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COUPLING EFFECTS OF WATER AND FERTILIZER ON THE GROWTH CHARACTERISTICS OF *CATALPA BUNGEI* SEEDLINGS

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Abstract

In pot experiments using a rotatable central composite design, the effects of soil water content and fertilizer application, and their connection to seedling stem height (SH), ground diameter (GD), biomass yield (BY), and leaf area (LA), were examined in seedlings of *Catalpa bungei* clone 004-1 using response surface methodology. Our results indicated that nitrogen application (*N*) and the soil water content (*W*) had positive effects on all four growth parameters. The size sequence of the effects was $N>W$; however, phosphorus application (*P*) exerted significantly positive effects only on BY and LA. A monofactor effects analysis indicated that changes in the four parameters with increases in *W*, *N*, and *P* were all parabolic. Moreover, the interactions of $W\times N$ were found to have significantly positive effects on the four growth parameters, which initially increased and then decreased with increasing *N* when *W* was fixed, and with increasing *W* when *N* was fixed. The interaction of $W\times P$ had a significantly positive effect on SH. The optimized combination was determined by establishing a multi-objective decision model based on the four parameters, when the actual *W*, *N*, and *P* values reached 74.605% of the field capacity, 4.710 g·plant^{-1}, and 2.009 g·plant^{-1}, respectively. Under these conditions, *C. bungei* seedlings achieved maximum growth. These recommendations will conserve water and improve production, and they provide a basic theory for seedling culture and afforestation of *C. bungei*. In addition, our model fitting results showed that the biomass yield of fine roots (diameter ≤2 mm) was closely correlated with SH and GD.

Key words: Soil water and fertilizer; Growth; *Catalpa bungei*; Root; Response surface methododogy.

Introduction

Catalpa bungei is a common high-quality timber tree species. The Chinese government plans to promote this species in suitable areas of the country; thus, it is important to determine appropriate water and fertilizer management strategies.

Soil water and fertilizer are key limiting factors for plant growth. Good soil moisture conditions and a reasonable nutrient content can boost plant growth and production (Dong *et al*., 2011; Haghjoo *et al*., 2013). Soil water is the solvent that facilitates nutrient absorption and transportation by plants. Furthermore, it has a major influence on the absorption of soil nutrients and directly affects root development (Toorchi *et al*., 2002; Christmann *et al*., 2007; Thind *et al*., 2008; Wang *et al*., 2013a). Hence, plants are generally affected by the interactions between water and fertilizer, including water and nitrogen (N) (Shangguan *et al*., 2000; Dong *et al*., 2011; Haghjoo *et al*., 2013; Hartmann *et al*., 2013; Porto *et al*., 2014) and water and phosphorus (P) (Rahbari *et al*., 2013). Significant differences in growth and production (Zhou *et al*., 2007; Dong *et al*., 2011; Porto *et al*., 2014), biomass allocation (Mao *et al*., 2013), root system growth and development (Wang *et al*., 2013a), and water and fertilizer use efficiency (Nesme *et al*., 2006; Deng *et al*., 2013; Liu *et al*., 2013; Wang *et al*., 2013b) have been documented in plants subjected to different soil water and fertilizer conditions.

Response surface methodology (RSM) can be used to design experiments, establish models, assess the significance of factors, and determine optimal conditions (Demirel and Kayan, 2012). It can also be used to conduct statistical analyses of how process variables affect the responses and to generate three-dimensional plots (Nasrollahzadeh *et al*., 2007; Dong *et al*., 2011). Rotatable central composite design (CCD) is a popular experimental design commonly used to study the relationship between plant growth and soil water or fertilizer (Dong *et al*., 2011; Lin *et al*., 2013).

C. bungei is common in warm temperate and sub-tropical regions of China due to its excellent material quality and aesthetics (Pan *et al*., 1991; Wang *et al*., 2012; Dong *et al*., 2013). In recent studies, soil moisture (Dong *et al*., 2013) and fertilizer application (Wang *et al*., 2012) have been shown to impact the growth of *C. bungei* seedlings. However, limited information is available on how soil water and fertilizer affect the growth characteristics of *C. bungei* seedlings. We addressed these questions using pot experiments and RSM based on a rotatable CCD. Recent studies have examined water and fertilizer consumption problems, including the excessive application of water and fertilizer, and water and fertilizer management of trees (Pires and Xavier, 2010; Rahbari *et al*., 2013; Porto *et al*., 2014). Many forest researchers believe that choosing suitable irrigation and fertilization strategies for seedling culture and field planting could save water and improve production (Dong *et al*., 2011; Haghjoo *et al*., 2013; Lin *et al*., 2013). The Chinese government plans to promote *C. bungei* planting on a large scale in appropriate areas; thus, information on irrigation and fertilizer application may be important. The present study examined the water and fertilizer needs of *C. bungei* seedlings and identified the optimal combination of irrigation and fertilizer application, thus providing a basic theory for seedling culture and afforestation of *C. bungei*.

