RESEARCH PAPER

Different growth responses of C₃ and C₄ grasses to seasonal water and nitrogen regimes and competition in a pot experiment

Shuli Niu¹, Weixing Liu¹,² and Shiqiang Wan¹,*

¹ State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, China
² Graduate University of Chinese Academy of Science, Yuquanlu, Beijing 100049, China

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Abstract

Understanding temporal niche separation between C₃ and C₄ species (e.g. C₃ species flourishing in a cool spring and autumn while C₄ species being more active in a hot summer) is essential for exploring the mechanism for their co-existence. Two parallel pot experiments were conducted, with one focusing on water and the other on nitrogen (N), to examine growth responses to water or nitrogen (N) seasonality and competition of two co-existing species Leymus chinensis (C₃ grass) and Chloris virgata (C₄ grass) in a grassland. The two species were planted in either monoculture (two individuals of one species per pot) or a mixture (two individuals including one L. chinensis and one C. virgata per pot) under three different water or N seasonality regimes, i.e. the average model (AM) with water or N evenly distributed over the growing season, the one-peak model (OPM) with more water or N in the summer than in the spring and autumn, and the two-peak model (TPM) with more water or N in the spring and autumn than in the summer. Seasonal water regimes significantly affected biomass in L. chinensis but not in C. virgata, while N seasonality impacted biomass and relative growth rate of both species over the growing season. L. chinensis accumulated more biomass under the AM and TPM than OPM water or N treatments. Final biomass of C. virgata was less impacted by water and N seasonality than that of L. chinensis. Interspecific competition significantly decreased final biomass in L. chinensis but not in C. virgata, suggesting an asymmetric competition between the two species. The magnitude of interspecific competition varied with water and N seasonality. Changes in productivity and competition balance of L. chinensis and C. virgata under shifting seasonal water and N availabilities suggest a contribution of seasonal variability in precipitation and N to the temporal niche separation between C₃ and C₄ species.

Key words: Chloris virgata, competition, growth, Leymus chinensis, nitrogen seasonality, water seasonality.

Introduction

Plant species in natural communities can utilize one or more limited resources separately from different soil depths and patches or in different seasons, minimizing competitive intensity and facilitating co-existence (Siverstown, 2004). For C₃ and C₄ species, the temporal niche separation, presenting as C₃ species flourishing in the cool spring and autumn while C₄ species being active during the hot summer, is possibly the main mechanism for their coexistence. Evidence for this temporal niche separation has been observed in community production (Redmann, 1975), community isotope (Ode et al., 1980), root growth (Nobel, 1997), photosynthetic activity (Tieszen, 1970; Williams and Markley, 1973; Kemp and Williams, 1980; Monson et al., 1983; Maragni et al., 2000), and normalized difference vegetation index (NDVI) (Tieszen et al., 1997).

Some mechanisms have been proposed to explain the temporal niche separation between C₃ and C₄ species. Different optimum temperature for photosynthesis, with C₃ species having lower temperature optima than C₄
species, has long been suggested as being responsible for the temporal niche separation (Kemp and Williams, 1980; Monson et al., 1983). Recent studies (Paruelo and Lauenroth, 1996; Winslow et al., 2003; Niu et al., 2005; Murphy and Bowman, 2007) show that seasonal water availability may also potentially contribute to the seasonal differentiation between C3 and C4 species, with wet summers promoting C4 expansion but wet winters increasing the abundance of C3 species. In addition, seasonal water regimes can cause seasonal fluctuations of soil N transformation (mineralization, nitrification, and denitrification) and thus N availability (Austin et al., 2004; Jankju-Borzelabad and Griffiths, 2006). With the concurrent seasonal fluctuations in temperature and soil water and N availability under natural conditions, it is difficult to distinguish between the roles of seasonal variability and amount of soil water and N availability as well as temperature in regulating the growth dynamics of C3 and C4 species. Therefore, it is unconvincing to attribute the temporal niche separation between C3 and C4 species solely to seasonal temperature changes. Considering the differences in water and N use efficiency between C3 and C4 species (Peary and Ehleringer, 1984), it is hypothesized that water and N seasonality may differentially affect C3 and C4 species growth and, consequently, contribute to the temporal niche separation between C3 and C4 species.

