


RESEARCH ARTICLE

Soil N enrichment mediates carbon allocation through respiration in a dominant grass during drought

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Abstract

1. Carbon (C) allocation strategy plays a critical role in plant adaptability, which are also important to assess the productivity stability under environmental change. Based on optimal partitioning theory, we asked two questions: (1) How is plant C allocation within tissues affected by nutrient enrichment (N addition)? And (2) does long-term N addition alter how plants allocate C under drought?
2. To address these questions, we conducted a greenhouse experiment using the widespread perennial C₃ grass, *Leymus chinensis*, under four treatments: 'Watered', 'Dry', 'Watered + N' and 'Dry + N'. ¹³C₂ pulse labelling was used to trace C transport through the plant–soil system.
3. We found that drought and N addition resulted in additive effects on C allocation. Greater above-ground biomass under N addition resulted in higher C loss via above-ground plant respiration, even under drought, which plays a more important role in the adjustment of root:shoot ratio than does the trade-off between above- and below-ground organs.
4. Compared to the concept of active phenotype adjustment for maximized growth rate in traditional optimal partitioning theory, our results imply that pre-drought allometry, which changes under long-term resource addition, also determines how plants respond to drought and their adaptability to changing environmental conditions.

KEYWORDS

¹³C pulse labelling; drought; *Leymus chinensis*, C allocation strategy, N addition, optimal partitioning model

1 | INTRODUCTION

How plants allocate carbon (C) in response to environmental change remains an unresolved question in plant ecology. The mass fraction of plant root systems is a key trait of interest related to soil

Bo Meng and Junqin Li authors are contributed equally to this work.

[Correction added on 10 April 2022, after first online publication: N corrected to N throughout.]

resource availability, as well as terrestrial C sequestration (Comas et al., 2013; Stockmann et al., 2013). Indeed, 46% of total terrestrial C fixation globally is allocated to below-ground production (Gherardi & Sala, 2020). Thus, how plants allocate resources to above- and below-ground production is essential for understanding how changing resource availability will affect plant adaptability and C turnover, both of which are needed to assess the stability of NPP and long-term ecosystem C balance (Gessler & Grossiord, 2019).

Optimal partitioning theory predicts that plants will adjust how they allocate resources internally (e.g. leaves versus roots) to maximize growth rate in response to variation in limiting resources (McConnaughay & Coleman, 1999; Poorter et al., 2012). Nevertheless, the degree to which plants are flexible in their C allocation strategies remains uncertain for many dominant species. One limitation in understanding the response of C allocation strategies to changing conditions is that the optimal partitioning model assumes variation in only one resource (Gleeson & Good, 2003). Thus, simultaneous increases in one resource and decreases in another make it difficult to clarify how plants may alter their allocation strategies under global environmental change. For instance, although a decrease in root:shoot ratio explained greater drought sensitivity by plants in response to N addition, rapid adjustments in biomass allocation stimulated by drought can simultaneously act as a counterbalance to increase drought tolerance (Meng, Li, et al., 2021).

While traditional approaches to measuring biomass allocation (e.g. allometric analyses) are valuable for understanding carbon allocation strategy in plants (Gedroc et al., 1996; Gleeson & Good, 2003; Noyce et al., 2019), biomass can be a poor proxy for plant C or energy partitioning (Litton et al., 2007). Biomass allocation can underestimate total C partitioning to below-ground processes, especially in plants with relatively low root:shoot ratios, because these plants tend to allocate more C to respiration or rhizodeposition (Kong & Fridley, 2019; Wang, Bicharanloo, et al., 2021). Indeed, up to 80% of fixed C can be lost via respiration (Carbone & Trumbore, 2007; Janssens et al., 2001), but the role that respiratory metabolic loss plays in C allocation remains unclear to date. As an alternative approach, isotopic tracers can provide important insights into plant–soil C processes and C allocation strategies (Brüggemann et al., 2011; Kuzyakov, 2006). Because the ambient amount of ^{13}C supplied to plant organs is low and known, pulse labelling can more

accurately quantify plant C allocation, as well as the trade-offs in energy and resource allocation between different plant organs and metabolic loss.

The Hierarchical Response Framework (Smith et al., 2009) predicted that plant phenotypic changes will characterize the initial response to global change drivers. Yet, the literature on optimal plant allocation strategies in response to multiple global change drivers is mixed. For example, Liebig's 'law of the minimum' states that the resource in least supply relative to requirements will most limit plant growth (von Liebig, 1841). As a consequence, plants should vary in physiology and morphology so as to avoid excess foraging for a non-limiting resource and to maximize effort expended in the acquisition of the most limiting resource (Gleeson & Tilman, 1992). In this regard, it could be hypothesized that severe drought stress can nullify or sharply reduce the effects of N addition on below-ground C allocation (Figure 1a). However, Shelford's 'law of tolerance' (Shelford, 1931) argues that resource limitations act in concert rather than in isolation. Furthermore, the concept of co-limitation suggests that allocation strategies for one resource may be independent of another, or the availability of one resource may impact the uptake of another (Harpole et al., 2011; Saito et al., 2008). In N-poor ecosystems, for instance, N supplies may co-limit plant growth under drought stress because of lower rates of mineralization and nutrient mobility in dry soils (Meisser et al., 2019; Sanaullah et al., 2012). In addition, drought-induced damage of membrane integrity will also reduce the ability of plants to take up N (Gessler et al., 2002, 2017), as reported in a recent meta-analysis (He & Dijkstra, 2014). Thus, based on the theory of multiple resource limitation and trade-offs, an alternative hypothesis would be that N enrichment can offset the effects of drought on C allocation strategies in plants (Figure 1b).

