



The Optimum Calcium Concentration for Seedling Growth of Mongolian Pine (*Pinus Sylvestris* Var. *Mongolica*) Under Different Soil Types in Northern Semi-Arid Areas of China

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Mongolian pine, as one of the major tree species of the Three-North Shelterbelt Project in the northern semiarid region of China, is very important for ensuring ecological and environmental security. Ca, as an imperative mineral element for plant development and a second messenger, partakes in photosynthesis and affects the growth of plants. However, the optimal calcium concentration for its growth in different soil types is still unclear. In this study, fifteen treatments with combinations of three different soils (e.g., sandy soil, cinnamon soil, dark brown soil) and five calcium concentration gradients (e.g., 0, 100, 200, 400, and 800 mg·kg⁻¹) were conducted to investigate this effect by measuring the indices of growth, biomass, photosynthetic pigment, gas exchange rate, photosynthates, chlorophyll fluorescence parameters and water use efficiency (IWUE) based on a pot experiment. The results showed that the optimal calcium concentration of Mongolian pine seedlings in sandy soil and cinnamon soil was 0–100 mg·kg⁻¹, and the optimal calcium concentration of Mongolian pine seedlings in dark brown soil was 100–200 mg·kg⁻¹. In other words, the results showed that there was an optimal calcium concentration for the growth of Mongolian pine seedlings, and the optimal calcium concentration was different under different soil types. And if the calcium concentration in the soil was too high, it would have an inhibitory effect on Mongolian pine seedlings, low calcium concentration maybe don't work. The addition of an appropriate amount of exogenous calcium could promote the growth of Mongolian pine seedlings in different soil types. The plant height, basal diameter and biomass of Mongolian pine seedlings all increased significantly after applying an appropriate amount of calcium ($p < 0.05$); the addition of an appropriate amount of exogenous calcium could promote the photosynthetic characteristics of Mongolian pine seedlings in different soil types. Under sandy soil and cinnamon soil, the peaks for Pn, Gs, Tr, accumulation of soluble sugar and starch of Mongolian pine seedlings occurred at 0–100 mg·kg⁻¹, and the decreasing trend of the Fv/Fm value was significant in the case of exceeding 200 mg·kg⁻¹ ($p < 0.05$), indicating that the growth of Mongolian pine seedlings was affected when calcium concentrations higher than 200 mg·kg⁻¹ were applied. Moreover, under

dark brown soil, the peaks for Pn, Gs, Tr, and accumulation of soluble sugar and starch of Mongolian pine seedlings occurred at 100–200 mg·kg⁻¹; similarly, the growth of Mongolian pine seedlings was affected when calcium concentrations higher than 200 mg·kg⁻¹ were applied. Compared with the treatment without calcium, after applying an appropriate amount of exogenous calcium, the water use efficiency of Mongolian pine seedlings in different soil types was significantly improved, though it was significantly reduced at 800 mg·kg⁻¹ ($p < 0.05$).

Keywords: calcium, semi-arid regions, soil types, biomass, photosynthetic characteristics, mongolian pine seedlings, chlorophyll fluorescence, water use efficiency

1 INTRODUCTION

The semiarid region has a dry climate, scarce precipitation, uneven distribution and an extremely fragile ecological environment that is very sensitive to human activities and global climate change (Zhang et al., 2020). Artificial forest plays a great role in preventing wind, fixing sand and conserving water and soil in this area. However, under such drought environmental stress, the problems of biological instability and environmental sensitivity and vulnerability of existing plantations are prominent. This can mostly be seen in the inability to resist abnormal environments, vulnerability to fungi and pest attacks, and susceptibility to soil fertility decline (Wang et al., 2021; Wu, 2021), factors that seriously restrict the development and sustainable management of plantations. Ca is an essential nutrient element for plant growth. The main functions of calcium in plant physiology include promoting cell elongation and division, stabilizing cell wall and cell membrane, balancing yin and yang ions, participating in signal transduction as a second messenger, affecting the absorption and utilization of other nutrient elements, participating in multiple physiological metabolic processes in plants, enhancing the ability of plants to resist stress, and playing an important role in protecting and promoting plant growth (Li, 2020; Mulaudzi et al., 2020; Liu, 2021). Therefore, calcium application can be used as a means to alleviate the decline of plantation, so as to realize the healthy development of plantations in semi-arid areas and promote the coordinated development of resources and environment in this area.

Having an appropriate calcium concentration can make plants grow better (Liu et al., 2013; Xu et al., 2013; Muhammad et al., 2015; Aras et al., 2021; Mazumder et al., 2021; Muhammad, 2021). The results showed that spraying calcium on leaves could improve the tolerance of Maize and Sugar Beet to drought stress (Naeem et al., 2018; Hosseini et al., 2019). By measuring the effects of calcium on the growth, photosynthesis and antioxidant response of *Zoysia japonica*, it showed that the application of appropriate calcium concentration improved the drought tolerance of *Zoysia japonica* to a certain extent (Xu et al., 2017). However, In general, Ca-deficiency symptoms in most plants manifest as rot or necrosis at the extremities, the leaf tips or fruits. These symptoms are thought to be due to the important role Ca plays in membrane integrity and in cell wall strengthening (Simon, 1978). For example, when the plant is short of calcium, the normal physiological activities of ginseng roots, stems and

leaves will be disturbed, resulting in the weakening of its resistance to adversity (Yang, 2015). Excessive Ca content may also lead to Ca toxicity. Excessive Ca in plant cells can form precipitation with phosphate, which will inhibit seed germination, interfere with photosynthesis and reduce plant growth rate (Li et al., 2021).

