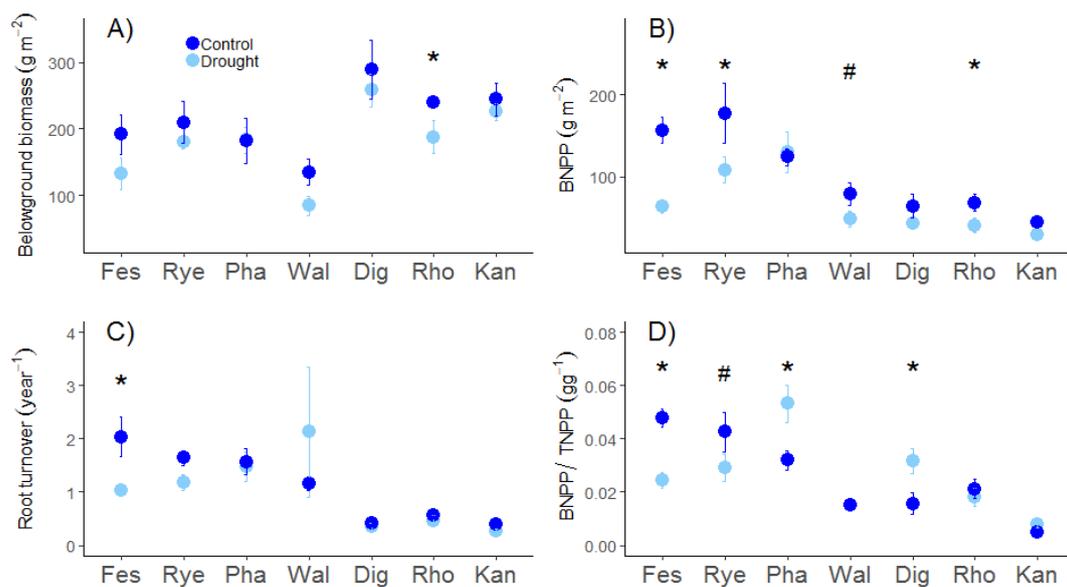
Fig 12. *A) Belowground biomass (root standing crop, November 2019), B) net primary productivity (BNPP) from May-Nov 2019, C) root turnover rate, and D) Root mass fraction – the ratio of belowground net primary productivity (BNPP) to total net primary productivity (TNPP) for plants grown under control (blue) and drought (light blue) conditions. * indicates significant treatment effects at P<0.05, # indicates marginal significance (P<0.1); species abbreviations match Fig 7.*

Fig 12 presents root data for grass monocultures, from Nov 2019, after all plots had been exposed to six months of extreme winter-spring drought. Drought significantly reduced total belowground biomass only for Rhodes (**Fig 12A**), and it reduced BNPP for Fescue, Ryegrass, Wallaby and Rhodes (**Fig 12B**). Root turnover was higher in Fescue than other species and was significantly reduced by drought (**Fig 12C**). Root standing crop measurements were also taken in Nov 2019 for all warmed

and droughted plots (**Fig A2**). Effects of warming were not generally significant, except for Fescue where warming - as well as drought - resulted in a substantial decline in total root biomass (**Fig A2**). Appendix **Table A2** provides a comparison of 2018 and 2019 total belowground biomass.

Changes in carbon allocation strategy, favouring belowground production compared to aboveground growth, or investing in thinner, less dense and/or longer roots, can increase water uptake relative to plant demand, and thus help alleviate drought stress. We found that Phalaris and Digit increased their relative investment in belowground growth (i.e. the proportion of whole plant biomass represented by roots) under drought (**Fig 12D**), whereas Fescue and Ryegrass reduced theirs. Temperate grasses generally had longer, less dense roots compared to tropical species (**Fig. 13**). Ryegrass, Digit and Rhodes increased specific root length and there was a general trend towards less dense root tissues when grown under drought. These results indicate that drought was driving an overall shift towards more resource-acquisitive belowground strategies, although Phalaris did not follow this trend, showing evidence of a more conservative rooting strategy (more dense roots, lower specific root length) in response to drought.
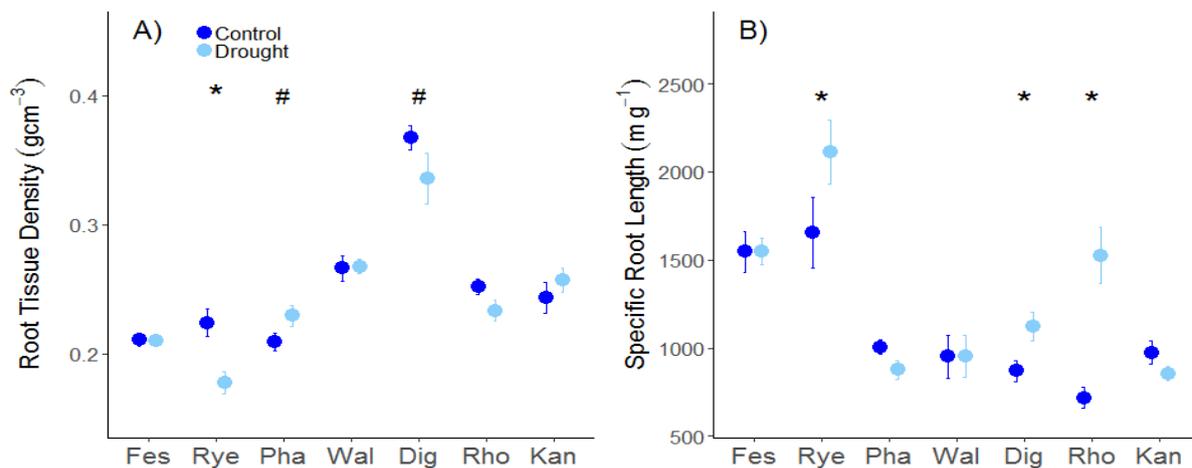
Fig 13. *Root tissue density (A) and specific root length (B) of roots produced under control (dark blue) and droughted (light blue) conditions during 2019. * indicates significant treatment effects at $P<0.05$, # indicates marginal significance ($P<0.1$), abbreviations match Fig 7. Error bars indicate standard error.*

It is interesting to note that there were also species differences in the extent of root mycorrhizal infection, and how this variable changed under drought. Although only five of the nine (single) species were analysed, all three C4 (tropical) grasses increased their investment in mycorrhizae (to increase water and nutrient uptake capacity) during the period of soil water stress, while the two C3 (temperate) species investigated did not (**Fig 14**). The tropical grasses generally had lower levels of mycorrhizal infection than temperate grasses (although note that Digit (57%) had higher levels of root mycorrhizal colonisation than Ryegrass (52%)). Plant investment of carbon to support greater mycorrhizal colonisation may be an adaptive response to drought for species which either have low levels of colonisation or root traits associated with a less (resource) acquisitive strategy (dense roots, low specific root length – see above). Links between plants' belowground carbon investment strategies (in terms of relative biomass allocation, root morphological traits and mycorrhizal colonisation), nutritional quality, drought sensitivity and recovery are a topic warranting further, more detailed investigation.
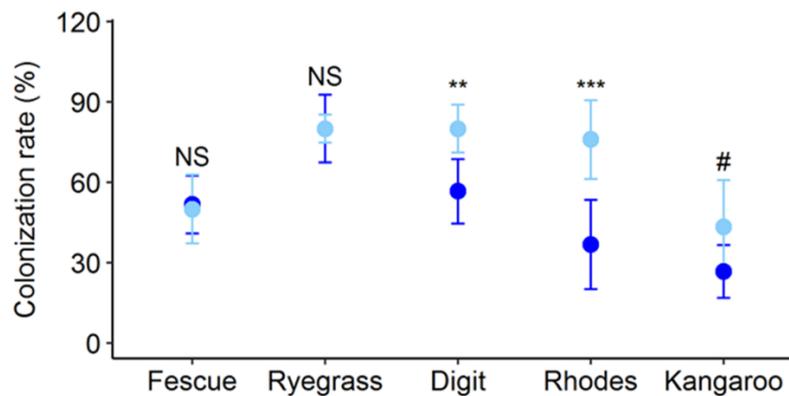
Fig 14. *Arbuscular mycorrhizal colonization rate (% root material infected) for five grasses grown under ambient rainfall (Con, light blue) and drought (Drt, dark blue) conditions during 2018. **/*** indicate significant treatment effects at P<0.01/0.001, # indicates marginal significance (P<0.1). Error bars indicate standard error.*

Species trait differences and trait-drought sensitivity relationships

In addition to examining individual root traits, we used a multivariate approach to probe relationships among these (and aboveground) traits and associated strategies. **Figure 15a** shows that the five species for which there was a full complement of above- and below- ground traits occupy distinct trait-space. The main axes (principal components) of this ordination plot explain approximately 50% of the variation in traits across species (33.7% and 15.9%, for axis 1 and 2, respectively). Ryegrass and Kangaroo are at opposite ends of axis 1, indicating distinct differences in measured traits. The other three species occupy more central locations along the first axis but separate along axis 2 - Digit at the top and Fescue closer to the bottom. The first PCA axis appears to reflect both root and shoot traits: higher root diameter, C:N ratios and water use efficiency are represented on the left side of axis 1 (low scores) while root biomass and specific root length are on the right side (high scores). The second axis (PC2) is associated with plant morphology and biochemistry, with greater root tissue density and specific leaf area represented at the top of this axis, and root diameter, NP ratios and silica concentrations at the opposite end (**Fig 15a)**.

Having established that these five species occupy quite distinct trait space, we then analysed relationships between individual traits and species' drought response (calculated as the "effect size", or proportional reduction in productivity under drought). Amongst these, two root traits - root tissue density and mycorrhizal colonization rate – were significantly related to the magnitude of drought treatment effects on aboveground biomass (**Fig 15b,c**). The relationship with mycorrhizal colonisation is, however, fairly weak and is partly driven by coarse differences in mycorrhizal colonisation levels between plant functional types (i.e. temperate grasses generally have higher % colonisation than tropical grasses). On the other hand, there is a much stronger relationship between root tissue density and biomass reduction under drought, suggesting that this root trait could potentially be a useful proxy for drought sensitivity. This is an interesting area that merits further work, including across a wider range of species and contexts, since it may help guide selection of more drought-tolerant species and genotypes.
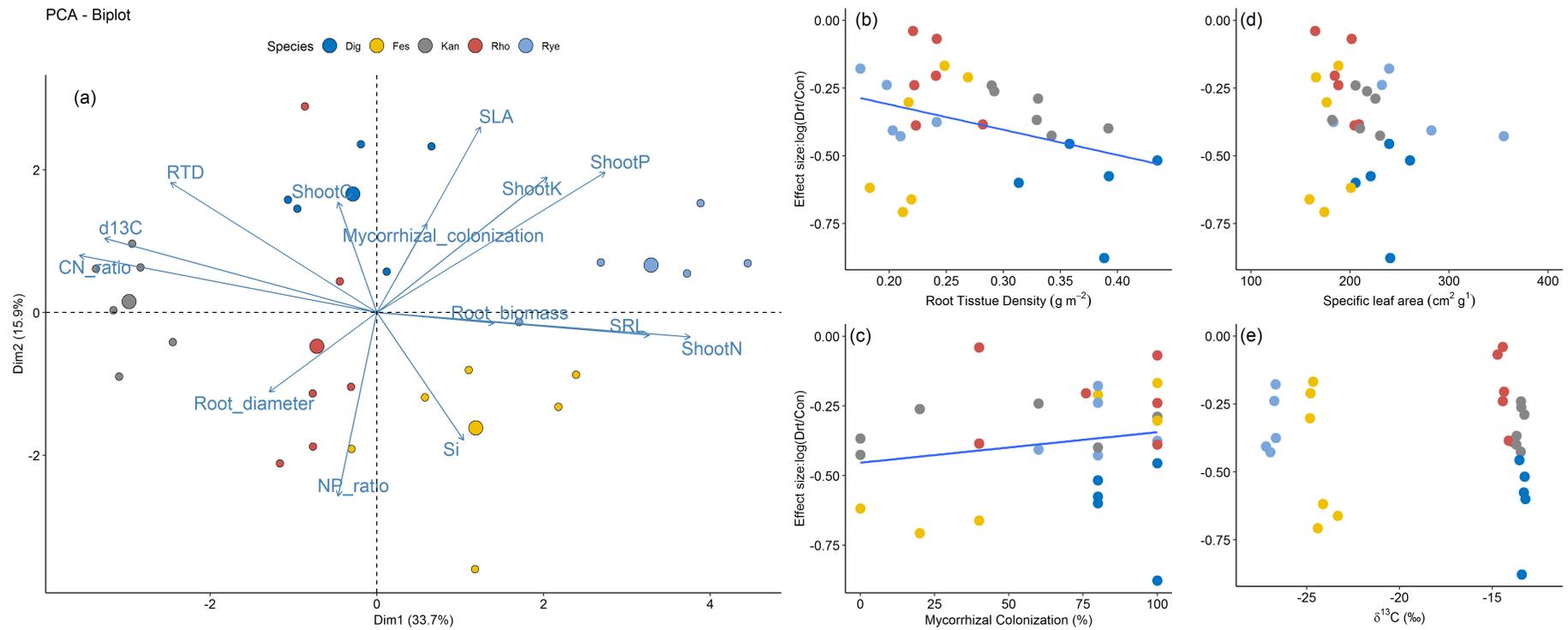
Fig 15. *Principal component biplot (a) of traits measured across the 60 subplots for the five selected species (Digit (blue), Fescue (yellow), Kangaroo (grey), Rhodes (red), Ryegrass (light blue)). Aboveground traits include specific leaf area (SLA), nutritional chemistry (shoot carbon (C), nitrogen (N), phosphorus (P), potassium (K), silicon (Si), C:N ratio, N:P ratio), water use efficiency (indicated by carbon isotopes: $\delta^{13}C$); belowground traits include root biomass, root mass fraction (RMF, calculated as $Root_{biomass}/Total_{biomass}$), specific root length (SRL), root diameter, root tissue density (RTD) and mycorrhizal colonization. Panels on the right illustrate relationships between plant functional traits and the effect size of drought on aboveground biomass (the log ratio of plant biomass in drought, relative to the control) for (b) root tissue density, (c) root mycorrhizal colonization rate, (d) specific leaf area and (e) water use efficiency ($\delta^{13}C$). Blue solid lines indicate significant relationships.*

