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Moderate Nitrogen Deposition Alleviates Drought Stress of *Bretschneidera sinensis*

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Abstract: Droughts are becoming more frequent and intense, and the nitrogen deposition rate is increasing worldwide due to human activities. Young seedlings of *Bretschneidera sinensis* Hemsl. are susceptible to mortality under drought conditions because their root tips have few root hairs. We studied the effect of nitrogen deposition on the physiological characteristics of two-year-old *B. sinensis* seedlings under drought stress. Seedlings were grown under no nitrogen deposition (control; N0), low nitrogen deposition (N30, 30 kg·hm^{-2} year^{-1}), medium nitrogen deposition (N60, 60 kg·hm^{-2} year^{-1}), and high nitrogen deposition (N90, 90 kg·hm^{-2} year^{-1}), and were subjected to either the normal watering regime (NW) or drought stress (DW). Under DW, the relative conductivity (RC) of seedlings receiving N60 was not significantly different from that of N0 seedlings, and the RC of seedlings receiving N90 was significantly higher than that of N0 seedlings. Under 10 d DW, N60 treatment increased antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities and content of soluble protein, chlorophyll a and a + b, with POD activity and soluble protein significantly increasing by 18.89% and 34.66%, respectively. Under DW, the proline (PRO) content of seedlings treated with N90 increased. Our data suggested that moderate nitrogen deposition could alleviate drought stress by decreasing cell membrane permeability, reducing cell membrane peroxidation, increasing the content of osmoregulatory substances, and reducing the tendency for chlorophyll to decline, whereas high nitrogen deposition increased the sensitivity of *B. sinensis* seedlings to drought stress and aggravated the degree of stress, thereby affecting growth.

Keywords: *Bretschneidera sinensis* Hemsl.; nitrogen deposition; drought; cell membrane permeability/peroxidation; osmoregulation; chlorophyll

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1. Introduction

Nitrogen deposition is part of the nitrogen cycle and is the process whereby reactive nitrogen emissions into the atmosphere enter the Earth's ecosystems. Since the beginning of the industrial era, nitrogen deposition fluxes in ecosystems have increased dramatically, mainly due to human activities [1]. Studies predict that total global nitrogen deposition will reach 50~80 Tg·year^{-1} by 2030 [2] and will continue to increase over time [3]. Nitrogen deposition is considered to be the third greatest threat to global biodiversity, and current nitrogen deposition levels have been shown to have significant negative effects on biodiversity [4]. An increase in the current atmospheric nitrogen deposition levels could influence food production, environmental quality, and climate change at regional and global scales [5,6]. For example, species richness declines as a linear function of the rate of inorganic nitrogen deposition, with a reduction of one species per 4 m^2 quadrat for every 2.5 kg·ha^{-1} year^{-1} of chronic nitrogen deposition [7]. Nitrogen deposition has rapidly increased since the beginning of industrialization and is influencing forest ecosystem processes and functions on a global scale [8,9]. A study by Etzold et al., suggested that nitrogen deposition is at least as important as the climate in regulating forest growth in continental Europe, with possible negative effects in areas of high nitrogen deposition [10].

Furthermore, Schulte-Uebbing has shown that nitrogen deposition enhances carbon (C) sequestration in woody biomass in only a third of global forests, mainly in the boreal region, and reduces C sequestration in 5% of forests, mainly in the tropics [11]. As nitrogen deposition increases, the impact on the ecosystem reaches a critical tipping point [12–14]. For example, a nitrogen deposition rate of 35 kg·ha^{-1} year^{-1} caused an 84% loss in the abundance of *Rhytidiadelphus squarrosus* (Hedw.) Warnst. [15], and a rate of 15 kg·ha^{-1} year^{-1} almost led to the disappearance of *Sphagnum balticum* (Russ) C. Jens. [16]. In some cases, a non-linear effect of nitrogen deposition has been observed in forest ecosystems. This is most pronounced in beech forests, which have a tipping point at 30 kg·ha^{-1} year^{-1} [10]. Furthermore, a study of Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) forest soils showed that although increasing nitrogen deposition enhanced C sequestration, nitrogen deposition above 20–30 kg·ha^{-1} year^{-1} could lead to nitrogen saturation [17]. Many countries have serious nitrogen deposition problems, including China. For example, the forest ecosystem in southern China is being damaged by long-term high-level nitrogen deposition [6,18].

Globally, the intensification of the hydrologic cycle is expected to increase the frequency and magnitude of climate extremes, such as severe drought and intense rainfall [19–21]. Global change-type drought occurs in conjunction with warmer temperatures due to climate change [22] and is already affecting forests on every wooded continent and driving regional tree mortality [23]. These extreme climate events are likely to interact with chronic environmental changes, such as nitrogen deposition [24,25]. Nitrogen deposition affects plant responses to drought [26–28]. For example, in a meadow steppe study, net ecosystem carbon dioxide exchange in water- and nitrogen-addition plots were more sensitive to drought stress than the control plots [29]. Nitrogen deposition also increases the sensitivity of the perennial grass *Molinia caerulea* (L.) Moench to drought [30]. However, moderate nitrogen deposition can partially offset the negative effects of drought stress on *Passiflora edulis* (Carr.) seedling development [31]. By contrast, Valliere et al. found that the effects of extended drought on shrubland ecosystems may be more severe under elevated nitrogen deposition [32]. In a study by Pivovaroff et al., nitrogen deposition affected the ability of native Southern Californian shrubs to respond to drought [33]. Many forests are experiencing increased nitrogen deposition and are expected to suffer in the future from increased drought frequency and intensity [34]. However, van der Graaf et al. found that nitrogen deposition did not have a consistent negative or positive effect on the response of forest productivity to drought at the FLUXNET forest site in Europe [35]. Interactions between seasonal drought and nitrogen deposition varied with drought severity. Furthermore, the interactive effects of these two drivers on gross primary productivity, ecosystem respiration, and net ecosystem productivity were additive under mild and moderate drought conditions but non-additive under severe drought [36]. However, long-term changes in precipitation regimes and nitrogen deposition may significantly alter the susceptibility of key ecosystem processes to drought stress. Therefore, understanding the interaction between nitrogen and water availability in variable environments and how plants respond to this rapid environmental change is important for predicting future ecosystem changes.

