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Interactions between soil water and fertilizer on leaf photosynthesis and δ^{13}C of *Catalpa bungei* seedlings

Abstract: Our objective was to identify combinations of irrigation and fertiliser application that promoted the photosynthetic productivity and water use efficiency (WUE) of *Catalpa bungei*. Using response surface methodology based on a rotatable central composite design, we conducted a pot experiment with *C. bungei* clone 004-1 seedlings. The experiment quantified the effects of interactions between soil water content (W), nitrogen application rate (N), and phosphorus application rate (P) on leaf net photosynthetic rates (Pn), SPAD values (SPAD-502 meter readings of leaf chlorophyll), and carbon isotope composition (δ^{13}C). N had significantly positive effects on the all three leaf parameters, while W had significantly negative effects on leaf δ^{13}C signatures but positive effects on leaf Pn and SPAD values. The magnitude of the N effect exceeded that of the W effect. P had no significant effects on any of the three leaf parameters. The $W \times N$ interaction had significant positive effects on Pn and SPAD, but significant negative effects on leaf δ^{13}C. Thus, irrigation and nitrogen application had different effects on seedling growth and WUE, and the two effects were interactive. *Catalpa bungei* growth was promoted by appropriately increasing nitrogen application and irrigation, but WUE was improved by increasing N and decreasing irrigation. Therefore, strategies for the irrigation and fertilisation of *C. bungei* plantations should be designed to appropriately balance plant growth and WUE.

Keywords: soil water and fertilizer, net photosynthetic rate, SPAD, δ^{13}C, *Catalpa bungei*

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Introduction

Soil water and nutrients are key limiting factors for plant growth; they have marked effects on physiological characteristics, growth, and productivity (Dong et al., 2011; Liu et al., 2013; Porto et al., 2014). In general, leaf functional traits and physiological characteristics, which are strongly influenced by environmental factors, provide key information on plant growth and **metabolic** status. Plant water use efficiency (WUE) has been well studied, and considerable attention has been focused on the interactive effects of soil water and fertiliser on plant WUE indices (Shangguan & Shao, 2000; Cabrera-Bosquet et al., 2007; Liu et al., 2013). Most of these studies have measured instantaneous WUEs, but few have measured the long-term interactive effects of soil moisture and fertiliser. Leaf carbon isotope composition (δ^{13}C) is a stable index which may be used to reliably estimate the long-term WUE of leaves (Farquhar et al., 1989) and their photosynthetic capacity (Flanagan & Farquhar, 2014). Leaf δ^{13} *C is influenced by a range of intrinsic and extrinsic factors, including plant genetics* (Prasolava et al., 2003), relative humidity (Liu et al., 2014), CO_2 concentration (Berry et al., 1997; del Amor, 2013), light (Berry et al., 1997), elevation (Van de Water et al., 2002; Zhou et al., 2013), and precipitation (Van de Water et al., 2002). However, few reports describe the effects of soil factors (water and fertiliser) on leaf δ^{13}C, and the specific functional mechanism has been inadequately documented.

Leaf performance determines plant growth and yield rates because leaves are the major photosynthetic organs. Leaf chlorophyll contents and photosynthetic rates are indicators of plant photosynthetic productive potential and realised output levels. The leaf photosynthetic mechanism is complicated and controlled by numerous intrinsic and environmental factors. As reported by Dong et al. (2011) and Liu et al. (2013), soil water and fertiliser regimes affect the net leaf photosynthetic rate (Pn) of plants markedly. Chlorophyll is an essential component of the plant photosynthesis mechanism, and the content of this pigment is closely related to photosynthetic capacity. Leaf SPAD values (SPAD-502 meter readings of leaf chlorophyll) are positive indicators of chlorophyll content (Uddling et al., 2007; Bielinis et al., 2015). Like Pn rates, leaf SPAD values are affected markedly by soil water and fertilisation regimes (Swiader & Moore, 2002; Azizian & Sepaskhah, 2014).

The cultivation of *Catalpa bungei* has expanded in China, where it is a valuable tree for landscaping, and timber production, and provides a source of medicinal compounds. Improved understanding of appropriate irrigation and fertiliser regimes will contribute to better cultivation procedures and expand the scientific knowledge base for this species. Numerous studies have examined the morphological and physiological responses of *C. bungei* to water stress (Dong et al., 2013) and nitrogen fertiliser application (Wang et al., 2013), but the interactive effects of these two factors on the growth, photosynthetic productivity, and WUE of *C. bungei* seedlings have rarely been studied. Previous investigations have emphasised problematic issues surrounding water and fertiliser use; both resources are often supplied at excessive rates (Pires & Xavier, 2010; Porto et al., 2014). *Catalpa bungei* plantations have expanded rapidly, especially in arid areas of China, and the massive use of water and fertiliser has become an issue of considerable controversy. Clearly, the time is ripe for investigations that will identify the most efficient irrigation and fertilisation protocols for sustainably maintaining the resources provided by this species.

