REGULAR ARTICLE

# Drought and phosphorus affect productivity of a mesic grassland via shifts in root traits of dominant species

Jeff Chieppa · Uffe N. Nielsen · David T. Tissue ·
Sally A. Power

## Abstract

*Aims* Precipitation and soil nutrients play an important role in regulating grassland productivity. However, little is known regarding the sensitivity of grasslands to changes in water and nutrient availability and the mechanisms driving productivity responses.

*Methods* We examined the effects of extreme drought (65% rainfall reduction) and phosphorus fertilization on aboveground net primary production (ANPP) and plant functional traits (PFTs) of four dominant mesic grassland species in a semi-natural grassland in southeast Australia. We used piecewise structural equation modelling to determine which PFTs contribute to the treatment effects on ANPP.

*Results* Reduced rainfall decreased ANPP by 29% while P addition increased ANPP by 62% at the community-level. Significant drought-related reductions in ANPP were apparent for *Setaria parviflora*, while *Cynodon dactylon* was the only species exhibiting increased ANPP under P addition. There was no interaction between rainfall and P addition. Structural equation modelling indicated specific root length was a key trait underpinning community-level ANPP responses to P; this was, however, primarily driven by a single dominant (~61% of community biomass) species (*Cynodon*).

*Conclusions* Our results indicate the negative impacts of drought on ANPP – driven primarily by *Setaria*– were not ameliorated by P addition. The positive effect of P addition on community-level ANPP was attributed to the response of the most dominant species, *Cynodon*, and mediated by changes in specific root length. This study highlights the importance of understanding the link between belowground traits and ANPP for predicting dominant species' response to global change drivers.

**Keywords** Plant functional traits · Grasslands · Phosphorus fertilization · Drought · Aboveground productivity

J. Chieppa (✉) · U. N. Nielsen · D. T. Tissue · S. A. Power
Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW, Australia

J. Chieppa
Penrith, Australia

## Introduction

Grasslands cover nearly 40% of the terrestrial surface (LeCain et al. 2002) and hold greater than one-third of the terrestrial carbon stock (Trumper 2009). Plant productivity and associated ecosystem processes in grasslands are primarily driven by rainfall patterns, with soil nutrient availability contributing to differences among

sites with similar climates (Suttie et al. 2005; Guo et al. 2012; Fay et al. 2015). Global climate models predict reductions in the frequency of rainfall events resulting in fewer events of greater magnitude with minimal change in total precipitation (Easterling et al. 2000; Seneviratne et al. 2012; IPCC 2013), which is likely to have negative impacts on the structure and function of grassland ecosystems (Knapp et al. 2001, 2002; Fay et al. 2011; Walter et al. 2012). These changes in precipitation will result in longer dry periods between rainfall events (Knapp et al. 2008), so investigating recovery of grasslands to rainfall events after prolonged rainfall deficits will be helpful in understanding how they may respond to future conditions. The absolute size of rainfall-related reductions in plant productivity will also depend on soil fertility and vary with management practices such as fertilization. For example, Hofer et al. (2017) observed that differing soil nitrogen (N) concentrations affected productivity responses of grasses and legumes following a severe drought.

The link between ANPP and rainfall is well established for terrestrial ecosystems (Huxman et al. 2004). Reduced water availability typically leads to down-regulation of photosynthesis resulting in lower biomass accumulation at the individual and community level (Medrano et al. 2002; Ripley et al. 2010). While this relationship has been widely studied, a framework for how and why communities vary in their sensitivity (i.e. ability to maintain ANPP under altered rainfall conditions compared to ambient conditions) of ANPP to changes in rainfall, remains elusive. In southeast Australia, predicted changes in rainfall include fewer days of rain, more days with heavy rain, and a greater contribution of those heavy rain events to the total rainfall received (Evans et al. 2017). These changes will generate longer dry periods followed by short-lived wet periods, similar to the pulse-dynamics experienced in more arid ecosystems (Austin et al. 2004).

Sensitivity of grassland ANPP to drought is likely mediated by the life history strategies and associated plant functional traits (PFTs, sensu Reich 2014), of the species found within those communities. For example, species that grow rapidly typically possess low tissue densities (i.e. mass to volume or area ratios), a result of limited carbon (C) investment into long-lived structural components. Species with high density tissues, associated with high longevity and resistance to biotic and abiotic stressors, typically undergo slow growth. This spectrum of plant form and function

has been described as the "resource-use" axis (Grime et al. 1997) and represents the inevitable co-occurrence between the ability of a plant to rapidly acquire resources and the increasing rate of tissue loss/turnover. Rapid-growing species are considered acquisitive, while slow-growing plants are considered as conservative. The relative abundance of acquisitive and conservative strategies in a community will lead to either resistance (i.e. ability to withstand disturbance, such as drought) or resilience (i.e. ability to recover following disturbance) of ANPP in grassland communities (Tardif et al. 2014).

Important PFTs associated with drought resistance include traits that allow the maintenance of growth during declining soil moisture levels, such as the possession of dense tissues for water retention to maintain photosynthesis or a high capacity for soil water uptake (i.e. high root mass fraction or high root length) (Volaire et al. 2014). Important PFTs associated with drought resilience include those that support rapid re-growth when soil water becomes available again, such as high specific leaf area (leaf area to leaf mass ratio). Therefore, the sensitivity of ANPP in grasslands to drought will depend on the abundance of PFTs related to acquisitive or conservative strategies to cope with drought.

Changes in root characteristics as a result of long-term fertilization in grasslands are likely to alter plant responses to drought. For example, there is evidence that increased soil P content increases root diameter and lowers specific root length (root length to mass ratio) resulting in less resource foraging potential (see Lambers et al. 2006 for review), which could negatively affect soil water absorption. In addition, high soil P availability may limit mycorrhizal colonization (Hetrick et al. 1990) and thus negatively affect water access (Ruiz-Lozano et al. 1995; Marulanda et al. 2003), although the soil P-mycorrhizae relationship is not universal across plant and fungal taxa (Osonubi 1994; Ruiz-Lozano et al. 1995; Augé 2001). Dos Santos et al. (2006) observed enhanced photosynthetic recovery following a mild water deficit of *Phaseolus vulgaris* L. when fertilized with P. Such morphological or physiological changes in grassland plant species as a result of P addition are likely to have consequences for ecosystem resistance and resilience to drought. There is also the potential for changes in soil nutrient availability to drive shifts across the acquisitive-conservative resource-use axis at the community scale that may moderate drought impacts on the system-level.

The overall objective of this study was to investigate the recovery of a mesic grassland community that had undergone P fertilization (no P and 10 g m$^{-2}$ y$^{-1}$ for four years) and reduced rainfall (65% reduction from ambient rainfall) following a summer drought period that had resulted in nearly total foliar dieback. Rainfall was applied artificially and occurred in tandem with natural events over forty days, with half the site receiving only 35% of those amounts (i.e. 65% reduction in rainfall) reflecting the most severe annual rainfall deficit for this site in the last 100 years. We used PFTs to understand potential mechanisms underlying species-specific responses. Specific questions we addressed included: (Q$_1$) does soil P addition alleviate the negative effects of reduced rainfall on community-level ANPP through changes in species-specific ANPP?, and (Q$_2$) do PFTs of dominant plant species within a community respond similarly to reduced water availability and P addition? Additionally, we asked: (Q$_3$) what PFTs mediate the ANPP response to rainfall and phosphorus in our mesic grassland community?

Providing a trait-based framework for these questions will help identify options for managing PFTs in grassland ecosystems to minimize the potential negative effects of anticipated future rainfall regimes. In addition, understanding how nutrient management and climate may affect grassland productivity will assist in developing more mechanistic and accurate predictions of terrestrial feedbacks on the global carbon cycle.

## Materials and methods

### Experimental site and design

The field site (area of 4800 m$^2$) was located at Western Sydney University's Hawkesbury campus (33.613586 S, 150.738047 E). Mean annual precipitation (1913–2013) is 807 mm (Australian Government Bureau of Meteorology, Richmond – UWS Hawkesbury Station, Station Number 67021) and summer (December to February) daily mean temperatures range between 18.8 and 29.4 °C, while winter daily mean temperatures range between 3.2 to 17.3 °C. The site was grazed by cattle for ~40 years until it was fenced in 2013 to exclude large herbivores. Historically, the site supported open grassy-woodlands (Benson and Howell 2002). The soil (90.2 ± 1.3% sand, 5.6 ± 1.4% clay) is Blackendon Sand with a hardpan at ~70 cm depth across the site. The most abundant species at the site are *Cynodon dactylon* (L.) Pers., *Setaria parviflora* (Poir.) Kerguélen, *Microlaena stipoides* (Labill.) R.Br. and *Eragrostis curvula* (Schrad.) Nees, which accounted for 61.4%, 17.3%, 11.8% and 2.8% of the plant community live ANPP (J. Chieppa, personal observation), respectively, averaged from 2014 to 2018 (total live ANPP contribution of 93.3% for all four species). All species are perennial C$_4$ grasses, except *Microlaena stipoides* which is a C$_3$ grass (Field and Forde 1990; Johnson et al. 2014). The next most abundant species, the C$_3$ forb *Commelina cyanea* R.Br. (Thorpe 1980), accounted for only 1.7% of live ANPP. In addition to these five species, a further 21 species were recorded – all representing <1% of the plot-level biomass – averaged from 2014 to 2018. Herein, species are referred to by their genus. During that time there were no significant changes in community composition. Therefore, while the majority of productivity at the site is comprised of non-native species, there is little evidence to suggest the community has experienced competitive exclusion (Grime 1973).