Bretschneidera sinensis Hemsl. is a monocotyledonous, endangered Tertiary relict plant that is endemic to China. This rare species, which is sporadically distributed in the mountainous forest areas of some provinces south of the Yangtze River in China, has been considered endangered for a long time owing to the destruction of its habitat, the scarceness of existing parent trees, the low survival rate of seedlings, and poor natural regeneration, combined with less fruitful under natural conditions, wildlife feeding on the seeds and human felling [37]. In 2021, it was listed as a Grade II protected wild plant in China. *B. sinensis* root tips have few root hairs and, therefore, the absorption of water and inorganic salts by epidermal cells alone is obviously weak. Seedlings without a good root system are therefore very sensitive to drought stress and are prone to die in the summer when there is little rain and high temperatures, which is one of the main reasons that this species is endangered [38]. The effect of atmospheric nitrogen deposition on the drought-resistance physiological characteristics of *B. sinensis* seedlings in the context of climate change has rarely been reported. Therefore, the aim of this study was to investigate the effects of different nitrogen deposition levels on the drought-resistance physiology of *B. sinensis* seedlings, including cell membrane permeability, the antioxidant system, and osmoregulatory substances. These data should provide a theoretical basis and technical guidance for *B. sinensis* cultivation in the future and may illuminate the mechanisms behind *B. sinensis* responses to environmental change.

2. Materials and Methods

2.1. Plant Materials and Fertilizer

Two-year-old *B. sinensis* container seedlings grown from seeds of the same mother plant were used as experimental material. In December 2021, seedlings (average height, 32.2 cm; average ground diameter, 5.1 mm) were selected and transplanted into pots (diameter 16.5 cm, height 25.0 cm) containing 2.0 kg of yellow soil, one seedling per pot. Soil physical and chemical properties were as follows: pH, 6.3; total nitrogen, 1.14 $g \cdot kg^{-1}$; alkali nitrogen, 103.74 $mg \cdot kg^{-1}$; total phosphorus, 0.68 $g \cdot kg^{-1}$; available phosphorus, 9.75 $mg \cdot kg^{-1}$; total potassium, 21.22 $g \cdot kg^{-1}$; available potassium, 429.83 $mg \cdot kg^{-1}$.

2.2. Experimental Treatments

The experiment was set up using a two-factor completely randomized design involving nitrogen deposition (Factor A) and water availability (Factor B). There were four nitrogen deposition levels: no nitrogen deposition, which acted as a control (N0); low nitrogen deposition (N30, 30 $kg \cdot hm^{-2}$ $year^{-1}$); medium nitrogen deposition (N60, 60 $kg \cdot hm^{-2}$ $year^{-1}$); and high nitrogen deposition (N90, 90 $kg \cdot hm^{-2}$ $year^{-1}$). There were two water supply conditions: normal water supply (NW) and drought stress (DW). Therefore, in total, there were eight treatments, with 45 replicates of each treatment, totaling 360 pots. The monthly NH_4NO_3 application rate per pot based on the planting opening area of the pot was calculated. NH_4NO_3 (purity > 99%) was dissolved in distilled water at room temperature. Complete dissolution was achieved after several hours of stirring at room temperature using a magnetic stirrer. The nitrogen deposition treatment began on 21 December 2021, and finished on 25 July 2022, with 50 mL of 0.000000, 0.003816, 0.007633, or 0.011449 $mol \cdot L^{-1}$ NH_4NO_3 solution applied per pot on the 21st day of each month, i.e., eight applications of the nitrogen deposition treatments in total. The nitrogen application levels used in this experiment were based on the nitrogen deposition levels in this region of China and those used in similar international studies [30]. Before the drought treatment began, the soil moisture content was maintained at a sufficient level. The drought treatment began on 21 June 2022. All pots were watered once and then allowed to dry out naturally. After four days, the water content of the soil reached the drought stress level (the relative water content of the soil reached a field water-holding capacity of 35 $\pm$ 5%). The weight of the container was measured between 16:00 and 18:00 h every day and the water consumed that day was replenished to control the water treatment conditions. The upper functional leaves (1.0 g) of five seedlings in each treatment were sampled on 5 July and 25 July 2022, and rapidly frozen in liquid nitrogen for later determination of their physiological indexes.

2.3. Determination of Cell Membrane Permeability

The upper functional leaves were washed with tap water and then rinsed three times with distilled water. Surface water was removed with filter paper before cutting the leaves into long strips of a suitable length (avoiding the main veins). Three fresh samples, 0.1 g each, were soaked at room temperature in 10 mL of deionized water for 12 h inside a tube sealed with a glass tube. The electrical conductivity of the extract was measured (R1) using a conductometer. The extract was then heated in a boiling water bath for 30 min before cooling to room temperature and shaking well. The electrical conductivity of the extract was measured again (R2). Relative conductivity (RC) = R1/R2 $\times$ 100%. The malondialdehyde (MDA) content was measured using kits produced by Suzhou Keming

Biological Technology Co., Ltd., Suzhou, Jiangsu, China. The 0.1 g samples were weighed and 1.0 mL of the extract was added. The samples were homogenized and then centrifuged at 8000 rpm at 4 °C for 10 min. The optical density (OD) value at 532 nm and 600 nm was measured by taking the supernatant. The MDA content in the fruit (μmol/g FW) was calculated according to the formula: MDA content (nmol/g FW) = 51.6 × (OD_{532} − OD_{600})/0.1 [39].

