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The Effects of Fertilization on the Growth and Physiological Characteristics of *Ginkgo biloba* L.

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Abstract: *Ginkgo biloba* L. is one of the most extensively planted and productive commercial species in temperate areas around the world, but slow-growth is the most limiting factor for its utilization. Fertilization is one of the key technologies for high quality and high forest yield. To better understand the impacts of fertilization on *Ginkgo* productivity, the effects of fertilization treatments (single fertilizer and combined fertilizer) on growth, nutrient content in *Ginkgo* leaves, and photosynthesis characteristics were studied in a 10-year-old *Ginkgo* plantation over two years. The single factor experiments suggested that DBH (diameter at breast height), H (height), NSL (length of new shoots), and V (trunk volume) showed significant differences between the different levels of single nitrogen (N) or phosphate (P) fertilizer application. Orthogonal test results showed that the nine treatments all promoted the growth of *Ginkgo*, and the formula (N: 400 $g \cdot tree^{-1}$, P: 200 $g \cdot tree^{-1}$, potassium (K): 90 $g \cdot tree^{-1}$) was the most effective. G_s (stomatal conductance) and P_n (net photosynthesis rate) showed significant differences between the different amounts of single N or P fertilizer application, while single K fertilizer only affected P_n. Combined N, P, and K fertilizer had significant promoting effects on C_i (intercellular CO_2 concentration), G_s and P_n. N and P contents in *Ginkgo* leaves showed significant differences between the different amounts of a single N fertilizer application. A single P fertilizer only improved foliar P contents in *Ginkgo* leaves. A single K fertilizer application improved N and K content in *Ginkgo* leaves. The effects of different N, P, and K fertilizer treatments on the nutrient content of *Ginkgo* leaves were different.

Keywords: *Ginkgo*; fertilization; growth; photosynthesis; nutrient content

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1. Introduction

Nutrient limitations develop when a stand's potential nutrient use cannot be met by the soil nutrient supply [1]. Improving stand nutrient supply through fertilization is a viable silvicultural option [2]. As an intervention strategy, large-scale application of fertilizers to forests has been implemented to accelerate growth of existing stands and shorten rotation times to overcome future projected timber shortfalls [3]. It is well established that fertilization, in particular nitrogen (N) and phosphate (P) applications, on nutrient limited sites increases tree productivity by increasing photosynthesis in the short term [4,5] and leaf area over the long term [6]. N is a vital constituent in proteins, nucleic acids, chlorophylls, and many secondary metabolites of plants [7]; therefore, it plays an essential role in the enzymatic activities of photosynthetic processes [8]. P plays a central role in almost all aspects of plant metabolism and is one of the nutrients that most commonly limits growth [9]. Potassium (K) is one of important nutrient elements and plays a major role in growth, modifying abundant enzyme activations and controlling cell osmoregulation [10]. Gross primary production (GPP) was also greatly increased by K fertilization as a result of lower stomatal and mesophyll resistance to CO_2 diffusion and higher photosynthetic capacity in the leaves [11,12]. In addition, potassium can also alleviate the harmful effects of abiotic stress [13–15].

The advantage of using chemical fertilizers is that nutrients are soluble and immediately available to the plants; therefore, the effect is usually direct and fast. Several studies have reported an increase in growing efficiency and higher enzymatic activities following fertilization [16,17]. Many studies have found that growing efficiency is largely unaffected by nutrient additions [18,19]. Different plants do not have the same nutrient demand and specific fertility targets [20]. The fast-growing broad-leaved tree *Populus alba* × *P. glandulosa* is more sensitive to increasing N availability [21], while the slow-growing *P. popularis* is more sensitive to decreasing N availability [22]. Among conifers, the fast-growing spruce families show more plasticity in biomass allocation than do the slowly growing ones under different nitrogen supplies [23], and the fast growing species *Pinus radiata* allocates more to the aboveground biomass under N and P fertilization [24].

Ginkgo is commonly referred to as Gongsun Tree or Duck Foot Tree, is one of the most ancient gymnosperms in the world, and is native to the temperate forests of China [25]. *Ginkgo* is an eco-economic tree species which is valuable for food, health care, medicine, timber, landscape, ecological protection, and scientific research. The great environmental adaptability and beauty of *Ginkgo* have made the tree a favorite species for planting as an ornamental tree throughout the world [26]. Its widespread use has been facilitated by its tolerance of air pollution and soil compaction. *Ginkgo* has a long history in leaf and nut production. However, it is still in the initial stage for timber cultivation in China. *Ginkgo* wood has also been highly valued for making furniture and handicraft articles, and *Ginkgo* wood is also an ideal material for making musical instruments [27]. Nowadays, *Ginkgo* wood is only used for carving and chopping blocks. The compression of *Ginkgo* wood was found to be stiffer than that of the other species [28]. Burgert et al. (2004) consequently concluded that there has been an evolutionary trend towards much more flexible compression wood [29]. Gong et al. (2009) reported higher lignin content in *Ginkgo* than in conifers [30]. *Ginkgo* wood was once used for pillars and rafters in the palaces and temples in ancient China. *Ginkgo* also has an anti-dust function, purifying the environment. One important role of *Ginkgo* is planting in rows in corridors, squares, or on both sides of a street as shade trees, as well as planting in gardens and vestibule entrances as landscape trees. *Ginkgo* wood has value for many purposes from the help of science and technology. In recent years, with the development of the *Ginkgo* industry, *Ginkgo* cultivation area continues to expand; many researchers have developed a variety of optimum fertilization schemes for *Ginkgo* plantations (especially leaf-producing plantations, nuts plantations, and seedlings) in China. However, studies concentrated on timber plantations are comparatively insufficient.

