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Stronger intra-specific competition aggravates negative effects of drought on the growth of *Cunninghamia lanceolata*

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Stronger intra-specific competition aggravates negative effects of drought on the growth of *Cunninghamia lanceolata*

Running title: Combined effects of competition and drought on growth

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Highlights

● Intra-specific competition becomes stronger under prolonged drought.

● Combined competition and drought greatly decline nitrogen uptake.

● Strong competition significantly lowers leaf $\delta^{13}C$ and ABA under prolonged drought.

● Combined competition and prolonged drought are highly harmful to growth.

Abstract

Plant-plant competition is a dynamic and complicated process that is strongly influenced by abiotic conditions. Drought is a critical threat to forests, particularly to young plantation forests. Temporal changes in competition combined with the effects of drought may dramatically influence the physiological traits of plants. *Cunninghamia lanceolata* plants exposed to intra-specific competition and no-competition conditions were investigated under two soil water levels (well-watered and drought). Changes in plant-plant competition relationships and nitrogen uptake rates were measured at different harvest times. The effects of drought and plant competition on physiological traits, for example, leaf nitrogen allocation, $\delta^{13}C$, and levels of abscisic acid (ABA), indole acetic acid (IAA) and jasmonic acid (JA), were also explored. Our results indicated that *C. lanceolata* shifted from intense neighbor competition to facilitation under well-watered conditions, whereas under drought neighbor competition became much stronger at the second harvest compared to the first harvest. Strong competition significantly decreased N uptake under

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drought. Leaf NH_4^+, NO_3^- and N allocation to water-soluble proteins increased under drought at the first harvest, but significantly declined under prolonged drought. Leaf, stem and root starch concentrations were enhanced by drought. However, during prolonged drought, the root starch concentrations, leaf $\delta^{13}C$, leaf ABA and starch content of *C. lanceolata* were much lower under strong neighbor competition than in no-competition conditions, which demonstrated that the combined effects of drought and strong competition were more harmful to plant growth and survival compared to single effects. Our study demonstrated that drought combined with competition strongly affected the N uptake, N allocation and physiological traits of plants. Intense competition imposed by neighbors is a great threat to the growth and survival of young *C. lanceolata* plantations under prolonged drought.

Keywords: forest plantation; nitrogen allocation; nitrogen uptake; prolonged drought

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1. Introduction

When the climate changes, forests experience more serious droughts at a higher frequency, which causes increased mortality and declined productivity, as observed during recent decades (Assal et al., 2016; Gessler et al., 2016). Competition is another crucial factor that limits plant growth and affects species' distribution patterns and adaptations (Baudis et al., 2014; Guo et al., 2018). A wealth of studies has focused on interactions between competition and drought. Many of them have suggested that competition among plants becomes weaker or even shifts to facilitation when soil water reduces (e.g. He et al., 2013; Baudis et al., 2014; Verwijmeren et al., 2019); on the contrary, many studies have reported an opposite pattern (e.g. Tielbörger and Kadmon, 2000; Hommel et al., 2016; Zhang et al., 2017). One possible reason for such contrasting results is that species' traits change along environmental stress gradients (He et al., 2016). Another likely reason is that plant-plant competition patterns change in the course of time (Biswas and Wagner, 2014; Ploughe et al., 2019).

Distinct competitive traits for capturing resources depend on the neighbor's identity and environmental conditions (Guo et al., 2016; Xia et al., 2020). Biomass allocation, nutrient uptake and storage are traits that are strongly impacted by drought and competition (Chen et al., 2014; Hommel et al., 2016; Walker et al., 2017; Han et al.,

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2019). In Chinese fir (*Cunninghamia lanceolata*), root traits, such as the total root length and root depth, are impacted by neighbors (Xia et al., 2019), implying variation in resource capture, e.g. soil nitrogen (Broadbent et al., 2018; Guo et al., 2018). Superior competitors are always more effective at nitrogen uptake when compared to their neighbors (Trinder et al., 2012; Walker et al., 2016). Both drought and competition promote plants to invest more energy in root growth and development to enhance access to limited soil water (Chen et al., 2014; Han et al., 2019) or to compete for soil nitrogen with their neighbors (Broadbent et al., 2018). However, nitrogen uptake and biomass accumulation rates are a dynamic process and vary during different growth stages, which leads to changes in plant-plant competition (Trinder et al., 2012).

Plant-plant competition affects plants' responses and resistance to environmental stresses (Andersen et al., 2000; He et al., 2016). Andersen et al. (2000) have reported that when grown in competition with grasses, soluble sugar concentrations of *Pinus ponderosa* significantly reduce and it becomes more susceptible to ozone. Soluble sugars, which are the main component of non-structural carbohydrates (NSC: the sum of soluble sugars and starch), play a crucial role in cell functioning, for example, in the maintenance of cell turgor or in membrane protection by an enhanced proline accumulation (Hüve et al., 2012; Hartmann and Trumbore, 2016). Plants adapt to water deficit by altering functions, such as osmotic adjustment, and increased soil water uptake and use efficiency (Flexas et al., 2016; Han et al., 2019). Plant-plant

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competition is a dynamic process across time (Trinder et al., 2012; Zhang et al., 2017). Plants suffering from strong competition may be more sensitive to drought (Lu et al., 2019), and their physiological responses, such as water use efficiency and hormone levels, may be influenced by changes in competition.

C. lanceolata is a widely planted fast-growing evergreen conifer species that provides timber and important ecological functions (Dong et al., 2019). During recent decades, drought has become frequent within its distribution region (Yang et al., 2012). In the present study, we firstly focused on changes in the intra-specific competition of *C. lanceolata* under drought. According to the stress gradient hypothesis, the intensity of plant-plant competition will become weaker or shift to facilitation under stress (Bertness and Callaway, 1994). Based on that, we hypothesized that intra-specific competition would be weaker under water deficit and much weaker or even shifting to facilitation during prolonged drought. Secondly, we investigated the effects of different competition intensities on N uptake, and N use efficiency, allocation and storage. Finally, we explored physiological responses to the combined drought and competition conditions, aiming to find out whether plants under competition are more affected by drought.

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2. Material and methods

2.1. Experimental design

One-year-old *C. lanceolata* seedlings were obtained from the Huitong Experimental Station of Forest Ecology, Chinese Academy of Sciences (26°40′-27°09′ N, 109°26′ -110°08′ E; 300-1000 m) and then transplanted into a greenhouse located in the Hangzhou Normal University in Zhejiang (30°19′ N, 120°23′ E), as detailed by Xia et al. (2019). Seedlings were planted in plastic cylindrical pots with a height and external diameter of 33 cm and 56 cm (about 35 kg soil), respectively. The chemical properties of the homogenized soil were as follows: soil organic matter 4 g kg^{-1}, total N 1.5 g kg^{-1} and pH 7.1 (the ratio of soil to $CaCl_2$ solution was 1:2.5).

We selected seedlings of *C. lanceolata* with a similar height and conducted competition experiments by planting two seedlings in each pot (a total of 20 pots). One seedling in a pot represented no-competition conditions (a total of 20 pots). All seedlings were planted in late December 2017. By the middle of May 2018, the surviving and well growing *C. lanceolata* plants included 18 pots representing competition and 20 pots being without competition. To monitor the dynamics of the soil water content, we selected 6 pots from those 38 pots and divided them into two groups, those exposed to well-watered or drought conditions. We collected soil with a

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soil sampler (diameter 0.5 mm) every four to five days and weighed the soil samples. All of them were dried at 75 ºC for 48 h, then weighed again and finally returned to original pots. These six pots were only used to monitor soil water conditions and not harvested. Finally, the remaining 16 pots representing competition and 16 pots being without competition were used to monitor plant growth in four different conditions: no competition under well-watered condition (WS), competition under well-watered condition (WC), no competition under drought (DS) and competition under drought (DC). The soil water levels were either 80% of field capacity (soil water ~28.48%) or 30% of field capacity (soil water 10.68%) to represent well-watered or drought conditions, respectively. The 30% field capacity treatment represented more extensive drought than the conditions in Dong et al. (2016) with 35% field capacity used in a study on *C. lanceolata*. The treatments began in the middle of May 2018. The information on soil water dynamics is displayed in Supplementary Fig. S1.