2.4. Determination of Protective Enzyme Activity

Protective enzyme activity was performed according to the reagent kits (Suzhou Keming Biological Technology Co., Ltd., Suzhou, China) method. After thawing the samples that had been frozen in liquid nitrogen, the samples were maintained at 2 °C to 8 °C. To 0.1 g of each of these samples, 1.0 mL of extract was added. The samples were homogenized and then centrifuged at 8000 rpm at 4 °C for 10 min. The supernatants were collected carefully, and the activities of protective enzymes were measured using enzyme-linked immunosorbent assay kits. The absorbance at 470 nm for 1 min and the absorbance after 2 min were recorded for the calculation of peroxidase (POD) activity. Measurement of absorbance at 450 nm was used to calculate superoxide dismutase (SOD) activity. The initial absorbance at 240 nm and the absorbance after 1 min were recorded for the calculation of catalase (CAT) activity [40].

2.5. Determination of Osmotic Adjustment Substance Content

About 0.1 g of each sample was homogenized in an ice bath with 1 mL of extract solution before placing in a 95 °C water bath for 10 min, centrifuging at 10,000 rpm at 25 °C for 10 min, and then measuring the proline (PRO) content in the supernatant. Next, 1 mL of distilled water was added to 0.1 g of each sample and ground to form a homogenate before pouring it into a centrifuge tube, which was then sealed and placed in a 95 °C water bath for 10 min. After cooling, the homogenate was centrifuged at 8000 rpm at 25 °C for 10 min. The supernatant was placed in a 10 mL test tube, made up to 10 mL with distilled water, shaken well, and then set aside for soluble sugar content determination. About 0.1 g of each sample was added to 1 mL of distilled water, homogenized in an ice bath, centrifuged at 10,000 rpm at 4 °C for 10 min, and the soluble protein content in the supernatant was then measured. PRO, soluble sugar, and soluble protein content were measured using kits produced by Suzhou Keming Biological Technology Co., Ltd., Suzhou, Jiangsu, China [40].

2.6. Determination of Chlorophyll Content

The upper functional leaves (0.2 g) of five seedlings in each treatment were sampled. Chlorophyll content was extracted by ethanol, measured using UV spectrophotometry, and calculated according to the following formula: chlorophyll a = 12.7 × OD663 − 2.69 × OD645; chlorophyll b = 22.9 × OD645 − 4.68 × OD663, where OD is optical density [41].

2.7. Determination of Growth Parameters

Before the nitrogen deposition treatment, ten plants were selected from each treatment and tagged with a number, and the initial seedling height and ground diameter were measured with a tape measure and vernier calipers, and the height and ground diameter of the tagged seedlings were measured again at the end of the nitrogen deposition treatment. The tagged seedlings were washed and dried with tap water, then placed in an oven at 105 °C for 20 min, then baked at 75 °C for 24 h to a constant weight and the dry mass of the different parts was weighed [42].

2.8. Statistical Analysis

Two-way analysis of variance (ANOVA) was used to compare treatment effects, and the treatments means were compared by Tukey's multiple-range test ($p < 0.05$). All data analyses were performed using Statistical Product and Service Solutions (SPSS) version 23.0. Figures and Tables were drafted using Microsoft Office 2016.

3. Results

3.1. Cell Membrane Permeability

Under DW, the MDA content and RC of seedlings were significantly higher than that of seedlings grown under NW (Figure 1). Under 10 d NW and 30 d NW, the MDA content of seedlings treated with N60 was significantly lower (10.50% and 11.39% lower, respectively) than that of N0 seedlings. By contrast, compared with N0 seedlings, the MDA content of seedlings treated with N90 was 6.65% higher under 30 d NW (Figure 1a). Although under 10 d DW and 30 d DW N30 had no significant effect on MDA content when compared with that of N0 seedlings, N90 significantly increased MDA content (19.14% and 13.48% higher, respectively). Under NW, seedlings treated with N30 or N60 had a lower RC value than that of N0 seedlings under NW (Figure 1b). RC values obtained under 10 d DW or 30 d DW and N30 or N60 were not significantly different from the RC value of N0 seedlings. Under 10 d DW or 30 d DW, the RC of seedlings that received N90 was significantly higher (17.13% and 18.25% higher, respectively) than that of N0 seedlings (Figure 1b). The MDA content and RC of seedlings that received N30 or N60 were either not significantly different from those of N0 seedlings or were significantly lower than those of N0 seedlings under NW and DW, indicating that N30 and N60 were beneficial for maintaining cell membrane permeability (Figure 1).

