

ARTICLE

Plant functional traits affect competitive vigor of pasture grasses during drought and following recovery

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Abstract

Grassland biomass production is strongly linked with the timing and intensity of precipitation events. While the direct effects of precipitation patterns on grasses are well-studied, less is known regarding plant–plant interactions during different phases of drought (i.e., dry down vs. recovery). Here, we examined how the intensity and timing of drought affected biomass production, traits related to growth rate and competitive vigor (specific leaf area [SLA], leaf dry matter content [LDMC], and height [HT]), and competitive effects in three common pasture grasses. Each species was grown alone (one individual per 45-L planter) or with competition (one individual of each species per 45-L planter) under three different drought types: (1) “short-term” drought where water was withheld until the first species reached stomatal closure; (2) “prolonged” drought where water was withheld until all three species reached stomatal closure; and (3) “repeated” short-term drought where water was withheld until the first species reached stomatal closure, plants were rewatered to capacity, and then, the drought was repeated. In all three drought types, replicates were assessed for biomass and traits pre- and post-rewatering to represent the “resistance” and “recovery” phases of drought, respectively. Overall, we found (1) competitive interactions during phases of drought were primarily mediated by plant HT and LDMC, not SLA; (2) the severity and frequency of drought were key factors in describing plant–plant interactions during phases of drought; and (3) interspecific differences in traits and trait responses to drought phases were key in predicting plant–plant competition. Such shifts in competition interactions associated with interspecific trait responses to drought and during recovery from drought are likely to have significant effects on the productivity and composition of multispecies, grass-dominated plant communities.

KEYWORDS

drought, interspecific competition, plant functional traits, productivity

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INTRODUCTION

The frequency, magnitude, and timing of rainfall events are primary drivers of plant productivity and functioning in grasslands (Huxman et al., 2004; Wilcox et al., 2017; Yang et al., 2008). So, changes in rainfall regimes are likely to have major consequences for the productivity and functioning in these ecosystems (Knapp et al., 2015; Power et al., 2016). In particular, altered soil moisture dynamics associated with less frequent rainfall events of greater magnitude will generate longer interpulse dry periods (Knapp et al., 2008). While intuitive that the onset of water limitation should increase competition between co-occurring plant species, the stress-gradient hypothesis (SGH) suggests that increasing facilitative interactions will occur with increasing environmental stress (Butterfield et al., 2016; Maestre et al., 2009). The increasingly facilitative interactions predicted by the SGH can be attributed to a differentiation in strategies used among co-occurring species with the goal of limiting resource competition (*sensu* Walter's two-layer hypothesis) (Holdo, 2013; Walter, 1971; Ward et al., 2013) or through increased space/resource availability resulting from mortality of stress susceptible individuals (He et al., 2013). While these mechanisms have been proposed to explain plant–plant interactions along resource gradients, few data exist to test them explicitly. New work is required to understand how altered precipitation patterns may affect plant–plant interactions, and potentially result in changes in key ecosystem processes such as biomass production.

Overall, interactions between co-occurring plants are predicted to become less competitive (i.e., increasingly facilitative) with increasing environmental stress (He et al., 2013; Michaletz et al., 2016; Ploughe et al., 2019). Nonetheless, while this prediction is common for dryland and alpine communities (Schöb et al., 2013; Soliveres et al., 2014; Soliveres & Maestre, 2014), few experiments have tested these assumptions in mesic species and ecosystems. Although some evidence suggests plant–plant interactions under varying water availability may not reflect dynamics of the SGH (Maestre et al., 2009), others have found the interplay between competition and facilitation to be equally important with respect to water availability in mesic ecosystems (Butterfield et al., 2016; Holmgren & Scheffer, 2010). Given the strong link between ecosystem function and precipitation patterns (Huxman et al., 2004; Morecroft et al., 2004), it is crucial to test assumptions of the SGH under future, more variable rainfall. Moreover, the extent to how severity and frequency of stressors affects plant–plant interactions remains unclear.

Trait-based approaches provide a valuable tool to assess plant–plant interactions under changing environmental conditions (Butterfield & Callaway, 2013). For example,

functional traits have been used to predict plant community responses to altered soil water dynamics (Comas et al., 2013; Skelton et al., 2015; Volaire, 2008; Volaire et al., 2014). Importantly, plant functional traits can be used as surrogates for plant physiological strategy, indicating species' locations on the resource-use axis (Grime et al., 1997), allowing us to discern whether plants are acquisitive or conservative with respect to their resource-use strategies. “Acquisitive” species are fast-growing, but have a high rate of tissue turnover and low stress tolerance. “Conservative” species, in comparison, are generally stress tolerant and slow-growing and maintain tissues as a result of greater carbon investment (Reich, 2014). For example, conservative species possess high leaf dry matter content (LDMC, ratio of leaf dry mass to fresh or saturated leaf mass, in milligrams per gram) and low specific leaf area (SLA, ratio of fresh/saturated leaf area to leaf dry mass, in square millimeters per milligram), while acquisitive species may possess comparably lower LDMC and greater SLA. The plant resource spectrum has been linked to plant heat tolerance (Michaletz et al., 2016), herbivory (Ruiz-Guerra et al., 2020) and soil nutrient conditions (Shen et al., 2019). Indeed, leaf and plant functional traits have been associated with performance during drought (Balachowski et al., 2016; Blumenthal et al., 2020; Bolger et al., 2005; Bristiel et al., 2019). Still, how trait-based responses to drought across species may affect plant–plant interactions is poorly understood (Ploughe et al., 2019; Wang & Callaway, 2021).

Plant functional traits can be useful for characterizing species' influence on ecosystem function (“effect” traits) or to describe species' responses to changes in the environment (“response” traits) (Suding et al., 2008). In the case of altered precipitation patterns and associated soil water dynamics (Knapp et al., 2008), traits may directly shape plant–plant interactions and subsequent outcomes for community productivity and composition. For example, species that exhibit shifts in traits (i.e., plasticity) conducive to conservative growth may exhibit superior resistance to the onset of water limitation (Figure 1). Yet, when rainfall does occur, species that have the capacity to shift to rapid growth (i.e., acquisitive) may be more competitive, relative to slower growing species. Indeed, differences in growth rates among neighboring species have previously been linked to competitive vigor (e.g., Schwinning et al., 2017). Such changes in traits and growth strategy in response to resource availability are likely to have large implications for plant–plant interactions. Given the importance of water availability for key grassland functions, it is crucial to understand the mechanisms that shape plant–plant interactions under predicted changes in rainfall regimes.

Using the “response and effect” framework to describe traits and their functions within plants (Suding et al., 2008), we can hypothesize how specific traits might influence