Competition is one of the important biotic factors regulating plant growth (Aarssen, 1989; Aguiar et al., 2001). Altered resource availability in an ecosystem can shift the competitive balance of plant species (Wedin and Tilman, 1993; Tielborger and Kadmon, 2000; Michalet, 2007). However, irrespective of the well-documented effects of resource availability on interspecific competition, knowledge of the effects of water and N seasonality on the competition between C3 and C4 species is still limited. Due to the differences in water and N use efficiency between C3 and C4 species, changes in temporal availability of soil water and N is expected potentially to alter the competitive outcome, contributing to the temporal niche separation of the two photosynthetic types. Since the competition between these two photosynthetic types could eventually result in altered species composition and a consequent change in carbon sequestration at the ecosystem scale (Still et al., 2003), understanding of how the growth traits of C3 and C4 species respond to competition under fluctuating resource availability will improve our knowledge of their co-existence mechanisms.

This pot study was conducted to examine whether water or N seasonality contribute to the temporal niche separation between two co-occurring C3 and C4 species. *Leymus chinensis* (C3) and *Chloris virgata* (C4) presented in the temperate steppe in northern China were planted in either monoculture or mixture. The two species were treated with the same amount of water or N but with three different seasonal patterns. The specific questions to be answered are: (i) how water and N seasonality and interspecific competition affect the growth of *L. chinensis* and *C. virgata* and (ii) will the interspecific competition between *L. chinensis* and *C. virgata* be shifted by water and N seasonality? In the previous studies of the same experiments, photosynthesis of *L. chinensis* and *C. virgata* were changed by water (Niu et al., 2005), but not N seasonality (Niu et al., 2006). Nevertheless, N seasonality affected N dynamics and use strategies of these two species (Yuan et al., 2007). Therefore, it is hypothesized that water or N seasonality will also impact growth of *L. chinensis* and *C. virgata* and their competition.

### Materials and methods

#### Experimental design

The details of the experiments have been described in Niu et al. (2005, 2006). In brief, the experiments were conducted at the Institute of Botany, Chinese Academy of Sciences (IBCAS), in Beijing, China (39°9′ N, 116°4′ E). The mean annual temperature is 13 °C, with the maximum mean monthly temperature of 27.3 °C in July and a minimum temperature of −3.7 °C in January, respectively. Mean annual precipitation is 507.7 mm. The monthly mean air temperature during the growing season presented as one peak model, with the highest temperature in July (also see Niu et al., 2005). All these meteorological dates were provided by the China Meteorological Administration. *L. chinensis* (C3) and *C. virgata* (C4) that coexist in the temperate grassland in northern China were selected. Plant seeds were collected in the field in autumn 2003. The two species are strict self-pollinated species. Therefore, it is impossible to introduce some intra-specific differences of genes in seeds. Seeds were germinated in March 2004 in shallow plastic trays covered with a 1–2 mm substratum of sand and peat (3:1 v:v) in a greenhouse at IBCAS (Niu et al., 2005, 2006). Seedlings with the fully expanded leaves of the first pair were transplanted to plastic pots (21 cm in diameter and 21 cm in height) with a capacity of 4.5 l and filled with 3 kg of soil on 21 April 2004. *L. chinensis* and *C. virgata* were planted in either monoculture (two individuals of one species per pot) or a mixture (two individuals including one *L. chinensis* and one *C. virgata* per pot).

Two parallel experiments were conducted in this study, with one focusing on water seasonality and the other on nitrogen seasonality. Completely random design was used in both the water and N experiments with two factors of water or N seasonality and species combination. There were three water or N treatments, including a two-peak model (TPM) with more water or N in the spring and autumn than in the summer, a one-peak model (OPM) with more water or N in the summer than in the spring and autumn, and an average model (AM) with water or N evenly distributed over the growing season (Fig. 1). In the water experiment, the total amount of water applied was 500 mm for all the three water treatments. Water was supplied at 5 d intervals from 1 May to 28 October 2004. In the nitrogen experiment, the total amount of N applied was 100 mg N kg⁻¹ soil for each of the three nitrogen treatments. Nitrogen was supplied in the form of NH₄NO₃ at 5 d intervals from 1 May to 28 October 2004.

