Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2022.021382

ARTICLE

N-Exponential Fertilization Could Affect the Growth and Nitrogen Accumulation of *Metasequoia glyptostroboides* Seedling in a Greenhouse Environment

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Received: 11 January 2022 Accepted: 23 February 2022

ABSTRACT

Metasequoia glyptostroboides (*M. glyptostroboides*) is a unique plant species related to relic flora in China. It plays a positive role in afforestation and its long-term protection with high paleoclimate research value. However, due to the nutrients-supply deficiency, it is a big challenge to cultivate the high-quality seedlings of *M. glyptostroboides.* In this study, a pot experiment in a greenhouse environment was carried out to identify the effect of N-exponential fertilization on the growth and nutrient distribution of *M. glyptostroboides* seedling. The *M. glyptostroboides* rooted seedlings with 12-month growth were chosen. Different N fertilizer levels with conventional fertilization (CF: 5.0 g seedling^{-1}), exponential fertilization including EF1, EF2, EF3 and EF4 were determined. The relevant growth indexes were measured after 210-day growth. The results indicated that non-significant differences in seedlings' height and ground diameter were found among the above treatments ($P > 0.05$); At the same time, N-exponential fertilization promoted the *M. glyptostroboides*'s biomass in different organs ($P < 0.05$), with the maximum total biomass under EF3 treatment. The N accumulation in root and stem of the N-exponential fertilization treatments were increased in to some extent ($P < 0.05$). The maximum N accumulation was also found under EF3 treatment. Therefore, steady-state nutrition and superior growth performance of *M. glyptostroboides* could be obtained by N-exponential fertilization of 5.0 g cutting^{-1}.

KEYWORDS

Metasequoia glyptostroboides seedling; nitrogen exponential fertilization; plant growth; biomass; nutrient supply

1 Introduction

The plant seedling's quality directly determines the survival of post-planting seedling especially in the afforestation process [1]. The high-quality seedlings with strong adaptability and rapid growth, are regarded as a sustainable management techniques. Normally, "nutrient-loaded" seedlings are chosen as one of the high-quality seedlings and are widely applied in the afforestation [2]. In that case, fertilizer application is a necessary measure to promote the quality of target seedlings [3]. Nitrogen (N) is a fundamental nutrient for plant survival [4]. Therefore, suitable N fertilizer application is important to improve the plant growth, however, excessive N application can lead to N loss which could further cause potential environmental problems such as eutrophication in water bodies [5].

Conventional fertilization (CF) regime supplies nutrients at the same rate during the fertilizer application period, inducing toxicity to young seedlings and nutrient loss in case the plant does not assimilate at its early-growth stage [3]. This application method also causes nutrient deficiencies at the growth stages, requiring more nutrients for seedling growth [4]. Compared to the conventional fertilization, the exponential fertilization can induce plant steady-state luxury nutrients consumption, which could effectively improve the nutrient uptake of plant and enhance the seedlings' competitiveness, so as to better adapt to the specific environment of the plantation [6–8]. At present, exponential fertilization has been studied on a variety of tree species. Previous research has shown that the biomass accumulation of Japanese *Larix kaempferi* seedlings in a certain range of N supply level increased with the increase of N supply, but decreases if the amount of N is excessive [9]. While other results show that the N content of seedlings increases exponentially or linearly under exponential fertilization, indicating that the growth and nutrient absorption of seedlings could well be matched through the exponential fertilization [10]. In terms of short-term effects, medium nitrogen load is the most effective in promoting nutrient uptake in nursery and improving transplanting reaction of conventionally fertilized young plants [11]. Meanwhile, researchers demonstrated that the use of exponential fertilization could well promote the growth of *Aralia elata* young plant, improve the nutritional status, and meet the nutrient requirements of seedlings in different periods. Therefore, the survival rate and growth effect of seedlings were much better [12]. However, there were no significant differences in *Tsuga heterophylla* seedling height, biomass and nutrient concentration, and the effects on biomass and nitrogen content of stem and leaf were not significant under exponential fertilization [13]. And the growth of *Pseudotsuga menziesii* seedlings after transplanting was not obvious under the condition of exponential fertilization as well [14]. Similar to the above studies, the research on the container seedlings of *Quercus variabilis* showed that the application of fertilizer had non-significant impact on the seedling height and ground diameter [15]. From the previous study, we can find that the plant species have different responses to the exponential fertilizer application which should be further studied.