2.2. Harvesting and measurements

Before planting in late December 2017, 10 additional even-sized seedlings of *C. lanceolata* were randomly selected and separated into leaves, stems and roots. Samples were dried at 75 ºC for 72 h and weighed. Then, the samples were ground into power to measure nitrogen concentrations. After water treatments, we conducted two harvests (four replicates of every treatment at each harvest), in the middle of September and in the middle of December 2018. All samples were separated into

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leaves, stems and roots, and dried at 75 ºC for 72 h and weighed. Fresh leaves and roots were preserved at -80 ºC until measurements. The relative growth rate was calculated based on biomass accumulation: relative growth rate at the first harvest = (ln ($biomass_1$) - ln ($biomass_0$)) / (t_1 - t_0); relative growth rate at the second harvest = (ln ($biomass_2$) - ln ($biomass_1$)) / (t_2 - t_1). $Biomass_0$, $biomass_1$ and $biomass_2$ represented *C. lanceolata* biomasses before transplantation, and at the first and second harvest, respectively.

The relative interaction index (RII) indicates changes in growth performance when grown with and without neighbors (Armas et al., 2004): RII = ($Biomass_C$ - $Biomass_S$)/ ($Biomass_C$ + $Biomass_S$), where $Biomass_C$ is the performance of a target plant with its neighbor, and $Biomass_S$ is the performance of a single plant without a neighbor. RII ranges from 1 (maximum facilitation) to -1 (maximum competition).

Dried materials were ground to fine powder to measure nitrogen, non-structural carbohydrates (soluble sugars and starch), $\delta^{13}C$ and $\delta^{15}N$. Then, 100 mg fine power was digested by H_2SO_4-H_2O_2 and measured by the semi-micro Kjeldahl method (Luo et al., 2015), and 50 mg fine powder was extracted in 80% (v/v) ethanol at 80 ºC for 30 min and centrifuged at 7000 g for 5 min. The supernatant was used to determine soluble sugars at 625 nm (LabTech, UV2100, USA) according to Yemm and Willis (1954). The residues were hydrolyzed with 9.2 M $HClO_4$ and then used to measure starch after centrifugation at 5000 g according to Guo et al. (2016). Leaf $\delta^{13}C$ and

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$\delta^{15}N$ were determined by measuring $^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively, by using an isotope ratio mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Inc., USA). The Pee Dee Belemnite (PDB) standard and N_2 were used as standards to calculate $\delta^{13}C$ and $\delta^{15}N$, respectively. Four replicates of each treatment were included in these measurements.

Fresh materials were ground into fine powder in liquid nitrogen before measurements. However, the amount of fine roots (diameter < 2 mm) at the first harvest was sufficient only for the measurements mentioned above. Therefore, root traits like NH_4^+, NO_3^-, peroxidase (POD) activity and proline were measured only at the second harvest. The measurements of leaf chlorophyll pigments were conducted according to Guo et al. (2018). The leaf and fine root NH_4^+ concentrations were measured based on the Berthelot reaction (Bräutigam et al., 2007) with minor modifications (Luo et al., 2015). Briefly, 100 mg fine power was extracted in a solution with 1 ml 100 mM HCl and 500 µl chloroform and shaken for 15 min at 4 ºC. Then, the aqueous proportion was transferred to a new tube, and 100 µl extraction solution was added into 500 µl 1% (v/v) phenol-0.005% (w/v) sodium nitroprusside solution followed by addition of 500 µl 1% (v/v) sodium hypochlorite-0.5% (w/v) sodium hydroxide solution. Finally, the mixture was incubated at 37 ºC for 30 min and measured at 620 nm (LabTech, UV2100, USA). Another 100 mg fine powder was extracted in 1 ml deionized water at 45 ºC to measure NO_3^- concentrations. Briefly, after adding 0.8 ml 5% (w/v) salicylic acid (SA) in concentrated H_2SO_4 to 0.2 ml of supernatant, the mixture was

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incubated at room temperature for 20 min, followed by the addition of 19 ml of 2 M NaOH to adjust pH > 12 and measurements at 410 nm (Luo et al., 2015).

Leaf nitrogen allocation to detergent-insoluble proteins ($N_{in\text{-}SDS}$), detergent-soluble proteins (N_S), water-soluble proteins (N_W) and other N was determined based on the methods of Takashima et al. (2004) and Liu et al. (2018). Other N concentration here represents non-protein N (N_{np}), which mainly contains inorganic N and small molecules, such as amino acids.

N_{np} = total N - $N_{in\text{-}SDS}$ - N_W - N_S.

In brief, 0.5 mg leaf powder was homogenized with 100 mM of Na phosphate buffer (pH 7.5, containing 0.4 M d-sorbitol, 2 mM $MgCl_2$, 10 mM NaCl, 5 mM iodoacetate, 5 mM phenylmethylsulphonyl fluoride, and 5 mM DTT). After centrifugation at 10000 *g* for 15 min at 4 ºC, the supernatant (N_W) was separated. The rest was mixed with 1 ml phosphate buffer, including 3% SDS, and heated in 90 ºC water for 5 min. The supernatant (N_S) collection was repeated three times. The residue ($N_{in\text{-}SDS}$) was washed with ethanol into a quantitative filter paper. To denature the proteins, 20% trichloroacetic acid was added to the supernatant mixture, followed by filtering with a quantitative filter paper. Finally, the quantitative filter papers were dried and digested with H_2SO_4-H_2O_2 and N concentrations were measured by the semi-micro Kjeldahl method.

The nitrogen uptake rate was calculated as described in Walker et al. (2017):

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nitrogen uptake rate at the first harvest = (Δ total N pool/t)*((ln(root mass$_1$) - ln(root mass$_0$))/ Δ root mass); nitrogen uptake rate at the second harvest = (Δ total N pool/t)*((ln(root mass$_2$) - ln(root mass$_1$))/ Δ root mass). Nitrogen use efficiency was calculated as the dry weight of the target plant divided by the total N uptake of the plant (Li et al., 2012).

The peroxidase (POD) activities were measured according to the method of Bi et al. (2020). For proline measurements (Han et al., 2019), 200 mg fine powder was extracted by 3% (w/v) aqueous sulfosalicylic acid solution. 1 ml supernatant was mixed with 2 ml acid ninhydrin and boiled for 60 minutes, and proline concentrations were determined at 520 nm using L-proline as a standard after adding 2.5 ml toluene.

Leaf hormones, including abscisic acid (ABA), indole acetic acid (IAA) and jasmonic acid (JA), were determined by the UPLC-ESI-MS/MS method using the ACQUITY UPLC H-Class system (Waters, USA). Briefly, 200 mg leaf powder was mixed with 1.5 ml extraction solution (methanol : water : formic acid = 7.9 : 2: 0.1) in a 5-ml tube. After 30-min ultrasound on ice, the samples were kept at 4 ºC for 12 h. The supernatant was collected after centrifugation at 4 ºC, 12000 rpm for 20 min. Samples were let to flow through a MAX SPE column (Waters, USA) followed by drip washing with 0.1 M ammonium hydroxide -60% (v/v) methanol solution and elution with 1.25 M formic acid -70% (v/v) methanol solution. The mobile phase consisted of 0.1% (v/v) formic acid (mobile phase A) and acetonitrile (mobile phase B) at a flow

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rate of 0.4 ml/min. The total run time was 3 min per sample and the injection volume was 1 μl. The standards of determined hormones were purchased from Sigma.