Sixteen plots (25 m$^2$ and a minimum 2 m apart) were established in 2014 in a factorial design including rainfall (ambient [ambient] and 65% reduced [reduced rainfall]) and phosphorus (no P addition [no P] and P addition [P+]). Four plots were fertilized in June 2014 at an annual rate of 10 g m$^{-2}$ y$^{-1}$ total P equivalent using Triple Superphosphate [P$_2$O$_5$] (Ameropa Australia, Melbourne VIC 3004) as described in the NutNet protocol (Nutrient Network, www.nutnet.umn.edu/, Morgan et al. 2016). In June 2015, another eight plots had rainfall exclusion shelters installed in the middle of each plot, and four of these plots were also fertilized (first fertilization in 2015) as above. Rainfall shelters (3 × 3 m), installed year-round, use clear acrylic plastic (Perspex) to exclude 65% of ambient rainfall, a level reflecting the lowest annual rainfall at this site in the last 100 years (268 mm in 1944). Slanted shelter roofs comprise 10 evenly-spaced corrugated plastic slats (each 300 cm × 19.5 cm) with gaps (each 300 cm × ~11.7 cm) allowing some rainfall to reach the ground in each plot. The central (~1.5 m$^2$) area under each shelter is used for all sampling and measurements, with a buffer of ~0.75 m around this to ensure that edge effects are avoided. A 1-in-100 year drought was chosen as the rainfall treatment because the field site is part of the international Drought Network (DroughtNet,

https://wp.natsci.colostate.edu/droughtnet/). The two fertilization treatments and two rainfall treatments were crossed in a factorial design across four blocks with one replicate per block.

Interception of photosynthetically active radiation (PAR) was recorded at 15 min intervals (Apogee sensors, model SQ-110, ICT International, Armidale, NSW, Australia) in one ambient and one sheltered plot; shelters intercepted <18% PAR. Soil moisture content was recorded at 5-min intervals starting November 11th 2015 using time-domain reflectometry (TDR) sensors (CS606, Campbell Scientific, Thuringowa, QLD, Australia). One TDR sensor was used in each plot and recorded the soil moisture content (±3% accuracy) of the top 15 cm of soil (Supplementary Fig. 1). Soil moisture sensors were checked periodically for accuracy using a handheld soil theta probe (Delta T Devices, Burwell, UK) which possesses ±1% accuracy. Rainfall data were obtained from the nearby EucFACE site (Ellsworth et al. 2017).

Total soil C, N and P were measured in each plot by homogenizing four cores (10 cm depth, 5 cm diameter). Dried, sieved (2 mm) soils were ground to a fine powder (MM400 Mixer Mill Grinder, Retsch GmbH, Haan, Germany). Soil was measured for total C and N content using an elemental analyzer (2400 II CHN elemental analyzer, Perkin-Elmer, USA) and total P was measured using X-ray fluorescence spectrometer (Epson $3^X$, PANanalytical, EA Almelo, The Netherlands) (Table 1).

Watering regime

In the first three years of the experiment (2014 to 2017), no artificial watering events were applied to the plots;

however, an unusually dry summer in 2018 necessitated supplemental water events (i.e. standing biomass was uniformly drought stressed across the site). From December 10th 2017 to February 8th 2018 (summer in the Southern Hemisphere), the site received 55 mm precipitation, with 4 mm in the last 15 days. Potential evaporation (mm) was 403.1 (bom.gov: RAAF, station 067105) over that time and mean site soil moisture content was $2.7 \pm 0.2\%$ as of early February. For reference, over a similar time period (December 10 to February 8) from 2015 to 2017 the site received 219, 350 and 146 mm, respectively (Supplementary Fig. 1). Following this long, dry (inter-pulse) period in 2018, plots were initially manually watered with 2 artificial events to assist the recovery of the vegetation which was supplemented by natural rainfall events (Fig. 1). Rainfall and soil moisture data from the previous years (Supplementary Fig. 1) indicated that two 20 mm events (~1 week apart) would raise soil water content in ambient plots to levels required to elicit a productivity response. The first 20 mm event was applied to ambient plots on February 8th and the second on February 13th 2018 (Fig. 1). Reduced (sheltered treatment) plots were irrigated with 65% less water than the ambient plots (i.e. 7 mm per event). Supplemental water was supplied from stored rainwater and applied at a rate of $12–18 \, l \, min^{-1}$, using a tank-and-pump system, and monitored using flow-meters.

Plant productivity

Aboveground NPP data were collected on March 5th 2018 to capture peak biomass (Chieppa, *unpublished data*). Prior to rainfall inputs in February 2018, the site had primarily standing dead material from the dry interpulse period. In each plot, two $10 \times 100$ cm rectangles (as per DroughtNet protocol) were clipped at ground level to determine live ANPP (following the watering events and natural rainfall events, Fig. 1). Sample locations were determined based on previous years' sampling positions (harvested strips had not been sampled in the previous year). In plots with rainfall shelters, material was collected from the centre 1.5 m$^2$ to ensure the selected vegetation was fully within the area of rainfall exclusion. The two samples were pooled, mixed and subsampled (30% of total sample) for sorting by species (dead material removed). Samples were dried at 60 °C for 72 h and weighed for dry mass.

**Table 1** Soil total carbon, nitrogen, and phosphorus concentrations in the four rainfall and phosphorus treatments from March 5th 2018

| Treatment | C ($\mu g \, g^{-1}$) | N ($\mu g \, g^{-1}$) | P ($\mu g \, g^{-1}$) |
|---|---|---|---|
| Ambient | $14{,}781 \pm 4798_a$ | $1367 \pm 461_a$ | $209 \pm 11_a$ |
| Ambient + P | $14{,}412 \pm 1875_a$ | $1233 \pm 197_a$ | $407 \pm 76_b$ |
| Shelter | $15{,}721 \pm 3061_a$ | $1323 \pm 269_a$ | $226 \pm 9_a$ |
| Shelter + P | $15{,}811 \pm 5176_a$ | $1237 \pm 420_a$ | $467 \pm 103_b$ |

Letters that are not shared within a column represent significant differences from Tukey post-hoc analysis. Soil P was analysed for significant differences using Kruskal-Wallis rank sum test followed by pairwise Wilcox rank sum test due to heterogeneity of variance
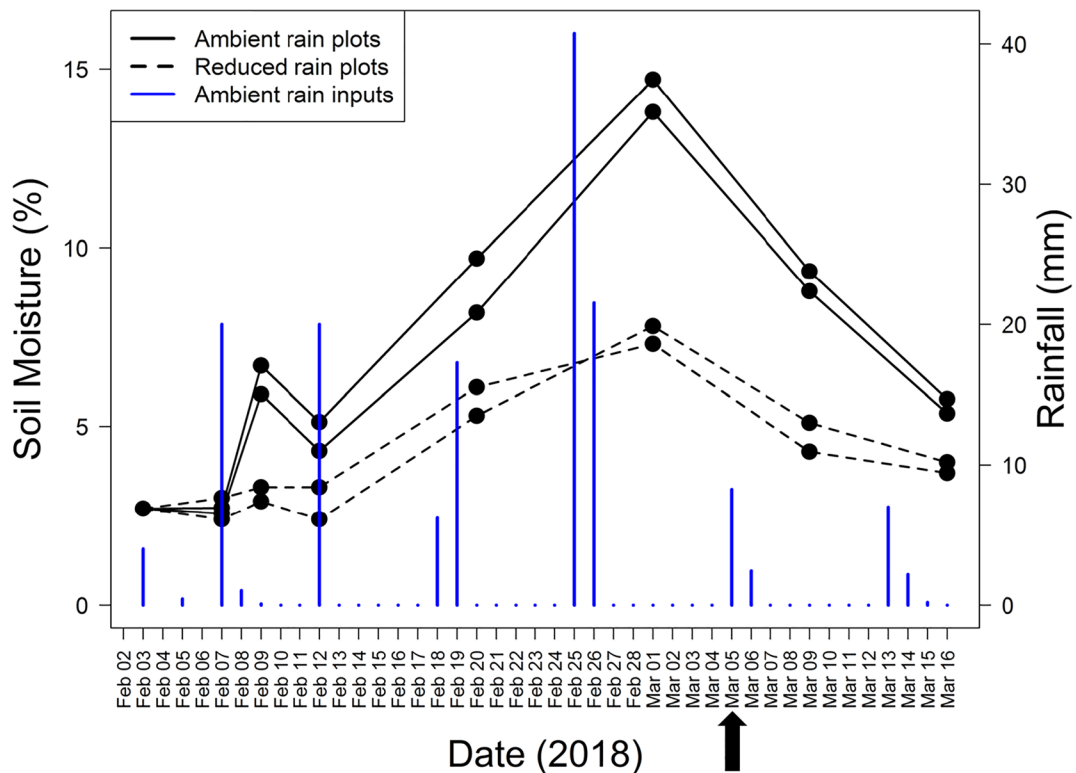
**Fig. 1** Soil moisture content for ambient rainfall (solid black lines, dots indicate day of sampling) and reduced rainfall (dashed lines, dots indicate day of sampling) plots. Blue vertical lines indicate ambient rainfall inputs including two experimentally applied 20 mm events (February 7th and 12th). Reduced rain plots received 35% of ambient rainfall amounts (i.e. 65% reduction). Aboveground net primary production harvests occurred on March 5th 2018 (denoted with black arrow). Plant functional trait data were collected March 5th to 16th 2018 on new growth

Plant functional trait collection

Functional trait data were collected from 5th to 16th March 2018 on new growth that followed the re-watering events, on unshaded, healthy (free of obvious insect damage or necrosis) individuals. Trait data were collected for the four most dominant species (mean live ANPP contribution of 93.3% from 2013 to 2018). Two individuals of each species were selected in each plot and measured for maximum height using a meter stick (Cornelissen et al. 2003). For each individual, two leaves were collected and placed in plastic vials, filled with deionized water and rehydrated for a minimum period of 6 h (Garnier et al. 2001). Water-saturated leaves were surface-dried, weighed and scanned using WinFolia (V 2015Pro, Regent Instruments Inc., Quebec, Canada) for leaf area determination. Leaves were subsequently dried at 60 °C for 72 h and weighed for dry mass. Leaf dry matter content was calculated as the ratio of dry mass to saturated mass (LDMC, mg g$^{-1}$). Specific leaf area was calculated as the ratio of saturated leaf area to dry mass (SLA, mm$^2$ mg$^{-1}$). Dried foliage was ground to a fine powder and measured for total C and N content using an elemental analyzer (2400 II CHN elemental analyzer, Perkin-Elmer, USA). Foliar C/N ratio was calculated as the ratio of total C content (%) to total N content (%) (FolCN, unitless).