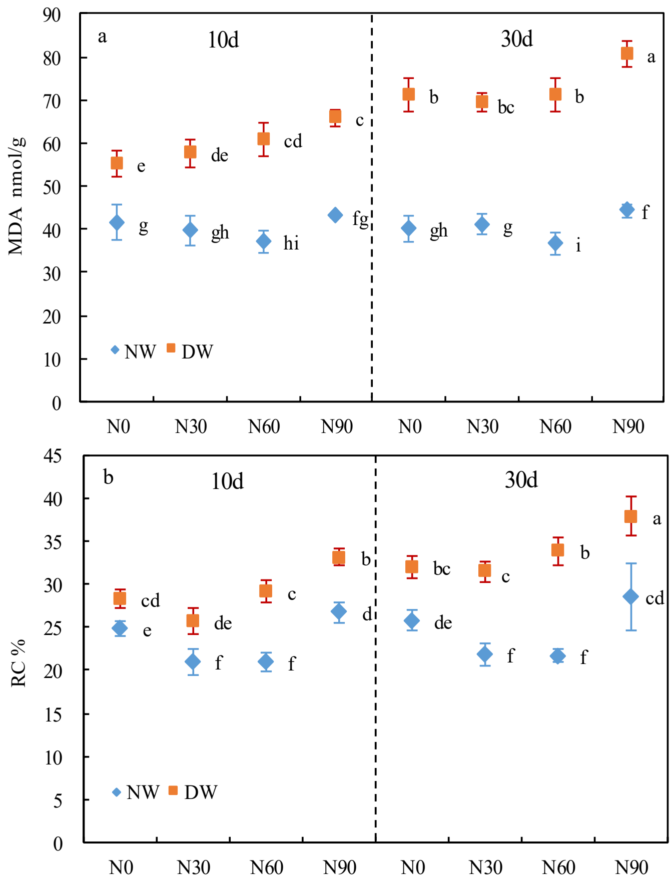


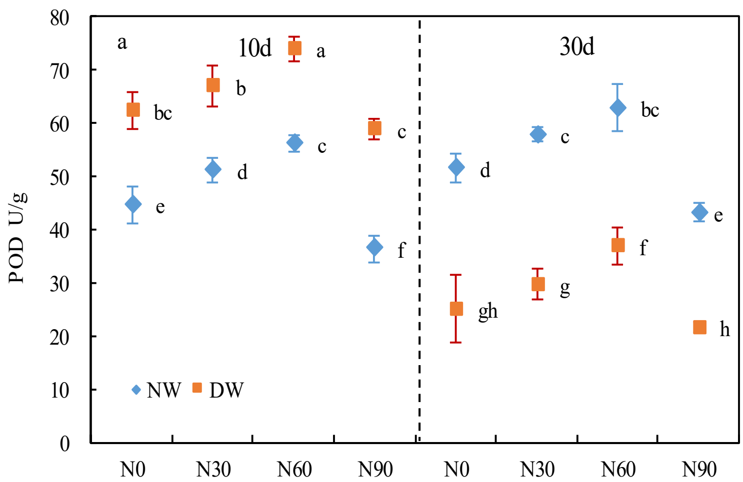
Figure 1. (**a**) Effect of drought stress and nitrogen deposition on MDA content; (**b**) effect of drought stress and nitrogen deposition on RC. Water supply treatments: normal water supply (NW) or drought stress (DW) for 10 d or 30 d. Nitrogen deposition treatments: control (N0, 0 kg·hm^{-2} year^{-1}), low nitrogen deposition (N30, 30 kg·hm^{-2} year^{-1}), medium nitrogen deposition (N60, 60 kg·hm^{-2} year^{-1}), or high nitrogen deposition (N90, 90 kg·hm^{-2} year^{-1}). Abbreviations: MDA, malondialdehyde; RC, relative conductivity. Data points represent means ± the SD; n = 5. Data points with the same lowercase letter are significantly different ($p < 0.05$) according to Tukey's test.

3.2. Protective Enzyme System

Under 10 d NW, seedlings had lower POD, SOD, and CAT activity than that of seedlings under 10 d DW; however, under 30 d DW, the results were reversed (Figure 2). Under 10 d DW or 30 d DW, the POD activity of seedlings treated with N60 was 18.89% and 46.94% higher, respectively, than that of N0 seedlings (Figure 2a). However, under 10 d DW or 30 d DW, the POD activity of seedlings treated with N30 or N90 was not significantly different from that of N0 seedlings (Figure 2a). Under 10 d NW, the SOD activity of seedlings treated with N60 was significantly higher than that of N0 seedlings. Under 30 d NW, the nitrogen deposition level had no significant effect on SOD activity (Figure 2b). By contrast, the SOD activity of seedlings treated with N90 was significantly lower (17.33% lower) than that of N0 seedlings when grown under 30 d DW (Figure 2b). The CAT activity of seedlings treated with N30, N60, or N90 was significantly higher than that of N0 seedlings under 10 d NW or 30 d NW (Figure 2c). Seedlings treated with N30, N60, or N90 had higher CAT activity under 10 d DW than N0 seedlings. Under 30 d DW, N30 and N60 had no significant effect on CAT activity; however, the CAT activity of seedlings treated with N90 was significantly lower (38.50% lower) than that of N0 seedlings (Figure 2c).

3.3. Osmoregulatory Substances

The DW treatment increased the PRO content of seedlings (Figure 3a). Under 10 d DW and 30 d DW, the PRO content of seedlings that received N90 was 6.19% and 13.98% higher, respectively, than that of N0 seedlings (Figure 3a). Under 10 d DW, the soluble sugar content of N30, N60, and N90 seedlings was not significantly different from that of the N0 seedlings (Figure 3b). Under 30 d DW, only seedlings treated with N60 had a significantly higher soluble sugar content (22.88%) than that of N0 seedlings (Figure 3b). The soluble protein content of seedlings treated with N30 and N60 was 38.18% and 34.66% higher, respectively, than that of N0 seedlings; however, the soluble protein content of seedlings treated with N90 did not differ significantly from that of N0 seedlings under 10 d DW (Figure 3c). Under 30 d DW, the soluble protein content of seedlings treated with N30 or N60 was significantly higher (61.69% and 56.01% higher, respectively) than that of N0 seedlings (Figure 3c). By contrast, under 30 d DW, the soluble protein content of seedlings treated with N90 was significantly lower than that of N0 seedlings (Figure 3c).



