Original Research

Water-Fertilizer Coupling Impacts Osmotic Regulation Substances in *Lonicera caeruleae* Seedlings

Yang Liu[1,2,3], Qichang Zhang[3]*, Yuling Li[1]**

[1]Heibei Agricultural University, Baoding, 071001 China
[2]Jilin Agricultural Science and Technology University, Jilin, 132013 China
[3]Beihua University, Jilin, 132013 China

Abstract

Other than lights, the main factors affecting seedling growth in nurseries are moisture and fertilizer. This study aimed to reveal the response mechanism of *Lonicera caeruleae* seedlings to the coupling effect of water and fertilizer and to provide a theoretical basis for improving *L. caeruleae* quality and yield. The cuttings of one-year-old seedlings were used in a pot experiment with a quadratic rotation regression design with five levels of three factors that was carried out in a greenhouse. The results show that nitrogen application amount, phosphorus application amount, and soil water content had significant positive effects on the content of various substances in seedlings, and water-nitrogen coupling and water-phosphorus coupling had significant effects on the content of osmotic regulatory substances in seedlings. The soluble proteins and sugars in seedlings were significantly positively affected, whereas both proline and malondialdehyde were significantly negatively affected. The model results provided an optimal solution to the equation, and the following combination of water and fertilizer substantially improved seedling growth: soil moisture at 71.3% of field water capacity, nitrogen fertilizer at 7.516 g/plant, and phosphorus fertilizer at 6.573 g/plant. In conclusion, using these parameter estimates could optimize water and fertilizer application for *L. caeruleae* seedlings.

Keywords: *Lonicera caeruleae* seedlings, osmotic regulation substance, water-fertilizer

Introduction

The coupling effect of water–fertilizer refers to how soil mineral elements and water interact to affect plant growth [1-3]. Unlike other abiotic factors, water plays a decisive role in plant growth [4, 5]; without

water, fertilizer is difficult to dissolve in the soil and difficult for plants to absorb. At the same time, fertilizer can improve the absorption of soil water by plants [6]. In the process of plant growth, once an imbalance between water and fertilizer manifests, the prospects for plant growth worsen considerably [7, 8]. Studies have found that when the soil moisture content is low, the nitrogen in the topsoil layer will be lost [9]. When the water use efficiency of plants decreases, the plants themselves can continue to grow by adjusting

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*e-mail: crossbridery@163.com
**e-mail: liyuling0425@126.com

other morphological or physiological traits [10]. This phenomenon has been observed in plants such as wheat [11] and maize [12]. Under the same level of nitrogen fertilizer, increasing the amount of fertilizer applied and increasing soil moisture can promote the growth and development of leaves [13]. However, the development of crops is significantly affected by different coupling modes of water and fertilizer. For example, under the drip irrigation and fertilization method, the root systems of crops grow well, and the absorption and utilization of nitrogen, phosphorus, and potassium by the root systems are enhanced [14, 15].

Lonicera caerulea is native to Europe, Asia, and the United States and is also distributed in northeast and north China. *L. caerulea* is a deciduous wild berry shrub with both edible and medicinal value [16]. Its fruit contains a variety of bioactive substances, such as anthocyanins, flavonoids, and vitamins [17, 18], whose medicinal properties include antioxidant activity, as well as aiding in digestion, lowering blood lipids, and preventing hypertension [19-21]. However, the planting and cultivation of *L. caerulea* is beset by low productivity due to many persistent and potential problems. In the planting process of honeysuckle transplanted from nurseries, water (insufficient rainfall and seasonal drought), nutrients (incorrect fertilization and excessive nitrogen addition), and other environmental conditions may become important limiting factors, restricting productivity [22].

The seedling stage is critical in the process of plant growth and recruitment into a reproductive size, and seedlings need to absorb many nutrients from the immediate external environment to sustain their growth rates [23, 24]. Factors including rainfall and water and fertilizer conditions play decisive roles in this process. Many studies have confirmed that soil water and fertilizer factors can affect water absorption and metabolism, nitrogen demand of plant [25-26]. Accordingly, the objectives of this study were to: (1) determine the effect of water and fertilizer coupling on osmotic-regulated substances in the seedling stage of *L. caerulea*; and (2) identify the best combination of water and fertilizer for seedling growth of this valuable shrub.

Materials and Methods

Study Site

The experiment was carried out in a greenhouse used to raise seedlings at Beihua University, Jilin Province, China. One-year-old *L. caerulea* cuttings of the variety 'Beihua No.1' were used as the test material. The measured height of the cuttings ranged from 4.07 to 5.61 cm.

Pre-Experiment Setting

The bulk density, field water capacity (28.27%), N, P, and K contents, and pH values of the cultivated soil prepared in house were determined. The contents of N, P, and K in the cultivated soil were 2.67, 1.07, and 24.97 mg/g, respectively, and the pH value was 6.23.