Our objective was to evaluate potential mechanisms underlying the interaction of long-term N addition and drought on plant–soil C

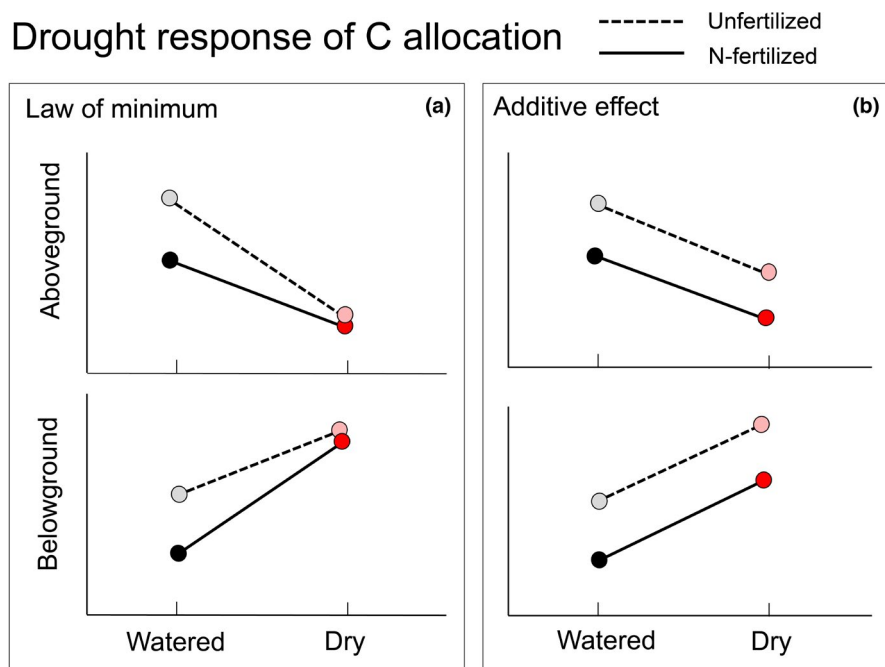


FIGURE 1 Hypothesized interaction between long-term N addition and drought on plant C allocation

allocation to examine whether C allocation strategy was driven by the single most-limiting resource (water) and if that allocation strategy could be additive (or offset), to some extent, by nutrient enrichment (nitrogen). To do so, we experimentally simulated an extended severe within-season drought event as part of a long-term (8-yr) N addition experiment. We pulse-labelled plants growing in intact field soil monoliths in the greenhouse using $^{13}\text{CO}_2$ and then traced the labelled ^{13}C for 31 days to investigate the allocation strategy of recently assimilated C in the plant–soil system.

2 | MATERIALS AND METHODS

2.1 | Study site

The field component of this study was carried out at the Jilin Songnen Grassland Ecosystem National Observation and Research Station, Jilin Province, China (44°34'25", 123°31'6"E). With a semi-arid continental climate, the average growing season (May to September) precipitation of this area is 411 mm over the past five decades. The maximum monthly mean temperature during the growing season is 28°C (July) and minimum is 9°C (May) (data from: <https://www.ncdc.noaa.gov/>). The main soil type of the study area is chernozem with a field capacity of 0.255 g/g and pH ranging from 8.5 to 9.5. Soil nitrogen (0.15%) and organic carbon (2.0%) are relatively low in this system. Vegetation was dominated by the C_3 rhizomatous perennial grass, *Leymus chinensis*, which accounted for more than 85% of above-ground biomass, along with less common perennials (*Phragmites australis* and *Kalimeris integrifolia*) and annuals, such as *Chloris virgata*.

2.2 | Experimental design and treatments

The long-term N addition treatment (Wang et al., 2018) was carried out in a fenced area with the saturating rate of N addition for stimulating ecosystem functions (10 g N/m² year⁻¹) using urea (Bai et al., 2010). Starting in 2011, urea was applied twice per year (5 g N/m² per application) in May and July annually. This long-term experiment and field measurements were permitted by the local government.

In April 2018, we moved 240 intact soil cylinders (monoliths) with diameter of 11 cm and depth of 30 cm from the field to an open-air greenhouse, in which temperature and relative humidity were similar to conditions outside the structure. Monoliths were carefully placed in plastic pots (depth of 32.5 cm) to preserve natural soil structure and vegetation status. All monoliths were randomly selected from relatively uniform vegetation with more than 95% cover of *L. chinensis*. Half of the monoliths were selected from treatment plots that had received N addition since 2011, and the other half were from control (unfertilized) plots. During the first 30 days of the experiment, all pots were weighed and watered every 3 days to maintain soil moisture at 60%–75% field capacity (0.15–0.19 g/g).

After the 30-day acclimation period, 200 pots were selected for the experimental treatments. In this stage (35 days), pots were randomly divided to five blocks (40 pots per block), and randomly assigned to Watered, Dry, Watered + N and Dry + N treatments (Figure S1). Soil moisture in the Watered treatment was kept at 60%–75% field capacity determined via weighting every 3 days. In the 'Dry' treatments, soil moisture was reduced to below 35% of field capacity within 5 days after the initiation of the drought treatment and maintained at 20%–35% of field capacity (0.05–0.09 g/g) throughout the drought period. This treatment created severe drought stress on the physiological functioning of *L. chinensis*, inferred by changes in C-fixation and carboxylation velocity (Xu & Zhou, 2011). Fertilized(+N) pots received 0.2 g N/m² during each watering event (the equivalent of 10 g N/m² year⁻¹).

2.3 | Pulse labelling procedure

The $^{13}\text{CO}_2$ pulse labelling experiments were immediately carried out after 35 days of drought treatment. Four pots from each of the treatments were randomly selected to measure natural background $\delta^{13}\text{C}$ of each C pool from each block. The rest of the 36 pots in each block were moved out of the greenhouse and each block was equipped with a polymethyl methacrylate chamber (0.9 m × 0.6 m × 0.7 m, 95% light permeability) before labelling. To stabilize the transparent chamber and reduce leakage of $^{13}\text{CO}_2$, an iron groove with a seal ring was fixed to the soil in advance of pulse labelling. In addition to ice packs and four symmetrical electric fans, temperature stabilization and air circulation in the chambers were achieved by an external air circulation system composed of a pump and pressure-tight cool box, which held air temperature < 38°C during the 90 min labelling period. CO_2 concentration and interior air temperature were monitored by an infrared gas analyzer (LI-6400, LiCor Inc., Lincoln, NE, USA) and thermocouple thermometer. During pulse labelling, $^{13}\text{CO}_2$ (>99.9% CO_2 with 99 atom% ^{13}C , Cambridge Isotope Laboratories, Andover, MA, USA) was repeatedly added over a period of 90 min to maintain CO_2 concentrations between 330 and 480 ppm. All pulse labelling was conducted on two consecutive sunny days between 8:30 a.m. and 10:30 a.m. To determine the physical $^{13}\text{CO}_2$ back-diffusion from the soil surface, we pulse labelled four extra pots together with the first block from which plants were removed.

