Effects of drought and nutrient deficit on the allocation of recently fixed carbon in plant-soil-microbe system

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Research Article

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Abstract

Background and Aims

Carbon (C) allocation plays important role in plant adaptation to water and nutrient stress. However, the effects of drought and nutrient deficit on the allocation of recently fixed C in plant-soil-microbe system remain largely unknown.

Methods

We studied the response of C allocation of *Sophora moorcroftiana* (an indigenous pioneer plant in Tibet) in plant-soil-microbe system to drought, nitrogen (N) and phosphorus (P) deficit using a microcosm experiment. The $^{13}CO_2$ continuous labeling was used to trace C transport through the plant-soil-microbe system.

Results

Drought significantly reduced C allocation to stem and root but increased allocation to soil. Deficit of N and P significantly increased C allocation to root under well-watered conditions, while P deficit significantly increased allocation to stem but decreased allocation to leaf under drought conditions. Carbon allocation to microbes was mainly affected by nutrient deficit, and ^{13}C amounts in microbial biomass was decreased by N deficit and increased by P deficit. Stem ^{13}C amount was positively related to net photosynthetic rate and leaf ^{13}C amount, suggesting that plants preferentially allocate C to stem. Soil ^{13}C amount decreased and ^{13}C amount in microbial biomass first decreased and then increased with increasing plant ^{13}C amount, indicating that high plant C supply did not drive high amounts of C transferred to soil and microbes.

Conclusion

We proved that drought and nutrient deficit interactively affected C allocation in plant-soil-microbe system, and revealed a U-shaped relationship between plant C supply and the amounts of C transferred to microbes.

Introduction

Resource deficit, especially water and nutrient deficit, is recognized as a critical stress for plant growth and production (Drenovsky and Richards 2004; Song et al. 2010). However, drought events are predicted to increase in future due to climate change (IPCC 2014; Berdugo et al. 2020). Moreover, soil nutrient availability is decreased under drought conditions as a result of reduction in nutrient mobility (He and

Dijkstra 2014; Wang et al. 2021). In response to stress of water and nutrient deficit, plants adjust the morphological (e.g. leaf area, stomatal aperture, root depth) and physiological (e.g. photosynthetic rate, osmotic adjustment) traits to maximize growth rate (Arndt et al. 2001; Nadeem et al. 2020). Resources are optimally allocated to plant organs for modifying these morphological and physiological traits (Poorter et al. 2012; Meng et al. 2022). Given the widespread drought and nutrient deficit across terrestrial ecosystems (Elser et al. 2007; Craine and Jackson 2010; Berdugo et al. 2020), knowledge of how plants allocate resources into different organs is essential for understanding the mechanism of plant adaptation to stressful conditions.

Carbon (C) allocation is one of the key mechanisms of plant adaptation to external environmental stresses (Gessler and Grossiord 2019; Meng et al. 2022). Effects of drought stress on plant C allocation have been investigated for decades under global climate changes (Ruehr et al. 2009; Brunn et al. 2022). Drought can directly affect plant C allocation by reducing C assimilation and decreasing C allocation speed (Brüggemann et al. 2011; Dannoura et al. 2019; Wang et al. 2021). But the responses of plant C allocation to drought stress remain controversial because results of C allocation are only valid for the specific conditions or specific species (Sanaullah et al. 2012; Li et al. 2020). Plant C allocation is also affected by drought indirectly through reduced soil nutrient availability (Wang et al., 2021). Previous studies have been focused on the response of C allocation to nutrient enrichment, which can counteract the effects of drought on C allocation (Wang et al. 2021; Meng et al. 2022). In contrast, the effects of nutrient deficit on C allocation are less known, and how nutrient deficit interact with drought to modulate C allocation have not been studied to our knowledge.

Response of plant internal C allocation to resource stress is unpredictable due to the complex physiological mechanisms of C transport (Brüggemann et al. 2011; Wang et al. 2021). Carbon allocation within plants is generally controlled by the balance between C supply and demand of different organs (Gessler and Grossiord 2019; Hartmann et al. 2020). Photosynthesis is always weakened under environmental stresses and thus proportionally less C is supplied from leaf (Ruehr et al. 2009; Verlinden et al. 2022). Moreover, environmental stress would reduce C demand for plant growth or respiration, which in turn restricts photosynthetic C assimilation (Palacio et al. 2014). However, more C might be demanded for root growth to take up limiting resources (Barthel et al. 2011). These various feedbacks between C supply and demand make it difficult to link C allocation and environmental stress. Based on the optimal partitioning theory, plants would adjust the internal C allocation to support growth, reproduction and defense in response to environmental stress (Meng et al. 2022). However, under nutrient deficit stress or a combination of drought and nutrient deficit stress, whether plant C would be preferentially allocated to root or to aboveground organs is unknown.

Environmental stresses not only alter plant internal C allocation, but also affect C transfer from plants to soil (Brüggemann et al. 2011; Pausch and Kuzyakov 2018). Root is the primary functional component that link plant-soil system by releasing C into soil, which provides energy for rhizosphere microbial activity (Pausch and Kuzyakov 2018; Bai et al. 2021). In turn, microbes mineralize soil organic matter and provide nutrients for plant growth (Shemesh et al. 2016). Under stressful conditions, the amount of C

released from root into soil would be reduced due to the reduction in photoassimilated C and C allocation to root exudates (Wang et al. 2021). Changes in C input into soil affect microbial structure and functions, and thus regulate C turnover and sequestration in soil C pool (Bai et al. 2021). However, microbes may also complete with plants for limited resources to maintain their own growth (Hodge et al. 2000; Zhu et al. 2017). Considering the complex interactions between plants and microbes, determining how plant transfer C to soil and microbes gives better insights into plant adaptation mechanism and C turnover process under resource deficit conditions (Pausch and Kuzyakov 2018; Gao et al. 2021).

Given that C allocation strategies are plant species-dependent (Bai et al. 2021), revealing the C allocation of tolerant species is critical for understanding plant adaptation to resource deficit. *Sophora moorcroftiana* is an indigenous pioneer shrub that distributed widely in the arid valley of Yarlung Zangbo River, Tibet, China (Liao et al. 2021). It exhibits strong tolerance to drought and nutrient deficit, and is considered as a key species for revegetation of degraded ecosystems in arid regions (Liu et al. 2006; Xin et al. 2021). Although *S. moorcroftiana* has received much attentions on its growth, distribution, and biomass allocation (Zhao et al. 2007; Cui et al. 2017; Xin et al. 2021), little information is available on the C allocation of *S. moorcroftiana* in response to drought and nutrient deficit. In this study, we investigated the effects of drought, N deficit and P deficit on recent C allocation of *S. moorcroftiana* in plant-soil-microbe system. Specifically, we aimed to answer the following questions: (a) how does nutrient deficit interact with drought to regulate allocation of recently fixed C in plant-soil-microbe system, (b) how do plants allocate C to organs (i.e. leaf, stem and root) and transfer C to soil and microbes under drought and nutrient deficit stress, and (c) whether does high plant C supply result in high amounts of C transferred to soil and microbes?

Materials And Methods

Soil and seeds

The soil samples were taken from the Basin of Niyang River, which is one tributary of the Yarlung Zangbo River (94.40°E; 29.65°N). The soil is sandy loam and has the following characteristics: field moisture capacity 24%, available phosphorus 10.66 g/kg, total nitrogen 0.58 g/kg. The seeds of *S. moorcroftiana* were collected from the Shigatse, Tibet Autonomous Region. Healthy and uniform seeds were selected, air-dried, and stored at laboratory temperature of 20–25 ^{o}C.

Experimental design and treatments

Seeds were surface-sterilized with 0.2% of sodium hypochlorite (NaOCl) for 10 min, and then washed and soaked using deionized water for five times and 9 h, respectively. The soaked seeds were germinated in germination apparatus that was irrigated with deionized water every day. The high quality and uniform seedlings were picked up and planted in microcosms (pots with 2 L volume, 13 cm bottom diameter, and 25 cm height) containing 700 g soils (three seedlings each pot). All the pots were arranged in the artificial climate chamber with temperature of 22 ^{o}C and relative humidity of 70%.

