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Optimal Soil Calcium for the Growth of Mulberry Seedlings Is Altered by Nitrogen Addition

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Abstract: Increasing soil N not only aggravates calcium (Ca) stress by stimulating Ca leaching from the soil but also impacts the sensitivity of plants to Ca stress. However, how increasing N influences the soil Ca demand of seedlings is largely unknown. We studied the influence of different concentrations of exogenous Ca (i.e., 0, 200, 400, 600, and 800 mg·kg^{-1} Ca^{2+}) on the growth, photosynthesis, Ca absorption, and intrinsic water use efficiency (iWUE) of mulberry (*Morus alba*) seedlings under two N levels (i.e., 200 and 600 mg·kg^{-1} NH$_4$NO$_3$). We found that there was an optimal concentration of soil Ca for the growth and net photosynthetic rate (Pn) of mulberry seedlings; the optimal Ca concentration was 200 mg·kg^{-1} under low N conditions and 400 mg·kg^{-1} under high N conditions. Therefore, the application of N fertilizer increased the optimal Ca concentration. Different from the unimodal relationship between biomass and Ca levels, the iWUE of mulberry was significantly and positively correlated with soil Ca levels. At the same time, except under the 800 mg·kg^{-1} Ca treatment, the soil Ca levels were reflected by foliar Ca concentrations. The N deposition, large-scale N fertilizer application, and drought increase Ca demand in plants, thus causing the application of Ca fertilizer to be necessary in low-Ca soil while alleviating Ca stress in high-Ca soil. The balance between the optimal Ca level needed for growth and drought resistance should be considered when determining the amount of Ca fertilizer required.

Keywords: Ca stress; nitrogen deposition; photosynthesis; biomass; intrinsic water use efficiency

1. Introduction

Increasing the nitrogen (N) supply through atmospheric deposition or fertilization may alter nutrient regimes in terrestrial ecosystems by leaching soil mineral nutrients [1]. Calcium (Ca) is an important mineral nutrient. It plays an important physiological role in plant growth [2,3] by maintaining the stability of cell walls, cell membranes, and membrane-bound proteins, as well as by regulating the process of stress resistance, which involves coordinating responses to an array of developmental and environmental challenges [4–8]. Ca leaching will inhibit plant growth and survival [9,10].

Both Ca deficiency and Ca excess in soil are problems that need to be dealt with for optimal plant growth [10,11]. H$^+$ that occurs simultaneously with N deposition and the processing of N by soil microbes can increase the weathering of Ca^{2+} from bedrock and displace Ca^{2+} from the soil exchange surface into pore water [12–14]. This influence on soil Ca availability is complex and varies in different soils. In low-Ca soil, increasing N can increase short-term Ca availability [14,15]; however, in the long term, aggravating Ca leaching will deplete available Ca and result in Ca deficiency [16]. In high-Ca soil, especially calcareous soils (high CaCO$_3$ and CaSiO$_3$ content), Ca stress will be further aggravated by the Ca released as a result of increasing N. N also has the potential to impact the sensitivity of plants to Ca stress; however, the influence of different Ca levels on plants under increasing concentrations of N has received little attention.

Soil Ca has been shown to affect the distribution and abundance of plant species. Some plant species are classified into calcifuges, which are found in acidic soil with low Ca levels, and calcicoles, which are found in calcareous soils with high Ca levels. Calcifuge and calcicole plants both grow well in their natural habitats, which differ markedly [17,18]. The planting of agricultural and forest crops typically does not account for the soil Ca. Ca spray (calcium chloride) has increasingly been used in the pre-harvest and post-harvest stages to improve the hardness, robustness, and shelf life of fleshy fruits in low-Ca soil [19–21]. Ca supplements in the form of $CaCO_3$ (lime) and $CaSiO_3$ (wollastonite) can also improve soil quality and increase plant productivity [9,22–25]. However, there are a few ways to improve the adaptability of plants to high Ca levels. Since N influences Ca availability, clarifying the Ca demand of seedlings under different N levels will help in finding methods to improve their adaptation to soil Ca stress.