2.4 | Sample collection

The incorporated ^{13}C in the leaves, stems, roots, soil and respiration were measured on samples taken immediately (0h), as well as 6 hr, 1 day, 2 days, 4 days, 7 days, 14 days, 21 days and 31 days after the end of the pulse labelling period. For the density of *L. chinensis*, the number of tillers was counted during each sampling period, and all above-ground plant parts in pots were harvested by clipping and divided into stems and leaves. Above-ground biomass (AGB) was calculated as the sum of the dry weight of stems and leaves.

Whole live roots were carefully washed out from the soil to measure below-ground biomass (BGB). All plant samples were immediately subjected to 105°C in a drying oven within a half hour of harvesting to stop metabolic activity, then kept at 70°C to a constant weight (approximately 48 hr). The root:shoot (R/S) ratio was calculated as BGB/AGB.

Before washing roots, a 60 g soil sample was collected from each pot to measure the $\delta^{13}\text{C}$ of soil total carbon (TC) and dissolved organic carbon (DOC). To avoid the effects of uneven vertical root distribution on measurement of the soil ^{13}C pool, the soil cylinder was divided into two parts (0–15 cm and 15–30 cm). Roots and other organic debris were removed using a 2-mm mesh stainless steel sieve. Half of the soil samples were oven-dried together with plant samples, and the other half were transported back to the laboratory in a cooler and stored in a freezer for DOC measurements within 5 days. DOC was measured in K_2SO_4 extracts (12.5 g of lyophilized soil was extracted with 50 ml of 0.5 M K_2SO_4) using a total organic carbon (TOC) analyzer (vario TOC, Elementar, Langensfeld, Germany). The lyophilized extracts were used to determine the $\delta^{13}\text{C}$ of DOC (Marhan et al., 2010). Carbon isotope composition and content of carbon compounds were determined by an elemental analyzer (vario EL cube, Elementar, Langensfeld, Germany) coupled to a mass spectrometer (Isoprime 100, Elementar, UK). The carbon isotope ratios are reported in parts per thousand relatives to Vienna Pee Dee Belemnite (VPDB) as:

$$\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000.$$

2.5 | Respiration measurements

Above-ground plant respiration and soil respiration were measured in parallel with sample collection. All clipped above-ground tissues in each pot were placed in an opaque CO_2 flux chamber (6400-9, LI-COR Inc., Lincoln, NE, USA) connected to an infrared gas analyzer (LI-6400, LiCor Inc., Lincoln, NE, USA) to measure above-ground plant respiration rates. Next, above-ground plant samples from each pot were immediately moved to another set of chambers where interior CO_2 had been removed by calcium hydroxide, and kept in the dark for 30 min. A 200 ml gas sample was collected from these chambers to determine the $\delta^{13}\text{C}$ of leaf respiration. In this case, we assumed that the detached shoots maintained physiological activity for a short period of time (Stutz et al., 2017). However, the measured ^{13}C loss rate via respiration was not used in the calculation of ^{13}C allocation, because that measurement in darkness may have overestimated respiration rate (Villar et al., 1995).

After clipping shoots, soil respiration rates were immediately determined using a portable CO_2 infrared gas analyzer (LI-6400, LiCor Inc.) with a soil CO_2 flux chamber (6400-9, LI-COR Inc.). After the measurement of soil respiration rate, the pots were covered by respiration chambers for 30 min, then a 200 ml gas sample was collected from each chamber to measure the $\delta^{13}\text{C}$ of soil respiration. For each measurement, a 200 ml air sample was collected to determine the background $\delta^{13}\text{C}$ of air. The $\delta^{13}\text{C}$ of soil respiration was

determined with a two end-member mixing model as follows (Werth & Kuzyakov, 2008):

$$\delta^{13}\text{C}_{\text{SR}} = \frac{\delta^{13}\text{C}_{\text{out}} \times [\text{C}_{\text{out}}] - \delta^{13}\text{C}_{\text{in}} \times [\text{C}_{\text{in}}]}{[\text{C}_{\text{out}}] - [\text{C}_{\text{in}}]}.$$

where $\delta^{13}\text{C}_{\text{SR}}$ is the $\delta^{13}\text{C}$ in soil respiration; $\delta^{13}\text{C}_{\text{out}}$ and $\delta^{13}\text{C}_{\text{in}}$ are $\delta^{13}\text{C}$ measured in the outlet (background $\delta^{13}\text{C}$ of air) and inlet of the respiration chamber, respectively; and $[\text{C}_{\text{out}}]$ and $[\text{C}_{\text{in}}]$ are the CO_2 concentrations of the same samples.

2.6 | Data analyses

The excess ^{13}C (Ex ^{13}C) atom% in a pool or flux at a certain time was calculated as the difference between the ^{13}C atom% of the respective pool or flux after labelling and its natural abundance measured before labelling.

$$\text{Ex } ^{13}\text{C atom\%}_{\text{sample}} = \left[\frac{100\%}{1 + \frac{1}{\left(\frac{\delta^{13}\text{C}_{\text{sample}}}{1000\%} + 1\right) R_{\text{PDB}}}} \right] - ^{13}\text{C atom\%}_{\text{natural}}.$$

where $R_{\text{PDB}} = 0.011,237$ and $^{13}\text{C}_{\text{natural}}$ atom% is the percentage of ^{13}C of total C in unlabelled pots. To estimate the percentage of total ^{13}C allocated to different pools and how much ^{13}C was lost, we calculated the proportions of newly fixed ^{13}C based on the following equation:

$$\text{Proportions} (\%) = \left(\frac{\text{Ex } ^{13}\text{C atom\%} \times W_{\text{sample}} \times [\text{C}_{\text{sample}}]}{\text{amount } \text{C}_{\text{fixed}}} \right) \times 100.$$

$$\text{amount } \text{C}_{\text{fixed}} = \sum_i \text{Ex } ^{13}\text{C atom\%}_i \times \% \text{C}_i \times W_i.$$

where W_{sample} is the dry weight of a certain pool; $[\text{C}_{\text{sample}}]$ is the C concentration of the sample; amount of ^{13}C fixed is the sum of ^{13}C in all measured compartments at 0h, including leaf, stem, root and DOC; %C is the elemental abundance of C in each compartment (i). Because a non-significant increase in $\delta^{13}\text{C}$ of soil TC pool after labelling (Figure S5a,c), the ^{13}C in DOC was used in place of TC in these calculations. Proportions of ^{13}C losses (via respiration mainly) were calculated by subtracting the sum of measured C pools from 100%.