This study was conducted to examine the relationship between fertilization and growth, photosynthesis characteristics, and foliage nutrient content of pure *Ginkgo* timber plantations. The main objectives were to assess the effect of fertilization on growth, nutrient content in *Ginkgo* foliage, and photosynthesis characteristics of new *Ginkgo* shoots in a 10-year-old *Ginkgo* plantation over two years. Information generated from this study is expected to be of great value for providing optimal fertilization measures for improving the yield of *Ginkgo* timber forests and realizing balanced nutrient management for its fast growth. In addition, the application of a suitable fertilizer ratio after foliage nutrient content analysis, photosynthesis, and tree growth characteristics are some of the most effective ways to reduce cost and fertilizer waste.

2. Materials and Methods

2.1. The Study Site

The experimental field was located in Yellow Sea Forest Park, the east-central region of the Jiangsu Province, Eastern China (32°51′ N, 120°51′ E, 5 m above sea level). The study area belongs to a transition zone between a subtropical zone and warm temperate zone, with seasonal pluvial heat and significant monsoon activity. The study area is characterized by a mean annual temperature 15.0 °C, an annual rainfall of 1061.2 mm, an annual sunshine duration of 2209 h, and an annual frost-free period of 220 days. For determining the site fertility, soil samples from the experimental field were collected for physical and chemical characteristics before actual experiments. The soil was characterized as coastal sandy saline-alkali soil with a pH of 8.42, a bulk density of 1.28 $g \cdot cm^{-3}$, a total nitrogen of 0.75 $mg \cdot g^{-1}$, a total phosphorus of 0.26 $mg \cdot g^{-1}$, and a total potassium of 5.25 $mg \cdot g^{-1}$.

2.2. Materials and Experimental Design

This study was carried out in 2014 and 2015. The test forest was pure *Ginkgo* forest planted in 2005, with a vine space and row space of 2 m × 8 m. The trees came from the same variety. The experiment with different fertilizer treatments was designed based on a single factor design (EXP.1) and an orthogonal design (EXP.2). Fertilizer was applied to a depth of approximately 10–15 cm (to prevent losses to rain or wind) in several spots in a 70 cm radius circle around each tree in early April 2014 and early April 2015.

Single factor designs (unit: $g \cdot tree^{-1}$): EXP.1 was designed as single-factor experiment. The dosage of four treatments of single N fertilizer was 600, 400, 200, and 100, respectively. The dosage of four treatments of single P fertilizer was 800, 600, 400, and 200, respectively. The dosage of four treatments of single K fertilizer was 200, 90, 40, and 15, respectively. The amounts of fertilizers were in descending order. The fertilizers used in the experiments were urea (N), superphosphate (P), and potassium sulfate (K). There were a total of 13 treatments, and one of them was CK_1 (control, without fertilizer application). Each treatment had 10 *Ginkgo* trees with three replications in the trial. There were a total of 39 plots and 390 *Ginkgo* trees (average diameter at breast height was 13.37 cm, average height was 8.04 m, and their growth was uniform.) in the single factor tests. Additionally, there were two guard rows around the test plots.

Orthogonal designs (unit: $g \cdot tree^{-1}$): Second, the experiment with different fertilizer treatments (EXP.2) was designed based on orthogonal designs with three levels of urea, superphosphate, or potassium sulfate, for a total of 10 treatments one of them was CK_2 (control), without fertilizer application, which differed from the CK_1 above. Treatments were replicated three times (Table 1). There were a total of 30 plots and 300 *Ginkgo* trees (average diameter at breast height was 11.62 cm, average height was 5.78 m, and their growth was uniform) in the orthogonal tests. Orthogonal tests plots were in close proximity to the single-factor test spots.

Table 1. Orthogonal trial fertilizer treatments.

Treatment No.	Fertilization Level ($g \cdot tree^{-1}$)		
	Urea	Superphosphate	Potassium Sulfate
1	100	200	15
2	100	400	40
3	100	600	90
4	200	200	40
5	200	400	90
6	200	600	15
7	400	200	90
8	400	400	15
9	400	600	40
CK_2	0	0	0

CK_2: control in EXP.2.