Mongolian pine (*Pinus sylvestris* var. *Mongolica*) is a pioneer tree species in the construction of wind prevention and sand control of the Three-North Shelterbelt. The introduction sites of Mongolian pine are divided according to soil types and mainly include zonal soils such as dark brown soil, brown soil, black soil, chernozem, cinnamon soil, sandy soil, red soil and nonzonal soils such as albic soil, meadow soil and saline alkali soil. Mongolian pine does not have strict requirements for soil. Whether it is the dark brown soil in the fertile Northeast Plain and mountains or geologically barren desert sand dunes in the northwest plain, Mongolian pine can grow normally. Nevertheless, in recent years, Mongolian pine trees in the Three-North Shelterbelt have declined earlier than those of the same age at other sites. Their decline is manifested as early capping, a shortened growth period, inhibition of normal physiological activities, reduced growth, serious pests and diseases, etc. Moreover, the land type with the decline in *Pinus sylvestris* in the Three-North Shelterbelt was mostly sandy soil, while the decline in *Pinus sylvestris* in other land types was not obvious (Zhu et al., 2003; Zhang et al., 2020). In response to the decline of Mongolian pine in semi-arid areas, many researchers put forward the reasons for the decline of Mongolian pine in sandy land from the aspects of climate, nutrients and water, diseases and pests, soil enzyme activity and so on (Yu, 2019; Li et al., 2020; Cao, 2021; Li, 2022). However, there are few studies on how calcium affects the decline of *Pinus sylvestris* var. *mongolica* and the optimal calcium concentration. This study is based on the fact that the absorption of calcium by plants varies according to soil type. After 2 years of planting young avocado trees, their dry weights of shoots and roots were significantly higher in the SL and S soils than in C soil (Bonomelli et al., 2019). Therefore, this study measured and analyzed the effects of exogenous calcium on the growth and photosynthetic characteristics of Mongolian pine seedlings under different soil types in this area, and discussed whether there was the best calcium concentration suitable for the growth of Mongolian pine seedlings. This study will provide a theoretical basis for systematically improving the decline of Mongolian pine seedlings under zonal soil and realizing better management of Mongolian pine forests in the future.

2 MATERIALS AND METHODS

2.1 Cultivation of Mongolian Pine Seedlings

This experiment was carried out at the Beishan experimental station of Shenyang Agricultural University. Three-year-old seedlings of Mongolian pine with uniform growth were selected as tested materials (the average plant height was 22.5 cm, and the average basal diameter was 7.1 mm). They were colonized on 15 June 2018. Different soils were screened to remove stones and impurities, and 3 kg of experimental soil (air-dried soil weight) was accurately weighed and mixed with 2 kg of quartz sand (60 mesh) as pot experimental soil. The substrates used were first mixed thoroughly and then washed with dilute hydrochloric acid before filling the pots to remove the possible Ca^{2+} . The pH of the soil-sand mixture after washing with hydrochloric acid was 6.3. The volume of each pot was 11.36 l, with a lower diameter of 19.4 cm, an upper diameter of 24.3 cm and a height of 26.5 cm (Zhu et al., 2005). One Mongolian pine seedling was colonized in each experimental pot. Plastic trays were set under the basin to prevent water loss. After half a month of recovery, calcium treatment was carried out on July 1, 2018, and the nutrient solution was changed every 7 days until the seedlings were collected on June 17, 2019. The nutrient solution was prepared with ultrapure water according to Xie's (2014) sand culture nutrient solution formula, and the pH value of the nutrient solution was adjusted to five to six with NaOH. The nutrients were 5 ml/L KNO_3 , 5 ml/L MgSO_4 , 5 ml/L KH_2PO_4 , 5 ml/L NaNO_3 and 5 ml/L EATA-Fe.

2.2 Experimental Design

In this experiment, Mongolian pine seedlings in each type of soil were divided into 5 Ca treatments, and each treatment was repeated 6 times. Three soil types: sandy soil, cinnamon soil and dark brown soil. The calcium was applied at five levels: 0, 100, 200, 400 and 800 $\text{mg}\cdot\text{kg}^{-1}$. The CaCl_2 solution was divided into several equal parts, and the soil was then irrigated to ensure a uniform distribution of CaCl_2 in the soil. The test sandy soil was taken from Tieling City, Liaoning Province (123°27'-125°06'E, 41°59'-43°23'N), the test cinnamon soil was taken from Chaoyang City, Liaoning Province (122°25'-123°48'E, 41°12'-42°17'N), and the test dark brown soil was taken from the Daxinganling region (121°12'-127°00'E, 50°10'-53°33'N). This region is located in the semiarid region of China and has an annual average temperature of 5.5–8.4°C and an annual average precipitation of approximately 400 mm. Soil samples between 0–40 cm soil depth were randomly collected from multiple points based on a standard site and then transported to the base of the university. The basic properties of organic matter content, total calcium and water-soluble calcium of mixed sandy soil samples were 5.51%, 1040 $\text{mg}\cdot\text{kg}^{-1}$ and 30 $\text{mg}\cdot\text{kg}^{-1}$, respectively. The basic properties of organic matter content, total calcium and water-soluble calcium of mixed cinnamon soil samples were 8.14%, 1980 $\text{mg}\cdot\text{kg}^{-1}$ and 60 $\text{mg}\cdot\text{kg}^{-1}$, respectively. The basic properties of organic matter content, total calcium and water-soluble calcium of mixed dark brown soil samples were 31.89%, 3580 $\text{mg}\cdot\text{kg}^{-1}$ and 80 $\text{mg}\cdot\text{kg}^{-1}$, respectively.

2.3 Determination of the Growth Indices of Mongolian Pine Seedlings

2.3.1 Growth of Mongolian pine seedlings

The basic plant heights and diameters were measured after planting in June 2018, and the plant heights and diameters of Mongolian pine seedlings were measured again when the seedlings were collected in June 2019. The plant heights were measured with a tape, accurate to 0.10 cm; the basal diameters were measured with a Vernier caliper, accurate to 0.01 mm.

2.3.2 Biomass of Mongolian pine seedlings

After the whole plant was completely removed from the pot, the seedlings with whole roots were carefully washed to remove the substrate. Subsequently, the whole plants were divided into roots, stems and leaves with pruning shears and were placed into envelopes for labelling. The envelopes were then placed in an oven at 105°C for 30 min and dried to a constant weight at 65°C. The dry weights of the roots, stems, leaves and total plant biomass were determined with an analytical balance.