4.1.7   Pasture water and carbon use strategies

We found significant variation in physiological traits relating to carbon fixation and water use (**Table 3**). Two tropical C4 grasses - Rhodes and Digit – had higher maximum levels of photosynthesis (reflecting a greater maximum photosynthetic rate under optimal conditions) than their temperate C3 counterparts (Fescue, Phalaris and Wallaby). However, average photosynthesis rates across the growing seasons were similar between the two groups. Native grasses (Kangaroo & Wallaby) had the lowest maximum and average rates of photosynthesis, while Lucerne had the highest mean values for both photosynthesis and stomatal conductance.

Table 3. Gas exchange traits of species studied, showing both the maximum and mean rates of light-saturated net photosynthesis, stomatal conductance and intrinsic water use efficiency (the ratio of average photosynthesis and the stomatal conductance).

Group	Species	Maximum Photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Mean Photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)	Water use efficiency ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)
Temperate	Wallaby	17.5	9.8	0.125	78.7
	Fescue	26.6	12.1	0.159	76.2
	Phalaris	30.9	15.6	0.289	54.0
	Lucerne	38.7	26.5	0.462	57.3
Tropical	Kangaroo	22.2	11.5	0.104	110.8
	Digit	38.5	12.4	0.065	190.7
	Rhodes	43.1	13.5	0.088	153.6

The hydraulic safety margin refers to the difference between the level of water stress experienced by the plant under nominal conditions and the point at which hydraulic damage occurs. These safety margins are key indicators of a species' drought response strategy and varied significantly amongst the species studied. **Figure 16** presents mean leaf water potentials of all measured species (averaged across measurement occasions), in relation to the species' threshold for hydraulic damage (P_{50} – the point at which 50% of water conducting xylem vessels collapse). This shows that Kangaroo and Digit had high hydraulic safety margins and were functioning with little risk of hydraulic collapse. Kangaroo achieved a high hydraulic safety margin by having a high threshold for hydraulic failure while Digit maintained a low midday leaf water potential. Safety margins for Phalaris, Rhodes and Fescue were far smaller.
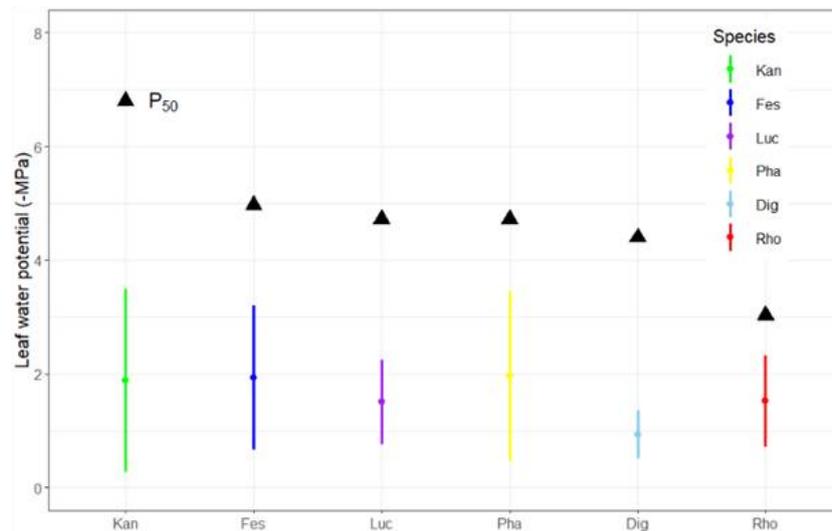
Fig 16. *Midday mean (and standard deviation) leaf water potential (vertical lines) and the point at which 50% of xylem function is lost (P_{50}) (Black Triangles).*

4.1.8 Greenhouse Gas fluxes under drought and warming

Nitrous oxide (N_2O) fluxes were measured in two pastures exposed to extreme winter-spring drought and continuous warming from July 2018 to June 2019. Fluxes were considerably higher in Lucerne plots, compared to Fescue, reflecting higher soil nitrogen availabilities for the former, driven by biological nitrogen fixation (**Fig A3-A5**). Non-droughted Fescue growing under ambient and elevated temperatures both showed net N_2O uptake (i.e. negative emissions) across the year (**Fig 17**), while drought was associated with a net flux of N_2O to the atmosphere; this effect was exacerbated in droughted plots exposed to +3°C warming, which had a net annual N_2O flux of 307 ± 123 g ha^{-1} yr^{-1}. Annual N_2O fluxes were very substantially increased by both warming and drought in Lucerne plots (averaging 521 g ha^{-1} yr^{-1}), compared to controls which had near-zero fluxes (21 g ha^{-1} yr^{-1}).
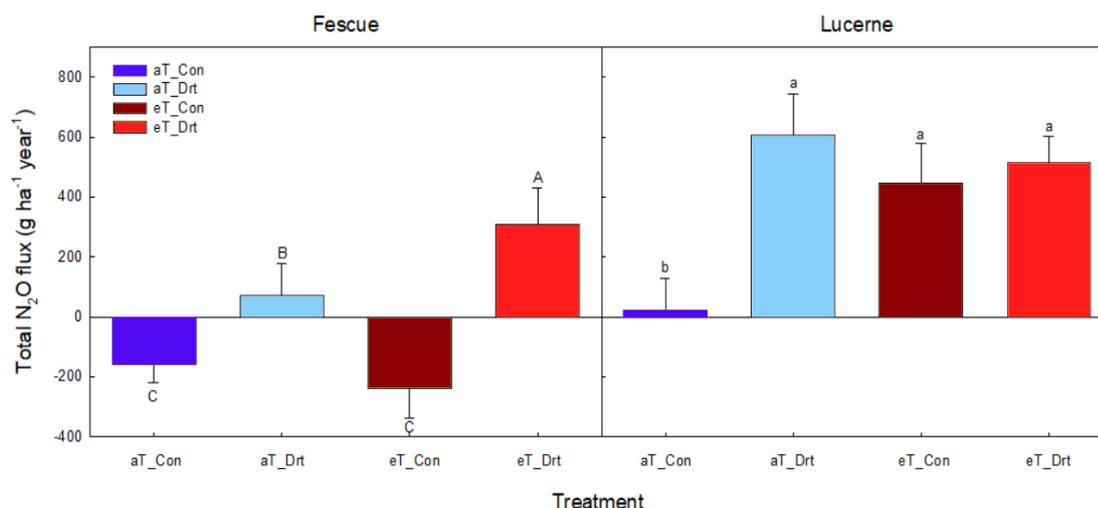
Fig. 17 *Annual N_2O flux associated with Fescue and Lucerne pastures exposed to winter-spring drought and continuous warming between Aug 2018 and Aug 2019.*

4.2   Glasshouse experiment 1: Warming, drought and the role of mycorrhizal fungi

Results from the first glasshouse experiment – examining the response of Fescue and Lucerne to warming and drought, and the role of soil mycorrhizal communities – have been presented in earlier milestone reports. Here we provide new data on nitrous oxide emissions (**Fig 18**) and nutrient leaching (**Fig 19**).
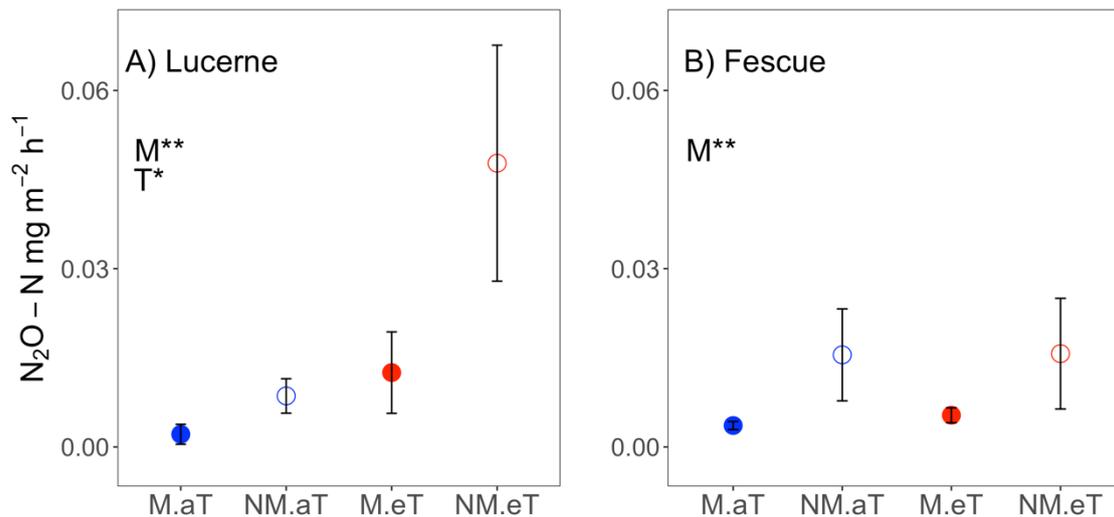
Fig 18. *N_2O fluxes of mycorrhizal (M, closed circle) and non-mycorrhizal (NM, open circle) Lucerne (A) and Fescue (B) grown under ambient (aT, blue) and elevated (eT, red) temperatures. Error bars indicate ± standard error (n = 8).*

Under ambient temperature (aT), we found that inoculation with arbuscular mycorrhizal (AM) fungi reduced N_2O fluxes by 76% for Lucerne and 77% for Fescue (**Fig 18**). Under elevated temperature (eT) AM fungi were responsible for a reduction in N_2O emissions that was six times bigger than that under aT for Lucerne. For Fescue, however, the impact of AM fungi on (reducing) N_2O emissions was not increased by warming. Of note here is that the extent of mycorrhizal infection in Fescue (but not Lucerne) roots was reduced by warming. This indicates that, for species where warming disrupts plant-mycorrhizal interactions, the capacity for mycorrhizae to reduce soil N_2O emissions as temperatures rise may be constrained.

AM fungi significantly reduced phosphorus (P) leaching by 46% for Lucerne, regardless of climate conditions. For Fescue, AM fungi reduced P loss under aT by 48%, but this effect disappeared under eT (**Fig 19a**, **b**). As indicated above, negative effects of warming on AM fungal colonisation rates in inoculated Fescue suggest that the nutrient capture benefits of mycorrhizal colonisation are also likely be lower as temperatures increase.