2.3. Tree Growth Indictors' Measurement

Tree diameter at breast height (DBH), height (H), and length of new shoots (NSL) were measured in November 2015. Tree height was measured using a hypsometer (SGQ-1, Harbin, China). DBH and NSL were measured using diameter tape. We selected 21 *Ginkgo* trees of different diameter classes (2, 4, 6, 8, 10, 14, and 20) around the experiment plots, cut them down, and measured their DBH and H. Then, according to the standard volume equation and formula (Equation (1)):

$$V = a_0 D^{a1} H^{a2} \quad (1)$$

we used the R programming language (version 3.0, The University of Auckland, Auckland, New Zealand) software to obtain the trunk volume determination formula (Equation (2)):

$$\mathrm{LnV} = 2.0135 \times \ln D + 0.5685 \times \ln H - 9.2452 \ (R^2 = 0.9819) \quad (2)$$

V: trunk volume (m^3), D: DBH (cm), H: height (m). We used this formula to calculate the *Ginkgo* trunk volume (V) on the test plots.

2.4. Determination of Photosynthetic Indexes

The photosynthetic characteristics were measured with a CIRAS-2 photosynthetic instrument (Hansatech, Norfolk, VA, USA). We selected sunny days in early August 2015 (08:30–11:30), and set the parameters: leaf temperature was not controlled, relative humidity was 85%, cylinder supply CO_2 concentration was 380 $\mu mol \cdot mol^{-1}$, and the light intensity of an artificial light source was 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (exceeding the *Ginkgo* light saturation point). Five trees were randomly selected from each replicate of each treatment on the test plots. Then, we selected mature healthy leaves of the fully-extended branches in the high position of the canopy. The net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) were measured.

2.5. Determination of Nutrient Concentration in Leaves

The sampling time was in August 2015. We selected five trees randomly from each replicate of each treatment in the test plots. We mined the new shoots in the middle part of the canopy with pole tree pruners. We collected the healthy 8–12 pieces of functional leaves without diseases and pest attack, and immediately put the leaves into an ice box, respectively. After the leaves were brought to the laboratory, the leaves were washed with deionized water, rapid fixed for 15 min at 105 °C, then dried to counterweigh at 70 °C, and, finally, crushed and passed through a 100-mesh sieve, severally (a total of 69 samples). Then, we weighed each sample to 0.1000 g and used concentrated H_2SO_4-$HClO_4$ to digest them. The nitrogen and phosphorus were analyzed with an AA3 continuous flow analytical system (Bran + Luebbe, Hamburg, Germany); potassium was analyzed via flame atomic absorption spectrometry (WFX-210, Beijing, China).

2.6. Statistical Analysis

Data are reported as the mean of three replicates ± standard deviation (SD), and all tests were performed using the Statistical Product and Service Solution statistical software program (IBM Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was conducted to compare the effect of fertilization treatments on growth, photosynthetic indexes, and the element contents in leaves. ANOVA was performed at the $p < 0.05$ level of significance. Duncan's multiple-range test was performed for each variable (note: different lowercases in the table showed significant differences at the 0.05 level. Treatment means with different lowercases among treatments in the figure are significantly different at the 0.05 level).

3. Results

3.1. Effects of Fertilizer on Growth Indicators

In Yellow Sea Forest Park, DBH, H, NSL, and V in EXP.1 showed significant differences between levels of single N or P fertilizer applications, suggesting that N fertilizer application in excess of 100 $g \cdot tree^{-1}$ or P fertilizer application in excess of 400 $g \cdot tree^{-1}$ may be suitable for tree growth (Table 2; $p < 0.05$). However, growth responses were small and none of the variables showed a significant growth response to K fertilizer (Table 2; $p > 0.05$). Maximum dosage tended to have the greatest influences on the growth indicators for single N, P, and K fertilizers (N = 600 g·tree^{-1}, P = 800 g·tree^{-1}, or K = 200 g·tree^{-1}). After two years of continuous fertilization, a single N fertilizer application rate at 600 g·tree^{-1} resulted in an increase in V greater than 17.7% compared with unfertilized plots (CK$_1$). Meanwhile, a single P fertilizer application rate at 800 g·tree^{-1} resulted in an increase in V greater than 9.7% compared with unfertilized plots (CK$_1$). Therefore, single N or P fertilizer treatments promoted *Ginkgo* growth indicators significantly, while the promotion effect of single K fertilizer treatment was not significant. Additionally, orthogonal tests in EXP.2 showed that combined N, P, and K fertilizer had significant promoting effects on growth indictors (Table 3; $p < 0.05$); the maximum effect on the growth of *Ginkgo* was achieved at a high dosage of N. After two years of continuous fertilization, Treatment 7 resulted in an increase in V greater than 27.7% compared with unfertilized plots (CK$_2$).

Table 2. Effects of two years in EXP.1 of N, P, and K fertilizer (F) on the diameter at breast height (DBH, cm), height (H, m), length of new shoots (NSL, cm), and trunk volume (V, m^3) of *G. biloba* in 2014 and 2015 in Yellow Sea Forest Park in China.