Ninety-four days after seedlings were planted in pots, drought and nutrient (N and P) deficit experiments were carried out. In order to study the interactive effects of drought and nutrient deficit on C allocation in plant-soil-microbe system, a three factorial experiment was established, including control (N1P1H), drought (N1P1L), N deficit (N0P1H), P deficit (N1P0H), N + P deficit (N0P0H), drought + N deficit (N0P1L), drought + P deficit (N1P0L), and drought + N + P deficit (N0P0L) treatments. Specifically, the experiment consisted two water (L and H with irrigation of 20 mL and 40 mL every 3 day), two N (N0 and N1 with 0 and 1.125 mg/kg every 3 day) and two P (P0 and P1 with 0 and 0.166 mg/kg every 3 day) treatments. Each treatment had 12 replicates, in total 96 pots. L and H represented drought and non-drought, respectively; N0 and N1 represented N deficit and N addition, while P0 and P1 represented P deficit and P addition, respectively.

N and P were applied with Hoagland's solution, and the components were 1.25 m*M* KNO_3, 1.25 m*M* $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 m*M* $MgSO_4 \cdot 7H_2O$, 0.25 m*M* KH_2PO_4, 11.6 μ*M* H_3BO_3, 4.6 μ*M* $MnCl_2 \cdot 4H_2O$, 0.19 μ*M* $ZnSO_4 \cdot 7H_2O$, 0.12 μ*M* $NaMoO_4 \cdot 2H_2O$, 0.02 μ*M* $FeSO_4 \cdot 7H_2O$, and 0.02 μ*M* EDTA-Na_2, with a pH of 6.5 ± 0.2. Four Hoagland's solutions were employed: Hoagland's solution with N and without P, with P and without N, with N and P, without N and P. The NO_3^- in Hoagland's solution without N was replaced by Cl^-, and PO_4^{3-} in Hoagland's solution without P was replaced by SO_4^{2-} (Zhang et al. 2014). The 15 mL solutions were diluted to 20 mL and 40 mL and added into pots every 3 days. The pots were regularly rotated throughout the experiment period to reduce edge effects (Song et al. 2010).

^{13}C labeling

Sixty days after drought and nutrient deficit experiments were carried out, the ^{13}C labeling experiments were performed. The detailed procedure for plant ^{13}C labeling is described in Zhao et al. (2019). Briefly, three pots from each treatment were randomly selected as unlabeled samples to measure the natural background $\delta^{13}C$ of leaf, stem, root, soil, microbial biomass C (MBC) and dissolved organic C (DOC). The rest pots were equipped with a plastic tube, which was inserted into soil before labeling. During labeling, 0.002 g 99% ^{13}C sodium carbonate ($Na^{13}CO_3$) was put in the plastic tube. Then pots were sealed with bottle stopper, which was equipped with triple valve. Finally, 5 mL hydrochloric acid (HCl) was injected into plastic tube using needle tubing from triple valve to generate $^{13}CO_2$, and the triple valve was closed immediately after HCl was injected. All labeling was conducted between 9:00 a.m. and 11:00 a.m. every day and lasted for 14 days. Drought and nutrient deficit experiments were still carried out for labeled and unlabeled pots during labeling and lasted for 74 days in total.

Sampling and measurements

Plant and soil samples were collected after ^{13}C labeling. Before sampling, the plant height was measured, and the photosynthetic rates of plants were measured using an LCi-T (ADC Bioscientific Ltd., Hoddesdon, UK) portable photosynthesis system. After sampling, the plants were washed using deionized water and separated into leaf, stem and root for morphological measurement and chemical analysis. Main root

length was measure using metal tape measure. Then all plant samples were oven-dried to constant mass at 80 C for 48 h and weighed the biomass. Aboveground biomass (AGB) was calculated as the sum dry mass of leaf and stem, and belowground biomass (BGB) was the dry mass of root. The root shoot ratio (R/S) was calculated as BGB/AGB. After being weighed, parts of plant and soil samples were ground and sieved through a 100 mesh sieve for analysis of total C and $\delta^{13}C$.

Fresh soil samples were stored in a freezer at -25 ^{o}C for MBC and DOC determination using $CHCl_3$ fumigation-extraction (Vance et al. 1987). After fumigation, 5 g fumigated and unfumigated soils were extracted with 20 mL 0.5 *M* K_2SO_4 solution. The mixtures were filtered after being shaken for 30 min. The extracts were immediately analyzed for DOC using a total organic C analyzer (vario TOC, Elementar, Langenselbold, Germany). The MBC was calculated as the difference between fumigated and unfumigated soils after correcting for extraction efficiency (k = 0.45) (Vance et al. 1987). DOC was determined as the total C in the extract of unfumigated soil. Thereafter, extracts were oven-dried at 60°C and ground to a fine powder and analyzed for $\delta^{13}C$ in MBC and DOC (Dijkstra et al. 2008). The isotopic value of MBC was calculated using a mass balance equation.

Total C amounts and $\delta^{13}C$ of leaf, stem, root, soil, MBC and DOC were analyzed with an Isotope Ratio Mass Spectrometer (IRMS Delta V Advantage, Thermo Scientific, Bremen, Germany).

Calculation and statistical analyses

The isotopic values were expressed relative to the international standard Vienna Pee Dee Belemnite (VPDB) reference as ^{13}C, and were computed using the following equations (Ruehr et al. 2009; Meng et al. 2022):

$$\delta 13\mathrm{C}(‰) = \frac{\mathrm{R_{sample}} - \mathrm{R_{VPDB}}}{\mathrm{R_{VPDB}}} \times 1000$$

$$\delta 13\mathrm{C}\left(‰\right)_{MBC} = \frac{\delta 13\mathrm{C}_F \times \mathrm{C_F} - \delta 13\mathrm{C_{UF}} \times \mathrm{C_{UF}}}{\mathrm{C_F} - \mathrm{C_{UF}}}$$

$$\delta 13\mathrm{C}\left(‰\right)_{DOC} = \delta 13\mathrm{C_{UF}}$$

where *R* is the $^{13}C/^{12}C$ ratio and the subscripts *sample* indicate leaf, stem, root, soil and K_2SO_4 extracts derived from fumigated and unfumigated soils; $\delta^{13}C(‰)_{MBC}$ and $\delta^{13}C(‰)_{DOC}$ are the $\delta^{13}C$ in MBC and DOC; $\delta^{13}C_F$ and $\delta^{13}C_{UF}$ are the $\delta^{13}C$ in the K_2SO_4 extracts derived from fumigated and unfumigated soils, respectively; C_F and C_{UF} are the C concentration in the extracts derived from fumigated and unfumigated soils, respectively.

The ^{13}C(atom%) in plants, soil and microbes were calculated as follows (Schönbeck et al. 2021; Wang et al. 2021):

$$13\mathrm{C}(\mathrm{atom}\%)=\frac{(\delta 13\mathrm{C}+1000)\times \mathrm{R}_{\mathrm{VPDB}}}{(\delta 13\mathrm{C}+1000)\times \mathrm{R}_{\mathrm{VPDB}}+1000}\times 100$$

where ^{13}C(atom%) represents the percent of ^{13}C atom of leaf, stem, root, soil, MBC and DOC in total C atoms.

The ^{13}C amounts derived from $^{13}CO_2$ labeling in plants, soil and microbes were calculated as (Wang et al. 2021):

$$13\mathrm{C\ amounts}=\frac{13\mathrm{C}(\mathrm{atom}\%)_{\mathrm{labeled}}\text{-}13\mathrm{C}(\mathrm{atom}\%)_{\mathrm{unlabeled}}}{100}\times\frac{\mathrm{C}_{\mathrm{sample}}}{100}$$

where ^{13}C amounts represent the difference in ^{13}C(atom%) between labeled and unlabeled samples; C_{sample} is the total C amounts in leaf, stem, root, soil, MBC and DOC.