2.3. Statistical analyses

Normality and homogeneity of variances of all data were checked and log-transformed when needed before analyses. Nitrogen uptake was analyzed with the Kruskal Wallis test, because it failed to meet these conditions. The effects of drought, competition and harvest time as well as their interactions were determined by three-way analyses of variance (ANOVA) followed by Tukey's tests as post hoc tests when a significant difference was found. For some measurements performed only at the second harvest, two-way analyses of variance (ANOVA) were applied to determine the effects of competition, drought and their interactions. The software Statistical Package for Social Science (SPSS) version 20.0 was used for data analyses.

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3. Results

3.1. Growth characteristic and competition intensity

At the first harvest, the leaf biomass of *C. lanceolata* declined under drought, while at the second harvest, drought strongly decreased its stem and total biomass and growth rate (Table 1, Supplementary Fig. S2). The interactive effect between competition and drought significantly affected the root biomass and root/shoot ratio (R/S). Specifically, at the second harvest, the root biomass and R/S of well-watered *C. lanceolata* exposed to intra-specific competition were significantly higher than in no-competition conditions, whereas the tendency was reverse under drought (Table 1). The relative interaction index (RII) also displayed these clear changes in competition: the strong intraspecific competition of *C. lanceolata* shifted to facilitation under well-watered conditions, while competition was very strong under drought at the second harvest (Fig. 1).

3.2. Nitrogen allocation and uptake rate

The leaf N concentration significantly decreased by competition (Fig. 2a), while the root N concentrations remained relative stable under competition and drought (Fig. 2b). The NH_4^+ concentration of leaves was lower under competition than without

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competition under drought at the first harvest, while no significant differences among treatments were detected at the second harvest (Fig. 3a). Drought significantly enhanced the NO_3^- concentration of leaves at the first harvest (Fig. 3b). The NH_4^+ and NO_3^- concentrations of roots were not impacted by drought and competition (Fig. 3c, d). $N_{in\text{-}SDS}$ and N_S concentrations were higher at the second harvest in all experiments (Fig. 4a, b). N_W was affected by drought, competition and their interactions (Fig. 4c). The N_W concentration of leaves was lower under competition than in no-competition conditions at the first harvest, but the situation became reversed at the second harvest (Fig. 4c). Similarly as the N concentration of leaves, N_{np} significantly decreased under competition (Fig. 4d).

Nitrogen uptake showed little variation among treatments at the first harvest. However, at the second harvest, N uptake was much higher in well-watered conditions than under drought, particularly when comparing WC and DC (Fig. 5a). The N use efficiency tended to be higher under competition than without competition (Fig. 5b). Drought significantly decreased $\delta^{15}N$ of leaves at the first harvest but increased it at the second harvest (Fig. 5c).

3.3. Physiological responses to drought and competition

The concentrations of leaf chlorophyll *a* and *b* were significantly lower in DS than in DC at the first harvest, while the total chlorophyll concentration in WC was

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significantly higher than that in WS at the second harvest (Supplementary Table S1). The proline concentration of leaves in WC was significantly lower than that in WS at the first harvest, while it was much higher in DC than in DS at the second harvest (Fig. 6a). POD of leaves showed little variation among treatments at the second harvest (Fig. 6b). The proline concentration of roots was lowest in WS at the second harvest (Fig. 6c). However, POD of roots significantly increased under drought and was significantly higher under competition regardless of the water availability (WC vs WS and DC vs DS) (Fig. 6d).

Drought, competition and their interactions significantly impacted the starch concentration of leaves and roots (Table 2). Drought significantly increased the starch concentration of stems and roots at each harvest time. The root starch concentration in DS was higher than that in DC under drought at the second harvest. Competition significantly increased $\delta^{13}C$, and $\delta^{13}C$ in DS was significantly higher than that in DC at the second harvest (Fig. 7).

The ABA concentration of leaves was significantly affected by competition and the interaction between competition and drought (Fig. 8a). ABA in DS was significantly higher than that in DC at the second harvest. IAA of leaves was affected by drought and its interaction with competition. The IAA concentration was much higher in WS than in WC at the second harvest (Fig. 8b). The JA concentration of leaves stayed stable in different treatments (Fig. 8c).

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3.4. Carbon and nitrogen storage

The amounts of nitrogen, starch and soluble sugars exhibited little variation at the first harvest, but they significantly declined under drought at the second harvest (Fig. 9). The amounts of starch and soluble sugars greatly increased during the experiments regardless of water availability. Nitrogen levels showed no significant differences under drought. Starch levels were significantly higher in DS than in DC at the second harvest (Fig. 9b).

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4. Discussion

4.1. Temporal changes in plant-plant competition and N uptake

Competitive traits of plants, for example, carbon assimilation and allocation, and nitrogen uptake and storage, vary across time (Trinder et al., 2012; Craine and Dybzinski, 2013; He et al., 2013; Guo et al., 2017, 2018). A higher competition intensity (indicated by more negative RII) under well-watered conditions, and a lower competition intensity (less negative RII) under water deficit conditions detected in *C. lanceolata* at the first harvest supported the stress gradient hypothesis of plant-plant interactions (Bertness and Callaway, 1994). However, at the second harvest, the plant-plant interactions shifted from intense competition to facilitation under well-watered conditions, while competition became stronger under water deficit conditions (Fig. 1). The total chlorophyll concentration became higher when plants were in lesser competition (Supplementary Table S1) indicating temporal changes in carbon assimilation affected by plant-plant competition (Guo et al., 2018). Therefore, our results failed to support the hypothesis that the intensity of intra-specific competition would be weaker under water deficit, possibly even shifting to facilitation under prolonged drought. Trinder et al. (2012) have argued that studies based on one-time harvest could not fully explain how and to what extent competition affects biomass. Instead, we should combine information on biomass accumulation and

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nutrient uptake across time. Our results also proved that competition relationships and N uptake of *C. lanceolata* changed across time under different soil water conditions (Fig. 1, 5). A greater root size likely enhances N uptake and competitive superiority belowground (Broadbent et al., 2018). Under prolonged drought, root biomass and R/S were greater in WC than in DC (Table 1), implying greater changes in N uptake under drought and competition.

Several studies have showed that a plant's lower N concentration or N uptake is caused by water deficit or competition (Walker et al., 2017; Guiz et al., 2018). In our study, *C. lanceolata* exposed to strong competition showed a lower N uptake rate (Fig. 5a). Previously, Miller et al. (2007) have found that plants under strong competition showed a 50% decrease in N uptake compared to plants without competition. Leaf $\delta^{15}N$ is associated with the presence and type of mycorrhizal associations (Stackpoole et al., 2008; Barthelemy et al., 2017). Higher $\delta^{15}N$ contributes to the presence of ectomycorrhizae and arbuscular mycorrhizae, but lower $\delta^{15}N$ associates with ericoid mycorrhizae (Craine et al., 2009; Barthelemy et al., 2017). Guo et al. (2019) have found that a decreasing competition intensity of *Larix kaempferi* is closely related to soil bacterial and fungal communities. For example, the fungal composition shifted from the *Basidiomycota* dominance to *Ascomycota* dominance, which is suggested to affect nitrogen absorption (Leroy et al., 2017). The $\delta^{15}N$ value was much lower at the first harvest but tended to be higher at the second harvest under drought (Fig. 5c), which implied that a decreasing N uptake caused by drought may be partly related to

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changes in soil microbial communities and consequent effects on plant-plant competition relationships.