F	DBH	H	NSL	V
N1 600	15.34 ± 0.396 a	9.85 ± 0.383 a	26.59 ± 1.204 a	0.0146 ± 0.000929 a
N2 400	15.11 ± 0.123 a	9.27 ± 0.492 b	25.56 ± 1.284 ab	0.0135 ± 0.000755 ab
N3 200	14.96 ± 0.301 a	9.17 ± 0.131 b	24.64 ± 2.840 ab	0.0133 ± 0.000448 b
N4 100	14.92 ± 0.407 a	9.14 ± 0.068 b	21.93 ± 2.635 bc	0.0132 ± 0.000369 b
CK$_1$ 0	14.35 ± 0.095 b	8.92 ± 0.158 b	20.23 ± 2.594 c	0.0124 ± 0.000256 b
P1 800	14.84 ± 0.063 a	9.45 ± 0.257 a	25.98 ± 1.33 a	0.0136 ± 0.000398 a
P2 600	14.42 ± 0.255 ab	9.13 ± 0.221 ab	24.02 ± 1.86 ab	0.0127 ± 0.000408 b
P3 400	14.23 ± 0.333 b	9.05 ± 0.182 ab	24.37 ± 0.688 ab	0.0124 ± 0.000514 b
P4 200	14.21 ± 0.292 b	8.83 ± 0.270 b	22.69 ± 0.419 bc	0.0121 ± 0.000464 b
CK$_1$ 0	14.35 ± 0.095 b	8.92 ± 0.158 b	20.23 ± 2.594 c	0.0124 ± 0.000256 b
K1 200	14.91 ± 0.499 a	9.01 ± 0.288 a	24.01 ± 0.844 a	0.0124 ± 0.000965 a
K2 90	14.65 ± 0.428 a	8.93 ± 0.195 a	23.71 ± 1.29 a	0.0117 ± 0.000445 a
K3 40	14.66 ± 0.553 a	8.88 ± 0.326 a	23.35 ± 2.14 a	0.0113 ± 0.000699 a
K4 15	14.38 ± 0.422 a	8.63 ± 0.459 a	24.49 ± 0.967 a	0.0114 ± 0.000993 a
CK$_1$ 0	14.35 ± 0.095 a	8.92 ± 0.158 a	20.23 ± 2.59 a	0.0124 ± 0.000256 a

Note: different lowercase letters in the table reflect significant differences between treatments. CK$_1$: control in EXP.1.

Table 3. Effects of two years in EXP.2 of combined N, P, and K fertilizer (F) on the diameter at breast height (DBH, cm), height (H, m), length of new shoots (NSL, cm), and trunk volume (V, m^3) of *G. biloba* in 2014 and 2015 in Yellow Sea Forest Park in China.

F	DBH	H	NSL	V
1	13.66 ± 0.908 bcd	7.45 ± 0.022 bc	24.15 ± 0.832 bcd	0.00983 ± 0.000675 cd
2	13.78 ± 0.825 abcd	7.49 ± 0.065 bc	26.606 ± 3.250 abc	0.00997 ± 0.000679 cd
3	13.2 ± 0.172 cd	7.42 ± 0.168 bc	26.00 ± 2.32 abc	0.00947 ± 0.000327 de
4	13.71 ± 0.233 bcd	7.65 ± 0.123 ab	25.90 ± 1.921 abc	0.01013 ± 0.000207 cd
5	13.88 ± 0.173 abc	7.73 ± 0.293 ab	25.26 ± 1.705 abc	0.01036 ± 0.000261 bc
6	13.94 ± 0.370 abc	7.55 ± 0.046 b	22.83 ± 0.656 cd	0.01017 ± 0.000304 cd
7	14.61 ± 0.347 a	8.01 ± 0.239 a	26.77 ± 4.070 abc	0.01130 ± 0.000302 a
8	14.50 ± 0.423 ab	7.81 ± 0.173 ab	28.89 ± 2.014 a	0.01094 ± 0.000240 ab
9	14.47 ± 0.208 ab	7.81 ± 0.205 ab	27.49 ± 1.980 ab	0.01092 ± 0.000304 ab
CK$_2$	12.92 ± 0.231 d	7.09 ± 0.0346 c	20.23 ± 2.594 d	0.00885 ± 0.000161 e

Note: different lowercase letters in the table reflect significant differences between treatments.

3.2. Effects of Fertilizer on Photosynthesis Indicators

In Yellow Sea Forest Park, P$_n$ and G$_s$ showed significant differences between levels of single N or P fertilizer application ($p < 0.05$), while T$_r$ and C$_i$ were not affected by fertilizer application (Table 4; $p > 0.05$). However, photosynthesis indicators showed no significant response to single K fertilizer treatment except P_n (Table 4; $p > 0.05$). After two years of continuous fertilization, a single N fertilizer application rate at 600 g·tree^{-1} resulted in an increase in P_n greater than 49.5% compared with unfertilized plots (CK_1). Meanwhile, single P fertilizer application rate at 800 g·tree^{-1} resulted in an increase in P_n greater than 27.6% compared with unfertilized plots (CK_1). Therefore, single N or P fertilizer treatments promoted *Ginkgo* P_n significantly, while the promotion effect of single K fertilizer was also significant at 90 g·tree^{-1} (K2). Additionally, orthogonal tests showed that combined N, P, and K fertilizer had a significant promoting effect on P_n and G_s ($p < 0.05$); while fertilization was not so important on T_r and C_i (Table 5; $p > 0.05$) in EXP.2, after two years of continuous fertilization, Treatment 9 resulted in an increase in P_n greater than 36.9% compared with unfertilized plots (CK_2).