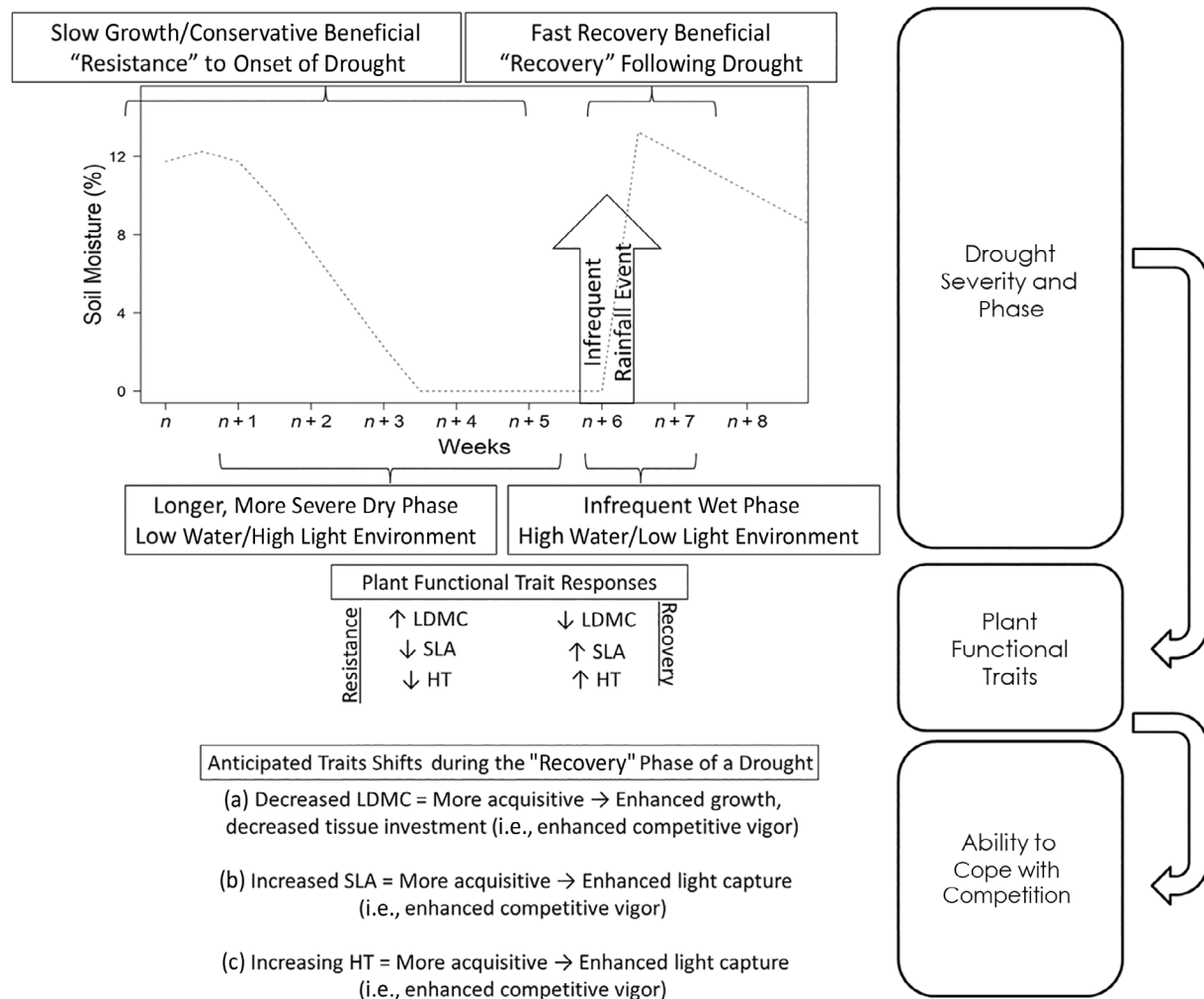


FIGURE 1 Altered soil moisture regimes associated with changing rainfall patterns may affect plant functional traits, underpinning competitive interactions within plant communities. The soil moisture diagram indicates that conservative traits might be beneficial during longer, drier periods, while acquisitive traits might be beneficial for responding to infrequent rainfall events. Below the soil moisture graph is prediction of trait values for leaf dry matter content (LDMC), specific leaf area (SLA), and height (HT) during the resistance phase and the recovery phase of a drought. Arrows adjacent to traits indicate whether a particular phase of drought might cause traits to increase (↑) or decrease (↓). Changes in traits associated with the drought phases would influence productivity when in competition (hypotheses a, b, and c included therein). Boxes to the right of the figure represent a simplified diagram of the variables and processes involved and provide a foundation for the piecewise structural equation modeling used to test our assumptions.

plant–plant interactions throughout the different phases (i.e., resistance or recovery) of drought. Aboveground plant biomass is often reduced during the onset of drought following a decrease in stomatal conductance and associated photosynthetic rate (Ilyas et al., 2021; Xu et al., 2009). Species-specific changes in plant stature could alter access to light, resulting in a size differential that will influence growth/composition during recovery from drought (Manea et al., 2016; Schwinning et al., 2017). Similarly, plant height (HT) is a useful measure to predict how species cope with light limitation (Farrior et al., 2013; Knapp, 1984) and is widely acknowledged for its relationship to competitive vigor (Falster & Westoby, 2003; Westoby, 1998). Thus, species that experience greater losses of biomass during drought may be outcompeted as the community recovers following drought,

when taller and larger plants outperform their smaller and/or shorter neighbors. Such changes in growth rate during the onset of, and recovery from, soil water limitation could be predicted by tissue-investment traits, such as LDMC (Díaz et al., 2016; Smart et al., 2017). Thus, plants that exhibit a decline in LDMC following recovery from drought should have high relative growth rates, potentially negating the negative effects of competition on postdrought growth. Another trait, SLA, may also be a useful proxy for plant responses to drought and potential plant–plant interaction. SLA, often more variable within species than LDMC (Wilson et al., 1999), exhibits a high degree of plasticity. So, the environmental fluctuation between water limitation (high light conditions from decreased vegetation growth) and water abundance (low light conditions from enhanced

vegetation growth) may drive changes in either the “area” or “mass” components of SLA. In the context of competition, increases in SLA associated with high water/low light conditions during drought recovery (i.e., increasing leaf area to increase light capture when water loss is not a primary concern) may have consequences for neighboring species (e.g., shading) (Craine & Dybzinski, 2013; Spitters & Aerts, 1983). Nonetheless, few data exist to test the role of traits in the outcome of plant–plant interactions in response to soil water availability. Moreover, how the severity and frequency of water limitation affects plant–plant interactions in a controlled environment is unstudied.

The goal of this study was to provide a trait-based framework for understanding whether changes in the severity and frequency of water limitation will directly or indirectly influence biomass production in pasture species. We address two overall themes regarding the impact of drought and competition on three common pasture grasses. First, we sought to understand how the severity and phase of drought affected biomass dynamics of plants grown in isolation and then the effects of competition among these species. Second, we sought to understand how drought severity and frequency would affect plant traits, and how such trait changes affect competitive outcomes. We hypothesized that (H1) competition would have a negative effect on target species’ biomass production, which would increase with increasing drought severity; (H2) competitive effects would be greater during the recovery phase of drought than the resistance phase, because active growth would intensify competitive interactions; and (H3) competitive vigor would be enhanced upon rewatering in species which shift to a more acquisitive strategy. We used a series of piecewise structural equation modeling (pSEM) to address three questions: (1) How does drought phase (i.e., during the dry down or following recovery) influence traits, and what are the effects on competition? (2) Do changes in the severity and frequency of drought alter the relative importance of different traits in competitive interactions among three pasture species? and (3) Are traits that mediate the drought–competition relationship in pasture grasses similar in all three species? Our objective was to increase understanding of the role that traits may play in mediating competitive interactions in grass-dominated communities under predicted scenarios of drought-associated changes in soil water dynamics.

MATERIALS AND METHODS

Experimental site, species, and design

The experiment was conducted at Western Sydney University’s Hawkesbury Campus using the “Large

Rainout Shelters” facility (www.westernsydney.edu.au/hie/facilities/rainout_shelters). The facility has six large shelters (12 m long \times 8 m wide \times 7 m tall) with roofs and curtains that automatically close during rain events to exclude ambient rainfall (see Dijkstra et al., 2016, for further description). In each shelter, there are eight overhead sprinklers for irrigation. In addition, each shelter has eight time-domain reflectometry (TDR) sensors that record soil moisture content ($\pm 3\%$ accuracy) at 15-min intervals in the top 15 cm of soil. Each half shelter can be irrigated and monitored for soil moisture independently, so while six shelters are present at the site, there are a total of 12 potential plots available for experimentation. The facility is equipped with a weather station that records temperature and rainfall.

From 1 to 7 December 2017, 45-L black polyethylene planter bags (410-mm HT, www.planterbags.com.au) were filled with sandy loam soil collected from Menangle, NSW, Australia (Drake et al., 2015, 2017; Zhang et al., 2018). In each plot, 16 bags were evenly spaced (for a total of 12 shelter plots, 16 bags in each plot, and thus an overall total of 192 planter bags). Ten seeds of *Phalaris aquatica* L. (cultivar: Holdfast GT), *Lolium perenne* L. (cultivar: Kidman), and *Festuca arundinacea* Schreb. (cultivar: Quantum II MaxP) were sown (5-cm depth) into planter bags on 12 March 2018 (Heritage seeds, Dandenong South, VIC, Australia). All three species are cool-season C₃ perennial grasses. *Phalaris* and *Festuca* are considered deep rooted (Norton et al., 2016; Voltaire & Lelièvre, 2001), while *Lolium* exhibits a shallower, fibrous root system (Zhang et al., 2019).

In planter bags with competition, seedlings were thinned over 100 days until one of each species remained in the bag at the commencement of the drought treatments (three individuals grown in 45 L of soil). In planter bags without competition, seedlings were thinned over that same period until a single individual remained in each bag (one individual grown in 45 L of soil). The protocol of manipulating the number of individuals in a fixed amount of soil has been used by others in recent years to examine the effects of competition/competition removal in tandem with drought on plant productivity (Fernando et al., 2019; Fitzpatrick et al., 2019; Loydi et al., 2018; Tom-Dery et al., 2018). This arrangement was selected over the “replacement” design for competition experiments where the total density of plants was maintained constant. Thus, the single-individual plants represent a “true” control where the only difference between mixtures and single-plant replicates is the presence of other species. This design permits a direct calculation of the effect of neighboring species on focal species (as described in Mahmoud & Grime, 1976).

Plants were given liquid fertilizer (12:1:4:7) (Seasol’s Powerfeed All Purpose, Bayswater, VIC) for the first

12 weeks of establishment, and thereafter, an initial application of slow-release fertilizer (28:0.4:5) was added on 4 June 2018 (Scotts Slow Release Lawn Fertilizer, Bella Vista, NSW). Fertilizer was applied at recommended rates to minimize potential nutrient limitation, allowing us to isolate potential water and light limitation. Competitive effects were observed during dry downs and upon rewatering (details to follow), while nutrient and space limitation were minimized, so water and/or light were the primary resources for plant competition.