Response surface methodology (RSM) provides three-dimensional plots which enable interpretation of the ways in which environmental variables affect plant responses (Nasrollahzadeh et al., 2007; Dong et al., 2011). We used an RSM based on a rotatable central composite design (RCCD) to study the interactive effects of soil water (*W*), nitrogen application (*N*), and phosphorus application (*P*) on the leaf Pn, SPAD, and δ^{13}C values of *C. bungei* clone 004-1 seedlings grown in pots. Our aim was to develop a mathematical model for quantifying the effects of *W*, *N*, and *P* and their interactions on leaf Pn, SPAD, and δ^{13}C values. Thus, we determined leaf Pn, SPAD, and δ^{13}C values of the seedlings each month during the growth periods in a crossed design of various treatment levels. Based on this work, we were able to construct a basic theory for *C. bungei* seedling culture and plantation cultivation.

Materials and Methods

Plant materials and growth conditions

The experiment was conducted in a plastic film greenhouse measuring 60.0 × 8.0 × 1.6 m. The arch height, arch space, and area of the growing space were 3.0 m, 1.0 m, and 480.0 m², respectively. The greenhouse was located at the Xiaolongshan Forestry Science and Technology Research Institution, Tianshui, Gansu Province, China (105°54'E, 34°28'N). In early March 2013, we transplanted two-year-old seedlings of *C. bungei* clone 004-1 into flowerpots (one seedling per flowerpot). The seedlings were well watered; we controlled pests and diseases of the stumps before treatments began. The flowerpots measured 35 × 35 × 30 cm and stood on plastic pallets. Each pot contained 15 kg of soil matrix made up to a 6:4 ratio of loess:peat (vol/vol). We measured soil field capacity (FC; [water volume/soil volume] × 100) and bulk density (BD) using the core cutter method (Lu,

2000); soil chemical properties were determined by the methods described by Lu (2000). The FC was 34.45%, the BD was 1.15 g cm^{-3}, soil pH was 7.47, the organic matter content was 29.62 g kg^{-1}, the total nitrogen (N) content was 1.55 g kg^{-1}, the total phosphorus (P) content was 0.81 g kg^{-1}, the total potassium (K) content was 18.68 g kg^{-1}, the available N content was 152.36 mg kg^{-1}, the available P content was 48.34 mg kg^{-1}, and the available K content was 103.06 mg kg^{-1}. The seedlings had an average stem height of 31.2 cm and an average diameter at ground level of 5.9 mm before treatments began. Seedlings were planted under average day/night temperatures of 31.6°C/15.8°C and maintained at a relative humidity of 35.8~65.2% during the experiment.

Experiment design

A central composite (uniform precision) response surface design was applied using SAS software (version 9.0, SAS Institute Inc., Cary, NC, USA). We randomly deployed 20 treatments, each of which was replicated fivefold; each experimental unit contained 100 seedlings. Five levels of each of the factors *W*, *N*, and P were assigned a code (Table 1) and incorporated into the design. The treatment combinations of factors and levels are listed in Table 2. N fertiliser and P$_2$O$_5$ were supplied via four holes in the soil surrounding each seedling. N fertiliser was applied in mid-May, mid-June, and mid-July (one third of the total dosage on each occasion), and all of the P$_2$O$_5$ was applied in mid-May. A single dose of K$_2$O was applied at a rate of 1.5 g plant^{-1} to all seedlings in mid-May. The soil water content was measured every 4 d using a handheld time domain reflectometer (FOM/mts type, Easy Test Ltd., Lublin, Poland). Seedlings were watered to reach the soil water content levels listed in Table 2. We calculated irrigation volume (IV, mL) using a 1000-mL measuring cylinder following the procedures of Dong et al. (2011) thus:

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

where FC and BD are soil field capacity and bulk density, respectively, and *W*, SW, and ρ are soil wa-

Table. 1 Coded and physical values of three experimental factors

Coded value	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	3.0	1.5
1	70	4.8	2.4
1.682	80	6.0	3.0

FC, soil field capacity.