Table 4. Average effects of two years N, P, and K fertilizer (F) in EXP.1 on the photosynthesis characteristics, net photosynthetic rate (P_n, μmol·m^{-2}·s^{-1}), transpiration rate (T_r, μmol·m^{-2}·s^{-1}), stomatal conductance (G_s, mol·m^{-2}·s^{-1}), and intercellular CO_2 concentration (C_i, μmol·mol^{-1}) in 2014 and 2015 in Yellow Sea Forest Park in China.

F	C_i	T_r	G_s	P_n
N1 600	278.56 ± 8.57 a	2.71 ± 0.252 a	167.64 ± 3.32 a	12.30 ± 0.472 a
N2 400	277.33 ± 4.98 a	2.68 ± 0.150 a	165.78 ± 2.55 a	11.74 ± 0.597 ab
N3 200	269.78 ± 5.10 a	2.43 ± 0.173 a	157.78 ± 4.43 a	10.25 ± 1.42 b
N4 100	267.79 ± 16.65 a	2.51 ± 0.284 a	156.67 ± 7.86 a	10.57 ± 0.635 b
CK_1 0	267.33 ± 21.73 a	2.53 ± 0.208 a	127.33 ± 15.5 b	8.23 ± 0.833 c
P1 800	289.89 ± 3.29 a	3.03 ± 0.153 a	156.56 ± 5.34 a	10.50 ± 1.19 a
P2 600	275.11 ± 8.26 a	2.80 ± 0.152 a	139.78 ± 4.17 b	10.22 ± 1.06 ab
P3 400	266.89 ± 5.23 a	2.62 ± 0. 135 a	129.89 ± 8.06 b	8.77 ± 1.72 abc
P4 200	275.33 ± 19.50 a	2.67 ± 0.333 a	133.88 ± 6.02 b	7.14 ± 0.635 c
CK_1 0	267.33 ± 21.73 a	2.53 ± 0.208 a	127.33 ± 15.50 b	8.23 ± 0.833 bc
K1 200	256.89 ± 1.92 a	2.60 ± 0.101 a	148.22 ± 11.5 a	10.39 ± 0.27 a
K2 90	251.00 ± 4.58 a	2.54 ± 0.081 a	133.11 ± 10.98 a	10.44 ± 0.44 a
K3 40	265.78 ± 7.93 a	2.46 ± 0.102 a	123.67 ± 15.94 a	8.37 ± 0.62 b
K4 15	266.79 ± 9.25 a	2.27 ± 0.095 a	121.56 ± 16.60 a	7.61 ± 0.37 b
CK_1 0	267.33 ± 21.73 a	2.53 ± 0.208 a	127.33 ± 15.50 a	8.23 ± 0.83 b

Note: different lowercase letters in the table reflect significant differences between treatments.

Table 5. Average effects of two years of N, P, and K fertilizer (F) in EXP.2 on the photosynthesis characteristics, net photosynthetic rate (P_n, μmol·m^{-2}·s^{-1}), transpiration rate (T_r, μmol·m^{-2}·s^{-1}), stomatal conductance (G_s, mol·m^{-2}·s^{-1}), and intercellular CO_2 concentration (C_i, μmol·mol^{-1}) in 2014 and 2015 in Yellow Sea Forest Park in China.

F	C_i	T_r	G_s	P_n
1	239.00 ± 10.40 b	2.56 ± 0.34 a	122.33 ± 13.39 c	9.00 ± 0.91 cd
2	246.78 ± 27.03 b	2.41 ± 0.20 a	127.33 ± 13.00 bc	9.22 ± 0.74 bcd
3	243.00 ± 25.32 b	2.41 ± 0.47 a	136.11 ± 7.31 abc	9.36 ± 1.02 bcd
4	254.00 ± 8.09 b	2.61 ± 0.24 a	133.56 ± 9.52 bc	9.48 ± 0.17 bcd
5	247.33 ± 5.20 b	2.59 ± 0.34 a	141.34 ± 3.51 abc	9.71 ± 1.51 abcd
6	272.11 ± 17.88 ab	2.74 ± 0.15 a	140.11 ± 4.40 abc	10.11 ± 0.23 abc
7	271.78 ± 16.68 ab	2.69 ± 0.25 a	142.67 ± 3.51 abc	10.68 ± 1.22 abc
8	272.55 ± 20.51 ab	2.69 ± 0.08 a	146.89 ± 12.98 ab	10.71 ± 0.71 ab
9	290.33 ± 16.18 a	2.79 ± 0.16 a	154.33 ± 12.20 a	11.27 ± 0.38 a
CK_2	265.33 ± 18.73 ab	2.50 ± 0.11 a	129.13 ± 12.50 bc	8.53 ± 0.58 d

Note: different lowercase letters in the table reflect significant differences between treatments.

