

Seedling growth, root development and nutrient use efficiency of Cypress clones in response to calcium fertilizer

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Research article

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Abstract

Background:

Cypress (*Cupressus funebris* Endl.) is an important tree species in the subtropics of China, it is also a major tree species for afforestation and forest land restoration under infertile site conditions. Cypress is considered to be a calcicolous tree, whose growth and development can be promoted significantly by exchangeable Calcium (Ca^{2+}) in the soil. However, most of the subtropical regions have infertile acidic soils, in which Ca^{2+} gradually becomes a limiting element for cypress growth.

Results:

In this study, different concentrations of Ca^{2+} fertilizer were added under fertile and infertile soil conditions. Cypress clones responded differently to Ca^{2+} addition in different soil conditions. In the infertile soil, the addition of $3 \text{ g}\cdot\text{kg}^{-1}$ Ca^{2+} advanced and prolonged the fast-growing period of seedling height growth, increased plant height and dry biomass, promoted the development of fine roots ≤ 1.5 mm in diameter, and improved accumulation efficiencies of nitrogen (N), phosphorous (P) and Ca by the roots in cypress clones; however, the addition of $6 \text{ g}\cdot\text{kg}^{-1}$ Ca^{2+} inhibited height growth and root development of cypress. In the fertile soil, Ca^{2+} addition delayed and shortened the fast-growing period for cypress height growth, but plant height and dry biomass did not differ significantly between treatments; Ca^{2+} addition also inhibited the development of fine roots. The clone with fast height growth had a larger proportion of roots with a diameter ≤ 1.5 mm and achieved higher N accumulation efficiency, while Ca accumulation efficiency showed genotypic differences only in the fertile soil.

Conclusions:

An appropriate level of Ca^{2+} can be added to infertile soil to promote cypress seedling growth, and clones with fast height growth and developed fine roots can be selected for cultivation and promotion in the fertile soil without Ca^{2+} application.

Background

Calcium (Ca^{2+}) is an essential nutrient required for plant growth and development [1, 2]. Plants generally have a Ca^{2+} content of 0.1–5.0%, which plays a role in maintaining the stability of plant cell walls, cell membranes and membrane-bound proteins, modulating inorganic ion transport and regulating various enzyme activities [3–6]. Co-application of Ca^{2+} and other elements, such as nitrogen (N), phosphorous (P) and potassium (K), can modulate intracellular Ca^{2+} levels, promote seedling growth and root development and enhance plant stress resistance [7, 8]. Applying an appropriate amount of Ca^{2+} fertilizer to forest trees and crops, such as Chinese fir [*Cunninghamia lanceolata* (Lamb.) Hook.] and peanut (*Arachis hypogaea* Linn.), can facilitate root development and increase N, P and K uptake by seedlings [2, 9]. Despite the various benefits of Ca^{2+} for plant growth, high concentrations of Ca^{2+} often have an inhibitory effect on plant growth and development [10].

The root is an important organ of plants for resource acquisition, and the spatiotemporal distribution of plant roots determines how much water and nutrients are absorbed for photosynthesis and harvest products [11, 12]. Additionally, the root acts as a supporting organ, which allows the plants to be fixed under the ground for a long time, ensuring their normal growth and development [13]. In different growth conditions, functional attributes such as root number and morphology have differential responses to changes in underground resources. For example, fine roots with a diameter of ≤ 1.5 mm are key for nutrient uptake, accounting for more than 80% of total root length and total root surface area [14]. When the soil environment is altered, the length growth rate for fine roots becomes faster or slower and the mean root diameter becomes thicker or thinner in a short amount of time; the duration of such changes varies [15]. By contrast, roots with a diameter of > 1.5 mm mainly play a role in transport and support, and they constitute a relatively low proportion of total root length and surface area [8]. Especially for tall arbores, their root growth and development status determine forest tree growth status in the next few years or even decades.

Plants growing in acidic or low base-saturation soils are prone to Ca^{2+} deficiency [16]. Ca^{2+} deficiency has gradually emerged as a limiting factor that influences the growth of crops and forest trees. In the subtropics of China, fast-growing plantations are primarily

distributed in large areas of hills and mountains, and these areas are mostly covered by infertile acidic red soils. Due to climate change and other reasons, acid deposition areas are continuously expanding along with a serious loss of base ions, especially Ca^{2+} in the soil. This results in a deficiency of soil nutrients and a decline in soil fertility, which in turn severely restricts the growth and development of forest trees and the productivity of forest stands.