Individuals were flagged to ensure above- and below-ground PFTs were collected on the same plants. Whole plants were carefully excavated (~25 cm depth) and stored in plastic bags in a cooler. Roots were kept at 4 °C until analysis, when they were gently washed over a 1-mm sieve before being scanned using WinRhizo (V 2013P, Regent Instruments Inc., Quebec, Canada). After scanning, roots were dried at 60 °C for 72 h and weighed. Specific root length was calculated as the ratio of total root length to dry mass (SRL, cm g$^{-1}$). Root tissue density was calculated as the ratio of root dry mass to total volume (RTD, mg mm$^{-3}$). Root diameter was considered as the mean diameter for the entire root

image (DIA, mm). Root branching intensity was calculated as the ratio of total root tips to total root length (RBI, branches $cm^{-1}$).

Data analysis

All analyses were conducted in R (R version 3.6.0, RStudio version 1.2.1335, R Core Team 2013). Data were log transformed when necessary to ensure homogeneity of variance determined using Levene's test (Katz et al. 2009). To test if P addition alleviated the community ANPP response to drought ($H_1$), the effects of 'rainfall', 'phosphorus', and their interaction on total ANPP (i.e. all species) and species-specific ANPP were tested using linear mixed models (*lme4* package). 'Block' was included as a random effect while 'rainfall' and 'phosphorus' were fixed. Residual degrees of freedom were estimated using Kenward-Roger Degrees of Freedom Approximation (Kenward and Roger 1997) which was also used for the estimation of *P* values (lmerTest package). *Post-hoc* Tukey 'single-step' comparison was employed when fixed effects were significant (*multcomp* package) (Newell and Douglas 2014; Bretz et al. 2016; Hothorn et al. 2017). To ensure the ANPP responses were not a result of space limitation (i.e. changes in relative productivity) we examined the effects of treatments on relative ANPP of each of the four dominant species, and the remaining species for their relative ANPP contribution, which yielded the same results as the raw ANPP values. This result indicates space-limitation had negligible effects on species and group-specific ANPP. The same analyses were completed for each of the eight PFTs included in this study to determine if PFTs of co-occurring grass species had similar PFT responses to rainfall, phosphorus, and their interaction ($H_2$).

Additionally, we modelled the direct effects of rainfall and phosphorus on the measured PFTs, and the direct effects of PFTs on ANPP using a piecewise structural equation model (SEM) performed in the *piecewiseSEM* package in R (Lefcheck 2016). Rather than testing the goodness-of-fit through a covariance matrix, we used Shipley's test of directed separation (Shipley 2000). Piecewise SEM is comprised of a set of linear equations which are then tested for the assumption of independence and whether there are variables missing that potentially explain the relationships across the set of linear equations (Duffy et al. 2016; Oliveira et al. 2016). The chi-square test for significance was set

at 0.05 while also considering Fisher's C statistic for goodness-of-fit (Shipley 2000). Linear equations were undertaken using the *nlme* package (Pinheiro et al. 2013; Lefcheck 2016). First, we created linear mixed-effects models to understand the relationship between 'rainfall', 'phosphorus' and their interaction on PFTs (Fig. 2, part i), then the effects of PFTs on ANPP (Fig. 2, part ii). 'Block' and 'species' were included as random effects in the original models (i.e. those encompassing all species, and multispecies models). The 'trait' component was analysed for each of the 8 PFTs (individually) to determine which traits provided a mechanistic link between treatments and ANPP ($H_3$). Finally, SEMs were analysed for individual species (as opposed to all species grouped together) to determine 1) if species-specific contributions were driving community-level responses; and 2) if co-occurring species possess similar PFTs underlying their productivity responses to rainfall and phosphorus.

## Results

Aboveground net primary production

Reduced rainfall decreased ANPP (29%, $P = 0.018$) and Phosphorus increased total ANPP (62%, $P = 0.003$) but there was no interaction between these treatments ($P = 0.103$, Table 2 and Fig. 3). At the species level, ANPP for *Setaria* decreased under reduced rainfall ($P = 0.004$), while *Cynodon* ANPP increased with P fertilization ($P = 0.003$, Fig. 3). *Microlaena* ANPP was not significantly affected by Rainfall or Phosphorus alone. There was, however, a significant two-way interaction for *Microlaena* ANPP ($P = 0.036$), although *post-hoc* analysis indicated that treatment means were not significantly different ($P = 0.074$).

Species' functional trait responses to drought and phosphorus

Specific leaf area decreased significantly in *Eragrostis* with P fertilization; however, the change appeared minor with respect to interspecific differences in SLA (Fig. 4a, Table 2). In *Microlaena*, SLA increased under reduced rainfall (Fig. 4a). Foliar C/N and LDMC were unaffected across treatments for all species (Fig. 4b and c, respectively). Height increased with P addition for
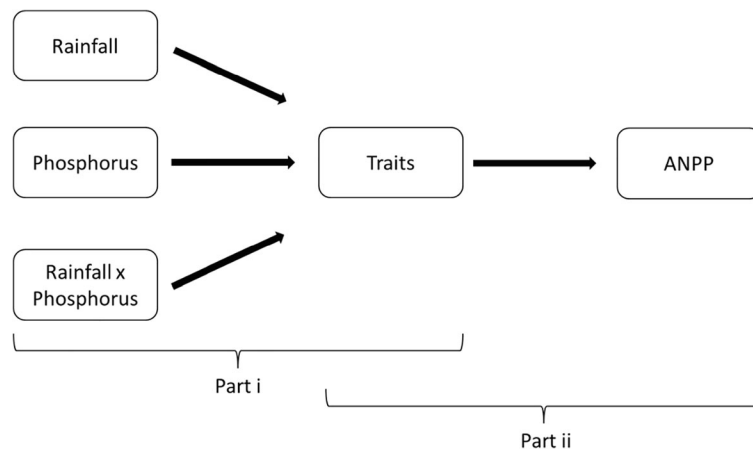
**Fig. 2** Structural equation model to test the causal relationships between 'rainfall', 'phosphorus' and their interactive effects on plant functional 'traits' (Part i), and 'traits' direct effects on aboveground net primary productivity ('ANPP') (Part ii). In the analysis, we used each of the 8 plant functional traits (individually) within the 'traits' component. We included the species as a random effect in the mixed model (i.e. multispecies model) to account for the four dominant species where we tested the causal relationships. Once significant traits were fitted to the overall model, we re-ran the model using data for each of the species individually (i.e. species-specific models) represented in the study

*Cynodon* and decreased for *Microlaena* under reduced rainfall (Fig. 4d).

In terms of belowground traits, phosphorus addition led to significant increases in RTD for *Cynodon* and *Eragrostis*, although it had the opposite effect in *Microlaena* (Fig. 4e). Phosphorus addition had opposing effects on SRL in *Cynodon* (decreased with P addition) and *Microlaena* (increased with P addition) (Fig. 4g). Reduced rainfall resulted in decreased SRL in *Eragrostis*. Like RTD, P addition had contrasting effects on RBI between species, with *Cynodon* increasing and *Microlaena* decreasing values of this trait under added P (Fig. 4h). Neither P addition nor reduced rainfall, or their interaction, had a significant effect on any PFTs measured for *Setaria* (Fig. 4).

Plant functional traits underlying changes in ANPP (structural equation models)

Based on the hypothesis that PFTs would mediate the effects of rainfall and phosphorus on ANPP (Fig. 2), we found SRL to be a key PFT in this relationship (Fig. 5, Table 3). Tests of directed separation indicated significant independence ($P > 0.106$). Path coefficients indicated phosphorus addition had a significant negative effect on SRL (−0.352), while increasing SRL had a negative effect on ANPP for the four species sampled (−0.347, Fig. 5). While the overall model fit was significant, adjusted $R^2$ values when considering random effects of 'block' and 'species' were greater than marginal effects (i.e. 'rainfall' and 'phosphorus') alone, indicating their importance. When using RTD in the model, we found phosphorus fertilization had a significant positive effect on RTD (path coefficient = 0.417); however, no significant relationship was observed between RTD and ANPP (Table 3). Similarly, we found reduced rainfall and phosphorus addition had independent positive effects on DIA (path coefficients = 0.327 & 0.394, respectively) with no significant relationship between DIA and ANPP. The importance of conducting species-specific SEMs was emphasized in the multispecies models in that adjusted $R^2$ values were greatly improved for all PFTs when using 'species' and 'block' as random effects (Table 3).