2.4 Determination of the Photosynthetic Characteristics and Water Use Efficiency of Mongolian Pine Seedlings

2.4.1 Gas exchange rates

The net photosynthetic rate (P_n), transpiration rate (T_r) and stomatal conductance (G_s) values of the plants were measured with a LI-COR 6400 system (LI-COR Inc, Lincoln, NE, United States) between 10:00 and 12:00 on each sampling day. The effective light intensity was set at 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and repeated 3 times for each treatment for each soil type.

2.4.2 Photosynthesis

To measure the soluble sugar content, oven-dried samples (50 mg) were macerated in 80% ethanol, and after centrifugation, the supernatant was reacted with anthrone reagent following the method of Shields and Burnett (1960). Calculation was performed from the standard curve of glucose. For starch analysis, 0.5 ml of starch extract and 4.5 ml of distilled water were added to a test tube. The test tube was placed into an ice bath, and then 10 ml of anthrone reagent was slowly added into the test tube. The tubes were placed in a boiling water bath for exactly 7.5 min before being immediately cooled in an ice bath. After cooling, the absorbance at 630 nm in 1 h using a UV-8000 spectrophotometer (Yuanxi, Beijing, China) was measured (Doan et al., 2019).

2.4.3 Chlorophyll fluorescence

To examine the influence of calcium on the minimal fluorescence F_0 , the maximum fluorescence F_m , the variable fluorescence F_v , and the maximal photochemical efficiency of photosynthesis system II, $F_v/F_m = (F_m - F_0)/F_m$, were measured by using a portable pulse modulated chlorophyll fluorescence metre (OS-5P+, United States) between 16:00 and 18:00 on each sampling

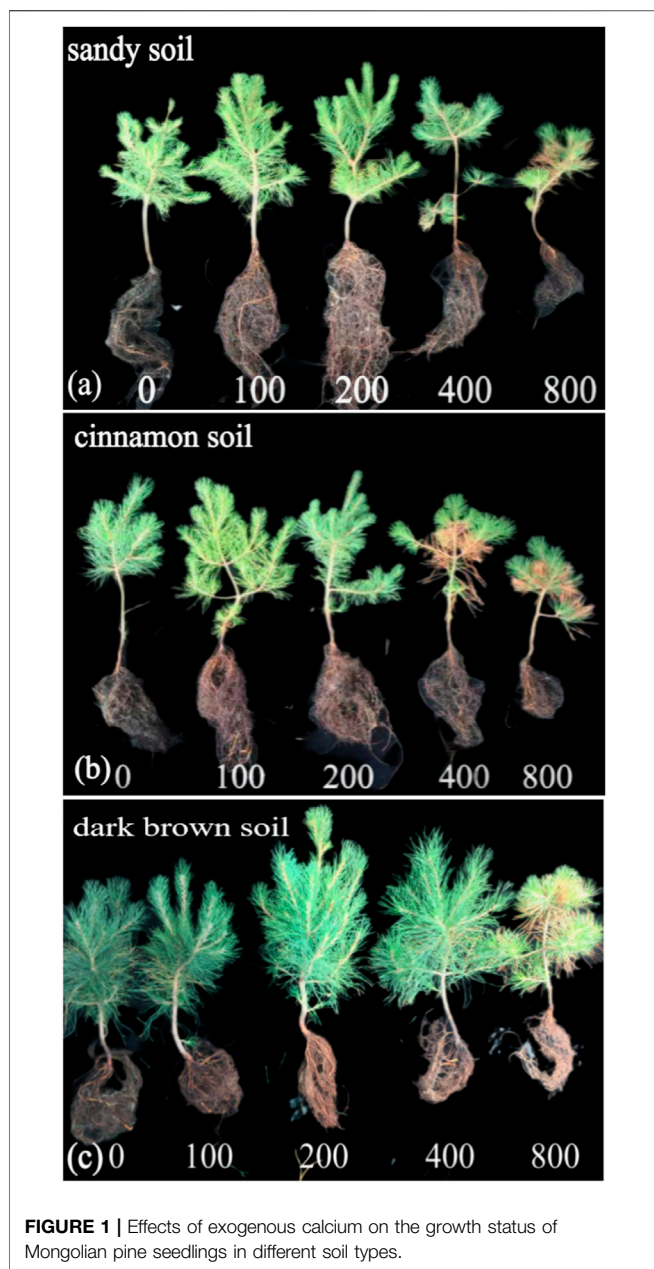


FIGURE 1 | Effects of exogenous calcium on the growth status of Mongolian pine seedlings in different soil types.

day. Before the measurements, the leaves were dark-adapted for 20 min by using light-exclusion clips.

2.4.4 Water use efficiency

The washed plant leaves were put in an oven at 105°C for 30 min, dried at 80°C for 2 h to a constant weight, and then ground through a 100-mesh sieve with a ball mill (Retsch200, Germany). Approximately 0.7 mg of each sample was taken, which was tightly wrapped with a tin boat, and 13°C was then measured using a stable isotope mass spectrometer (DELTA V Advantage Isotope Ratio Mass Spectrometer). Then, the long-term water use efficiency value (WUEL) was calculated from 13°C and used to characterize the overall water use efficiency (WUE) of the Mongolian pine seedlings.