4.2. Effects of competition and drought on N allocation

Leaves are more sensitive to drought and competition compared to non-photosynthetic organs, but root modifications strongly impact the physiological processes of leaves during periods of drought (Hommel et al., 2016; Puértolas et al., 2017). A lower leaf carbon/nitrogen content caused by drought (McDowell et al., 2011) or competition (Guiz et al., 2018) implies changes in plants' physiological and metabolic processes (Chen et al., 2014; Sardans et al., 2015). We found that N, NH_4^+ and NO_3^- concentrations of leaves are more sensitive to drought and competition than those of roots (Fig. 2, 3). A higher leaf NH_4^+ concentration and R/S in DS indicated that those plants enhanced water absorption more than plants in DC at the first harvest (Table 1, Fig. 3a). However, as N uptake was strongly limited under prolonged drought, the dramatic decline in leaf NH_4^+ and NO_3^- possibly indicated translocation to the cell wall ($N_{in\text{-}SDS}$) and cell membrane (N_s) or allocation to produce related drought-resistant compounds like proline (Fig. 4, 6). The results illustrated that when N uptake greatly declines, *C. lanceolata* has to enhance N use efficiency and allocate limited internal N resources to maintain functions and to resist prolonged drought (Fig. 5c, 7).

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Leaf N contains four fractions: water-soluble proteins, SDS-soluble proteins, SDS-insoluble proteins and other N (Takashima et al., 2004; Liu et al., 2018). Nitrogen allocated into SDS-insoluble proteins ($N_{in\text{-}SDS}$), which represent cell wall proteins (Takashima et al., 2004), showed little effects caused by drought and competition. However, the allocation of nitrogen into SDS-soluble proteins (N_s), which contain soluble enzymes in stroma and cytosol and membrane-associated proteins (Evans and Seemann, 1989), was much more sensitive to the interactions of drought and competition. Drought damages cell membrane systems, which is visible in the cellular ultrastructure (Chen et al., 2014; Han et al., 2019). The lowest N_s of DC at the second harvest implied that prolonged drought combined with competition may further destroy membranes. Nitrogen participating in water-soluble proteins (N_w) (about one-half is represented by RuBPCase, Takashima et al., 2004) was greatly affected by drought and competition (Fig. 4c).

Drought increases soluble proteins in leaves, which together with proline and antioxidants (POD) function as osmoprotectants contributing to the cellular osmotic adjustment, stabilization of enzymes and protection of membrane integrity (Ahmad et al., 2019). At the first harvest, *C. lanceolata* exposed to weaker competition under drought (DC) had higher N_w than plants exposed to stronger competition under well-watered conditions (WC). This result implied that *C. lanceolata* may have an increased soluble protein content in response to drought (Ahmad et al., 2019). However, at the second harvest, N_w was higher in WC than in DC when competition

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was stronger. The soluble proteins, which are an important internal N source, may be decomposed and then allocated to SDS-soluble proteins (Fig. 4b) or to proline (Fig. 6a), since the N uptake was severely inhibited by interactions between drought and competition under prolonged drought (Liu et al., 2018).

4.3. Combined effect of drought and competition

Plants adapt to water deficit by increasing soil water uptake and water use efficiency (Puértolas et al., 2017; Han et al., 2019). Competition combined with drought causes different physiological responses in photosynthetic traits, leaf water potential and long-term water use efficiency ($\delta^{13}C$), as shown by Chen et al. (2014). We found that *C. lanceolata* enhances its water use efficiency under drought regardless of competition and harvest time, as indicated by higher leaf $\delta^{13}C$ in those conditions (Fig. 7). However, the leaf $\delta^{13}C$ of *C. lanceolata* exposed to competition was much lower than that in plants kept in isolation under prolonged drought. In addition, leaf ABA was lower under competition than in isolation at the second harvest (Fig. 8a). A higher ABA level has been shown to promote drought resistance (Puértolas et al., 2017; Song et al., 2019). The primary functions of NSC are to provide building components and energy storage during different growth stages. Many studies have demonstrated that plants with higher NSC show a better ability to resist drought (Niinemets, 2010; Dong et al., 2016; Hesse et al., 2019). As a crucial energy storage, starch plays an important role in buffering environmental changes under long-term drought periods by releasing

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soluble sugars when photosynthesis is limited (MacNeill et al., 2017). To survive better under drought, plants accumulate more starch (Hesse et al., 2019). We discovered that drought greatly promoted starch accumulation in *C. lanceolata* stems and roots, particularly at the second harvest (Table 2). The amount of starch in *C. lanceolata* roots in DC was significantly lower than that in DS (Table 2, Fig. 9b). Therefore, the combined drought and competition effects impact more seriously the physiological processes of *C. lanceolata* under prolonged drought.

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5. Conclusions

Our study demonstrated a temporal change in the competition relationships of *C. lanceolata* under different soil water conditions. At the early growth stage of *C. lanceolata*, when having neighbors, light availability had a minor effect, while competition for underground resources was evidently the major force influencing plants' performance. Intense competition imposed by neighbors was a great threat to the growth and survival of young *C. lanceolata* plants under prolonged drought, because they were seriously damaged by the combined effects of drought and competition.

Author contributions Qingxue Guo had the main responsibility for data collection, analysis and writing, Xiaoyi Wu contributed to data collection, Helena Korpelainen contributed to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for experimental design and project management.

Conflict of interest The authors declare that they have no conflict of interest.

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Table 1 Effects of competition on biomass and biomass allocation of *C. lanceolata* under well-watered and drought conditions (mean ± S.E., n = 4). The first and second harvest were in September and December, respectively.

Harvest time	Treatment	Leaf (g)	Stem (g)	Root (g)	Total (g)	R/S
First harvest	WS	9.35 ± 0.81^{b}	5.37 ± 0.36^{b}	3.29 ± 0.32^{c}	18.00 ± 1.46^{b}	0.22 ± 0.01^{d}
	WC	9.21 ± 0.57^{ab}	5.06 ± 0.38^{b}	3.96 ± 0.45^{c}	18.23 ± 1.20^{b}	0.28 ± 0.02^{d}
	DS	6.58 ± 0.38^{d}	3.79 ± 0.22^{b}	3.81 ± 0.24^{c}	14.19 ± 0.67^{b}	0.37 ± 0.01^{c}
	DC	6.65 ± 0.42^{d}	4.36 ± 0.22^{b}	3.18 ± 0.16^{c}	14.19 ± 0.50^{b}	0.29 ± 0.02^{d}
Second harvest	WS	17.10 ± 0.52^{a}	13.44 ± 0.75^{a}	7.88 ± 0.26^{b}	38.42 ± 0.53^{a}	0.26 ± 0.01^{d}
	WC	15.25 ± 0.29^{a}	13.66 ± 0.89^{a}	12.98 ± 0.57^{a}	41.88 ± 1.53^{a}	0.45 ± 0.01^{ab}
	DS	7.53 ± 0.61^{abc}	5.25 ± 0.35^{b}	6.24 ± 0.47^{b}	19.03 ± 0.87^{b}	0.49 ± 0.03^{a}
	DC	6.75 ± 0.39^{bc}	4.58 ± 0.29^{b}	4.48 ± 0.10^{c}	15.81 ± 0.52^{b}	0.40 ± 0.01^{bc}
P value	D	<0.001	<0.001	<0.001	<0.001	<0.001
	C	0.088	0.893	0.004	0.876	0.106
	T	<0.001	<0.001	<0.001	<0.001	<0.001
	D×C	0.412	0.989	<0.001	0.033	<0.001
	D×T	<0.001	<0.001	<0.001	<0.001	0.689
	C×T	0.105	0.622	0.005	0.996	0.012
	D×C×T	0.576	0.230	<0.001	0.046	0.003

Different letters denote significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$. *P* values of three-way ANOVAs indicate the significance of drought (D), competition (C), harvest time (T) and their interaction effects on biomass and biomass allocation. WS and WC represent *C. lanceolata* grown in isolation and competition under well-watered conditions, respectively. DS and DC represent *C. lanceolata* grown in isolation and competition under drought, respectively.

