GROWTH, PHOTOSYNTHESIS, AND NUTRIENT UTILIZATION RESPONSES OF *SALIX INTEGRA* TO FERTILIZATION IN A PLANTATION ALONG THE HUAIHE RIVER IN CHINA

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(Received 21st Jun 2024; accepted 9th Dec 2024)

Abstract. *Salix integra* Thunb. (Salicaceae) is a perennial deciduous shrub that grows mainly in the riparian areas of the Huaihe River basin in China. where it plays a vital role in conserving water and soil. To optimize the development of *S. integra* production and devise appropriate fertilization strategies for plantation management, we studied the plant's responses to different nitrogen (N) and phosphorus (P) fertilizer levels in *S. integra* in the lowland areas in Bangang in Yingshang County, Anhui Province, China. Fertilization experiments were conducted on two-year-old *S. integra* stands under six treatment levels: T1 (30 g $N \cdot m^{-2}$), T2 (20 g $N \cdot m^{-2}$ + 15 g $P \cdot m^{-2}$), T3 (30 g $N \cdot m^{-2}$ + 15 g $P \cdot m^{-2}$), T4 (45 g $N \cdot m^{-2}$ + 15 g $P \cdot m^{-2}$), T5 (60 g $N \cdot m^{-2}$ + 15 g $P \cdot m^{-2}$), T6 (15 g $P \cdot m^{-2}$) and a control (CK). The results showed that fertilization significantly enhanced the shrub's growth and photosynthesis. T5 represented the optimal nutrient input for desirable *S. integra* growth, at which fertilization level the biomasses of the stems, roots, and root stumps were at their maximum. The total biomass under the different treatments was ranked as CK (20.42 $t \cdot hm^{-2}$) < T2 (21.76) < T1 (22.67) ≈ T3 (22.95) ≈ T6 (22.96) < T4 (30.20) < T5 (37.64). The applied ratios also considerably impacted the distributions of carbon, nitrogen, phosphorus, potassium, calcium, and magnesium in the roots, root stumps, stems, and foliage of the *S. integra* stands. Significant differences in soil-available nutrients were also observed across various fertilizer types and amounts, with T5 resulting in the highest NH^{4+}-N and NO^{3-}-N concentrations. These findings indicate that supplemental 60 g $N \cdot m^{-2}$ and 15 g $P \cdot m^{-2}$ could be applied to young *S. integra* plantations along the Huaihe River for optimal forest management.
Keywords: *nitrogen, phosphorous, fast-wood plantation, forest management, Salicaceae, fertilization regime biomass*

Introduction

Salix integra Thunb. (Salicaceae) is a perennial fascicular deciduous shrub that exhibits wide adaptability under adverse conditions, such as cold weather, waterlogging, and mild saline-alkali soils with pH values below 8.5 and salt contents below 0.3%. Salix integra is primarily located in the riparian zone along the Huaihe River in China, and fulfills essential roles in water and soil conservation. Plantations of *S. integra* in the lowland areas can effectively protect riverbanks, reduce the risk of natural disasters, and limit the damage to and adverse effects on agricultural production as a result of natural hazards. As a multipurpose shrub, *S. integra* is also harvested as a raw material for willow wicker crafts due to its high sprouting rate and long, flexible stems. Its ability to thrive in diverse environments makes it a critical species for soil erosion control, phytoremediation, and bioenergy production. Optimizing fertilization for Salix integra is crucial to enhance its growth, improve its biomass yield, and maximize its utility in these applications. Despite its potential, there is limited research on the specific nutrient requirements of this species, leading to suboptimal management practices that may

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

hinder its ecological and economic benefits. Understanding the fertilization needs of Salix integra not only supports sustainable agricultural and environmental practices but also contributes to the global push for renewable resources and ecosystem restoration (Perttu et al., 1997; Kopp et al., 2001; Volk et al., 2009).

Due to its high economic, social, and ecological significance, coupled with its unregulated exploitation, the cultivation of *S. integra* has garnered growing attention, leading to the identification of numerous precious varieties (Li et al., 1995; Liu, 1998). Two cultivars, *S. integra* cv. qingpi and cv. hongpi, are the main cultivars along the Huaihe River. Currently, the annual productivity of *S. integra* can reach 26000 kg per hectare when properly managed. Therefore, establishing fast-growing and high-yield *S. integra* plantation programs throughout China holds practical importance. The cornerstone of this widespread implementation lies in developing a strong forest management system, backed by scientifically-sound production guidelines. There have not been many studies on *S. integra* worldwide, and previous investigations focused primarily on high-yield cultivation techniques and intercropping plantations (Hou, 2002; Yang et al., 2004; Wang, 2008). Little is known of young *S. integra* forests and the optimal fertilization regime for productivity.

Fertilization management is crucial in mitigating land degradation, maintaining soil quality promoting fast forest growth, and enhancing productivity (Stone, 1984; Manna et al., 2007). It has been employed by many countries with developed forestry industries as a necessary and important means of developing “fast-wood” plantations and enhancing the production of renewable resources (Adams et al., 1987; Bolstad and Allen, 1987). Based on theory and practical developments, the methods for forest-calculated fertilization can be divided into four types, including the empirical method, field fertilization experimentation, nutrient diagnosis, and formulated fertilization (such as soil testing and fertilizer recommendation) (Baule and Fricker, 1970; Pritchett, 1980; Brown and Driessche, 2002). Existing research on forest fertilization has mainly concentrated on fast-growing timber species with high economic value in combination with directional cultivation, such as *Populus* × *tomentosa* Carrière, *Paulownia tomentosa* Steud., *Eucalyptus robusta* Sm., Bambusoideae, and *Cunninghamia lanceolata* (Lamb.) Hook. in China (Chen et al., 1998; Wang and Zhang, 2000; Qiu et al., 2001; Jin et al., 2007). Nitrogen (N) is required in greater quantities than any other soil nutrient for plant growth. Low N levels can limit forest growth, and thus N is the most common fertilizer for successful commercial production (Waring, 1969). However, incorrect fertilization practices, particularly the overuse of nitrogen (N) and phosphorus (P), can result in the depletion of organic carbon (C) and N from the soil, such as through priming effects (Dai et al., 2016). The specific fertilizer application quantity depends on the soil fertility of the cultivation location. Lime concretion black soils in the study site along the Huaihe River are characterized by insufficient organic matter content and generally have very low N and P nutrient reserves, but are relatively abundant in potassium (K).