Monthly mean temperatures in 2018 were 13.8°C (May), 11.6°C (June), 11.0°C (July), 11.9°C (August), 15.0°C (September), 18.0°C (October), and 20.5°C (November), with 99% of daily mean temperatures between 7.0 and 26.5°C. Over the course of the study, all rainfall was excluded from the plots using automated rainfall exclusion curtains on the shelters (as described above). For reference, evaporation in 2018 was 79.4 (May), 53.9 (June), 76.2 (July), 103.9 (August), 122.6 (September), 111.8 (October), and 161.5 mm (November) (709.3 mm total evaporation), while total rainfall at the site was 281.2 mm during the study.

Determination of drought treatments

We used the 12 shelter plots to impose three drought treatments. Three plots were designated as well-watered controls, with each plot corresponding to a specific drought treatment (Appendix S1: Figure S1). Each of the three drought treatments was replicated in three plots (3 plots \times 3 drought treatments = 9 plots, plus the 3 control plots = 12 plots in total). Rather than using environmental events to determine the severity and frequency of drought (e.g., 40 or 100 days without water), or trying to manipulate the rainfall received/soil moisture content (e.g., 30% or 60% reduction), drought treatments were based on physiologically relevant thresholds. Specifically, drought treatments were based on species-specific differences in the time until stomatal closure (i.e., minimal stomatal conductance) of the grasses, as advocated by others to better understand drought impacts on plant performance (Jaleel et al., 2009; Medrano et al., 2002; Miyashita et al., 2005). In all three treatments, we measured stomatal conductance weekly (g_s in millimoles per square meter per second; details below) to determine the severity and frequency of soil water deficits.

Within the experiment, we conducted three concurrent drought treatments: short-term drought (three plots), prolonged drought (three plots), and a repeated drought (three plots). There was also a separate well-watered control (three plots; Figure 2). The severity and frequency of each treatment was based on stomatal closure (i.e., minimal g_s values). In the “short-term” drought treatment (Figure 2a),

water was withheld in all plants (except well-watered controls) until the first of the three species exhibited zero g_s for ~2 weeks. At that time, droughted plants were harvested for biomass and trait assessment, representing the “resistance” phase of the drought. The remaining planter bags were then rewatered to capacity and allowed 2 weeks of recovery, at which time plants in the “recovery” planter bags were harvested. Well-watered controls for the short-term drought treatment were harvested at the midpoint between resistance and recovery phases.

The second drought treatment (hereafter, “prolonged” treatment) examined the resistance and recovery phases following a drought that continued until all three species reached zero g_s for a 2-week period; however, in the prolonged treatment the resistance and “recovery” phase harvests occurred 4 weeks apart (Figure 2b). Thus, the short-term drought is considered mild, where few members of the plant community were affected by soil water limitation. The long-term drought is considered severe, where all members of the community were affected by soil water limitation. The final drought treatment (hereafter, “repeated” treatment) examined the effect of two short-term droughts (as seen in the short-term treatment), with the resistance and recovery phase harvests also occurring 4 weeks apart (Figure 2c). This drought was intended to determine whether “priming” to drought (i.e., previous exposure) altered the trait dynamics of the experimental species.

For all droughted plants, watering was withheld beginning 20 June 2019. In the short-term treatment (Figure 2a), the first species to attain zero g_s for 2 weeks was *Lolium*, after 79 days of water being withheld (Appendix S1: Figure S2 and Table S1). In the prolonged treatment (Figure 2b), the last of the three species to reach zero g_s was *Festuca*, after 118 days without water (Appendix S1: Figure S3 and Table S1). In the repeated treatment (Figure 2c), the first species to reach zero g_s was *Lolium*, after 79 days without water (as observed in the short-term treatment) (Appendix S1: Figure S4 and Table S1). Following 3 days of rewatering, it took an additional 47 days for the first species to have zero g_s which occurred in *Lolium* and *Phalaris*, simultaneously.

Stomatal conductance, traits, and productivity measurements

Stomatal conductance (g_s , in millimoles of H_2O per square meter per second) was measured on a weekly basis for the duration of the experiment using a portable leaf porometer (Model SC-1, Decagon Devices, Pullman, WA, USA). Measurements were taken between 9:30 AM and 2:30 PM on sunny days with minimal wind. Plants grown in the absence of competition (i.e., one individual of one species

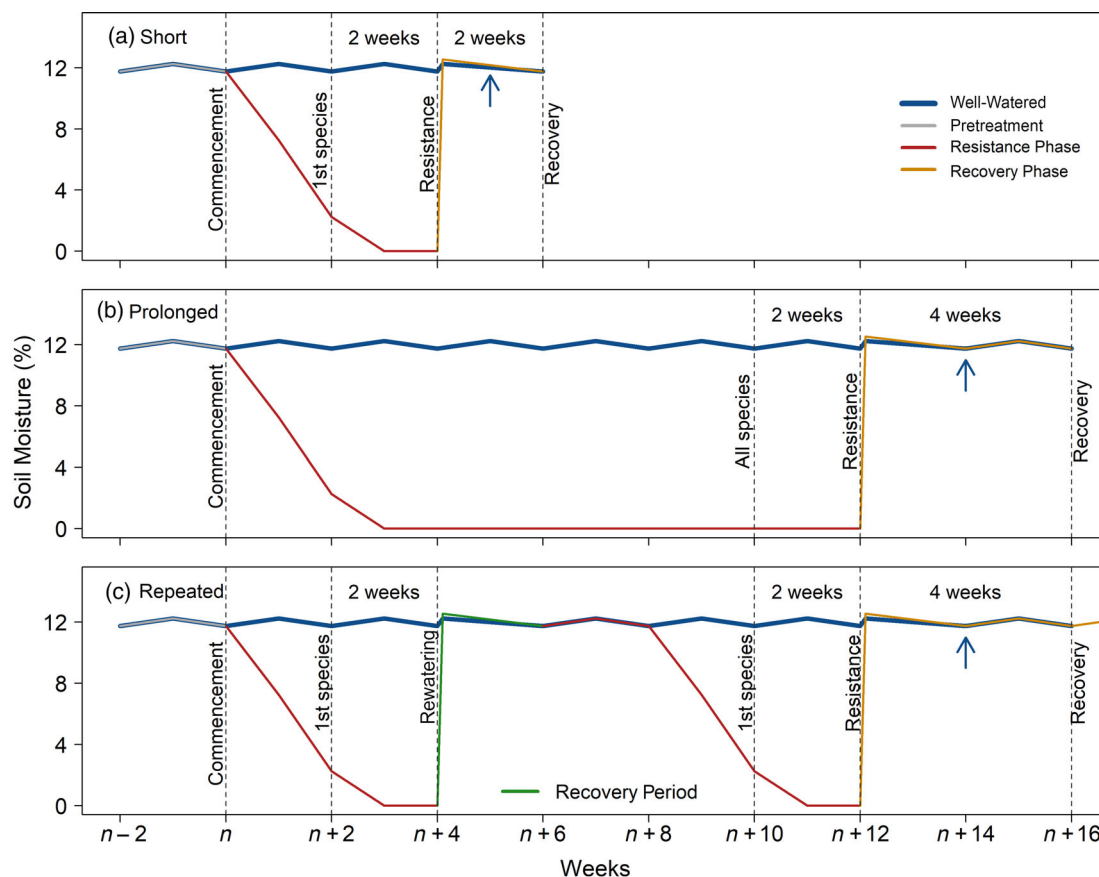


FIGURE 2 Experimental design outlining the soil moisture regimes for the short-term (panel a) prolonged (panel b) and repeated (panel c) drought treatments. For each treatment, the resistance phase (red lines) begins at the onset of water limitation. Recovery periods (orange lines) begin when plants are rewatered to field capacity prior to final recovery measurements. The duration of treatments was set to biologically relevant events, namely, minimal stomatal conductance (g_s) or closure of stomata. For example, in the short-term drought treatment (a), droughted plants are permitted to dry down until one of the three species in the experiment reaches near-zero g_s (i.e., first species). Two weeks following this date, a subset of droughted replicates were harvested, to determine how plants resisted the onset of water limitation. Remaining plants were rewatered and maintained as soil moisture capacity for 2 weeks prior to harvesting, to determine how plants recovered from the dry-down period. Blue arrows denote when well-watered control plants were harvested (i.e., at the midpoint between resistance and recovery harvests).

per planter) in soils with TDR sensors were measured (Appendix S1: Figures S2–S4). We chose g_s to determine drought intensity/duration because it is an important surrogate for physiological activity (Jaleel et al., 2009) and is indicative of droughts severe enough to elicit responses in traits and productivity.