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Table 2 Effects of competition on non-structural carbohydrate concentrations (starch and soluble sugars) of *C. lanceolata* under well-watered and drought conditions (mean ± S.E., n = 4). The first and second harvest were in September and December, respectively.

Harvest time	Treatment	Starch concentration			Soluble sugar concentration		
		Leaf	Stem	Root	Leaf	Stem	Root
First harvest	WS	4.94 ± 0.93^{e}	7.70 ± 1.11^{c}	4.48 ± 1.08^{e}	58.04 ± 7.23^{c}	40.39 ± 2.41^{b}	26.82 ± 1.86^{b}
	WC	5.86 ± 0.38^{de}	7.88 ± 0.28^{c}	7.04 ± 1.31^{de}	68.86 ± 0.12^{c}	46.71 ± 1.80^{b}	32.53 ± 1.60^{b}
	DS	8.54 ± 0.75^{cd}	12.52 ± 1.17^{c}	15.41 ± 1.67^{d}	73.69 ± 4.98^{bc}	38.59 ± 4.97^{b}	26.61 ± 3.30^{b}
	DC	4.48 ± 0.58^{e}	13.78 ± 1.22^{c}	9.90 ± 1.10^{de}	73.95 ± 8.11^{bc}	49.27 ± 6.04^{b}	34.06 ± 1.03^{b}
Second harvest	WS	11.11 ± 0.11^{abc}	25.59 ± 1.93^{b}	60.36 ± 1.68^{b}	108.72 ± 7.49^{a}	75.96 ± 4.79^{a}	70.85 ± 5.53^{a}
	WC	9.94 ± 0.75^{bc}	24.31 ± 1.09^{b}	47.27 ± 2.43^{c}	110.74 ± 5.40^{a}	83.30 ± 8.39^{a}	57.98 ± 3.42^{a}
	DS	14.00 ± 0.83^{a}	32.85 ± 1.37^{a}	90.66 ± 3.90^{a}	100.85 ± 5.74^{ab}	94.75 ± 4.16^{a}	63.12 ± 3.97^{a}
	DC	12.38 ± 0.50^{ab}	34.80 ± 2.03^{a}	63.90 ± 2.36^{b}	115.32 ± 3.51^{a}	96.17 ± 5.91^{a}	63.26 ± 3.42^{a}
P value	D	0.001	<0.001	<0.001	0.308	0.047	0.899
	C	0.005	0.590	<0.001	0.113	0.108	0.961
	T	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	D×C	0.009	0.277	0.001	0.910	0.920	0.105
	D×T	0.117	0.082	<0.001	0.164	0.057	0.669
	C×T	0.855	0.841	<0.001	0.749	0.597	0.007
	D×C×T	0.026	0.583	0.343	0.183	0.511	0.210

Different letters denote significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$. *P* values of three-way ANOVAs indicate the significance of drought (D), competition (C), harvest time (T) and their corresponding interaction effects. WS and WC represent *C. lanceolata* grown in isolation and competition under well-watered conditions, respectively. DS and DC represent *C. lanceolata* grown in isolation and competition under drought, respectively.

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Figure legends

Figure 1 Relative interaction index (RII) of *C. lanceolata* under well-watered and drought conditions. The white bars indicate first harvest in September, while the black bars indicate second harvest in December. WC: competition under well-watered conditions; DC: competition under drought. Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$.

Figure 2 Leaf and root nitrogen concentrations of *C. lanceolata* under well-watered and drought conditions. The white bars indicate first harvest in September, while the black bars indicate second harvest in December. WS: no competition under well-watered conditions; WC: competition under well-watered conditions; DS: no competition under drought; DC: competition under drought. Significant or marginally significant *P* values show drought, competition, harvest time and their interaction effects, as analyzed by three-way analysis of variance (ANOVA). D: drought effect; C: competition effect; D×T: drought and harvest time interaction effect. Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$.

Figure 3 Leaf and root NH_4^+ and NO_3^- concentrations of *C. lanceolata* under well-watered and drought conditions. The white bars indicate first harvest in

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September, while the black bars indicate second harvest in December. WS: no competition under well-watered conditions; WC: competition under well-watered conditions; DS: no competition under drought; DC: competition under drought. Significant or marginally significant *P* values show drought, competition, harvest time and their interaction effects, as analyzed by three-way analysis of variance (ANOVA). D: drought effect; C: competition effect; T: harvest time effect; D×C: drought and competition interaction effect; C×T: competition and harvest time interaction effect; D×T: drought and harvest time interaction effect; D×C×T: drought, competition and harvest time interaction effect. Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$. Note that root NH_4^+ and NO_3^- concentration measurements were conducted only at second harvest, as described in material and methods, and were analyzed by two-way analysis of variance (ANOVA).

Figure 4 Leaf nitrogen allocation of *C. lanceolata* under well-watered and drought conditions. The white bars indicate first harvest in September, while the black bars indicate second harvest in December. $N_{in\text{-}SDS}$: leaf nitrogen allocation to detergent-insoluble proteins, N_S: leaf nitrogen allocation to detergent-soluble proteins, N_W: leaf nitrogen allocation to water-soluble proteins; N_{np}: leaf nitrogen allocation to others. WS: no competition under well-watered conditions; WC: competition under well-watered conditions; DS: no competition under drought; DC: competition under drought. Significant or marginally significant *P* values show drought, competition,

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harvest time and their interaction effects, as analyzed by three-way analysis of variance (ANOVA). D: drought effect; C: competition effect; T: harvest time effect; D×C: drought and competition interaction effect; C×T: competition and harvest time interaction effect; D×T: drought and harvest time interaction effect; D×C×T: drought, competition and harvest time interaction effect. Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$.

Figure 5 Nitrogen uptake, use efficiency and $\delta^{15}N$ of *C. lanceolata* under well-watered and drought conditions. The white bars indicate first harvest in September, while the black bars indicate second harvest in December. WS: no competition under well-watered conditions; WC: competition under well-watered conditions; DS: no competition under drought; DC: competition under drought. Significant or marginally significant *P* values show drought, competition, harvest time and their interaction effects, as analyzed by three-way analysis of variance (ANOVA). D: drought effect; C: competition effect; T: harvest time effect; D×T: drought and harvest time effect. Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$. Nitrogen uptake was analyzed using Kruskal Wallis test, as described in material and methods.

Figure 6 Proline concentrations and POD activities of *C. lanceolata* under well-watered and drought conditions. The white bars indicate first harvest in

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September, while the black bars indicate second harvest in December. WS: no competition under well-watered conditions; WC: competition under well-watered conditions; DS: no competition under drought; DC: competition under drought. Significant or marginally significant *P* values show drought, competition, harvest time and their interaction effects, as analyzed by three-way analysis of variance (ANOVA). D: drought effect; C: competition effect; T: harvest time effect; D×C: drought and competition interaction effect; C×T: competition and harvest time interaction effect; D×T: drought and harvest time interaction effect; D×C×T: drought, competition and harvest time interaction effect. Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$. Note that root proline concentration and POD activity measurements were conducted only at second harvest, as described in material and methods, and were analyzed by two-way analysis of variance (ANOVA).