Materials and Methods

Plant materials and growth conditions: Stumplings of *C. bungei* clone 004-1 were transplanted into flowerpots (one stumpling per flowerpot) in early March of 2013. The stumplings were removed from two-year-old *C. bungei* seedlings cultivated at the Xiaolongshan Forestry Science and Technology Research Institute (Tianshui, Gansu province, China; 105 º 54 ′ E, 34 º 28 ′ N) at an elevation of 1160 m. This area is a temperate zone with a semi-humid monsoon climatic region with an average annual temperature of 11^{o}C, average annual rainfall of 600–800 mm, evaporation capacity of 1290 mm, and average annual frost-free period of 180 days. The size of each flowerpot was 35 cm × 35 cm × 30 cm. Plastic pallets were placed under each flowerpot to prevent water loss and soil erosion. The soil matrix was loess:peat soil (6:4), and the weight of soil in each flowerpot was 15 kg. The soil field capacity (FC) and bulk density (BD) were measured using the core cutter method and the chemical properties of the soil were determined as described by Lu (2000). The soil property data are provided in Table 1. The average stem height (SH), ground diameter (GD), biomass yield (BY), and leaf area (LA) of the seedlings reached 31.2 cm, 5.9 mm, 64.99 g, and 1142.36 cm^2, respectively, before treatment. From March to September of 2013, the trees were planted in a greenhouse with a high arched roof and a size of 60.0 m (length) × 8.0 m (width) × 3.0 m (height). During the entire experiment the average daytime temperature was 31.6^{o}C, the average nighttime temperature was 15.8^{o}C, and the relative humidity was 35.8–65.2%.

Growth parameter measurements: SH, GD, LA, and BY were selected as parameters to evaluate plant growth. SH and GD were determined every 15 days from mid-May. When the increase in SH was zero (early September in our experiment), further determination of the SH and GD, harvesting, and determination of the LA were stopped. LA was determined using an LI-3000 meter (LI-COR Inc., Lincoln, NE, USA). Seedlings were harvested to estimate the BY using the oven-drying method of Singh and Singh (2006), and the leaves, stems, fine roots (diameter ≤2 mm), and coarse roots (diameter >2 mm) were oven-dried for 72 h at 80^{o}C. Their dry weight was calculated and recorded, respectively. In our study, the dry weight of fine roots and coarse roots represents the BY of fine roots (BY_{fr}) and coarse roots (BY_{cr}), respectively. The total root BY (BY_{tr}) was calculated as follows: $BY_{tr}=BY_{fr}+BY_{cr}$.

Experimental design: In this study, the rotatable CCD described by Dong *et al*. (2011) was used; a central composite (uniform precision) response surface design was applied using SAS software (version 9.0; SAS Institute Inc., Cary, NC, USA) with 3 factors, 5 levels, 20 runs, and 6 center points. The coded values of the five levels ranged from -1.682 to 1.682; the five levels of *W*, *N*, and *P* were applied as shown in Table 2. In this study, the five levels of *W* were designed according to the response of *C. bungei* seedlings to different severities of soil water stress as reported by Dong *et al*. (2013), and the five levels of *N* were set based on the growth performance of *C. bungei* seedlings in response to different amounts of N fertilizer, as described by Wang *et al*. (2012). The five levels of *P* were designed based on our single-factor (*P*) pilot experiment. A total of 100 seedlings subjected to 20 treatments with five replications were arranged randomly (Table 3). Our experiment started on May 15, 2013. Fertilizer was placed in holes near the root area of the seedlings; the respective actual values of N and P_2O_5 were calculated are shown in Table 2. N was applied three times (1/3N, May 15th; 1/3N, June 15th; and 1/3N, July 15th), whereas P_2O_5 and K_2O (1.5 g·plant^{-1}) were applied on May 15th. The soil water content was determined using a hand-held TDR (FOM/mts type; Easy Test Ltd., Lublin, Poland) every four days. Beginning on May 15th, the irrigation project was performed when the soil moisture level was close to the lower limit of the *W* threshold (Table 3), and the increase in the upper limit of *W* values by irrigation was defined as 100%FC. The irrigation volume (IV) was calculated using a measuring cylinder (range=1000 ml) with the following expression (Dong *et al*., 2011):

$$IV\ (ml) = [(FC-W)/BD] \times SW/\rho,$$

where FC, BD, *W*, SW, and ρ represent the field capacity, soli bulk density, soil water content, soil weight in the flowerpot, and density of water, respectively.

Table 1. Soil field capacity (FC), bulk density (BD), and chemical properties.