During harvests for each of the three treatments and associated well-watered controls (Appendix S1: Table S1), we measured plant HT (in millimeters), SLA, and LDMC, in addition to aboveground and belowground biomass. We chose these three traits because HT is strongly correlated with light capture and competitive vigor (Falster & Westoby, 2003; Farrior et al., 2013; Knapp, 1984; Westoby, 1998), SLA integrates aspects of resource-use strategy and competitive vigor while being commonly recorded worldwide (Craine & Dybzinski, 2013; Spitters & Aerts, 1983), and LDMC is strongly correlated to resource-use strategy

(Díaz et al., 2016; Smart et al., 2017). Plant HT was recorded as stretched length using a meter ruler (Cornelissen et al., 2003). For each plant, two recently expanded leaves (free of obvious insect damage or necrosis) were collected and rehydrated in deionized water for a minimum of 6 h (Garnier et al., 2001). Senescence, which is triggered by abscisic acid accumulation following stomatal closure (Thimann & Satler, 1979; Tombesi et al., 2015), had not occurred at the points of sampling. So, leaves were assumed to exhibit full rehydration. Saturated leaves were scanned to determine leaf area using WinFolia (V 2015 Pro, Regent Instruments Inc., Quebec, Canada) and weighed for saturated leaf mass. Leaves were subsequently dried for 72 h at 60°C for dry matter determination. Specific leaf area was calculated as the ratio of saturated leaf area to dry mass (SLA, in square millimeters per milligram). Leaf dry matter content was calculated as the ratio of leaf dry mass to saturated leaf mass (LDMC, in milligrams per gram).

Aboveground biomass was harvested from each planter bag and sorted by species for those plants grown in competition. Root biomass was quantified following sieving (2 mm) and washing of roots. It was not possible to sort root biomass by species in mixtures due to root entanglement, as others have observed using considerably smaller planters than the 45-L bags used here (e.g., Schwinning et al., 2017, using ~9-L pots and Montazeaud et al., 2018, using ~11-L pots). All biomass was dried for 72 h at 60°C before weighing.

Data analysis

Of the 192 planters used in this study (Appendix S1: Figure S1), 64 were allocated to each of the drought treatments (short-term, prolonged, and repeated). Of the 64 planters in each treatment, 16 were well-watered controls. There were four replicates of each competition treatment: four planters of *F. arundinacea* grown without competition (one individual per planter); four planters of *Phalaris* grown without competition (one individual per planter); four planters of *Lolium* grown without competition (one individual per planter); and four planters grown with one individual of each species in competition (one individual per species per planter and three individuals in total). Of the 48 remaining planters in each drought treatment, 24 (six replicates of four planter types) were harvested following stomatal closure of select species (resistance phase) and the remaining 24 (six replicates of four planter types) were harvested after rewatering (recovery phase) according to the previously mentioned timelines and criteria (Appendix S1: Table S1 and Figure S1).

All tests of significance were performed with R (3.6.0, R Core Team, 2013) in R Studio (1.0.143). In all cases, α was set at 0.05. To examine how competition and drought affected species-specific biomass production dynamics and functional traits (SLA, LDMC, and HT), we conducted a set of linear mixed effects models (*nlme* package) (Lefcheck, 2016; Pinheiro et al., 2013). Data were grouped by species and drought treatment for analyses. The phase of drought (resistance, recovery, and well-watered control) and whether the plant was grown in competition (0 or 1 dummy variable) were included as fixed effects with plot included as a random effect. Due to the complex magnitude and directional responses of traits across species, drought types, phase of drought, and competition trait data are presented log response ratios. For each species grown with or without competition from each drought type, we calculated the log response ratio ($\pm 95\%$ CI) for each trait, specifically the response to rewatering (i.e., trait change from resistance phase to recovery phase). For each species and drought type, we considered the effect of competition on trait responses to

rewatering significant if CI did not overlap. Log response ratios were calculated using the *singlecaseES* package (Swan & Pustejovsky, 2018).

Belowground biomass could not be separated to species in mixed species planters, so we analyzed these data by phase of drought alone, using plot as a random effect. Species-specific belowground biomass from planters with one individual (i.e., without competition) were also analyzed individually. Residual df were calculated using Kenward–Roger approximation to estimate *p*-values (*LMERTEST* package) (Kenward & Roger, 1997). Post hoc Tukey “single-step” comparison was employed when fixed effects resulted in significance ($\alpha = 0.05$) (*multcomp* package) (Bretz et al., 2016; Hothorn et al., 2017; Newell & Douglas, 2014). Data were log transformed as necessary to ensure homogeneity of variance using Levene’s test (Katz et al., 2009) in the *car* package.

To determine how the presence of neighboring species affected biomass of the target species, we calculated the competitive effect as:

$$\log CE_X = \log(\overline{B_X}) - \log(B_{X,N}), \quad (1)$$

where $\overline{B_X}$ represents the average biomass for the focal species when grown without competition (i.e., one individual per planter), and $B_{X,N}$ is the biomass of individuals of that focal species in planters with competition (i.e., three individuals and one of each species) (Goldberg et al., 1999; Schwinning et al., 2017). So, if *Lolium* produced more biomass without competition ($\overline{B_X}$) than when in competition with *Festuca* and *Phalaris* ($B_{X,N}$), the competitive effect would be greater than zero. Thus, the lower the value of competitive effect, the greater competitive vigor (i.e., ability to maintain productivity when in competition comparable to amounts when grown without competition) for that species. Competitive effect data were calculated for all individuals grown in competition, using average biomass data pooled from each combination of drought treatment (short, prolonged, and repeated) and phase of drought (resistance, recovery, and well-watered controls). The competitive effects on aboveground biomass were analyzed as outlined above; however, competitive effects on belowground biomass could not be calculated because it was not possible to separate belowground biomass and assign it to a single species. Values for our competitive effect index are nearly identical, although inversely, to the relative interactions index (Armas et al., 2004).

Finally, we used piecewise structural equation modeling (pSEM) to determine whether the relationship between drought (either drought type or phase) and competitive effects on aboveground biomass was mediated by

traits. The pSEM relies on directed separation (independence) of exogenous variables, while endogenous variables contribute to the overall goodness-of-fit which is determined by χ^2 test and Fisher's C statistic (Duffy et al., 2016; Oliveira et al., 2016; Shipley, 2000). Analyses were conducted using linear mixed models (*nlme* package) and the *piecewiseSEM* package (Lefcheck, 2016; Pinheiro et al., 2013). The SEMs were created by combining two linear mixed model components, namely, “drought recovery → traits” and “traits → competitive effects.” The pSEM cannot handle factors as fixed effects, so we substituted 0 and 1 for the resistance and recovery phases of drought. We ran the models in three ways. First, we used all available data to determine whether traits underlying competitive vigor during drought were common across species and drought types (i.e., severity and duration). Second, to test what traits were important for competitive vigor across all species, we ran the pSEM

for each drought type. Third, to test what traits were important for each species, regardless of drought type, we ran the pSEM for each species.

RESULTS

Aboveground biomass response to drought recovery and competition

For aboveground biomass, we observed a significant “phase” (i.e., well-watered, resistance, and recovery) × competition (with and without) effect for all species in all drought types (Appendix S1: Table S2). Within the short-term drought, aboveground biomass increased with rewatering in all species; however, in no species did rewatering lead to aboveground biomass production comparable to the well-watered controls. During the onset of