Metasequoia glyptostroboides (*M. glyptostroboides*) is a unique plant species of the *taxaceae* family in China. It was discovered in Lichuan City, Hubei Province in the 1940s, and has been included in the 2013 IUCN red list of endangered species [16]. *M. glyptostroboides* belongs to the tree species which is relatively resistant to water flooding, and its planting can not only improve the soil quality but also promote the growth and development of *M. glyptostroboide*'s protection [17,18]. Therefore, *M. glyptostroboide* is the main afforestation tree species in coastal shelter-belt and plain river network in east China [19]. It is also grown in countries other than China, such as Canada and Australia [20]. Studies have found it to be well developed in both the eastern and western United States [21]. At present, research has been done on *M. glyptostroboides* seeding, seedling cultivation, cuttage propagation, etc. [22–24], but there were few studies on the effects of different fertilization on. *M. glyptostroboide.* In our study, different fertilization on seedling height, ground diameter, biomass, nitrogen content and accumulation of leaves, stems and roots of 1-year *M. glyptostroboides* seedlings were studied under potted conditions. We hypothesized that N-exponential fertilization promoted the M. glyptostroboides's biomass and increased N accumulation in root and stem. Our study revealed the law of nitrogen requirement of *M. glyptostroboides* seedlings and provide a basis for nutrient management for *M. glyptostroboides* seedlings.

2 Material and Methods

2.1 Study Site

The study site was located in the forestry station seedling breeding base of Tongxiang City, Zhejiang Province (30°41′52″N, 120°32′38″E), with a subtropical monsoon climate. The annual average precipitation is 1212.3 mm, with annual average sunshine hours of 1983.4 h, annual average temperature of 16.9°C, and annual frost-free period of 244 d [25]. The experiment was carried out in a greenhouse with a temperature of 25°C to 35°C and the relative air humidity of 55% to 75%.

2.2 Study Material

In April, 2019, a total of 225 one-year-old uncultivated *M. glyptostroboides* seedlings with basically the same growth were selected, the average stem and seedling height were 0.42 and 63.0 cm, respectively. They were planted in pots of 28 cm × 23 cm × 24 cm (height × base diameter × upper diameter). 10 kg paddy soil was filled for every experimental pot. The soil belonged to Ferralsols based on the Food and Agriculture Organization (FAO) [26]. The physical and chemical properties of soil were measured (pH: 6.6, organic carbon: 4.8 g kg^{-1}, alkaline nitrogen: 22.7 mg kg^{-1}, available phosphorus: 0.9 mg kg^{-1}, available potassium: 55.5 mg kg^{-1})

2.3 Experimental Design and Fertilization Regimes

A completely randomized design was adopted, with conventional fertilization (CF, 5.0 g $seedling^{-1}$) and 4 exponential fertilization (EF1, EF2, EF3 and EF4: 1.0, 3.0, 5.0, 8.0 g $cuttling^{-1}$, respectively). The P_2O_5 was 14% superphosphate (10.0 g $seedling^{-1}$) and K_2O was 50% potassium sulfate (5.0 g $cuttling^{-1}$), which were mixed together as the base fertilizer when preparing the culture substrate. There were 15 seedlings for each treatment with 3 repetitions. The conventional fertilization provided N for 1.00 g $seedling^{-1}$ per month. The exponential fertilization provided N based on exponential fertilization theory, which is described by Ingestad et al. [27]. And the corresponding fertilization dose are listed in Table 1.