In the current study, we explored the ecological and physiological reactions of *S. integra* to various dosages and proportions of N, P, or combined N + P fertilizers in the low-lying regions along China’s Huaihe River. The main objectives of this study were to (1) determine the fertilizer treatment that optimizes the growth and production of young *S. integra* forests; and (2) understand the associated physiological mechanisms by characterizing the impact of fertilization on photosynthetic properties, nutrition allocation, and soil nutrients. These objectives to set up for the optimal and sustainable management of fast-growth and high-yield *S. integra* plantations.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

Material and methods

Study site and experimental design

The experiment was conducted at Bangang, which is located in the south of Yingshang County, on the north shore of the Huaihe River in Anhui Province (32°29’23”N–32°32’30”N, 116°11’19”E–116°17’23”E). This region has a warm temperate semi-humid monsoon climate that is characterized by four distinct seasons. The annual precipitation at the study site is around 900 mm, the average annual evaporation is 1604.2 mm, the sunshine duration is 2252.5 h, and the percentage of sunshine is 51%, with a 222 frost-free-day period. The soil was identified as lime concretion black soil. In the 0–50 cm soil layer, the pH was 6.87; the content of organic matter was 10.46 g/kg; the total N was 1.65 g/kg; the available N (NH_4^+-N + NO_3^--N) was 1.79 mg/kg; the available phosphorus (AP) was 4.44 mg/kg; and the K, calcium (Ca), and magnesium (Mg) concentrations were 132.74, 15.77, and 76.69 mg/kg, respectively. Two-year-old (2a) *S. integra* cv. *qingpi* stands with uniform growth status were selected for the fertilization trial. The test fertilizers consisted of urea containing 47% nitrogen and superphosphate containing 12% P_2O_5. Six treatment levels for N and P (*Table 1*) were applied once in April to 2 m×2 m randomized plots and replicated in three blocks. The unfertilized plots (CK) were similarly treated with fresh water as a control. Other management procedures were the same as for typical productive young forests in the area.

Table 1. *Fertilization treatments for the field experiment*

	Fertilization level (g·m^{-2})						
	CK	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
N	0	30	20	30	45	60	0
P	0	0	15	15	15	15	15

CK is the control without fertilizer application.

Harvesting and measurements

The shoot height was documented with a precision of 0.1 cm using a measuring tape, while the base diameter was gauged twice by a Vernier caliper accurate to 0.01 cm, and the average of the two measurements was calculated. The leaves, stems, roots, and root stumps were collected and then oven-dried at 60°C until a constant weight was achieved. Subsequently, the dried samples were weighed using electronic scales to ascertain their dry weights, and from these measurements, the biomasses were calculated (Farquhar and Sharkey, 1982).

Using an open gas exchange system (the Li-6400 Photosynthesis System from LI-COR, Lincoln, NE, USA), the photosynthetic properties of the various treatment groups were assessed on a sunny July day between 9:00 and 13:00, under natural light levels ranging from 1000 to 1500 μmol·m^{-2}·S^{-1}. The system underwent calibration before each gas exchange measurement, following the manufacturer’s guidelines. During the measurements, the air temperature inside the leaf chamber was kept at 30°C, while the relative humidity was maintained between 40% and 60%. Chlorophyll was extracted from the leaves using 80% acetone, and the absorbance of the solution from each sample was measured with a UV-Visible spectrophotometer. By analyzing

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

the pigment peaks, the concentrations of chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophyll (Chl (a + b)) were calculated according to the Aron revised method (Lee, 2000).

The plant samples were dried at 65–70°C to a constant weight and then weighed. The samples were then ground to pass through a 100-mesh sieve for the determination of nutrient contents. The N concentration was determined by the Kjeldahl method (Magomya et al., 2014); the P concentration was determined using the molybdenum-antimony colorimetric method (Zhang et al., 2020); and the K, Ca, and Mg concentrations were determined by the nitric acid-perchloric acid digestion followed by the atomic absorption spectrophotometer method (Ishikawa et al., 2018). Soil profiles were obtained for 0–10 and 10–20 cm layers; samples were air-dried and ground to pass through a 2 mm sieve before storage. The soil available phosphorus (AP) content was determined using the hydrochloric acid-ammonium fluoride extraction method.

Statistical analysis

All statistical analyses were conducted using Statistical Product and Service Solutions (SPSS) software provided by IBM, located in Armonk, NY, USA. Repeated measures ANOVA (Analysis of Variance) was employed for evaluation. The strength of the associations between growth and physiological parameters was analyzed through Pearson's correlation coefficient tests. Hypotheses were tested at a 5% significance level ($P < 0.05$).

Results

Growth and biomass

The mean base diameter (MBD) and mean height (MH) were 0.85–1.05 cm and 184.19–217.12 cm, respectively, for the six treatments and the control before fertilization, showing no significant differences (*Table 2*). After fertilization, however, the base diameter and shoot height of *S. integra* responded differently to the fertilizer treatments. For the mean base diameter, T2 and T3 exhibited a marginally enhanced base diameter relative to T1 and CK; T4 demonstrated a considerable 1.09-fold increase compared to T3 but was not significantly different from T5 and T6; and the mean base diameter of T6 was 1.13-fold greater than that of CK. In terms of shoot height, all fertilizer treatments resulted in evident improvements relative to CK. The mean height did not differ significantly among T1, T2, T3, T4, and T6; however, T5 exhibited a significant increase compared to the other treatments. The base diameter increment of CK was 32.25%, which was higher than in T1, T2, T3, and T4, but lower than in T5 and T6. This trend was consistent with the shooting height, where only T5 and T6 showed greater increases than CK (45.17%) (*Table 2*).

The biomass of the *S. integra* foliage, stem, root, and root stump samples responded differently to fertilization (*Table 3; Fig. 1*). The ratio of aboveground biomass to total biomass was 79.62%–82.15%, and the organ biomass was ranked as stem > root stump > leaf > root. The total biomass was ranked as T1 ≈ T2 ≈ T3 ≈ T6 < T4 < T5. T5 yielded the maximum stem (28.68 $t \cdot hm^{-2}$), root (1.40 $t \cdot hm^{-2}$), root stump (5.32 $t \cdot hm^{-2}$), aboveground (30.92 $t \cdot hm^{-2}$), and total (37.64 $t \cdot hm^{-2}$) biomasses. In addition, the root biomass of T4 (1.41 $t \cdot hm^{-2}$) was close to that of T5. In contrast to T5, which was

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

beneficial for stem and overall growth, T4 increased the foliar biomass the most (3.97 t·hm^{-2}) among all treatments (*Fig. 1*). Interestingly, the CK stands had the smallest stem, root, root stump, aboveground (i.e., leaves + stems), and total biomasses, but exhibited a significantly larger foliar biomass than T1, T2, T5, and T6. Therefore, the fertilizer combinations evidently promoted biomass accumulation in the stems, roots, and root stumps as well as the aboveground organs and total biomass.