FC (%)	BD (g·cm^{-3})	pH	OM (g·kg^{-1})	TN (g·kg^{-1})	TP (g·kg^{-1})	TK (g·kg^{-1})	AN (mg·kg^{-1})	AP (mg·kg^{-1})	AK (mg·kg^{-1})
34.45	1.15	7.47	29.62	1.55	0.81	18.68	152.36	48.34	103.06

Note: OM, TN, TP, TK, AN, AP, and AK represent soil organic matter content, total nitrogen (N) content, total phosphorus (P) content, total potassium (K) content, available N content, available P content, and available K content, respectively

Table 2. Coded and physical values of three experimental factors.

Coded values	Soil water content (*W*, %FC)	Nitrogen application (*N*, g·plant^{-1})	Phosphorus application (*P*, g·plant^{-1})
-1.682	30	0	0
-1	40	1.2	0.6
0	55	3	1.5
1	70	4.8	2.4
1.682	80	6	3

Table 3. Experimental design matrix.

Treatments	Coded values of factors			Treatments	Coded values of factors		
	x_1	x_2	x_3		x_1	x_2	x_3
T1	-1	-1	-1	T11	0	-1.682	0
T2	-1	-1	1	T12	0	1.682	0
T3	-1	1	-1	T13	0	0	-1.682
T4	-1	1	1	T14	0	0	1.682
T5	1	-1	-1	T15	0	0	0
T6	1	-1	1	T16	0	0	0
T7	1	1	-1	T17	0	0	0
T8	1	1	1	T18	0	0	0
T9	-1.682	0	0	T19	0	0	0
T10	1.682	0	0	T20	0	0	0

Note: *W*, *N*, and *P* are represented by x_1, x_2, and x_3, respectively

Mathematical modeling and statistical analysis: The rotatable CCD can provide novel data for regression analyses by establishing linear polynomial models (Bajić *et al*., 2010). In this study, the three-factor polynomial model was of the following form:

$$y=b_0+b_1x_1+b_2x_2+b_3x_3+b_{12}x_1x_2+b_{13}x_1x_3+b_{23}x_2x_3+b_{11}x_1^2+b_{22}x_2^2+b_{33}x_3^2,$$

where y is the response, x_1, x_2, and x_3 represent the coded values of variables *W*, *N,* and *P*, respectively, and the *b* coefficients are the regression coefficients of the optimization model. Insignificant coefficients were eliminated by checking the significance (Rigas *et al*., 2000). The absolute value of the coefficients indicates the importance degree of the variable, and the function direction of the variables is given by the symbol "+" or "-" (Zhou *et al*., 2007).

In this study, three non-linear regression models were selected to fit the BY of the roots (including total roots and fine roots) with SH and GD using the algorithm of Levenberg-Marquardt and Universal Global Optimization (LM+UGO) in 1st Opt software (version 1.5; 7D-Soft High Technology Inc., Xian, China). These models, which were described by Cheng (2007), were expressed as follows:

$$\text{Lorentzian model: } B = \frac{a}{[1 + ((SH-b)/c)^2] \times [1 + ((GD-g)/h)^2]}$$

$$\text{Paraboloid model: } B=a+b \cdot SH + c \cdot GD + g \cdot SH^2+h \cdot GD^2;$$

$$\text{Caussian model: } B = a \cdot e^{-0.5} [((SH-b)/c)^2 + ((GD-g)/h^2]$$

where B contains BY_{tr} and BY_{fr} and *a*, *b*, *c*, *g*, and *h* represent the regression coefficients of the models. The root mean square error (RMSE) was calculated to evaluate the prediction accuracy of the models (smaller RMSE values indicate greater prediction accuracy).

All data are shown as the means ± standard deviation of five replications. The significance of SH, GD, BY, and LA were examined using a one-way analysis of variance (ANOVA). A multiple non-linear regression analysis was used to construct polynomial models using the R^2 test for ANOVA. The significance of the regression coefficients was checked by an ANOVA and *F*-test. Probability (*p*) values <0.05 were considered statistically significant. All statistical analyses were performed using SAS software (version 9.0; SAS Institute Inc.). The regression models were optimized using LINGO software (version 11.0; LINDO SYSTEMS Inc., Chicago, IL, USA). Figures were generated using Sigma Plot 10.0 (Systat Software, Inc., San Jose, CA, USA**)**.

Results

Growth analysis: The results of our examination of four growth parameters under different conditions of seedling growth are shown in Figure 1. According to our one-way ANOVA results, SH, GD, BY, and LA were significantly different according to the growth conditions (p<0.01). As seen in Figure 1, *C. bungei* seedlings grown under T7 and T8 achieved larger SH, GD, BY, and LA values than those grown under other conditions, whereas the growth of seedlings under T11 was restrained by the limited water and inadequate fertilizer application.