There were nine treatments (three water or N seasonality treatments and three species combinations) in the water and N experiments. Each of the nine treatments with six replicates for
of the three biomass parameters of *C. virgata* (*P* > 0.05; Table 1). Compared with those under the OPM treatment, the final shoot and total biomass of *L. chinensis* under the TPM and AM regimes were 118–137% higher in monoculture and 76–85% higher in the mixture (*P* < 0.05; Fig. 3). Final root biomass of *L. chinensis* was 134–171% higher (*P* < 0.05) under the TPM and AM than under the OPM water treatment in monoculture, but did not differ among the three water treatments in the mixture (Fig. 3).

The main effects of N seasonality were statistically significant on all the shoot, root, and total biomass results in both *L. chinensis* and *C. virgata* (Table 1). Across the whole growing season, all shoot, root, and total biomass of both species were greater under the TPM and AM than under the OPM treatment (Fig. 2). Compared with those under the OPM regime, the final shoot and total biomass of *L. chinensis* under the TPM and AM treatments were 28–76% and 33–67% higher in monoculture and mixture, respectively (all *P* < 0.05; Figs 2, 3). However, the final root biomass of *L. chinensis* did not differ among the three N treatments. In monoculture, final root and total biomass of *C. virgata* were 27% and 14% higher (*P* < 0.05) under the TPM than under the OPM treatment, respectively (Fig. 3). Sampling date interacted with the water or N treatments to affect plant biomass in both species (Table 1; Fig. 2).

In the water experiment, competition significantly impacted shoot, root, and total biomass of *L. chinensis* (*P* < 0.001; Table 1), with greater biomass in the monoculture than in the mixture (Figs 2, 3). The final shoot, root, and total biomass of *L. chinensis* in monoculture was 56–92%, 73–242%, and 61–134% higher (all *P* < 0.001), respectively, than in the mixture, irrespective of the water treatments (Fig. 3). The magnitude of the competition effect on plant biomass of *L. chinensis* varied with water seasonality (*P* < 0.05; Table 1). Although shoot and total biomass of *C. virgata* was significantly influenced by competition across the whole growing season (Table 1), no difference in the final biomass of *C. virgata* was observed between the monoculture and the mixture regardless of plant parts and water treatments (Fig. 3).

In the nitrogen experiment, the main effect of competition was significant on shoot, root, and total biomass of *L. chinensis* (*P* < 0.001), but not on *C. virgata* (*P* > 0.05; Table 1). The final shoot, root, and total biomass of *L. chinensis* was 169–236%, 265–274%, and 207–248% higher in the monoculture than in the mixture regardless of N regimes (*P* < 0.001). N seasonality significantly influenced biomass responses to competition in both species (*P* < 0.05; Table 1). For example, final biomass of *C. virgata* was significantly higher in monoculture than in the mixture under the AM treatment, but there was no difference in final biomass between monoculture and mixture under the OPM and TPM N regimes. The effects of competition on biomass of both species in the

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**Fig. 1.** Water and nitrogen treatment models during the growing season. Arrows represent the six sampling dates. OPM: one-peak model, TPM: two-peak model, AM: average model.

**Biomass measurements**

One of the 12 blocks in the water or nitrogen experiment was randomly selected for biomass measurement by harvesting at 30 d intervals from 15 May to 16 October. The soil was carefully rinsed until the roots disentangled. Plant material in each pot was rinsed until the roots disentangled. Plant material in each pot was divided into above- and below-ground parts. All plant parts were washed, oven-dried at 65 °C for 48 h, and weighed. The relative growth rate (RGR) was calculated as per cent changes in plant dry weight over a certain time period.

**Statistic analysis**

Repeated Measures ANOVA (SPSS 11.0 for windows, USA) were used to examine water and nitrogen treatment and competition effects on biomass and relative growth rate in *L. chinensis* and *C. virgata* over the growing season. Between-subject effects were evaluated as water or N seasonality and competition and their interactions, and within-subject effects were sampling data and its interactions with treatments. One-way ANOVA (Duncan test) was used to analyse the significant differences in final biomass among water or N treatments and between monoculture and mixture.

**Results**

**Biomass**

Logistic growth functions described well the growth dynamics of both *L. chinensis* and *C. virgata* (*P* < 0.001; Fig. 2). During the growing season, *C. virgata* showed significantly higher biomass than *L. chinensis* (*P* < 0.0001).