For species-specific SEMs, *Cynodon* was the primary species of interest (Table 4). Specifically, phosphorus effects on ANPP were mediated by HT, SRL and RBI in *Cynodon*. Phosphorus addition resulted in greater HT and RBI (path coefficients = 0.819 & 0.991, respectively) resulting in greater ANPP (path coefficients = 0.553 & 0.781, respectively). For SRL, as observed in the multispecies SEM, phosphorus fertilization resulted in lower SRL of *Cynodon* (path coefficient = −0.793), while increasing SRL had a negative effect on ANPP (path coefficient = −0.746). The interactive effects of rainfall and phosphorus had a significant effect on *Cynodon*'s FolCN, and phosphorus alone affected its RTD; however, these changes in PFTs were unrelated

**Table 2** Summary statistics for the effects of rainfall (ambient vs reduced) and phosphorus (no P added vs. P added) treatments, and their interaction, from linear mixed models

| Response Variable | Group/Species | Rainfall | | Phosphorus | | Rain*Phosphorus | |
|---|---|---|---|---|---|---|---|
| | | F-value | *P* value | F-value | *P* value | F-value | *P* value |
| ANPP | **Total Live** | **8.42** | **0.018*** | **16.16** | **0.003**** | 3.30 | 0.103 |
| | ***C. dactylon*** | 1.63 | 0.234 | **14.85** | **0.004**** | 1.64 | 0.232 |
| | *E. curvula* | 1.41 | 0.266 | 0.43 | 0.529 | 0.55 | 0.479 |
| | ***M. stipoides*** | 0.59 | 0.461 | 0.90 | 0.367 | **6.10** | **0.036*** |
| | ***S. parviflora*** | **15.80** | **0.003**** | 0.14 | 0.719 | 0.12 | 0.733 |
| | Non-dominant | 0.00 | 0.972 | 0.68 | 0.431 | 1.44 | 0.260 |
| SLA | *C. dactylon* | 0.02 | 0.883 | 1.19 | 0.279 | 0.00 | 0.987 |
| | ***E. curvula*** | 0.788 | 0.377 | **7.04** | **0.009**** | 0.30 | 0.583 |
| | ***M. stipoides*** | **10.37** | **0.002**** | 0.07 | 0.794 | 0.47 | 0.497 |
| | *S. parviflora* | 0.03 | 0.855 | 3.59 | 0.062 | 2.78 | 0.099 |
| FolCN | *C. dactylon* | 1.48 | 0.235 | 3.36 | 0.079 | 2.89 | 0.101 |
| | *E. curvula* | 0.27 | 0.606 | 0.04 | 0.846 | 0.16 | 0.696 |
| | *M. stipoides* | 0.02 | 0.888 | 0.10 | 0.752 | 0.10 | 0.756 |
| | *S. parviflora* | 0.00 | 0.955 | 0.48 | 0.494 | 0.16 | 0.694 |
| LDMC | *C. dactylon* | 0.09 | 0.769 | 1.97 | 0.168 | 0.41 | 0.525 |
| | *E. curvula* | 1.68 | 0.202 | 0.74 | 0.395 | 0.50 | 0.483 |
| | *M. stipoides* | 0.02 | 0.887 | 1.26 | 0.271 | 0.00 | 0.942 |
| | *S. parviflora* | 1.57 | 0.217 | 0.382 | 0.540 | 1.13 | 0.293 |
| HT | ***C. dactylon*** | 0.03 | 0.869 | **42.55** | **< 0.001***** | 1.43 | 0.237 |
| | *E. curvula* | 0.08 | 0.782 | 0.00 | 0.946 | 2.27 | 0.138 |
| | ***M. stipoides*** | **10.3** | **0.003**** | 0.00 | 0.890 | 0.687 | 0.413 |
| | *S. parviflora* | 2.62 | 0.111 | 0.00 | 0.977 | 1.01 | 0.318 |
| RTD | ***C. dactylon*** | 1.91 | 0.207 | **38.74** | **< 0.001***** | 0.616 | 0.456 |
| | ***E. curvula*** | 1.34 | 0.277 | **14.03** | **0.005**** | 0.00 | 0.973 |
| | ***M. stipoides*** | 0.98 | 0.356 | **5.95** | **0.045*** | 0.05 | 0.823 |
| | *S. parviflora* | 0.00 | 0.979 | 0.86 | 0.377 | 0.07 | 0.803 |
| DIA | *C. dactylon* | 1.83 | 0.214 | 1.14 | 0.319 | 0.14 | 0.717 |
| | *E. curvula* | 2.38 | 0.157 | 2.82 | 0.127 | 0.70 | 0.425 |
| | *M. stipoides* | 0.76 | 0.411 | 0.22 | 0.650 | 2.83 | 0.129 |
| | *S. parviflora* | 0.03 | 0.860 | 1.23 | 0.297 | 0.02 | 0.901 |
| SRL | ***C. dactylon*** | 1.05 | 0.338 | **59.84** | **< 0.001***** | 0.32 | 0.590 |
| | ***E. curvula*** | **8.75** | **0.016*** | 2.99 | 0.118 | 0.08 | 0.784 |
| | ***M. stipoides*** | 0.30 | 0.599 | **13.17** | **0.008**** | 0.11 | 0.753 |
| | *S. parviflora* | 0.02 | 0.902 | 1.26 | 0.292 | 0.13 | 0.727 |
| **RBI** | ***C. dactylon*** | 0.71 | 0.428 | **161.04** | **< 0.001***** | 0.07 | 0.802 |
| | *E. curvula* | 3.73 | 0.086 | 0.14 | 0.718 | 1.17 | 0.308 |
| | ***M. stipoides*** | 4.54 | 0.069 | **15.35** | **0.005*** | 0.63 | 0.448 |
| | *S. parviflora* | 4.24 | 0.070 | 0.11 | 0.748 | 2.67 | 0.119 |

Bolded rows are those with significant effects for ease of reading. *Abbreviations ANPP* aboveground net primary productivity (g m$^{-2}$ y$^{-1}$), *SLA* specific leaf area (mm$^2$ mg$^{-1}$), *FolCN* foliar C to N ratio, *LDMC* leaf dry matter content (mg g$^{-1}$), *HT* height (mm), *RTD* root tissue density (mg mm$^{-3}$), *DIA* root diameter (mm), *SRL* specific root length (cm g$^{-1}$), *RBI* root branching intensity (branch #·cm). Nominator degrees of freedom are 1 in all cases. Denominator degrees of freedom (ddf) were 9, estimated using Kenward-Rogers Degrees of Freedom Approximation. Asterisks next to *P* values denote level of significance (*** < 0.001, ** < 0.01, * < 0.05)

**Fig. 3** Live aboveground net primary production by rainfall (left panel) and phosphorus (right panel) treatments, separated for: all species (Total Live), dominant species (*Cyodon dactylon*, *Eragrostis curvula*, *Microlaena stipoides*, *Setaria parviflora*) and non-dominant species (Other spp.). Plots with asterisks represent significant differences at $P < 0.05$. Error bars represent standard errors
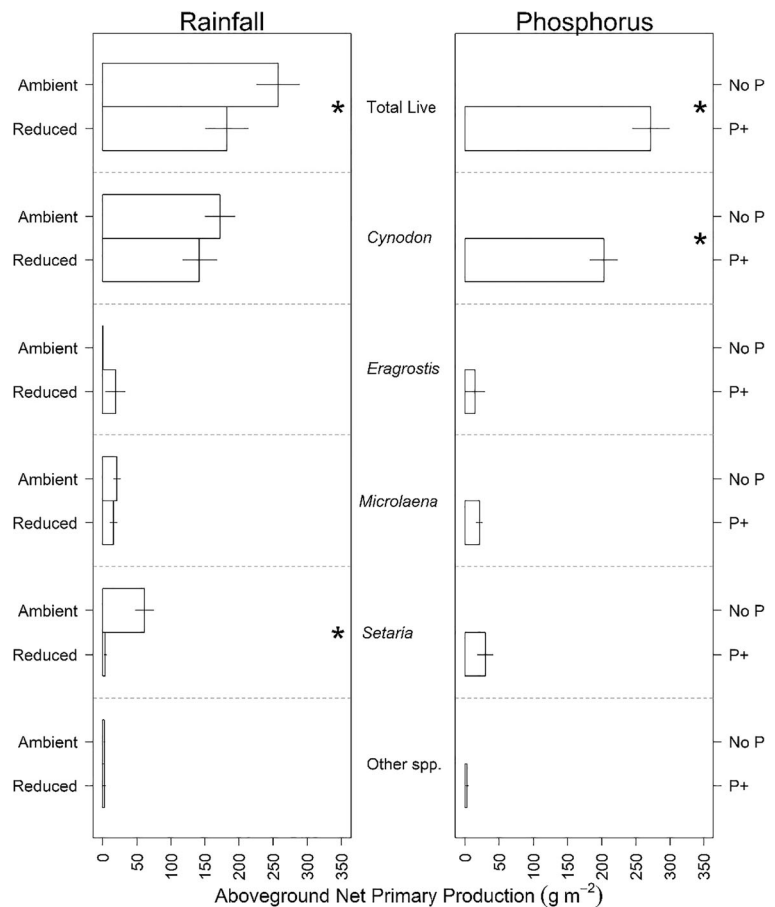


to ANPP. Similarly, HT in *Microlaena* and RTD in *Eragrostis* were significantly affected by treatments, but these had no significant relationship with ANPP.

## Discussion

In this study, we addressed the potential impact of drought and soil P addition on ecosystem functioning (ANPP) and associated PFTs of the dominant species in a mesic grassland. A 65% reduction in rainfall reduced ANPP by 29% while P addition increased total ANPP by 62%, a response primarily driven by a single, dominant grass species (*Cynodon*). Studies involving P (Bennett and Adams 2001) or NPK (Morgan et al. 2016) fertilisation elsewhere in Australia have reported a lack of ANPP response to fertiliser addition, although Morgan et al. (2006) observed a fertiliser-driven shift in the relative cover of native and non-native species across four grasslands. The lack of interaction between

nutrient and drought treatments in our study indicates that P addition did not alleviate the negative effects of drought on grassland productivity. This is in line with Yu et al.'s (2009) observation for a semi-arid grassland in inner Mongolia, although contrasts with the positive response of ANPP to P fertilization during drought reported in dominant grasses in North American prairies (La Pierre et al. 2016) and other sites outside of Australia (Lee et al. 2010; Yahdjian et al. 2011). It is important to note that rainfall intensity and frequency can govern the rate of N mineralization (Homyak et al. 2017) and P transformation (e.g. sorption/desorption, Tiessen 2008), which may restrict plant uptake of nutrients under drought conditions (Alam 1999; Hu and Schmidhalter 2005).