Cypress (*Cupressus funebris* Endl.) is an important tree species in the subtropics of China, mainly occurring in the Yangtze River Basin and to the south. Cypress is highly adaptive and can grow in a variety of site conditions, especially on limestone mountains (including acidic or weakly alkaline soils); thus, it is also a major tree species for afforestation and forest land restoration under infertile site conditions. Cypress is calcicole, whose growth and development can be promoted significantly by exchangeable Ca^{2+} in the soil. However, most of the subtropical regions have infertile acidic soils, in which Ca^{2+} gradually becomes a limiting element for cypress growth. Previous research has found substantial variation in the root length of cypress when the soil environment is disturbed; this effect is long lasting and, in particular, greater for the number, morphology and function of fine roots [17]. However, there are diverse types of soil with a wide variety of physicochemical properties in areas where cypress is distributed. Little has been reported on root development and nutrient use of cypress in response to Ca^{2+} across different soil environments. Research is therefore needed to clarify Ca^{2+} fertilizer requirements for the seedling growth and root development of cypress, as the results play major indicative roles in improving seedling quality and forest tree productivity [18, 19].

In this study, different concentrations of Ca^{2+} fertilizer were added under fertile and infertile soil conditions. The objectives of this study were to explore (1) the seedling height growth of cypress clones in response to Ca^{2+} fertilizer; (2) the effects of Ca^{2+} addition on the roots of different diameter classes in terms of their length, surface area, and volume; and (3) the variation in N, P and Ca accumulation efficiencies of cypress under different nutrient conditions.

Results

Cypress seedling height and height growth rhythm

The seedling height of the cypress clones appeared to be different after Ca^{2+} was added to the 2 soils. In the fertile soil, seedling height of cypress did not differ significantly among the Ca^{2+} treatments. In the infertile soil, there were significant differences in seedling height among the Ca^{2+} treatments ($P < 0.01$). The treatment with $3 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$ significantly promoted seedling height growth, resulting in 34.22% and 32.19% increases in seedling height for clones C1 and C2, respectively, compared with the $0 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$ treatment. By contrast, seedling height showed a decrease following the addition of $6 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$.

In both soil conditions, seedling height exhibited a “slow-fast-slow” growth process (Fig. 1). In the fertile soil, the fast-growing period for seedling height began at 61 and 69 days under the $3 \text{ g}\cdot\text{kg}^{-1}$ and $6 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$ treatments, respectively; the beginning date was delayed by 5 and 13 days, while the duration of the fast-growing period was shortened by 9 and 14 days, respectively, compared with that of $0 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$ treatment (Table 2). In the infertile soil, the fast-growing period for seedling height began at 76 days after the addition of $3 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$; the beginning date was advanced by 5 days, while the duration of the fast-growing period was prolonged by 6 days, compared with that of $0 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$ treatment. By contrast, the fast-growing period for seedling height began at 82 days after the addition of $6 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$; the beginning date was delayed by 1 day, while the duration of the fast-growing period was prolonged by 4 days compared with that of control group ($0 \text{ g}\cdot\text{kg}^{-1} \text{ Ca}^{2+}$ treatment).

Table 1
Physical and chemical properties of potted soil

Nutrient elements	Total N ($\text{g}\cdot\text{kg}^{-1}$)	Total P ($\text{g}\cdot\text{kg}^{-1}$)	Hydrolytic N ($\text{mg}\cdot\text{kg}^{-1}$)	Available K ($\text{mg}\cdot\text{kg}^{-1}$)	Available P ($\text{mg}\cdot\text{kg}^{-1}$)	Organic matter ($\text{g}\cdot\text{kg}^{-1}$)	Exchange Ca ($\text{mg}\cdot\text{kg}^{-1}$)	Exchange Mg ($\text{mg}\cdot\text{kg}^{-1}$)	pH value
Average content	0.75±0.09	0.32±0.05	53.5±4.70	18.5±1.12	0.99±0.14	15.8±1.89	128±12.5	9.24±0.85	4.65±0.21

Table 2

Parameters of fitting curve of height growth of *C. funebres* under different Ca²⁺ treatments

Nutrient environment	Ca ²⁺ treatment	k	a	b	R ²	t ₁	t ₂
Fertile soil conditions	0 g·kg ⁻¹	31.61	14.04	0.024	0.9814	56	176
	3 g·kg ⁻¹	30.84	16.33	0.024	0.9834	61	172
	6 g·kg ⁻¹	33.51	21.17	0.025	0.9892	69	175
Infertile soil conditions	0 g·kg ⁻¹	15.32	35.61	0.027	0.9846	81	177
	3 g·kg ⁻¹	19.40	27.05	0.026	0.9854	76	178
	6 g·kg ⁻¹	14.12	32.85	0.026	0.9848	82	182

Dry biomass

In the fertile soil, Ca²⁺ addition had no significant effects on dry matter accumulation in the roots, stems or leaves of cypress clones C1 and C2. Both clones had significantly higher root-shoot ratios under the 0 g·kg⁻¹ Ca²⁺ treatment than under the other treatments. In the infertile soil, Ca²⁺ addition had significant effects on dry biomass in the roots, stems, leaves and whole plants of C1 and C2 seedlings ($P < 0.05$). The highest dry biomass of various organs was always obtained from plants under the 3 g·kg⁻¹ Ca²⁺ treatment. In terms of the root-shoot ratio, C1 had the highest ratio under the 6 g·kg⁻¹ Ca²⁺ treatment, and C2 had the highest ratio under the 3 g·kg⁻¹ Ca²⁺ treatment (Fig. 2).