To estimate mean residence times (MRT) of the ^{13}C excess in each compartment, we fitted the following exponential decay function:

$$N_{(t)} = N_0 e^{(-\lambda t)}.$$

where t is the time in days after the peak; N_0 is the initial amount of Ex ^{13}C atom% at peak; λ is the decay constant and $N_{(t)}$ is the amount of ^{13}C after time t . The MRT was calculated as the reciprocal of λ .

Effects of N addition and drought treatment on above-ground biomass (AGB), below-ground biomass (BGB), root:shoot ratio,

respiration rate, $\delta^{13}\text{C}$ value and the percentage of ^{13}C in different pools after labelling were assessed using a repeated measures model with drought and N addition treatment as the fixed effects and sampling timepoint embedded in block as a repeated effect. Given that the physical $^{13}\text{CO}_2$ back-diffusion was depleted within 24 hr (Figure S2), we excluded measurements of $\delta^{13}\text{C}$ of respiration rate at 0 and 6 hr from the repeated measures model. Additionally, we performed the two-way ANOVA without a time effect to analyse the effects of drought and N addition and their interaction on MRT in each C pool. All analyses were conducted using 'nlme' package in R (Pinheiro et al., 2021). Data are presented as means \pm 1 SE ($n = 5$); level of significance: $p < 0.05$.

3 | RESULTS

3.1 | Vegetation and biomass

Average tiller numbers across all sampling periods for each pot was 14.6 ± 0.5 in the Watered treatment, 11.4 ± 0.4 in the Dry treatment, 23.8 ± 1.0 in the Watered + N treatment and 15.3 ± 0.6 in the Dry + N treatment. Additionally, long-term N addition significantly increased AGB by 186% ($F_{1,132} = 334$, $p < 0.01$), and BGB by 73% ($F_{1,132} = 145$, $p < 0.01$, Figure 2). We observed the greatest drought-induced decrease in biomass in the N addition treatment, as well as a significant interaction between N addition and drought on biomass (Figure 2). For instance, drought significantly reduced AGB by 41% in the unfertilized treatment, and by 52% in the +N treatment ($F_{1,132} = 154$, $p < 0.01$). Drought significantly increased the R/S ratio

by 65% ($F_{1,132} = 102$, $p < 0.01$), whereas N addition reduced the R/S ratio by 47% ($F_{1,132} = 86.9$, $p < 0.01$), also with a significant interaction ($F_{1,132} = 4.54$, $p = 0.03$).

3.2 | Temporal changes of ^{13}C value

Our pulse labelling treatment effectively increased the $\delta^{13}\text{C}$ values of leaves to $1036 \pm 92\text{‰}$ in the Watered treatment, $807 \pm 54\text{‰}$ in the Dry treatment, $1110 \pm 114\text{‰}$ in the Watered + N treatment and $670 \pm 49\text{‰}$ Dry + N treatment at the end of labelling period (Figure S3). Overall, N addition tripled the total accumulation of ^{13}C ($F_{1,176} = 208$, $p < 0.01$, Figure S4), whereas the drought obviously reduced ^{13}C peak accumulation in leaves, stems and roots, and soil DOC (Figures S3 and S5). There was a significant interaction between the drought and nitrogen treatments on the $\delta^{13}\text{C}$ values of leaves ($F_{1,132} = 4.63$, $p = 0.03$) and stems ($F_{1,132} = 14.6$, $p < 0.01$), but not root tissues ($F_{1,132} = 0.33$, $p = 0.57$, Figure S3). In all treatments, the tracer content ($\delta^{13}\text{C}$) in roots reached a peak within 2 days after the labelling pulse.

3.3 | ^{13}C allocation and dynamics in the plant-soil system

We estimated the allocation and turnover rate of ^{13}C in each C pool 31 days after the labelling. The proportion of newly fixed ^{13}C allocated to leaves decreased sharply to about 10% within the first 2 days after labelling in all treatments, but no significant differences were detected among the treatments (Figure 3). Nitrogen addition