Table 2. *Mean base diameter and shoot height of S. integra under different fertilization treatments*

Treatment	Before fertilization		After fertilization		Increment	
	Mean base diameter (cm)	Mean height (cm)	Mean base diameter (cm)	Mean height (cm)	Base diameter (%)	Height (%)
T1	0.85 (0.2)a	217.12 (32)a	1.12 (3.76)a	302.27 (55.78)a	31.76	39.22
T2	0.97 (0.19)a	211.69 (33)a	1.16 (3.20)b	299.39 (48.37)a	19.59	41.43
T3	0.95 (0.26)a	215.74 (33)a	1.17 (3.12)b	284.63 (57.40)a	23.16	31.93
T4	1.05 (0.19)a	204.32 (30)a	1.27 (3.36)c	295.18 (55.57)a	20.95	44.47
T5	0.89 (0.24)a	185.20(45)a	1.20 (3.23)c	278.98 (56.22)b	34.83	50.63
T6	0.90(0.25)a	203.67 (43)a	1.26 (3.00)c	298.76 (53.45)a	40	46.69
CK	0.93 (0.24)a	184.19 (56)a	1.12 (3.38)a	267.40 (57.28)c	32.25	45.17

Values in parentheses represent the standard deviation; different letters in each column indicate a significant difference at $P < 0.05$ among the treatments; n = 6

Table 3. *Effects of different fertilization treatments on S. integra biomass*

Treatment	Leaf (t·hm^{-2})	Stem (t·hm^{-2})	Root (t·hm^{-2})	Root stump (t·hm^{-2})	Aboveground (t·hm^{-2})	Total (t·hm^{-2})
T1	2.09 (0.88)a	15.20 (2.94)a	1.13 (0.11)a	4.17 (0.25)a	17.29a	22.67a
T2	2.07 (0.16)a	15.02 (1.60)a	1.32 (0.14)b	3.34 (0.31)b	17.09a	21.76a
T3	2.67 (0.42)d	15.02 (3.49)a	1.17 (0.14)a	5.09 (0.56)c	17.69a	22.95a
T4	3.97 (1.02)b	20.00 (4.88)b	1.41 (0.11)b	4.81 (0.42)c	23.97b	30.20b
T5	2.24 (0.65)c	28.68 (1.70)c	1.40 (0.09)b	5.32 (0.18)c	30.92c	37.64c
T6	2.21 (0.63)c	15.14 (6.83)a	1.38 (0.12)b	4.23 (0.21)a	17.35a	22.96a
CK	2.45 (0.26)d	13.81 (6.02)d	0.97 (0.05)c	3.19 (0.22)b	16.26d	20.42d

Values in parentheses represent the standard deviation; different letters in each column indicate a significant difference at $P < 0.05$ among the treatments

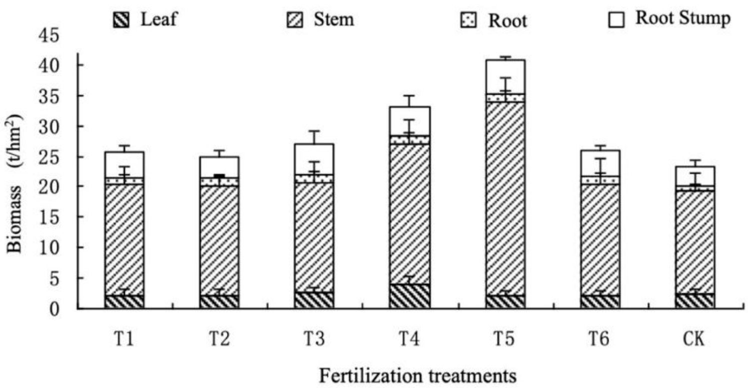

Figure 1. *Effects of fertilization on the biomass distribution of Salix integra stands. The values presented are the average plus or minus the standard deviation. An asterisk (*) denotes statistical significance with a P-value less than 0.05*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

Physiological responses

Pn (net photosynthetic rate), Gs (stomatal conductance), Ci (intercellular CO_2 concentration), and Tr (transpiration rate) are key photosynthetic properties (Farquhar and Sharkey, 1982), differ depending on the heredity of forest trees as well as the environmental conditions (Roháček, 2002). The fertilizer treatment significantly affected the net photosynthetic rate of *S. integra* (*Table 4*). T3 (13.69 $\mu mol \cdot m^{-2} \cdot s^{-1}$) displayed the maximal Pn, followed by T4 and T5, while CK showed the minimal Pn of 8.58 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which did not significantly differ from T1, T2, or T6. Similarly, T3 resulted in the highest G_s in the *S. integra* leaves, which was followed by T5, whereas no significant differences in G_s were observed among CK, T1, T4, and T6 (*Table 4*). Moreover, C_i was highest in T2 and was 9.83% higher than in CK. The C_i of T3, T4, and T5 did not differ evidently from CK, but was significantly greater than in T1 and T6 (*Table 4*). T_r also responded differentially to the fertilization treatments, with T3 resulting in the maximal T_r of 6.76 $mmol \cdot m^{-2} \cdot s^{-1}$. Except for T6, T_r under all fertilizer treatments was markedly higher than that of CK, exhibiting an increase of 19.76–59.06% (*Table 4*).

Table 4. *Effects of fertilization treatments on the photosynthetic properties of S. integra*

Treatment	Net photosynthetic rate (Pn) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance (Gs) ($mmol \cdot m^{-2} \cdot s^{-1}$)	Intercellular CO_2 concentration (Ci) ($\mu mol \cdot m^{-2} \cdot s^{-1)}$)	Transpiration rate (Tr) ($mmol \cdot m^{-2} \cdot s^{-1}$)
T1	9.76 (1.33)a	0.12 (0.02)a	227.44 (13.36)a	5.09 (0.58)a
T2	9.72 (2.47)a	0.13 (0.04)cd	260.45 (21.71)b	5.67 (1.20)b
T3	13.69 (2.73)b	0.16 (0.04)e	241.98 (17.95)bc	6.76 (1.39)c
T4	12.37 (2.92)c	0.12 (0.02)ac	223.44 (27.63)ad	5.23 (0.86)ab
T5	11.34 (2.87)c	0.14 (0.04)de	241.06 (22.00)cd	5.76 (1.31)b
T6	10.23 (2.88)a	0.10 (0.03)ab	219.80 (44.25)a	4.34 (0.95)d
CK	8.58 (1.28)a	0.10 (0.02)ab	237.12 (15.05)cd	4.25 (1.23)d

Values in parentheses represent the standard deviation; in each column, different letters signify statistically significant differences among treatments at a P-value less than 0.05

The chlorophyll content (Chl) and specific leaf weight (SLW) were both considerably influenced by the tested fertilization regimes, with the highest values detected in T5 and the lowest values recorded in CK (*Table 5*). The fold change in the chlorophyll content was 1.32, 1.45, 1.43, 1.44, 1.50, and 1.20 for T1 to T6, respectively, compared to CK. The SLW of CK was equal to 93.07% of T1 and 91.78% of T2, and the fold increase relative to CK was 1.12, 1.37, 1.43, and 1.60 for T2, T3, T4, and T5, respectively (*Table 5*).