Table. 2 Physical and coded values of experimental factors in 20 treatments

Treatments	*W* (%FC)	*N* (g plant^{-1})	*P* (g plant^{-1})
1	40 (−1*)	1.2 (−1)	0.6 (−1)
2	40 (−1)	1.2 (−1)	2.4 (1)
3	40 (−1)	4.8 (1)	0.6 (−1)
4	40 (−1)	4.8 (1)	2.4 (1)
5	70 (1)	1.2 (−1)	0.6 (−1)
6	70 (1)	1.2 (−1)	2.4 (1)
7	70 (1)	4.8 (1)	0.6 (−1)
8	70 (1)	4.8 (1)	2.4 (1)
9	30 (−1.682)	3.0 (0)	1.5 (0)
10	80 (1.682)	3.0 (0)	1.5 (0)
11	55 (0)	0 (−1.682)	1.5 (0)
12	55 (0)	0 (1.682)	1.5 (0)
13	55 (0)	3.0 (0)	0 (−1.682)
14	55 (0)	3.0 (0)	3.0 (1.682)
15	55 (0)	3.0 (0)	1.5 (0)
16	55 (0)	3.0 (0)	1.5 (0)
17	55 (0)	3.0 (0)	1.5 (0)
18	55 (0)	3.0 (0)	1.5 (0)
19	55 (0)	3.0 (0)	1.5 (0)
20	55 (0)	3.0 (0)	1.5 (0)

*Values in parentheses are coded values. *W*, soil water content; *N*, nitrogen application; *P*, phosphorus application.

ter content, soil weight (15 kg), and water density (1 g cm^{-3}), respectively. The total IVs applied were 37.66, 40.15, 54.48, 67.52, and 76.27 L to reach soil water content levels of 30, 40, 55, 70, and 80% FC, respectively.

Leaf photosynthetic parameter measurements

Leaf net photosynthetic rate (Pn) and SPAD values were selected as parameters to evaluate plant growth and photosynthetic capacity. Pn and SPAD were determined monthly (mid-May, mid-July, and mid-August 2013) in the late mornings (09:00~11:00) on the fourth fully expanded leaf (from the apex) of each plant. Pn values were recorded with a portable photosynthesis system (LI-6400; LI-COR Inc., Lincoln, NE, USA); the photosynthetic photon flux density (PPFD) and temperature at the leaf surface were set at 1200 μmol m^{-2} s^{-1} and 35°C respectively, under the following conditions: relative humidity 44.7% and a reference carbon dioxide (CO$_2$) concentration of 396.3 μmol mol^{-1}. SPAD values were determined with a portable chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd., Tokyo, Japan).

Leaf sample collection and preparation

Leaf samples were collected in mid-June, mid-July, and mid-August. Each sample comprised 3~5 whole fresh leaves (fourth and fifth fully expanded

leaves from the apex]) of each plant. After washing, the samples were oven dried at 80°C for 72 h, collected in numbered sample bags, crushed, and sieved through a 100-mesh screen. These samples were used for measuring leaf $\delta^{13}C$ values

Leaf $\delta^{13}C$ measurements

Leaf $\delta^{13}C$ values were determined in a stable isotope laboratory at the Chinese Academy of Forestry (Beijing, China). As described by Bidartondo et al. (2004) and Yang et al. (2012), leaf samples were analysed in a continuous flow elemental analyser (Flash EA1112 HT; Thermo Finnigan, Waltham, MA, USA) coupled with a mass spectrometer under the following operating conditions: oxidising furnace temperature 900°C, reducing furnace temperature 680°C, and pillar temperature 40°C. The CO_2 emitted was purified in a vacuum line and injected into a Finnigan MAT Delta V advantage spectrometer (Thermo Fisher Scientific) fitted with a double inlet and collector systems. The isotope signatures were measured in units of $\delta^{13}C$ relative to a standard in the conventional δ per thousand notation as follows:

$$\delta^{13}C = [((^{13}C/^{12}C) \text{ sample}) / ((^{13}C/^{12}C) \text{ standard}) - 1] \times 1000,$$

where $^{13}C/^{12}C$ is the isotopic ratio (sample/PDB ratio [PDB, Peedee standard from the Belemnite Formation, South Carolina, USA]). The overall analytical precision was ±0.1‰ for both sample preparation and analyses.

Mathematical modelling and statistical analysis

Based on the RCCD, we constructed three-factor polynomial models to quantify the relationships between the response (y) and independent variables (x) via regression analyses. The mathematical model was structured as follows:

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2,$$

where y is the response variable, x_1, x_2, and x_3 are the coded values of W, N, and P, respectively, and b values are regression coefficients. We deleted the statistically insignificant coefficients. The effect sizes (magnitudes) of the variables were ranked by the absolute values of b; the symbols "+" and "−" indicate the functional directions of the response variables.

All values presented here are means ± SD (n = 5). We employed SAS software (version 9.0; SAS Institute Inc.) to perform multiple non-linear regression analyses that were used to construct polynomial models based on the R^2 test for ANOVA; the significance levels of the regression coefficients were checked by ANOVA and F-tests. The effects of treatment and month on leaf Pn, SPAD, and $\delta^{13}C$ values were examined by two-way ANOVAs using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Probability (p) values <0.05 were considered statistically significant. The regression models were optimised using LINGO software (version 11.0; LINDO SYSTEMS Inc., Chicago, IL, USA). Figures were generated using SigmaPlot 10.0 software (Systat Software, Inc., San Jose, CA, USA).