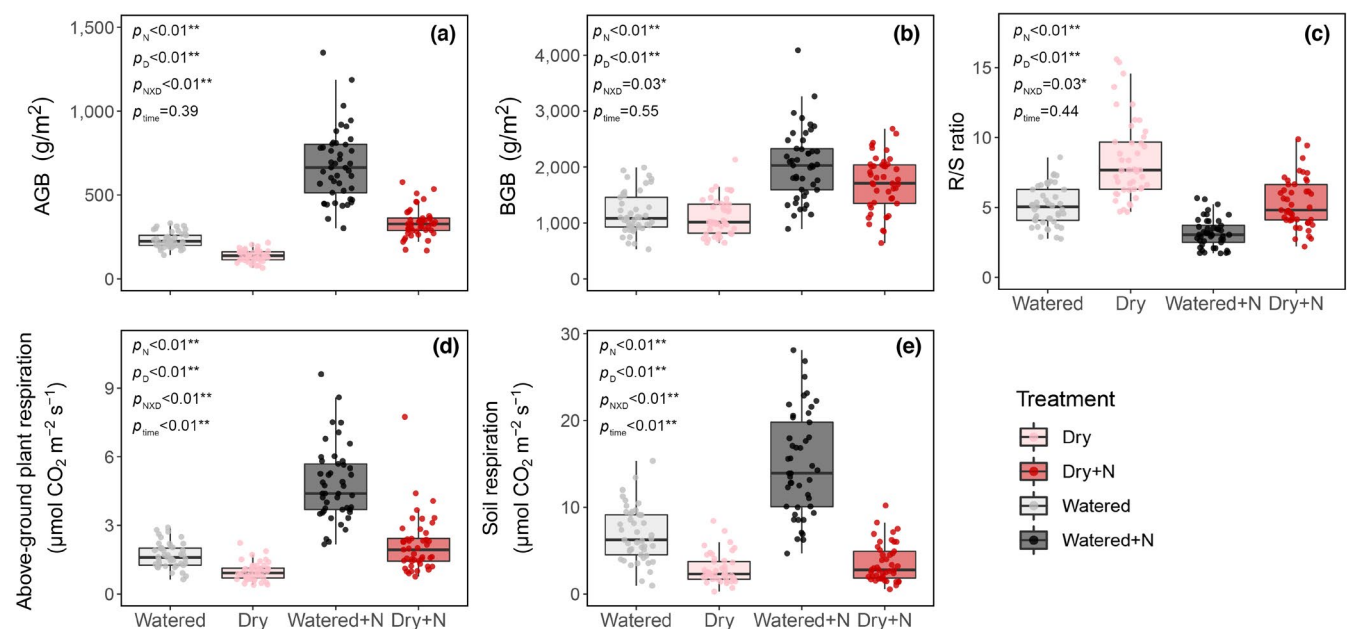


FIGURE 2 Boxplots of drought (D) and nitrogen (N) addition effects on above-ground biomass (AGB, a), below-ground biomass (BGB, b), root:shoot (R/S) ratio (c), above-ground plant respiration rate (d) and soil respiration rate (e). Median and first and third quartile are shown. Data points include all plots in each treatment ($n = 45$). p values for repeated-measures ANOVA are provided. Asterisks indicate significant treatment effect. **: $p < 0.01$; *: $p < 0.05$

significantly increased C allocation to stems ($F_{1,132} = 26.7, p < 0.01$). A significantly larger fraction of newly fixed ^{13}C was incorporated into roots in the Dry treatment ($F_{1,132} = 51.5, p < 0.01$). In contrast, N addition significantly reduced C allocation to roots ($F_{1,132} = 36.6, p < 0.01$). Similar patterns were also found in soil DOC. However, no significant interactive effects were observed between drought and N addition on ^{13}C allocation (Figure 3), which resulted in additive responses on the ^{13}C allocation to each pool. For example, in day-2 and day-7 sample periods, drought increased ^{13}C allocation to roots by 13% and 10% but N addition reduced ^{13}C allocation to roots by 10% and 9.4% (Figure 4). Moreover, drought significantly increased mean residence time by 23% in stem, 40% in root and 48% in the shallow soil DOC pool. In contrast, N addition tended to reduce mean residence time in each C pool, but the effects were not statistically significant, and no significant interaction between drought and N addition was found (Table 1).

3.4 | Respiration loss

Drought significantly decreased above-ground plant respiration by 41% ($F_{1,132} = 141, p < 0.01$) and soil respiration by 58% ($F_{1,132} = 260, p < 0.01$), whereas N addition increased above-ground plant respiration by 188% ($F_{1,132} = 230, p < 0.01$) and soil respiration by 117% ($F_{1,132} = 83.3, p < 0.01$, Figure 2). Between 60% and 80% of newly fixed ^{13}C was eventually consumed by respiration or other biological processes over the monitoring period. Drought significantly reduced the proportion of ^{13}C loss ($F_{1,132} = 89.4, p < 0.01$), yet N addition tended to enhance ^{13}C loss in general ($F_{1,132} = 8.31, p < 0.01$, Figure 3d). Results further showed that N addition significantly increased ^{13}C loss rate via above-ground plant respiration under drought stress compared to drought alone (Figure 5).

4 | DISCUSSION

The technique of isotopic labelling allowed us to determine how a dominant and widespread grassland species, *Leymus chinensis*, allocated carbon in response to changes in soil water and nitrogen availability, two resources that are likely to change simultaneously under global environmental change. By tracing the pulse labelling-derived ^{13}C in above- and below-ground plant organs and respired CO_2 , our results demonstrate that long-term N addition mediated plant C allocation in response to drought.

4.1 | C allocation strategy in response to drought

Decreased total C assimilation and increased allocation of carbon to roots under drought stress as predicted by optimal partitioning theory have been previously observed for a number of species resulting in a reduction in above-ground growth (Karlowsky et al., 2018; Poorter et al., 2012). However, our data indicated that

the proportional increase in root allocation did not occur at the expense of above-ground C allocation. In our case, drought significantly increased ^{13}C allocation to roots, while the proportion of ^{13}C allocated above-ground did not decrease, likely because of lower C loss via plant respiration (Figure 3d). This result implies that downregulation in metabolic loss plays a more important role in the adjustment of R/S ratio than does the trade-off between above- and below-ground compartments. As the allocation of ^{13}C to roots gradually increased over time, the proportional allocation to soil DOC also significantly increased in the drought treatment in both shallow and deep soil layers (Figure S5). Nevertheless, the $\delta^{13}\text{C}$ value of soil DOC remained significantly lower than in the Watered treatment (Figure S5), which implies that drought reduced new carbon accumulation in the soil carbon pool. Moreover, consistent with previous studies (Brüggemann et al., 2011; Fuchslueger et al., 2014; Gavrícková et al., 2018), we found that drought slowed the rate of below-ground C turnover (Table 1).

4.2 | N addition effects on the dynamics of ^{13}C partitioning

Long-term N addition tripled the amount of ^{13}C assimilated relative to the Watered treatment after pulse labelling (Figure S4). Higher available soil N can theoretically increase leaf N concentration and chlorophyll content, which likely increases leaf photosynthetic capacity allowing the plant to accumulate more biomass (Reich et al., 1995). As expected, N addition significantly decreased proportional allocation of recently assimilated C to roots because more newly fixed C was allocated to stems and plant respiration (Figure 3b and d). This is consistent with results from a global synthesis of C processes in trees (Li et al., 2019). Not only did leaf respiration increase, but we also found that ^{13}C loss rate via soil respiration doubled compared to the Watered treatment (Figure 5) either directly through the stimulation of microbial activity or indirectly via more below-ground C input (Meng, Ochoa-Hueso, et al., 2021). Compared to drought effects, N addition tended to accelerate below-ground C turnover due to more soil microbial activity in this N-poor ecosystem (Chen et al., 2017; Grandy et al., 2013).