Mulberry (*Morus alba* L.) is an important economic tree in China. Its leaves are foraged for rearing silkworm (*Bombyx mori*) and livestock [26,27]; in addition, its leaves, stems, roots, and fruits are used in traditional medicine [28]. Meanwhile, mulberry shows strong adaptability to drought, cold, low temperatures, and other abiotic stresses. Thus, mulberry is widely cultivated all over the world in sites with different soil Ca levels. However, the influence of exogenous Ca treatments on the various morpho-physiological parameters of mulberry under different N levels is poorly understood. Here, we investigated the influence of exogenous Ca on the morpho-physiological characteristics and responses of mulberry seedlings under different soil N conditions to evaluate the impact of N levels on plant Ca demand. The hypotheses of the investigation were that (1) each specific nutrient environment has an optimal Ca level and (2) the optimal Ca level is higher in high-N soil.

2. Materials and Methods

2.1. Materials and Experimental Design

The experiment was conducted in the greenhouse of the Forestry College of Shenyang Agricultural University (41°48′ N, 123°25′ E, Shenyang, China). During the experiment, the average temperature was 18–25 °C, with a light intensity of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a relative humidity of 60%. The soil was carefully excavated from a mulberry garden in Liaoning Province, China, in March 2017. The basic soil parameters are listed in Table 1. The mulberry variety used was "*qinglong*", which is widely planted in northern China. The mulberry seeds were provided by the Institute of Silkworm, Heilongjiang Academy of Agricultural Sciences.

Table 1. Chemical characteristics of the nursery soil before seeding.

Organic C concentration ($g \cdot kg^{-1}$)	18.02 ± 0.53
Total N concentration ($g \cdot kg^{-1}$)	1.47 ± 0.09
Total P concentration ($g \cdot kg^{-1}$)	1.18 ± 0.10
Available N concentration ($mg \cdot kg^{-1}$)	174.2 ± 7.32
Available P concentration ($mg \cdot kg^{-1}$)	13.07 ± 0.13
Water-extractable Ca concentration ($mg \cdot kg^{-1}$)	113.62 ± 5.22
Exchangeable Ca concentration ($mg \cdot kg^{-1}$)	4567.32 ± 54.70
Maximum water-holding capacity (%)	30.5 ± 0.33

The seeds were sterilized with 1% sodium hypochlorite for 15 min and then washed several times with distilled water before they were germinated in moist gauze at 20 °C in the dark. When radicles began to appear, five seeds were planted in pots (bottom diameter: 18.3 cm; outer diameter: 22.5 cm; and height: 21.5 cm) containing 5 kg of soil. Distilled water was used to maintain the moisture level to prevent the introduction of exogenous Ca from tap water. All the seedlings were grown in the soil for one month, after which four seedlings were removed so that only one seedling was left in each pot.

A 2 × 5 factorial greenhouse experiment with 6 replicates per treatment was established. A total of 60 seedlings were examined (two N levels × five Ca levels × six seedlings) from April 2017 (start of experiment) to November 2017 (end of experiment). The seedlings were treated with different levels of N and Ca by dissolving $CaCl_2$ and NH_4NO_3 in distilled water

and then adding the mixture into the pots. The levels of N included low N (200 mg·kg^{-1} NH_4NO_3) and high N (600 mg·kg^{-1} NH_4NO_3). The Ca levels included Ca0 (0 mg·kg^{-1} Ca^{2+}); Ca200 (200 mg·kg^{-1} Ca^{2+}); Ca400 (400 mg·kg^{-1} Ca^{2+}); Ca600 (600 mg·kg^{-1} Ca^{2+}); and Ca800 (800 mg·kg^{-1} Ca^{2+}).

2.2. Plant and Soil Analysis

2.2.1. Determination of Net Photosynthetic Rate

From 08:00 to 11:00 in July 2017, the net photosynthetic rate of the leaves was measured using a portable photosynthesis system Li-6400 (LI-6400XT, LI-COR Biosciences, Lincoln, NE, USA) (one leaf per plant, three plants per replicate). A gas exchange system was used to carry out the measurements, with a leaf chamber temperature of 25 ± 1 °C and the inner photo flux density set to 1000 μmol·m^{-2}·s^{-1}.

2.2.2. Growth Characteristics

The height of the seedlings was measured in November 2017 before harvesting. The height was measured from the cotyledon scar to the tip of the terminal bud. The harvested seedlings were washed with distilled water, divided into aerial and root portions, and then dried to a constant weight at 80 °C. The biomass of the shoot and root was measured with a digital balance (0.1 mg). After weighing, the dried tissue was ground with an electric mill and passed through a 100-mesh sieve before being sealed and stored in a valve bag.