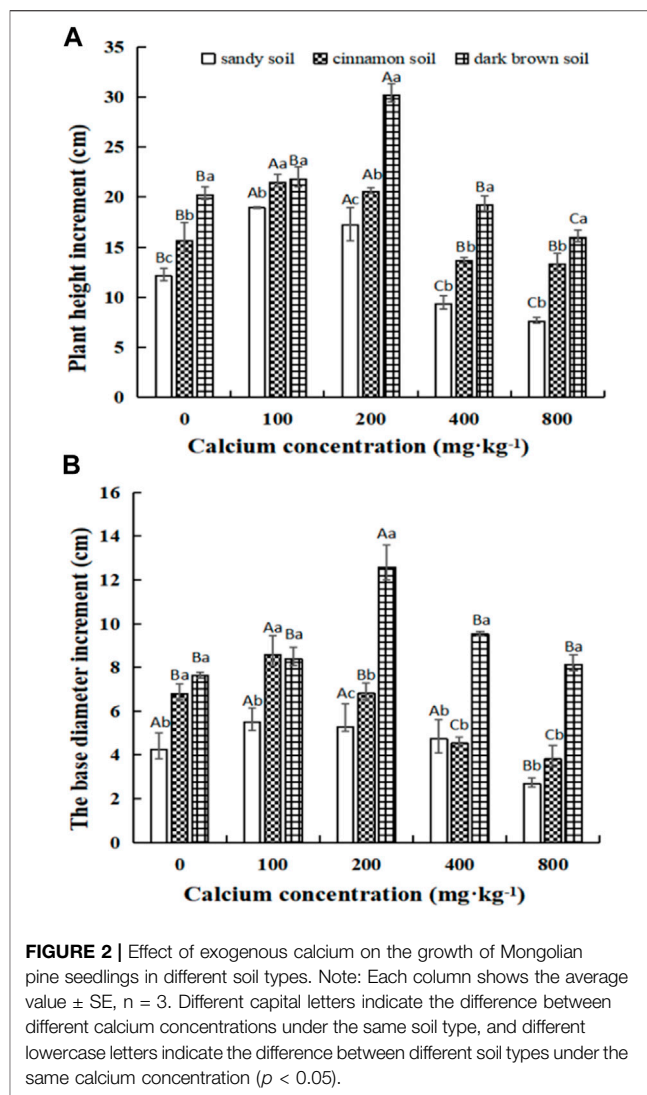


FIGURE 2 | Effect of exogenous calcium on the growth of Mongolian pine seedlings in different soil types. Note: Each column shows the average value \pm SE, $n = 3$. Different capital letters indicate the difference between different calcium concentrations under the same soil type, and different lowercase letters indicate the difference between different soil types under the same calcium concentration ($p < 0.05$).

2.6 Statistical Analysis

All results are expressed as the mean \pm standard error (SE) of three replications. The effects of different calcium treatments on the growth and physiological characteristics of Mongolian pine seedlings were analysed by one-way ANOVA and Duncan's new multiple extreme difference method. Different capital letters indicate the difference between different calcium concentrations under the same soil type, and different lowercase letters indicate the difference between different soil types under the same calcium concentration. The software packages SPSS 19.0 (IBM, Chicago, United States) and Excel 2019 were used to conduct the statistical analyses and to draw charts.

3 RESULTS

3.1 Growth Status of Mongolian Pine Seedlings

The growth status of the Mongolian pine seedlings in different soil types is shown in **Figure 1a-c**. For sandy soil and cinnamon

TABLE 1 | Effects of exogenous calcium on the biomass of Mongolian pine seedlings in different soil types.

Soil Type	Calcium Treatment (mg·kg ⁻¹)	Root Biomass (g)	Longest Root (cm)	Stem Biomass (g)	Leaf Biomass (g)	Total Biomass (g)
Sandy soil	0	26.91 ± 0.03Ab	67.65 ± 0.03Aa	17.867 ± 0.06Cc	24.904 ± 0.03Cb	69.681 ± 0.12Cc
	100	26.845 ± 0.07Ac	50.74 ± 0.03Cb	23.745 ± 0.09Ab	31.14 ± 0.32Ac	81.73 ± 0.16Ac
	200	23.015 ± 0.02Bc	53.59 ± 0.25Bb	23.218 ± 0.01Bb	28.975 ± 0.08Bc	75.208 ± 0.11Bc
	400	18.498 ± 0.01Cb	43.3 ± 0.02Dc	13.618 ± 0.04Db	13.264 ± 0.05Db	45.38 ± 0.08Db
	800	9.666 ± 0.01Dc	42.8 ± 0.64Db	8.561 ± 0.04Ec	9.267 ± 0.24Ec	27.494 ± 0.20Ec
Cinnamon soil	0	29.123 ± 0.08Ba	42.81 ± 0.04Db	22.359 ± 0.06Ba	24.653 ± 0.23Cb	76.1351 ± 0.21Cb
	100	27.809 ± 0.18Cb	37.62 ± 0.02Ec	23.769 ± 0.01Ab	40.455 ± 0.04Ab	92.033 ± 0.16Ab
	200	30.7 ± 0.06Ab	71.15 ± 0.08Aa	20.077 ± 0.01Cc	39.979 ± 0.11Bb	90.756 ± 0.17Bb
	400	16.839 ± 0.01Dc	47.3 ± 0.09Bb	9.058 ± 0.06Ec	10.817 ± 0.09Ec	36.714 ± 0.04Dc
	800	15.522 ± 0.03 Eb	46.21 ± 0.05Ca	10.007 ± 0.06Db	11.454 ± 0.11Db	36.983 ± 0.15Db
Dark brown soil	0	25.94 ± 0.05Cc	40.52 ± 0.10Dc	20.448 ± 0.08Db	46.363 ± 0.04Da	92.751 ± 0.02Da
	100	34.561 ± 0.11Ba	75.92 ± 0.15Aa	28.498 ± 0.01Ba	53.103 ± 0.11Ca	116.162 ± 0.21Ba
	200	44.958 ± 0.08Aa	44.61 ± 0.14Cc	48.287 ± 0.07Aa	72.82 ± 0.08Aa	166.065 ± 0.23Aa
	400	24.585 ± 0.02Da	67.2 ± 0.03Ba	25.846 ± 0.04Ca	63.567 ± 0.07Ba	113.998 ± 0.09Ca
	800	19.391 ± 0.12Ea	31.51 ± 0.18Ec	13.384 ± 0.07Ea	31.67 ± 0.05Ea	64.445 ± 0.00Ea

Each column shows the average value ±SE, n = 3. Different capital letters indicate the difference between different calcium concentrations under the same soil type, and different lowercase letters indicate the difference between different soil types under the same calcium concentration ($p < 0.05$).

soil, the growth status was good at 0–100 mg·kg⁻¹, while for dark brown soil, the growth status of the Mongolian pine seedlings was good at 100–200 mg·kg⁻¹. When the calcium concentration was not appropriate in the different soil types, Mongolian pine seedlings grew poorly or even died.