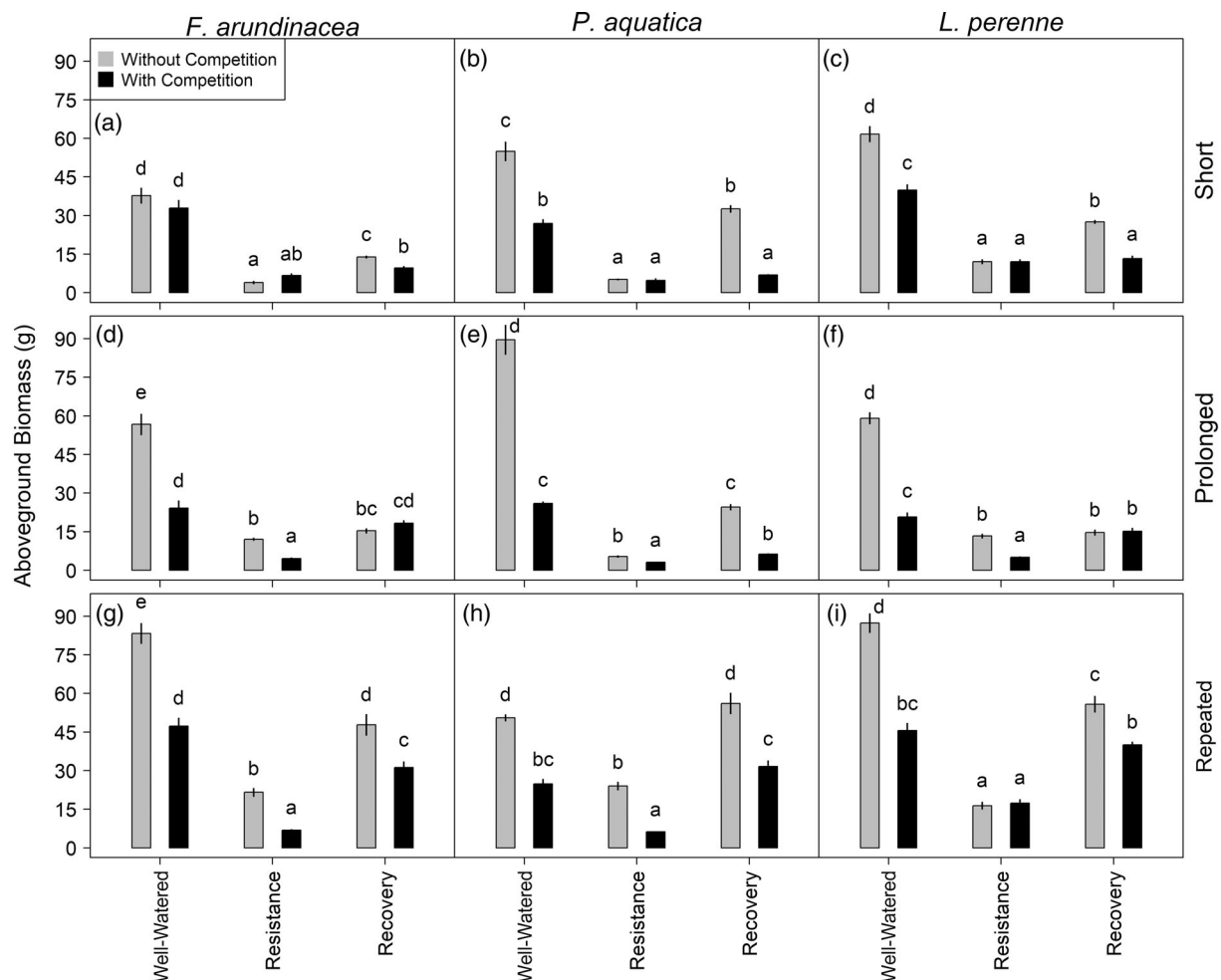


FIGURE 3 Aboveground biomass for each species when grown in isolation (gray bars) and with competition (black bars). Species-specific data were separated by drought treatment. The x-axis indicates the phase of drought (resistance and recovery) when plants were harvested and are grouped with their respective treatment's well-watered control plants. Letters within each panel that are shared indicate no significant difference between those amounts of aboveground biomass. Error bars are SE.

the short-term drought (i.e., resistance phase), aboveground biomass was similar with and without competition in all species. Yet, following recovery, biomass was suppressed when grown with neighbors in all three species (Figure 3a–c). Under well-watered conditions, aboveground biomass production of *Phalaris* and *Lolium* was inhibited by the presence of neighbors when compared to those species grown in isolation (Figure 3b,c).

In the prolonged drought, the presence of neighbors suppressed aboveground growth in all species during the resistance (droughted) phase (Figure 3d–f). After rewatering, aboveground biomass was suppressed by competition in *Phalaris* (Figure 3e), but not the two other species. When grown in isolation, no species recovered levels of aboveground biomass that were comparable to well-watered controls following rewatering. When grown in competition, only *Festuca* exhibited aboveground mass following the

recovery period that was comparable to well-watered *Festuca* grown in mixture (Figure 3d). In both short-term and prolonged drought treatments, biomass produced following rewatering (i.e., during recovery) did not match or exceed aboveground production in the well-watered control plants, with the exception of *Festuca* grown under competition.

In the repeated drought, the presence of neighbors suppressed aboveground production during the onset of drought in *Festuca* (Figure 3g) and *Phalaris* (Figure 3h), but not in *Lolium* (Figure 4i). Still, all species exhibited superior growth following the recovery period when grown in isolation compared to when grown with competition. Aboveground productivity during recovery was comparable to well-watered controls in *Phalaris* and *Lolium* when grown with competition (Figure 4h,i). In the absence of competition, only *Phalaris* had aboveground biomass

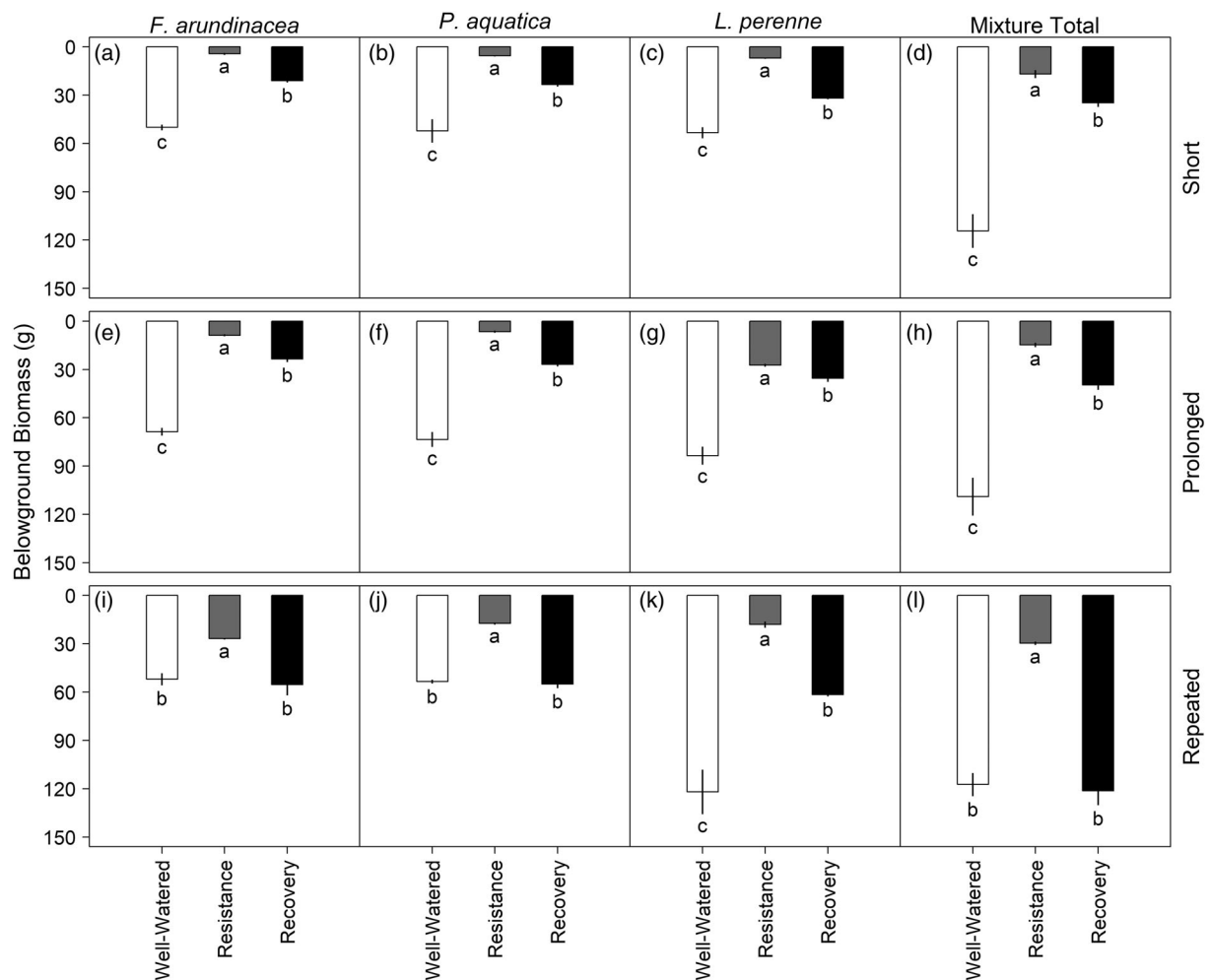


FIGURE 4 Belowground biomass for species grown without competition (*Festuca arundinacea*, *Phalaris aquatica*, and *Lolium perenne*) and with competition (mixture total, representing one individual of each species). Data were separated by drought treatment. The x-axis indicates the phase of drought (resistance and recovery) when plants were harvested and are grouped with well-watered control plants. Letters within each panel that are shared indicate no significant difference between those amounts of belowground biomass. Error bars are SE.

production comparable to the well-watered controls following recovery (Figure 4h).