2.2.3. Determination of Soil Ca

The soil remaining after collecting the roots was evenly mixed and air-dried. Half the soil was passed through a 0.85 mm (20-mesh) sieve for the determination of water-extractable Ca and exchangeable Ca in the soil. The water-extractable Ca^{2+} was determined as follows: 5.00 g sub-sample of the 20-mesh soil sample was weighed in a 100 mL conical flask and 25 mL of double-distilled water was added to the soil. The soil suspension was shaken in a rotary shaker for 15 min. The extractant solution was spiked with 0.5 mL of 3% $SrCl_2$. The Ca concentration was then measured using a flame atomic absorption spectrometer (ZA3000, Hitachi, Tokyo, Japan). The exchangeable Ca^{2+} was determined as follows: 0.5 g sub-sample of the 100-mesh soil sample was weighed in a 100 mL conical flask, and 50 mL of NH_4OAc (1 mmol·L^{-1}) was added to the soil. The soil suspension was shaken in a rotary shaker for 30 min and then filtered. The extractant solution was spiked with 1 mL of 3% $SrCl_2$. Finally, the Ca concentration was measured using a flame atomic absorption spectrometer.

2.2.4. Shoot Ca Concentration

A sub-sample of the 100 mg ground sample was digested in 8 mL of 3:1 (HNO_3: $HClO_4$) acid mixture. Before diluting to 50 mL with double-distilled water, 2 mL of 3% $SrCl_2$ was added to all the digested solutions. The Ca concentration was measured using a flame atomic absorption spectrometer.

2.2.5. Shoot N Concentration

The N concentration in mulberry leaves was analyzed using the Dumas combustion method with an elemental analyzer (Elemental vario PYRO cube, Frankfurt, Germany).

2.2.6. Leaf $\delta^{13}C$ and iWUE

The plant water use efficiency (WUE) can be reflected by the plant's $\delta^{13}C$ value, which is linearly and closely related to intrinsic water use efficiency (iWUE) [29]. To evaluate the long-term WUE, the $\delta^{13}C$ value of the plants was determined using an elemental analyzer coupled with an isotope ratio mass spectrometer (IsoPrime 100 Isotope Ratio Mass Spectrometer, Germany).

The isotopic discrimination of C ($\Delta^{13}C$) in the leaves associated with carbon fixation by C$_3$ plants was calculated as shown in Equation (1) [30]:

$$\Delta^{13}C = (\delta^{13}C_a - \delta^{13}C_{plant})/(1 + \delta^{13}C_{plant}) \tag{1}$$

where $\delta^{13}C_a$ and $\delta^{13}C_p$ represent the $\delta^{13}C$ values of atmospheric CO_2 and plant tissues, respectively. The carbon discrimination of C_3 plants during CO_2 fixation is linearly related to the ratio of intercellular to atmospheric CO_2 concentration (c_i/c_a) by the following equation:

$$\Delta^{13}C = a + (b - a)\, c_i/c_a \tag{2}$$

where a (4.4‰) indicates the part diffused through the stomata, b (27‰) denotes the isotope discrimination caused by carboxylation [31], and c_i and c_a are the intercellular and environmental CO_2 concentrations, respectively.

Finally, Equation (3) relates these parameters to the iWUE of plants by using a scale factor of 1.6, which is the ratio of water vapor to CO_2 diffusivity. The iWUE was calculated using the following equation [32]:

$$iWUE = A/g_s = (c_a - c_i)/1.6 = c_a(1 - c_i/c_a)/1.6 = c_a(b - \Delta^{13}C)/1.6(b - a) \tag{3}$$

where A is net photosynthesis and g_s is stomatal conductance.

2.3. Statistical Analysis

The data analysis was carried out using the SPSS 22.0 statistical package (SPSS, Inc., Chicago, IL, USA) and OriginPro 2020 was used to draw charts. The results are expressed as mean $\pm$ SE (standard error). The significance of the effects of the Ca and N treatments and their interaction on the measured variables related to the plants and soil was tested using a two-way ANOVA. We compared a one-way analysis of variance (ANOVA) using a Duncan's multiple comparison test ($p < 0.05$) to compare the values of water-extractable Ca, exchangeable Ca, shoot biomass, root biomass, total biomass, height, Pn, and shoot N concentration among the five Ca treatments under the two N levels. When only two groups were compared, we used the Student's *t*-test. A correlation analysis was used to investigate the relationship between the Ca addition and plant Ca concentration and between the Ca addition and iWUE in aerial parts.