While the impacts of nutrient addition on plant C allocation strategies shown here are consistent with classic optimal partitioning theory and previous empirical studies (e.g. Li et al., 2019; Poorter et al., 2012; Wang, Cavagnaro, et al., 2021), some inconsistent results in field experiments have been reported. For instance, Wang et al. (2019) conducted a ^{13}C pulse labelling experiment in a semi-arid temperate grassland, and found that more rather than less C was allocated to roots with experimental N addition. The inconsistent results in C allocation below-ground between these two studies may have resulted from either direct water limitation or the 'luxury consumption' of soil nutrients (Van Wijk et al., 2003) in the field experiment. Thus, as the major limiting factor in semi-arid and arid ecosystems, water availability will likely regulate how plants respond to other global change drivers.

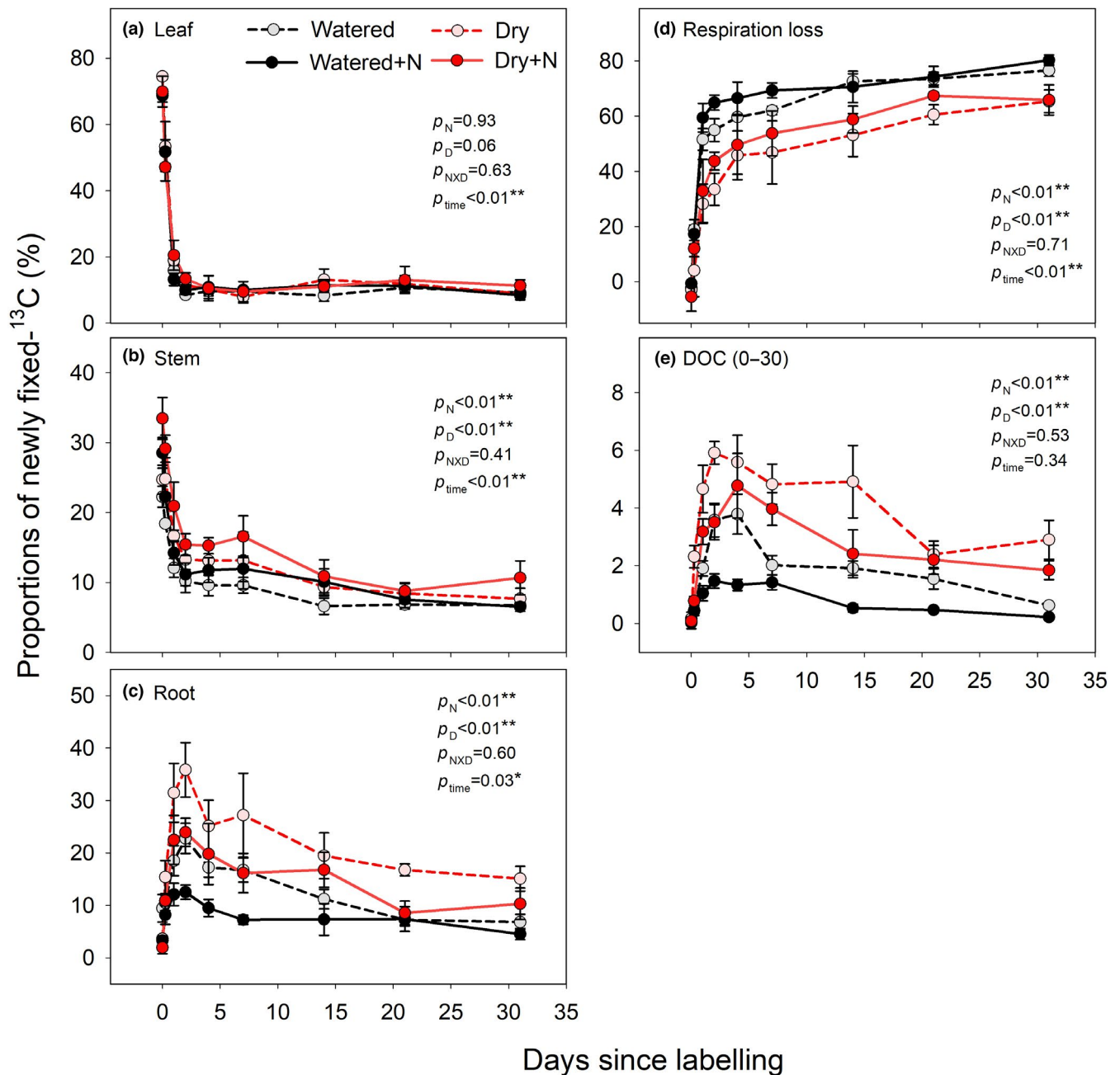


FIGURE 3 Temporal changes in the proportions of total newly fixed ^{13}C allocated to leaf (a), stem (b), root (c) tissues, respiration losses (d) and soil dissolved organic carbon (DOC) (e) after the pulse labelling. Respiration losses were calculated by subtracting the sum of measured C pools from 100%. Data are reported as mean \pm 1 SE ($n = 5$). p values for repeated-measures ANOVA are provided. Asterisks indicate significant treatment effects. **: $p < 0.01$; *: $p < 0.05$

4.3 | Interactive effects of drought and N addition on C allocation strategy

In line with Wang, Cavagnaro, et al. (2021) who suggested that N addition could reverse drought effects on below-ground C allocation, our results further showed that drought and N addition exhibited additive effects on C allocation at two key time points (Days 2 and 7; Figure 4), which resulted in no significant interaction between drought and N addition on the proportional allocation of newly fixed ^{13}C (Figure 3). This is also consistent with prior research showing that

drought does not interact with fertilization to affect biomass allocation or partitioning patterns of tree and shrub seedlings (Kleczewski et al., 2010; Wu et al., 2008). Generally, these results support our alternative hypothesis that enriched resources will regulate plant C allocation strategy even if growth is most limited by other resources.