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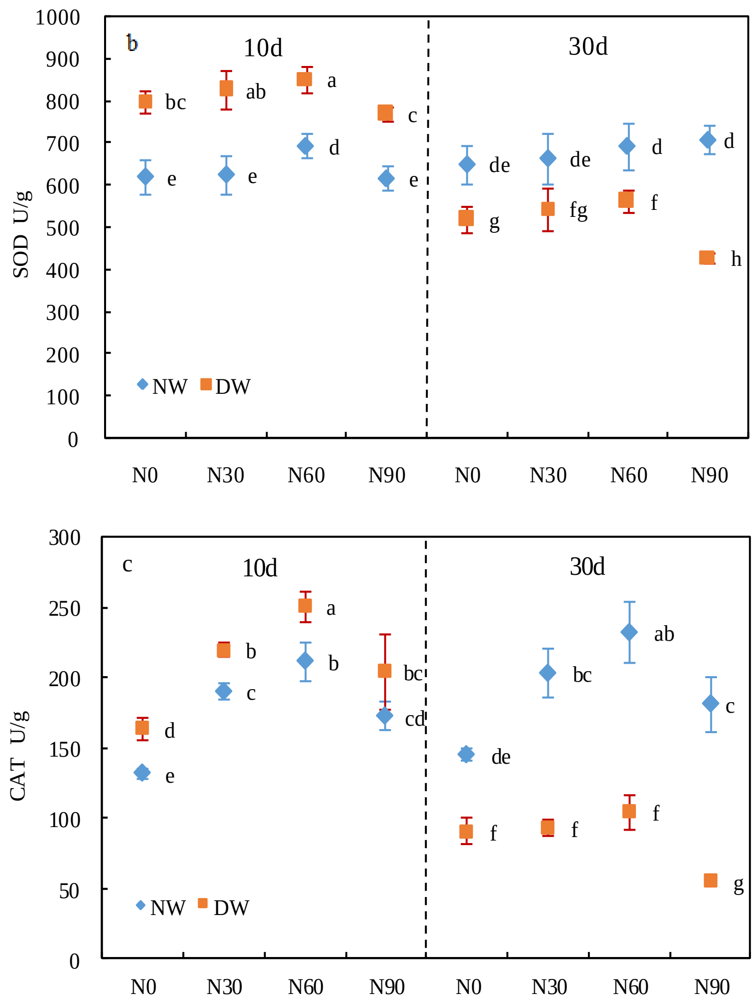

Figure 2. (**a**) Effect of drought stress and nitrogen deposition on POD activity; (**b**) effect of drought stress and nitrogen deposition on SOD activity; (**c**) effect of drought stress and nitrogen deposition on CAT activity. Water supply treatments: normal water supply (NW) or drought stress (DW) for 10 d or 30 d. Nitrogen deposition treatments: control (N0, 0 kg·hm^{-2} year^{-1}), low nitrogen deposition (N30, 30 kg·hm^{-2} year^{-1}), medium nitrogen deposition (N60, 60 kg·hm^{-2} year^{-1}), or high nitrogen deposition (N90, 90 kg·hm^{-2} year^{-1}). Abbreviations: POD, peroxidase; SOD, superoxide dismutase; CAT, catalase. Data points represent means ± the SD; n = 5. Data points with the same lowercase letter are significantly different ($p < 0.05$) according to Tukey's test.

3.4. Chlorophyll Content

Water supply had a significant effect on the chlorophyll a, b, a + b content and chlorophyll a/b of seedlings (Table 1). Under 10 d NW and 30 d NW, the chlorophyll a content of seedlings that received N60 was 19.3% and 18.6% higher, respectively, than that of N0 seedlings. The chlorophyll a and a + b content of seedlings grown under DW that received N60 were significantly higher than that of N0 seedlings (Table 1). By contrast, the chlorophyll a and a + b content of seedlings treated with N30 and N90 were either not significantly different from those of N0 seedlings or were significantly lower than those of N0 seedlings, indicating that medium nitrogen deposition could alleviate the negative effects of drought on chlorophyll content (Table 1).