Regression model: The reliability and degree of fit of the models were investigated by an ANOVA and *F*-test. As shown in Table 4, the regression models were extremely significant (p<0.01); the linear correlation coefficient (R^2) ranged from 0.967–0.991. Thus, these regression models were well-adjusted to the experimental data and all of the growth parameters were closely correlated with *W*, *N*, and *P*. After eliminating the insignificant regression coefficients, the models were expressed as follows:

$y_{SH} = 145.639 + 2.826x_1 + 21.730x_2 - 5.209x_1^2 + 6.413x_1x_2 - 2.663x_1x_3 - 12.192x_2^2 - 1.833x_3^2$ .... (1),

$y_{GD} = 20.200 + 1.081x_1 + 2.485x_2 - 0.654x_1^2 + 1.663x_1x_2 - 1.503x_2^2 - 0.566x_3^2$ .... (2),

$y_{BY} = 402.556 + 12.831x_1 + 64.265x_2 + 11.843x_3 - 14.202x_1^2 + 25.574x_1x_2 - 45.109x_2^2 - 10.102x_3^2$ .... (3), and

$y_{LA} = 13891.010 + 485.518x_1 + 2198.831x_2 + 466.312x_3 - 483.445x_1^2 + 847.979x_1x_2 - 1767.563x_2^2 - 389.782x_3^2$ .... (4),

where y_{SH}, y_{GD}, y_{BY}, and y_{LA} represent the values of dependent variables (SH, GD, BY, and LA) and x_1, x_2, and x_3 represent the coded values of *W*, *N*, and *P*, respectively.

Table 4. Significance test of the regression coefficients and R^2 for the regression models after an ANOVA.

Variance source	y_{SH}		y_{GD}		y_{BY}		y_{LA}	
	***F* ratio**	***p* value**	***F* ratio**	***p* value**	***F* ratio**	***p* value**	***F* ratio**	***p* value**
x_1	12.36	0.0056	35.48	0.0001	7.85	0.0187	7.48	0.0210
x_2	730.79	<0.0001	187.39	<0.0001	197.02	<0.0001	153.47	<0.0001
x_3	2.69	0.1318	4.63	0.0569	6.69	0.0271	6.90	0.0253
x_1^2	44.32	<0.0001	13.71	0.0040	10.15	0.0097	7.82	0.0189
x_1x_2	37.28	<0.0001	49.12	<0.0001	18.27	0.0016	13.37	0.0044
x_1x_3	6.43	0.0296	0.02	0.8775	0.01	0.9138	<0.01	0.9501
x_2^2	242.77	<0.0001	72.31	<0.0001	102.44	<0.0001	104.65	<0.0001
x_2x_3	1.62	0.2316	0.02	0.8775	<0.01	0.9943	<0.01	0.9698
x_3^2	5.48	0.0412	10.26	0.0009	5.13	0.0468	5.09	0.0477
Model	117.83	<0.0001	40.19	<0.0001	37.58	<0.0001	32.25	<0.0001
R^2	**0.991**		**0.973**		**0.971**		**0.967**	

Note: y_{SH}, y_{GD}, y_{BY}, and y_{LA} represent the response equations of SH, GD, BY, and LA

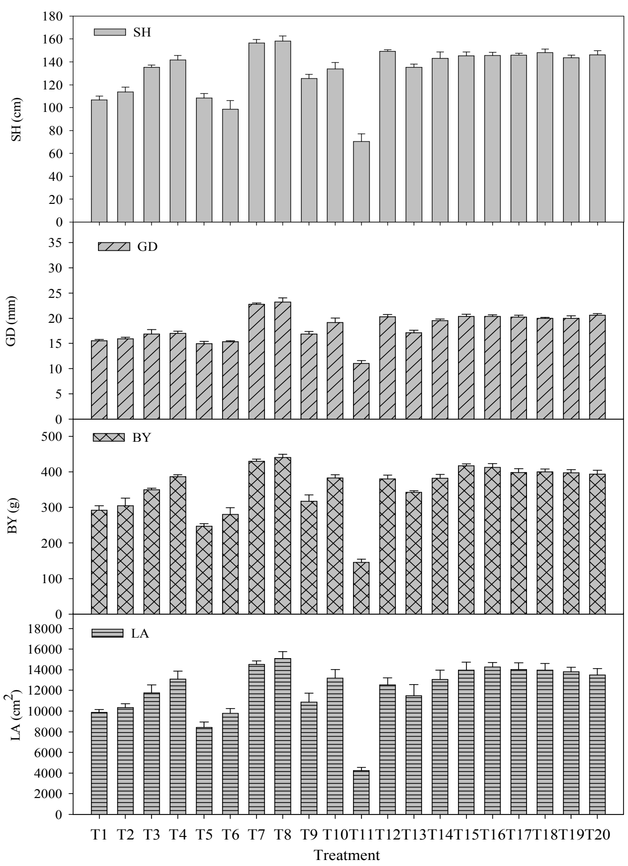

Fig. 1. Stem height (SH), ground diameter (GD), biomass yield (BY), and leaf area (LA) of *Catalpa bungei* seedlings.