3. Results

3.1. Available Concentrations of Ca in Soil

The exogenous $CaCl_2$ increased the water-extractable Ca concentration ($p < 0.01$) and exchangeable Ca concentration ($p < 0.01$) in the soil (Table 2). For each Ca treatment, the high N treatment increased the water-extractable Ca concentration but decreased that of the exchangeable Ca (Table 3). The water-extractable Ca concentrations were significantly different in the Ca0 and Ca400 treatments at different N levels, while the exchangeable Ca concentrations were significantly different in the Ca600 treatment at different N levels.

Table 2. Statistical significance of the effects of Ca and N treatments and their interaction on plant and soil parameters as determined using two-way ANOVA. *p* values < 0.05 are bolded.

Variables	Ca		N		Interaction	
	F	*p*	**F**	*p*	**F**	*p*
Water-extractable Ca^{2+}	84.302	**<0.01**	3.078	0.095	0.197	0.937
Exchangeable Ca^{2+}	9.328	**<0.01**	11.704	**<0.01**	1.596	0.214
Pn	30.053	**<0.01**	7.390	**<0.05**	70.486	**<0.01**
Shoot biomass	29.129	**<0.01**	21.770	**<0.01**	9.199	**<0.01**
Root biomass	32.139	**<0.01**	27.142	**<0.01**	7.306	**<0.01**
Total biomass	23.323	**<0.01**	8.722	**<0.01**	5.335	**<0.01**
Height	9.341	**<0.01**	0.015	0.903	5.392	**<0.01**
N concentration in shoot	3.496	**<0.05**	96.979	**<0.01**	7.966	**<0.01**
Ca concentration in shoot	53.774	**<0.01**	9.575	**<0.01**	14.350	**<0.01**
iWUE	3.050	**<0.05**	0.013	0.909	0.768	0.558

Table 3. Effect of different Ca and N treatments on water-extractable Ca and exchangeable Ca concentrations in soil.

Ca Treatment (mg·kg^{-1})	Water-Extractable Ca (mg·kg^{-1})		Exchangeable Ca (mg·kg^{-1})	
	Low N	High N	Low N	High N
0	61.4 ± 2.5 [Eb]	91.9 ± 2.3 [Ea]	4264.8 ± 47.5 [Ba]	4188.4 ± 50.7 [Ba]
200	225.1 ± 6.5 [Da]	257.7 ± 4.9 [Da]	4279.4 ± 57.8 [Ba]	4246.4 ± 24.1 [ABa]
400	379.2 ± 10.5 [Cb]	436.5 ± 11.6 [Ca]	4326.5 ± 93.1 [Ba]	4287.8 ± 94.5 [ABa]
600	595.6 ± 8.8 [Ba]	620.8 ± 16.1 [Ba]	4533.9 ± 64.3 [Aa]	4259.9 ± 43.8 [ABb]
800	715.3 ± 13.1 [Aa]	804.0 ± 14.5 [Aa]	4595.2 ± 38.8 [Aa]	4445.2 ± 77.5 [Aa]

Capital letters denote significant differences among different Ca treatments within the same N level. Lowercase letters denote significant differences between the different N levels within the same Ca treatment.