Fertilizing grasslands may lead to declines in species richness (DiTommaso and Aarssen 1989; Gough et al. 2000; Clark and Tilman 2008). However, this was not observed under P addition in our study over 4 years (Chieppa *unpublished data*), although it has been found
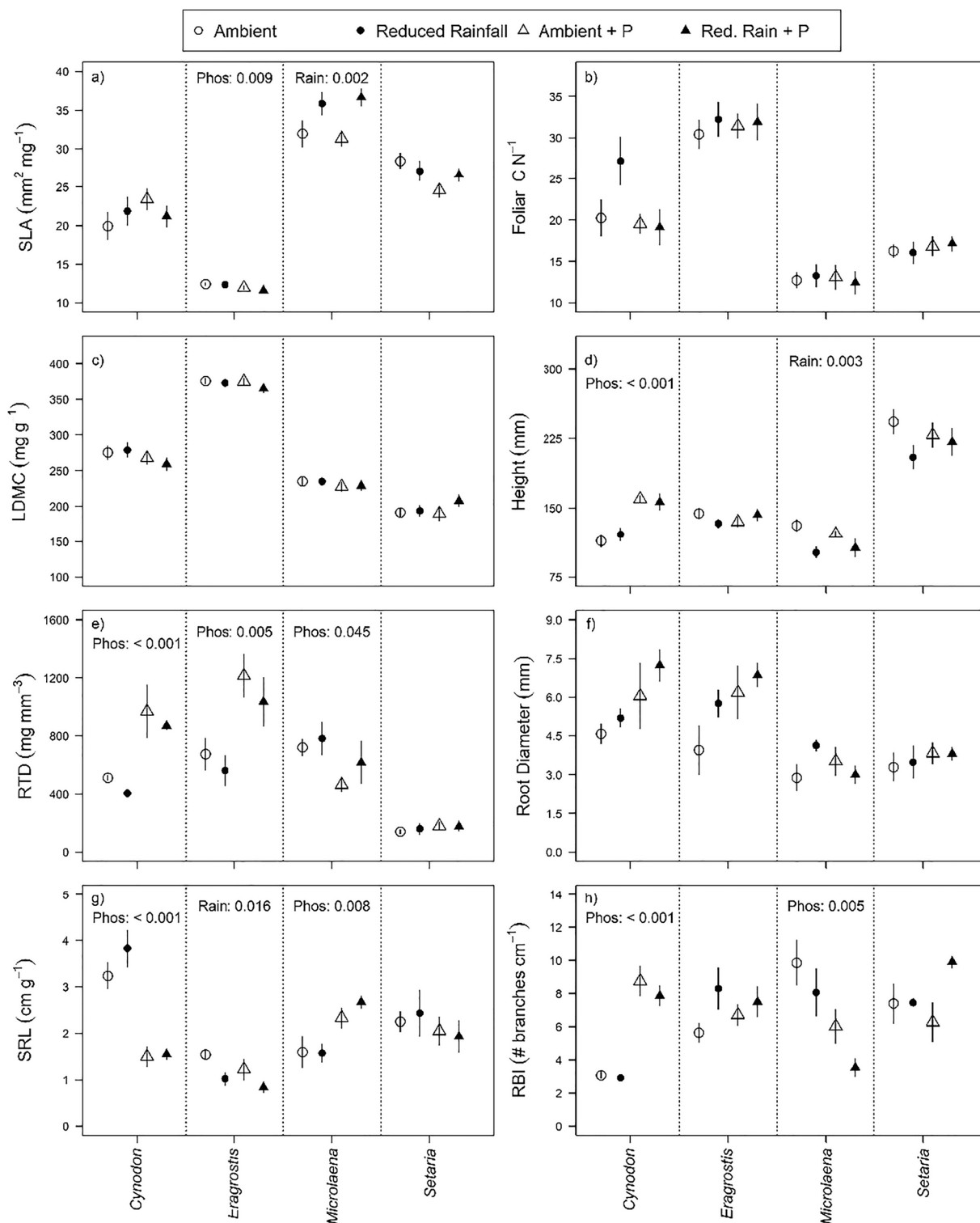
◄ **Fig. 4** Plant functional traits in different treatments for the four dominant species. Abbreviations labelling y-axes represent (panel **a**) specific leaf area, (**c**) leaf dry matter content, (**e**) root tissue density, (**g**) specific root length, and (**h**) root branching intensity. "Phos" denotes the *P* value resulting from linear mixed models (LMMs) for the phosphorus treatment. "Rain" denotes the *P*-value resulting from LMMs for the rainfall treatment. *P* values below 0.05 are considered significant; however, *P* values between 0.05 and 0.10 are also reported for clarity. Error bars indicate standard errors

in other studies (Crawley et al. 2005; Hejcman et al. 2007; Ceulemans et al. 2014). The link between fertilization and declining species richness may reflect competition and ultimate displacement of weaker species by superior competitors for the newly available resource (McKane et al. 2002; Lambers et al. 2004; Clark and Tilman 2008; Gazol et al. 2016). Few studies have observed shifts in PFTs (i.e. changes in functional diversity, but see Bernhardt-Römermann et al. 2011), which could be occurring even in the absence of species turnover (i.e. hierarchical response framework, Smith et al. 2009). Recently, the importance of root PFTs was highlighted in understanding community compositional changes induced by N enrichment (Zheng et al. 2019), yet no studies to our knowledge have examined the consequences of changes in PFTs for alternative abiotic

disturbances such as drought. Under future rainfall regimes, the key will be selecting appropriate PFTs which both underpin responses to drought (Griffin-Nolan et al. 2018) and additional abiotic agents such as fertilization.

We found that ANPP of multiple plant species in a single community did not respond similarly to reduced water availability and P addition. In fact, at the species level, only *Setaria* decreased ANPP under reduced rainfall and only *Cynodon* increased ANPP with P addition. *Setaria* was the species most sensitive to extreme drought in our system, perhaps because it did not adjust PFT values in response to low soil water availability, which accounted for the decline in productivity under dry conditions (Albert et al. 2010). In contrast, the positive effect of P addition on ANPP of *Cynodon*, and on four of its eight measured traits, suggests that species that readily acclimate to changes in resource-availability may do best under variable environments. Fort et al. (2015) found acquisitive strategies in legumes were associated with reduced negative effects of water and P limitation, but this was not observed in *Cynodon*. Specifically, the most conservative species (*Eragrostis*), was observed to have no negative effects of drought or positive responses to P addition. P deficiency may decrease root hydraulic conductance per root surface area
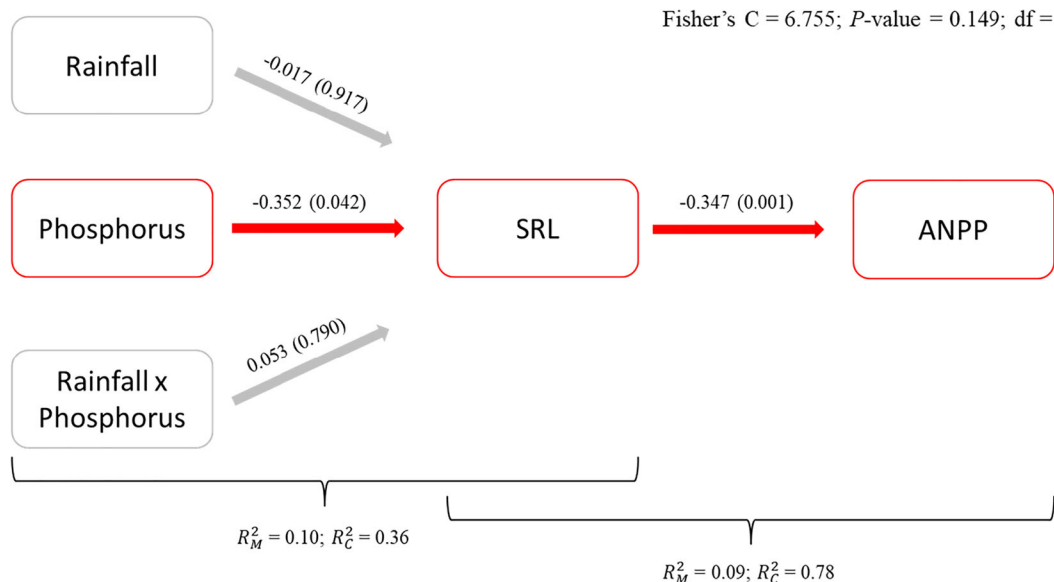
Fisher's C = 6.755; *P*-value = 0.149; df = 4



**Fig. 5** Structural equation model results for the four dominant species measured, examining the effects of 'rainfall', 'phosphorus' and their interaction on specific root length ('SRL') and the relationship between 'SRL' and aboveground net primary production ('ANPP'). Grey boxes and arrows indicate non-significant pathways while red boxes and arrows indicate significant pathways.