3.2 Growth and Biomass of Mongolian Pine Seedlings

In general, the growth indicators (plant height and basal diameter) of Mongolian pine seedlings of different soil types increased first and then decreased with increasing calcium concentration (Figure 2A,B). Calcium had different effects on the organs and total biomass of Mongolian pine seedlings in different soil types (Table 1).

The peaks for the average plant height increment and the average basal diameter increment of sandy soil and cinnamon soil occurred at 100 mg·kg⁻¹, while the peaks for the average plant height increment and the average basal diameter increment of dark brown soil occurred at 200 mg·kg⁻¹ (Figure 2).

Regarding the biomass of Mongolian pine seedlings (Table 1), the influence of calcium on the stems, leaves and total biomass of the three soils was consistent. The peaks for sandy soil and cinnamon soil occurred at 100 mg·kg⁻¹, and the peak for dark brown soil occurred at 200 mg·kg⁻¹. Subsequently, the downwards trends for the stem, leaf and total biomass of the three soils were shown with the increase in calcium concentration ($p < 0.05$). The three lowest values of stem, leaf and total biomass in sandy soil and dark brown soil occurred at 800 mg·kg⁻¹, while the three lowest values of stem, leaf and total biomass in cinnamon soil occurred at 400 mg·kg⁻¹. The peaks for root biomass in cinnamon soil and dark brown soil occurred at 200 mg·kg⁻¹, while the peak in sandy soil occurred at 0–100 mg·kg⁻¹, and the three lowest values for the three soils occurred at 800 mg·kg⁻¹. The peak for the longest root biomass in sandy soil occurred at 0, that in cinnamon soil was at 200 mg·kg⁻¹, and that in dark brown soil was at 100 mg·kg⁻¹, while the three lowest values also appeared at different calcium gradients.

3.3 Gas Exchange Rates of Mongolian Pine Seedlings

In general, the gas exchange rates (Pn, Gs, Tr) showed a trend of first increasing and then decreasing with increasing calcium concentration in different soil types (Figure 3A–C).

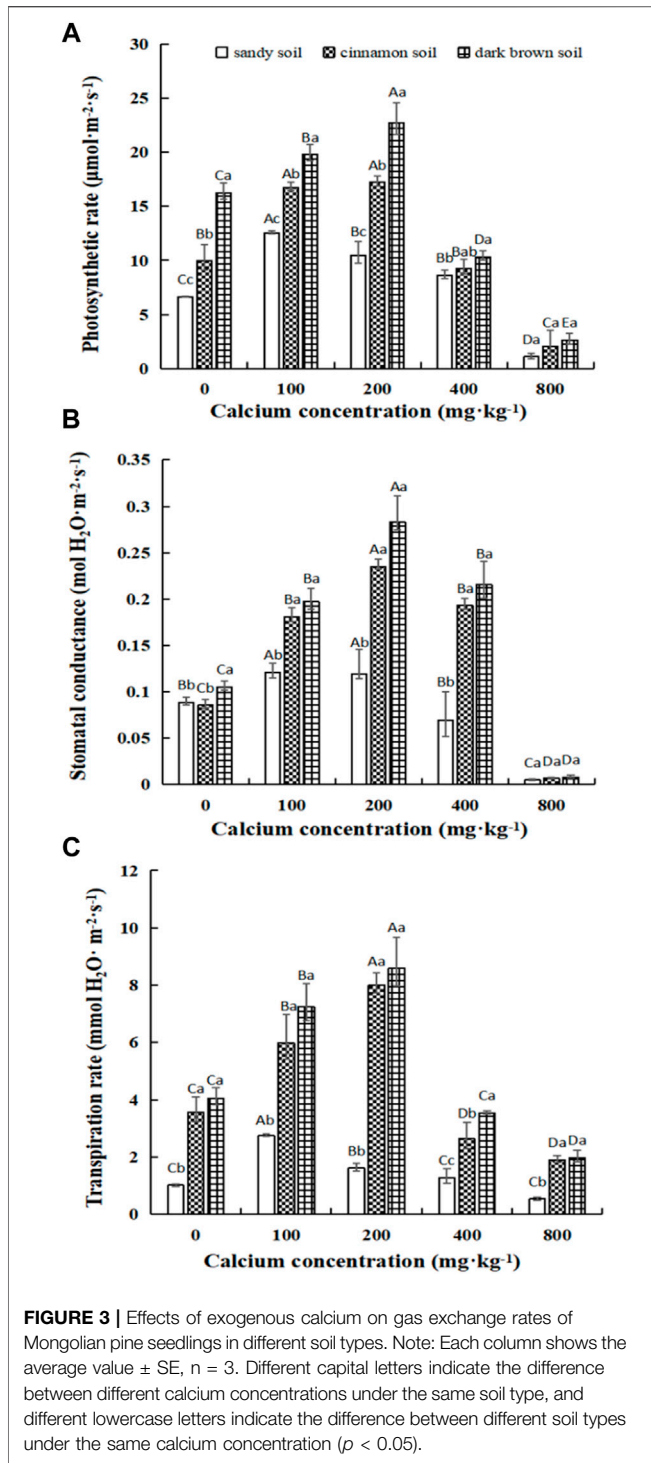
First, the peaks for the gas exchange rates (Pn, Gs, Tr) of sandy soil all occurred at 100 mg·kg⁻¹, which were 12.55 μmol·m⁻²·s⁻¹, 0.12 molH₂O·m⁻²·s⁻¹, and 2.74 mmolH₂O·m⁻²·s⁻¹ and were 89.01%, 50.01% and 168.62% higher, respectively, than when external calcium was not applied ($p < 0.05$); the peak (17.21 μmol·m⁻²·s⁻¹) for the Pn of cinnamon soil occurred at 100 mg·kg⁻¹, which was 72.44% higher than that without external calcium ($p < 0.05$). Nevertheless, the peaks for the Gs and Tr of cinnamon soil occurred at 200 mg·kg⁻¹, which were 0.23 molH₂O·m⁻²·s⁻¹ and 8.01 mmolH₂O·m⁻²·s⁻¹ and were 187.5% and 125.63% higher, respectively, than when external calcium was not applied ($p < 0.05$); dark brown soil was similar to cinnamon soil, and the peak (22.71 μmol·m⁻²·s⁻¹) for the Pn occurred at 100 mg·kg⁻¹. The peaks for Gs and Tr occurred at 200 mg·kg⁻¹, which were 0.28 molH₂O·m⁻²·s⁻¹ and 8.59 mmolH₂O·m⁻²·s⁻¹ and were 154.54% and 112.1% higher, respectively, than those without exogenous calcium ($p < 0.05$).