3.2. Mulberry Growth

Both the Ca and N treatments significantly affected the net photosynthetic rate (Pn) with significant interactions between the two factors (Table 2). Under low N conditions, the Pn was the highest under the Ca200 treatment and significantly higher than in any other Ca treatment seedlings (Figure 1). Under high N conditions, the Pn increased as the Ca treatment increased from Ca0 to Ca400, and the maximum value was maintained in the Ca400 treatment, being significantly higher than that in any other Ca treatment. The Pn was significantly lower in high N conditions than under low N conditions in the Ca0 and Ca200 treatments and was significantly higher in the Ca400 and Ca600 treatments.
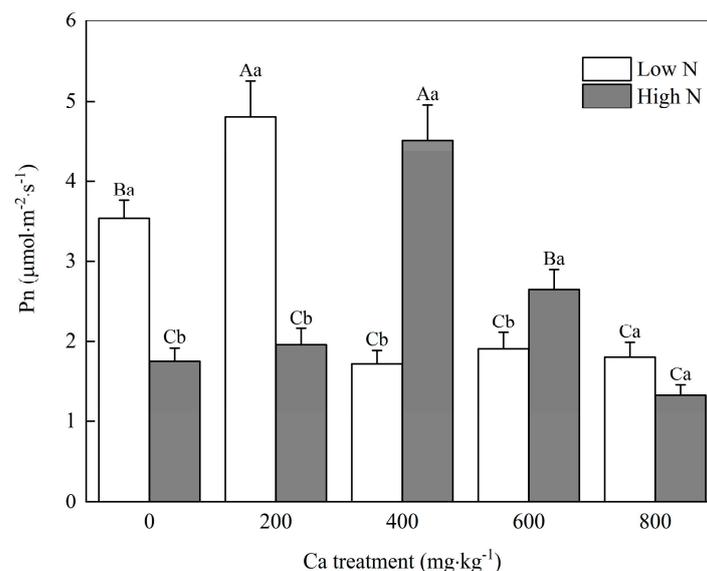

Figure 1. Effect of Ca treatments on the net photosynthetic rate of mulberry seedlings under different N levels. Capital letters denote significant differences among different Ca treatments within the same N level. Lowercase letters denote significant differences between the different N levels within the same Ca treatment.

Both the Ca and N treatments had significant effects on shoot, root, and total biomass, with significant interactions between the two factors (Table 2). Under low N conditions, the maximum biomass occurred in the Ca200 treatment; Ca treatments higher than 200 mg·kg^{-1} significantly decreased the mulberry seedling biomass and the minimum value occurred in the Ca800 treatment (Figure 2). Under high N conditions, the trend of the relationship between the biomass and Ca treatments was similar to that under low N conditions; however, the three parameters all reached a maximum in the Ca400 treatment. The shoot biomass was significantly higher in high N conditions than in the low N conditions at the Ca400 and Ca600 treatments. On the other hand, the root biomass was significantly lower under high N conditions than under low N conditions at the Ca0 and Ca200 treatments.

Finally, total biomass was significantly higher in the high N conditions than in the low N conditions at the Ca400 treatment.
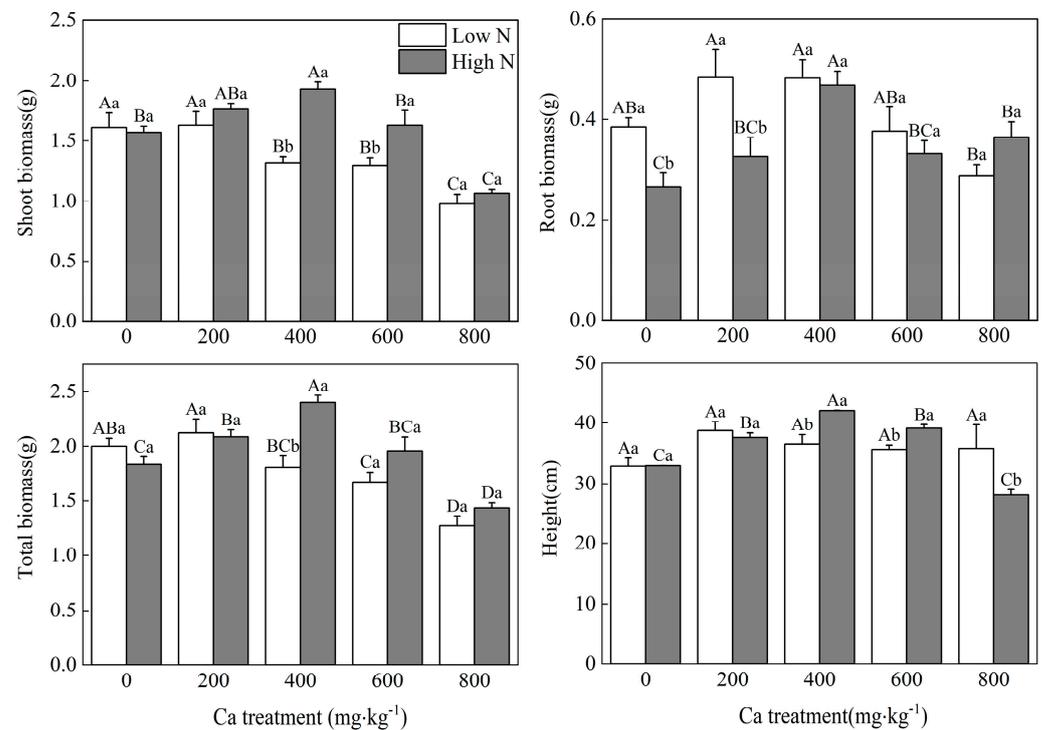

Figure 2. Effect of Ca treatments on the growth of mulberry seedlings under different N levels. Capital letters denote significant differences among different Ca treatments within the same N level. Lowercase letters denote significant differences between the different N levels within the same Ca treatment.