Table 5. *Effects of different fertilizations on the chlorophyll content and specific leaf weight of S. integra*

Treatment	Chlorophyll ($mg \cdot g^{-1}$)		Specific leaf weight ($g \cdot cm^{-2}$)	
	After fertilization	Compared to CK (fold)	After fertilization	Compared to CK (fold)
T1	2.79 (0.09)a	1.32	121.14 (15.35)a	1.07
T2	3.07 (0.19)b	1.45	126.74 (13.44)a	1.12
T3	3.03 (0.23)b	1.43	154.99 (13.41)c	1.37
T4	3.05 (0.10)b	1.44	161.06 (15.68)c	1.43
T5	3.17 (0.04)c	1.50	180.95 (24.31)d	1.60
T6	2.54 (0.08)a	1.20	122.85 (18.96)a	1.09
CK	2.12 (0.11)d	1.00	112.75 (16.08)c	1.00

Values in parentheses represent the standard deviation; distinct letters within each column denote significant variations among treatments at a *P*-value of less than 0.05

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

The correlation relationships between the parameters of *S. integra* growth and the physiological parameters are shown in *Table 6*. The shoot height and base diameter were positively and significantly correlated under the tested fertilization regimes ($P < 0.05$). P_n had positive associations with T_r and G_s ($P < 0.05$), with correlation coefficients of 0.77 and 0.76, respectively. G_s was positively and strongly correlated with T_r ($P < 0.01$), with a correlation coefficient of 0.99.

Table 6. *Pearson's correlation coefficients between the growth and physiological parameters*

Correlation coefficient	BD	SH	Pn	Gs	Ci	Chl	LA	Tr	AB
BD	1.00	0.83*	0.46	−0.11	−0.49	0.26	0.29	−0.07	0.44
SH		1.00	0.11	−0.03	−0.18	0.19	0.28	0.05	−0.18
Pn			1.00	0.76*	−0.07	0.39	0.61	0.77*	0.38
Gs				1.00	0.50	0.54	0.23	0.99**	0.32
Ci					1.00	0.04	0.04	0.51	−0.04
Chl						1.00	0.54	0.17	0.61
LA							1.00	−0.41	0.39
Tr								1.00	0.26
AB									1.00

Base diameter (BD), shoot height (SH), net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), chlorophyll content (Chl), leaf area (LA), transpiration rate (T_r), aboveground biomass (AB). Asterisks denote significant relationship. *, $P < 0.05$; **, $P < 0.01$

Nutritional properties

Table 7 shows the nutrient concentrations of the *S. integra* leaves under the different treatments. The foliar C concentration was greatest in T5 but lowest in T6, and the fold increase in foliar C concentration compared to CK was 1.12, 1.13, 1.16, 1.17, 1.18, and 0.98 for T1 to T6, respectively. The foliar N and P concentration displayed similar trends as C, with T5 resulting in the highest concentrations, with the lowest concentrations detected in CK. The fold increase in foliar N relative to CK was 1.16, 1.29, 1.32, 1.39, 1.39, and 1.07 for T1 to T6, respectively. For foliar P concentration, the fold increase compared to CK was 1.02, 1.10, 1.06, 1.11, 1.13, and 1.02 for T1 to T6, respectively (*Table 7*). The effect of fertilization on the nutrient concentrations in the *S. integra* stems is shown in *Table 8*. T5 yielded the highest C and N concentrations in the stem, while CK exhibited the lowest C concentration and T6 obtained the lowest N concentration. The P concentration in the stem was found to be highest in T5 and T6 and lowest in CK and T1 (*Table 8*). The responses of nutrient concentrations in the *S. integra* roots to the tested fertilizers are shown in *Table 9*. The C concentration in the roots was found to be greatest under the T6 treatment and lowest under CK or T1. For N concentration in the roots, T3 obtained the highest value, while T1 resulted in the lowest value, and T5 fell in-between and differed significantly from the other treatments. The impact of different fertilizations on the nutrient concentrations in the *S. integra* root stumps is displayed in *Table 10*. The concentrations of both C and N in the root stumps were greatest in T5. T2 was associated with the lowest C, and T1 led to the lowest N concentration in the root stumps. There was no clear trend in P concentration in the roots and root stumps under the tested regimes (*Tables 9* and *10*). No apparent

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

regularity was observed for K, Ca, and Mg in the leaves, stems, roots, and root stumps of *S. integra* under the tested conditions (*Tables 7–10*).

Table 7. *Nutrient concentrations in the leaves of S. integra under different treatments*

Leaf	C (g·kg^{-1})	N (g·kg^{-1})	P (g·kg^{-1})	K (g·kg^{-1})	Ca (g·kg^{-1})	Mg (g·kg^{-1})
T1	366.32 (51.47)a	36.27 (5.97)a	1.17 (0.22)a	30.75 (0.74)a	5.53 (1.12)a	1.79 (0.55)a
T2	369.62 (30.40)a	40.46 (4.16)b	1.27 (0.65)b	31.08 (1.09)a	3.51 (0.84)b	1.73 (0.33)a
T3	379.73 (28.79)b	41.44 (3.87)b	1.22 (0.15)b	31.82 (2.93)a	3.51 (0.64)b	1.89 (0.95)b
T4	383.38 (43.15)b	43.40 (4.99)c	1.28 (0.15)b	31.39 (1.22)a	6.54 (1.08)c	1.80 (0.85)a
T5	386.45 (51.23)b	43.67 (3.27)c	1.30 (0.25)b	31.50 (1.68)a	8.50 (1.13)c	1.91 (0.17)b
T6	318.86 (43.71)c	33.40 (7.89)d	1.17 (0.44)a	31.72 (3.50)a	7.52 (2.03)c	1.62 (0.71)c
CK	326.53 (60.93)c	31.32 (5.90)b	1.15 (0.04)a	30.84 (1.69)a	5.22 (1.10)a	1.98 (0.02)d