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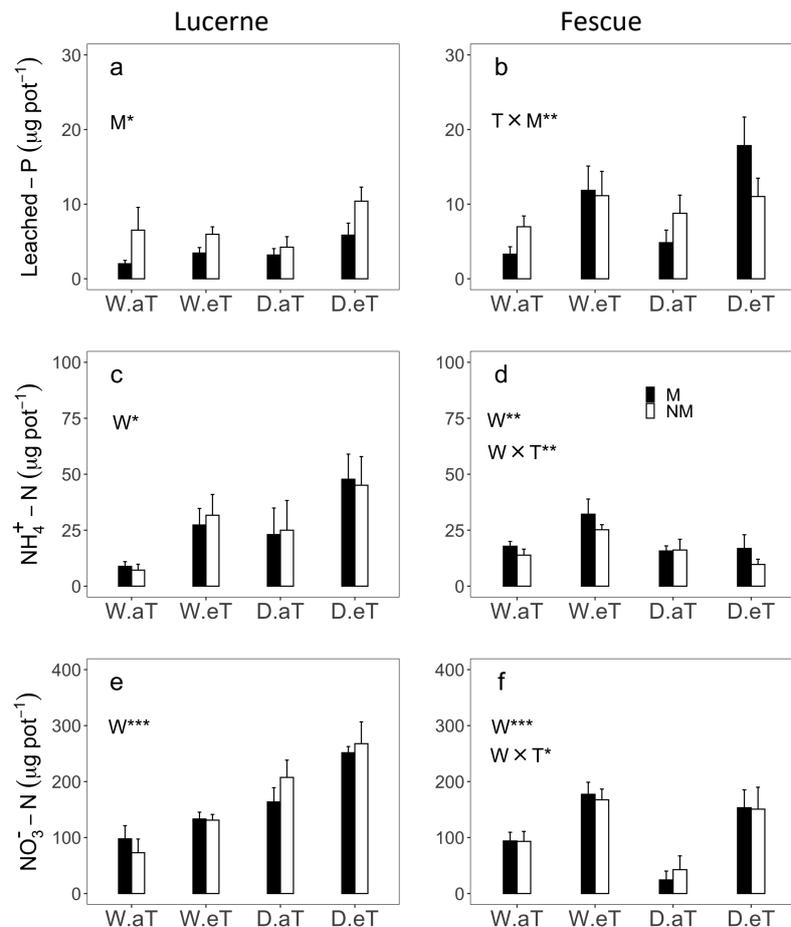
Fig 19. *Total amount of leached P (**a, b**), NH$_4^+$-N (**c, d**) and NO$_3^-$-N (**e, f**) for each pot for mycorrhizal (M, black bars) and non-mycorrhizal (NM, white bars) Lucerne (left column) and Fescue (right column) grown under different water (well-watered, W; drought, D) and temperature (ambient, aT; elevated, eT) conditions.*

4.3   Glasshouse experiment 2: Elevated CO$_2$ and tropical grass-legume mixtures

Desmodium/Panic mixed pots produced 35% greater biomass than monocultures of the contributing individuals, under ambient CO$_2$ conditions – evidence of over-yielding for this species pair. There was, however, no evidence of over-yielding for Burgundy bean/Rhodes when grown together in ambient air. Aboveground biomass <u>per plant</u> clearly demonstrated a competitive trade-off in mixtures, compared to monocultures; in both species-pairs the grass grew more while the legume grew less, with this pattern being stronger for the Desmodium/Panic pair (**Fig 20**).
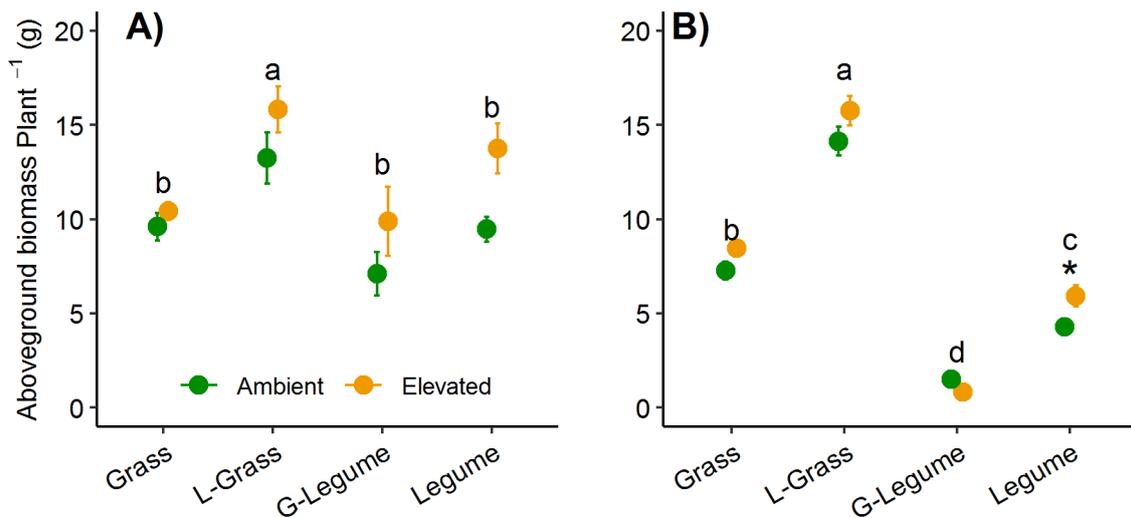
Fig 20. *Aboveground biomass of tropical pasture species grown in monoculture (Grass and Legume in legend) and mixtures (grass grown with a legume: L-Grass, and legumes grown with a grass: G-Legume) under ambient and elevated CO_2 conditions for A) Burgundy bean and Rhodes grass and B) Desmodium and Panic grass. Values shown are means ± 1 SE. Same letter designations indicate differences among plant types within a species pair and * indicates a significant effect of elevated CO_2 within a plant type.*

Elevated CO_2 concentrations resulted in modest, species-dependent increases in biomass; these benefits were generally smaller for tropical C4 grasses than C3 legumes, as expected. Significant CO_2-related increases in growth in Burgundy bean ($P<0.05$) were apparent when the species was growing in monoculture, but were slightly lower ($P<0.10$) when it was grown alongside Rhodes (39%- mixed pot, 45%- monoculture pot; **Fig 20A**). In contrast, Desmodium growth declined under elevated CO_2 when grown with Panic (-45%; effect not significant), while experiencing a strong fertilization effect from elevated CO_2 in monoculture (+38%; **Fig 20B**). The CO_2-associated increase in growth of Burgundy bean was reduced when grown with Rhodes, compared with monoculture pots, although neither increase was statistically significant (39%- mixed, 45%- monoculture; **Fig 20A**). In contrast, Desmodium growth declined under elevated CO_2 when grown with Panic (-45%; effect not significant), while experiencing a strong fertilization effect from elevated CO_2 when grown in monoculture (+38%; **Fig 20B**).

Legumes had considerably greater tissue nitrogen concentrations than grasses and, while grasses did tend to increase %N concentrations when grown with legumes, these increases were not significant. Elevated CO_2 resulted in decreased %N for both grasses and legumes, with the biggest reduction seen for Burgundy bean (1.48% in eCO_2, compared to 1.78% in ambient CO_2, $P<0.05$; **Table 4**).

Table 4. Mean aboveground plant tissue concentrations of N (%) for individual pasture species grown singly and in mixture under ambient (aCO₂) and elevated CO₂ (eCO₂) conditions

Species Pair	Species	Pot type	Treatment	
			aCO₂	eCO₂
Burgundy Bean – Rhodes grass	Burgundy bean	Monoculture	1.78 ± 0.08	1.48 ± 0.06
		Mixed	1.70 ± 0.07	1.20 ± 0.10
	Rhodes grass	Monoculture	0.33 ± 0.02	0.30 ± 0.02
		Mixed	0.38 ± 0.03	0.36 ± 0.02
Desmodium-Panic grass	Desmodium	Monoculture	1.12 ± 0.09	1.06 ± 0.09
		Mixed	1.10 ± 0.10	0.94 ± 0.11
	Panic grass	Monoculture	0.38 ± 0.03	0.30 ± 0.02
		Mixed	0.47 ± 0.10	0.29 ± 0.02
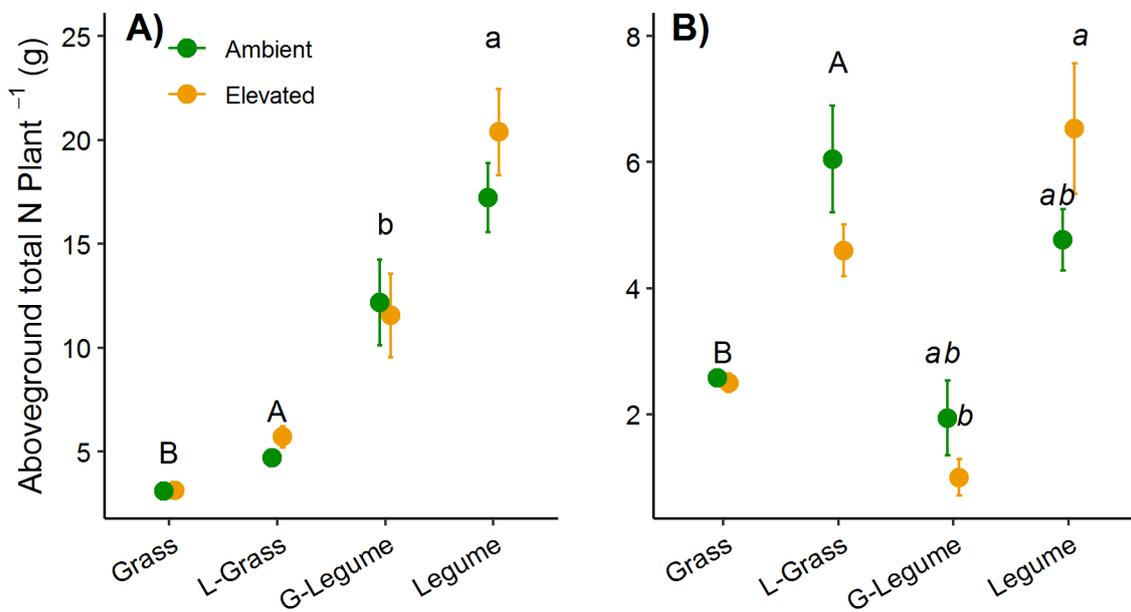
Fig 21. Aboveground biomass total nitrogen for tropical grasses and legumes grown in monoculture (Grass and Legume in legend) and together in a two-species mixture (grass grown with a legume: L-Grass, and legume grown with a grass: G- Legume) under ambient and elevated CO₂ conditions. A) Burgundy bean and Rhodes and B) Desmodium and Panic grass. Values shown are means ± 1 SE. Same letter designations indicate non-significant differences between mixed and monoculture pots for grasses (upper case) and legumes (lower case). Significant interactions between plant type (monoculture/mixture) and CO₂ are indicated by italics.

Despite limited changes in aboveground %N when species were grown in mixture, (compared to monocultures) - or in response to elevated CO₂ - total plant-level N contents (i.e. biomass multiplied by %N) provide strong evidence of nutrient facilitation. When growing with legumes, grasses had significantly higher total N contents (Rhodes: +51%, Panic: +135%). In contrast, legumes had lower values of total N when grown with a grass (Burgundy bean -29%, Desmodium -59%), suggesting strong competition for nutrients. Elevated CO₂ conditions had markedly different effects on the two

species pairs. Burgundy bean and Rhodes had a very small (non-significant) increase in total N under eCO$_2$ (**Fig 21A**) while, in contrast, eCO$_2$ tended to decrease the N content of both Panic and Desmodium when they were growing together, reflecting the poor growth performance of this legume in mixtures.
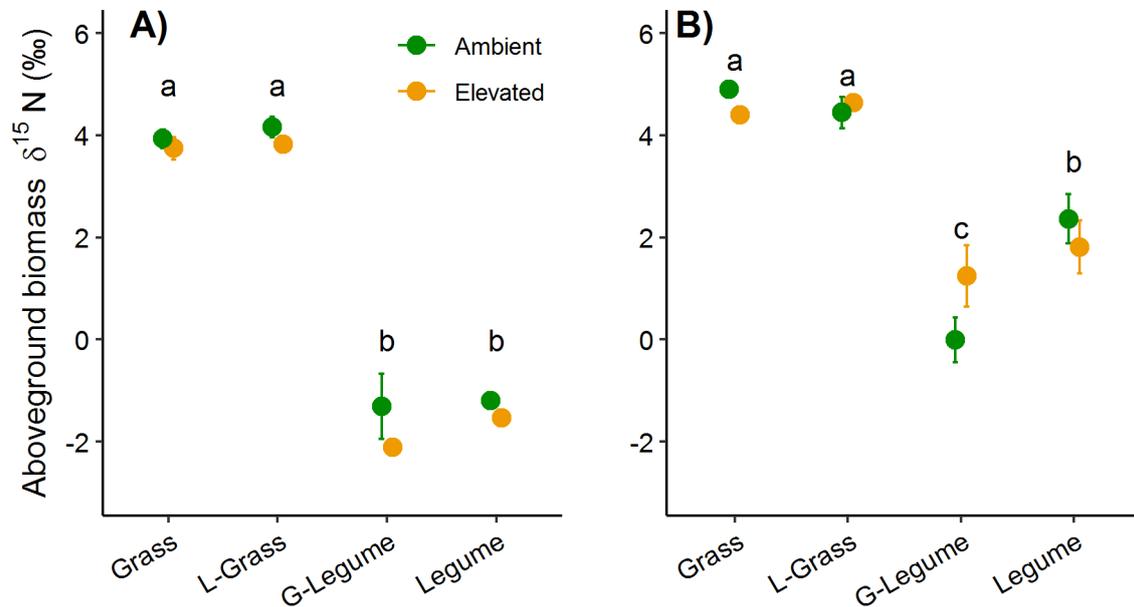
Fig 22. Aboveground biomass δ^{15}N signature, indicating the relative contributions of soil-derived versus biologically-fixed nitrogen for A) Burgundy bean and Rhodes grass and B) Desmodium and Panic grass. Values shown are means ± 1 SE. Same letter designations indicate non-significant differences among plant types. Lower values are associated with a greater contribution of biologically fixed N to the plant N budget.