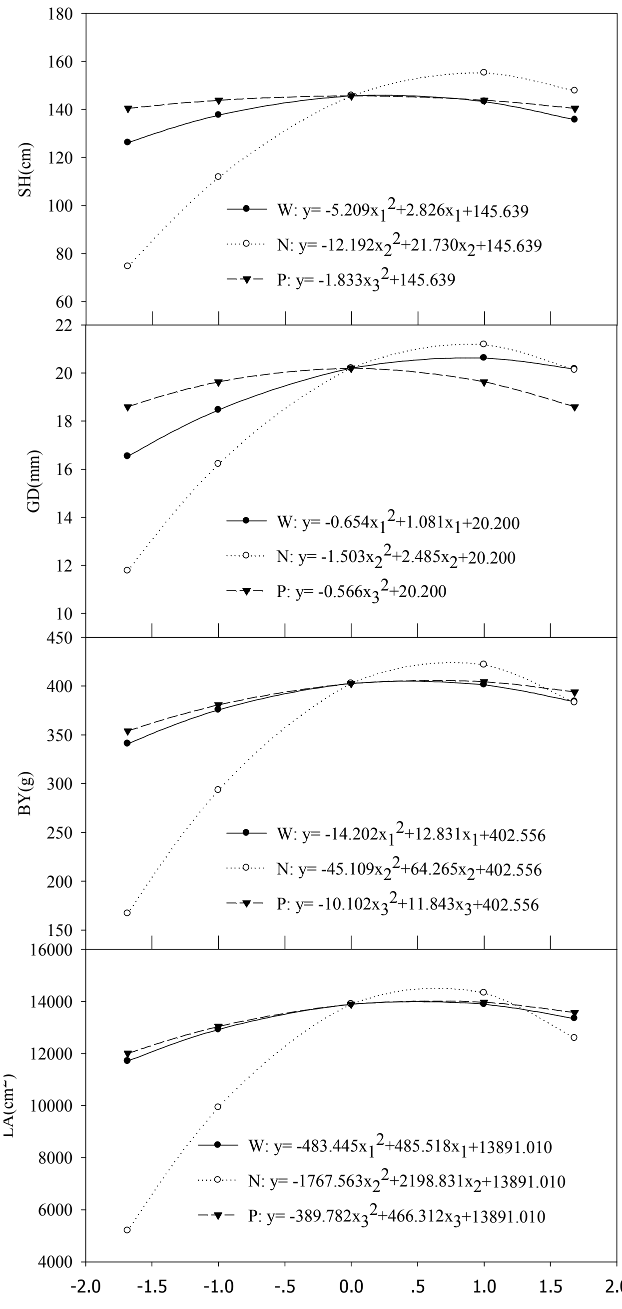

Fig. 2. Monofactor effects of *W*, *N* and *P* on SH, GD, BY, and LA of *C.bungei* seedlings.

Main factor effects and monofactor effects analysis: As shown in models 1–4, x_1 and x_2 were positive, demonstrating that *W* and *N* had significantly positive effects on the SH, GD, BY, and LA of *C. bungei* seedlings. The size sequence of the effects was *N*>*W*. *P* had a significantly positive effect only on BY and LA because the coefficients for x_3 were positive in models 3 and 4. As indicated in Table 4, all x_1x_2 values were positive and the *p* values were <0.05, demonstrating that the interaction of *W*×*N* had a significantly positive effect on the SH, GD, BY, and LA of the seedlings. In addition, the x_1x_3 value in model 1 was significantly positive, demonstrating that the interaction of *W*×*P* only significantly affected SH.