Three-way ANOVA was performed to test the main and interactive effects of drought, N deficit and P deficit on C allocation. First, the effects of drought and nutrient deficit on ^{13}C amounts in various C pools (i.e. leaf, stem, root, soil, MBC and DOC) were evaluated using multivariate ANOVA. Then the effects on ^{13}C amounts in each C pool were analyzed using univariate ANOVA. The normality of data and homogeneity of variance were tested using Kolmogorov-Smirnov and Levene's test, respectively. Multiple comparisons were performed with Duncan's multiple range tests at the significance level of 0.05. Linear and quadratic regressions were used to determine the relationships among variables. All the statistical analyses were performed in SPSS 18.0 (SPSS Inc.).

Results

Effects of drought and nutrient deficit and their interaction on ^{13}C allocation

Multivariate ANOVA based on ^{13}C amounts in various pools showed that drought and N deficit had significant main and interactive effects on C allocation (P< 0.001, Table 1). For P deficit, only N × P × drought significantly affected C allocation (P< 0.05, Table 1). Univariate analyses further indicated that ^{13}C allocation to leaf, stem, root, soil, MBC and DOC responded differently to drought and nutrient deficit (Table 2). Drought significantly affected ^{13}C amounts in most C pools except DOC (P< 0.05), while N deficit had significant effects on ^{13}C amounts in root, MBC and DOC (P< 0.001), but P deficit had no significant effects on ^{13}C amounts in any C pools (P> 0.05). The N × drought significantly affected ^{13}C amounts in leaf, root and soil (P< 0.01), while N × P and N × P × drought had significant effects on root ^{13}C amount, and P × drought had significant effects on leaf ^{13}C amount (P< 0.05).

Table 1

Multivariate ANOVA for testing the main and interactive effects of drought and nutrient deficit on C allocation based on the ^{13}C amounts in various pools (i.e. leaf, stem, root, soil, MBC, and DOC) at different treatments.

Treatments	Wilks's Lambda	Hypothesis df	Error df	F-values	Sig.
Drought	0.177	6.00	59.00	20.25	0.000***
N	0.372	6.00	59.00	16.59	0.000***
P	0.892	6.00	59.00	1.20	0.321
N × P	0.896	6.00	59.00	1.14	0.353
N × drought	0.646	6.00	59.00	5.38	0.000***
P × drought	0.866	6.00	59.00	1.52	0.189
N × P × drought	0.762	6.00	59.00	3.07	0.011*

Notes: Asterisks indicate significant treatment effect. *: $P < 0.05$; ***: $P < 0.001$; N: nitrogen deficit; P: phosphorus deficit.

Table 2

Univariate ANOVA for testing the main and interactive effects of drought and nutrient deficit on ^{13}C amounts in each pool at different treatments.

Treatments	Leaf	Stem	Root	Soil	MBC	DOC
Drought	8.195**	25.817***	108.387***	11.719**	4.325*	3.439
N	3.772	1.163	24.068***	0.607	36.026***	15.421***
P	2.078	0.76	1.139	1.545	0.484	1.76
N×P	0.848	0.009	5.858*	0.121	0.01	1.186
N × drought	13.439**	0.215	7.881**	18.979***	1.511	0.143
P × drought	4.444*	2.394	1.657	0.394	0.145	1.069
N × P × drought	3.195	3.711	14.583***	0.249	0.09	0.702

Notes: Asterisks indicate significant treatment effect. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; N: nitrogen deficit; P: phosphorus deficit.

Plant biomass, morphological traits and photosynthetic rates

Drought significantly decreased aboveground biomass by 50% and belowground biomass by 53% ($P < 0.05$; Fig. 1a and b), but did not significantly affect the ratio of aboveground and belowground biomass (except under P deficit stress), plant height, root length, and net photosynthetic rate ($P > 0.05$; Fig. 1c, d, e and f). N and N + P deficit showed no significant effects on plant biomass, morphological traits and photosynthetic rates ($P > 0.05$; Fig. 1), while P deficit caused the significant increase of net photosynthetic rates by 65% ($P < 0.05$; Fig. 1f). Interacted with drought stress, nutrient (N, P, N + P) deficit significantly decreased aboveground and belowground biomass ($P < 0.05$; Fig. 1a and b), while N + P deficit significantly decreased plant height by 24% ($P < 0.05$; Fig. 1d).

Plant internal ^{13}C allocation

Drought significantly decreased $\delta^{13}C$ allocation to stem, but increased $\delta^{13}C$ allocation to leaf ($P < 0.05$; Fig. 2a, b and d). N + P deficit significantly increased $\delta^{13}C$ allocation to root ($P < 0.05$; Fig. 2c). Interacted with drought, N deficit significantly increased $\delta^{13}C$ allocation to leaf and decreased $\delta^{13}C$ allocation to stem ($P < 0.05$; Fig. 2a and b). The interaction between N + P deficit and drought increased $\delta^{13}C$ allocation to leaf, and P deficit interacted with drought decreased $\delta^{13}C$ allocation to stem ($P < 0.05$; Fig. 2a and b).

Drought and nutrient deficit showed no significant effects on ^{13}C amount in leaf ($P > 0.05$; Fig. 2d). Drought significantly decreased ^{13}C amount in stem by 42% and root by 65% ($P < 0.05$; Fig. 2e and f). N deficit showed no significant effects on ^{13}C allocation to plant organs ($P > 0.05$; Fig. 2d, e and f). P deficit caused the significant increase of ^{13}C amount in leaf by 60%, while N + P deficit significantly increased ^{13}C amount in root by 65% ($P < 0.05$; Fig. 2d and f). Interactions between drought and nutrient (N, P, N + P) deficit were observed in stem (decreased by 33%-51%) and root (increased by 43%-58%) ($P < 0.05$; Fig. 2e and f).

Drought and its interaction with nutrient deficit significantly decreased total ^{13}C amounts fixed in plants ($P < 0.05$; Fig. 3a), which was attributed to the decrease of ^{13}C in stem and root ($P < 0.05$; Fig. 2e and f). However, nutrient deficit showed no significant effects on the total ^{13}C amount in plants ($P > 0.05$; Fig. 3a). When expressed as a proportion of total ^{13}C amount in plants, we observed that drought only significantly decreased the proportion of ^{13}C allocated to root ($P < 0.05$; Fig. 3b). Single N and P deficit showed no significant effects on the proportion of ^{13}C allocated to leaf, stem and root ($P > 0.05$), while N + P deficit significantly increased the proportion of ^{13}C allocated to root ($P < 0.05$; Fig. 3b). Drought + P deficit significantly increased the proportion of ^{13}C allocated to stem ($P < 0.05$; Fig. 3b).

^{13}C in soil and microbes

Drought and drought + P deficit significantly increased $\delta^{13}C$ allocation to soil and MBC and increased ^{13}C amount in soil ($P < 0.05$; Fig. 4a, b and d). Moreover, N deficit and drought + N deficit significantly

increased $\delta^{13}C$ and ^{13}C amount in DOC ($P < 0.05$; Fig. 4c and f). However, drought and nutrient deficit did not significantly alter ^{13}C amount in MBC ($P < 0.05$; Fig. 4e).

When expressed as a proportion of total ^{13}C amount in soil, MBC-to-soil ratio of ^{13}C amount was significantly decreased by N deficit and increased by P deficit ($P < 0.05$; Fig. 4g), while DOC-to-soil ratio of ^{13}C amount was significantly increased by drought + N deficit ($P > 0.05$; Fig. 4h).

Correlations between plant, soil and microbial C

Net photosynthetic rate was significantly related to ^{13}C amount in stem ($P < 0.05$; Fig. 6b). Significantly positive correlation in ^{13}C amount was observed between leaf and stem ($P < 0.05$; Fig. 6d), but not observed between leaf and root ($P > 0.05$; Fig. 6e). Aboveground biomass was positively related to aboveground ^{13}C amount ($P < 0.05$; Fig. 6f), while belowground biomass had significant positive correlation with ^{13}C amount in root ($P < 0.05$; Fig. 6i). Plant height had significant correlation with ^{13}C amount in stem ($P < 0.05$; Fig. 6g), and root length had no significant correlation with ^{13}C amount in root ($P > 0.05$; Fig. 6h).