We observed significant effects of Ca ($p < 0.01$) and the combination of Ca and N on the height of the plants ($p < 0.01$) (Table 2). Under low N levels, Ca had less of an effect on the height; there was no significant difference between Ca treatments, and the maximum height was reached at the Ca200 treatment. Ca first caused a height increase and then a decrease under high N conditions, where the maximum height was reached at the Ca400 treatment, being significantly higher than any other Ca treatment.

3.3. Uptake of N and Ca in Mulberry

The Ca and N treatments significantly affected the Ca and N concentrations in shoots with significant interactions between the two factors ($p < 0.01$) (Table 2). The N concentration in the mulberry seedlings under the high N treatment were significantly higher than that under the low N treatment. Our results showed that the N concentration was lowest at the Ca200 treatment under low N conditions and the Ca400 treatment under high N conditions (Figure 3).

Under low N conditions, the Ca concentration in the shoots increased significantly with Ca treatment from Ca0 to Ca400 (Figure 4); the maximum was achieved at the Ca600 treatment, and the concentration decreased significantly at the Ca800 treatment. Under high N conditions, the Ca concentration in the shoots increased significantly with Ca treatment, increasing from Ca0 to Ca600, and also significantly decreased at the Ca800 treatment. High N conditions significantly increased the shoot Ca concentrations under the Ca600 treatment and significantly decreased the shoot Ca concentrations under the Ca0 and Ca800 treatments. It was found that, except for in the Ca800 treatment, the level of water-extractable Ca in the soil was significantly and positively correlated with the Ca concentration in mulberry seedlings under both N conditions.
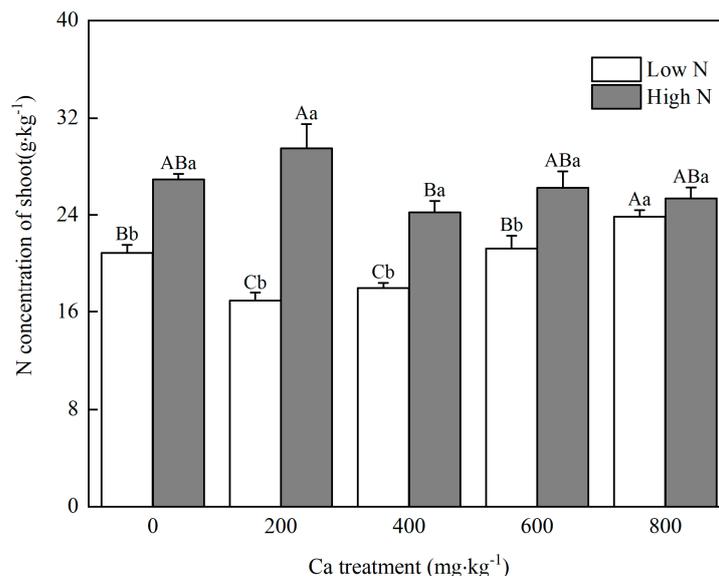
Figure 3. Effect of Ca treatments on the N concentration in shoots under different N levels. Capital letters denote significant differences among different Ca treatments within the same N level. Lowercase letters denote significant differences between the different N levels within the same Ca treatment.
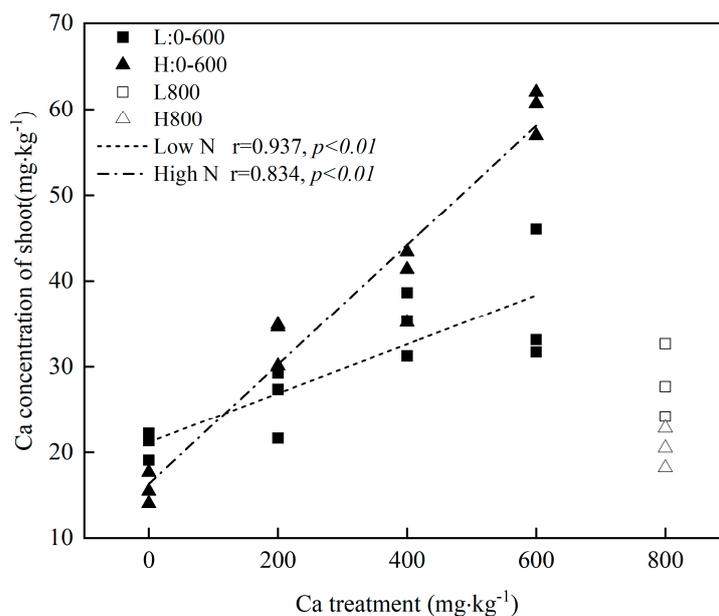