Values in parentheses represent the standard deviation; within each column, varying letters represent statistically significant differences at $P < 0.05$ between the treatments

Table 8. *Nutrient concentrations in the stem of S. integra under different treatments*

Stem	C (g·kg^{-1})	N (g·kg^{-1})	P (g·kg^{-1})	K (g·kg^{-1})	Ca (g·kg^{-1})	Mg (g·kg^{-1})
T1	423.64 (52.33)a	27.13 (5.91)a	0.41 (0.07)a	4.83 (3.30)a	277.28 (4.37)a	13.94 (2.67)a
T2	430.78 (59.95)a	26.69 (5.78)a	0.51 (0.01)b	5.35 (3.58)b	276.18 (26.87)a	11.31 (1.75)b
T3	434.85 (58.53)a	27.55 (4.87)a	0.59 (0.07)b	5.03 (2.86)c	312.59 (36.16)b	11.99 (1.43)b
T4	449.06 (51.91)b	28.54 (5.37)a	0.48 (0.08)b	3.88 (2.26)d	315.44 (13.94)b	12.09 (2.35)b
T5	458.09 (39.15)c	30.58 (4.21)b	0.59 (0.11)b	4.85 (3.23)a	248.99 (29.60)c	10.83 (0.44)c
T6	425.03 (49.21)a	26.59 (3.87)a	0.41 (0.02)a	5.94 (2.41)e	286.27 (31.09)a	9.95 (0.67)c
CK	402.19 (57.30)d	27.41 (2.91)a	0.41 (0.01)a	6.19 (2.54)f	294.60 (39.90)d	11.12 (1.02)b

Values in parentheses represent the standard deviation; in each column, different letters mark a significant difference at the $P < 0.05$ level among the various treatments

Table 9. *Nutrient concentrations in the roots of S. integra under different treatments*

Root	C (g·kg^{-1})	N (g·kg^{-1})	P (g·kg^{-1})	K (g·kg^{-1})	Ca (g·kg^{-1})	Mg (g·kg^{-1})
T1	470.13 (82.52)a	20.35 (2.86)a	1.14 (0.02)a	13.29 (2.11)a	11.58 (1.08)a	49.80 (6.66)a
T2	489.27 (86.72)b	21.26 (2.86)b	1.02 (0.12)b	14.14 (3.14)b	11.55 (2.34)a	49.80 (7.68)a
T3	510.76 (77.53)c	24.88 (2.97)c	1.31 (0.23)c	13.29 (2.78)a	10.85 (3.42)b	50.96 (8.72)b
T4	482.44 (76.47)b	24.72 (2.93)c	1.21 (0.04)d	12.74 (2.56)c	11.60 (1.56)a	46.88 (6.33)c
T5	508.47 (76.08)c	22.58 (3.04)d	1.46 (0.03)e	14.64 (3.83)b	11.88 (2.68)a	52.13 (8.92)d
T6	511.87 (85.17)c	21.25 (2.86)a	1.48 (0.10)e	14.39 (4.12)b	13.00 (3.22)c	43.67 (5.43)c
CK	470.13 (82.52)a	21.66 (2.86)a	1.21 (0.24)d	15.48 (2.56)d	12.88 (2.38)c	53.59 (6.81)d

Values in parentheses represent the standard deviation; in each column, distinct letters signify a statistically significant difference at $P < 0.05$ among the treatments

Table 10. *Nutrient concentrations in the root stump of S. integra under different treatments*

Root stump	C (g·kg^{-1})	N (g·kg^{-1})	P (g·kg^{-1})	K (g·kg^{-1})	Ca (g·kg^{-1})	Mg (g·kg^{-1})
T1	527.67 (81.45)a	20.68 (2.91)a	0.99 (0.01)a	8.04 (2.15)a	10.04 (2.22)a	24.72 (6.29)a
T2	527.06 (69.90)b	20.71 (3.04)a	1.06 (0.20)b	9.54 (3.55)b	10.56 (1.48)b	36.38 (5.38)b
T3	542.70 (72.97)c	20.82 (3.55)a	1.27 (0.38)c	7.89 (2.36)c	10.71 (2.67)b	25.01 (4.41)a
T4	535.28 (68.92)c	20.52 (3.53)a	1.21 (0.28)c	8.39 (1.21)d	11.09 (3.11)c	26.76 (3.46)c
T5	582.55 (73.12)d	21.25 (3.04)b	1.38 (0.14)d	8.29 (2.67)d	10.68 (2.56)b	25.60 (5.65)a
T6	536.22 (81.45)a	21.05 (3.09)b	1.26 (0.28)c	10.09 (3.99)e	11.60 (3.32)d	32.59 (6.11)d
CK	527.67 (66.87)a	20.99 (2.89)b	1.20 (0.77)e	10.54 (2.68)e	11.27 (4.13)e	27.64 (3.89)c

Values in parentheses represent the standard deviation; within each column, different letters denote a significant difference at $P < 0.05$ among the treatments

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DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

The nutrient accumulation in different components of the *S. integra* stands under the tested fertilization regimes is indicated in *Table A1*. T4 resulted in the highest C, N, P, K, Ca, and Mg accumulation in the foliage compared to the other treatments. In contrast, in the stem, root, and root stump of the *S. integra* stands, T5 resulted in the largest C and P content. The most remarkable difference was observed in the stem and root stump, where C or P accumulation was almost doubled in T5 relative to CK. The P allocation in the stem and root stump of *S. integra* responded significantly to the tested combinations, where there was a trend of enrichment with increased fertilizer amount, and the interaction between N and P fertilizers was significant ($P < 0.05$). Additionally, T5 led to the highest N accumulation in the stem (2.10-fold of CK) and root stump (1.69-fold of CK). The K accumulation was also superior in T5, and the fold increase was 1.47, 1.36, and 1.31 relative to CK, respectively, in stem, root, and root stump. The N and K distribution in different organs of *S. integra* responded differentially to the treatments, which may result from the interaction between fertilizer type and amount. The multiple comparison results revealed that fertilizer type, fertilizer amount, and their interaction had a significant impact on Ca accumulation in the leaf and stem ($P < 0.05$). Moreover, Mg allocation in the stem and root stump of *S. integra* was found to respond more greatly to the treatments than in other organs.