A U-shaped relationship between plant ^{13}C amount and the amount of ^{13}C allocated to microbes was observed ($P < 0.05$; Fig. 7a). Specially, the ^{13}C amount in microbial biomass was negatively related to ^{13}C amount in root ($P < 0.05$; Fig. 7e), but had no significant correlations with ^{13}C amounts in leaf and stem ($P > 0.05$; Fig. 7c and d). In contrast, the ^{13}C amount in soil was negatively related to ^{13}C amounts in plant, leaf and stem ($P < 0.05$; Fig. 7b, f and g), but no significant correlation was observed between soil ^{13}C and root ^{13}C ($P > 0.05$; Fig. 7h).

Discussion

Effects of drought and nutrient deficit on plant internal C allocation

Based on the optimal partitioning theory, more C would be allocated to root under drought stress to improve water potential (Karlowsky et al. 2018; Schönbeck et al. 2021; Brunn et al. 2022). However, our data indicated that *S. moorcroftiana* decreased C allocation to root in response to drought stress, both in terms of the absolute ^{13}C amount in root and the relative proportion within plants (Fig. 3). One possibility would be that our drought condition with soil moisture of 6.54% was a severe drought (Fig. S1), which resulted in the significant reduction in both aboveground and belowground biomass (Fig. 1). Under the severe drought conditions, reduced C allocation to root could be attributed to dynamics of C supply, transport, demand, and output (Brüggemann et al. 2011; Pausch and Kuzyakov 2018; Gessler and Grossiord 2019). Primarily, reduced aboveground biomass indicated the small size of plant C pool under drought stress, thus less C were supplied for root (Wang et al. 2021). Secondly, our drought conditions might disable transport of C from aboveground organs to root (Schönbeck et al. 2021), which was in line

with the decrease in stem ^{13}C (Fig. 2e). Moreover, the metabolic activity of root might have been impaired by drought and thus C demand was reduced (Schönbeck et al. 2021). Alternatively, even more C was allocated to root, but a higher proportion of C would be exuded by root under severe drought conditions (Williams and de Vries 2020), and thus decreased ^{13}C amounts in root.

Interestingly, P deficit and N + P deficit affects C allocation to aboveground (i.e. leaf and stem) and belowground organs (i.e. root), respectively. In contrast with drought, joint N and P deficit significantly increased C allocation to root under well-watered conditions (Fig. 3a). Previous studies have suggested that nutrients addition reduced C allocation to root (Schönbeck et al. 2021; Meng et al. 2022). On the contrary, under non-limiting water and limiting nutrient conditions, plants invest more C to root for nutrient acquisition as predicted by optimal allocation theory (Hartmann et al. 2020). However, our results indicated that the effects of nutrient deficit on plant internal C allocation are dependent on water (Fig. 5). The effects of N + P deficit on C allocation to belowground root were not observed under drought conditions (Fig. 3), which primarily due to the impeding of C transfer from aboveground organs to root under water deficit stress (Schönbeck et al. 2021). Instead, single P deficit played critical roles in regulating aboveground C allocation (Fig. 3), which could partly be attributed to changes in net photosynthetic rate. Net photosynthetic rate was increased under P deficit conditions (Fig. 1f), and thus caused the increase of total ^{13}C amounts in plants (Fig. 3b). However, we found that C allocation to leaf was decreased rather than increased because more C was allocated to stem (Fig. 3a), which strongly influences water and nutrients transportation from soil to leaf (Zhang and Cao 2009).

Effects of drought and nutrient deficit on C allocation in soil and microbes

Soil is the major C pool within plant-soil system, and the C allocation to soil was increased under drought stress. In general, an osmotic adjustment occurs in response to drought by exuding relatively labile C compounds in the rhizosphere to draw water flow towards the root (Hasibeder et al. 2015; Wang et al. 2021). Furthermore, increase in C allocation to soil could also be a result of higher rhizodeposition rate and less microbial decomposition (Fuchslueger et al. 2014). However, the effects of microbial activity could be excluded, because drought had no significant effects of on C allocation to MBC and DOC (Fig. 4e ~ h). Therefore, our results indicated that more C would be stored in soil rather than respired by microbes under drought stress as suggested in previous studies (Brüggemann et al. 2011; Fuchslueger et al. 2014; Wang et al. 2021). Moreover, there was no significant effect of drought on ^{13}C amount in microbial biomass under drought stress (Fig. 4e), suggesting that drought was not the primary driver of microbial activity under nutrient rich conditions.

Under well-watered conditions, nutrient deficit had no significant effects on the absolute ^{13}C amounts in soil, but C allocation to microbial biomass was altered by nutrient deficit. Specifically, N and P deficit showed opposing effects on the C allocation to microbial biomass (Fig. 4g), which was closely associated with microbial processes of biomass synthesis and metabolism (Poeplau et al. 2016). C allocation to microbial biomass in our study was decreased under N deficit, which was in line with the

previous findings that N availability constrained the size of MBC across global soils (Hartman and Richardson 2013). N deficit inhibits microbial growth because N is critical for biosynthesis of N-rich proteins and reduces microbial demand for C (Poeplau et al. 2016; Dong et al. 2022). Although P is also crucial for protein synthesis, the limitation of P deficit on microbial metabolism seemed to outweigh the decreased microbial growth. Previous studies found that microbial metabolic quotients (microbial respiration: biomass ratio) decreased with lower P availability (Hartman and Richardson 2013; Poeplau et al. 2016), which indicated the higher microbial C use efficiency under P deficit, and thus more C would be stored in microbial biomass.

The effects of nutrient deficit on C allocation to soil and microbes were regulated by water availability. There was no significant effect of nutrient deficit on soil ^{13}C amount under well-watered conditions, while N deficit decreased ^{13}C amount in soil under drought conditions (Fig. 4d). The result indicated that drought would enhance the negative effects of nutrient deficit C allocation to soil, which was determined by the balance between plant C input and C mineralization (Bai et al. 2021). On the other hand, drought might inhibit the effects of nutrient deficit on microbial growth and activity, because no significant effects of nutrient deficit on C allocation to MBC were observed under drought conditions (Fig. 4g). Instead, we found that C allocation to DOC was increased in response to N deficit under drought conditions (Fig. 4h). Therefore, our results indicated that more C was stored as DOC rather than utilized by microbes when C amount in soil was limited. Furthermore, the increase in C allocation to DOC could be attributed to the high root exudation rates or the reduced activity of root associated microbes under N deficit conditions, which leaded to C accumulation in DOC (Sanaullah et al. 2012).

Trade-off between C supply and demand

According to the priorities concept, C is used first by higher priority tissues and followed by the next priority (Weinstein et al. 1991; Litton et al. 2007). We found that net photosynthetic rate and leaf ^{13}C amount were positively related to stem^{13}C amount (Fig. 6b and d), suggesting that recently fixed C would be used first by stem. Increased C supply from leaf resulted in the higher source-sink turgor difference, and thus drove the transport of C compounds via phloem to sink organs for growth and/or storage (van Bel 2003; Brüggemann et al. 2011). Consequently, more C was demanded for stem growth to enhance phloem loading in response to higher C supply (Ainsworth and Bush 2011). Moreover, we observed the positive relationships between stem ^{13}C amount and plant height and between aboveground ^{13}C amount and biomass (Fig. 6), indicating the C allocation to aboveground organs is demanded for increasing plant biomass and adjusting morphological traits. However, root adjusted its phenotypes in response to resource stress using different C allocation strategy from aboveground organs (Freschet et al. 2018). Our results showed that root C was mainly allocated to build root biomass rather than to extend root length (Fig. 6h and i), which supported the previous findings that root biomass is more sensitive to environmental changes than root morphological traits (Freschet et al. 2015; Kramer-Walter and Laughlin 2017).