The regulating effect of enriched N in the drought treatment could be driven by multiple mechanisms. First, our pulse labelling experiment showed that N addition led to more ^{13}C allocation to stems even under drought (Figure 3b), which resulted from light competition as a consequence of greater above-ground biomass and tiller

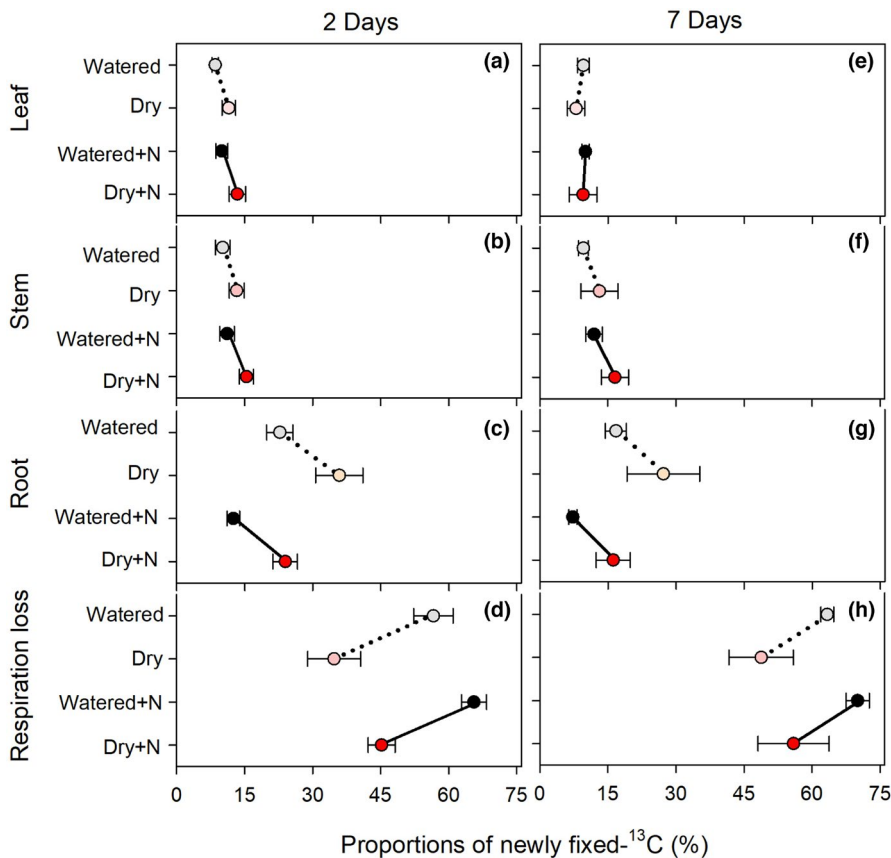


FIGURE 4 Proportion of newly fixed ^{13}C allocated to leaf (a, e), stem (b, f), root (c, g) tissues and respiration losses (d, h) 2 and 7 days after the pulse labelling. Dashed lines represent the unfertilized treatment, and solid lines represent the N-fertilized treatment. Red circles represent the drought treatment. Data are reported as mean \pm 1 SE ($n = 5$)

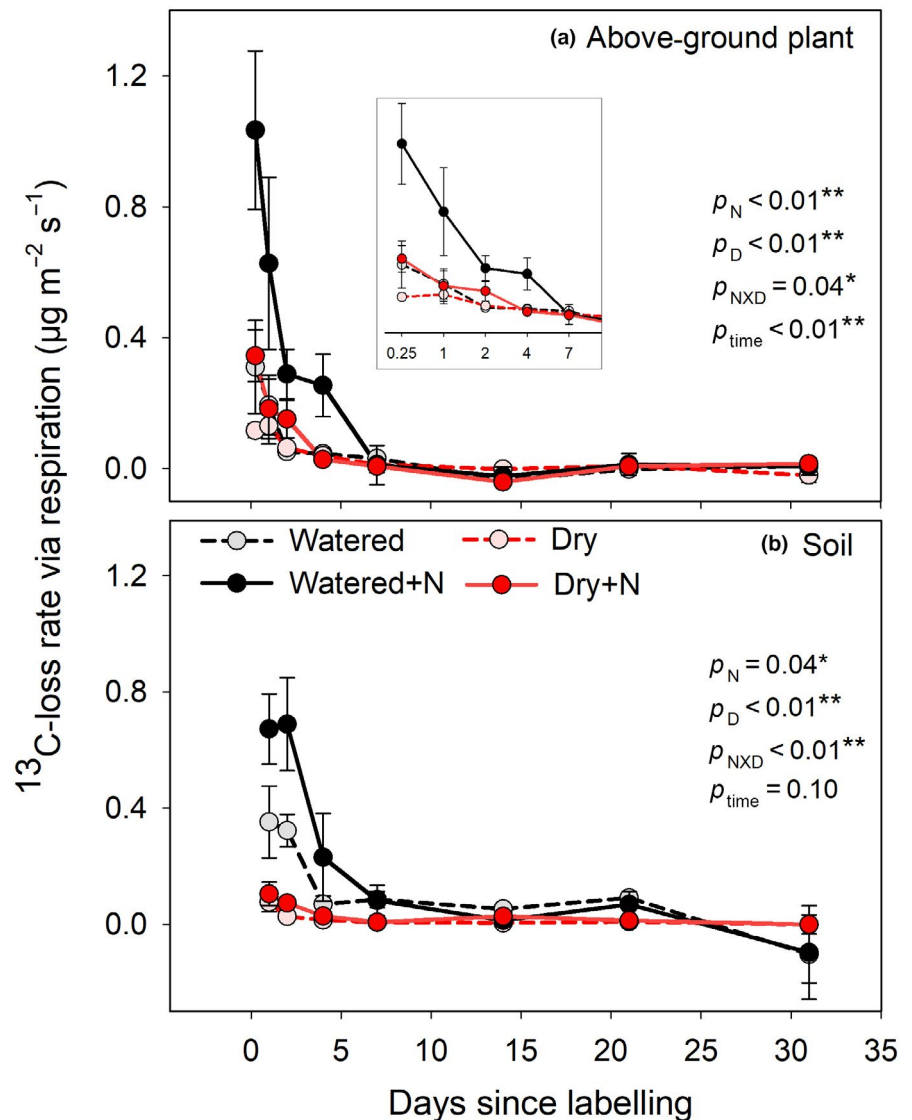
	Leaf	Stem	Root	DOC (0–15 cm)	DOC (15–30cm)
Peak	0 h	0 h	2 day	2 day	4 day
MRT (days)					
Watered	0.78 \pm 0.1	9.81 \pm 1.9	18.9 \pm 2.1	14.7 \pm 2.4	30.6 \pm 4.7
Dry	0.93 \pm 0.1	12.1 \pm 3.8	26.4 \pm 1.9	21.7 \pm 1.4	34.5 \pm 6.2
Watered + N	0.79 \pm 0.1	5.34 \pm 2.8	18.6 \pm 2.4	12.3 \pm 1.9	20.3 \pm 5.9
Dry + N	1.1 \pm 0.2	15.1 \pm 3.5	23.8 \pm 1.0	18.8 \pm 3.5	39.0 \pm 7.3
F-value					
N	$F_{1,4} = 2.01$	$F_{1,4} = 0.44$	$F_{1,4} = 2.59$	$F_{1,4} = 0.72$	$F_{1,4} = 0.72$
D	$F_{1,4} = 3.70$	$F_{1,4} = 12.2^*$	$F_{1,4} = 17.0^*$	$F_{1,4} = 12.9^*$	$F_{1,4} = 6.05$
N \times D	$F_{1,4} = 0.28$	$F_{1,4} = 6.64$	$F_{1,4} = 0.01$	$F_{1,4} = 0.25$	$F_{1,4} = 2.37$