Root growth and development

In the infertile soil, root length, root surface area and root volume of the diameter classes D1–D4 (except for D5) differed significantly among the Ca²⁺ treatments. The addition of 3 g·kg⁻¹ Ca²⁺ significantly promoted root development of classes D1–D4 in clone C1, and the resulting root lengths were 1.16-, 1.17-, 1.15- and 1.30-fold that under the 0 g·kg⁻¹ Ca²⁺ treatment, respectively; the root lengths of classes D1–D4 in clone C2 were 1.15-, 1.36-, 1.29- and 1.06-fold that under the 0 g·kg⁻¹ Ca²⁺ treatment, respectively (Fig. 3d). The corresponding root surface area and root volume also showed similar variation, with a 1.05–1.35-fold increase (Fig. 3e,f). When the concentration of Ca²⁺ added was 6 g·kg⁻¹, root length, root surface area, and root volume of classes D1–D4 all decreased compared with those under the 0 g·kg⁻¹ Ca²⁺ treatment. The sum of the root length of classes D1–D3 accounted for 97.1%, 98.7% and 98.3% of total root length across the three Ca²⁺ treatments (0, 3, and 6 g·kg⁻¹), respectively; among these, the root length of class D1 accounted for 63.26%, 66.32% and 60.53% of total root length, respectively.

In the fertile soil, root length, root surface area and root volume of the diameter classes D1–D5 all significantly decreased in clone C2 following Ca²⁺ addition (Fig. 3a,b,c). By contrast, Ca²⁺ addition had no significant effects on root length, root surface area or root volume in clone C1. The sum of the root lengths of classes D1–D3 accounted for 96.6%, 97.1%, and 97.0% of total root length across the three Ca²⁺ treatments (0, 3, and 6 g·kg⁻¹), respectively; among these, the root length of class D1 accounted for 59.34%, 55.22%, and 52.18% of total root length, respectively.

N, P and Ca accumulation efficiencies

In the two soils, accumulation efficiencies of N, P and Ca all differed significantly among the Ca²⁺ treatments. In the fertile soil, N accumulation efficiency for both clones exhibited a downward trend with increasing Ca²⁺ concentration, and the highest N accumulation efficiency was achieved under the 0 g·kg⁻¹ Ca²⁺ treatment. The P use efficiency for clone C1 was the highest under the 3 g·kg⁻¹ Ca²⁺ treatment, while that of clone C2 was the highest under the 6 g·kg⁻¹ Ca²⁺ treatment. Both clones achieved their highest Ca accumulation efficiency under the 6 g·kg⁻¹ Ca²⁺ treatment. In the infertile soil, both C1 and C2 achieved their highest N, P, and Ca accumulation efficiencies under the 3 g·kg⁻¹ Ca²⁺ treatment (Fig. 4).

Clone effect

In the fertile soil, the mean seedling height of clone C1 was 56.45 cm, and the mean dry biomasses of its roots, stems and leaves were 5.53, 5.97 and 10.46 g, respectively; these mean values were 30%, 87%, 86% and 64% higher than those for clone C2, respectively. The lengths of total roots (D1–D5) for clone C1 were 26.5%, 108.0% and 67.4% longer than those for clone C2 across the three Ca²⁺ treatments (0, 3 and 6 g·kg⁻¹), respectively. Root surface area and root volume for C1 were also significantly higher than those for C2. N and Ca accumulation efficiencies differed significantly between clones. In terms of N, the accumulation efficiencies for C1 were 3.04%, 13.52% and 10.64% higher than those for C2 across the three Ca²⁺ treatments (0, 3 and 6 g·kg⁻¹), respectively. In terms of Ca, the accumulation efficiencies for C1 were 16.94%, 6.84% and 10.39% higher than those for C2 across the three Ca²⁺ treatments, respectively. However, no significant difference was detected in P accumulation efficiency between clones.

In the infertile soil, the mean seedling height of clone C1 was 38.38 cm, and the mean dry biomasses of its roots, stems and leaves were 3.58, 2.23 and 4.50 g, respectively; these mean values were 15.71%, 10.05% and 3.12% higher than those of clone C2. Root length, root surface area and root volume of D1, D2 and D3 differed significantly between clones, but no significant differences were detected in D4 and D5 between clones. Root lengths of D1, D2 and D3 in clone C1 were 11.5%, 7.0% and 25.1% higher than those in clone C2 across the 3 Ca²⁺ treatments (0, 3 and 6 g·kg⁻¹), respectively. Similar trends were observed for root surface area and root volume. C1 achieved significantly higher N accumulation efficiency than did C2, while P and Ca accumulation efficiencies did not differ significantly between clones.