Although plant C input is a major contributor to soil C (Bai et al. 2021), we found that ^{13}C amounts in soil and MBC did not increase with increasing plant ^{13}C amount. Changes in soil C was determined by the balance between C input and output (Gougoulias et al. 2014; Feng et al. 2022). Plant C input would stimulate microbial respiration and decomposition of organic C which caused C output from soil (Fontaine et al. 2004). Moreover, based on the U-shaped relationship between ^{13}C amounts in plants and MBC (Fig. 7a), we inferred that the highest priority of C allocation is kept in plants for maintaining their basic metabolic processes, and only surplus C would be exported to soil and utilized by microbes when fixed C amounts were high enough (Prescott et al. 2020). When fixed C amounts were limited, more C would be utilized for plants, which further complete for resources with microbes (Zhu et al. 2017). Carbon and resources deficit inhibited microbial growth and activity, and thus resulted in the decreasing C amount in microbes with plant growth. Root C was the major source of C for microbes and could stimulate microbial growth through the input of root exudates (Feng et al. 2022). But we found that the ^{13}C amount in MBC was significantly but negatively related to root ^{13}C (Fig. 7e), which was not consistent with previous studies (Bai et al. 2021). This might be attributed to rhizodeposition, microbial decomposition and respiration (Ingrisch et al. 2020; Bai et al. 2021), which were not considered in our study. Consequently, further studies considering both C input and output processes should be conducted to facilitate the evaluation of the interactions between plant and soil C.

Conclusion

Drought and nutrient deficit interactively affected C allocation in plant-soil-microbe system. Drought decreased C allocation to root and increased allocation to soil, but N + P deficit counteracted the effects of drought by allocating more C to root for nutrients uptake. Drought and nutrient deficit mainly affected belowground C allocation, and only P deficit regulated aboveground C allocation under drought conditions. Effects of nutrient deficit on C allocation were transferred from belowground root to aboveground organs under drought conditions. Moreover, drought enhanced the effects of nutrient deficit on C allocation to soil, but weakened the effects on C allocation to microbes. We found that only stem ^{13}C amount was positively related to net photosynthetic rate and leaf ^{13}C amount, suggesting recently fixed C would be first used by stem for resource transport. In addition, large plant C supply did not result in the high C amounts in soil and microbes. We revealed a U-shaped relationship between plant C supply and the amounts of C transferred to microbes. More studies are needed to analyze the opposing effects of different resource stress and the negative correlations between plant and soil C, and further to determine the applicability of C allocation strategies for *S. moorcroftiana* to other plant species or to other resources.

Declarations

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Competing interests:

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Author contributions:

H.X. and T.Z. analyzed the data and wrote the manuscript. T.Z. and X.L. carried out the experiments and conducted analysis. T.H., X.W. and J.Z. contributed to the interpretation of the results and the improvement of the manuscript. K.Z. designed the research, conducted analysis, and wrote the manuscript. All authors assisted in discussion of the results and preparation of the manuscript.

Data Availability:
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

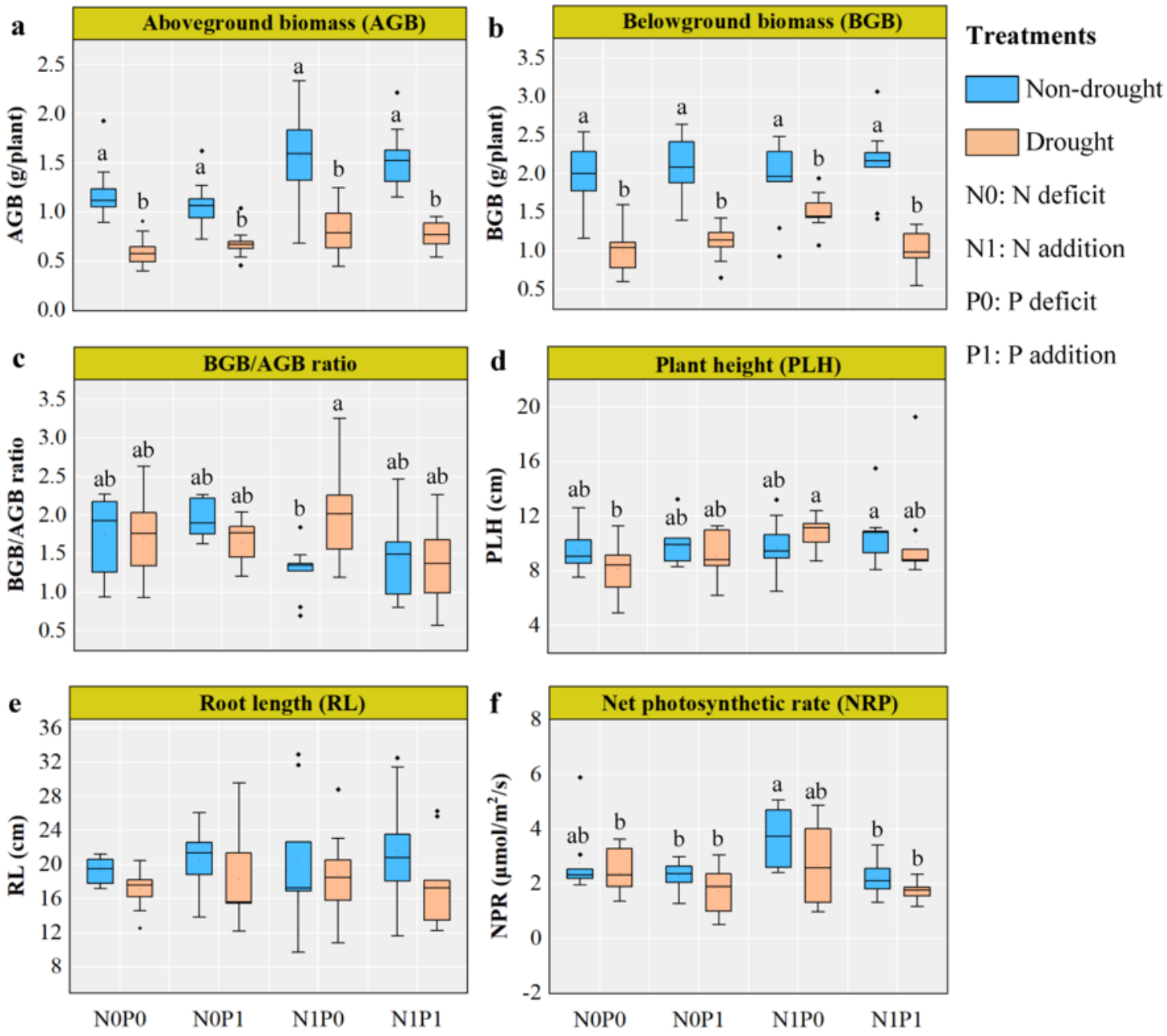

Figure 1

Effects of drought and nutrient deficit on biomass (a, b, c), morphological (d, e) and photosynthetic rates (f). Error bars represent the 10th and 90th percentiles; black lines within the boxes indicate median values and the box limits indicate values in the 25-75th percentile range. Different letters were labeled above boxes to show significant differences among treatments.