Water seasonality significantly affected shoot, root, and total biomass of *L. chinensis* (*P* < 0.001), but not on any of the three biomass parameters of *C. virgata* (*P* > 0.05; Table 1). Compared with those under the OPM treatment, the final shoot and total biomass of *L. chinensis* under the TPM and AM regimes were 118–137% higher in monoculture and 76–85% higher in the mixture (*P* < 0.05; Fig. 3).
Fig. 2. Shoot, root, and total biomass in *L. chinensis* (*L. c*) and *C. virgata* (*C. v*) grown in monoculture (mono) and mixture (mix) under the water (left panels) and nitrogen (right panels) treatments. See Fig. 1 for treatment abbreviations.
water and N experiments were all time-dependent (Table 1; Fig. 2).

**Relative growth rate**

*L. chinensis* and *C. virgata* showed similar seasonal dynamics in relative growth rate (RGR) regardless of water or N seasonality or competition treatments, with higher values at the first measuring date and then gradually declining until the end of the growing season (Fig. 4). RGR of *C. virgata* was significantly higher than that of *L. chinensis* (Fig. 4).

Both water and N seasonality significantly affected RGR of the two species (*P* < 0.05; Table 1). The highest seasonal mean RGR of *L. chinensis* and *C. virgata* was

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### Table 1. The effects of water and nitrogen (N) seasonality (S), competition (C), and sampling date on shoot, root, and total biomass, and relative growth (RGR) in *L. chinensis* and *C. virgata* (Repeate...
observed under the TPM and AM treatments, respectively, in the water experiment. However, both *L. chinensis* and *C. virgata* had the greatest seasonal mean RGR under the OPM N treatment. Interspecific competition profoundly influenced RGR of both species in the N experiment, but not in the water experiment (Table 1). The RGR of *L. chinensis* in monoculture was 35–77% higher than that in the mixture under the three N treatments across the growing season (Fig. 4). N seasonality significantly interacted with competition to affect RGR of *C. virgata* (P < 0.05; Table 1). For example, RGR of *C. virgata* in monoculture was 23% lower under the TPM N treatment, but 99% higher under the OPM N treatment than that in the mixture. The effects of N seasonality and competition were dependent on sampling dates in both species (Table 1).

Discussion

**Effect of seasonal water and N regimes on plant growth**

Consistent with our expectation, changing water seasonality affected the growth of *L. chinensis* and *C. virgata* differently. The TPM and AM water seasonality were favourable while the OPM was unfavourable for *L. chinensis* to accumulate biomass. However, water seasonality did not significantly change biomass of *C. virgata* (Table 1). Our results partly supported the field observations by Paruelo and Lauenroth (1996) that dry winters and wet summers (like the TPM treatment in our study) increase the abundance of C₃ species. Some other studies have also suggested that seasonal water availability may play an important role in regulating the growth of C₃ and C₄ species (Winslow et al., 2003; Winslow and Hunt, 2004; Murphy and Bowman, 2007). The previous conclusions have been drawn from field observations where there are simultaneous changes in the amount and temporal distribution of precipitation. Therefore, it is difficult to distinguish between the roles of availability versus seasonal variability of precipitation or water availability in affecting the growth of C₃ and C₄ species. In our experiment, the same amount of water was kept, but the seasonal distribution was changed, so we could examine the specific effects of seasonal dynamics of water availability.

Our previous studies (Niu et al., 2005, 2006) in the same experiments and other studies have observed greater photosynthesis of C₃ species in the spring and autumn than in the summer. Greater stomatal conductance associated with greater photosynthesis implies greater transpiration. Thus greater soil water availability is needed to meet the water requirement of the C₃ species in the spring and autumn. The TPM water treatment, therefore, provides a favourable water supply for *L. chinensis*, which has been reflected by the changes in leaf water content under the water treatments in the experiment (Niu et al., 2005). Variability of plant water status among the three water treatments has resulted in a higher photosynthetic rate of *L. chinensis* under the TPM relative to the OPM water treatment in the late growing season (Niu et al., 2005). Consequently, the final biomass of *L. chinensis* is
much higher (129%) under the TPM than under the OPM treatment, directly supporting the conclusion that water seasonality influences C3 species growth. Compared with L. chinensis, C. virgata has higher photosynthesis in summer than in spring and autumn (Niu et al., 2005, 2006). Therefore, it is expected that the OPM water treatment would favour biomass accumulation in comparison with the TPM and AM treatments. Contrary to our expectation, no differences in final biomass of C. virgata were detected among the three water treatments (Fig. 3). The results suggest that C. virgata is relatively insensitive to the seasonal water variability, probably due to its higher water use efficiency (2–4 times that of the C3 species L. chinensis). The differential responses of the two species to varying water seasonality may facilitate their coexistence, and partially contribute to their temporal niche separation.