Numbers next to arrows indicate standardized path coefficients and *P* values (in parentheses). The overall model fits are included in the top right corner. Adjusted $R^2$ with (conditional, $R_C^2$) and without random effects (marginal, $R_M^2$) for each section of the model are included in the brackets below the variables

**Table 3** Goodness-of-fit and path significance from the structural equation model (SEM) analysis

| Trait | Goodness-of-fit | | | df | Rainfall | Phosphorus | Rainfall*Phosphorus | Trait | | ANPP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | Fisher's C | $P$ value | | $P$ value | $P$ value | $P$ value | $R^2_M$ | $R^2_C$ | $P$ value | $R^2_M$ | $R^2_C$ |
| LDMC | 4 | 10.982 | 0.027 | 33 | 0.811 | 0.716 | 0.577 | 0.01 | 0.89 | 0.365 | 0.13 | 0.63 |
| SLA | 4 | 11.052 | 0.026 | 33 | 0.796 | 0.496 | 0.565 | 0.03 | 0.58 | 0.056 | 0.00 | 0.57 |
| FolCN | 4 | 10.709 | 0.030 | 33 | 0.663 | 0.783 | 0.192 | 0.01 | 0.89 | 0.277 | 0.08 | 0.60 |
| HT | 4 | 7.775 | 0.100 | 33 | 0.490 | 0.116 | 0.775 | 0.06 | 0.53 | 0.234 | 0.05 | 0.66 |
| **SRL** | **4** | **6.755** | **0.149** | **33** | **0.917** | **0.042*** | **0.790** | **0.10** | **0.36** | **0.001*** | **0.09** | **0.78** |
| **RTD** | **4** | **7.655** | **0.105** | **33** | **0.940** | **0.006*** | **0.405** | **0.12** | **0.55** | **0.106** | **0.04** | **0.61** |
| **DIA** | **4** | **11.193** | **0.024** | **33** | **0.037*** | **0.016*** | **0.391** | **0.15** | **0.47** | **0.155** | **0.15** | **0.47** |
| RBI | 4 | 10.001 | 0.040 | 33 | 0.316 | 0.111 | 0.665 | 0.09 | 0.09 | 0.316 | 0.01 | 0.61 |

Goodness-of-fit $P$ values below 0.05 are not good fits through violations of independence. The significance of 'rainfall', 'phosphorus', or their interaction are denoted with asterisks in the 6th, 7th, and 8th columns. The adjusted $R^2$ values from the mixed models of their effects on the specified functional 'trait' are included without random effects (marginal, $R^2_M$) while the conditional $R^2$ values ($R^2_C$) include the random effects of 'species' and 'block'. The same data, including $P$ values, are included for the relationship between the 'traits' and aboveground net primary productivity ('ANPP'). Bolded rows indicate SEMs which adhere to all criteria and are significant ($\alpha = 0.05$) for both parts of the model

(Trubat et al. 2006), which could have negative implications for drought tolerance. It has been observed that legumes, grasses and small trees have very different responses to P and drought, suggesting very different plant strategies to drought and fertilization based on plant functional type (Baldocchi et al. 2004; Volaire 2008; Onipchenko et al. 2012). In our study, the intensity of the drought applied (i.e. the percentage of irrigation/rainfall which was reduced) was severe and expected to elicit declines in ANPP in the community (Beierkuhnlein et al. 2011; Jung et al. 2014; Knapp et al. 2015; Copeland et al. 2016). However, this grass-dominated ecosystem was found to be very resistant to drought, perhaps reflecting historic exposure, and associated plant community adaptation, to high inter-annual variability in rainfall.

Overall, we found strong differences amongst our dominant grassland species with respect to PFT responses to reduced rainfall and P addition. While many traits were unaffected by drought or P addition, we observed contrasting directional responses of PFTs across species. For example, SRL increased with P addition in *Microlaena*, but decreased in *Cynodon*; this is not unexpected since differences in grass root response to P addition are often species-specific (Fort et al. 2014). While we attribute these changes to the direct effects of P addition, we did not investigate the indirect effects of P addition which could potentially alter PFT responses (e.g. intransitive dynamics or complementarity, Soliveres et al. 2015). For example, with limited niche space for root exploitation, a direct effect of P addition on SRL of *Cynodon* could result in a shift in root traits of *Microlaena*, via competition. Alternatively, potential unique P requirements between *Microlaena* and *Cynodon* could result in opposite responses to fertilization (Lambers et al. 2006). Others have observed P uptake to be directly related to root traits (namely specific root hair cylinder volume) which has consequences for productivity responses to fertilization in both grass and legumes (Haling et al. 2016). These results highlight the complexity of trait-environmental relationships in (semi-)natural plant communities and warrants further study, ideally through the use of coordinated experiments to determine how site-specific characteristics may influence PFTs underpinning drought and fertilization responses (Fraser et al. 2013; Ploughe et al. 2018).

For the whole community, SEM indicated decreasing SRL was associated with greater ANPP; however, upon further inspection, this was only true for the most abundant species, *Cynodon*. We can assume that the response of *Cynodon*, which represented >50% of community

**Table 4** Goodness-of-fit and path significance from the structural equation model analysis of individual plant functional traits for specific species

| Species | Trait | Goodness-of-fit | | | df | Rainfall | Phosphorus | Rainfall*Phosphorus | Trait | | | ANPP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | df | Fisher's C | P value | | P value | P value | P value | | $R^2_M$ | $R^2_C$ | P value | $R^2_M$ | $R^2_C$ |
| *C. dactylon* | HT→\|→ | 4 | 6.598 | 0.159 | 9 | 0.675 | **0.019** | 0.755 | | 0.61 | 0.61 | **0.047** | 0.32 | 0.32 |
| | Path Coefficients: | | | | | NS | **0.819** | NS | | | | Path Coef: | | **0.553** |
| | SRL→\|→ | 4 | 1.086 | 0.896 | 9 | 0.171 | 0.002 | 0.355 | | 0.86 | 0.86 | **0.004** | 0.58 | 0.58 |
| | Path Coefficients: | | | | | NS | **−0.793** | NS | | | | Path Coef: | | **−0.746** |
| | RBI→\|→ | 4 | 2.994 | 0.559 | 9 | 0.869 | **< 0.001** | 0.541 | | 0.90 | 0.92 | **0.002** | 0.63 | 0.63 |
| | Path Coefficients: | | | | | NS | **0.991** | NS | | | | Path Coef: | | **0.781** |
| | FOLCN→\| | 4 | | | 9 | 0.253 | 0.698 | **0.036** | | 0.22 | 0.82 | 0.320 | 0.09 | 0.09 |
| | Path Coefficients: | | | | | NS | NS | **−0.575** | | | | Path Coef: | | NS |
| | RTD→\| | 4 | | | 9 | 0.516 | **0.016** | 0.973 | | 0.67 | 0.67 | 0.337 | 0.08 | 0.08 |
| | Path Coefficients: | | | | | NS | **0.781** | NS | | | | Path Coef: | | NS |
| *M. stipoides* | HT→\| | 4 | | | 9 | **0.004** | 0.059 | **0.035** | | 0.26 | 0.28 | 0.838 | 0.01 | 0.01 |
| | Path Coefficients: | | | | | **−0.852** | NS | **0.207** | | | | Path Coef: | | NS |
| *E. curvula* | RTD→\| | 4 | | | 9 | 0.582 | **0.026** | 0.693 | | 0.56 | 0.56 | 0.269 | 0.10 | 0.10 |
| | Path Coefficients: | | | | | NS | **0.757** | NS | | | | Path Coef: | | NS |

Only models with significant paths are included. Significant *P* values and associated path coefficients are bolded. Arrows and bars under each trait indicate if the first (treatment effects on traits) and second paths (trait effects on ANPP) were significant. Bolded values indicate significance at $\alpha = 0.05$

ANPP, and its PFTs, will likely dictate community-level responses to future change (i.e. the biomass-ratio hypothesis, Grime 1998). Interestingly, greater ANPP was associated with more conservative values of SRL (less root length per mass) as more acquisitive species typically have higher productivity (Reich 2014). It is possible that greater resource availability led to a more conservative resource-use strategy which manifested as reduced tissue water loss in roots (i.e. thicker and denser roots preventing dehydration to drier soils). Thus, having reduced water loss from roots would limit mortality (Huang and Wilkinson 2000; Huang and Gao 2000) and healthier root systems would allow for greater ANPP in the field; however, this was not tested directly (Manschadi et al. 2006). Alternatively, changes in the ratio of root to shoot biomass can have knock-on effects on aboveground productivity under drought (Akram et al. 2007). Because of the heterogeneous nature of mesic grasslands, determining belowground biomass by species is difficult, although this aspect would clearly benefit from further investigation. Other important PFTs relating to water and nutrient uptake, such as rooting depth and root mass fraction, could explain why some species performed better than others under reduced rainfall (Volaire 2008; Comas et al. 2013; Volaire et al. 2014). However, such measures are difficult to undertake in mixed field communities, are highly destructive, and not suited to long-term experiments.

RBI and HT were also important traits underlying productivity and significantly affected by P addition in *Cynodon*. The changes in RBI, however, were counterintuitive given P addition was associated with more conservative SRL values (low length to mass ratio) yet RBI values were more acquisitive (high number of branches per unit length) with added P. It should be noted that root traits are multidimensional (Kramer-Walter et al. 2016) with aspects of surface area, length, branching and tissue density indicting unique aspects of the functionality of roots as organs (Fitter 1991; Bardgett et al. 2014). While complex, their use as key indicators of grassland responses to drought and fertilization will be crucial under future climate and management scenarios.

## Conclusion

We assessed the response of a mesic grassland to severe drought, the role of P fertilization in alleviating drought, and the mechanisms (PFTs) underpinning plant responses. Drought generated a reduction in ANPP for one of the four most abundant species, and the addition of P increased ANPP for a different species, but P addition did not alleviate the negative drought effect, despite significant effects of P on PFTs. Interspecific and contrasting shifts in PFT values of co-occurring perennial grassland species create uncertainty regarding the use of PFTs to explain community responses to drought and fertilization. We found SRL to be an important trait underlying response of ANPP in the community; however, this could be mostly attributed to the single dominant species, *Cynodon* with shifts towards more conservative SRL values from P addition resulting in greater ANPP. While we observed no interactive effects of drought and P fertilization on PFTs or ANPP, we hypothesize that SRL may be a key PFT underpinning future drought sensitivity of grasslands given its importance to the acquisition and conservation of water.