Figure 7 Leaf $\delta^{13}C$ of *C. lanceolata* under well-watered and drought conditions. The white bars indicate first harvest in September, while the black bars indicate second harvest in December. WS: no competition under well-watered conditions; WC: competition under well-watered conditions; DS: no competition under drought; DC: competition under drought. Significant or marginally significant *P* values show drought, competition, harvest time and their interaction effects, as analyzed by three-way analysis of variance (ANOVA). D: drought effect; C: competition effect; T: harvest time effect; D×C: drought and competition interaction effect; D×C×T: drought,

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competition and harvest time interaction effect. Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$.

Figure 8 Abscisic acid (ABA), indole acetic acid (IAA) and jasmonic acid (JA) levels in the leaves of *C. lanceolata* under well-watered and drought conditions at second harvest. WS: no competition under well-watered conditions; WC: competition under well-watered conditions; DS: no competition under drought; DC: competition under drought. Significant or marginally significant *P* values show drought, competition and their interaction effects, as analyzed by two-way analysis of variance (ANOVA). D: drought effect; C: competition effect; D×C: drought and competition effect; Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$.

Figure 9 Nitrogen, starch and soluble sugar amounts of *C. lanceolata* under well-watered and drought conditions. The white bars indicate first harvest in September, while the black bars indicate second harvest in December. WS: no competition under well-watered conditions; WC: competition under well-watered conditions; DS: no competition under drought; DC: competition under drought. Significant or marginally significant *P* values show drought, competition, harvest time and their interaction effects, as analyzed by three-way analysis of variance (ANOVA). D: drought effect; C: competition effect; T: harvest time effect; D×C: drought and

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competition interaction effect; D×C×T: drought, competition and harvest time interaction effect. Different letters indicate significant differences among treatments according to Tukey's HSD test at a significance level of $P < 0.05$.