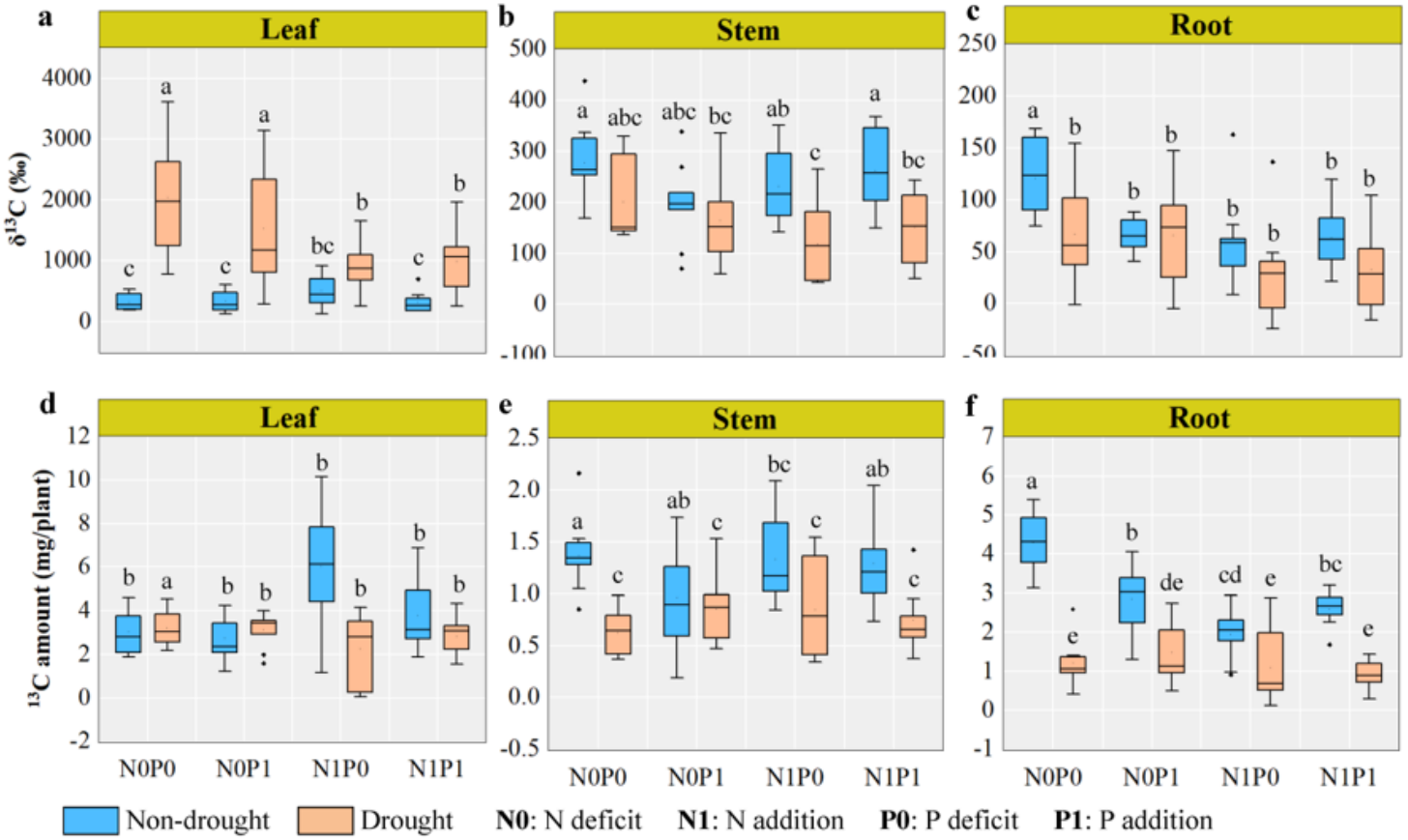

Figure 2

Effects of drought and nutrient deficit on $\delta^{13}C$ and ^{13}C collation in leaf (a, d), stem (b, e) and root (c, f). Error bars represent the 10^{th} and 90^{th} percentiles; black lines within the boxes indicate median values and the box limits indicate values in the 25-75^{th} percentile range. Different letters were labeled above boxes to show significant differences among treatments.

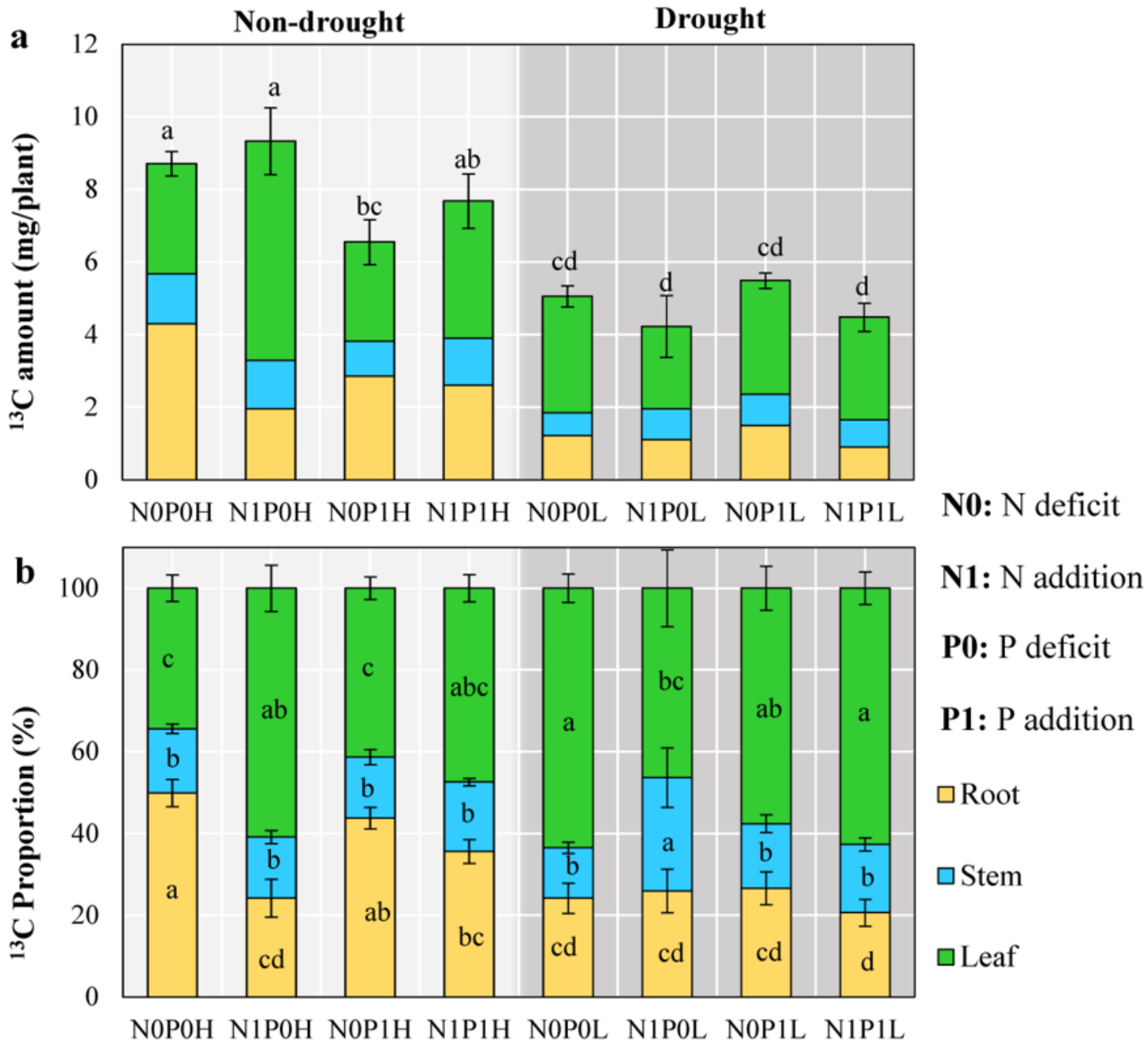

Figure 3

Effects of drought and nutrient deficit on the absolute amount (a) and proportion (b) of ^{13}C in leaf, stem and root. Error bars represent standard error (n=9); different lowercase letters were labeled to show significant differences among treatments.