3.3. Effects of Fertilizer on Leaf N, P, and K Contents

In Yellow Sea Forest Park, N and P contents in leaves showed significant differences between levels of single N fertilizer application ($p < 0.05$), while K content was not affected by N fertilizer application (Figure 1; $p > 0.05$). N and K contents showed no significant response to single P fertilizer ($p > 0.05$), while P content showed a significant difference (Figure 2; $p < 0.05$). N and K contents showed significant differences between the different amounts of single K fertilizer application ($p < 0.05$), while P content was not affected by K fertilizer application (Figure 3; $p > 0.05$). After two years of continuous fertilization, a single N fertilizer application of 600 g·tree^{-1} resulted in an increase in N content greater than 16.4% and an increase in P content greater than 19.9% compared with unfertilized plots (CK_1). Meanwhile, a single P fertilizer application of 800 g·tree^{-1} resulted in an increase in P content greater than 23.8% compared with unfertilized plots (CK_1). A single K fertilizer application of 200 g·tree^{-1} resulted in an increase in N content greater than 12.2% and an increase in K content greater than 21.8% compared with unfertilized plots (CK_1). Therefore, single N fertilizer treatment promoted *Ginkgo* N and P content significantly, single P fertilizer treatment only promoted *Ginkgo* P content significantly, and the promotion effect of single K fertilizer treatment on N and K content were significant. Additionally, orthogonal tests in EXP.2 showed that combined N, P, and K fertilizer treatment had a significant promoting effect on N, P, and K content (Figure 4; $p < 0.05$). After two years of continuous fertilization, Treatment 9 resulted in an increase in N content greater than 13.4% compared with unfertilized plots (CK_2). Treatment 6 resulted in an increase in P content greater than 21.5% compared with unfertilized plots (CK_2). Treatment 7 resulted in an increase in K content greater than 16.7% compared with unfertilized plots (CK_2).

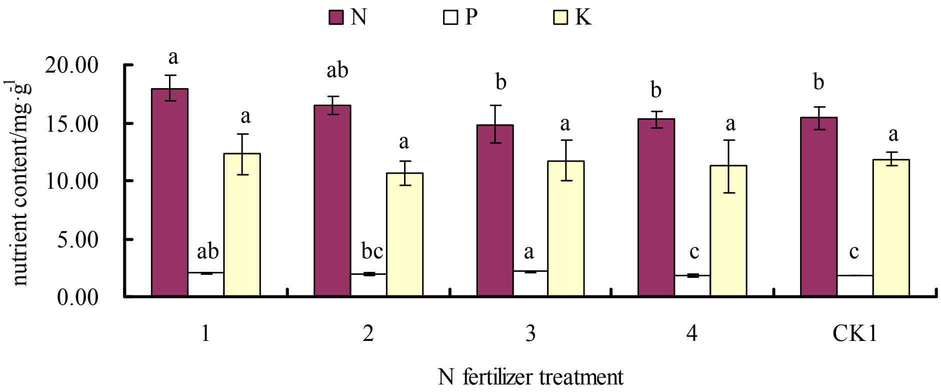

Figure 1. Effects of single N fertilizer application on *G. biloba* leaf N, P, and K contents in EXP.1.

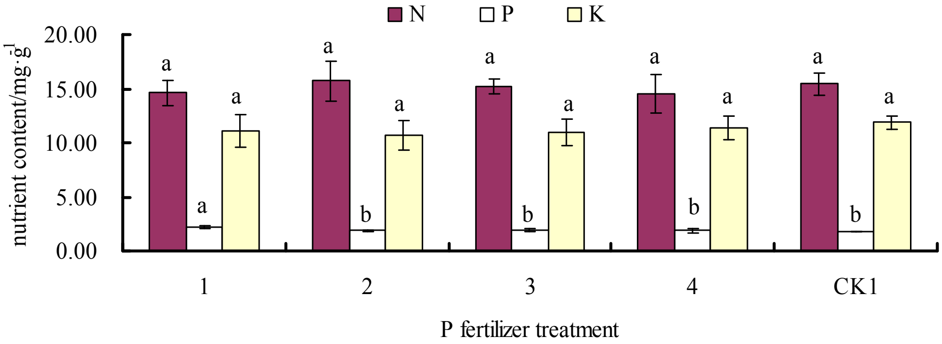

Figure 2. Effects of single P fertilizer application on *G. biloba* leaf N, P, and K contents in EXP.1.

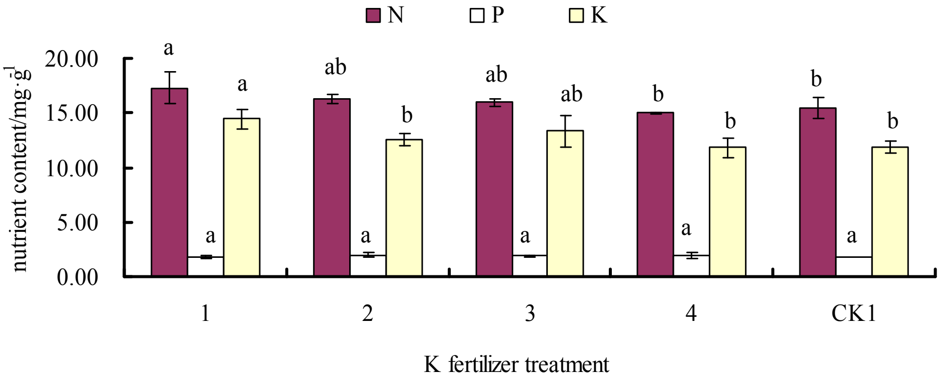

Figure 3. Effects of single K fertilizer application on *G. biloba* leaf N, P, and K contents in EXP.1.