**Author contributions** JC conceived the ideas, acquired data, analysed data and wrote the manuscript. UN, DT and SP designed

the experimental site and contributed critically to manuscript development.

**Compliance with ethical standards**

**Conflict of interest** The authors declare no conflicts of interest for the work presented

## References

Akram NA, Shahbaz M, Ashraf M (2007) Relationship of Photosynthetic Capacity and Proline Accumulation with the Growth of Differently Adapted Populations of Two Potential Grasses (*Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L.) to Drought Stress. Pak J Bot 39(3):777–786

Alam SM (1999) Nutrient uptake by plants under stress conditions. Handbook Plant Crop Stress 2:285–313

Albert CH, Thuiller W, Yoccoz NG, Soudant A, Boucher F, Saccone P, Lavorel S (2010) Intraspecific functional variability: extent, structure and sources of variation. J Ecol 98(3):604–613

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal Symbiosis. Mycorrhiza 11(1):3–42

Austin AT, Yahdjian L, Stark JM, Belnap J, Porporato A, Norton U, Ravetta DA, Schaeffer SM (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141(2):221–235

Baldocchi DD, Xu L, Kiang N (2004) How plant functional-type, weather, seasonal drought, and soil physical properties Alter water and energy fluxes of an oak–grass savanna and an annual grassland. Agric For Meteorol 123(1):13–39

Bardgett RD, Mommer L, De Vries FT (2014) Going underground: root traits as drivers of ecosystem processes. Trends Ecol Evol 29(12):692–699

Beierkuhnlein C, Thiel D, Jentsch A, Willner E, Kreyling J (2011) Ecotypes of European grass species respond differently to warming and extreme drought. J Ecol 99(3):703–713

Bennett LT, Adams MA (2001) Response of a perennial grassland to nitrogen and phosphorus additions in sub-tropical, semi-arid Australia. J Arid Environ 48(3):289–308

Benson D, Howell J (2002) Cumberland plain woodland ecology then and now: interpretations and implications from the work of Robert Brown and Others. Cunninghamia 7:631–650

Bernhardt-Römermann M, Römermann C, Sperlich S, Schmidt W (2011) Explaining grassland biomass–the contribution of climate, species and functional diversity depends on fertilization and mowing frequency. J Appl Ecol 48(5):1088–1097

Bretz F, Westfall P, Hothorn T (2016) Multiple comparisons using R. Chapman and Hall/CRC, London

Ceulemans T, Stevens CJ, Duchateau L, Jacquemyn H, Gowing DJG, Merckx R, Wallace H, Van Rooijen N, Goethem T, Bobbink R (2014) Soil phosphorus constrains biodiversity across European grasslands. Glob Chang Biol 20(12):3814–3822

Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451(7179):712

Comas L, Becker S, Cruz VMV, Byrne PF, Dierig DA (2013) Root traits contributing to plant productivity under drought. Front Plant Sci 4. https://doi.org/10.3389/fpls.2013.00442

Copeland SM, Harrison SP, Latimer AM, Damschen EI, Eskelinen AM, Fernandez-Going B, Spasojevic MJ, Anacker BL, Thorne JH (2016) Ecological effects of extreme drought on Californian herbaceous plant communities. Ecol Monogr http://onlinelibrary.wiley.com/doi/10.1002/ecm.1218/full

Cornelissen JHC, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich DE, Reich PB et al (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust J Bot 51(4):335–380

Crawley MJ, Johnston AE, Silvertown J, Dodd M, de Mazancourt C, Heard MS, Henman DF, Edwards GR (2005) Determinants of species richness in the park grass experiment. Am Nat 165(2):179–192

DiTommaso A, Aarssen LW (1989) Resource manipulations in natural vegetation: a review. Vegetatio 84(1):9–29

dos Santos MG, Ribeiro RV, de Oliveira RF, Machado EC, Pimentel C (2006) The role of inorganic phosphate on photosynthesis recovery of common bean after a mild water deficit. Plant Sci 170(3):659–664

Duffy JE, Lefcheck JS, Stuart-Smith RD, Navarrete SA, Edgar GJ (2016) Biodiversity enhances reef fish biomass and resistance to climate change. Proc Natl Acad Sci 113(22):6230–6235

Easterling DR, Evans JL, Groisman PY, Karl TR et al (2000) Observed variability and trends in extreme climate events: a brief review. Bull Am Meteorol Soc 81(3):417

Ellsworth DS, Anderson IC, Crous KY, Cooke J, Drake JE, Gherlenda AN, Gimeno TE, Macdonald CA, Medlyn BE, Powell JR (2017) Elevated $CO_2$ does not increase eucalypt Forest productivity on a low-phosphorus soil. Nat Clim Chang 7(4):279

Evans JP, Argueso D, Olson R, Di Luca A (2017) Bias-corrected regional climate projections of extreme rainfall in south-East Australia. Theor Appl Climatol 130(3–4):1085–1098

Fay PA, Blair JM, Smith MD, Nippert JB, Carlisle JD, Knapp AK (2011) Relative effects of precipitation variability and warming on tallgrass prairie ecosystem function. Biogeosciences 8(10):3053–3068

Fay PA, Prober SM, Harpole WS, Knops JMH, Bakker JD, Borer ET, Lind EM, MacDougall AS, Seabloom EW, Wragg PD (2015) Grassland productivity limited by multiple nutrients. Nature Plants 1:15080

Field TRO, Forde MB (1990) Effects of Climate Warming on the Distribution of C4 Grasses in New Zealand. Proc N Z Grassland Assoc 51:47–50

Fitter AH (1991) Characteristics and functions of root systems. Plant Roots: Hidden Half 2:1–29

Fort F, Cruz P, Jouany C (2014) Hierarchy of root functional trait values and plasticity drive early-stage competition for water and phosphorus among grasses. Funct Ecol 28(4):1030–1040

Fort F, Cruz P, Catrice O, Delbrut A, Luzarreta M, Stroia C, Jouany C (2015) Root functional trait syndromes and plasticity drive the ability of grassland Fabaceae to tolerate water and phosphorus shortage. Environ Exp Bot 110:62–72

Fraser LH, Henry HAL, Carlyle CN, White SR, Beierkuhnlein C, Cahill JF, Casper BB et al (2013) Coordinated distributed experiments: an emerging tool for testing global hypotheses in ecology and environmental science. Front Ecol Environ 11(3):147–155

Garnier E, Shipley B, Roumet C, Laurent G (2001) A standardized protocol for the determination of specific leaf area and leaf dry matter content. Funct Ecol 15(5):688–695

Gazol A, Uria-Diez J, Elustondo D, Garrigó J, Ibáñez R (2016) Fertilization triggers 11 Yr of changes in community assembly in Mediterranean grassland. J Veg Sci 27(4):728–738

Gough L, Osenberg CW, Gross KL, Collins SL (2000) Fertilization effects on species density and primary productivity in herbaceous plant communities. Oikos 89(3):428–439

Griffin-Nolan RJ, Bushey JA, Carroll CJW, Challis A, Chieppa J, Garbowski M, Hoffman AM, Post AK, Slette IJ, Spitzer D (2018) Trait selection and community weighting are key to understanding ecosystem responses to changing precipitation regimes. Funct Ecol (32)7 1746–1756

Grime JP (1973) Competitive exclusion in herbaceous vegetation. Nature 242(5396):344

Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. J Ecol 86(6):902–910

Grime JP, Thompson K, Hunt R, Hodgson JG, Cornelissen JHC, Rorison IH, Hendry GAF, Ashenden TW, Askew AP, Band SR (1997) Integrated screening validates primary axes of specialisation in plants. Oikos 79, 259–281

Guo Q, Hu Z, Li S, Li X, Sun X, Yu G (2012) Spatial variations in aboveground net primary productivity along a climate gradient in Eurasian temperate grassland: effects of mean annual precipitation and its seasonal distribution. Glob Chang Biol 18(12):3624–3631. https://doi.org/10.1111/gcb.12010

Haling RE, Yang Z, Shadwell N, Culvenor RA, Stefanski A, Ryan MH, Sandral GA, Kidd DR, Lambers H, Simpson RJ (2016) Root morphological traits that determine phosphorus-acquisition efficiency and critical external phosphorus requirement in pasture species. Funct Plant Biol 43(9):815–826. https://doi.org/10.1071/FP16037

Hejcman M, Klaudisová M, Štursa J, Pavlů V, Schellberg J, Hejcmanová P, Hakl J, Rauch O, Vacek S (2007) Revisiting a 37 years abandoned fertilizer experiment on Nardus grassland in the Czech Republic. Agric Ecosyst Environ 118(1–4):231–236

Hetrick BAD, Wilson GWT, Todd TC (1990) Differential responses of C3 and C4 grasses to mycorrhizal Symbiosis, phosphorus fertilization, and soil microorganisms. Can J Bot 68(3):461–467

Hofer D, Suter M, Buchmann N, Lüscher A (2017) Nitrogen status of functionally different forage species explains resistance to severe drought and Post-drought overcompensation. Agric Ecosyst Environ 236:312–322

Homyak PM, Allison SD, Huxman TE, Goulden ML, Treseder KK (2017) Effects of drought manipulation on soil nitrogen cycling: a meta-analysis. J Geophys Res Biogeosci 122(12):3260–3272

Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S, Hothorn MT (2017) Package 'Multcomp.' Obtenido de http://cran.stat.sfu.ca/web/packages/multcomp/multcomp.pdf