Soil-available nutrients

According to *Figure 2*, the soil concentrations of NH_4^+-N, NO_3^--N, and available phosphorus (AP) did not significantly differ in the *S. integra* stands before the fertilization treatments. The concentration of NH_4^+-N at the outset was 0.75–1.35 mg·kg^{-1} in the 0–10 cm soil layer and 0.19–0.36 mg·kg^{-1} at the depth of 10–20 cm (*Fig. 2a, b*). These concentrations increased remarkably after the first fertilization, with T5 showing the maximum and CK displaying the minimum. This trend was consistent after the second fertilization, with NH_4^+-N accumulating further. The concentration of NO_3^--N at the outset was 1.36–2.51 mg·kg^{-1} in the 0–10 cm soil layer and 1.00–2.35 mg·kg^{-1} in the 10–20 cm layer (*Fig. 2c, d*). The NO_3^--N concentrations for the different treatments after the first fertilization were ranked as T5 > T3 > T4 > T2 > T1 > CK > T6, and a similar pattern was observed after the second fertilization: T5 > T4 > T3 > T1 > T2 > T6 > CK. The concentrations of AP in the 0–20 cm soil layer after the first fertilization were ranked as T1 < CK < T2 < T3 < T4 < T6 < T5; this order was mostly the same after the second fertilization, where T1 and CK exerted less effect than the other five treatments (*Fig. 2e, f*).

Discussion

Effect of fertilization on S. integra growth

In field experiments, the base diameter and height are commonly employed to ascertain the impact of fertilizer on plant growth and performance (Bolstad and Allen, 1987). In addition, community biomass can reflect the levels of community productivity and is the basis for studying material cycling in forest ecosystems. Measuring tree species biomass is important for evaluating and improving forest management. In the present study, combined fertilizer application resulted in remarkable increases in volume and biomass in young *S. integra* forest (*Tables 2* and *3; Fig. 1*). Mean base diameter and shoot height improved as the N amount was increased in the trial, indicating that the demand for N had not been saturated in these stands when combined

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DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

with 15 $g \cdot m^{-2}$ P fertilizer. This is in agreement with a previous report on *S. integra* (Song et al., 2009), which observed substantial productivity improvements by fertilization in late May to mid-June, with a 30–40% increase using 300 kg per hectare ammonium nitrate. Our findings are also in line with a field fertilization experiment in Boxing County on a 2a *Salix suchowensis* Cheng cv. *hongpi* stand at a density of 5 cm × 25 cm (Wang et al., 2007) supplemented with 50 kg NPK fertilizer (the ratio of urea:P_2O_5:KCl was 3:2:1), 30 kg N (urea with 46% N), 30 kg P (superphosphate with 16% P_2O_5), and 15 kg K (KCl with 50% K_2O). The results indicated that NPK, N, P, or K fertilizer could obviously promote growth, with yield increases of up to 155%, 132%, 118.8%, and 108.4%, respectively. Furthermore, a previous comparative trial (Li et al., 2006) suggested that the combination of urea and diammonium phosphate at a ratio of 1:1 exerted the greatest influence on *S. integra* yield (an increment of 36%), followed by urea only (an increment of 29%). Similarly, research on a 1a *Salix fragilis* plantation demonstrated that the suitable fertilizer application amount for N, P, and K was 133.82–148.69 g, 57.13–71.42 g, and 41.22–66.76 g per seedling, respectively. Moreover, the N:P:K ratio for optimal height, breast diameter, and volume growth was 2:1:1, 2.8:1.1:1, and 3.4:1.7:1, respectively (Li, 2012).

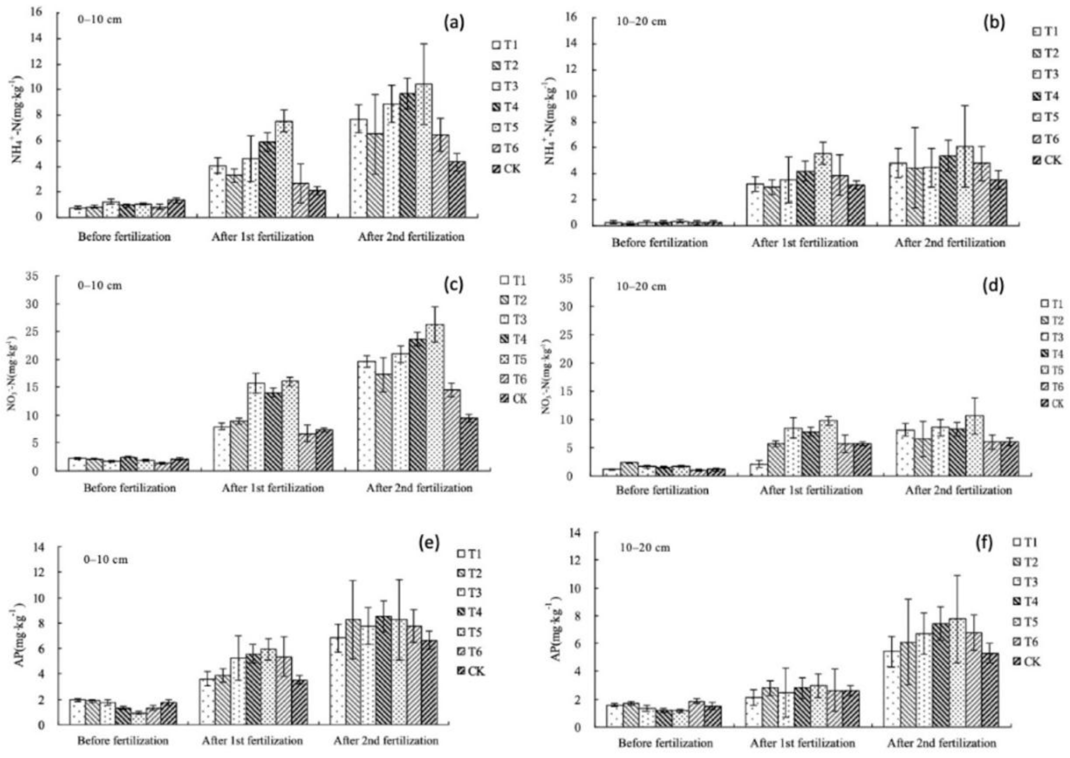

Figure 2. *Effects of fertilization on soil NH_4^+-N, NO_3^--N, and available phosphorus (AP) in Salix integra stands. The values shown are the mean accompanied by the standard deviation. A star (*) signifies a statistically significant difference with a P-value below 0.05*