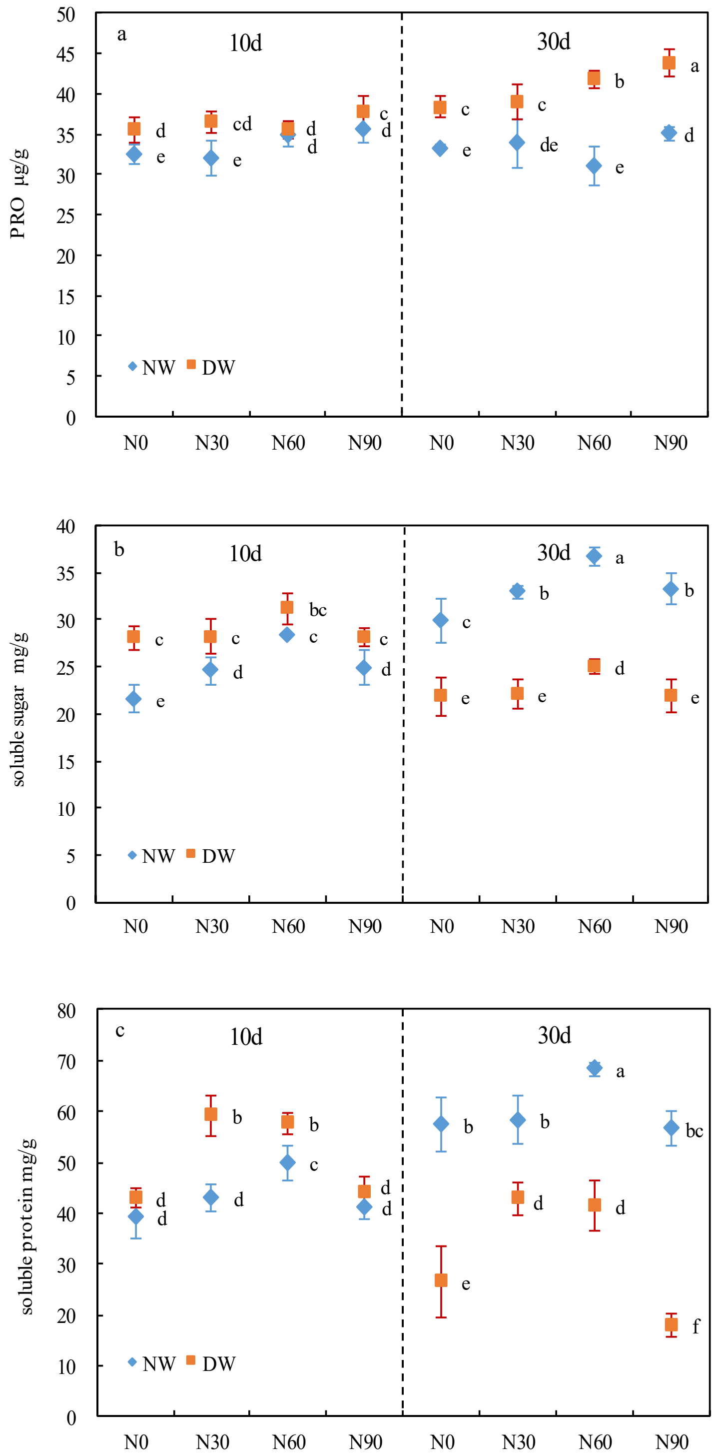

Figure 3. (**a**) Effect of drought stress and nitrogen deposition on PRO content; (**b**) effect of drought stress and nitrogen deposition on soluble sugar content; (**c**) effect of drought stress and nitrogen deposition on soluble protein content. Water supply treatments: normal water supply (NW) or drought stress (DW) for 10 d or 30 d. Nitrogen deposition treatments: control (N0, 0 kg·hm^{-2} year^{-1}), low nitrogen deposition (N30, 30 kg·hm^{-2} year^{-1}), medium nitrogen deposition (N60, 60 kg·hm^{-2} year^{-1}), or high nitrogen deposition (N90, 90 kg·hm^{-2} year^{-1}). Abbreviations: PRO, proline. Data points represent means ± the SD; n = 5. Data points with the same lowercase letter are significantly different (p < 0.05) according to Tukey's test.

Table 1. Effect of water supply and nitrogen deposition level on the chlorophyll a and b content of *Bretschneidera sinensis* seedlings.

Treatment			Chl a (mg·g^{-1})	Chl b (mg·g^{-1})	Chl a + b (mg·g^{-1})	Chl a/b
10 d	NW	N0	1.46 ± 0.04 c	0.43 ± 0.03 bc	1.89 ± 0.07 b	3.42 ± 0.05 ab
		N30	1.51 ± 0.05 bc	0.47 ± 0.01 b	1.98 ± 0.05 b	3.25 ± 0.03 bc
		N60	1.61 ± 0.11 b	0.49 ± 0.04 b	2.10 ± 0.17 ab	3.32 ± 0.11 bc
		N90	1.14 ± 0.07 de	0.34 ± 0.02 c	1.48 ± 0.02 d	3.45 ± 0.09 a
	DW	N0	1.02 ± 0.06 e	0.30 ± 0.02 cd	1.32 ± 0.02 e	3.44 ± 0.06 a
		N30	0.97 ± 0.04 e	0.29 ± 0.01 d	1.26 ± 0.05 e	3.40 ± 0.12 ab
		N60	1.17 ± 0.05 d	0.34 ± 0.01 c	1.52 ± 0.05 cd	3.46 ± 0.07 a
		N90	1.01 ± 0.10 e	0.29 ± 0.02 d	1.30 ± 0.03 e	3.46 ± 0.03 a
30 d	NW	N0	1.45 ± 0.06 c	0.44 ± 0.05 bc	1.89 ± 0.08 b	3.32 ± 0.07 bc
		N30	1.56 ± 0.05 b	0.48 ± 0.01 b	2.04 ± 0.06 b	3.29 ± 0.03 bc
		N60	1.89 ± 0.18 a	0.60 ± 0.05 a	2.49 ± 0.18 a	3.19 ± 0.15 c
		N90	1.25 ± 0.02 d	0.36 ± 0.01 c	1.61 ± 0.03 c	3.49 ± 0.08 a
	DW	N0	1.03 ± 0.02 e	0.30 ± 0.01 cd	1.33 ± 0.03 e	3.42 ± 0.09 ab
		N30	0.84 ± 0.07 f	0.24 ± 0.01 d	1.07 ± 0.07 f	3.54 ± 0.19 a
		N60	0.98 ± 0.03 e	0.28 ± 0.01 d	1.26 ± 0.04 e	3.46 ± 0.05 a
		N90	0.70 ± 0.08 g	0.20 ± 0.02 d	0.90 ± 0.10 f	3.56 ± 0.04 a

Values represent means ± the standard error (SE) of the mean; $n = 5$. Values followed by different letters within the same column are significantly different ($p < 0.05$) according to Tukey's test. Water supply treatments: NW, normal water supply; DW, drought stress. Nitrogen deposition treatments: N0, 0 kg·hm^{-2} year^{-1}, control; N30, 30 kg·hm^{-2} year^{-1}, low nitrogen deposition; N60, 60 kg·hm^{-2} year^{-1}, moderate nitrogen deposition; N90, 90 kg·hm^{-2} year^{-1}, high nitrogen deposition.

3.5. Growth Indicators

The DW treatment decreased the growth of seedlings (Figure 4). The height net growth of N0 seedlings under DW was significantly lower (25.67% lower) than that of N0 seedlings under NW. However, the height net growth of N60 seedlings under NW and DW was not significantly different (Figure 4). Under DW, the height and ground diameter net growth of seedlings that received N60 was 72.37% and 66.67% higher, respectively, than that of N0 seedlings (Figure 4). The root dry weight of seedlings treated with N0, N30, and N90 under DW was significantly lower (19.10%, 15.37%, and 20.22%, respectively, lower) than that of seedlings under NW (Figure 4). The DW treatment significantly reduced the shoot dry weight of seedlings treated with different nitrogen deposition levels (Figure 4).

3.6. ANOVA Results

Nitrogen deposition and soil water content had a significant effect on MDA content and RC (Table 2). Nitrogen deposition had a significant effect on soluble protein (Table 2). Water supply level and nitrogen deposition level had a significant effect on chlorophyll a and b content but not on chlorophyll a/b (Table 2).

Table 2. ANOVA results of the effects of water supply and nitrogen deposition levels and their interactions on the physiological characteristics of *Bretschneidera sinensis* seedlings.