With the predicted increases in seasonal variability of rainfall under global change (Austin et al., 2004), the production and growth of L. chinensis are expected to change, consequently leading to shifts in its competitive ability, relative dominance, and distribution.

Previous results have only addressed the effect of N amount, but provided less evidence for the effect of N seasonality on growth of C3 and C4 species (Sage et al., 1999). This study demonstrates that nitrogen seasonality can impact the biomass production of L. chinensis and C. virgata in different ways. The OPM N treatment was unfavourable for L. chinensis to accumulate biomass (Table 1; Figs 2, 3). Nitrogen use strategies under the altered N seasonality could explain the biomass responses of L. chinensis to the N treatments. The OPM N supplement is opposite to the seasonal dynamics of photosynthesis of L. chinensis (Niu et al., 2006), causing an unfavourable N supply situation to meet the photosynthetic N requirement. The temporal mismatch between N supply and photosynthetic N requirement under the OPM N regime leads to the lowest N productivity, N-use efficiency, and N-resorption efficiency of L. chinensis among the three N treatments (Yuan et al., 2007). As a consequence, L. chinensis accumulated the least biomass under the OPM treatment (Fig. 3). Irrespective of the greatest N productivity, use efficiency, and resorption efficiency of C. virgata under the OPM treatments (Yuan et al., 2007), the final biomass of C. virgata under the OPM N regime is not as high as expected (actually lower in most cases) than under the TPM and AM N regimes (Fig. 3). The inherently higher NUE of C. virgata (Yuan et al., 2007) might have helped to explain its N insensitivity. A growing body of evidence has demonstrated that the relative abilities of coexisting species to capture N are different and depend on the seasonal timing of N pulses (Bilbrough and Caldwell, 1997; Gebauer and Ehleringer, 2000; James and Richards, 2006). Our results provide direct evidence for the different growth responses to N seasonality between the coexisting C3 and C4 species, which can facilitate their coexistence and potentially lead to their niche separation.

It has been reported that in arid and semi-arid ecosystems, nitrogen is limiting and mainly available to plants in brief pulses following precipitation events (Austin et al., 2004; James and Richards, 2006). The synchronous effect of water and nitrogen is therefore expected to affect plant growth. In our study, biomass of the C3 species is lower under the OPM than AM and TPM regimes in both the water and N experiments (Figs 2, 3), suggesting that the temporal synchronicity of water and N resource with photosynthesis is critical for growth and biomass production of C3 species. However, the case is different for C4 species. Water seasonality did not change and the TPM N treatment favoured biomass production of the C4 species relative to the AM and OM N treatments (Figs 2, 3), suggesting that water and N seasonality asynchronously affect C4 species production. The results indicate a differentiation between the coexisting species in their response to the synchronization of water and nitrogen availability, which may facilitate the coexisting species to capture resources at different times. The temporal differentiation in resource use may help to maintain species diversity and to minimize competition for resources in plant communities (Tilman, 1982; McKane et al., 2002).