Given the shifts in productivity for grasses and legumes when grown together, as well as evidence of nutritional benefits in terms of the total amount of N in aboveground biomass for grasses, at the apparent expense of the legumes, we examined the source of nitrogen used by the different plant species in both monoculture and mixture. This was accomplished based on differences in δ^{15}N for grasses grown in monoculture, relying 100% on soil N (values ranging from 0-14‰), and for biologically fixed N (BFN) by rhizobial symbionts of legumes (where δ^{15}N values are typically much lower; **Fig 22**). Isotopic signatures suggest that Burgundy bean and Desmodium differ in the amount of total plant N they each derive from BFN when grown on their own (Burgundy bean: 77% BFN, Desmodium: 37% BFN; low δ^{15}N values). Both legumes increased reliance on BFN as a source of N when grown with grasses (Burgundy bean mixed: 81% BFN, Desmodium mixed: 71% BFN) suggesting strong competition for soil nutrients (lower δ^{15}N values). Furthermore, despite growing alongside legumes for 11 weeks, grasses in mixed pots still relied strongly on soil N (Rhodes: 97%, Panic: 92%; high δ^{15}N values). Elevated CO$_2$ conditions had relatively little impact on the source of plant N for any species or mixture, although Desmodium did increase reliance on soil N (higher δ^{15}N value) under eCO$_2$ when grown with Panic (51%, compared to 29% in monoculture).

5 Discussion

5.1 PACE field experiment

5.1.1 Aboveground productivity and nutritional quality

After two successive years of winter/spring drought – in combination with warming for some species - we have been able to evaluate productivity and nutritional responses for a range of pasture species and two native grasses. A consistent pattern is that, despite strong (species-specific) yield reductions during the period of imposed drought, most species exhibited high resilience (i.e. rapid recovery to control plot levels). Fescue and Kangaroo were less resilient than the other species in the first year, exhibiting carry-over effects of winter/spring drought on summer/autumn production. This was associated with reduced tiller numbers (stem densities) and, for Fescue, plant death, particularly in warmed x droughted plots. Clearly having relatively few tillers or living plants at the end of a very dry (and/or hot) spring makes it impossible for a species to bounce back strongly during the summer. Notably, however, recovery from the second drought was strong, with no significant carry-over yield reductions seen in summer/autumn 2020 for any species, and even some evidence of increased yield in previously droughted plots.

The importance of the timing of periods of severe water stress in relation to crop phenology (especially peak productivity) is apparent from our results. For those species that produce most of their biomass during the warmer months (Digit, Rhodes), the effects of a winter/spring drought on total annual (June-May) productivity was generally lower than for species that are productive either predominantly in the cooler months (Ryegrass, Biserrula, Phalaris, Wallaby) or year-round (Lucerne, Fescue, Kangaroo). **Table 5** provides a simple, visual overview of species' relative sensitivity to drought and warming, based on responses across the full experimental period. Digit comes out as being the most affected by drought during the winter/spring period, but because it recovers quickly and produces much of its annual biomass in the warmer months its overall (annual) sensitivity is quite low. The same is true for Phalaris and Rhodes. Fescue is considered moderately drought-sensitive during the winter/spring (i.e. droughted) period, but high levels of tiller and plant death after the first drought – particularly in warmed plots – meant it had almost no capacity to recover and thus was the most sensitive species overall in terms of annual biomass production in the first year of this study. This is somewhat at odds with reports of it being relatively drought tolerant (Clark et al., 2016) but is likely explained by the severity of the imposed drought occurring before plants had had a full year to establish. Of interest, though, are finding from the second year of the experiment that show much smaller (non-significant) carry over effects of the second drought, resulting in non-significant treatment effects on Fescue's annual productivity in year 2.

Greater growth reductions in the second year of drought treatment for Wallaby, Biserrula and Phalaris are likely due to the longer drought duration, and thus greater absolute amount of water withheld, in 2019 relative to 2018. However, it is also possible that there was a cumulative effect of successive years of water stress, either directly (for perennial species, including Kangaroo) or indirectly (via changes in the soil, for annual species). Increasing effect size over time may be related to changes in soil biology and chemistry that can have indirect feedbacks to subsequent plant performance, for example promoting invasion by non-target pasture species (Meisner et al., 2013; Bennett and Klironomos, 2019). Fescue and Ryegrass were, however, interesting exceptions to this pattern, experiencing lower (although still statistically significant) yield reductions in the second

year. Possible explanations for this response may include greater soil nutrient or water availability in formerly droughted (and warmed) plots at the beginning of the second winter period, associated with earlier, strong vegetation dieback (and thus both reduced plant uptake and increased root mortality). Indeed, there is evidence that both soil water content and extractable nutrient concentrations were higher in previously-droughted plots in autumn for Fescue **(Fig 2A, Fig A5)**; higher levels of soil resource availability may have offset some of the treatment effects on productivity in winter/spring 2019 as plants entered the second six-month period of drought. Another possibility for the difference in sensitivity between 2018 and 2019 could be the timing at which the combination of temperature and soil water required to initiate strong growth occurred in each of the years. Or, alternatively, rooting depth, and thus access to deeper soil water, may have increased over time. The destructive nature of rooting depth sampling in small experimental plots has, however, meant that we were unable to test this hypothesis in the field.

Table 5. *Categorisation of species' sensitivity to drought and/or heat, based on 2 years' productivity responses in winter/spring (in both 2018 and 2019), summer/autumn (2019 and 2020) and across the full 24-month period from June 2018-May 2020.*

Species	Productivity (winter/spring)		Productivity (summer/autumn)		Productivity (overall – 24 months)	
	Drought	Heat	Drought	Heat	Drought	Heat
Fescue	[light orange]	[light orange]	[orange-brown]	[light orange]	[orange-brown]	[light orange]
Kangaroo/Wallaby	[orange-brown]	[blue]	[yellow]	[yellow]	[light orange]	[blue]
Lucerne	[light orange]	[yellow]	[light orange]	[yellow]	[light orange]	[yellow]
Phalaris/Sub clover	[light orange]	[yellow]	[yellow]	[light orange]	[yellow]	[yellow]
Rhodes	[light orange]		[blue]		[yellow]	
Ryegrass	[light orange]		N/A		[yellow]	
Digit	[red]		[blue]		[yellow]	
Biserrula	[light orange]		N/A		[light orange]	
Digit/Biserrula	[orange-brown]		[blue]		[yellow]	
Kangaroo	[orange-brown]		[yellow]		[light orange]	
Wallaby	[light orange]		[blue]		[yellow]	
Phalaris	[light orange]		[blue]		[light orange]	

Colours represent yield reductions <u>averaged over two years</u> for cool season (Jun-Nov 2018 and 2019), warm season (Dec 2018-May 2019, Dec 2019-May 2020) and overall (Jun 2018-May 2020) productivity: BLUE < 5% yield decline; YELLOW: 5-25% yield decline; LIGHT ORANGE 26-45% yield decline; ORANGE-BROWN 46-60% yield decline; RED >60% yield decline. N/A reflects species where crops were fully re-sown following end-of-spring (November) harvests.

Over two years, the warming treatment has provided limited evidence of productivity benefits, with the exception of Kangaroo/Wallaby plots in the first winter/spring period. Indeed, productivity generally declined in warmed plots across the year, and there were significant warming x drought interactions. Of particular note was the additive effect of warming and drought for Fescue (-61%) and Phalaris (-40%), which experienced the biggest overall yield declines when exposed to this treatment combination. Lucerne experienced modest cool season biomass reductions in response to warming, and the combination of drought and warming. It should be noted that the negative effects of warming on species' productivity we report here are, of course, in the context of the local

ambient temperatures. In this study, the +3°C warming treatment resulted in an additional 29/10 days when canopy temperatures exceeded 35°C (in summer 2018/19 and 2019/20, respectively) and a reduction in the number of days with winter minimum temperatures below 5°C (43 fewer days in 2018/19 and 32 fewer in 2019/20). We expected that warmer winter temperatures would be associated with increased cool-season productivity, via removal of low temperature constraints on growth (Cullen *et al.*, 2012). However, our findings of winter/spring yield declines in field-grown C3 grasses and legumes exposed to +3°C suggest a complex interplay between temperature constraints on biological activity and temperature-driven increases in evapotranspiration, and thus reduced soil water availability. Clearly it is important to consider both the direct (physiological) and indirect (soil moisture, nutrient availability and microbial) mechanisms by which elevated temperature – both on its own and in combination with changes in rainfall – will affect seasonal pasture production under future, more extreme climate conditions.

Fescue (Quantum II MaxP) was found to experience the biggest yield declines under winter/spring drought in our study, especially under warmer conditions. Furthermore, its capacity to recover was constrained by the death of a high proportion of individual plants, so this species is likely to require re-sowing following prolonged, severe drought. At the other end of the scale, Rhodes appears to be drought-resilient. This species, together with Digit, was highly productive in the warm season following each year's winter/spring drought and may therefore be considered useful alternative forage species, filling the summer feed gap even in drier years. Their generally low nutritional quality is, however, noted.

It is clear that climate extremes strongly affect the ability of pastures to support livestock production by reducing pasture productivity, in some cases by more than 50%. However, pasture quality was also affected by drought and warming, with some species showing improved and others decreased quality. For example, Lucerne and Biserrula (in mixture) had lower crude protein concentrations under drought, as did Phalaris in mixture with Sub clover. These observations might be explained by reduced rates of rhizobial N fixation. In contrast, drought and/or warming caused crude protein to increase in several grasses.

Forage produced by tropical grasses under drought was more digestible than under control conditions, but not sufficiently so to compensate for reduced production, whereas temperate grasses maintained relatively higher levels of digestible dry matter under all conditions. Food intake by livestock is depressed under heat stress, and this can be ameliorated by diets that contain less fibre (fibre favours the energetically inefficient, and highly thermogenic production of acetate, over propionate; Beale et al. 2018). The observed treatment-related changes in digestibility and fibre content will generally mitigate the impact of heat stress on livestock nutrition.

Drought alters growth rates and phenology of pasture species in two ways which have contrary effects on nutritional quality. In several species, drought accelerated leaf senescence, reducing the ratio of live:dead material and thus overall digestibility (Appendix 9.1.3.1; Appendix **Table A4**). However, drought can also reduce the stature and delay the phenology of pasture species, resulting in plants with fewer, shorter stems and flowering parts and a higher leaf:stem ratio, which is associated with higher digestibility and crude protein content. Therefore, choosing varieties with delayed onset of flowering may allow for improved digestibility in drought conditions, by increasing the leaf:stem ratios.

5.1.2 Mixed pastures

Grass-legume mixtures are often considered to be more productive, and of higher nutritional quality, than grass-only monocultures - a phenomenon that is widely observed in both the agricultural and ecological literature (Nyfeler et al., 2011; Zuppinger-Dingley et al., 2014; Suter et al., 2015). Although there was some evidence for greater biomass production in mixed species plots (e.g. Digit-Biserrula in year 2), differences in planting densities between mixtures and monocultures made this difficult to detect in the field. For example, Phalaris growing in combination with sub-clover had a lower plant density and lower plot-level yields than Phalaris-only plots. However, per-plant yields were higher in the mixed plots, demonstrating evidence of legume facilitation at the individual plant level (Nyfeler et al., 2011).

In the first year of the experiment, there was evidence that mixtures provided a degree of "climate insurance", with smaller yield reductions under drought, compared to the respective single species pastures. This effect disappeared in 2019, which is likely the result of the grass-legume mixtures experiencing a longer (full 6-month) drought, compared to 2018, and consequently greater (negative) impacts on legume yield and N-fixing activity. The benefits of legumes in mixed species swards thus appears to be dependent on the duration of drought – as well the precise combination of grasses and legumes (see glasshouse 2 results) – and further research in this area is, therefore, needed.

Nutritional benefits of grass-legume mixtures (compared to grass monocultures) were also apparent, with both Digit and Phalaris having higher crude protein concentrations when grown alongside a legume (Biserrula and sub-clover, respectively). The benefits of species diversity to maintain productivity under climate stress ("the insurance hypothesis" Yachi and Loreau, 1999) has been the subject of much research in the field of ecology (e.g. Isbell et al., 2015). While we only included three sets of two species mixtures in this study the potential benefits of combining multiple species may well be greater than we report here, given the longer period during which legume facilitation can occur with perennial species.