Effects	MDA	RC	SOD	POD	CAT	PRO	Soluble Protein	Soluble Sugar	Chl a	Chl b	Chl a + b	Chl a/b
Water supply level (W)	***	***	ns	ns	*	**	ns	ns	***	***	***	***
Nitrogen deposition level (N)	*	***	ns	ns	ns	ns	*	ns	***	***	***	ns
W × N	ns	ns	ns	ns	ns	ns	*	ns	***	**	***	ns

Values represent the means ± the SE; $n = 5$. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. ANOVA factors: nitrogen deposition level—no deposition (control), low nitrogen deposition, medium nitrogen deposition, high nitrogen deposition; water availability level—normal water supply, drought stress. Abbreviations: MDA, malondialdehyde; RC, relative conductivity; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; PRO, proline.

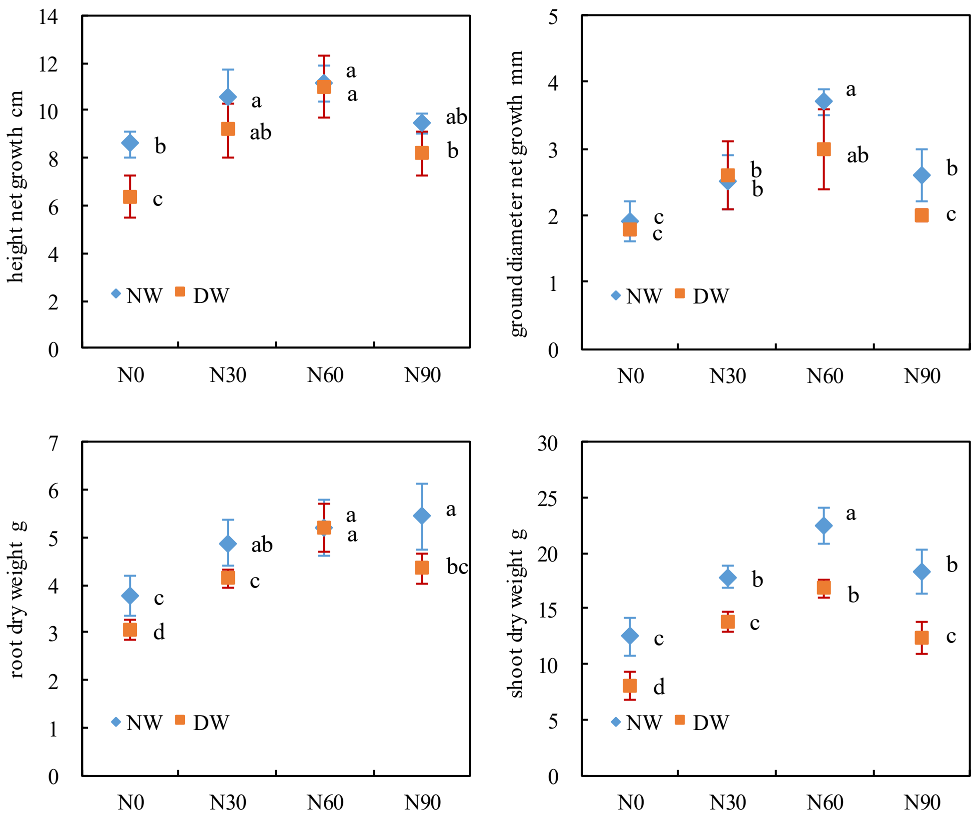

Figure 4. Effect of drought stress and nitrogen deposition on growth indicators. Water supply treatments: normal water supply (NW) or drought stress (DW) for 10 d or 30 d. Nitrogen deposition treatments: control (N0, 0 kg·hm^{-2} year^{-1}), low nitrogen deposition (N30, 30 kg·hm^{-2} year^{-1}), medium nitrogen deposition (N60, 60 kg·hm^{-2} year^{-1}), or high nitrogen deposition (N90, 90 kg·hm^{-2} year^{-1}). Data points represent means ± the SD; $n = 5$. Data points with the same lowercase letter are significantly different ($p < 0.05$) according to Tukey's test.

4. Discussion

Drought stress can lead to severe dehydration of the protoplasm, causing physiological and biochemical metabolic disorders that slow or stop cell division, affecting growth [43]. Malondialdehyde is the final breakdown product of membrane lipid peroxidation and, hence, MDA content can reflect the degree of stress injury to plants [44,45]. The relative electrical conductivity of plant leaves is a basic index reflecting the permeability of the plant cell membrane [46]. Under normal circumstances, the plant cell membrane has the ability to select the permeability of substances. However, when plants are affected by adverse environmental conditions, the cell membrane is damaged and, therefore, membrane permeability increases, causing cell electrolyte extravasation, and the electrical conductivity of the cell extract increases [47]. In this study, the content of MDA and RC in leaves of *B. sinensis* seedlings increased under drought stress and increased over the drought stress period. This indicates that the structure and function of cell membranes in the leaves of these seedlings were damaged under drought stress, increasing cell membrane permeability, resulting in electrolyte outflow. Under drought stress, the MDA content and RC of seedlings treated with N30 and N60 were not significantly different from those of N0 seedlings. By contrast, the MDA content and RC of seedlings treated with N90 increased significantly, which indicated that a high concentration of nitrogen deposition aggravated the degree of drought stress experienced by *B. sinensis* seedlings which in turn affected growth. Tao et al. [48] also found that a high concentration of nitrogen deposition tended to aggravate the degree of drought stress in *Phyllostachys edulis* (Carriere) J. Houzeau seedlings.