In addition, the downwards trends of Pn, Gs and Tr of sandy soil were shown in the case of reaching and exceeding 100 mg·kg⁻¹, and the downwards trends of Pn, Gs and Tr of cinnamon soil and dark brown soil were shown in the case of reaching and exceeding 200 mg·kg⁻¹.

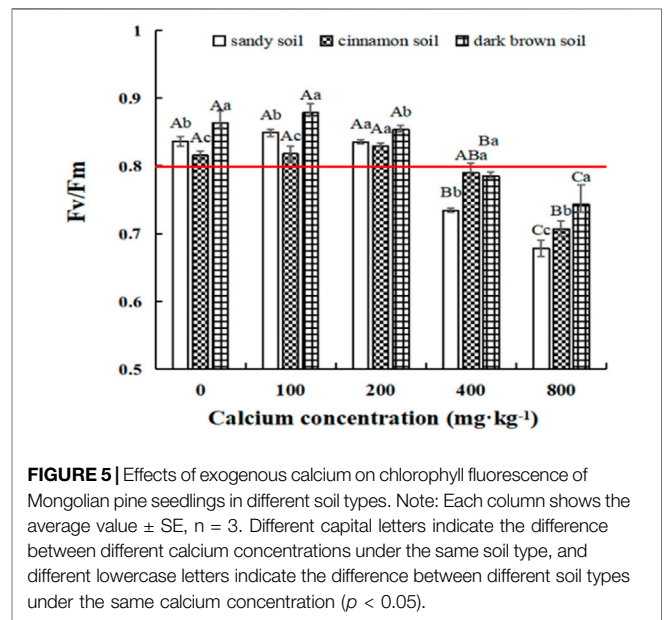
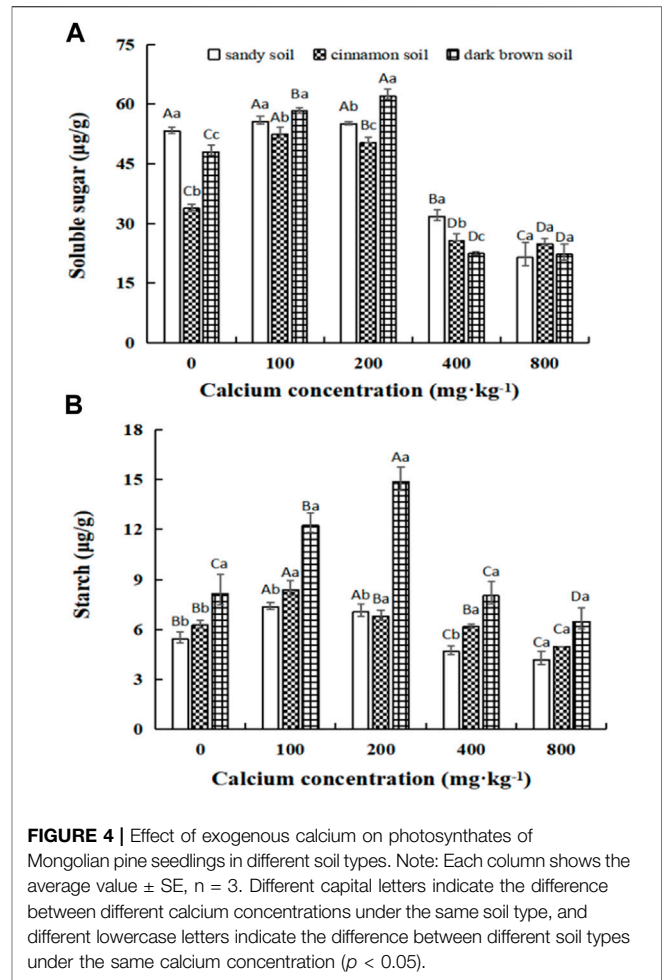
3.4 Photosynthesis of Mongolian Pine Seedlings

In general, the photosynthates (soluble sugar, starch) showed a trend of first increasing and then decreasing with increasing calcium concentration in different soil types (Figure 4A,B).