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Figure 1

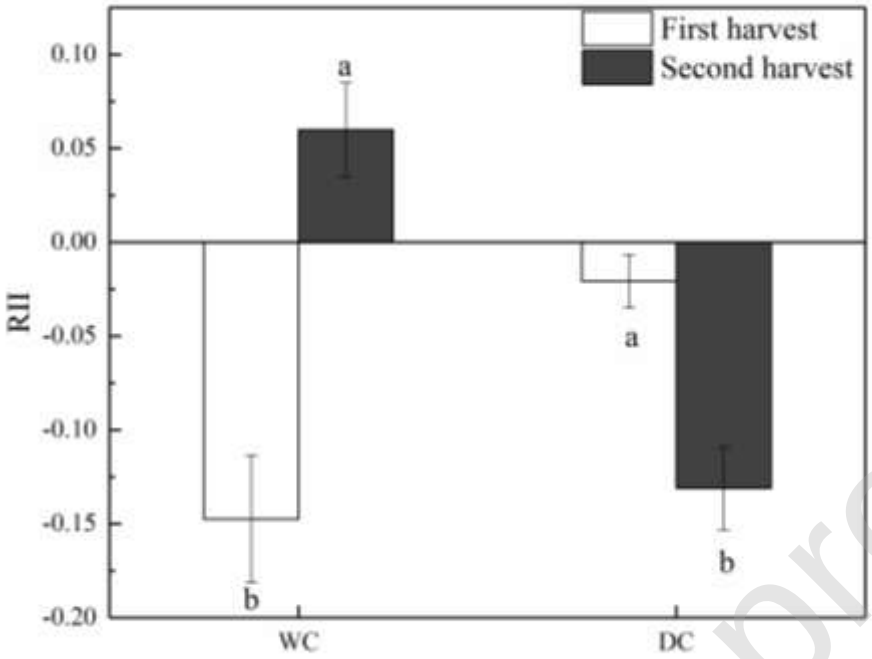

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Figure 2

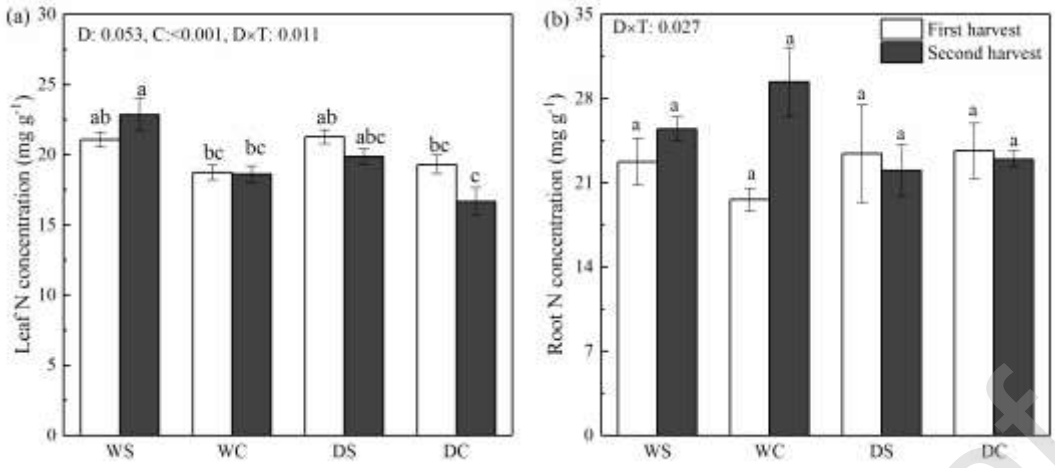

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Figure 3

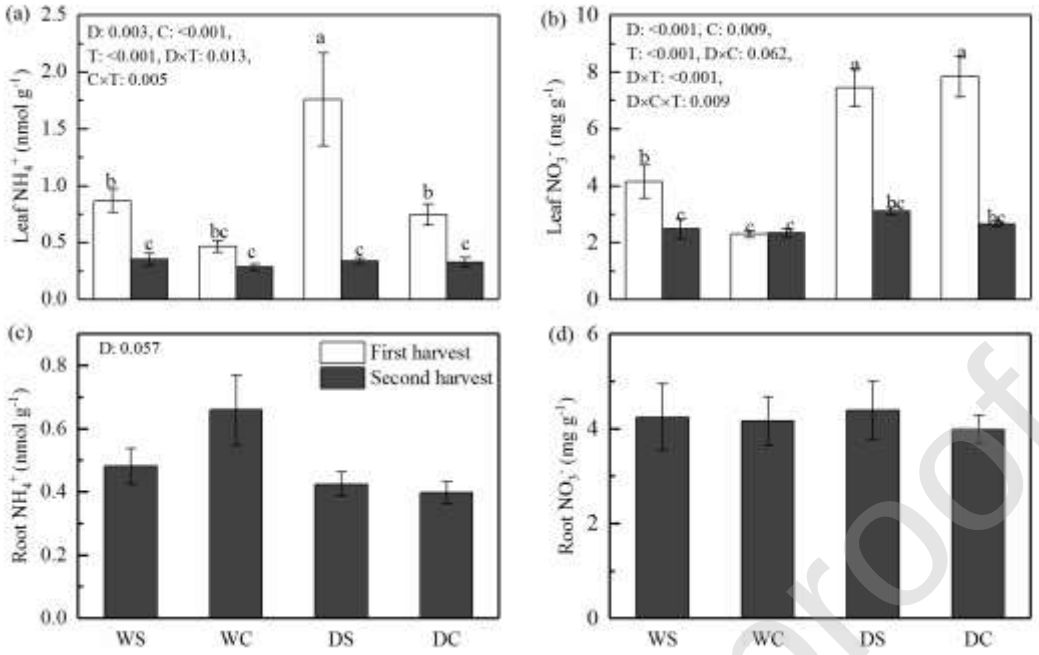

Figure 4

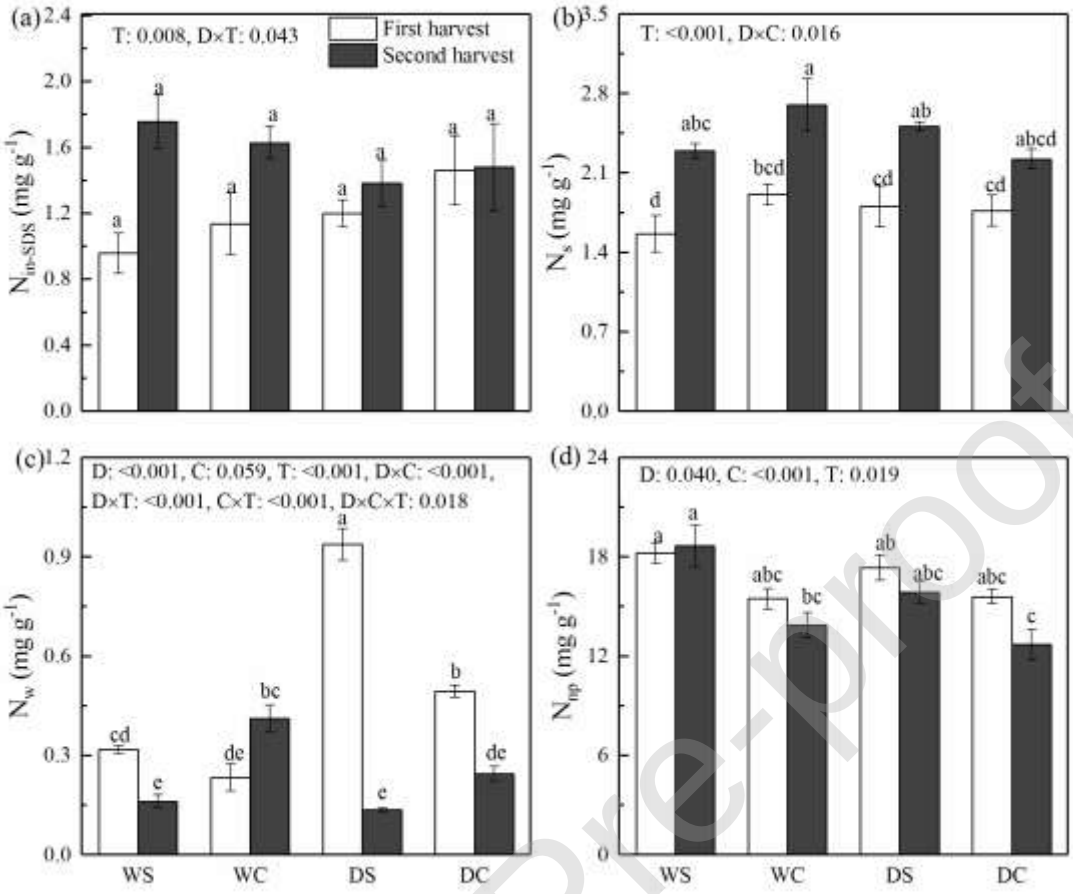

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Figure 5

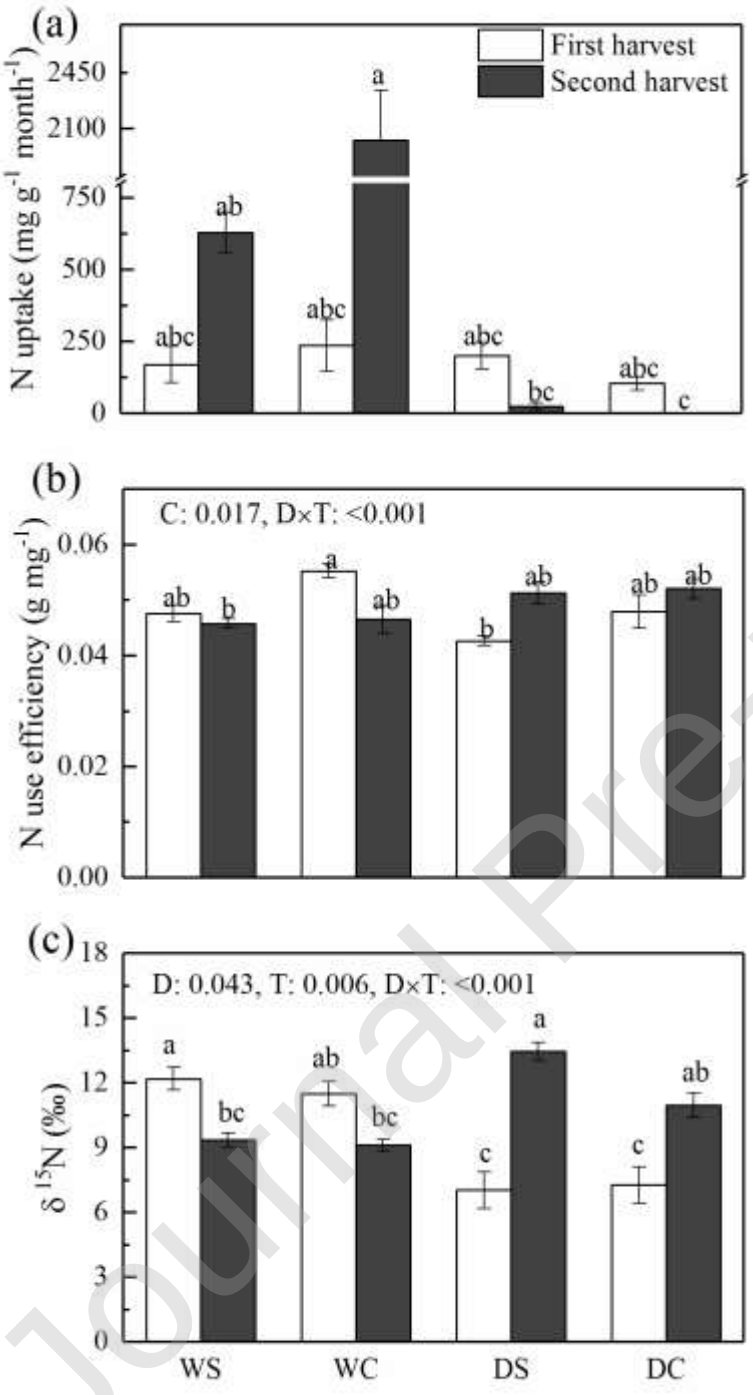