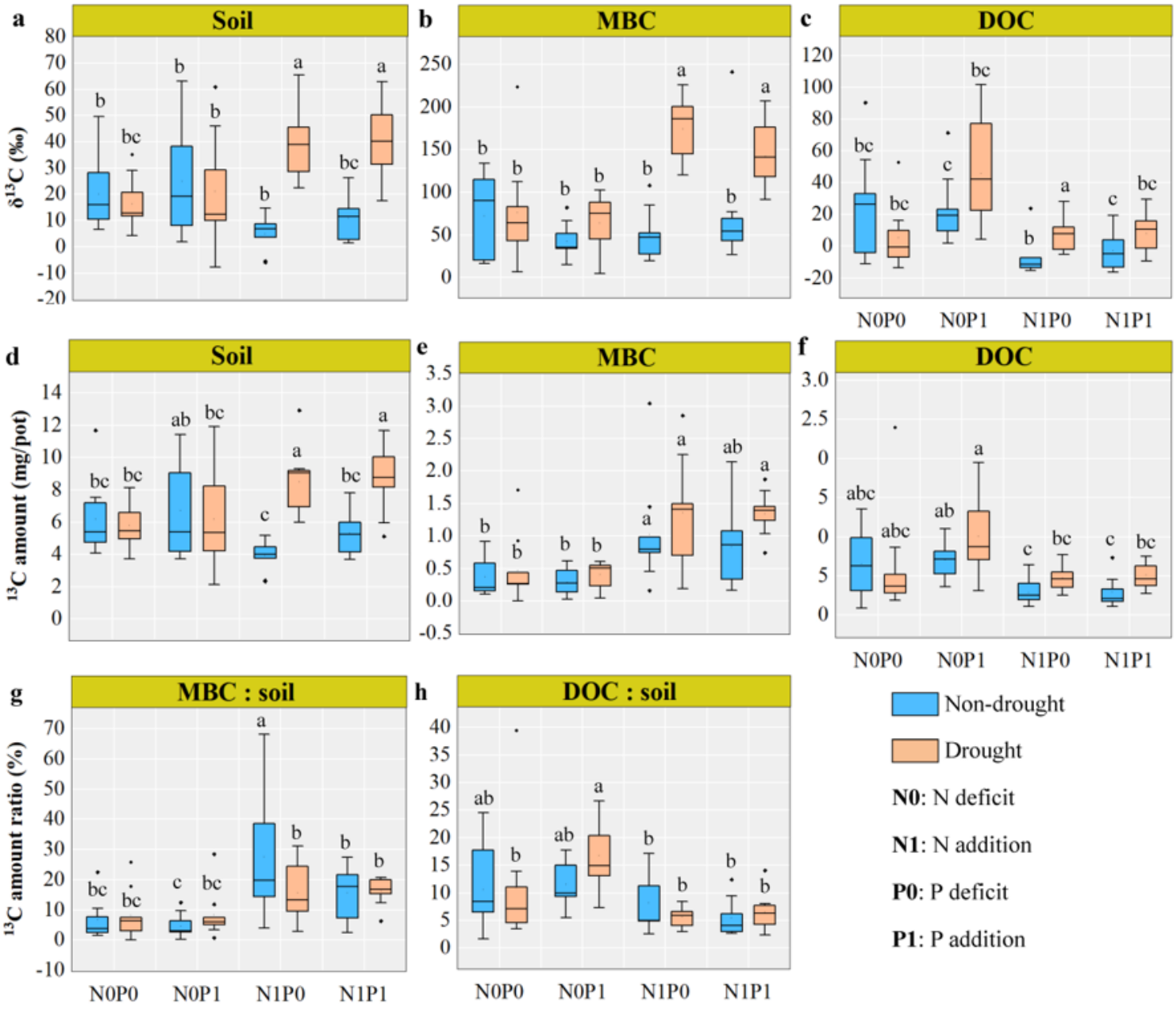

Figure 4

Effects of drought and nutrient deficit on $\delta^{13}C$, ^{13}C amount, and ratio to soil of ^{13}C amount in soil (a, d), microbial biomass C (MBC) (b, e, g) and dissolved organic C (DOC) (c, f, h). Error bars represent the 10^{th} and 90^{th} percentiles; black lines within the boxes indicate median values and the box limits indicate values in the 25-75^{th} percentile range (n=9). Different letters were labeled above boxes to show significant differences among treatments.

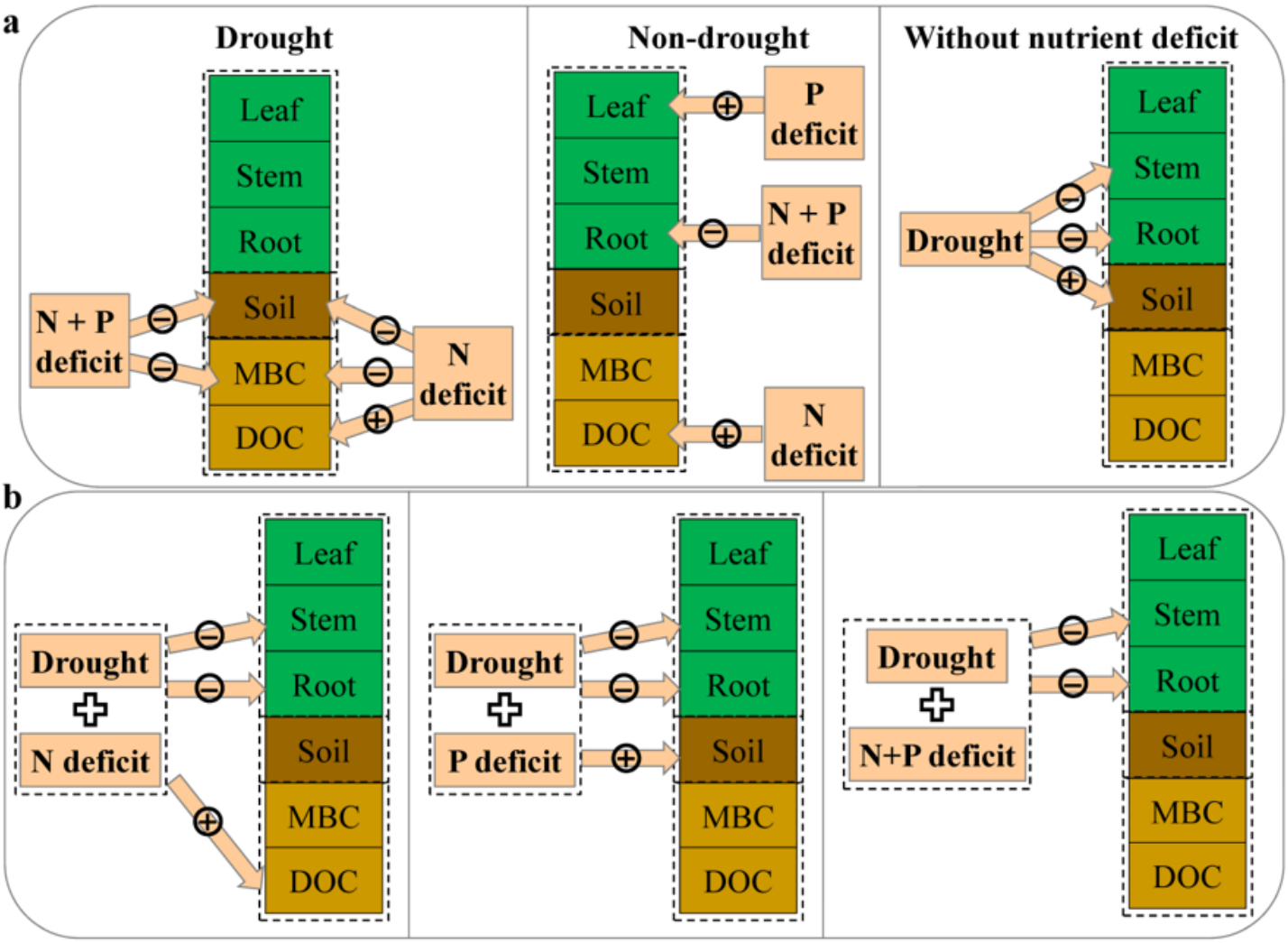

Figure 5

Effects of nutrient deficit and drought (a) and their interaction (b) on ^{13}C amount in leaf, stem, root, soil, MBC and DOC. N: nitrogen; P: phosphorus; MBC: microbial biomass C; DOC: dissolved organic C; ⊕: increase ^{13}C allocation to the given C pool; Θ: decrease ^{13}C allocation to the given C pool.