The growth responses of tree species to nutrient availability can vary widely, ranging from growth inhibition due to nutrient deficiency, through optimal growth within an ideal nutrient range, to a state of luxury consumption where additional nutrients no longer enhance growth, and finally to reduced growth caused by toxic nutrient

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DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

concentrations when levels become excessively high (Chapman, 1973; Larcher, 2003). The nutrient range of the fertilization treatments assessed in the present study suggested that T5 had the approximated optimal nutrient input for desirable *S. integra* growth. However, a previous investigation suggested an optimal N amount of 30 kg per 667 m^2, while excessive N fertilizer restrained the growth of 2a *S. integra* cv. hongpi (Wang et al., 2007). Urea fertilizer was applied at N1 15 kg, N2 22.5 kg, N3 30 kg, N4 37.5 kg, and N5 45 kg per 667 m2, which generated 120.6%, 129.0%, 135.7%, 135.0%, and 134.0% more yield relative to the control. The productivity peaked at N3 and declined with higher quantity, which may be caused by toxicity from disproportionate soluble salts beyond a certain limit. Wider ranges of nutrient availability may need to be tested to quantify fertilization more accurately under varying conditions at a specific site, in particular in relation to climatic or soil characteristics. Under medium to poor soil fertility with a lower content of N, P, and organic matter in our study, the rational maximization of N might be an approach for enhancing the growth performance of young *S. integra*.

Effect of fertilization on the photosynthesis of S. integra

Photosynthesis is the basis for plant growth and development and is a decisive factor for productivity (Heilman and Fu-Guang, 1994; Xu, 2001). The addition of fertilizers may facilitate high photochemical performance in *S. integra* forest restoration due to the importance of nutrients in physiological processes. Recent studies have demonstrated that nitrogen (N) enhances the photosynthetic carbon fixation capacity and facilitates the accumulation of biomass in roots, stems, leaves, and overall. (Bulgarelli et al., 2017; Dong et al., 2018). Although increased fertilization may benefit seedling growth, negative effects could result from carbohydrate depletion due to increased respiration. Pn is a sensitive photosynthetic indicator and was significantly improved by the T3, T4, and T5 treatments. T3 had the greatest impact on Gs, followed by T5. Intercellular CO_2 in the leaves serves as the photosynthetic substrate, and T2 was found to result in the highest Ci, while T1 and T6 had only marginal effects. Transpiration rate was closely related to stomatal conductance, and T3 led to the largest Tr, while T6 showed no significant difference compared to the control, indicating that N or N + P fertilizer had a positive influence on Tr, whereas P fertilizer alone had no impact. According to *Table 5*, fertilization remarkably elevated the chlorophyll content and specific leaf weight. While fertilizers containing only N or P did not show obvious impacts ($P > 0.05$), the beneficial effects on chlorophyll content and specific leaf weight improved as the N amount increased during the simultaneous application of N and P fertilizers. This finding is consistent with the observations in a two-factor experiment for fertilizer type and amount, which suggested that 225 $kg \cdot hm^{-2}$ of urea produced the largest stem and aboveground biomasses in *S. integra*, while 75 $kg \cdot hm^{-2}$ ammonium phosphate resulted in the highest Pn (Liu, 2011).

The accumulative attributes of nutritional elements in S. integra

The mineral nutrition status in plants is a direct reflection of the supply capacity of soil nutrients, and nutrient analysis of tissues represents a reliable way to ascertain the amounts or even seasonal patterns of plant nutrient uptake (Teixeira et al., 2002). Nutrient requirements depend on the nutrients needed for new tissue production as well as the nutrient quantity reallocated from existing plant tissues. In more competitive

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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environments, nutrients can be retranslocated from older tissues to actively growing tissues. Nutrient reallocation can occur within plants, such as from storage tissues (e.g., woody stem) in early spring and from senescing leaves in autumn, or externally from decomposition such as senesced roots and pruned wood. The boost of nutrients by fertilizer addition directly impacted the concentration allocation of nutrients (*Table 7–10*) and mainly affected the foliar concentrations of C, N, and P in *S. integra*. Although fertilization with N or P alone did not result in a considerably different effect ($P > 0.05$), during the simultaneous supplementation of N and P, the foliage C and N increased as the amount of N increased, while the P concentration did not show much change. The application of N fertilizer has been reported to result in increased growth and foliar N levels in many studies (Neilsen et al., 1992; Sayer et al., 2004; Van Den Driessche et al., 2008). However, the nutritional responses of *S. integra* to various fertilization rates have not been well examined, and further investigations on the poplars and willows of the Salicaceae family are thus needed. The N application rate was reported to be the main limiting factor for poplar plantation growth (Heilman et al., 1990). A nutrient experiment in *Populus deltoides* × *Populus balsamifera* and *Populus deltoids* × *Populus petrowskyana* demonstrated a significant correlation of height and diameter growth with foliage P and S content (Brown and Driessche, 2002). In addition, nutrition research on the 2a *Populus deltoides* “luxi-69” indicated that 4.937 kg N, 0.593 kg P, 0.015 kg K, 0.017 kg Ca, and 0.004 kg Mg were absorbed for the production of 1 t wood, and the nutrient distribution, except for P in the organs, was leaf > bark > trunk > branch (Zhu et al., 1995). Research has shown that applying phosphorus fertilizer can alter how nutrients are distributed among plant parts, possibly restricting the buildup of root biomass (Lin et al., 2016). In the present study, greater growth performance was induced by the highest N rate and increased nutrition reserves in most organs except the leaves. Inconclusive performance across studies suggests that comprehensive field experiments may help determine rational fertilization requirements for meeting a particular site condition. K, Ca, and Mg are usually abundant in the low-lying beach stands subjected to occasional flooding; and N and P are required in large quantities to supply organic matter in sandy soils along the Huaihe River in order to fulfil nutrient demands (Liu, 2011).