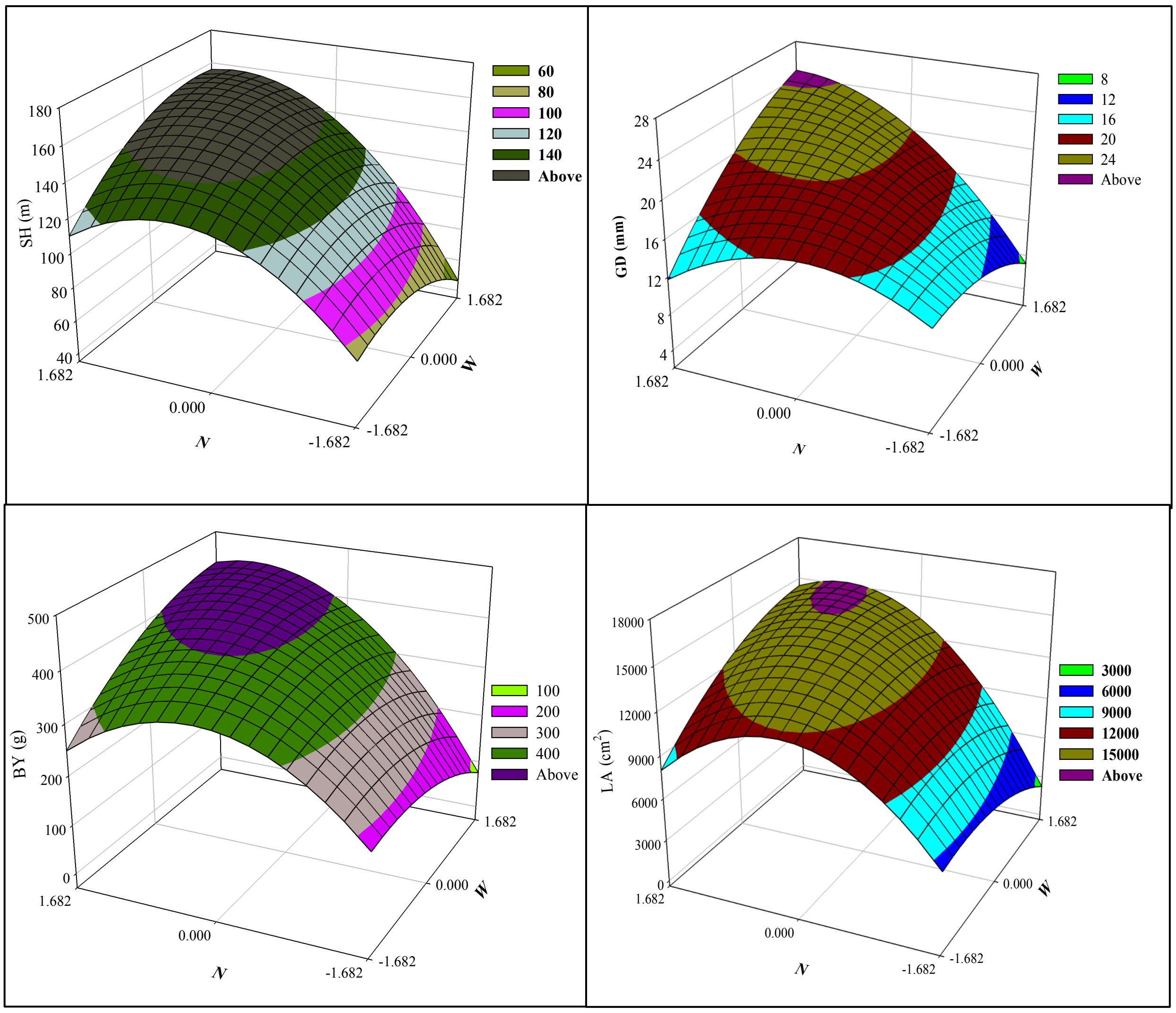

Fig. 3. Interactions of *W* and *N* on seedlings SH, GD, BY, and LA.

In models 1–4, when the two factors were fixed at zero and the other factor was set as a single independent variable, the quadratic equations with one unknown were respectively obtained as Figure 2. As shown in Figure 2, all parameters first increased and then decreased with increasing *W*, *N*, and *P* values (i.e., the changes in SH, GD, BY, and LA of the *C. bungei* seedlings with increases in *W*, *N*, and *P* were all parabolic). Our results indicate that the growth and biomass accumulation of *C. bungei* seedlings would be promoted by increased irrigation and fertilizer application, but that it would be limited in seedlings subjected to excessive irrigation and fertilizer application.

Interactions between *W* and *N*: The interactions between *W* and *N* were shown to have significantly positive effects on SH, GD, BY, and LA in *C. bungei* seedlings (models 1–4). Moreover, when x_3=0 the regression models were expressed as follows:

y_{SH} = 145.639+2.826x_1+21.730x_2-5.209x_1^2+6.413x_1x_2-12.192x_2^2 .... (5),

y_{GD} = 20.200+1.081x_1+2.485x_2-0.654x_1^2+1.663x_1x_2-1.503x_2^2 .... (6),

y_{BY} = 402.556+12.831x_1+64.265x_2-14.202x_1^2+25.574x_1x_2-45.109x_2^2 .... (7), and

y_{LA} = 13891.010+485.518x_1+2198.831x_2-483.445x_1^2+847.979x_1x_2-1767.563x_2^2 .... (8)

As shown in Figure 3, all four parameters initially increased and then decreased with increasing *N* when *W* was fixed, and also with increases in *W* when *N* was fixed. When x_1=-1.682 and x_2=-1.682, SH=73.249 cm, GD=12.804 mm, BY=177.434 g, and LA=5406.592 cm^2. With increasing values of *W* and *N*, the values of SH, BY, and LA increased rapidly; their maximum values (SH=159.499 cm, BY=448.229 g, and LA=15247.050 cm^2) appeared at the following *W* and *N* levels: x_1=0.978, x_2=1.148; x_1=1.468, x_2=1.128, x_1=1.327, and x_2=0.940. When *N* and *W* continued to increase, the values of SH, BY, and LA decreased. When the value of GD peaked, both *W* and *N* increased to their highest level (when x_1=1.682 and x_2=1.682).