Beyond the potential benefits in terms of productivity and total protein yield of mixed pastures, we found evidence that grass-legume mixtures derived a higher proportion of their total N supply from biological N fixation, compared to the respective monocultures. Isotopic analyses revealed that Digit obtained some of its N from neighbouring Biserrula, and that N fixation was upregulated in mixed species plots, compared to the legume monoculture. Our findings here supported the stress-gradient hypothesis (SGH), which predicts that facilitation (as opposed to competition) will be more common in conditions of high abiotic stress, relative to more benign conditions (Maestre et al., 2009). Future work should focus on further tests of the extent of facilitation, particularly under climate stress, across different grass-legume mixtures. Clearly legume N fixation can reduce inorganic N fertiliser requirements for productive mixed pastures, compared to grass-only systems, resulting in greater N-use efficiency, economic benefits and, potentially, reduced emissions of greenhouse gasses such as nitrous oxide. Indeed, the evidence of enhanced N_2O emissions under drought (PACE field plots) – and their suppression by mycorrhizal fungi (PACE glasshouse experiment) – from this study suggests that incorporation of drought-tolerant perennial legumes into pasture mixtures may be particularly beneficial for improving the efficiency of fertiliser use and reducing associated greenhouse gas emissions as global temperatures increase.

5.1.3   Plant resource allocation strategies

Shifts in carbon allocation strategy in favour of root investment is one mechanism by which plants can increase their water uptake and reduce transpiration water loss, and is thus a commonly reported response to soil water stress (Harrison and LaForgia, 2019). Species with a high root mass fraction (proportional investment in belowground biomass), deep rooting systems and/or acquisitive root morphologies (such as long, thin roots and low tissue density) are, therefore, generally associated with greater tolerance of drought conditions (Wasaya et al., 2018). In our study, we found overall evidence of reduced root production and species differences in carbon allocation strategies in response to drought. However, there were also changes in root morphology amongst temperate species (towards longer and thinner roots) in droughted plots, in line with studies elsewhere revealing a more acquisitive root strategy under climate stress (Fitter, 2002). Additionally, while temperate grasses generally had higher levels of root mycorrhizal colonisation than tropical species, the latter group increased their levels of mycorrhizal colonisation under droughted conditions, bringing them more in line with levels seen in temperate species'. Taken together, these findings show that, although root production is generally lower in response to drought (and, for Fescue and Lucerne, also warming), species differed in their carbon allocation responses to water stress (in the form of reduced water inputs, or heat-associated soil warming). Our results indicate that optimised C allocation strategies in favour of belowground C investment and more acquisitive root morphologies will improve resistance and resilience to climate stress.

5.1.4   Water use strategies under drought

Given the trade-off between water loss and carbon uptake at the leaf level, stomatal strategy – which determines how they regulate stomatal CO_2 and water flux – influences both photosynthetic rates and water use. In the context of drought, stomatal strategies can range from 'conservative' (minimise water loss, but also low carbon gain) to 'risky' (maximise carbon gain, but may also have large water loss). The conservative strategy is usually associated with early stomatal closure and lower productivity, along with hydraulic (water transport) traits that provide high thresholds to withstand water stress (Li et al., 2019). In contrast, the risky strategy is usually associated with keeping stomata open for long periods of time, generating high productivity, but such plants also have hydraulic traits that provide lower thresholds to withstand water stress. These strategies provide different benefits dependent on the environmental conditions and partly reflect the evolutionary history of the species based on the climate-of-origin. Determination of stomatal and hydraulic traits in this study allows us to position species along axes that relate to their water use strategy and provides mechanistic insight into species' responses to drought.

Figure 23 shows the relative positions of seven pasture species in terms of their resistance to water stress based on their stomatal and hydraulic strategies. Kangaroo is the most resistant to hydraulic failure (cell collapse and tissue death) but it closes its stomata earlier than some species (a conservative strategy) and is vulnerable to significant reductions in productivity under severe drought. At the opposite end of the spectrum is Rhodes, which keeps its stomata open making itself vulnerable to hydraulic failure under periods of prolonged water stress. Rhodes experienced one of the smaller productivity declines under drought, which is likely due to high rates of photosynthesis even as water availability declined, an indication that the level of drought imposed was below a critical threshold for this species. Wallaby and Digit both have somewhat risky water use strategies.

However, Digit experienced the bigger decline in productivity across the winter/spring period in both years but recovered rapidly once full watering was resumed. This suggests that Digit diverted carbon away from aboveground growth, towards storage in roots and crowns, to support rapid regrowth once favourable conditions resumed. Carbohydrate analyses of roots and crowns will be undertaken over the coming months, to test this hypothesis.

It is important to note that drought duration and severity will generate different impacts on pasture species depending on their water use strategies. For example, conservative plant strategies that minimise water loss are very useful in long duration and severe droughts but are disadvantageous in less stressful conditions because this strategy is associated with lower photosynthesis and productivity. On the other hand, riskier plant strategies that maximise carbon gain are very useful in wetter conditions but are disadvantageous as conditions become progressively hot and dry. As such, it is important to balance the potential productivity gains from risky species in wetter conditions against the probability of increased frequency and severity of drought, along with a background of rising temperatures and greater water demand.
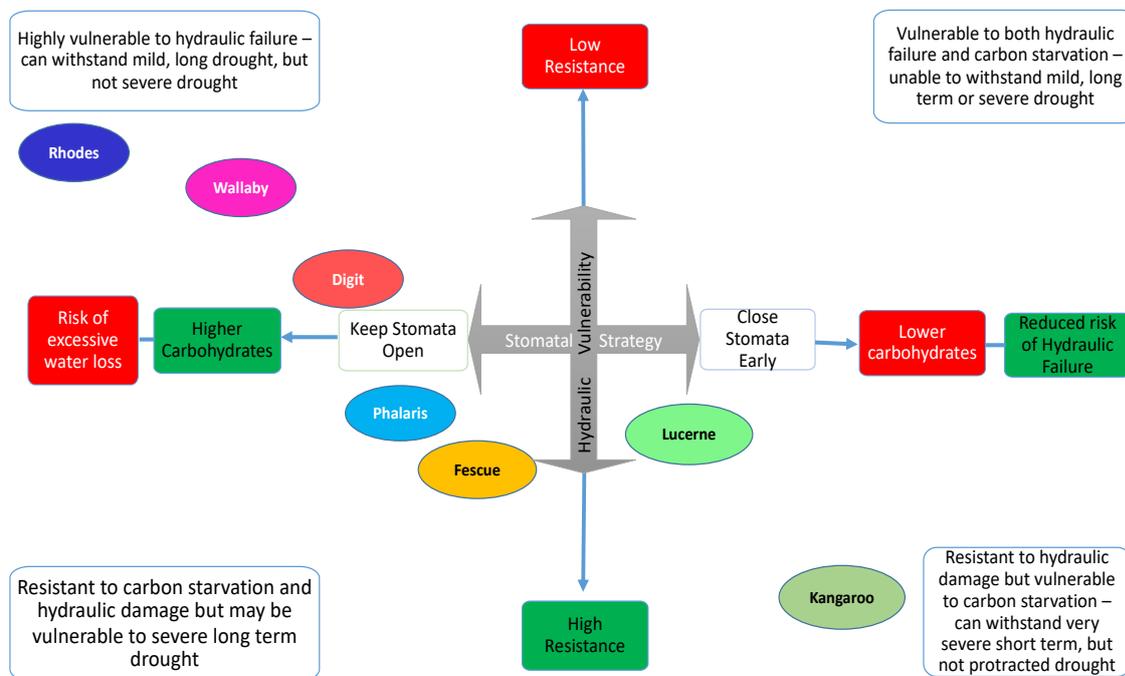
Fig 23. Species positions along axes reflecting their stomatal strategy and hydraulic vulnerability.

5.1.5   Relationships between plant traits and sensitivity to drought

Plant functional traits are increasingly used to predict ecological consequences of climate change, yet few experiments have established direct linkages between plant traits and observed climate-driven plant population and community changes (Nelson et al., 2017). From our analysis of 2018 trait data), root tissue density (RTD) and mycorrhizal colonization rates had the strongest relationships with species' drought sensitivity. Species with higher RTD and lower levels of mycorrhizal colonization were associated with greater biomass reduction under drought. The high carbon cost of constructing dense roots appears to divert carbon away from aboveground growth when plants are exposed to drought. Mycorrhizal fungi have been shown to increase plant drought tolerance via

increased uptake of water and nutrients, and our findings support this observation (Augé, 2001). Whilst there is still work needed to maximise the power of this type of multi-trait analysis, it does nevertheless provide unique insight into species differences in key traits related to the uptake and use of water and nutrients, and can help explain observed differences in plant sensitivity to climate extremes.

Additional traits, such as specific root length and root diameter, also have direct hypothesised links with water uptake and/or carbon allocation strategies under drought, although we did not see a significant relationship here. However, further investigation of trait data from 2019, including trait plasticity in response to treatments, and incorporation of data from other species is warranted. Such an approach would enable more powerful analysis of trait-sensitivity relationships that can be used to inform species and cultivar choice, as well as opportunities for breeding more drought-resistant varieties. The important role of mycorrhizae in boosting productivity and offsetting climate impacts suggests that management strategies aimed at enhancing soil health and belowground biodiversity may increase the tolerance of pasture species to climate stress.

5.1.6 Summary

Responses to imposed climate manipulations differed considerably among pasture species, reflecting variation in above and belowground traits and associated mechanisms relating to carbon allocation strategies and the acquisition and use of water and nutrients. Understanding trait-sensitivity relationships can be valuable for predicting responses of other species and cultivars to future, more extreme climates.

Based on productivity responses across the full two years of this study, species can be categorised according to their drought resistance[#], as follows:

Drought Resistance: **High**: Rhodes, Digit, Wallaby
                        Medium: Ryegrass, Phalaris, Lucerne, Kangaroo
                        Low: Fescue, Biserrula

[#]Resistance categories correspond to overall yield reductions (all harvests across the 24-month study period): High <20% yield reduction, Medium 20-40% reduction, Low >40% reduction.

Amongst the perennial species, annual yields in plots exposed to 6 months of winter/spring drought were greatest for Kangaroo, lucerne, Digit and Rhodes, followed by Wallaby, Fescue and Phalaris; the highest yielding species under drought were those that had either moderate drought resistance and a long growing season (e.g. Lucerne, Rhodes), or lower resistance combined with strong recovery (high resilience) and inherently fast growth rates (e.g. Digit, Kangaroo). Biserrula's low drought resistance may be associated with strong negative effects on the size and activity of its N-fixing nodules, particularly when exposed to prolonged drought.

While many of our results focus on the effects of drought, it needs to be emphasized that warming frequently had negative effects on plant performance, leading to additive or less-than-additive effects in the combined treatments. Interactive effects of seasonal drought and warming on soil water and nutrient availability need to be incorporated into model forecasts, and experiments like PACE provide the data required for parameterizing such models. In many cases, these combined

treatments can have non-linear, threshold effects that are difficult to recognize in the absence of factorial experiments.

5.2 PACE glasshouse experiments

5.2.1 Experiment 1: Warming, drought and the role of mycorrhizal fungi

We designed the first glasshouse experiment to investigate how mycorrhizal fungi mediate nutrient losses (N_2O fluxes and nutrient leaching) under different climate scenarios. N_2O is a strong greenhouse gas and contributes to rising temperatures (Butterbach-Bahl et al., 2013). AM fungi are present at varying abundance in soils (Storer et al., 2018) and have the potential to mitigate N_2O emissions from agricultural (Teutscherova et al., 2019) and natural (Liang et al., 2019) ecosystems. We observed that warming greatly increased N_2O fluxes from soil but that inoculation with AM fungi greatly reduced those fluxes and, in some cases, negated them (**Fig 17**). The benefit associated with AM fungi under warming was greater with Lucerne than with Fescue, which was likely due to a negative effect of warming on AM fungal abundance when associated with Fescue. Therefore, agricultural management practices that promote abundance of AM fungi and increase their resilience and resistance to environmental stress may have important benefits for reducing greenhouse gas emissions.

Similar context-dependent results were observed with regard to another key ecosystem function, reduction in nutrient leaching. Other studies have demonstrated that AM fungi can reduce leaching of nutrients in agricultural and natural ecosystems (Cavagnaro et al., 2015; Köhl and van der Heijden, 2016; Martínez-García et al., 2017), but our results suggest that this benefit could be reduced under warmer temperatures for some plants. AM fungi reduced leaching of mineral P under both ambient and elevated temperatures in the presence of Lucerne (**Fig 18a, b**). This benefit was, however, only observed under ambient temperatures for Fescue and was lost under elevated temperature. Since we did not find any effects of AM fungi on NO_3^- and NH_4^+ leaching in this study, N acquisition and interception by AM fungi may have been relatively low in this experiment, which is in line with previous studies (Reynolds et al., 2005; Van Der Heijden, 2010; Bender et al., 2015).