Discussion

Ca²⁺ can promote the growth and development of plants, while Ca²⁺ deficiency or excess negatively affects the growth and development of plants—this is related to the growth environment [2]. It is generally thought that the plant is not deficient in Ca²⁺ when the exchangeable Ca²⁺ content in soil is greater than 400 mg·kg⁻¹. In the present study, the experimental soil contained an exchangeable Ca²⁺ content of 128 mg·kg⁻¹, which was lower than the cutoff value without Ca²⁺ application, thus indicating Ca²⁺-deficient soil [20]. Compared with that of the infertile soil, the seedling height of cypress growing in the fertile soil responded less to Ca²⁺, and the Ca use efficiency decreased by 21.7%, 31.4% and 30.2% under the 0, 3 and 6 mg·kg⁻¹ Ca²⁺ treatments, respectively. The addition of Ca²⁺ fertilizer reduced N use efficiency in cypress and inhibited the development of fine roots (diameter ≤ 1.5 mm), while Ca use efficiency reached its highest level under the 6 mg·kg⁻¹ Ca²⁺ treatment. These observations indicate that N and P nutrients were sufficient in the fertile soil and that such nutrient accumulation aggravated the deficiency of available Ca in soil; consequently, exchangeable Ca²⁺ was exchanged by a large amount of NH₄⁺ and K⁺, which facilitated the desorption of exchangeable Ca²⁺, and N use efficiency decreased with increasing Ca²⁺ concentration [21]. Moreover, exchangeable Ca²⁺ and PO₄³⁻ underwent irreversible ion exchange, and the increase in P promoted the conversion of water-soluble and exchangeable Ca²⁺ to unavailable non-acid-soluble Ca, which also resulted in a decrease in the exchangeable Ca²⁺ in soil [4, 22]. This is the possible reason why Ca use efficiency was higher under the application of a high Ca²⁺ concentration; however, the exact process still requires further investigation. In the infertile soil, the seedling height of cypress increased by adding an appropriate amount of Ca²⁺ (3 g·kg⁻¹), and the highest N, P and Ca accumulation efficiencies were all achieved under the 3 g·kg⁻¹ Ca²⁺ treatment, with a synergy between Ca²⁺ fertilizer versus N and P accumulation efficiencies. However, when the Ca²⁺ concentration was increased, seedling growth of cypress clones decreased under the 6 g·kg⁻¹ Ca²⁺ treatment. These results indicate that the synergy had a range of adaptation to the rate of Ca²⁺ applied. That is, an appropriate amount of Ca²⁺ promoted plant N and P uptake, while an excessively high concentration of Ca²⁺ fertilizer exhibited an inhibitory effect [23, 24]. In a study conducted on coniferous species such as pine (*Pinus massoniana* Lamb.), good adaptation was also observed in the soil environment with Ca²⁺ supplied at 1–2 mmol·L⁻¹, while the plant height growth of pine seedlings decreased after the Ca²⁺ supply exceeded this concentration [25]. Therefore, full consideration should be given to the tolerance of tree species when applying Ca²⁺ to promote seedling growth.

Root architecture refers to the spatial distribution of plant roots in the soil, and it reflects the special root characteristics of plants that are formed during evolution for adaptation to the environment [26, 27]. In the infertile soil, the addition of 3 g·kg⁻¹ Ca²⁺ promoted root development of classes D1–D4 (diameter ≤ 2.0 mm) in clone C1; when the Ca²⁺ concentration was increased to 6 g·kg⁻¹, root length, root surface area and root volume increases of classes D1–D4 were inhibited in this clone. Moreover, Ca²⁺ addition inhibited root

length, root surface area and root volume increases of classes D1–D5 in clone C2. These results demonstrate that the roots of different diameter classes have distinct reactions to Ca^{2+} [27, 28]. In cypress seedlings growing in the two soils, fine roots of classes D1–D3 (diameter ≤ 1.5 mm) accounted for more than 96.6% of the total root length and more than 88% of the root surface area. As the key parts of the plants for nutrient uptake, fine roots have small diameters and low lignification levels, with high sensitivity to changes in soil nutrients [26]. Compared with coarse roots with relatively large diameters, the root length and root surface area of fine roots enable plants to respond to changes in the soil environment more easily [27]. Moreover, the main functional unit of the roots for nutrient uptake is closely related to the root tip region, and no anatomical structure related to nutrient uptake has been found in roots with a diameter > 2.0 mm [29, 30]. In the fertile soil, D1 roots (diameter ≤ 0.5 mm) accounted for 59.34%, 55.22% and 52.18% of the total root length across the three Ca^{2+} treatments (0, 3 and $6 \text{ g}\cdot\text{kg}^{-1}$), respectively. Lateral root growth was inhibited with the increase in Ca^{2+} concentration, which indicates that cypress roots were under Ca^{2+} stress in the fertile soil and that they adapted to this Ca^{2+} environment by reducing lateral root growth and soil contact area. The corresponding proportions of D1 roots were even higher in the infertile soil, reaching 63.26%, 66.32% and 60.53% across the three Ca^{2+} treatments (0, 3 and $6 \text{ g}\cdot\text{kg}^{-1}$), respectively. This result indicates that cypress can adjust the morphology of its fine roots to adapt to different Ca^{2+} environments. When the site condition was relatively infertile, cypress formed more roots with a diameter ≤ 0.5 mm to improve nutrient acquisition [11].