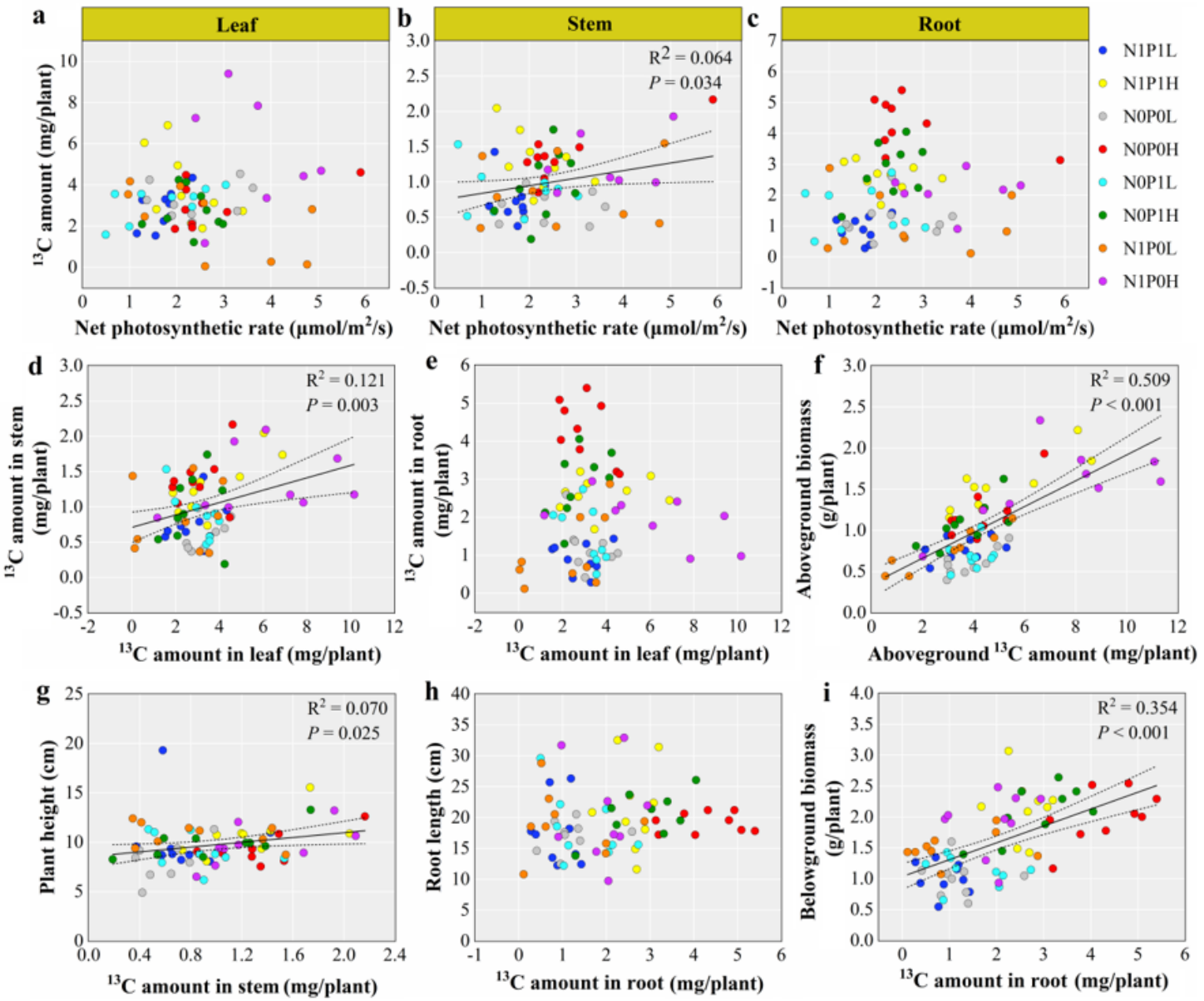

Figure 6

Correlations between plant aboveground biomass, belowground biomass, plant height, root length, net photosynthetic rate and ^{13}C amounts in leaf, stem and root. The solid black lines represent the fitted regression lines, and the dotted lines indicate 95% confidence bands. N: nitrogen; P: phosphorus; L: drought; H: non-drought; N0: N deficit; N1: N addition; P0: P deficit; P1: P addition.

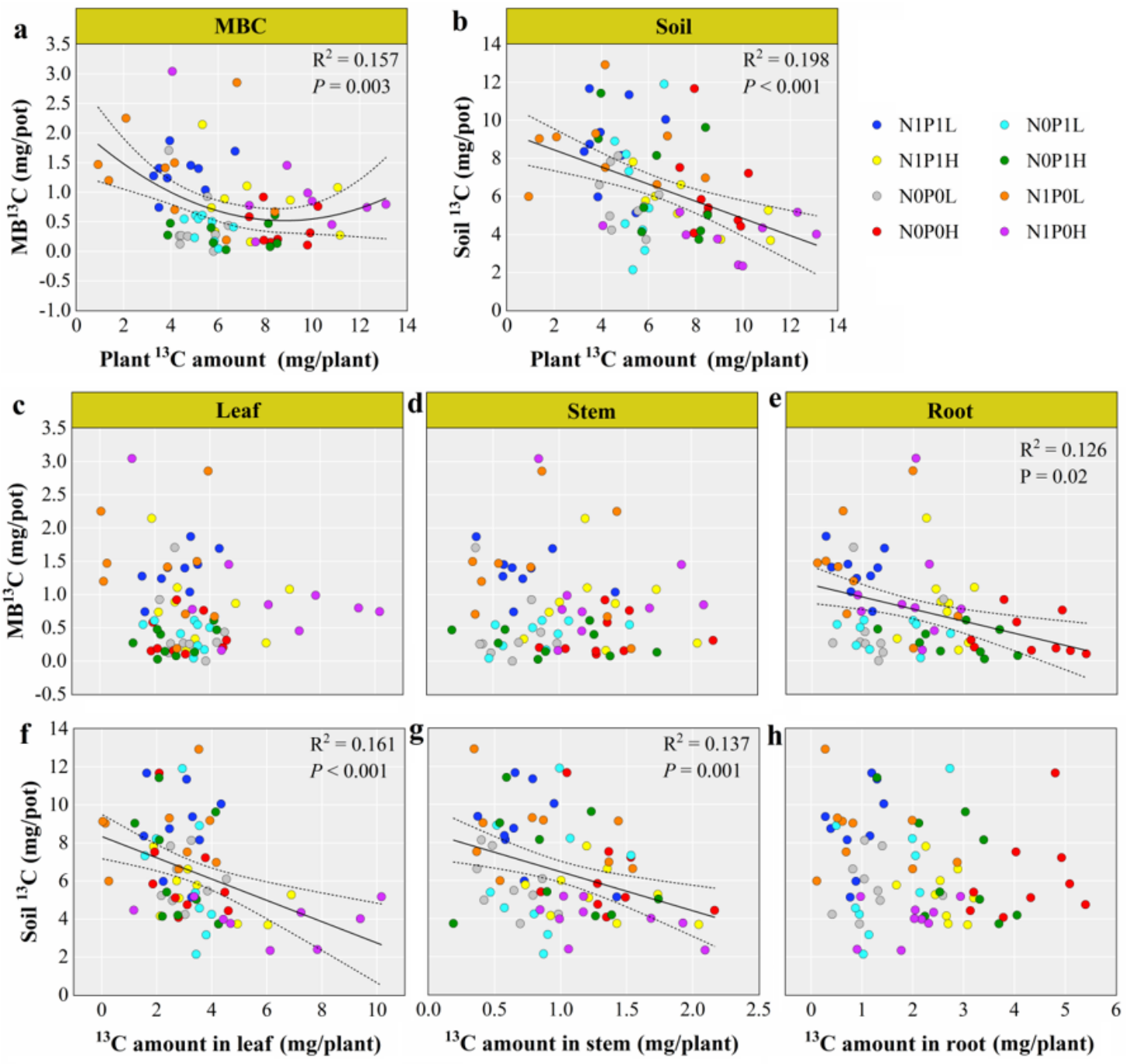

Figure 7

Correlations between ^{13}C amount in plant, leaf, root, soil and MBC. The solid black lines represent the fitted regression lines, and the dotted lines indicate 95% confidence bands. N: nitrogen; P: phosphorus; L: drought; H: non-drought; N0: N deficit; N1: N addition; P0: P deficit; P1: P addition.

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