Results

Leaf photosynthetic parameters and $\delta^{13}C$ values analysis

The values of leaf Pn, SPAD, and $\delta^{13}C$ in the seedlings are depicted in Figure 1. All parameter values were significantly different between months ($p <$ 0.001, Table 3). *Catalpa bungei* seedlings treated with different soil water and fertiliser factor combinations had significantly different leaf Pn, SPAD, and $\delta^{13}C$ values ($p < 0.001$). The interaction term (treatment × month) was also significant (Table 3), indicating that the directions of the response variables (leaf Pn, SPAD, and $\delta^{13}C$ values) differed among months.

Regression model

The average values of leaf parameters determined in June, July, and August were selected as the response variables (y) for regression analyses. The reli-

Table 3. Summary statistics for a two-way ANOVA testing the effects of treatment and month on seedling leaf parameters. See Table 1 for treatment factors and levels. Pn, leaf net photosynthetic rate; SPAD, SPAD-502 meter reading of leaf chlorophyll; $\delta^{13}C$, stable carbon isotope signature

Effect	Pn			SPAD			$\delta^{13}C$		
	df	F ratio	p value	df	F ratio	p value	df	F ratio	p value
Corrected model	59	40.885	<0.001	59	40.589	<0.001	59	33.426	<0.001
Treatment	19	36.326	<0.001	19	91.358	<0.001	19	67.123	<0.001
Month	2	711.365	<0.001	2	199.466	<0.001	2	198.911	<0.001
Treatment × Month	38	7.876	<0.001	38	6.842	<0.001	38	7.867	<0.001
Error	240			240			240		

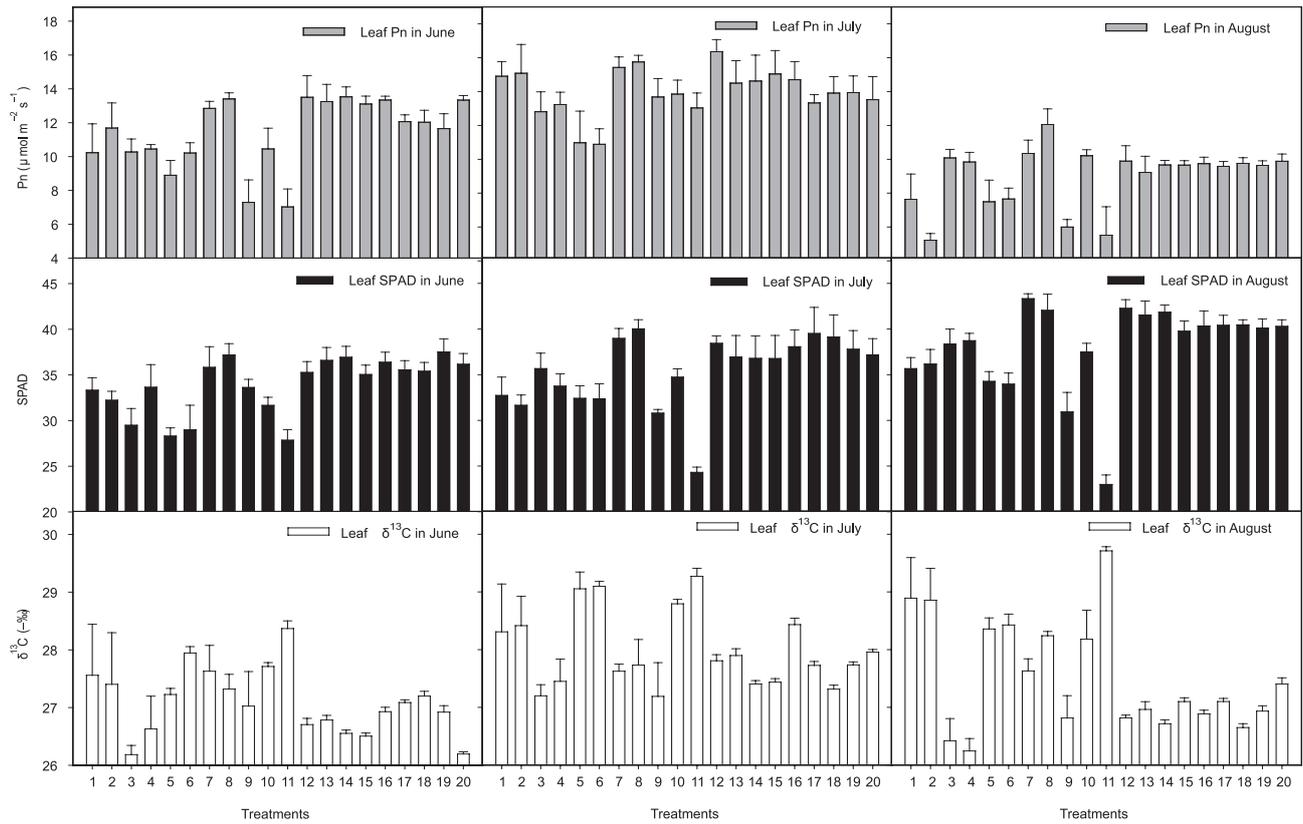Fig. 1. Leaf net photosynthetic rates (Pn), SPAD (SPAD-502 meter readings of leaf chlorophyll), and δ¹³C values of seedlings in June, July, and August. See Table 2 for a list of treatment combinations. Values are means + SD (n = 5)