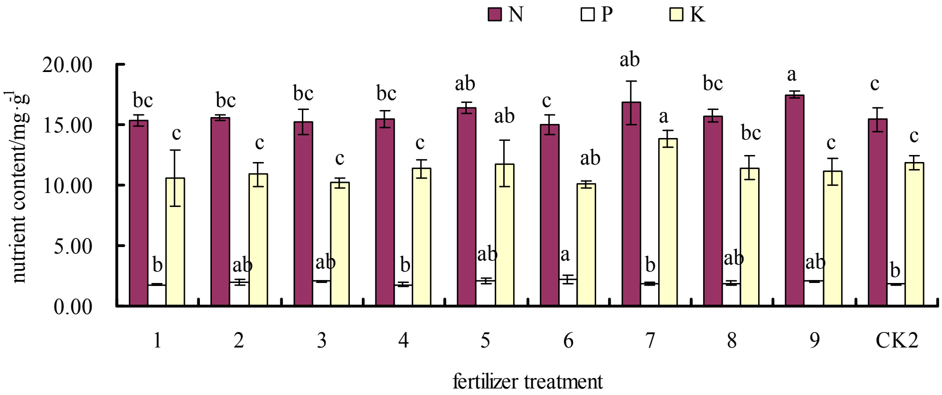

Figure 4. Effects of combined N, P, and K fertilizer application on *G. biloba* leaf N, P, and K contents in EXP.2.

4. Discussion

Nutrient deficiency is generally considered the major factor limiting growth, and fertilization is the main way to supplement the lack of nutrition. The fertilization effects have shown different results under different quantities of fertilizer, the number of years since fertilization treatment, and the nutrition status of the forest site [31]. Our study was carried out in 2014 and 2015. The single factor experiments suggested that DBH, H, NSL, and V showed significant differences between the different levels of single N or P fertilizer application. The orthogonal test results showed that the nine treatments all promoted the growth of *Ginkgo*, and Treatment 7 (N: 400 g·tree^{-1}, P: 200 g·tree^{-1}, K: 90 g·tree^{-1}) was the most effective one. The response to fertilization in *Ginkgo* plantations depended on the degree of mismatch between nutrient supply from the soil and the plant nutrient demand [32]. With an increment in the N fertilizer, there was also an increment in the growth, foliar nutrient status, and photosynthesis rate of the *Ginkgo* plantation. Phosphorus supply affected photosynthesis, leaf metabolites, and allocation to roots versus leaves and growth. In our study, the growth indicators improved with the P fertilizer dosage. However, excess P supply in the soil is a major environmental concern. The accumulation of P in the soil from applications of chemical fertilizer exceed the requirement of plant and can increase the risk of P movement to surface and groundwater [33]. Thus, we should be very circumspect in the application of P fertilizer. Many investigations have shown that improved N and P nutrition increased growth and yield in many tree species [34]. N and P deficiency cause negative effects on plants [35,36]. However, Gotore et al. (2014) proposed that single N application had negative effects on tree growth, while single P had positive effects on *Pinus patula* growth at Charter Forests. This may be because N is not a limiting factor to tree growth in the first few years after planting and may be readily available in the soil [19]. Additionally, Moilanen et al.

(2004) reported clearly that N fertilization is unnecessary on the nitrogen-rich sites, and additional N increased growth only at the most barren sites [37]. In our study, the promotion effect of a single K fertilizer on *Ginkgo* growth indicators was not significant, though it was better than the unfertilized plots. A previous study showed that fertilization with K was causing a decreased allocation to roots, which enables increased growth in height and leaf number [38]. Moreover, the viewpoint that an optimal potassium nutrition status can reduce the effects of abiotic stresses, such as drought, heat, high light intensity, or salinity has been well established [14,39,40]. The characteristic of our study site is coastal sandy saline–alkali soil; thus, K fertilizer is essential in Yellow Sea Forest Park. In general, N played a key role in the growth of *Ginkgo*, and P and K were important nutrient elements playing a major role in *Ginkgo* growth and development. According to the classification standard of soil nutrients in China, the analysis of soil elements in our experimental plots reveals that contents of N, P, and K are basically at low levels. Therefore, supplementary fertilization may be required on the experimental plots, particularly as the soil nutrient availability cannot meet the demand of the trees.