Figure 4. Relationship between Ca treatments and shoot Ca concentrations. L: 0–600 denotes the treatments of 0, 200, 400, and 600 mg·kg^{-1} Ca under low N conditions; L:800 denotes the treatment of 800 mg·kg^{-1} Ca under low N conditions; H:0-600 denotes the treatments of 0, 200, 400, and 600 mg·kg^{-1} Ca under high N conditions; H:800 denotes the treatment of 800 mg·kg^{-1} Ca under high N conditions.

3.4. Relationship between iWUE and Ca

The exogenous Ca increased the iWUE value of mulberry seedlings (Figure 5). iWUE was significantly and positively correlated with exogenous Ca. Although the Pn and biomass were strongly inhibited by Ca800 treatment, the iWUE was the highest at the Ca800 treatment.
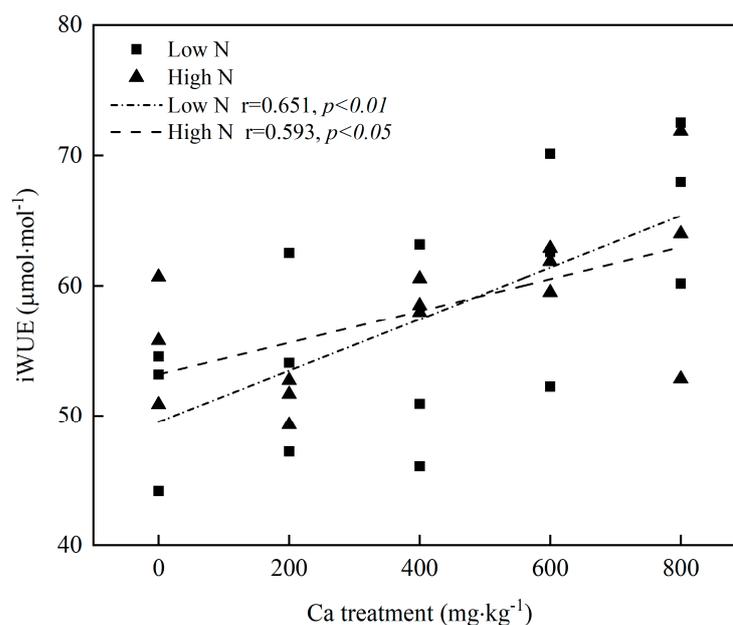
Figure 5. Effect of Ca treatments on the iWUE of shoots under different N levels.

4. Discussion

We analyzed the effects of different levels of exogenous Ca on the growth, photosynthesis, iWUE, and Ca absorption of mulberry seedlings under two N levels. It was found that there was an optimal Ca level for the growth of mulberry seedlings and the optimal Ca level was higher under high N conditions. It is reasonable to speculate that: (1) Ca fertilization is necessary for agriculture and forestry with the increase in N input. (2) It is possible that N deposition is altering the distribution of plant species by increasing Ca demand, at least in regions with calcareous soils. In addition to selecting seedling species or varieties suitable for low-/high-Ca soil, the application of Ca in low-Ca soil and N in high-Ca soil is another way to mitigate Ca stress. At the same time, the change in soil Ca was reflected in the absorption of Ca by mulberry seedlings, and the iWUE of mulberry seedlings was significantly positively correlated with soil Ca, suggesting that Ca fertilizer can be used as a measure to increase the water use efficiency in drought-prone regions.