**Competition effect on growth of the C3 and C4 species**

With the large biomass difference between L. chinensis and C. virgata, asymmetrical competition between these two species is expected. As anticipated, competition significantly decreased the biomass of L. chinensis but not C. virgata, irrespective of the water treatments, suggesting divergent responses of competitive abilities for water resource between the two species. The biomass responses are consistent with their photosynthesis responses to interspecific competition (Niu et al., 2005). Our results disagree with previous studies conducted in the field (Amundson et al., 1994; Winslow et al., 2003) where in years with a moist spring and a dry summer, C3 grasses became more competitive, whereas C4 species become more competitive in years with a dry spring and a wet summer. The biomass of C. virgata was ten times that of L. chinensis (Fig. 2), which greatly over shadowed L. chinensis when they grew together. Therefore, light might be the predominant limiting resources for competition. In order to capture more light, L. chinensis allocates more resources above-ground, which is reflected by the lower root/shoot ratio in the mixture than in the monoculture (Fig. 3). Nitrogen plays an important role in the competitive balance of C3 and C4 species. Experimental N additions in humid temperate grassland have generally favoured C3 grasses and forbs at the expense of C4 grasses (Wedin and
In the N experiment of this study, interspecific competition only negatively affected *L. chinensis* in terms of productivity and RGR (Table 1; Figs 2, 4), suggesting asymmetric competition for N between the two species. Some studies have also found that C₄ species are more competitive for N than C₃ species (Sage and Pearcy, 1987). The above observations could primarily be ascribed to the fact that C₄ species have inherently higher N use efficiency (NUE) than C₃ species (Yuan et al., 2007). High NUE species will capture more available N in a mixture relative to their low NUE counterparts (Wedin and Tilman, 1993), resulting in the competitive advantage of *C. virgata* relative to *L. chinensis*. The asymmetrical competition of the two species could also be attributable to the overshadowing effect of *C. virgata* on *L. chinensis*, which was discussed above. Although the asymmetric competition patterns between the two species did not change, the magnitude of the competition effect on plant biomass was influenced by N seasonality (Table 1; Fig. 3), suggesting the important role of N in plant competition response (Boyer and Zedler, 1999; Sage et al., 1999).

### Temporal niche separation between C₃ and C₄ species

The temporal niche separation between C₃ and C₄ plants originally proposed is based on the observation of contrasting seasonal dynamics of leaf photosynthesis (Kemp and Williams, 1980). Similar seasonal patterns of leaf photosynthesis for the C₃ and C₄ species have also been found in our experiments (Niu et al., 2005, 2006). In addition, substantial variability of final biomass has been observed among the three seasonal water and N treatments. Nevertheless, neither absolute biomass accumulation nor relative growth rates of either of the two species used in our experiments show the same seasonal dynamics with their temporal dynamics of photosynthesis. Biomass accumulation is the results of net carbon (C) balance between uptake (photosynthesis) and release (respiration) at the individual level. Given the dependence of plant respiration on photosynthesis for substrate supply (Krömer, 1995), greater photosynthesis may be offset by the associated greater plant respiration, leading to inconsistent seasonal patterns of net C balance and biomass accumulation with those of plant photosynthesis. However, the inherently logistic increases in biomass and declining relative growth rates of the two species observed in our experiments (Figs 2, 4) do not necessarily counteract with those findings in previous studies at the community and ecosystem levels, i.e. temporal differentiation between C₃ and C₄ plants in terms of production (Redmann, 1975), isotope (Ode et al., 1980), root growth (Nobel, 1997), and normalized difference vegetation index (Tieszen et al., 1997). Natural plant communities consist of many coexisting C₃ and C₄ plants. Differences in phenology between the multiple coexisting C₃ and C₄ plants over the growing season can contribute to the observed phenomena in the field. Therefore, findings in our greenhouse experiments with two species only cannot directly be extrapolated to natural ecosystems without modification. In addition, the temperature was not controlled in this study, which may impact the water or N responses of C₃ and C₄ species. In order to understand the explicit roles of temperature, water, and nutrient in regulating the temporal niche separation between these two functional types, future research needs to be conducted in natural plant communities with multiple C₃ and C₄ species coexisting and concurrent with manipulations of the above three environmental factors.

### Conclusions

In this study, biomass accumulation and growth rate of one C₃ and one C₄ grass species were investigated under altered water or N seasonality but with the same total amount. Seasonal water and N regimes affected productivity and growth rate of *L. chinensis* more than *C. virgata*. The differential responses between *L. chinensis* and *C. virgata* to water or N seasonality suggest resource-based niches between the two coexisting species. The species-specific responses to the synchronization of water and nitrogen effects can facilitate the coexisting species to capture resources across the temporal scale. The seasonal distributions of precipitation and nitrogen could, potentially, contribute to the temporal niche separation between C₃ and C₄ species. Our findings indicate that productivities of the C₃ and C₄ species and their competition magnitude will change under the altered seasonal precipitation and nitrogen regimes associated with global climate change.

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