TABLE 1 The effect of drought (D) and nitrogen (N) treatments on mean residence time (MRT, days) and the peak of ^{13}C content in leaf, stem, root and soil dissolved organic carbon (DOC) at two depths. Data are reported as mean \pm 1 SE ($n = 5$). F values for two-way ANOVA are provided. Asterisks and bold values indicate significant treatment effect. $**p < 0.01$; $*p < 0.05$

density (Poorter et al., 2012). But more importantly, an obviously greater proportion of recently fixed ^{13}C was lost via respiration in the Dry + N treatment (Figure 3d). Our results implied that pre-drought allometric growth patterns (the absolute and relative biomass of shoots) in response to long-term N addition could play an important role in how plants allocate C under drought stress. Specifically, we found greater ^{13}C loss from above-ground respiration compared to soil respiration (Figure 5) under drought stress due to disproportionate above-ground biomass accumulation under long-term N addition (Figure 2). This hypothesis is consistent with the idea that the highest priority for C allocation under stress is for maintenance respiration

rather than C accumulation to ensure that the plant can maintain basic metabolic processes (Hartmann et al., 2013). This response also illustrates why respiration generally shows much lower sensitivity to soil drying than photosynthesis (Clair et al., 2009; Maseyk et al., 2008). Although N addition increased soil respiration by 24%, the difference in the ^{13}C loss rate via soil respiration was not significant between the drought and the drought plus nitrogen treatments (Figure 5b). This likely occurred because of lower below-ground biomass allocation in the +N treatment and the leading role of soil water in below-ground metabolic processes (Liu et al., 2009). Similar drought-induced changes in below-ground C dynamics were also

FIGURE 5 Temporal changes in ^{13}C loss rate via leaf respiration (a) and soil respiration (b) after the pulse labelling. Data are reported as mean \pm 1 SE ($n = 5$). P values for repeated-measures ANOVA are provided. Asterisks indicate significant treatment effects. ** $p < 0.01$; * $p < 0.05$



reported from experiments in other grasslands (Ingrisch et al., 2020; Meeran et al., 2021; Slette et al., 2021). This C allocation strategy reflects the preferential allocation of sucrose for storage in roots and serves as a mechanism for osmotic adjustment under drought stress regardless of enriched resource (N). Moreover, the $\delta^{13}\text{C}$ value of soil total C did not change during this one-time pulse labelling experiment (Figure S5), which is not surprising given the very large soil ^{12}C pool. Thus, we cannot infer how the interaction of N and water availability affects the soil TC pool via the addition of recently fixed C.

4.4 | Implications

It is increasingly important to consider the potential interactive effects of multiple global change factors to predict optimal partitioning strategies that allow plants to adapt to precipitation variability. If co-limited multiple resources are taken up simultaneously under the same allocation strategy, such as soil water and nutrients, one may predict synergistic effects on plant resource allocation

strategies. Our results, on the other hand, provide evidence for contrasting effects of different resources on allocation strategy, in that the response to the main limiting resource (water) could be partly counteracted by enrichment in the availability of other essential resources (nitrogen). In our case, *L. chinensis* did not quickly adjust its adaptive response to reduce C loss as predicted by theory after the R/S ratio was changed by long-term N addition, which increased above-ground productivity under ambient soil moisture conditions. Lack of adjustment could explain the destabilizing effects of long-term resource enrichment on productivity, which is independent of species variability (Hautier et al., 2020). Contrasting resource effects on carbon allocation within plants have received limited attention to date. Thus, many questions remain regarding how plants will respond to interactions among various press and pulse disturbances. Clearly, more studies are needed to determine whether the additive or offsetting responses that we observed in a widespread dominant grass will apply to changes in other drivers, such as warming and elevated CO_2 , as well as in different ecosystems and plant functional types.

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CONFLICT OF INTERESTS

The authors have no relevant financial or non-financial conflict of interest to disclose.

AUTHORS' CONTRIBUTIONS

B.M. and W.S. designed the experiment; B.M., J.L., Y.Y., H.C. performed the field and laboratory work; B.M. and J.L. analysed the data; B.M., S.L.C. and W.S. wrote the manuscript; D.H.W. and J.B.N. provided valuable comments and suggestions on manuscript.

DATA AVAILABILITY STATEMENT

The data used for this study were submitted to the Dryad Digital Repository <https://doi.org/10.5061/dryad.x95x69pjj>. (Meng, 2021). [Correction added on 10 April 2022, after first online publication: Citation and Reference for dataset added.]

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