The results from this experiment indicate that AM fungi can be important moderators of key soil nutrient processes under changing climates, although further study is needed to determine why and to what extent AM fungal-mediated soil nutrient retention is affected by climate stress for other species. Additional benefits may be possible for plant breeders, agronomists and producers if we can identify the specific traits in plants and their fungal symbionts that are responsible for improving soil nutrient retention and plant nutrient-use efficiency under present and future climates.

5.2.2 Experiment 2: Elevated CO_2 and tropical grass/legume mixtures

Tropical grasses are being increasingly used in sub-tropical/temperate locations, due to their high performance under warm conditions and their inherently high water-use-efficiencies. However, their low nutritional quality compared to many temperate species means that it is important to explore cost-effective ways of improving their nutritional value for livestock. Intercropping grasses with legumes can generate over-yielding (increased productivity for the species mixture over monoculture) and there are several potential mechanisms via which this occurs (Ashworth et al., 2018). Possible mechanisms include a) facilitation e.g. by donation of biologically fixed N from

legumes to grasses, or b) complementary N use (i.e. the avoidance of competition for common nutrient pools, such that grasses access more soil N and legumes rely more on biological N fixation (BNF)). Many studies have demonstrated an increase in BNF in legumes grown under eCO_2 (Edwards et al., 2006; Lam et al., 2012) and, with global CO_2 concentrations rising steadily, it is possible that BNF could play a bigger role in supporting growth of legumes and neighbouring grasses in the future.

This study revealed clear benefits of growing tropical grasses with a companion legume, both in terms of actual biomass and the total amount of N in plant material. There was, however, only limited evidence of an overall benefit of eCO_2 to legume-grass facilitation; although legumes did benefit from eCO_2 themselves, the associated increase in growth of companion grasses was modest, such that productivity in mixed species pots (while up to 35% greater than in single species pots for Desmodium-Panic) was not significantly increased by eCO_2.

Stable isotope analysis provided some important insights into the role of biological N fixation (via legume nodules) in the growth facilitation of grasses observed in our glasshouse experiment. When grown together, legumes increased their reliance on BNF for their N requirements, while grasses did not. This suggests that the increase in productivity of grasses in mixture with legumes derives from greater access to soil N. Taken together, these findings suggest that, whilst growing tropical grasses with companion legumes can boost their yield, effects on their tissue N concentration are small. Further, whilst eCO_2 can result in increased legume productivity (and that of their companion grasses, in mixture), these effects are small. A final consideration is that grass-legume facilitation – and responses to eCO_2 – varied between species pairs. Further investigation for additional species mixtures would, therefore, provide more information about opportunities for boosting the performance of tropical pastures, including under elevated CO_2. It should, however, be noted that these responses are also likely to be modified by other elements of climate change, such as drought, warming and climate extremes.

We anticipate that a large number of scientific and technical publications will result from the PACE project. The first three for journal submission, below, are in an advanced state of preparation:

1) Zhang, H, Powell, JR, Plett, JM, Churchill, A C, Power, SA, Macdonald, CA, Jacob, V, Kim, G, Pendall, E, Tissue, DT, Catunda, K M, Igwenagu, C, Carrrillo, Y and Anderson, IC. Arbuscular mycorrhizal fungal reduction of P nutrient leaching may be partially negated by climate warming. Submitted to *Soil Biology and Biochemistry*.

2) Churchill, AC, Zhang, H, Fuller, K, Amiji, B, Anderson, IC, Barton, CVM, Carrillo, Y, Catunda, KM, Chandregowda, M, Igwenagu, C, Jacob, V, Kim, G, Macdonald, CA, Medlyn, BE, Moore, B, Pendall, E, Plett, J, Post, AK, Powell, JR, Tissue, DT, Tjoelker, MG and Power, SA. Consequences of climate extremes for plant productivity, and associated recovery, among diverse pastures. For submission to *Agriculture, Ecosystems and Environment*.

3) Zhang, H, Powell, JR, Power, SA, Churchill, A ., Plett, JM, Macdonald, CA, Jacob, V, Kim, G, Pendall, E, Tissue, DT, Catunda, KM, Igwenagu, C., Carrillo, Y, Moore, BD and Anderson, IC. Benefit of N_2O reduction from arbuscular mycorrhizal fungi persists under future warming for pastures. For submission to *Soil Biology and Biochemistry*.

6   Conclusions and recommendations

This project has made significant advances in understanding of how key pasture species respond to climate extremes, and some of the mechanisms associated with their sensitivity. Our research provides guidance for selection of species – and associated traits – to maximise productivity and pasture nutrition in the context of extreme seasonal drought and predictions of increased temperatures.

All species were significantly affected by the winter/spring drought treatment, although there were considerable differences in the extent to which cool-season productivity (i.e. biomass produced during the imposed drought) was reduced. Despite this, most perennial species were resilient and exhibited rapid recovery following rewatering. The warming treatment generally reduced productivity, with the only exception being for the native grass mixture (Kangaroo/Wallaby) in the first winter. There was also evidence that warming increased the negative impacts of drought for some species and time periods, highlighting the importance of co-occurring climate stresses for pasture systems productivity.

Plant carbon allocation strategies in response to drought and warming differed between species, with evidence of a proportional increase in belowground investment and/or shifts towards more acquisitive rooting strategies (including increased root mycorrhizal colonisation for tropical grasses) under more extreme climates. There is a clear association between species' root traits and their response to drought and warming, with low root densities associated with greater resistance to climate extremes. Further work in the area of plant trait-climate sensitivity relationships may be effective for predicting sensitivities to future climates for a wide range of species and cultivars, based on their morphological and physiological characteristics.

Observations of large increases in greenhouse gas emissions (N_2O) associated with both drought and warming flags this as a key issue for the sector under future climates. Evidence that mycorrhizae can reduce or even fully offset this increase in emissions points to the important role of soil biology for sustainable pasture management and achievement of industry goals in relation to carbon neutrality.

Practical application of findings. Annual yields in plots exposed to severe winter/spring drought were greatest for Kangaroo, Rhodes, Lucerne and Digit, followed by Wallaby, Phalaris and Rye. The highest yielding species under drought were those that had either relatively high drought resistance and a long growing season (e.g. Rhodes, Lucerne), or lower resistance combined with strong recovery and inherently fast growth rates (e.g. Digit, Kangaroo). Combinations of relatively drought-resistant legumes (e.g. Lucerne) with resilient grasses (e.g. Digit) represent an opportunity to secure more consistent, high quality biomass production across multiple seasons, even during periods of prolonged drought.

Fescue's performance in experimental plots was modest, compared to reported field productivity values, likely reflecting lower plant densities and the high temperatures experienced at the field site. This species was, however, one of the least sensitive to drought and warming in the second year of the study, implying that its tolerance of repeated droughts may increase over time.

Field and glasshouse experiments provided evidence of increased digestibility and higher crude protein concentrations of grasses grown alongside a legume, compared to grass monoculture; there

was also evidence of lower drought-related yield reductions in mixtures (i.e. "climate insurance"), compared to grass-only monocultures, during the first year of the project. However, given differing (plant growth and root nodule) sensitivity to winter/spring drought, along with differences in growth phenology, choice of legumes for mixed pastures will be a strong determinant of their ability to facilitate growth of neighbouring grasses. The generally perceived benefits of increased atmospheric CO_2 concentrations for tropical legumes are not likely to result in large productivity increases.

Adaptation strategies – Matching plant species and cultivars to future, potentially more extreme climates is key to farm-scale adaptation. Those species that do not tolerate extreme heat (e.g. Ryegrass, Fescue) or prolonged, severe drought (e.g. Biserrula) can either be replaced, or alternative cultivars targeted, to reduce climate risk. Inclusion of tropical grasses which, in our study were the biggest biomass producers, even under drought conditions, may help offset climate impacts on pasture productivity. However, the generally lower nutritional value of tropical species, compared to temperate grasses, means that companion planting with legumes, additional fertiliser or feed supplementation would be necessary to realise the full benefits of productivity gains and climate resilience associated with tropical grasses.

Inclusion of N-fixing legumes in mixed pastures has the potential to increase productivity, improve nutritional quality and reduce fertiliser requirements, with knock on reductions in greenhouse gas emissions and increased fertiliser use efficiency. Mixed pastures may also be more resistant to severe drought and warmer temperatures, and recover more rapidly when conditions improve. Since mycorrhizal fungi appear to play a role in drought responses of tropical grasses, and were linked to reductions in greenhouse gas emissions and soil nutrient leaching, it seems clear that practices aimed at supporting and maintaining healthy soils with diverse, functional microbial communities are a key strategy for boosting productivity and minimising the global warming potential of pasture systems under future climates.

Future R&D – Trait-sensitivity relationships offer a means of predicting species' or cultivar responses to climate change, including extremes of heat or drought. Expansion of these datasets to include more species and a more comprehensive suite of plant traits would provide a powerful means of trait-based predictions of climate sensitivity to inform planting recommendations across a wide range of environmental conditions.

Plant hydraulics data suggest that the imposed drought was not that extreme relative to the capacity of the plants to adjust to the reduction in soil water availability. Given the likelihood of increased severity and duration of future drought, in combination with increasing temperatures, there is still a need to expose species to harsher climate conditions to determine thresholds beyond which they are unable to recover. Such information for pasture species that currently dominate within the major dairy and livestock regions will strengthen the evidence base for targeted adaptation efforts around species selection.

Mixed pasture systems seem to offer a degree of climate insurance, both in terms of productivity and nutritional quality, relative to single species plantings. The ability of legumes to facilitate grass growth and boost sward nutrition is key to the success of mixed pastures, yet their N-fixing ability can be strongly affected by climate extremes. Knowledge of how keystone legumes respond to climate extremes is a key area for further research. Furthermore, the potential for mixed perennial

pastures to sequester carbon in soils may be increased by shifts in belowground carbon allocation and altered root traits – as suggested in this study. Since plant carbon allocation strategies will be altered by management practices such as grazing intensity and fertiliser regime, understanding the role of management in above- and belowground plant performance is a key area for further research, especially in the context of CN30.

Plant-microbial interactions are key to sustainable pasture productivity under variable and extreme climates. Our findings of detrimental impacts of drought and warming on legume rhizobia, and an important role for mycorrhizal fungi in reducing greenhouse gas emissions, underlines the importance of healthy soils for productive, resilient pastures. Further research is needed to explore these relationships in order to make informed recommendations regarding soil management.

7 Key messages

1. Species varied considerably in the magnitude of their response to drought and warming; those species that completed most of their growth during the winter/spring drought period generally experienced bigger reductions in annual productivity compared to those that continued to grow strongly during the summer/autumn. Consideration of species' phenology in relation to local climate predictions is, therefore, important for minimising climate risk.

2. Tropical grasses were, on average, more productive under prolonged winter/spring drought than temperate species. Whilst tropical species are generally of lower nutritional quality than their temperate counterparts, their crude protein concentrations and digestibility were significantly increased under drought conditions and there was also evidence of improved nutrition when grown alongside legumes.

3. Warming was generally associated with reduced biomass production across the year. This is likely the result of greater evaporative demand under higher temperatures, as well as decreased rates of photosynthesis as species exceed their optimal temperatures during summer. Of note, however, warming did result in higher summer/autumn crude protein and digestible dry matter concentrations, partially offsetting warm season yield reductions.

4. Plants that had less dense roots performed better under drought conditions. Cultivars with these root traits are likely to be associated with greater resistance to future, more extreme climates.

5. Emissions of nitrous oxide – one of the most potent greenhouse gases – will increase as temperatures rise. Use of best-practice fertiliser management practices (e.g. timing inputs to match peak crop demand or use of enhanced efficiency fertilisers) along with practices that promote healthy soils (with high levels of mycorrhizal fungi) will reduce the global warming potential of pasture systems as temperatures continue to rise.

Areas for future research have been identified in the discussion and conclusions sections, but key priorities for further work are as follows:

6. Improved understanding of relationships between plant traits and climate sensitivity will make it possible to identify trait combinations associated with climate resilience across a wider range of species than have been tested so far. Such knowledge can inform on-ground species and cultivar choice as well as breeding programmes for cultivars that can perform well under future climates.

7. The use of pasture models to predict yield and manage climate risk is currently limited by a lack of information on how the key physiological and morphological traits that underpin model performance are themselves modified by climate extremes. Incorporation of new trait data from manipulation experiments will improve the capacity of existing models to simulate pasture species' responses to extreme climate conditions.

8. Mixed perennial pastures, including legumes and tropical grasses, have the potential to offer climate insurance, through niche differentiation and facilitation. More research is needed to understand how key pasture legumes will perform – particularly in relation to their N-fixing capacity – under warmer, drier conditions, and which grass-legume combinations offer the best opportunity to optimise multi-season production and nutrition, under more extreme climate conditions.

9. Plant belowground carbon allocation strategies including root traits such as root to shoot ratio, carbon to nitrogen ratios and rooting depths are known to respond to climate conditions. Quantifying how intrinsic plant strategies and responses to altered climate affect soil organic matter accumulation and greenhouse gas emissions is a crucial area for research, especially in the context of industry commitments to CN30, improved land management and reduced greenhouse gas emissions.