Effect of fertilization on soil-available nutrients in S. integra stands

The taproot of *S. integra* has the potential to extend to a depth of 100 cm, whereas its lateral roots are predominantly spread across the 0-40 cm soil stratum. The soil concentrations of NH_4^+-N, NO_3^--N, and AP exhibited a remarkable increase after fertilization treatment, and the amount was higher in the 0–10 cm soil layer than the 10–20 cm layer (*Fig. 2*). Additionally, T5 resulted in the highest NH_4^+-N and NO_3^--N concentrations. In contrast, soil AP was not influenced by single N fertilizer application, and the increased N gradients from T1 to T5 in the N + P combinations did not have much effect on AP, suggesting that N addition promoted *S. integra* growth as well as the utilization and absorption of P from soils (Goodale et al., 2000; Lilleskov et al., 2002). The economics of fertilization will depend on the capacity to obtain sufficient N into the ecosystem in a form that provides adequate mineralization to supply forests, and the amount the soil can hold varies with the soil characteristics. Although excessive N and P can increase productivity, excessive fertilizer may increase the production of root exudates and litter, as well as microbial populations and decomposition processes (Bremer and Kuikman, 1997). Furthermore, chemical fertilizers may also have negative

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

environmental impacts, such as soil acidification, nutrient runoff, and waterway pollution. Therefore, minimizing these tradeoffs relies on the efficient application of fertilizer inputs. Effective fertilizer management requires a good understanding of plant biological characteristics and nutritional demands, in terms of both the nutrient amount and the timing in which each nutrient is needed most. The sprout growth of *S. integra* starts before Qingming (early April), and the peak growth period is between May and July, when the average height increment can reach 1.9 m. The development of height and base diameter stops around the autumnal equinox. The growing speed of *S. integra* differs according to the cultivar, site conditions, as well as management. Supplying sufficient nutrient load to young seedlings during the early exponential growth stage may offer a means of realizing *S. integra* nutrient demands more precisely.

Conclusions

The promotion of the *S. integra* enterprise can improve land resource utilization, maintain the ecological balance, and enhance the sustainable development of regional economy. Appropriate combinations of N and P fertilizer can enhance plant growth and biomass accumulation, as well as maximize soil quality. This study investigated various fertilization combinations to ascertain the best application rates of nitrogen (N) and phosphorus (P) for enhancing plant growth. and nutrient utilization in young *S. integra* forest development. Our results showed that the tested regime exerted substantial and beneficial effects on base diameter, shoot height, and photosynthesis. T5 resulted in the greatest stem, root, and root stump biomasses. The total biomass under the different treatments was ranked as CK (20.42 $t \cdot hm^{-2}$) < T2 (21.76) < T1 (22.67) ≈ T3 (22.95) ≈ T6 (22.96) < T4 (30.20) < T5 (37.64). The different supplemental ratios also considerably impacted the distributions of C, N, P, K, Ca, and Mg among the roots, root stumps, stems, and foliage of the *S. integra* stands. There was also a significant difference in soil-available nutrients among the different fertilizer types and amounts, with T5 resulting in the highest NH_4^+-N and NO_3^--N concentrations. Therefore, fertilization with 60 g $N \cdot m^{-2}$ and 15 g $P \cdot m^{-2}$ was deduced to be the optimum nutrient input for desirable growth performance of 2a *S. integra* in the lowland areas along the Huaihe River. These findings hold practical importance in devising fertilization plans for young forests in the field and guiding the best management practices for fertilized *S. integra* plantations. Further research may focus on improving the N and P fertilization regime in order to maintain or enhance long-term soil fertility, as well as adjusting cultural approaches to agricultural practices. It is suggested that in future forest management, reasonable tillage and fertilization should be utilized to improve soil conservation and nutrient supply for the sustainable development of *S. integra* stands on soils with low organic matter content.

Funding. This work was supported by “The Key Discipline Project ‘Landscape Architecture’ of Ordinary Higher Education Institutions in Shaoxing City, Zhejiang Province”(SXSZY202412); “The young and middle-aged academic team Project of Zhejiang Shuren University”(KBY0119604); “The research project of Zhejiang Shuren University” (KXJ0218105).

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APPENDIX

Table A1. *Nutrient accumulation in different components of S. integra stands under the fertilization treatments*

	Treatment	Leaf ($kg \cdot hm^{-2}$)	Stem ($kg \cdot hm^{-2}$)	Root ($kg \cdot hm^{-2}$)	Root stump ($kg \cdot hm^{-2}$)
C	T1	765.61a	7742.87a	531.25a	2200.38a
	T2	765.11a	7764.81a	645.84b	1760.38b
	T3	1013.88b	7836.65a	597.59a	2762.34a
	T4	1522.02c	10332.42b	680.24b	2574.70a
	T5	865.65d	14513.21c	711.86c	3099.17c
	T6	704.68a	7711.74a	706.38c	2268.21a
	CK	800.00a	6762.42d	456.03d	1683.27b
N	T1	75.80a	495.86a	23.00a	86.24a
	T2	83.75b	481.09a	28.06b	69.17b
	T3	110.64c	496.49a	29.11b	105.97c
	T4	172.30d	656.68b	34.86c	98.70d
	T5	97.82b	968.84c	31.61c	113.05c
	T6	73.81a	482.45a	29.33b	89.04a
	CK	76.73a	460.87a	21.01a	66.96b
P	T1	2.87a	9.38a	1.28a	4.14a
	T2	2.42a	9.17a	1.35a	3.53a
	T3	3.79b	10.56b	1.53b	6.47b
	T4	4.69c	10.99b	1.71b	5.84b
	T5	2.91a	18.84c	2.04c	7.35c
	T6	2.78a	10.74b	2.04c	5.33b
	CK	3.80b	10.17b	1.18a	3.82d

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DOI: http://dx.doi.org/10.15666/aeer/2302_19992015

K	T1	72.63a	88.28a	15.02a	33.53a
	T2	72.62a	96.37b	18.66b	31.86a
	T3	84.96b	90.65b	15.55a	40.16b
	T4	124.61c	89.27a	17.96b	40.36b
	T5	70.57a	153.55c	20.50c	44.10c
	T6	70.10a	107.84d	19.86b	42.68d
	CK	75.55d	104.13d	15.02a	33.62a
Ca	T1	0.22a	5067.85a	13.08a	41.86a
	T2	0.14b	4978.20a	15.24b	35.25b
	T3	0.18b	5633.28b	12.69a	54.51c
	T4	0.51c	7257.96c	16.36b	53.33c
	T5	0.39d	7888.50d	16.64b	56.82d
	T6	0.10b	5194.14a	17.94c	49.09c
	CK	0.36d	4953.46a	12.49a	35.95b
Mg	T1	0.09351a	0.25472a	0.05627a	0.10308a
	T2	0.08940a	0.20386b	0.06574b	0.12151b
	T3	0.12673b	0.21608b	0.05962a	0.12730b
	T4	0.17878c	0.27818a	0.06610b	0.12872b
	T5	0.10676b	0.34301c	0.07298c	0.13619c
	T6	0.08964a	0.18053d	0.06026a	0.13786c
	CK	0.12152b	0.18697d	0.05198d	0.08817d

Distinct letters in each column signify a statistically significant difference at $P < 0.05$ among the various treatments

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