Table 5. Model information for the dependent variable B (BY_{tr} and BY_{fr}) and independent variables SH and GD.

Dependent variable	Model	Equation	R^2	RMSE
BY_{tr}	Paraboloid	$B = -148.090 + 1.008SH + 22.510GD - 0.004SH^2 - 0.428GD^2$	0.745	14.368
	Lorentzian	$B = \frac{230.499}{[1 + ((SH - 128.026)/112.700)^2] \times [1 + ((GD - 26.899)/18.504)^2]}$	0.739	14.541
	Caussian	$B = 222.230 \times e^{-0.5} [((H - 127.963/96.773)^2 + ((D - 26.2989)/14.095)^2]$	0.742	14.441
BY_{fr}	Paraboloid	$B=-19.821+0.153SH+2.755GD-0.0005SH^2-0.022GD^2$	0.834	2.968
	Lorentzian	$B = \frac{66.509}{[1 + ((SH - 140.381)/125.704)^2] \times [1 + ((GD - 35.231)/18.055)^2]}$	0.821	3.078
	Caussian	$B = 61.083 \times e^{-0.5} [((SH - 145.875/114.236)^2 + ((GD - 37.678)/18.618)^2]$	0.826	3.040

Note: BY_{tr} and BY_{fr} represent biomass yield of total roots and biomass yield of fine roots respectively

Multi-objective decision model analysis: According to the partial least squares method, the weight values of SH, GD, BY, and LA were $\omega_{SH=}0.25$, $\omega_{GD=}0.25$, $\omega_{BY=}0.25$, and $\omega_{LA=}0.25$, while their optimal values were SH=160.688 cm, GD=24.800 mm, BY=451.700 g, and LA=15386.520 cm^2. The following multi-objective decision model resulted in:

$$y = 0.25(y_{SH}-160.688)^2+0.25(y_{GD}-24.800)^2+0.25(y_{BY}-451.700)^2+0.25(y_{LA}-15386.520)^2$$

Our results show that when x_1=1.307, x_2=0.950, and x_3=0.565, all parameters reached their optimal values. Hence, when the actual conversion values reached *W*=74.605%FC, *N*=4.710 g·plant^{-1}, and *P*=2.009 g·plant^{-1}, respectively, the optimal values of the four parameters for *C. bungei* seedlings were SH=155.473 cm, GD=23.383 cm, BY=450.621 g, and LA=15385.620 cm^2.

Correlation between root BY, SH, and GD: As indicated in Table 5, R^2 in the models fitting the dependent variable BY_{tr} and independent variables H (SH) and D (GD) ranged from 0.739 to 0.745, and the RMSE ranged from 14.368 to 14.541. The models for BY_{fr} and SH and GD showed a stronger correlation and better goodness of fit because the value of R^2 in these models was 0.821–0.834 and the RMSE ranged from 2.968 to 3.078. In terms of the model fitting results, the BY of roots (especially fine roots) was closely correlated with SH and GD. Thus, in this study the root system made a significant contribution to *C. bungei* seedling growth, and the development and growth of fine roots were found to have a significant influence on SH and GD.

Discussion

Plant growth can be affected by specific factors such as the soil moisture, N, or P content (Noorka *et al*., 2009; Vessella *et al*., 2010; Vasileva *et al*., 2011; Sharif *et al*., 2014), and these factors play different roles in plant growth. Recent studies of *C. bungei* showed that the species exhibited limited growth and/or died under conditions of extreme drought stress (Dong *et al*., 2013), and that plant growth increased rapidly only when N fertilizer application was appropriate (Wang *et al*., 2012). Moreover, these studies indicated that water and fertilizer have a significant influence on photosynthetic capacity, biomass accumulation, and allocation and root development in *C. bungei* seedlings. In our study, *N* and *W* had a significant effect on the growth of *C. bungei* seedlings, with a size sequence of *N*>*W*, while studies of other tree species confirmed that *W* has a more significant effect on plant growth than *N* (Dong *et al*., 2011; Lin *et al*., 2013). However, these observations require further study. These conflicting results may be attributed to differences between tree species in response to soil water and fertilizer conditions. Specifically, *C. bungei* seedlings may have relatively stronger drought resistance and lower water consumption, and *N* rather than *W* may be the most important limiting factor in the growth of *C. bungei* seedlings.

Furthermore, the interactions of soil moisture and fertilizer application with the growth characteristics of plants have been confirmed in numerous studies (Dong *et al*., 2011; Lin *et al*., 2013; Porto *et al*., 2014). It was observed that the interaction of *W*×*N* affected the growth of *C. bungei* seedlings, as described by Dong *et al.* (2011) and Lin *et al.* (2013). In previous studies, soil water was reported to determine soil N availability and transportation; hence, N absorption and utilization by plants could be promoted by increasing irrigation (Hu *et al*., 2009; Al-Kaisi & Yin, 2003). In addition, increasing the application of N can promote water use efficiency and absorption for the expansion and development of roots. Hence, the interaction of *W*×*N* can positively affect the expansion and development of roots, as well as the absorption and utilization of water and N, thus promoting the growth and biomass allocation of plants for stronger leaf photosynthesis. In addition, it was noted that *P*, *W*×*P*, and *N*×*P* had relatively limited effects on the growth of *C. bungei* seedlings. However, the reason for these effects on the growth of *C. bungei* seedlings remains unclear.