A previous study has shown that there are interaction effects of genotype and environment on root development and nutrient accumulation efficiency in cypress seedlings. This finding suggests the growth of cypress clones has various adaptations to Ca^{2+} addition. In our fertile soil, the C1 genotype with fast height growth achieved its highest P accumulation efficiency under the $3 \text{ g}\cdot\text{kg}^{-1}$ Ca^{2+} treatment, while clone C2 achieved its highest P accumulation efficiency under the $6 \text{ g}\cdot\text{kg}^{-1}$ Ca^{2+} treatment. In the infertile soil, accumulation efficiencies of N, P and Ca were all the highest under the $3 \text{ g}\cdot\text{kg}^{-1}$ Ca^{2+} treatment. These results demonstrate that Ca^{2+} application increased the use efficiency of P and Ca in cypress seedlings. According to another study on Chinese fir, which was also conducted in the subtropics, there may exist a synergy between P and Ca uptake in low-P environments. Here, the N/P ratios of cypress seedlings were 11.31, 10.59 and 9.11 under the 0, 3 and $6 \text{ g}\cdot\text{kg}^{-1}$ Ca^{2+} treatments, respectively. This result suggests that the P uptake by cypress increased with increasing Ca^{2+} concentration, and in order to maintain the same N/P ratios as the soil, metabolic mechanisms were regulated, which possibly reduced N uptake.

Materials And Methods

Experimental site and materials

The experiment was conducted in a greenhouse of Laoshan Forestry Farm in Zhejiang Province, China. One-year-old cutting seedlings of Cypress (*Cupressus funebris* Endl.) were cultivated as the experimental material. The scions used for cutting came from elite individual plants of clone C1 (fast height growth) and clone C2 (slow height growth) in the full-sib progeny. For each clone, robust and disease-free cutting seedlings aged 1 year old were selected. At the time of planting, seedlings were selected based on their plant height (5.15 ± 0.05 cm) and ground diameter (0.17 ± 0.01 cm). Then, they were planted in a container 30 cm in height and 20 cm in diameter. The potting soil was an acidic red soil collected from forestland, and the soil layer was 0–20 cm thick. The controlled-release fertilizer used in the experiment was a nursery fertilizer (APEX).

Experimental design

NPK fertilizer was added at 3 and 0 g per kg of soil to simulate fertile and infertile soils, respectively. For Ca^{2+} fertilizer, CaSO_4 was added at 0, 3 and 6 g per kg of soil. Both NPK fertilizer and CaSO_4 were mixed with their respective soil, stirred uniformly and placed into the containers. The experiment involved 6 treatments. Treatment 1: $3 \text{ g}\cdot\text{kg}^{-1}$ NPK fertilizer + $0 \text{ g}\cdot\text{kg}^{-1}$ CaSO_4 ; treatment 2: $3 \text{ g}\cdot\text{kg}^{-1}$ NPK fertilizer + $3 \text{ g}\cdot\text{kg}^{-1}$ CaSO_4 ; treatment 3: $3 \text{ g}\cdot\text{kg}^{-1}$ NPK fertilizer + $6 \text{ g}\cdot\text{kg}^{-1}$ CaSO_4 ; treatment 4: $0 \text{ g}\cdot\text{kg}^{-1}$ NPK fertilizer + $0 \text{ g}\cdot\text{kg}^{-1}$ CaSO_4 ; treatment 5: $0 \text{ g}\cdot\text{kg}^{-1}$ NPK fertilizer + $3 \text{ g}\cdot\text{kg}^{-1}$ CaSO_4 ; and treatment 6: $0 \text{ g}\cdot\text{kg}^{-1}$ NPK fertilizer + $6 \text{ g}\cdot\text{kg}^{-1}$ CaSO_4 . The physicochemical properties of the soil are provided in Table 1. The experiment used a completely randomized block design. Twenty cutting seedlings were planted per treatment per clone, with three replicates each; therefore, 720 potted seedlings were planted. All seedlings were maintained in a greenhouse under conventional management.