10. Given the importance of forage legumes within the meat, livestock and dairy industries, a key question is how will legumes perform under future climates? This relates to both current, widely used species and new, tropical legumes with the potential to play a more prominent future role in southern pasture systems. Identifying legumes that can perform strongly under severe climate stress could help maintain pasture production, as well as reducing the sector's carbon and nitrogen footprints (for example, via reduced ruminant methane emissions (*Desmanthus* spp) and decreased fertiliser requirements).

11. Commercially available smart fertilisers and microbial amendments have the potential to reduce nutrient leaching and greenhouse gas emissions, while increasing pasture productivity. Research is needed to evaluate how smart fertiliser formulations perform under future climates.

12. The inevitability of warmer temperatures and more frequent, longer duration and recurrent droughts means that understanding how prolonged climate stress affects perennial species' performance is a key area for future research. High temporal resolution of plant responses to heatwaves can identify tipping points of soil water content or cumulative days of excessive heat beyond which plant death occurs, and thus climate thresholds for species' persistence.

8   Bibliography

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9 Appendix

9.1 Field drought and warming treatments 2018-2020
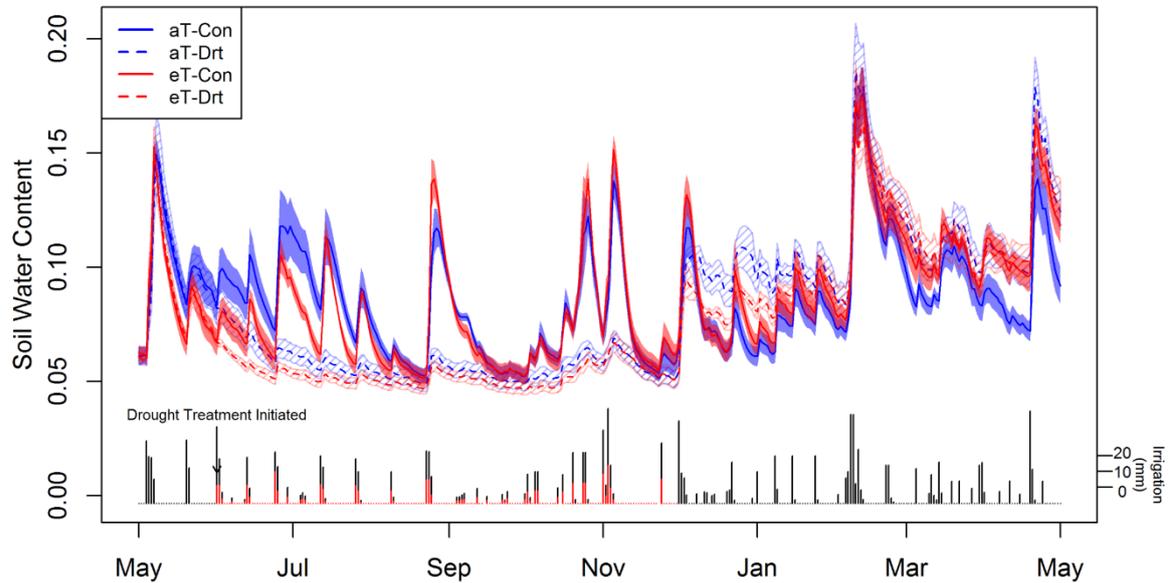
Fig A1*. Soil moisture content in Fescue plots during the 2018 winter-spring drought and the summer-autumn recovery period in 2019.*

Table A1. *Total number of days during Year 1 of the field experiment (June 1st 2018- May 31st 2019) and Year 2 (June 1st 2019- Feb 1st 2020) where canopy surface temperatures in droughted and warmed plots exceeded specified thresholds*

	Year	aT-Con	aT-Drt	eT-Con	eT-Drt
Days < 0 °C	Year 1	3	4	0	0
	Year 2	2	0	0	0
Days < 5 °C	Year 1	63	61	20	20
	Year 2	48	47	16	11
Days > 35 °C	Year 1	112	148	151	168
	Year 2	103	129	113	138
Days > 40 °C	Year 1	64	93	91	119
	Year 2	66	95	76	105
Days > 45 °C	Year 1	22	53	49	72
	Year 2	29	54	34	72

9.2   Root biomass in response to 2019 winter-spring drought
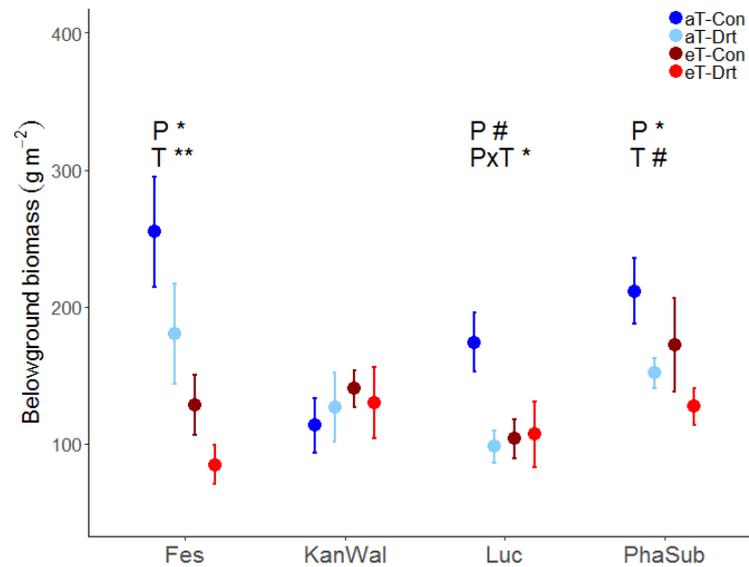
Fig A2. *Belowground biomass for plants grown under drought (Con/Drt) and warming (aT/eT) conditions based on soil cores collected during Nov 2019, following six months of drought. All notations and species abbreviations match Fig 7.*

**Table A2.** *Belowground root standing crop (g m^{-2}) for samples collected at the end of the first drought period (Nov. 2018, Year 1), six months after the drought ended (May 2019) and at the end of the second winter/spring drought (Nov. 2019, Year 2).*

Pasture	Treatment	Drt period Year 1	Recovery	Drt period Year 2
Biserrula	aT-Con	144.8 ± 23.9	NA	67.3 ± 10.0
	aT-Drt	108.0 ± 18.5	NA	66.7 ± 8.1
Digit	aT-Con	269.5.0 ± 47.6	157.1 ± 31.3	289.5 ± 44.3
	aT-Drt	305.6 ± 43.4	148.0 ± 14.2	258.0 ± 25.0
Digit/ Biserrula	aT-Con	295.1 ± 26.1	NA	286.5 ± 24.8
	aT-Drt	271.2 ± 40.8	NA	235.8 ± 27.7
Kangaroo	aT-Con	197.6 ± 39.0	124.0 ± 9.3	244.4 ± 24.5
	aT-Drt	236.0 ± 40.9	94.2 ± 8.1	225.7 ± 13.2
Phalaris	aT-Con	NA	86.2 ± 10.5	181.8 ± 33.8
	aT-Drt	NA	132.7 ± 19.4	183.0 ± 19.0
Rhodes	aT-Con	286.9 ± 26.6	165.6 ± 12.2	240.7 ± 9.7
	aT-Drt	299.4 ± 31.4	140.0 ± 14.4	187.8 ± 24.9
Ryegrass	aT-Con	347.5 ± 46.5	83.0 ± 16.4	209.9 ± 31.1
	aT-Drt	306.1 ± 37.7	66.6 ± 6.8	180.5 ± 10.6
Wallaby	aT-Con	NA	46.5 ± 9.1	134.7 ± 19.5
	aT-Drt	NA	21.4 ± 4.3	84.7 ± 14.9
Fescue	aT-Con	473.7 ± 48.5	82.6 ± 15.9	254.8 ± 40.3
	aT-Drt	303.6 ± 22.9	65.1 ± 11.3	180.4 ± 36.4
	eT-Con	349.8 ± 54.5	59.6 ± 9.7	128.4 ± 21.9
	eT-Drt	263.0 ± 33.3	42.9 ± 5.2	84.7 ± 13.9
Kangaroo/ Wallaby	aT-Con	158.2 ± 17.1	NA	113.3 ± 19.7
	aT-Drt	134.0 ± 21.8	NA	126.8 ± 25.1
	eT-Con	124.6 ± 12.9	NA	140.1 ± 13.2
	eT-Drt	154.0 ± 16.0	NA	129.7 ± 25.8
Lucerne	aT-Con	NA	NA	174.0 ± 21.5
	aT-Drt	NA	NA	98.0 ± 11.6
	eT-Con	NA	NA	103.7 ± 14.2
	eT-Drt	NA	NA	106.9 ± 23.9
Phalaris/ Sub clover	aT-Con	343.9 ± 28.1	NA	211.4 ± 24
	aT-Drt	369.0 ± 40.7	NA	151.4 ± 11
	eT-Con	328.3 ± 50.2	NA	172.4 ± 34
	eT-Drt	343.7 ± 51.8	NA	127.1 ± 13.7

9.3   Soil nutrient availability under drought and warming

The availability of soil nutrients is an important constraint on plant nutrient uptake that ultimately dictates plant growth and forage quality. There were important differences in soil nitrogen availability between species, with Lucerne having the highest levels. We found that drought reduced soil nitrogen availability by close to 50% in Digit and Digit/Biserrula mixed plots (**Fig A3a**). As these pastures aboveground biomass were the most strongly affected by drought during the recent winter-spring (2019) period, the reduction in available soil nitrogen may play a role in drought-related productivity declines for this species. On-going analyses will examine whether increasing plant or microbial uptake and immobilisation of nitrogen accounts for the decline in soil availability under drought. Drought significantly decreased available soil phosphorus concentrations by a similar magnitude as nitrogen (up to 50%) for a range of species, including Rhodes, Digit, Digit/Biserrula mix, Fescue, Ryegrass and the Phalaris/Sub mix (**Fig A3b**). These effects of drought on nitrogen and phosphorus availability could not explain any of the observed effect on above- or below-ground productivity. Ongoing analysis will examine whether the observed effects on soil nutrient availability are related to plant nutrient status and will examine the role of nutrient availability in pasture recovery from drought.
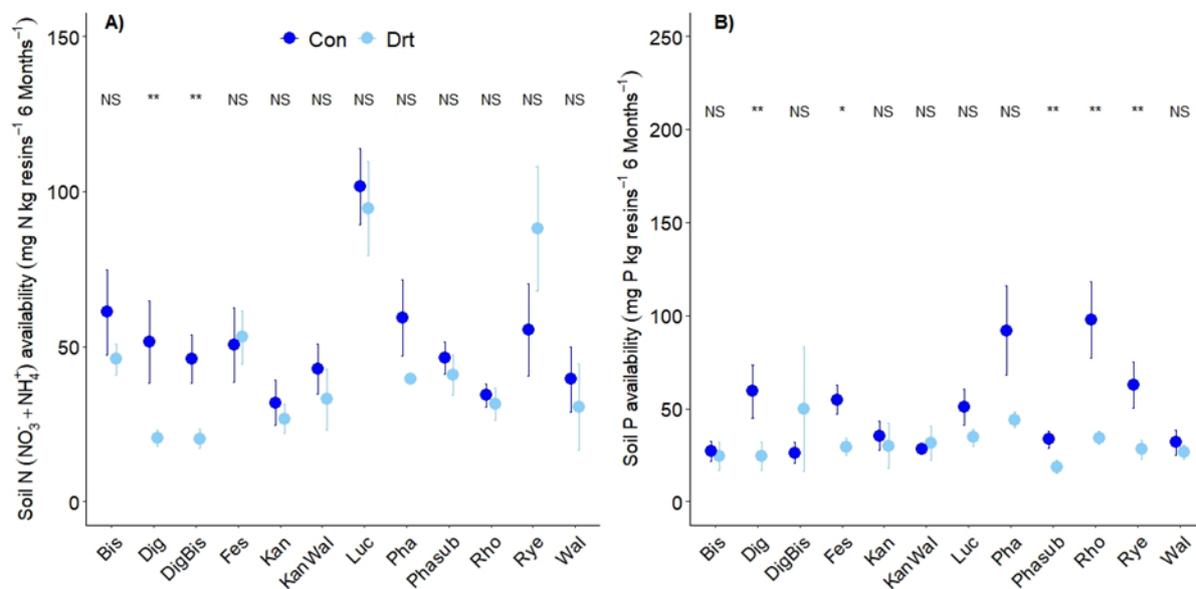