Belowground biomass response to drought recovery

Under short-term and prolonged drought, recovery of belowground biomass exceeded observed values from the resistance phase (Figure 4a–h; Appendix S1: Table S3); however, in all cases the biomass produced following recovery did not match (or exceed) production of well-watered control plants. This was true for plants grown without competition (Figure 4a–c,e–g) and for those grown with competition (Figure 4d,h).

Under repeated drought, belowground biomass production increased upon rewatering (i.e., from resistance to recovery phases) to match production in the well-watered controls for *Festuca* and *Phalaris* when grown without competition (Figure 4i,j), as well as when plants experienced competition (Figure 4l). While belowground biomass of *Lolium* increased upon rewatering, it did not match belowground biomass produced under well-watered conditions (Figure 4k).

Note that we were unable to separate belowground biomass by species in mixtures. None of the species grown with or without competition produced >120 g of belowground biomass during the recovery phase, except the mixture under repeated drought. Compared to the maximum root production of any treatment combination (~120 g; Figure 4k,l), this indicates plants were not strongly limited by rooting space during drought phases. Thus, effects of competition on aboveground production were likely a result of aboveground interactions or differences in water/resource consumption strategies.

Competitive effects on aboveground biomass during drought recovery

The negative effects of neighboring species on focal species' aboveground production (i.e., competitive effect) varied by drought type and species. In all instances, we observed a significant effect of drought phase on the magnitude of competitive suppression (Appendix S1: Table S3). Importantly, competition was not equal across species during phases of drought. For example, during the onset of short-

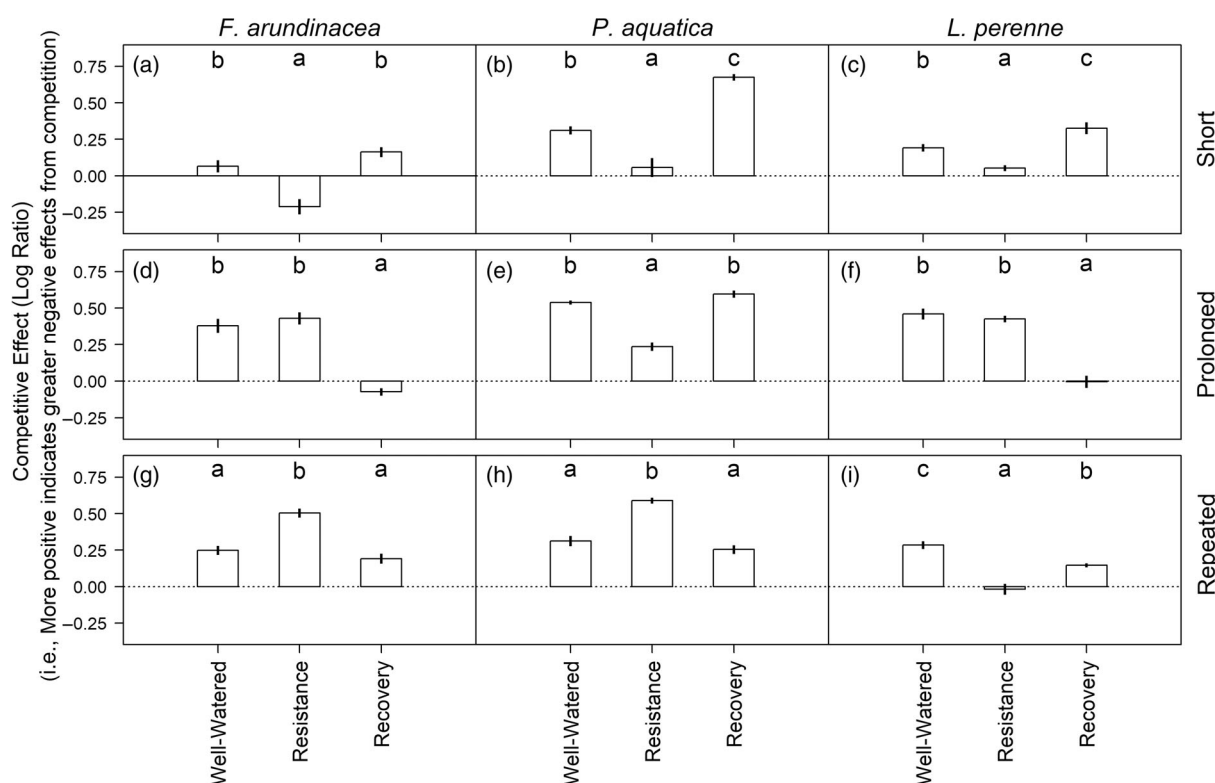


FIGURE 5 Competitive effects on aboveground biomass production by species (columns), treatment (rows), and drought phase (x-axis). Before and after rewatering (resistance and recovery, respectively) plants are compared to their respective well-watered control plants. Competitive effects >0 indicate an increasingly negative effect of competition on aboveground biomass, while competitive effects <0 reflect a positive effect of competition on aboveground biomass production. Letters within each panel that are shared indicate no significant difference between aboveground biomass. Error bars represent SE.

term drought, *Festuca* aboveground biomass was enhanced by neighboring species, while the competitive effect was near-zero for *Phalaris* and *Lolium* (Figure 5a–c). Interestingly, drought type had a large effect on competitive dynamics of each species. For example, as noted above, *Festuca* exhibited enhanced growth with competition during the onset of the short-term drought (Figure 5a), yet aboveground biomass was suppressed during the onset of the prolonged drought (Figure 5b). This highlights how severity and frequency of drought are important in shaping plant–plant interactions and composition of communities.

During the prolonged drought, the competitive effect on aboveground production was seen predominantly in all three species during the resistance phase (Figure 5d–f). Still, we observed near-zero effects of competition on aboveground production in *Festuca* and *Lolium* following recovery from prolonged drought (Figure 5d,f) when competitive effects were strong for *Phalaris* (Figure 5e).

During the repeated drought, competitive effects were high for *Festuca* and *Phalaris* following the second dry down but were partially alleviated upon rewatering

(Figure 5g,h). Still, competition was near-zero for *Lolium* during the onset of the second dry down, but increased upon rewatering (Figure 5i).

Trait responses to drought and competition

Variation in traits was strongly tied to the presence/absence of competition and drought phase (Appendix S1: Tables S4 and S5). To simplify the directionality and magnitude of trait responses to rewatering, we present these data as log response ratios (Figure 6). In *Festuca*, rewatering generally led to a positive or neutral (i.e., CI of response ratio overlapped with zero) effect on SLA across drought types (Figure 6a–c). Still, the presence of neighbors did not alter the response of SLA to rewatering compared to *Festuca* grown alone. In *Phalaris*, SLA increased upon rewatering when grown in isolation in the short-term and prolonged droughts (Figure 6a,b) but declined in the repeated drought (Figure 6c). In all three drought types, the response of SLA following rewatering was significantly different in *Phalaris*