First, the peaks for the soluble sugar and starch contents of sandy soil occurred at 100 mg·kg⁻¹, which were 55.65 μg/g and



7.35 $\mu\text{g/g}$ and were 4.51% and 35.35% higher, respectively, than those without external calcium. Cinnamon soil is similar to sandy soil. The peaks for the soluble sugar content and starch content of cinnamon soil occurred at 100 $\text{mg}\cdot\text{kg}^{-1}$, which were 52.39 $\mu\text{g/g}$ and 8.37 $\mu\text{g/g}$ and were 55.18% and 33.92% higher, respectively, than those without exogenous calcium. Unexpectedly, the peaks for the soluble sugar content and starch content of dark brown



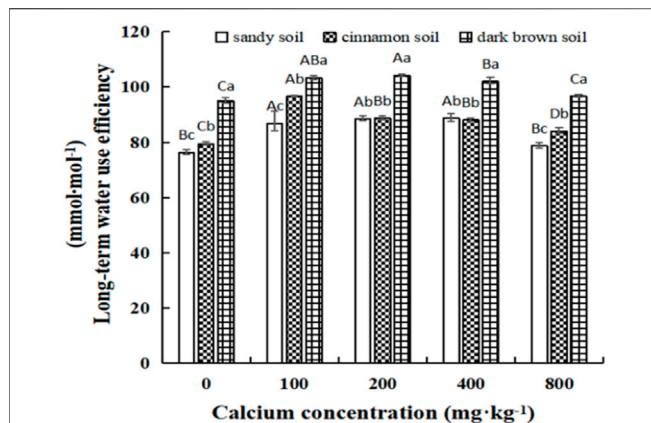


FIGURE 6 | Effects of exogenous calcium on the long-term water use efficiency of Mongolian pine seedlings in different soil types. Note: Each column shows the average value \pm SE, $n = 3$. Different capital letters indicate the difference between different calcium concentrations under the same soil type, and different lowercase letters indicate the difference between different soil types under the same calcium concentration ($p < 0.05$).

soil occurred at 200 mg·kg⁻¹, which were 62.09 μ g/g and 14.86 μ g/g and were 29.3% and 82.33% higher, respectively, than those without exogenous calcium.

In addition, under sandy soil and cinnamon soil, the downwards trends of the soluble sugar content were shown in the case of reaching and exceeding 400 mg·kg⁻¹, and the downwards trends of the starch content were shown in the case of reaching and exceeding 200 mg·kg⁻¹; under dark brown soil, the downwards trends of the soluble sugar content and starch content were shown in the case of reaching and exceeding 400 mg·kg⁻¹.

3.5 Chlorophyll Fluorescence of Mongolian Pine Seedlings

In general, the Fv/Fm values showed a decreasing trend with increasing calcium concentration in the different soil types (Figure 5).

First, the Fv/Fm values of sandy soil, cinnamon soil and dark brown soil were close to those of exogenous calcium at 0, 100 and 200 mg·kg⁻¹, and the changes were relatively gradual. Second, for the three soils, when the exogenous calcium content was greater than or equal to 400 mg·kg⁻¹, the Fv/Fm values were all lower than 0.8, indicating that the seedling growth of Mongolian pine was in a stressed state. The differences were that the Fv/Fm values of sandy soil were reduced by 12.04% and 19.27% at 400 and 800 mg·kg⁻¹, respectively, compared with no exogenous calcium and showed significant differences ($p < 0.05$). The Fv/Fm value of cinnamon soil was reduced by 13.58% at 800 mg·kg⁻¹ compared with no exogenous calcium and showed a significant difference ($p < 0.05$). The Fv/Fm values of dark brown soil were reduced by 10.46% and 13.95% at 400 and 800 mg·kg⁻¹, respectively, compared with no exogenous calcium and showed significant differences ($p < 0.05$).

3.6 Water Use Efficiency of Mongolian Pine Seedlings

In general, the long-term water use efficiency (WUEL) showed a trend of first increasing and then decreasing with increasing calcium concentration in the different soil types (Figure 6).

First, the peak (88.66 mmol·mol⁻¹) for the WUEL of sandy soil occurred at 400 mg·kg⁻¹, which was 16.32% higher than that without exogenous calcium ($p < 0.05$); the peak (96.56 mmol·mol⁻¹) for the WUEL of cinnamon soil occurred at 100 mg·kg⁻¹, which was 21.75% higher than that without exogenous calcium ($p < 0.05$); and the peak (104.14 mmol·mol⁻¹) for the WUEL of dark brown soil occurred at 200 mg·kg⁻¹, which was 9.75% higher than that without exogenous calcium ($p < 0.05$).

In addition, downwards trends of the WUEL of sandy soil, cinnamon soil and dark brown soil were shown in the case of reaching and exceeding 800, 200 and 400 mg·kg⁻¹, respectively.

4 DISCUSSION

4.1 An Optimal Calcium Concentration for the Growth of Mongolian Pine Seedlings

Calcium is essential not only for maintaining membrane integrity and improving cell structure, but it is also a signal for protecting plant cell walls and a second messenger for membrane stabilizers. At the same time, according to changes in the external environment, calcium can also provide timely feedback to the plants. (Singh and Pandey, 2020; Martins et al., 2021; Raina et al., 2021; Zhao et al., 2021). Our results showed that the appropriate amount of exogenous calcium promotes the plant height, base diameter and biomass of Mongolian pine seedlings in different soil types. Studies have shown that when plants are deficient in calcium, they often show the characteristics of blocked growth, short internodes, short and soft plant heights, yellow leaves, necrotic growth points, and little or no fruit (Liu et al., 2019; Hagagg et al., 2020; Aras et al., 2021). However, our results are slightly different from this, showing that the leaves of Mongolian pine seedlings are more likely to turn yellow at high calcium, while the performance of Mongolian pine seedlings is not obvious at low. Our results showed that an appropriate amount of exogenous calcium could improve the photosynthetic characteristics of Mongolian pine seedlings, and improve their adaptability to the environment. This is consistent with previous research results. Salinity radically slowed down growth of rice seedlings and Ca²⁺ noticeably improved growth performances. Exogenous application of Ca²⁺ (10 mM CaCl₂) increased the total chlorophyll content, while the 15 mM CaCl₂ sometimes showed negative effect on the aspect of mitigating effect of salinity in rice (Rani et al., 2019). It may be that when the calcium content in the external environment is too high, the excessive calcium in the plant inhibits the absorption and utilization of other elements, which causes the plant to receive calcium stress, which leads to slow growth and development (Madani et al., 2013; Peng et al., 2020; Feng et al., 2021; Wang, 2021). Foliar application of calcium reduces the effects of low night temperature (LNT) stress on the growth and photosynthetic characteristics of *Arachis hypogaea* and significantly improves its cold resistance (Wu et al., 2020). Supplementation with Ca²⁺ helps to