Fig A3. *A) Total soil inorganic nitrogen (NH_4^+ and NO_3^-) and B) phosphorus availability among pastures exposed to winter+spring drought (2019). Data are from soil resin extractions installed at 0 – 10 cm soil depth during the winter-spring 2019 drought period. All notations and abbreviations match Fig 7.*
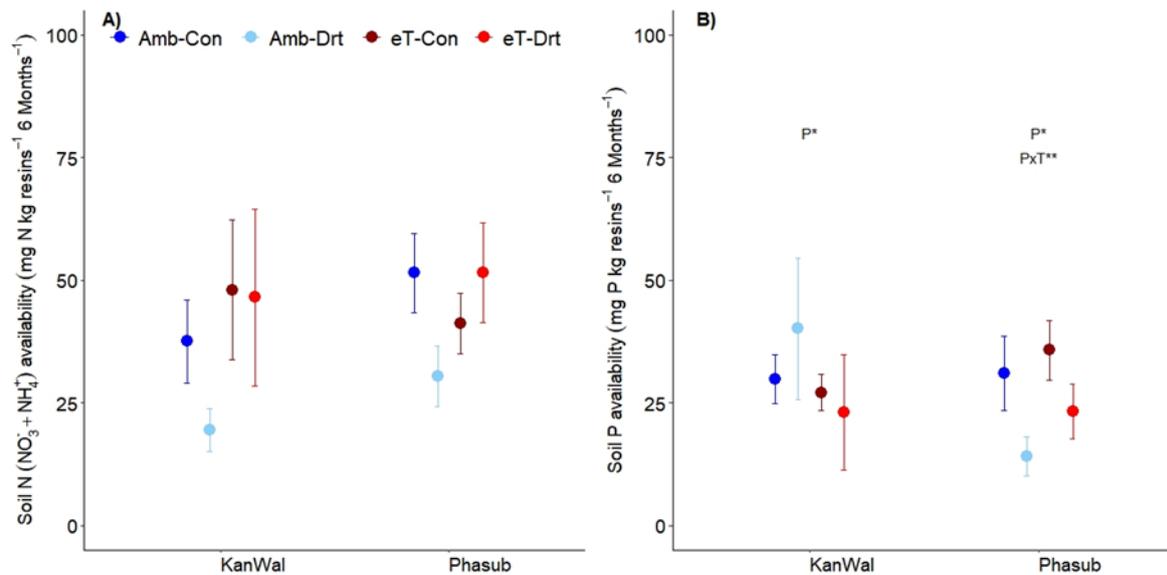
Fig A4. *A) Total soil inorganic nitrogen (NH_4^+ and NO_3^-) and B) phosphorus availability among pastures exposed to both drought and warming treatments from soil resin extractions installed at 0 – 10 cm soil depth during the winter-spring 2019 drought. All notations and abbreviations match Fig 7.*

In general, neither warming nor the combined warming+drought treatment had a significant effect on soil N availability during the winter/spring 2019 season (**Fig A4a**). However, soil phosphorus availability was increased by warming in Phalaris/Sub clover mixed plots, possibly driven by increased P demand in legume nodules, and this warming-induced increase in P was stronger in droughted plots (**Fig A4b**). Ongoing analysis will determine whether increased plant uptake and/or microbial immobilisation account for these observed effects under warming.
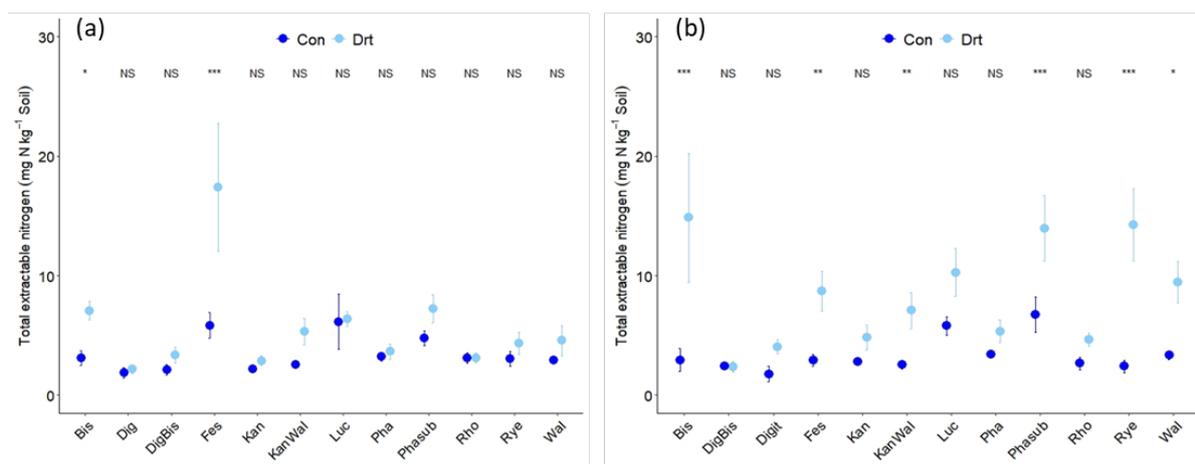
Fig A5. *Total extractable soil inorganic nitrogen (NH4+ and NO3-) (a) August 2019 (early-drought) and (b) November 2019 (end of drought) among pastures exposed to winter+spring drought (2019). Data are from soil extractions (K_2SO_4) from 0 – 10 cm. Significant treatment effects are indicated as * $P < 0.05$, ** $P < 0.01$ and $P < 0.001$. NS is not significant. Error bars indicate ± one standard error (n = 6). All notations and abbreviations match Fig 7.*

9.4   Species' nutritional quality under drought and warming

Table A3. *Nutritional metrics for species grown under control, drought, warming and drought + warming treatments for plants harvested at the end of the 2018 drought period, and recovery post-drought in 2019.*

Pasture	Treatments	Crude Protein (% of DM)		Neutral Detergent Fibre (% of DM)		Digestible Dry Matter (% of DM)	
		Drought	*Recovery*	*Drought*	*Recovery*	*Drought*	*Recovery*
Legumes							
Biserrula#	Control	15.3±0.1	NA	53.1±0.6	NA	60.0±1.1	NA
	Drought	16.0±0.9	NA	54.5±1.6	NA	58.8±0.9	NA
Biserrula (D/B)#	Control	11.9±2.5	NA	64.4±10.5	NA	55.6±7.4	NA
	Drought	8.6±0.4	NA	66.7±5.0	NA	52.5±3.9	NA
Lucerne#	Control	18.6±0.7	13.9±0.6	47.4±2.5	50.0±0.4	64.0±0.7	62.5±0.1
	Drought	16.7±0.5	14.8±0.2	45.5±0.4	47.6±2.0	64.8±0.5	64.5±1.7
	Warming	18.8±1.0	14.2±0.5	47.2±1.1	45.4±0.8	64.0±1.0	66.0±0.5
	D+W	16.3±0.8	15.1±0.98	48.9±1.3	46.4±2.3	63.0±0.5	65.1±1.2
Temperate Grasses							
Fescue	Control	10.0±0.7	13.3±0.6	63.7±1.2	53.5±0.6	64.6±0.5	68.6±0.2
	Drought	12.2±0.6	17.5±1.2	64.8±1.4	48.5±0.5	64.3±0.7	71.1±0.3
	Warming	11.8±0.7	15.7±1.4	62.2±1.0	51.2±1.3	65.5±0.4	69.8±0.7
	D+W	12.0±0.5	17.0±0	62.5±1.0	50.0±0	64.5±0.6	69.9±0
Phalaris (P/S)#	Control	13.8±1.2	17.6±1.4	61.4±0.2	56.0±0.3	63.6±0.5	70.8±0.4
	Drought	11.0±0.5	18.3±1.0	63.5±0.2	55.8±1.1	61.6±0.1	70.2±0.1
	Warming	13.2±0.1	21.6±0.2	62.3±1.2	54.2±0.9	63.1±0.6	71.5±0.5
	D+W	11.3±0.8	22.3±1.2	62.2±1.2	53.7±0.3	62.6±0.7	72.4±0.5
Phalaris#	Control	15.7±2.0	15.4±0.3	61.7±2.4	56.0±0.8	64.6±1.7	67.2±0.5
	Drought	15.6±0.2	14.2±0.7	62.5±0.4	55.9±0.7	65.6±0.3	67.6±0.4
Ryegrass#	Control	12.8±0.6	NA	59.4±0.7	NA	64.7±0.4	NA
	Drought	15.0±1.2	NA	59.6±1.3	NA	64.9±1.2	NA
Wallaby#	Control	7.9±1.0	10.4±1.2	76.7±1.8	68.6±1.0	54.6±0.8	60.0±0.3
	Drought	6.8±0.5	11.8±0.5	74.7±1.2	67.2±0.2	55.8±0.7	60.5±0.4
Tropical grasses							
Digit	Control	7.9±0.9	6.5±0.8	74.0±1.4	68.9±1.7	57.0±1.3	60.9±1.4
	Drought	8.2±1.2	7.1±0.3	70.8±1.6	70.9±0.4	60.8±1.0	60.8±0.3
Digit (D/B)#	Control	9.4±0.2	7.3±0.6	68.4±1.0	68.4±1.2	60.0±0.6	62.0±0.9
	Drought	12.0±0.1	8.7±1.0	65.2±1.9	67.5±0.4	63.3±0.2	62.8±0.4
Kangaroo	Control	5.4±0.1	6.6±0.9	78.2±0.5	71.0±0.6	56.4±0.6	61.7±0.5
	Drought	5.0±0.2	7.1±0.5	76.7±0.8	70.7±1.0	58.5±0.2	62.2±0.6
Kangaroo (K/W)	Control	3.4±0.8	6.3±0.5	79.4±1.5	73.7±1.1	52.9±0.9	61.7±0.8
	Drought	3.7±0.6	7.2±0.7	74.2±1.4	73.6±0.6	58.3±1.1	63.0±0.4
	Warming	3.8±0.5	7.6±1.2	76.7±1.1	74.0±1.0	54.5±1.0	62.4±1.1
	D+W	4.8±0.3	7.8±1.0	71.2±2.7	72.7±2.1	59.3±1.7	63.1±1.3
Rhodes	Control	6.7±0.4	7.5±0.6	72.8±0.8	71.5±1.1	61.8±0.1	64.2±0.7
	Drought	7.0±0.7	7.8±0.6	69.3±0.9	69.5±0.5	65.3±0.6	65.1±0.1

Values shown are mean ± 1 standard error. # indicates species that did not experience a full 6-month drought in 2018. NA means insufficient sample for analysis. D/B refers to the Digit-Biserrula, mixture, P/S the Phalaris and Sub-clover mix and D/B the Digit-Biserrula mix. "Drought" refers to the Nov. 2018 harvest. "Recovery" refers to the first harvest of each species in 2019, following cessation of the winter-spring 2018 drought. Digestible dry matter (DDM) was calculated using the formula: DMD = 88.9-(0.779*Acid detergent fibre percentage of dry matter) (Linn & Martin, 1989).

9.5   Factors influencing plant nutritional quality

Comprehensive nutritional analysis of samples from the end of the second drought period in November 2019 is still underway, but proportions of live (c.f. dead) and leaf (c.f. stem) material are summarised in Appendix **Table A4**. These data can help to explain observed changes in nutritional quality across pasture species and treatments. For Phalaris (in mixtures) and Fescue, and, to a lesser extent Digit and Rhodes, drought reduced the proportion of live material relative to control plots. Warming had no effect on the proportion of live material for any species. The relative proportion of live (green) and dead (dry) material of the aboveground biomass will influence the forage quality as green pasture will always be higher quality (60-85% digestibility) than dead herbage (35-60%). (DPI, 2018). Drought most commonly increased the proportion of leaf, relative to stem, in harvested material, as did warming (Kangaroo and Phalaris mixtures). This may be explained by greater plant heights and more advanced phenology in control pastures. Stems can be expected to be associated with more fibre, increased forage toughness and reduced digestibility (Terry and Tilley, 1964).

Table A4. Treatment effects on proportion of live and leaf material of pastures species at the end of drought period in November 2019.

Pasture	Drought effects		Warming effects		Drought + Warming effects	
	Live %	*Leaf %*	*Live %*	*Leaf %*	*Live %*	*Leaf %*
Biserrula	-3%	8%	-	-	-	-
Digit	-9%	13%	-	-	-	-
Digit (D/B)	-20%	23%	-	-	-	-
Kangaroo	0%	5%	-	-	-	-
Phalaris	1%	2%	-	-	-	-
Rhodes	-27%	-15%	-	-	-	-
Ryegrass	-3%	1%	-	-	-	-
Wallaby	6%	4%	-	-	-	-
Fescue	-62%	5%	-7%	4%	-59%	1%
Kangaroo (K/W)	-6%	40%	-2%	24%	-2%	46%
Lucerne	0%	0%	0%	-2%	-1%	-7%
Phalaris (P/S)	-62%	17%	2%	21%	-66%	21%

D/B = Digit & Biserrula mix; K/W = Kangaroo & Wallaby mix; P/S = Phalaris & Sub-Clover mix.