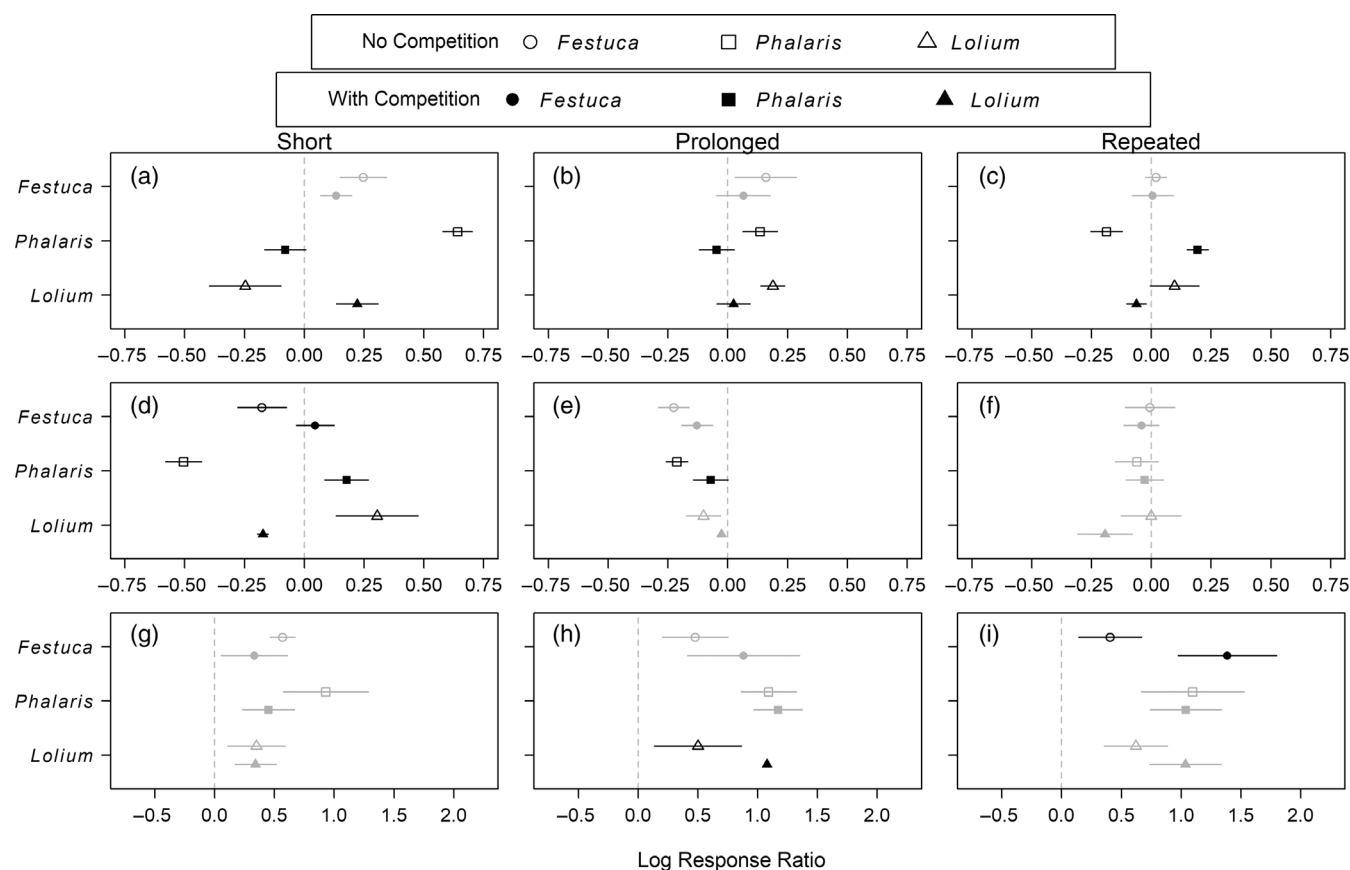


FIGURE 6 Log response ratios ($\pm 95\%$ CI) of specific leaf area (a–c), leaf dry matter content (d–f), and plant height (g–i) in response to rewatering following three drought types (short, prolonged, and repeated) in three grasses grown with (filled symbols) and without (empty symbols) competition. 95% CI that overlap zero (vertical dashed line) indicate no effect of rewatering on trait values. Gray symbols denote no significant difference between plants grown with and without competition within a species, while black symbols denote a significant difference in trait responses to rewatering between individuals within a species with and without competition.

grown alone compared to those grown with competition. *Lolium* exhibited negative, positive, and slightly positive SLA responses to rewetting when grown alone in the short, prolonged, and repeated droughts, respectively. Still, responses of SLA in *Lolium* to rewetting were significantly altered by the presence of neighbors (Figure 6a–c).

In *Festuca*, rewetting led to neutral or negative effects on LDMC (Figure 6d–f), yet only in the short-term drought did competition result in a significant change in the response of SLA under competition compared to *Festuca* grown alone (Figure 6d). In *Phalaris* grown alone, we observed a strong negative response of LDMC upon rewetting in the short-term drought, but under competition LDMC exhibited an increase (Figure 6d). Competition had no effect on LDMC of *Phalaris* upon rewetting in the prolonged or repeated drought types, where we observed a negative and neutral response, respectively (Figure 6e,f). Leaf dry matter content increased with rewetting in the short-term drought in *Lolium* grown alone, with a significantly negative response when grown in competition (Figure 6d). Competition had no effect on LDMC of *Lolium*

upon rewetting in the prolonged or repeated drought types, where we observed slightly negative to neutral responses overall (Figure 6e,f).

Height significantly increased in all species in all drought types, in both monoculture and mixtures. Still, competition had no effect on any species under short-term drought (Figure 6g). Upon rewetting in the prolonged drought, HT exhibited a stronger increase for *Lolium* plants growing in competition compared to those grown alone (Figure 6h). Similarly, upon rewetting in the repeated drought, HT exhibited a stronger increase in competition for *Festuca* compared to when grown alone (Figure 6i).

Using traits to explain how drought recovery affects competition

Across all species and treatments, we found no single trait explained competitive vigor under drought (Appendix S1: Table S6) and SLA was unimportant for “across species”

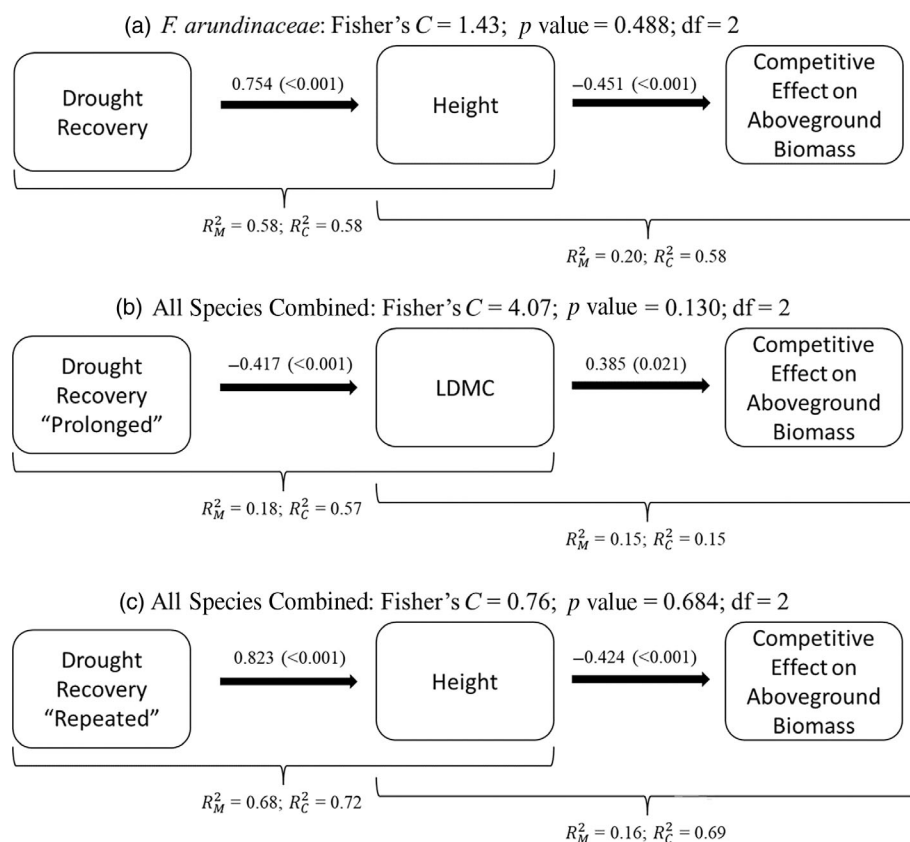


FIGURE 7 Piecewise structural equation model results examining how drought recovery affected key plant traits underlying competitive vigor for *Festuca* across all drought types (a) and across all species in the prolonged drought (b) and repeated drought (c). Overall, goodness-of-fit p -values indicate independence of drought recovery and competitive effects. R^2_M and R^2_C indicate R^2 for component linear mixed models with fixed effects only (R^2_M), and fixed and random effects together (R^2_C). Numbers above arrows indicate the path coefficient and p value (in parentheses). All species were combined for the (b) prolonged and (c) repeated drought treatments. LDMC, leaf dry matter content.

and “across drought type” models. Still, across all drought types (short-term, prolonged, and repeated), *Festuca* exhibited a strong increase in HT upon rewatering (coefficient = 0.754), which resulted in enhanced competition vigor (coefficient = −0.451; Figure 7a). For neither *Phalaris* nor *Lolium* did we find traits which mediated drought-competition responses (Appendix S1: Table S6).

Across all species, we observed a significant decline in LDMC upon rewatering in the prolonged drought (coefficient = −0.417), which aligned with our third hypothesis (Figure 1); however, the decline in LDMC resulted in greater effects of competition on aboveground production (i.e., reduced competitive vigor) (Figure 7b; Appendix S1: Table S7). Under repeated drought, we found rewatering resulted in greater HT growth, with taller individuals exhibiting enhanced competitive vigor (Figure 7c), generally aligning with third hypotheses.