Table 1
Physical and chemical properties of potted soil

Nutrient elements	Total N (g·kg ⁻¹)	Total P (g·kg ⁻¹)	Hydrolytic N (mg·kg ⁻¹)	Available K (mg·kg ⁻¹)	Available P (mg·kg ⁻¹)	Organic matter (g·kg ⁻¹)	Exchange Ca (mg·kg ⁻¹)	Exchange Mg (mg·kg ⁻¹)	pH value
Average content	0.75 ± 0.09	0.32 ± 0.05	53.5 ± 4.70	18.5 ± 1.12	0.99 ± 0.14	15.8 ± 1.89	128 ± 12.5	9.24 ± 0.85	4.65 ± 0.21

Cultivation, harvest and analysis

The experiment started on April 2, 2018, with plant height and ground diameter measured for all plants. Thereafter, plant height was measured for the same plants once every 20 days. Measurements were completed in November 2018, and continuous data for plant height were used to analyse the plant height growth rhythm of cypress. Seedlings were harvested on November 23. Whole plants were collected and divided into roots, stems and leaves, with each organ harvested separately. First, the roots were separated from the soil, washed with deionized water and stored. Root diameter was classified as follows: class D1 (root diameter range: 0–0.5 mm), class D2 (0.5–1.0 mm), class D3 (1.0–1.5 mm), class D4 (1.5–2.0 mm) and class D5 (> 2.0 mm) (Liu et al. 2018). Root length, surface area, and root volume of each diameter class were measured using the image analysis software WinRHIZO Pro STD1600+ (Regent Instruments, Canada). Next, the roots, stems and leaves were deactivated in an oven at 105 °C for 30 min and then dried at 80 °C until a constant weight was achieved, in order to obtain the dry biomass of each part. The N content of each organ was measured using a FOSS (Foss Sossanalytical a-s., Ahlleroed, Denmark) nitrogen analyser [31]. The P content was measured by molybdenum antimony anti-colorimetry [32]. The Ca content was measured by atomic absorption spectrophotometry [2]. The N, P and Ca contents were multiplied by the dry biomass of the whole plant to obtain N, P and Ca accumulation. N accumulation efficiency = dry biomass accumulation of whole plant/N uptake of whole plant (g·mg⁻¹); P and Ca accumulation efficiencies were calculated following the same method as that used for N accumulation efficiency.

Data analysis

Logistic regression was used to fit seedling height growth rhythm of cypress under different Ca²⁺ treatments; the fitting equation was $y = k / (1 + a \cdot e^{-bt})$, where y is cumulative growth of seedling height, t is the growth time, k is the theoretical upper limit of height growth, and a and b are the undetermined coefficients. One-way analysis of variance (ANOVA) was used to test significant differences in seedling growth, root morphological characteristics, and nutrient accumulation efficiency under Ca²⁺ treatments in fertile and infertile soils. All statistical analyses were performed using IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA).

Abbreviations

Ca²⁺: Calcium; N: Nitrogen; P: Phosphorous; D1: Root diameter range(0–0.5 mm); D2: Root diameter range(0.5–1.0 mm); D3: Root diameter range(1.0–1.5 mm); D4: Root diameter range (1.5–2.0 mm); D5: Root diameter range (> 2.0 mm).

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional information fles.

Ethics approval and consent to participate

Masson pine sampling was carried out under the permission of the Laoshan Forest Farm of Chun'an Country. The treatment of the Masson pine during the experimental procedures were approved by the Chinese Academy of Forestry.

Consent to publish

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

ZZ conceived the work and conducted the experiment. GQJ analyzed the data and were responsible for funding acquisition. ZCZ invested the work. ZZ wrote the first draft of the manuscript