Photosynthesis is the basis of growth and yield formation, and the effects of nutrition on growth may be driven primarily by photosynthesis [41]. Fertilization can increase the photosynthetic efficiency, prolong the duration of photosynthesis, and enlarge the leaf area index (LAI). In the present study, G_s and P_n showed significant differences between the different levels of single N or P fertilizer applications, while single K fertilizer only affected P_n. Combined N, P, and K fertilizers had significant promoting effects on C_i, G_s, and P_n. N played a vital role in the enzymatic activities of photosynthetic processes, and N addition improved the *Ginkgo* photosynthesis rate and growth response. Among species there is often a strong positive correlation between maximum rates of photosynthesis and N [42]. Greater application of N fertilizer and greater allocation of N to metabolically active tissues, such as leaves and new stems, in the previous study promoted P_n by increasing LAI and chlorophyll concentration of leaves, which, in turn, supported better plant growth [43]. There was also a strong connection between P and the maximum rate of photosynthesis [44]; a partial explanation may lie in a positive relationship between the concentration of P and the amount of Rubisco. Inorganic phosphate (Pi) is a prerequisite for RuBP and also a competitive inhibitor of RUBP [45,46]. Additionally, there was a strong correlation between K concentrations and Rubisco [21]. Zorb et al. (2014) also reported that rates of photosynthesis were positively correlated with application rates of K [47]. K deficiency reduces photosynthesis by decreasing stomatal conductance [48]. However, the differences of stomatal conductance were not significant between different amounts of K fertilizer in our research. Possibly owing to the high mobility of K in both soils and plants, growth is expected to improve in response to added K, as shown in sandy soils [49]. Under this circumstance, deficiency of K may be so severe that fertilization is necessary to sustain tree growth until the end of rotation [50,51]. Transpiration and leaf water status are coordinated to minimize plant water loss and avoid hydraulic failure [52]. T_r of different levels of fertilization had no significant difference in our study. Probably because the climate characteristic of the site belongs to seasonal pluvial heat and significant monsoon, the rainfalls mainly occur in summer. Many studies have shown that growth of *Populus* was related to increasing water availability and fertilization on nutrient-limited soils [53,54]. Thus, sufficient rainfall and appropriate fertilization contributed to better growth in *Ginkgo* plantations in Yellow Sea Forest Park.

Nutrient concentrations of foliage have been accepted as adequate indicators of growth and soil fertility in forest stands [55,56]. Maintaining near-optimal foliar nutrient status of trees is especially critical to the maintenance of growth, the establishment of cold tolerance, and the evaluation of soil fertility at sites [56,57]. Our results suggested that N and P contents showed a significant influence of single N fertilizer application. Single P fertilizer only improved foliar P content, and high P fertilizer dosage had an inhibiting effect on K content. Single K fertilizer application improved N and K content in *Ginkgo* leaves. Effects of different combined N, P, and K fertilizer treatments on the nutrient content of *Ginkgo* leaves were different. In this study, we found that foliar N concentration was significantly correlated with N fertilizer application, which might result primarily from the supply status of soil nutrient elements. Moreover, foliar N concentration was significantly correlated with K fertilizer application, which might result from K-increased N use efficiency [58]. A previous study has showed that the maximum photosynthetic carbon assimilation rate positively correlated with foliar N concentrations [59]. A greater allocation of N to metabolically-active tissues, such as leaves and new stems in the fertilization treatment, increases the LAI and chlorophyll concentration of leaves, further supporting a better growth of *Ginkgo* plantations. In N-poor sites, particularly during cold growing seasons, the availability of N for trees may also be low and N deficiencies may be quite common [60,61]. Thus, it is an adaptation to store N in excess of current requirements for photosynthesis that can later be recycled and utilized during periods of lower N supply from the soil. P fertilization improved foliar P contents, and a high P fertilizer dosage had an inhibiting effect on K content. Our findings were consistent with the trend of a previous study of *Phoebe bournei* seedlings, where foliar N and P concentrations increased, but K concentration decreased as the phosphorus addition increased [62]. The results from Crous et al. (2008) showed a trend of increasing foliar K with an increase in the application of K when no phosphate fertilizer was applied. When P was applied with K on the plots with residual fertilizer, no such trend was observed [63]. Nakashgir (1992) reported nitrogen utilization of maize was accentuated when K application was supplemented [64]. We found that foliar K concentration was significantly correlated with K fertilizer application, which might owe to the high mobility of K in plants and soil. Additionally, light affects the internal redistribution of potassium, leading to higher concentrations in sun-exposed branches than in shaded ones [65,66]. The previous studies reported that potassium plays an important role in stomatal function by maintaining turgor pressure [67]. Growth is expected to improve in response to added K as shown in sandy soils [49]. According to the nutrient content contained in the *Ginkgo* leaves, the largest demand is N, followed by K, then P in this study. These results suggest that a new compound ratio of fertilizer is needed to optimize the growth of *Ginkgo* timber forest planted in coastal sandy saline–alkali soil because of different foliar N, P, and K concentrations under different fertilization treatments. According to the actual situation, we can suggest an optimal fertilization formula for improving the yield and quality of *Ginkgo* timber forests in Yellow Sea Forest Park.

5. Conclusions

The beneficial influences of single N, P, and K fertilization and combined N, P, and K fertilization on *Ginkgo* nutrition, photosynthesis, and growth were very obvious. This study confirmed the positive foliar nutrient content and the increased photosynthesis and growth responses with sufficient data, which indicated significantly better growth in fertilized stands compared to unfertilized stands. Single N, P, and K fertilizers and the combined N, P, and K fertilizer had positive effects on the growth of *Ginkgo*. Combined N, P, and K fertilizer and optional formulation are the better choices for achieving balanced nutrition of *Ginkgo* on our experimental plots. Sufficient nutrition supplements in barren places are significant to guarantee the growth of the forests. Efficient fertilizer regimes require an accurate knowledge of the nutrient status during the growth period to avoid any unforeseen deficiencies.

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