DISCUSSION

We examined how the severity and frequency of drought affected biomass production and allocation of three common pasture species grown independently or in competition. In addition, we examined how drought or recovery from drought affected traits and competitive vigor. Aligning with our first hypothesis, we found that competition reduced aboveground biomass production of all species; however, the severity and frequency of drought affected species-specific biomass responses to competition. Belowground biomass production dynamics in mixtures were like those observed in single species planter bags, indicating minimal effects of competition on belowground biomass, except for the repeated drought treatment where biomass upon rewatering matched belowground biomass production under well-watered conditions. Still, the negative effects of competition on aboveground biomass production were species-specific and depended on the harvest phase (resistance or recovery) and drought treatment (short, prolonged, and repeated). In general, competition intensified upon recovery from drought, aligning with our second hypothesis. Path analysis indicated plant HT and LDMC (not SLA) were key traits that responded to drought phase and play a significant role in competitive interactions between species. Results for HT aligned with our third hypothesis; however, a downward shift in LDMC upon rewatering led to reduced competitive vigor. Overall, shifts in traits in response to drought, and during recovery from drought, are likely to have significant effects on biomass production in multispecies grass-dominated plant communities. In this case, we observed reduced negative effects of competition (i.e., facilitative effect) on biomass production in *Festuca*, but this only occurred under short-term drought. Finally, as nutrient and

soil volume limitations during drought phases were minimized, we can conclude that water and light are the primary resources for which plants competed in this experiment (Figure 1). Likely, interspecific differences in gas-exchange responses to soil water drove competitive effects observed in the resistance phase of drought, while increasing light limitation drove competitive effects in the recovery phases.

Competition is widely recognized as a fundamental driver of plant community composition and productivity (Callaway & Walker, 1997; Newbery & Newman, 1978). However, less is known regarding the nature of competitive interactions under changing environmental conditions, and consequences for ecosystem productivity (Maestre et al., 2009; Ploughe et al., 2019). A field study by Provendier and Balandier (2008) found dry periods resulted in reduced growth of *Fagus sylvatica* tree seedlings when grown in competition with grasses; however, the negative effects of competition on the tree seedlings were alleviated following rainfall events. Another study investigating drought and competition between two grasses (*Bromus erectus* and *Brachypodium pinnatum*) observed that removal of competition had a significant positive effect on productivity for each species, but drought substantially altered those competitive effects (Corcket et al., 2003). Here, we found soil water availability to greatly influence the magnitude of competitive effects on aboveground biomass production. The SGH predicts reduced competition under increased abiotic stress, in which we observed during the onset of short-term drought; however, competition was intensified upon rewatering. The lack of facilitative effects within our experiment is not surprising. Given all three grasses are of similar functional type (C_3 perennials) and possess similar climatic requirements (mesic grassland species), the degree to which they are competing for common resources is likely to result in competitive interactions in most scenarios. Nonetheless, the SGH provides a platform to examine coexistence mechanisms in resource-limited plant communities. We found that the reduction in competition associated with resource limitation (e.g., low soil water) may only be evident under mildly stressful conditions, with prolonged stress increasing competitive interactions in plant communities. So, while some evidence suggests plant–plant interactions under varying water availability may not reflect dynamics of the SGH (Maestre et al., 2009), our results support the application of the SGH to mesic grasses under varying water availability (Butterfield et al., 2016; Holmgren & Scheffer, 2010).

We hypothesized that the negative effects of competition on aboveground biomass would be enhanced with greater severity/duration of drought, although we found this to be generally untrue. Rather, during the short-term and prolonged droughts, the magnitude of competitive effects differed between the onset of drought and the period of drought recovery. Similar to Provendier and

Balandier (2008), we found that rainfall following a dry period alleviated competition in *Festuca* and *Lolium* under prolonged drought. However, this was not the case for *Phalaris*, where rewatering had no significant effect on competition compared to well-watered control plants. Under repeated drought, there was no effect of competition on recovery from drought for *Festuca* and *Phalaris*, yet competition decreased upon rewatering in *Lolium*. These contrasting results highlight the complexity of the differential impacts of drought timing and intensity on species-specific biomass production under competition.

In a review of grassland sustainability under future, drier conditions, Voltaire et al. (2014) concluded that competitive interactions, and underlying traits associated with water availability, should be a priority when investigating the response of grass-dominated communities to future rainfall scenarios. The onset of drought and recovery from drought are likely to select for species with contrasting growth strategies (resistance and avoidance/recovery strategies, respectively), so we hypothesized that traits indicative of resource-use strategy would be important to understand grassland dynamics in the future. Here, we provide evidence that exposure to drought and subsequent recovery upon rewatering altered both plant HT and LDMC, which explained resistance/susceptibility to the effects of competition. Specifically, across all treatments, recovery from drought resulted in increased plant HT, with taller plants experiencing less competition (as hypothesized). The change from water-limited (high light) to water abundant (low light) scenarios and the response of HT suggest that species, which rapidly increase stature following this transition may benefit from less negative effects of competition. Others have observed this size-related dynamics to competitive vigor in invasive species' success (Dickson et al., 2012; Schwinning et al., 2017; Van Kleunen et al., 2010) and shifts in community composition more broadly (Weiner, 1990). Thus, traits such as HT may be crucial for understanding which species will become more abundant following extreme disturbances and droughts, when many species may undergo mortality.

We found that rewatering resulted in decreased LDMC, indicating a shift from conservative to acquisitive strategies, during transition from resistance to recovery phases of drought (Figure 1). Contrary to our hypothesis, more acquisitive values of LDMC were associated with reduced ability to maintain productivity when grown under competition. Under prolonged drought, this was true across all species. This is a particularly interesting result because it was only observed in the prolonged drought, while HT was the primary trait of importance under repeated drought. Hence, we conclude that extreme rainfall scenarios may require shifts in whole-plant carbon allocation strategies (i.e., reflected through LDMC), while milder droughts will likely promote shifts in traits related to associated

transitions in light availability. Specific leaf area has been observed to increase with growing season precipitation (Dwyer et al., 2014). Others have attributed increased SLA to enhanced competitive interactions along an increasing N gradient, which could be associated with light competition (Knops & Reinhart, 2000). However, here SLA had no significant influence on competition quantified in our study. We hypothesized SLA would increase (i.e., reflecting a more acquisitive strategy) upon rewatering (which was true [Appendix S1: Table S3], but positive path coefficient of 0.123 not shown); however, this had no effect on competitive vigor. These results highlight that LDMC and HT were key traits mediating the relationship between drought and competitive interactions. Leaf dry matter content and HT were differentially important under contrasting drought treatments, indicating that the severity and frequency of drought will affect traits and species response to drought, particularly with respect to competition with neighboring species. Finally, we found that LDMC and HT responses were not species-specific under prolonged and repeated drought, respectively. Hence, these traits may be suitable for use in a variety of grassland ecosystems, reinforcing the value of trait-based approaches for comparative studies across ecosystems with differing community compositions (Funk et al., 2017; Lavorel & Garnier, 2002).

Overall, the SGH predicts increased facilitative interactions (i.e., decreased competition) under stress; however, we found positive competitive effects for all species under well-watered conditions. Furthermore, there were several instances where drought resistance or recovery phases resulted in greater competitive effects than well-watered conditions. As noted in the sections above on root responses, there were few instances where rooting space became limiting under drought phases, indicating that competitive effects can largely be attributed to aboveground interactions or differences in water-/resource-use strategies. However, under well-watered conditions mixtures exhibited root production that could have resulted in space limitation. Thus, competitive effects under well-watered conditions should be interpreted with caution.

CONCLUSIONS

In this study, we applied three drought treatments (short, prolonged, and repeated) to three common pasture grasses (*Festuca*, *Phalaris*, and *Lolium*) grown independently and in competition. We found that, generally, competition resulted in negative effects on biomass production of a target species. However, the magnitude of competitive effects was dependent on species, drought severity, and frequency of drought, as well as the phase of drought (i.e., during the dry down or upon rewetting). In addition, we found belowground biomass dynamics

were similar in individuals and mixtures, except in the repeated drought treatment. This highlights a potential research need because changes in community root biomass amounts and species' contributions will likely influence success of grassland communities in accessing soil water under future, drier conditions. For the three species used in this experiment, *Festuca* had a competitive advantage under prolonged drought due to an increase in HT upon rewatering. Hence, *Festuca* may be a potential candidate for pasture use under future rainfall scenarios in order to minimize the negative effects of competition and drought on aboveground production. More generally, we found that plants shifting to a more acquisitive strategy following recovery from prolonged drought were associated with less intense competition. Also, increasing HT following recovery from repeated drought may be a useful strategy to minimize the negative effects of competition with other pasture grasses. In conclusion, these results highlight the importance of the impact of potential future soil water regimes, associated with less frequent rainfall events of greater magnitude, on key traits underpinning competitive effects on biomass production in pastures and grass-dominated ecosystems.

AUTHOR CONTRIBUTIONS

Jeff Chieppa, Sally A. Power, Uffe N. Nielsen, and David T. Tissue conceived the ideas and designed the experiment. Jeff Chieppa collected/analyzed the data and wrote the initial manuscript. Sally A. Power, Uffe N. Nielsen, and David T. Tissue contributed to manuscript development.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Chieppa, 2022) are available from Zenodo: <https://doi.org/10.5281/zenodo.6498042>.

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