ability and degrees of fit of the models were investigated by ANOVA and *F*-tests. The regression models were highly significant for leaf Pn, SPAD, and δ¹³C, ($p < 0.01$, Table 4). Thus, the model fits to the experimental data were good; all of the leaf parameters were closely correlated with *W*, *N* and *P*. After deleting the insignificant regression coefficients, the models were formulated as follows:

$$y_{PN} = 12.181 + 0.419x_1 + 1.208x_2 + 0.907x_1x_2 - 0.664x_1^2 - 0.438x_2^2 \quad (1),$$

$$y_{SPAD} = 38.094 + 0.748x_1 + 3.015x_2 + 1.613x_1x_2 - 1.591x_1^2 - 2.086_2^2 \quad (2),$$

$$y_{CIC} = 27.191 - 0.314x_1 + 0.570x_2 - 0.244x_1x_2 - 0.176x_1^2 - 0.351x_2^2 \quad (3),$$

where y_{PN}, y_{SPAD}, and y_{CIC} are the average values of leaf Pn, SPAD, and δ¹³C determined in mid-May, mid-July, and mid-August 2013, respectively, and x_1, x_2, and x_3 are the coded values of *W*, *N*, and *P*, respectively.

Table. 4 Significance of the model regression coefficients and R^2 determined by ANOVA

Variance source	y_{PN}		y_{SPAD}		y_{CIC}	
	F ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value
x_1	10.43	0.0009	5.73	0.0377	45.27	<0.0001
x_2	86.60	<0.0001	93.19	<0.0001	149.12	<0.0001
x_3	0.88	0.3713	0.04	0.8428	<0.01	0.985
x_1^2	27.62	0.0004	27.38	0.0004	14.99	0.0031
x_1x_2	28.60	0.0003	15.62	0.0027	13.49	0.0043
x_1x_3	1.17	0.3047	<0.01	0.9762	0.30	0.5964
x_2^2	12.01	0.0061	47.07	<0.0001	59.55	<0.0001
x_2x_3	0.34	0.5753	0.27	0.6138	0.01	0.9062
x_3^2	0.90	0.3651	0.76	0.4030	0.28	0.6074
Model	18.61	<0.0001	20.75	<0.0001	31.11	<0.0001
R^2	0.944		0.950		0.966	

y_{PN}, y_{SPAD}, and y_{CIC} refer to Pn, SPAD, and δ¹³C, respectively.

Main factor effects, magnitudes of experimental effects, and directions of the effects

x_2 was positive in models 1–3, showing that N had significantly positive effects on leaf Pn, SPAD, and $\delta^{13}C$ values. In models 1–2, W had significantly positive effects on leaf Pn and SPAD (x_1 values were positive), but in model 3, x_1 was negative, indicating that W had a significantly negative effect on leaf $\delta^{13}C$. Both W and N significantly influenced the three leaf parameters; however, the magnitude of the N effect

exceeded that of W. P had no significant effects on any of the three leaf parameters. All x_1x_2 terms were positive (models 1–3) and their p values were <0.05 (Table 4), indicating that the interaction of $W \times N$ had significantly positive effects on all leaf parameters. The interaction of $W \times P$ had no significant effects on the leaf parameters ($p > 0.05$). We therefore focused on the effects of W and N on the response variables.

A set of simple quadratic equations was obtained by setting one factor to zero in models 1–3 and using the other factor as an explanatory variable (Fig. 2). The trajectories of leaf Pn, SPAD, and $\delta^{13}C$ responses to increases in N were all parabolic (Fig. 2). Thus, leaf parameter increases were diminished by excessive fertilisation. The plotted trajectories of leaf Pn, SPAD, and $\delta^{13}C$ responses to increasing W levels were also parabolic (Fig. 2). Thus, cautious irrigation increases had positive effects on leaf performance parameters.