Table 1: Fertilization time and content under different treatments

Treatment	Fertilization time and application amount/N (g $seedling^{-1}$)					
	May 5th	June 5th	July 5th	August 5th	September 12th	Total
CF	1.00	1.00	1.00	1.00	1.00	5.00
EF1	0.04	0.09	0.14	0.30	0.42	1.00
EF2	0.16	0.28	0.48	0.83	1.26	3.00
EF3	0.26	0.44	0.75	1.34	2.20	5.00
EF4	0.42	0.70	1.21	2.09	3.59	8.00

2.4 Plant Sampling and Chemical Analysis

At the end of our experiment, 5 seedlings of different treatments were collected by the whole harvest method with 3 repetitions. The height and ground diameter of seedlings were measured by tapeline and vernier caliper. The roots, stems and leaves were washed with clean water and then rinsed with deionized water. Their roots, stems, and leaves were obtained, respectively. Finally, placed in envelopes and placed in an oven at 105°C for 30 min, then dried at 70°C to a constant weight, and the dry matter mass of different organs, namely biomass, was measured and calculated as described as Wu et al. [3].

After the dried plant samples were crushed, they were sifted through a 0.5 mm sieve and determined by elemental analyzer (Germany Elementer, uario EL cube).

$$\text{Nitrogen accumulation } (\text{mg seedling}^{-1}) = \text{ nitrogen mass fraction of different organs } (\text{mg g}^{-1}) \times \text{ biomass of corresponding organs } (\text{g}) \tag{1}$$

$$\text{Biomass harvesting index } (\text{g g}^{-1}) = \text{ biomass/total N application amount} \tag{2}$$

$$\text{N harvest index (\%)} = \text{N accumulation/total N application amount} \times 100 \quad (3)$$

2.5 Data Analysis

The data are presented as average ± standard deviation (SD). Statistical variance analysis was carried out using one-way ANOVA, and the least significant difference (LSD) method was used for multiple comparisons in a SPSS version 21.0 [28,29]. The 0.05 for significant test was applied. The homogeneity test for raw data was conducted [30,31]. The graphs of statistical results were produced suing Origin 8 software.

3 Results

3.1 Seedling Height and Ground Diameter of M. glyptostroboides under Different N-Fertilization

Compared with CF, the seedling height of the four EF treatments increased by 6.3%–17.0%, and the ground diameter increased by 2.0%–7.1% (Fig. 1). The average seedlings height treated with EF1 was 96.8 cm, which was obviously higher than that of CF ($P < 0.05$). However, there was no significant difference in seedlings' ground diameter among treatments ($P > 0.05$).

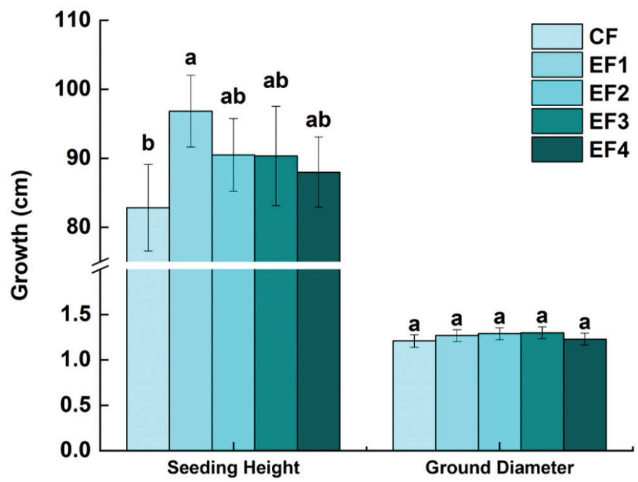

Figure 1: The external properties of seedlings with different treatments

3.2 The Biomass of M. glyptostroboides Seedlings under Different Fertilization Treatments

In Table 2, EF treatments promoted the roots and stems biomass accumulation of *M. glyptostroboides.* Compared with the CF, EF treatments significantly increased the root biomass, total biomass and root-to shoot ratio by 45.1%–78.0%, 20.4%–22.5% and 22.4%–46.9% ($P < 0.05$), respectively. The stem biomass of EF1, EF2 and EF3 was also significantly higher than that of CF ($P < 0.05$), including EF3 root biomass (48.4 g cutting^{-1}) and the root-to-shoot ratio (0.72) significantly higher than EF1, EF2, EF4 ($P <$ 0.05). The leaf biomass of *M. glyptostroboides* was between 22.4 and 25.5 g cutting^{-1}, and there was no significant difference among different fertilization treatments ($P > 0.05$).