Hu Y, Schmidhalter U (2005) Drought and salinity: a comparison of their effects on mineral nutrition of plants. J Plant Nutr Soil Sci 168(4):541–549

Huang B, Wilkinson RE (2000) Role of root morphological and physiological characteristics in drought resistance of plant. In Plant-environment interaction, New York, Marcel Decker. pp. 39–64

Huang B, Gao H (2000) Root physiological characteristics associated with drought resistance in tall fescue cultivars. Crop Sci 40(1):196–203

Huxman TE, Smith MD, Fay PA, Knapp AK, Shaw MR, Loik ME, Smith SD et al (2004) Convergence across biomes to a common rain-use efficiency. Nature 429(6992):651–654

IPCC, 2013: Climate Change (2013) The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, and Midgley PM (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, pp 1535. https://doi.org/10.1017/CBO9781107415324

Johnson SN, Lopaticki G, Hartley SE (2014) Elevated atmospheric $CO_2$ triggers compensatory feeding by root herbivores on a C3 but not a C4 grass. PLoS One 9(3):e90251

Jung V, Albert CH, Violle C, Kunstler G, Loucougaray G, Spiegelberger T (2014) Intraspecific trait variability mediates the response of subalpine grassland communities to extreme drought events. J Ecol 102(1):45–53

Katz GS, Restori AF, Lee HB (2009) A Monte Carlo study comparing the Levene test to other homogeneity of variance tests. N Am J Psychol 11(3):511–521

Kenward MG, Roger JH (1997) Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. Biometrics 53(3):983–997

Knapp AK, Briggs JM, Koelliker JK (2001) Frequency and extent of water limitation to primary production in a Mesic temperate grassland. Ecosystems 4(1):19–28

Knapp AK, Fay PA, Blair JM, Collins SL, Smith MD, Carlisle JD, Harper CW, Danner BT, Lett MS, McCarron JK (2002) Rainfall variability, carbon cycling, and plant species diversity in a Mesic grassland. Science 298(5601):2202–2205

Knapp AK, Beier C, Briske DD, Classen AT, Luo Y, Reichstein M, Smith MD et al (2008) Consequences of more extreme precipitation regimes for terrestrial ecosystems. BioScience 58(9):811–821. https://doi.org/10.1641/B580908

Knapp AK, Carroll CJW, Denton EM, La Pierre KJ, Collins SL, Smith MD (2015) Differential sensitivity to regional-scale drought in six central US grasslands. Oecologia 177(4):949–957

Kramer-Walter KR, Bellingham PJ, Millar TR, Smissen RD, Richardson SJ, Laughlin DC (2016) Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic Spectrum. J Ecol 104(5):1299–1310

La Pierre KJ, Blumenthal DM, Brown CS, Klein JA, Smith MD (2016) Drivers of variation in aboveground net primary productivity and plant community composition differ across a broad precipitation gradient. Ecosystems 19(3):521–533

Lambers JHR, Harpole WS, Tilman D, Knops J, Reich PB (2004) Mechanisms responsible for the positive diversity–productivity relationship in Minnesota grasslands. Ecol Lett 7(8):661–668

Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006) Root structure and functioning for efficient Acquisition of Phosphorus: matching morphological and physiological traits. Ann Bot 98(4):693–713

LeCain DR, Morgan JA, Schuman GE, Reeder JD, Hart RH (2002) Carbon exchange and species composition of grazed pastures and Exclosures in the shortgrass steppe of Colorado. Agric Ecosyst Environ 93(1):421–435

Lee M, Manning P, Rist J, Power SA, Marsh C (2010) A global comparison of grassland biomass responses to CO2 and nitrogen enrichment. Philos Trans Royal Soc B 365(1549):2047–2056

Lefcheck JS (2016) PiecewiseSEM: piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol Evol 7(5):573–579

Manschadi AM, Christopher J, Hammer GL (2006) The role of root architectural traits in adaptation of wheat to water-limited environments. Funct Plant Biol 33(9):823–837

Marulanda A, Azcon R, Ruiz-Lozano JM (2003) Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by Lactuca Sativa plants under drought stress. Physiol Plant 119(4):526–533

McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski BL, Laundre JA (2002) Resource-based niches provide a basis for plant species diversity and dominance in Arctic tundra. Nature 415(6867):68

Medrano H, Escalona JM, Bota J, Gulías J, Flexas J (2002) Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. Ann Bot 89(7):895–905

Morgan JW, Dwyer JM, Price JN, Prober SM, Power SA, Firn J, Moore JL, Wardle GM, Seabloom EW, Borer ET (2016) Species origin affects the rate of response to inter-annual growing season precipitation and nutrient addition in four Australian native grasslands. J Veg Sci 27(6):1164–1176

Newell PD, Douglas AE (2014) Interspecies interactions determine the impact of the gut microbiota on nutrient allocation in drosophila melanogaster. Appl Environ Microbiol 80(2):788–796

Oliveira BF, Machac A, Costa GC, Brooks TM, Davidson AD, Rondinini C, Graham CH (2016) Species and functional diversity accumulate differently in mammals. Glob Ecol Biogeogr 25(9):1119–1130

Onipchenko VG, Makarov MI, Akhmetzhanova AA, Soudzilovskaia NA, Aibazova FU, Elkanova MK, Stogova AV, Cornelissen JHC (2012) Alpine plant functional group responses to Fertiliser addition depend on abiotic regime and community composition. Plant Soil 357(1–2):103–115

Osonubi O (1994) Comparative effects of vesicular-arbuscular mycorrhizal inoculation and phosphorus fertilization on growth and phosphorus uptake of maize (Zea mays L.) and Sorghum (Sorghum bicolor L.) plants under drought-stressed conditions. Biol Fertil Soils 18(1):55–59

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2013) Nlme: nonlinear mixed-effects models. R Package, 3–1, 104

Ploughe LW, Jacobs EM, Frank GS, Greenler SM, Smith MD, Dukes JS (2018) Community response to extreme drought

(CRED): a framework for drought-induced shifts in plant-plant interactions. New Phytol 222(1) 52–69

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna Austria. http://www.R-project.org/

Reich PB (2014) The world-wide 'fast–Slow'Plant economics Spectrum: a traits manifesto. J Ecol 102(2):275–301

Ripley B, Frole K, Gilbert M (2010) Differences in drought sensitivities and photosynthetic limitations between Co-Occurring C3 and C4 (NADP-ME) panicoid grasses. Annals of Botany 105(3):493–503

Ruiz-Lozano JM, Azcón R, Gomez M (1995) Effects of arbuscular-mycorrhizal Glomus species on drought tolerance: physiological and nutritional plant responses. Appl Environ Microbiol 61(2):456–460

Seneviratne SI, Nicholls N, Easterling D, Goodess CM, Kanae S, Kossin J, Luo Y, Marengo J, McInnes K, Rahimi M (2012) Changes in climate extremes and their impacts on the natural physical environment. In Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation Chapter 3. A special report of working groups I and II of the Intergovernmental Panel on Climate Change

Shipley B (2000) A new inferential test for path models based on directed acyclic graphs. Struct Equ Model 7(2):206–218

Smith MD, Knapp AK, Collins SL (2009) A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. Ecology 90(12):3279–3289

Soliveres S, Maestre FT, Ulrich W, Manning P, Boch S, Bowker MA, Prati D, Delgado-Baquerizo M, Quero JL, Schöning I (2015) Intransitive competition is widespread in plant communities and maintains their species richness. Ecol Lett 18(8):790–798

Suttie JM, Reynolds SG, Batello C (2005) Grasslands of the World. Food & Agriculture Organization of the United Nations

Tardif A, Shipley B, Bloor JMG, Soussana J (2014) Can the biomass-ratio hypothesis predict mixed-species litter decomposition along a climatic gradient? Ann Bot 113(5):843–850

Thorpe N (1980) Accumulation of carbon compounds in the epidermis of five species with either different photosynthetic systems or stomatal structure. Plant Cell Environ 3(6):451–460

Tiessen H (2008) Phosphorus in the Global Environment. In The Ecophysiology of Plant-Phosphorus Interactions, Dordrecht, the Netherlands: Springer, 1–7

Trubat R, Cortina J, Vilagrosa A (2006) Plant Morphology and Root Hydraulics Are Altered by Nutrient Deficiency in Pistacia lentiscus (L.). Trees 20(3):334

Trumper K (2009) The natural fix?: the role of ecosystems in climate mitigation: a UNEP rapid response assessment. United Nations Environment Programme (United Nations Environment Programme-World Conservation Monitoring Centre, Cambridge)

Volaire F (2008) Plant traits and functional types to characterise drought survival of Pluri-specific perennial herbaceous swards in Mediterranean areas. Eur J Agron 29(2):116–124

Volaire F, Barkaoui K, Norton M (2014) Designing resilient and sustainable grasslands for a drier future: adaptive strategies, functional traits and biotic interactions. Eur J Agron 52:81–89

Walter J, Grant K, Beierkuhnlein C, Kreyling J, Weber M, Jentsch A (2012) Increased rainfall variability reduces biomass and forage quality of temperate grassland largely independent of mowing frequency. Agric Ecosyst Environ 148:1–10

Yahdjian L, Gherardi L, Sala OE (2011) Nitrogen limitation in arid-subhumid ecosystems: a meta-analysis of fertilization studies. J Arid Environ 75(8):675–680

Yu Z, Zeng D, Jiang F, Zhao Q (2009) Responses of biomass to the addition of water, nitrogen and phosphorus in Keerqin Sandy grassland, Inner Mongolia, China. J For Res 20(1):23–26

Zheng Z, Bai W, Zhang W (2019) Root Trait-Mediated Belowground Competition and Community Composition of a Temperate Steppe under Nitrogen Enrichment. Plant and Soil 437(1-2):1–14