Interactions between soil water and nitrogen application

The $W \times N$ interaction had significantly positive effects on the leaf parameters. We used models 1–3 to obtain the response surface plots depicted in Fig. 3. Leaf Pn increased with increasing N values when W was fixed, and with increasing W when N was fixed; a maximum value was reached when x_1 = 1.682 and x_2 = 1.682 (Fig. 3a). Leaf SPAD initially increased, but then decreased with increasing N when W was fixed, and with increasing W when N was fixed; SPAD values were lowest when x_1 = −1.682 and x_2 = −1.682. As W and N increased, SPAD values increased rapidly, reaching a maximum when x_1 = 0.841 and x_2 = 841; as N and W levels continued to increase, SPAD values declined (Fig. 3b). Leaf $\delta^{13}C$ values increased with increasing N when W was fixed and with decreasing W when N was fixed (Fig. 3c). At the peak value of $\delta^{13}C$, W was at its lowest level and N at its highest level (when x_1 = −1.682 and x_2 = 1.682). Thus, the responses of the three leaf performance parameters to interactions between W and N were not consistent.

Discussion

Soil moisture content and mineral nutrient concentrations are generally considered to have different effects on plant photosynthetic productivity and growth performance (Shangguan et al., 2000; Liu et al., 2013; Guo et al., 2014). Hence, plant growth rates vary in a complex manner among different soil conditions. Recent studies showed that the photo-
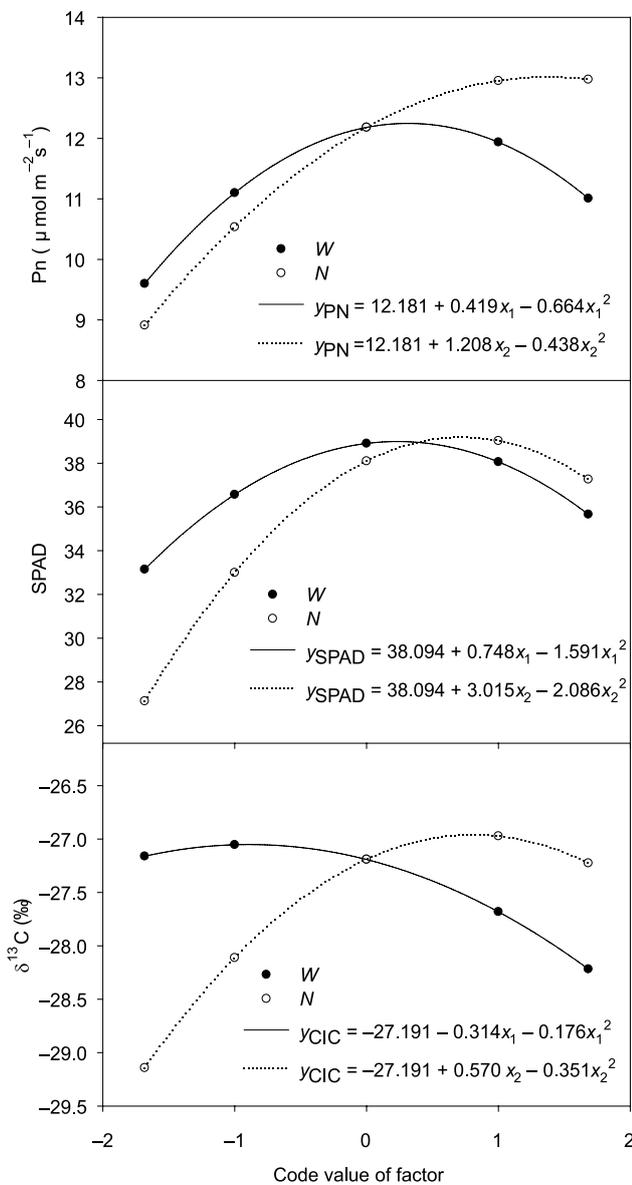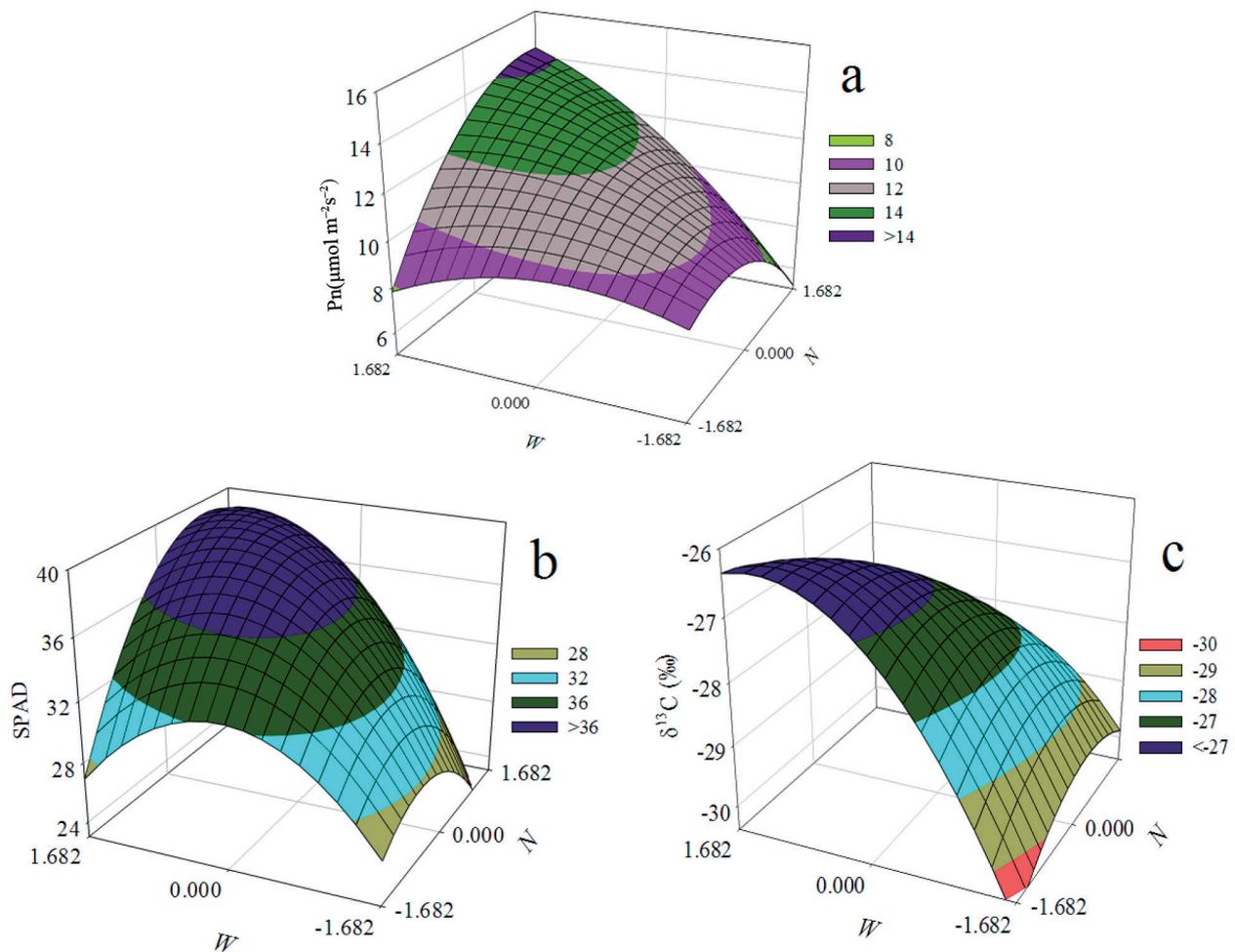