Peroxidase, SOD, and CAT are important antioxidant enzymes in plants [49]. The POD is an enzyme with high activity that is associated with respiration, photosynthesis, and auxin oxidation [50]. The SOD plays a key role in scavenging free radicals in plants, catalyzing their conversion to less damaging components, i.e., molecular oxygen and H_2O_2 and, hence, SOD plays an important role in maintaining physiological activity [51]. Catalase scavenges H_2O_2, catalyzing the breakdown of H_2O_2 into molecular oxygen and water so as to protect cells from H_2O_2 poisoning [52]. In this study, the activities of POD, SOD, and CAT were increased under short-term drought stress but decreased under long-term drought stress. In order to maintain the redox balance in the cells, seedlings could eliminate excessive free radical reactive oxygen species at the early stages of drought by increasing the activity of antioxidant enzymes. However, under prolonged drought stress, antioxidant regulation cannot continue to resist the decline of membrane lipid peroxidation, and antioxidant enzyme activity declines, leading to the accumulation of free radical reactive oxygen species in the plant cell membrane [53]. After 10 d of drought, the POD, SOD, and CAT activities of seedlings treated with N60 were significantly higher than those of N0 seedlings, and the activities of POD, SOD, and CAT in seedlings treated with N90 were significantly lower than those of N0 seedlings after 30 d of drought. These results indicate that moderate nitrogen deposition could increase the activities of antioxidant enzymes and enhance the ability of antioxidant regulation; however, excessive nitrogen deposition could increase the sensitivity of *B. sinensis* seedlings to drought stress, and then inhibited growth. These findings support those of a previous study showing that a nitrogen supply of less than 20 $g \cdot m^{-2}$ $year^{-1}$ promoted the physiological performance of *Quercus variabilis* Blume and *Quercus mongolica* Fisch. ex Ledeb. under drought conditions. However, *Q. variabilis*, which has more plastic responses than *Q. mongolica*, was more sensitive to nitrogen deposition than *Q. mongolica* [54]. Plant age should be taken into account when assessing plants' susceptibility to nitrogen deposition. Zhang et al. [55] found that the 3- and 5-year-old bamboo seemed to be less tolerant to extremely high nitrogen deposition than 1-year-old bamboo since they were saturated at a lower nitrogen addition.

Osmoregulation is an adaptive response of plants to drought stress and is an important physiological mechanism of drought tolerance [56]. Under drought conditions, cells maintain turgor pressure by accumulating osmotic regulators to maintain the normal physiological activities of plants [57]. Proline, soluble sugar, and soluble protein are important osmotic regulators. Under drought stress, the PRO content of *B. sinensis* seedlings was correlated with the MDA content and increased gradually as the period of drought stress increased. Zhou et al. [58] have also shown that PRO content was significantly correlated with MDA and plasma membrane permeability. Under drought stress, the PRO content of seedlings treated with N90 was significantly higher than that of N0 seedlings, indicating that excessive nitrogen deposition aggravated the degree of drought stress in *B. sinensis* seedlings. The soluble sugar and soluble protein content of *B. sinensis* seedlings increased during the early stages of drought. This suggests that drought stress activated the osmotic regulation mechanism of *B. sinensis* seedlings by accumulating soluble protein and soluble sugar to reduce the cell osmotic potential, which increases the water-holding capacity of cells, to help seedlings resist the damage caused by drought stress. However, the soluble sugar and soluble protein content of drought-stressed seedlings decreased under long-term drought conditions. This may be because long-term drought stress on the one hand increased the consumption of carbohydrates by plants, forcing seedlings into low energy consumption metabolism to resist drought stress and, on the other hand, inhibited photosynthesis, reducing the synthesis of carbohydrates [59]. Under drought stress, the soluble sugar and soluble protein content levels of seedlings treated with N60 were significantly higher than those of N0 seedlings. These results suggest that moderate nitrogen deposition was beneficial for maintaining water content and alleviating drought injury by regulating soluble sugar and soluble protein content levels. However, drought stress inhibited the chlorophyll synthesis of *B. sinensis* seedlings, and the content of chlorophyll decreased as the drought stress period increased. Under drought conditions, the chlorophyll a and chlorophyll a + b content levels in seedlings treated with N60 were significantly higher than those of N0 seedlings, indicating that moderate nitrogen deposition could alleviate the degree of drought stress by regulating the chlorophyll content.

Nitrogen is a major component of enzymes, the most active and ubiquitous important substances in plant physiological metabolism, as well as an important component of nucleic acids, chlorophyll, and hormones. Nitrogen application can significantly improve the osmoregulatory capacity of plants, which is related to the fact that nitrogen nutrition promotes the accumulation of osmoregulatory substances such as PRO and soluble sugar. *B. sinensis* seedlings without a good root system are therefore very sensitive to drought stress. The effect of atmospheric nitrogen deposition on the drought resistance physiological characteristics of *B. sinensis* seedlings in the context of climate change is important for seedling breeding in response to climate change. In this study, a certain degree of nitrogen deposition can alleviate drought stress, the reason may be that nitrogen directly affects the hydration status of cellular protoplasm and the state of the protoplasmic colloid system, increases the hydration of protoplasts, improves the water retention capacity of protoplasts, and also increases the activity of leaf protective enzyme systems, thus reducing the degree of membrane lipid peroxidation, maintaining the integrity of cell membranes under drought stress, and facilitating plant growth. Excessive nitrogen deposition can increase the sensitivity of *B. sinensis* seedlings to drought stress and aggravate the degree of stress. It may be due to the induction of the production of other types of reactive oxygen species that harm membrane lipids. Excess nitrogen causes disruption of chloroplast ultrastructure, in the presence of more than 2 $mg \cdot L^{-1}$ of nitrogen, reactive oxygen species production in chloroplasts increases significantly, and the abundance of six proteins involved in photosynthesis decreases in response to excess nitrogen [60].

5. Conclusions

Drought is an important environmental stressor affecting the growth of *B. sinensis* seedlings. A certain degree of nitrogen deposition can alleviate drought stress by decreasing cell membrane permeability, reducing cell membrane peroxidation, increasing the content of osmoregulatory substances, and reducing the tendency of chlorophyll to decline. However, excessive nitrogen deposition can increase the sensitivity of *B. sinensis* seedlings to drought stress and aggravate the degree of stress. Therefore, in practice, measures to improve the drought tolerance of *B. sinensis* seedlings should be based on the atmospheric nitrogen deposition conditions and soil background levels.

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