3.3 Nitrogen Contents and Nitrogen Accumulation of M. glyptostroboidesunder Different Fertilization Treatments

The N mass fraction of *M. glyptostroboides* seedlings roots ranged from 28.7 to 32.3 g kg^{-1} (Table 3), and EF1 was significantly higher than that of CF and EF4 ($P < 0.05$). The N content of the stem ranged from 16.7 to 19.0 g kg^{-1}, and non-significant difference was observed among different treatments ($P > 0.05$). The N mass fraction of the leaves ranged from 29.2 to 32.3 g kg^{-1}. Compared with CF, the nitrogen mass fraction of EF reduced by 3.0% to 9.7%, but the difference was not significant ($P > 0.05$).

Table 2: Biomass of *M. glyptostroboides* by different treatments

Treatment	Root (g seedling^{-1})	Stem (g seedling^{-1})	Leaf (g seedling^{-1})	Total biomass (g cutting^{-1})	Root/shoot ratio
CF	26.25 ± 4.71c	32.32 ± 1.22c	23.42 ± 2.13a	81.92 ± 4.91b	0.51 ± 0.02c
EF1	39.52 ± 3.31b	39.63 ± 1.52b	22.51 ± 1.82a	101.64 ± 12.63a	0.65 ± 0.04b
EF2	41.62 ± 2.53b	44.13 ± 1.51a	25.54 ± 1.72a	111.22 ± 2.23a	0.61 ± 0.05b
EF3	48.45 ± 2.62a	43.12 ± 1.73a	23.71 ± 1.43a	115.24 ± 6.85a	0.71 ± 0.03a
EF4	40.58 ± 2.13b	36.46 ± 2.02c	23.02 ± 2.21a	99.91 ± 6.33ab	0.67 ± 0.06ab

Table 3: *M. glyptostroboides's* N contents with different treatments

Treatment	Nitrogen mass fraction (g kg^{-1})			Nitrogen accumulation (mg seedling^{-1})			
	Root	Stem	Leaf	Root	Stem	Leaf	Total
CF	28.71 ± 2.12b	17.03 ± 1.62a	32.32 ± 3.14a	781.95 ± 55.26c	566.62 ± 43.84b	725.24 ± 65.15a	2073.75 ± 164.25b
EF1	32.34 ± 0.52a	19.03 ± 1.63a	29.23 ± 2.74a	1275.32 ± 66.94b	750.81 ± 56.28a	656.92 ± 61.34a	2683.42 ± 184.56a
EF2	29.83 ± 2.22ab	17.31 ± 1.52a	31.22 ± 2.84a	1238.91 ± 75.82b	764.52 ± 66.34a	796.12 ± 72.63a	2799.55 ± 214.79a
EF3	29.84 ± 2.02ab	17.52 ± 1.43a	31.35 ± 2.86a	1441.92 ± 84.63a	752.91 ± 59.11a	744.13 ± 67.24a	2938.96 ± 210.98a
EF4	28.83 ± 2.45b	16.73 ± 1.74a	30.46 ± 3.27a	1166.73 ± 67.35b	606.92 ± 50.43b	697.72 ± 56.93a	2471.34 ± 174.71b