4.1. N Influences the Optimal Soil Ca Level for Mulberry Seedlings

We found a unimodal relationship between the biomass and soil Ca levels. Soil Ca is heterogeneous [33–35]; large areas of acidic soil with low Ca levels and calcareous soils with high Ca levels are common on a regional scale [36,37]. In a natural environment, Ca can be the limiting factor in Ca-deficient soil [12,38]. At the same time, high Ca levels also restrict plant growth [39]. The optimal soil Ca level can be used as an additional indicator when finding specific plant species for soils with various Ca levels.

The nutrient use efficiency can be described by the dry leaf weight per unit of nutrient production and calculated as the reciprocal of the foliar nutrient concentration [40]. In this study, mulberry seedlings had the highest biomass, Pn, and N use efficiency under the optimal Ca level. In summary, the optimal Ca concentration was 200 mg·kg^{-1} under low N conditions and 400 mg·kg^{-1} under high N conditions; thus, N fertilizer increased the optimal Ca concentration. Exogenous N is widely used to increase productivity in agriculture and forestry [41]. Higher productivity means faster Ca consumption, and a higher soil Ca concentration will be needed under the same soil moisture. Ca fertilizer application should be required in soils with relatively low Ca levels and repeated biomass harvest systems to prevent the growth limitations caused by Ca and to achieve the full production increasing function of N fertilizer. High-Ca soil, which is usually accompanied by a lack of N and P, is another limiting factor for plant growth and distribution. The results

of this study show that exogenous N can raise the optimal soil Ca level, suggesting that N deposition will alleviate Ca stress and enhance species distribution to high-Ca soils.

In this study, the Pn and root biomass were significantly lower under high N conditions than under low N conditions at the Ca0 and Ca200 treatments, indicating that the photosynthesis of mulberry seedlings was limited more severely by Ca deficiency under high N treatment than under low N treatment. Similarly, Reyer et al. [42] found that N deposition led to an increase in forest productivity in northern Europe, an increase or decrease in central Europe, and a decrease in southern Europe. Overdosing N can inhibit the photosynthesis of *Cryptomeria japonica*, *Pinus densiflora*, and *Fraxinus mandshurica* [43,44], and low Ca (in this study) and other environmental factors can become limiting factors, resulting in excess N. The increase in Pn after Ca application may be due to the adjustment of Ca and the imbalance of N caused by Ca application, alleviating the influence of excessive N.

4.2. Soil Ca Influences Plant Ca Uptake

Ca is absorbed through an extracellular pathway, and Ca absorption is mainly determined by transpiration water consumption and soil Ca availability [2,4,45], which is a characteristic that leads to a positive correlation between the extractable Ca concentration in the soil and Ca concentration in the shoots. Furthermore, excess Ca can induce stoma closure and inhibit transpiration [46], while excess Ca^{2+} ions will reduce water potential in soil and inhibit water absorption. This could explain why the Ca800 treatment inhibited photosynthesis and transpiration more severely, resulting in the Ca concentrations in the shoots of these seedlings being significantly lower than those under the Ca400 treatment.

4.3. Ca Increases Plant iWUE and Drought Resistance

The plant iWUE reflects the relationship between plant water consumption and dry matter production and is an important indicator of plant drought resistance [47,48]. This study showed that exogenous Ca could increase iWUE even when the Ca level was higher than the optimal level. Ca plays a crucial role in allowing seedlings to adapt to drought stress, not only by stabilizing the cell wall and cell membrane as an essential nutrient but also by the transduction of drought signals, the regulation of stomatal closure, and the control of a series of enzyme activities as a secondary messenger [49–52]. Thus, Ca can increase iWUE, which is positively correlated with the carbon isotopic ratio [29]. At the same time, under a global background, N deposition, and N fertilizer application will promote Ca deficiency and inhibit drought resistance.

5. Conclusions

We found an optimal concentration of Ca for the growth and net photosynthetic rate of mulberry seedlings; we observed that high N levels could increase the optimum concentration of Ca in the soil. The iWUE of mulberry seedlings was significantly and positively correlated with the soil Ca level. Considering the role of Ca in plant growth and drought resistance, we found that Ca fertilizer is required to meet the demand for Ca caused by N deposition and N fertilizer, which is meaningful given the vulnerability of agriculture and forestry throughout the world to the warm and drought-prone climate predicted for the future.

Informed Consent Statement: Not applicable.

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

Conflicts of Interest: The authors declare no conflict of interest.

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