Fig. 2 Single factor effects of soil water content (W) and nitrogen fertilisation (N) on leaf net photosynthetic rates (Pn), SPAD-502 meter reading of leaf chlorophyll (SPAD), and stable carbon isotope signatures ($\delta^{13}C$); see Table 1 for an explanation of the coded values of the factors

Within the figure:

$y_{PN} = 12.181 + 0.419x_1 - 0.664x_1^2$

$y_{PN} = 12.181 + 1.208x_2 - 0.438x_2^2$

$y_{SPAD} = 38.094 + 0.748x_1 - 1.591x_1^2$

$y_{SPAD} = 38.094 + 3.015x_2 - 2.086x_2^2$

$y_{CIC} = -27.191 - 0.314x_1 - 0.176x_1^2$

$y_{CIC} = -27.191 + 0.570x_2 - 0.351x_2^2$

Axis labels: Pn ($\mu mol\ m^{-2}\ s^{-1}$); SPAD; $\delta^{13}C$ (‰); Code value of factor

Fig. 3 Effects of the interaction between soil water content and nitrogen fertilisation ($W \times N$) on (a) leaf net photosynthetic rates (Pn), (b) SPAD-502 leaf chlorophyll meter reading (SPAD), and (c) stable carbon isotope signatures

synthetic capacity of *C. bungei* seedlings increased rapidly when N fertiliser was applied appropriately (Wang et al., 2013), but growth rates were limited by extreme drought stress (Dong et al., 2013); both studies indicated that water and fertiliser are clearly limiting factors for the photosynthesis and growth of these seedlings. *W* significantly positively influenced leaf Pn and SPAD values of *C. bungei* seedlings, probably because increasing soil water content promotes the absorption and transportation of water and nutrients (Hu et al., 2009), which in turn elevate leaf chlorophyll content (hence, SPAD values) and stomatal conductance. We also found that *N* had similar effects on leaf Pn and SPAD values, which we attributed to increases in leaf chlorophyll content as *N* supply was increased. *N* had a greater effect than *W* on the growth of *C. bungei*; however, in other studies on *Populus tomentosa* (Dong et al., 2011) and *Eucalyptus urophylla × E. grandis* (Lin et al., 2013) *W* had a greater effect on growth. The discrepancy among studies may be attributed to species-specific effects of soil water and fertiliser conditions. *Catalpa bungei* may have higher drought resistance and lower wa-

ter consumption than the other two species, which in turn would reduce its sensitivity to soil moisture levels. Experimental evidence is required to substantiate this postulate.