Exponential fertilization significantly increased the accumulation of nitrogen in the roots of *M. glyptostroboides* seedlings. Compared with CF, the accumulation of nitrogen in EF treatment significantly increased by 49.2%–84.4% ($P < 0.05$), and the accumulation of nitrogen in EF3 treatment was significantly higher than that in EF1, EF2 and EF4 (Table 3). The nitrogen accumulation of EF1, EF2 and EF3 treated stems was significantly higher than CF ($P < 0.05$), increasing by 32.5%~34.9%. The accumulation of nitrogen in leaves ranged from 656.9 to 796.4 mg· plant^{-1}, with no significant difference among different treatments ($P > 0.05$). The total nitrogen accumulation of plants was the highest by EF3 treatment, reaching 2938.9 mg cutting^{-1}.

3.4 Nitrogen Distribution of M. glyptostroboides with Different Fertilization

The order of N distribution rate in each organ of the seedling of the *M. glyptostroboides* was as follows: root > stem > leaf, the proportion of N accumulation in root, stem and leaf ranged from 37.7% to 49.1%, 24.6% to 28.0%, and 24.5% to 35.0%, respectively (Fig. 2). Compared with CF, nitrogen accumulation in EF treated roots increased significantly, while nitrogen accumulation in leaves decreased significantly ($P < 0.05$).

3.5 Nitrogen Utilization Efficiency of M. glyptostroboides Seedlings under Different Fertilization

As can be seen from Table 4, the biomass and nitrogen harvest index of EFs (EF1, EF2 and EF3) were higher than CF ($P < 0.05$), while no significant difference was found between the CF and EF4 treatment whatever the biomass harvest index and nitrogen harvest index ($P > 0.05$).

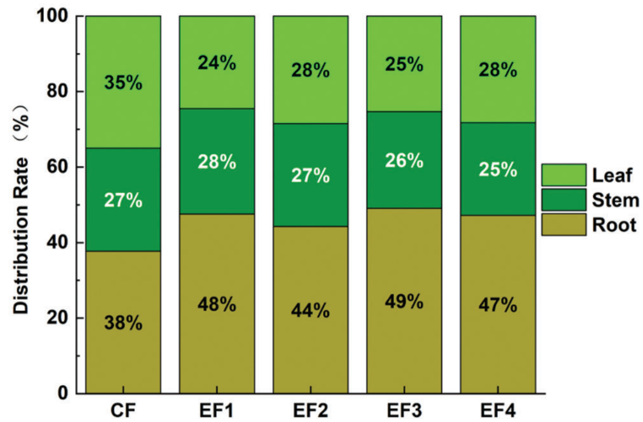

Figure 2: *M. glyptostroboides's* N-distribution with different treatments

Table 4: *M. glyptostroboides's* N-uptake and efficiency with different treatments

Treatment	Biomass harvest index (g g^{-1})	Nitrogen H index (%)
CF	16.67 ± 2.13d	51.52 ± 4.02d
EF1	101.64 ± 9.85a	268.31 ± 27.12a
EF2	37.12 ± 4.83b	93.31 ± 9.83b
EF3	23.12 ± 3.11c	58.83 ± 3.24c
EF4	12.54 ± 1.83d	30.91 ± 2.74d

4 Discussion

Since the external indicators such as height and ground diameter are essential to judge the status of plant growth, they are always determined to reflect the quality of seedlings. Compared with CF, the seedling height of EF1 treatment increased significantly ($P < 0.05$). There was no significant difference in the seedling height and ground diameter between other treatments. Song et al. [32] also showed the effect of exponential fertilization on the ground diameter of *Cyclobalanopsis glauca* (Thunb.) *Oerst.* was not significant. While Wu et al. [3] found that compared to the no N fertilizer application treatment, the external properties of N-fertilized seedlings of Chinese fir were significantly higher ($P < 0.05$), with EF2 having the best properties. Therefore, the proper N supply can improve the growth of *M. glyptostroboides* cuttings [33,34].