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Figures

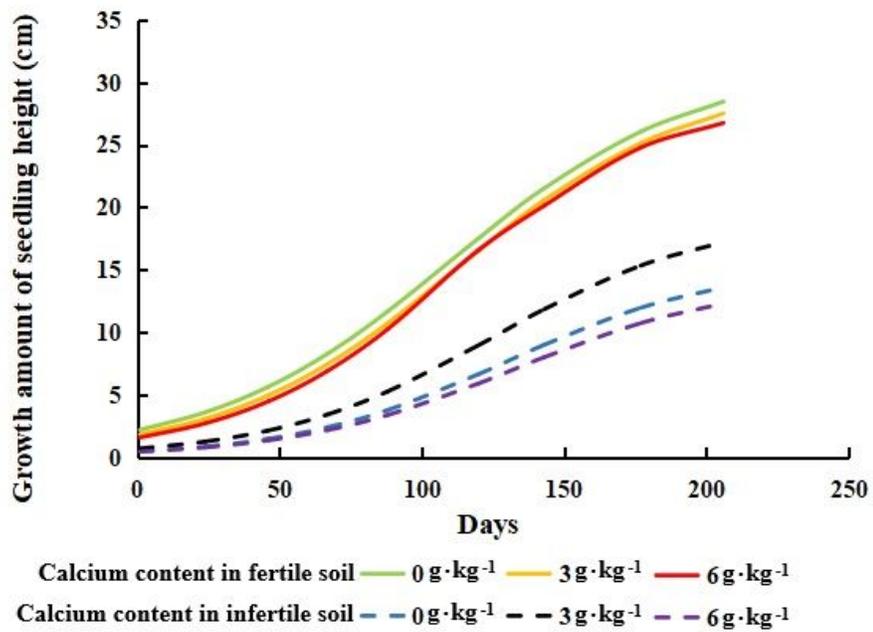


Figure 1

Seedling height growth curve of *C. funebris* under different calcium treatments. The dotted line in the picture indicates infertile soil and the solid line indicates fertile soil.

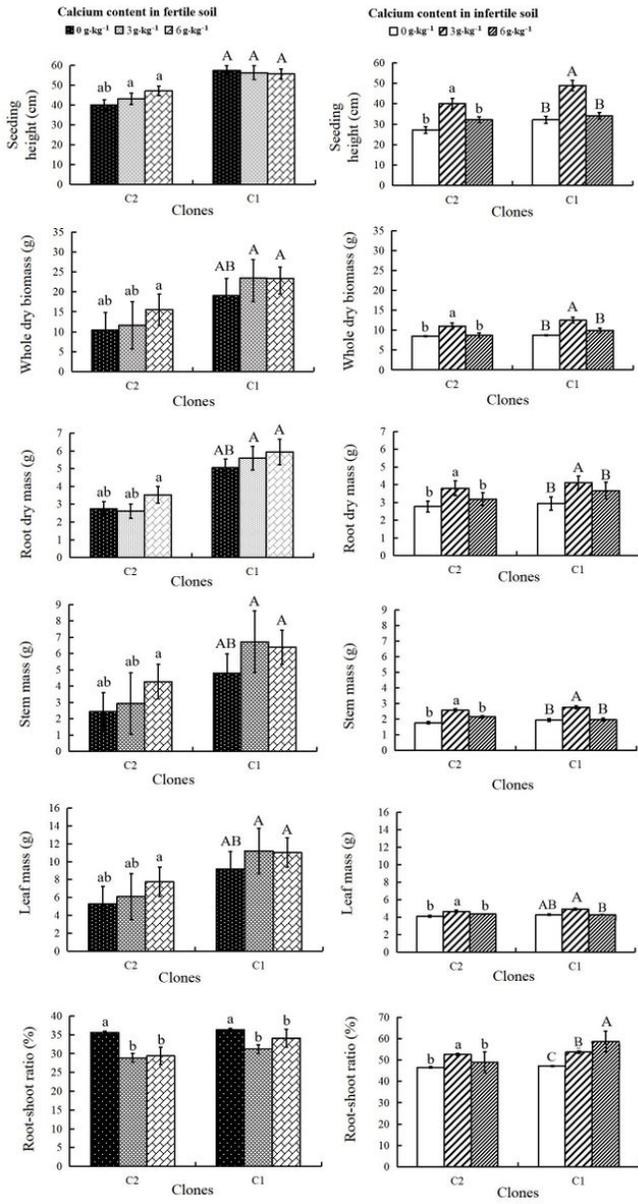


Figure 2

Differential growth of *C. funebris* clones in different solid environment and calcium treatments. Means followed by different lower- and upper-case letter (s) are significantly different ($p < 0.05$).