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Figure 6

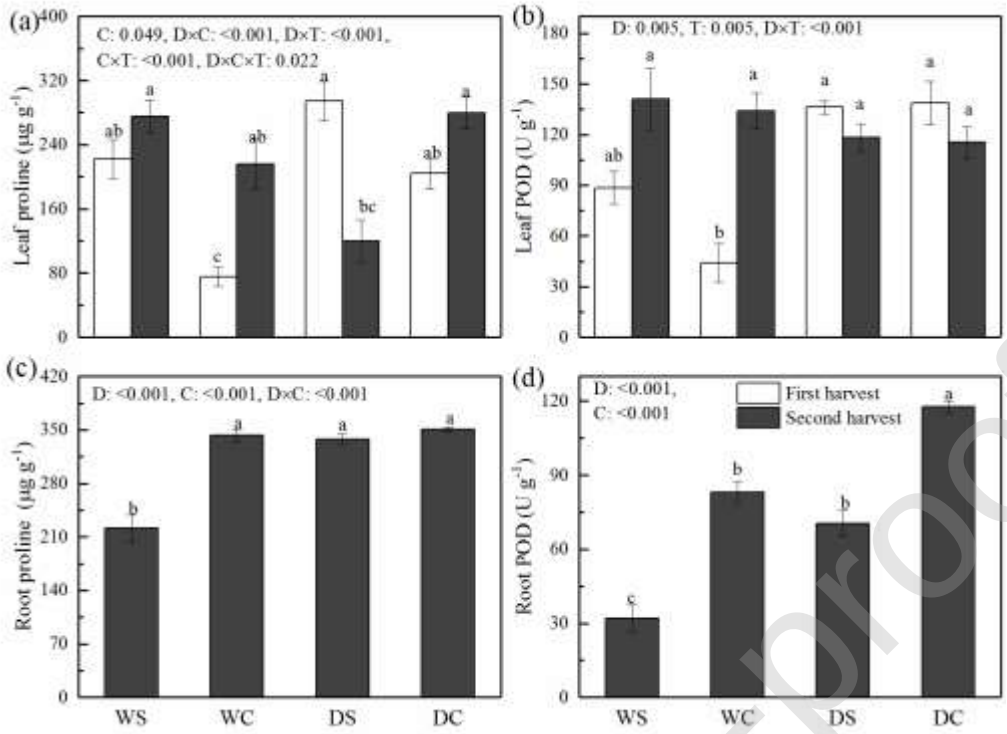

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Figure 7

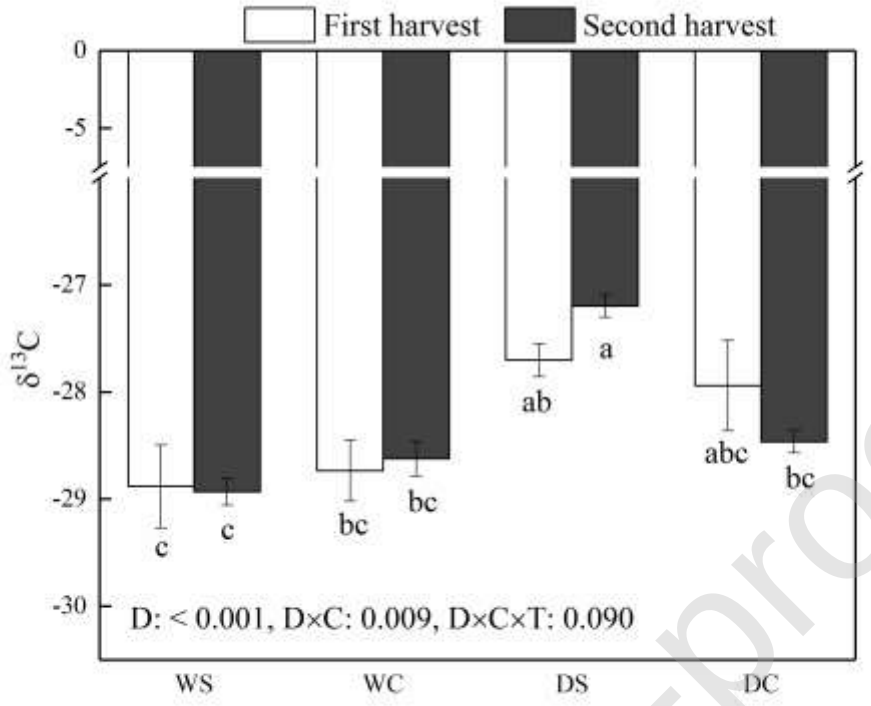

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Figure 8

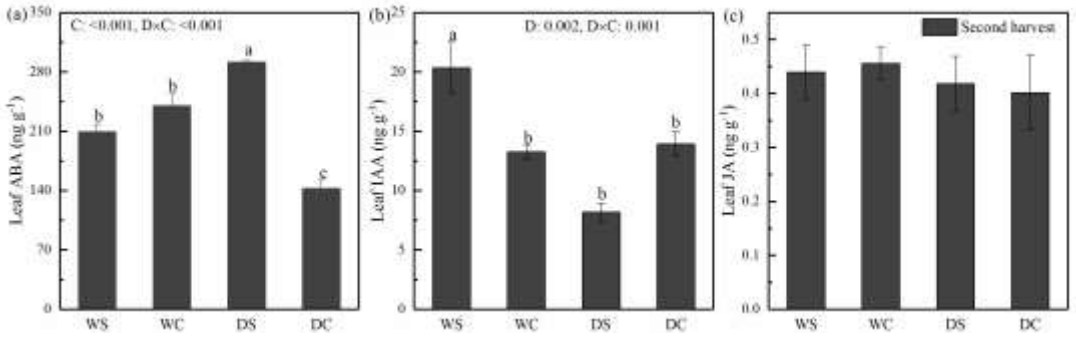

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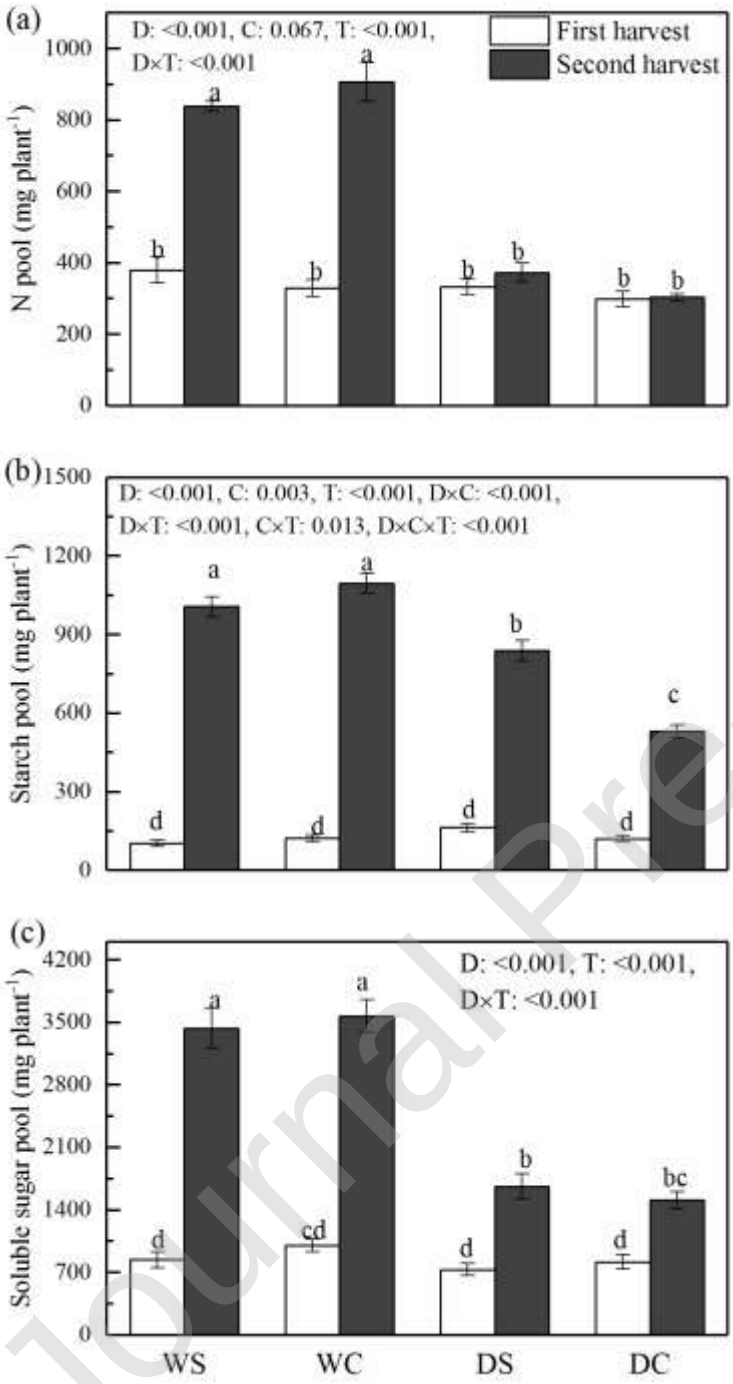
Figure 9



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