The biomass is regarded as another external indicator to reflect plant productivity. The applied nitrogen often affects the distribution of biomass in different parts of plant. Compared with CF, EF treatment significantly improved the accumulation of root and stem biomass (Table 2). Compared with conventional fertilization, the fertilizer using efficiency of exponential fertilization is higher, which indicates that exponential fertilization can effectively convert the N absorbed by plants into plant biomass [35]. In the exponential fertilization, the biomass of different organs increased first and then decreased with the increase of nitrogen application, among which the biomass of stem and leaf was the highest after EF2 treatment (3.0 g $seedling^{-1}$), while the biomass of root and total biomass was the largest after EF3 treatment (5.0 g $seedling^{-1}$). When nitrogen application continued to increase, the plant biomass decreased slightly. For example, compared to other EFs, the EF4 decreased slightly the root biomass. Such phenomena could be related to the N supply, as N was insufficient, plants roots would increase the N absorption and further promote the root biomass [3]. An appropriate amount of exponential fertilization can promote the growth of seedlings [36] and significantly increase the dry matter mass of target plant seedlings [15,37]. On the contrary, excessive nitrogen application often causes mild poisoning of seedlings [10,12], thus inhibiting the growth and biomass accumulation of seedlings [30]. At the final harvest stage, the highest ratio (0.71) of root to shoot biomass was observed in EF3, while the lowest (0.51) was found in CK. Boivin et al. [38] reported that the growth rate of root was much higher than that of shoot during the late fertilization phase. This difference may be due to the different experimental varieties and fertilization application method [39].

Fertilization is a main factor affecting N concentration and the utilization effciency of seedlings. The improvement of N utilization efficiency can promote the quality of seedlings, thus increasing the survival rate of afforestation. The nutritional status of seedlings *in vivo* is an internal physiological indicator for evaluating the quality of seedlings. And nitrogen mass fraction and accumulation amount of seedlings under different fertilizers are different [40]. And after seedling afforestation, the effect of afforestation depends on the initial nutrient status of seedling, because the nutrient absorbed by root system from the soil is limited [41]. The nitrogen mass fraction in the roots of *M. glyptostroboides* seedlings treated with EF1 was significantly higher than that of CF and EF4, while the difference in nitrogen mass fraction of stems and leaves was not significant among different treatments, mainly because *M. glyptostroboides* was a deciduous tree. When sampling on October 20, the leaves had turned slightly yellow, and part of nitrogen would be transferred to roots and stems. It is also possible that N element index fertilization changed the morphology of seedling roots and enhanced the ability of seedling roots to absorb nutrients [42]. Nitrogen accumulation in roots and stems treated by EF was significantly higher than CF ($P < 0.05$), and increased first and then decreased with the increase of nitrogen application, among which, nitrogen accumulation in root was the highest with EF3 treatment. While nitrogen accumulation in stem and leaf was highest by EF2 treatment. Salifu et al. [43] believed that when nitrogen supply was insufficient, the nutrient content of different organs of seedling would increase with the nitrogen supply increasing. When nitrogen supply surpasses seedling demand, nutrient content in different organs decreases [44].

5 Conclusions

Nitrogen exponential fertilization effectively promoted the roots and stems growth and biomass accumulation of *M. glyptostroboides*, and improved the nitrogen nutrient status and nutrient carrying capacity of seedling roots and stems. The total biomass and nitrogen accumulation of EF3 treatment (5.0 g seedling^{-1}) reached the maximum value of 115.24 g seedling^{-1} and 2938.96 mg seedling^{-1}, respectively. So it was the best exponential fertilization method for *M. glyptostroboides* greenhouse seedling cultivation.

Authorship: The authors confirm contribution to the paper as follows: study conception and design: Wu J. S., Lin H.; data collection: Ye Z. H.; Jin J., analysis and interpretation of results: Wu J. S.; Tong G.; Guo R.; draft manuscript preparation: Wu J. S. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgement: We gratefully acknowledge Miss Jiemiao Liu for the field work.

Funding Statement: This study was supported by the Natural Science Fundation of Zhejiang Province (LY20C160004).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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