alleviate the effect of Cd stress on the photosynthetic light response of rice (Sebastian and Prasad, 2019). In the study of exogenous calcium on plant chlorophyll fluorescence, it has been found that supplementation with 20 or 25 mM Ca^{2+} can increase the maximum quantum yield (Fv/Fm) of PSII to greater than 0.8, thereby enhancing the freezing resistance of spinach (Min et al., 2021). A concentration of 5 mM Ca^{2+} can significantly increase the Fv/Fm value and most effectively alleviate the damage of drought stress to Tung tree seedlings (Li et al., 2017). Our results showed that applying an appropriate amount of exogenous calcium significantly increased the photosynthates of Mongolian pine seedlings. This is consistent with previous research results. Calcium can increase the activity levels of various enzymes under cadmium stress by inducing the biosynthesis of nitric oxide (NO) and hydrogen sulfide (H_2S), thereby increasing carbohydrate accumulation (Khan et al., 2020). Similarly, in terms of water use efficiency, our results were consistent with the previous research results. It was considered that exogenous calcium could significantly improve the water use efficiency of Mongolian pine seedlings (Ren et al., 2020). In summary, the seedlings of Mongolian pine in our experiment had the most suitable calcium concentration for growth. The optimal calcium concentration can promote the growth of Mongolian pine seedlings under different soil types and enable them to improve their own gas exchange rates, increase the accumulation of their own photosynthates, improve their own water use efficiency, and enhance their own drought resistance.

4.2 The Optimal Calcium Concentration Varies Among Different Soils

Our results showed that the surface of the sandy soil layer dries faster under the same temperature and light conditions, the water in the soil flows into the tray set at the bottom faster, and the water storage capacity of dark brown soil is significantly stronger than that of cinnamon soil and stronger than that of sandy soil. Although the three soil types of Mongolian pine seedlings were uniformly watered and irrigated in our experiment, due to the different soil aggregate structures of sandy soil, cinnamon soil and dark brown soil, their soil water retention capacities were also different. Studies have revealed that soil water content plays a vital role in plant growth (Reich et al., 2018; Zhou et al., 2019; Sutinen and Middleton, 2020). Studies have emphasized that the observed soil moisture conditions are responsible for the degradation of Mongolian pine (Dang et al., 2021). Therefore, the phenomenon that Mongolian pine seedlings is more prone to decline in sandy soil can be explained to a certain extent.

Our research finds that there is an optimal calcium concentration for seedling growth of Mongolian pine, the optimal calcium concentration of Mongolian pine seedlings is different under different soil types, and the optimal calcium concentration required by Mongolian pine seedlings in dark brown soil is higher than those of sandy soil and cinnamon soil. The author speculates that the occurrence of this phenomenon is related to the structural properties of the soil itself (Cristina et al., 2020; Zhao et al., 2021; Bonomelli et al., 2019); the reason may be that during the cultivation of Mongolian pine seedlings, the seedlings grown in dark brown soil show stronger water retention capacity. In the experiment, exogenous calcium was added to the soil by dissolving it in water, which caused

more calcium to be stored in the soil in the dark. This is in line with the experimental results: Mongolian pine seedlings have the longest root system in the dark brown soil environment. The root system is an important nutrient organ of the plant and plays important roles in the absorption of water and nutrients in the plant body and in improving the soil structure (Griffiths et al., 2021; Suzzdaleva et al., 2021; Xiao et al., 2021; Xiao et al., 2020). The soil water retention capacity of Mongolian pine seedlings on sandy soil is weak, which results in less calcium content in the soil compared with dark brown soil. From the data point of view, its root system growth is not as developed as that of dark brown soil, and ultimately, the growth status is not as strong as that of dark brown soil. Therefore, the tests show that the optimal calcium concentration of Mongolian pine seedlings in sandy soil is lower than that in dark brown soil. Many research results are similar to our results, that is, plants need calcium within a certain concentration range, and there is an optimal calcium concentration to make plants grow better. For example, 80 mmol·L⁻¹ is the optimal calcium concentration to increase the germination rate of corn under low-temperature stress, which is conducive to the development of cold-tolerant corn varieties, and 20 mmol·L⁻¹ is an appropriate calcium concentration to increase corn yield in coal mining areas under the synergistic effect of AMF (Li et al., 2013; Zhang et al., 2020). In summary, during the cultivation of Mongolian pine seedlings in semiarid areas, according to the physical and chemical properties and structure of the soil itself, different concentrations of exogenous calcium should be applied to the soil to achieve the optimal growth effect.

5 CONCLUSION

Exogenous calcium promoted plant growth in semiarid regions. Adding an appropriate amount of exogenous calcium could enhance the plant height, basal diameter, biomass, photosynthetic characteristics, photosynthates and water use efficiency of Mongolian pine seedlings in different soil types. However, excessive exogenous calcium inhibited the growth of Mongolian pine seedlings and an insufficient concentration of exogenous calcium don't work. The results showed that there was an optimal calcium concentration for the growth of Mongolian pine seedlings, and the optimal calcium concentration was different under different soil types. The optimal calcium concentration of Mongolian pine seedlings in sandy soil and cinnamon soil was 0–100 mg·kg⁻¹, and the optimal calcium concentration of Mongolian pine seedlings in dark brown soil was 100–200 mg·kg⁻¹.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HL: Conceptualization, investigation, methodology, visual-ization, Writing—review and editing. XL: Data analysis and sorting.

testing, writing—original draft, cultivating seedlings. GZ: Cultivating seedlings, Writing—review and editing. XW, Cultivating seedlings, Writing—review and editing. SH, Cultivating seedlings, testing. YZ, Conceptualization, Methodology. SZ: Methodology. LL: Methodology. JP: Methodology, Writing—review and editing; Conceptualization.

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