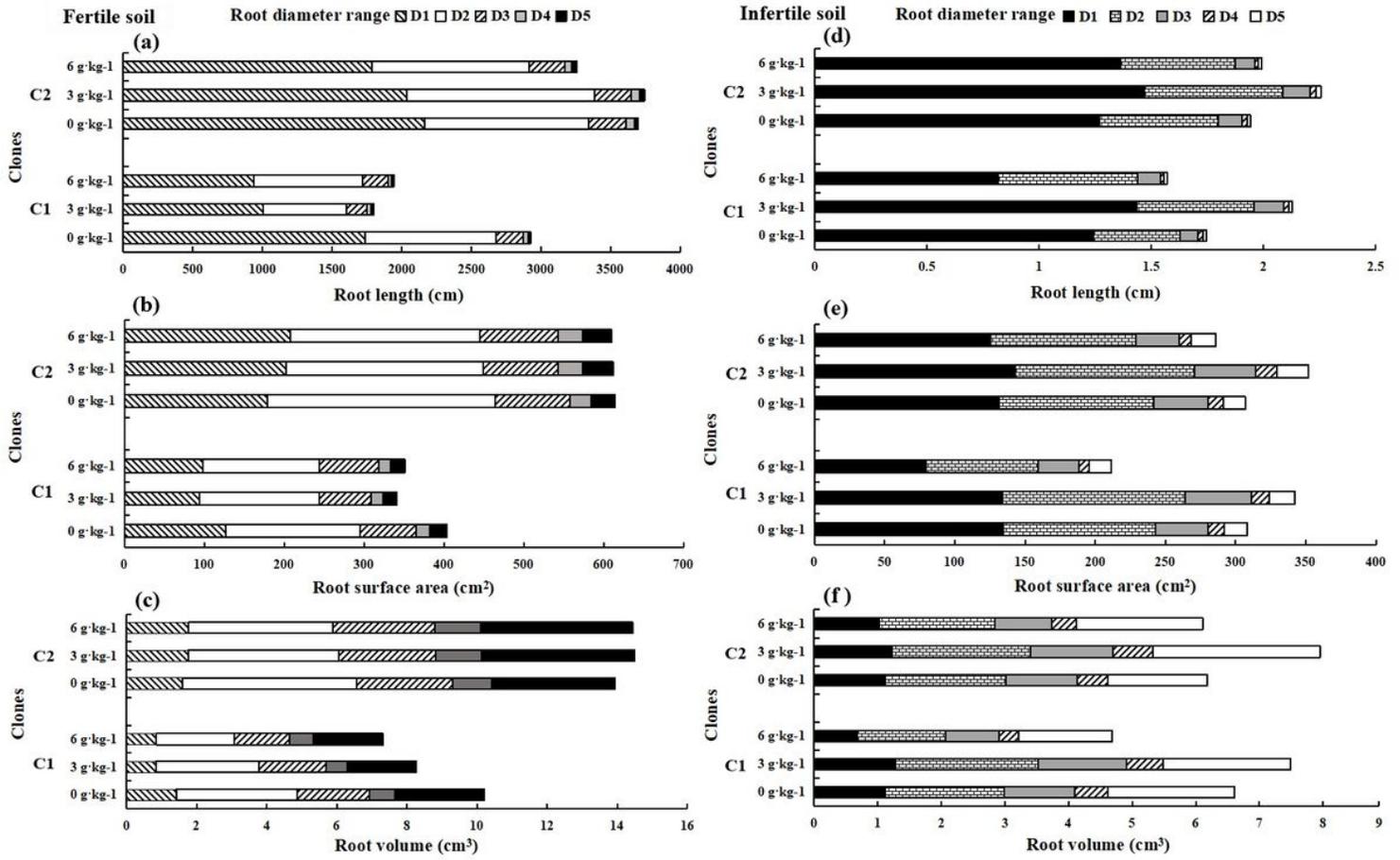


Figure 3

Effects of calcium supply levels on root morphology of different families of *C. funebris* under two soil fertility levels

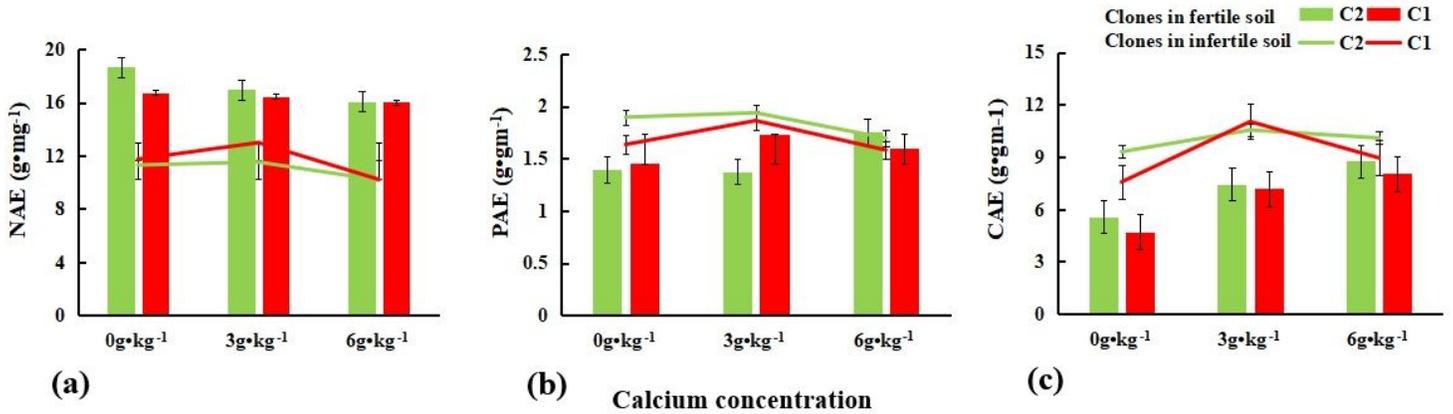


Figure 4

Effects of calcium supply levels on absorption efficiency of nitrogen, phosphorus and calcium of *C. funebris* under two soil fertility levels