Recent studies of the coupling effects of soil moisture and fertilizer on woody plants revealed obvious differences between species based on the optimal combinations of soil moisture and applied fertilizer, among which *Eucalyptus urophylla* × *E. grandi* seedlings were optimized when W=78.10% FC, N=4.48 g·plant^{-1}, and P=1.67 g·plant^{-1} (Dong *et al*., 2011), and maximum growth of *Populus tomentosa* Carr. seedlings was observed when W=78.10% FC, N=2.32 g·plant^{-1}, and P=1.51 g·plant^{-1} (Lin *et al*., 2013). The amounts of water and fertilizer required for *C. bungei* seedlings were similar to those for *P. tomentosa* Carr. seedlings. Thus, many of the techniques used for the seedling culture and afforestation of *P. tomentosa* could be applied to *C. bungei.*

The development of root systems has been demonstrated to be strongly associated with soil moisture and fertilizer (Toorchi *et al*., 2002; Christmann *et al*., 2007; Wang *et al*., 2012); these same studies revealed that the growth of plant vegetative organs, including roots, increased rapidly under sufficient soil water. However, the statement that root development and expansion could be promoted when plants experienced a certain level of drought stress has generally been accepted (Toorchi *et al*., 2002; Christmann *et al*., 2007). In addition, fertilizer and soil water had similar effects on root systems; root growth increased rapidly when the amount of fertilizer was adequate, whereas root expansion to obtain nutrients was observed under nutrient-deficient conditions (Trubat *et al*., 2006; Wang *et al*., 2012; Holub *et al*., 2013). Thus, the effect of water and fertilizer on roots was double-sided and even contradictory; furthermore, the combined effects of water and fertilizer on roots were complex. The processes whereby roots physically absorb water and nutrients are distinct; however, the physical and chemical processes and microorganisms and plant physiological processes throughout the soil were easily affected by the soil water level; thus, soil moisture and nutrients are closely linked (Majdi and Andersson, 2005). The mechanisms involved in the response of roots to balanced soil moisture and fertilizer remain unclear, but our results may be used to explain the interactions between soil moisture and fertilizer during plant growth. Moreover, coupling effects of soil moisture, N, and P application on root growth characteristics were observed by Shen *et al*. (2013). Hence, the coupling effects of soil moisture and fertilizer on root characteristics, including BY, length, surface area, and volume, should be studied in the future. In this study, fine roots were observed to have a strong correlation with the growth of aboveground parts of *C. bungei* seedlings. This may be explained as follows: fine root growth was considered to have a close correlation between soil fertilizer application and irrigation. The root system is known to be divided into fine roots and coarse roots (Cheng *et al*., 2005). Fine roots are mainly responsible for the absorption of water and nutrients, while coarse roots mainly contribute to the transport and storage of water and nutrients (Vogt *et al*., 1986). Thus, fine roots respond more rapidly to changes in soil moisture and nutrient content.

Many recent studies have explored the issues surrounding water consumption and fertilizer application in fast-growing *C. bungei* plantations. Therefore, controlling water and fertilizer application is important for *C. bungei* plantation management. Irrigation and fertilizer application in *C. bungei* plantations should be explored in the future.

Conclusions

RSM based on a rotatable CCD can increase our understanding of the coupling effects of soil water and fertilizer on the growth characteristics of *C. bungei* seedlings. Our results indicated that W and N both had positive effects on the growth characteristics (SH, GD, BY, and LA) of *C. bungei* seedlings, and the size sequence of the effects was $N>W$, whereas P only had positive effects on BY and LA. The results of a monofactor effects analysis indicated that the changes in SH, GD, BY, and LA of *C. bungei* seedlings with increases in W, N, and P were parabolic. Moreover, the interactions of $W\times N$ had a significantly positive effect on the growth characteristics of seedlings, while the interaction of $W\times P$ had a positive effect only on SH. *C. bungei* seedlings showed maximum growth when their actual irrigation and N and P application values reached W=74.605% FC, N=4.710 g·plant^{-1}, and P=2.009 g·plant^{-1}, respectively. These results could be used to recommend the appropriate fertilizer application for field management according to the appropriate planting density of *C. bungei*. On the other hand, the results of the fitting model revealed a close correlation between root (especially fine root) BY and SH and GD. Furthermore, soil water and fertilizer application had a significant coupling effect on root system development and affected the growth of the aboveground parts of *C. bungei* seedlings.

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