Dong et al., 2011, Lin et al., 2013, and Porto et al., 2014 showed that soil moisture and fertiliser interactively influence the growth characteristics of plants. We showed that the $W \times N$ interaction significantly affected leaf Pn and SPAD values in *C. bungei* seedlings. Soil water reportedly determines soil N availability and transportation; hence, N absorption and utilisation by plants may be promoted by increased irrigation (Hu et al., 2009; Al-Kaisi & Yin, 2003). Increasing *N* fertilisation to *C. bungei* seedlings increases WUE and absorption, thereby promoting the expansion and development of roots. The interaction of *W* and *N* promoted the absorption and utilisation of both water and N, which enhanced leaf photosynthesis. *P*, $W \times P$, and $N \times P$ had relatively limited effects on the growth of *C. bungei* seedlings, perhaps because the P fertiliser was released slowly; species-specific P requirements may also have played a role. Further experimental studies are required.

The $\delta^{13}C$ signature of plant is determined by a complex series of functions that which begin with the import of atmospheric $^{13}CO_2$ through the stomata followed by its incorporation into the tissues through a chain of physical and biochemical processes. This sequence of steps is strongly influenced by soil water and fertiliser availability, leaf nutrient status, and water potential (Ehleringer et al., 1991; Welker et al., 1993; Kao & Tsai, 1998; Zhang et al., 2011) through effects on photosynthetic rate and the stomatal mechanism. Plants reduce transpiration through stomatal closure when soil water availability and leaf water potentials are low, thereby increasing leaf $\delta^{13}C$ values (Cao et al., 2009). We found that high W levels had a significantly negative effect on leaf $\delta^{13}C$ values. The effects of N and P on seedling leaf $\delta^{13}C$ differed. N had a significantly positive effect on leaf $\delta^{13}C$ values, perhaps because ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), chlorophyll content, and other functional biochemical molecules related to the carbon assimilation and fixation process in the leaves of C_3 plants are influenced directly by leaf N composition (Sparks & Ehleringer, 1997). According to this explanation, increases in N would have increased leaf N concentration by increasing the absorption of available N in the soil, which in turn would have increased leaf carbon assimilation and fixation processes, thereby increasing leaf $\delta^{13}C$ values. Leaf P status is closely related to the process of ATP formation and photosynthetic capacity`, which should influence leaf $\delta^{13}C$ values, but P had no significant effects on the leaf $\delta^{13}C$ values of *C. bungei* seedlings. Further research is required to explain this experimental outcome.

In our experiments, the effects of W on photosynthetic parameters (Pn and SPAD values) and WUE ($\delta^{13}C$) were positive and negative, respectively (Fig. 3). These contradictory effects complicate the design of strategies for irrigation and fertiliser application in *C. bungei* plantations. We recommended that irrigation and N application should be increased to promote growth in humid regions; in arid regions, irrigation should be reduced according to the soil water status, and the WUE of *C. bungei* seedlings should be promoted by increased N application. However, the photosynthetic productivity of *C. bungei* seedlings was reduced when we subjected them to excess irrigation and N (Fig. 2), hence cautious irrigation and N application should promote the growth of *C. bungei* seedlings while reducing wasteful use of water and fertiliser resources. *Catalpa bungei* is among a range of fast-growing species (e.g. *Populus* and *Eucalyptus*) cultivated widely in plantations across China; the managers of these stands are often accused of excessive water use and fertiliser consumption. The interactive effects of irrigation and N application on the growth and WUE of *C. bungei* may be extrapolated to the other tree species in question. Chinese poplar, for example, has growth characteristics (fast-growing, deciduous, broad-leaved tree species) and planting zones (arid areas in northern China) that are similar to those of *C. bungei*; hence, the main findings of our research may have broad application to plantation species.

Our work on the leaf parameters of *C. bungei* seedlings was performed under controlled experimental conditions. Extrapolation to plantation management will require experimental studies in commercial forests.

Conclusions

We used RSM based on RCCD to improve understanding of effects of the interactions between soil water and fertiliser application on the leaf parameters of *C. bungei* seedlings. Both factors markedly influenced the photosynthetic productivity and WUE of the seedlings, but the effects differed between factors, which operated interactively. Photosynthetic parameters and $\delta^{13}C$ values responded differently to the $W \times N$ factor interaction. Seedling growth was enhanced by appropriate increases in N application and irrigation, but WUE was promoted by increasing the N application rate and decreasing irrigation. Therefore, strategies for controlling water and nutrient supply should be developed according to site-specific differences in soil water and nutrient status, and management demands (e.g. increased growth *vs*. higher WUE). Our study provides basic data required for the manipulation of irrigation and fertilisation to increase the growth and WUE of *C. bungei*.

Acknowledgements

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