Experimental Design

In this experiment, three abiotic factors, each having five levels, were adopted in a quadratic regression universal rotation design. The three factors were irrigation quota, nitrogen fertilizer application amount, and phosphorus fertilizer application amount. Table 1 shows these tested factors and their respective level coding (see Supplementary Table S1 for the test structure matrix and Supplementary Table S2 for the actual input values of each treatment). A pot experiment was set up; one plant was planted in each pot, with a total of 20 treatments and eight pots per treatment, for a total of n = 160 plants in 20 treatments (i.e., T1-T20). Pots were placed on flat terrain, with eight pots in each row for a total of 20 rows. The size of each pot was 30 cm (inner diameter of the upper opening) × 25 cm (height). Pastoral soil was used in each pot in the greenhouse, and all soil was disinfected before being placed into the pot. An electronic scale was used to keep the weight of each pot limited to 10 kg. Phosphatic fertilizer (main component $Ca(H_2PO_4)_2$) containing 16% P, and potassium fertilizer (main component K_2SO_4) containing 50% K were used. Potassium and phosphatic fertilizers were applied once on May, 26, 2018. Urea (containing 46% N) fertilization was carried out using the whole method. Fertilization was carried out in four equidistant places around seedlings, and the amount of fertilizer was approximately the same in each place. Fertilization was applied once every 25 days, on May 26, June 20, and July 15. The experiment began in April 2018 and lasted 4 months. Samples were taken on August 30, and during this process destructive samples were taken from both plants and soil. After the samples were collected, laboratory experiments were carried out to measure the roots and leaves of *L. caerulea* seedlings. These experiments were repeated with three leaves and three roots in each group of 20 samples. Supplementary Table S1 shows the test structure matrix, and Supplementary Table S2 shows the actual input values for the 20 groups of treatments.

Index Detection

1) Determination of soluble protein content

Coomassie brilliant blue G-250 was used to detection soluble protein [27]. Bovine serum albumin (25 mg) was dissolved in distilled water (100 mcg/mL). A constant volume was obtained in a 100-mL volumetric flask, and the solution was drawn from a 40-mL volumetric flask. Next, 60 mL of distilled water was added to the 100-mL

Table 1. Soil moisture content and fertilizer application at each treatment level.

Code value	−1.682	−1	0	1	1.682
W (%)	35	45	60	75	85
N (g/per plant)	0	2.17	5.22	8.26	10.43
P (g/per plant)	0	3.125	7.5	11.875	15

Note: 'W' is rated water content; 'N' is the nitrogen application rate; 'P' is phosphate.

Table 2. Amount of each reagent for the standard curve.

Tube code	1	2	3	4	5	6
Standard protein (μg /mL)	0	0.20	0.40	0.60	0.80	1.00
Distilled water (mL)	1.00	0.80	0.60	0.40	0.20	0
Protein (μg)	0	20	40	60	80	100

volumetric flask. The liquid standard protein solution was then added to the volumetric flask. Each reagent, in the order given in Table 2, was added to six test tubes and sealed with a plug (20 mL). Five milliliters of the G-250 solution of Coomassie blue was added, and the tubes were shaken. The tubes were left to sit for 5 min before their absorbance was measured at 595 nm. The data was collected, and a standard curve was derived in MS Excel.

For each sample's determination, 0.3 g of sample was ground with a mortar and pestle. The absorbance was measured according to the specific experimental steps [27], and the protein content was checked against the standard curve. The obtained value was substituted into the following formula:

$$\textit{Soluble protein content} \text{ (mg/g)} = (C \times VT) / (VS \times W \times 1000),$$

where C is the protein content obtained from the regression equation (μg); VT is the total extraction liquid volume (mL); VS is the volume of sample solution (mL); and W is the weight of the leaf (g).

2) Determination of soluble sugar content

Anthrone colorimetry was used to detect soluble sugar content [28]. To draw the standard curve, 1.000 g of pure sucrose was dried at 80ºC to a constant weight, and an appropriate amount of water was added. The solution was transferred to a volumetric flask

(100 mL), and 0.5 mL of concentrated sulfuric acid was added at a constant volume. Graduated test tubes (20 mL) were numbered, and the solutions were added in the order given in Table 3.

In each tube, 0.5 mL of anthracene ketone and 0.5 mL of ethyl acetate were added, followed by 5 mL of sulfuric acid. The tubes were plugged and fully oscillated. In vitro ligation with rubber was initiated in a boiling water bath. One minute after cooling, the absorbance value (630 nm) was determined (Excel was used to establish the standard curve).

To determine the soluble sugar content, each sample was weighed and divided into three parts (0.3 g each). The absorbance was measured via anthrone colorimetry. The sugar content (μg) was estimated from a regression equation and substituted into the following formula:

$$\textit{Soluble sugar content} \text{ (mg/g)} = (C \times VT \times N)/(VS \times W \times 1000),$$

where C represents the amount of sugar (μg) obtained from the regression equation; N is the dilution factor; VT is the total volume of the extract (mL); VS is the volume of the absorbed sample solution (mL); and W is the fresh weight of the sample (g).

3) Determination of free proline (Pro) content

The triketohydrindene hydrate method was used to detect free proline [29]. Proline (25 mg) was added to a 250-mL volumetric flask. Original Pro solution

Table 3. Amount of each reagent for the standard curve.

Reagent	Tube code					
	0	1, 2	3, 4	5, 6	7, 8	9, 10
Sucrose solution (100 μg/L)	0	0.2	0.4	0.6	0.8	1.0
Distilled water (mL)	2.0	1.8	1.6	1.4	1.2	1.0
Sucrose (μg)	0	20	40	60	80	100

was added to six clean 50 mL volumetric flasks at volumes of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL, and then the corresponding concentrations of proline were added to each flask: 1, 2, 3, 4, 5, and 6 μg/mL, respectively. In six 20-mL test tubes, 2 mL of the above solutions, 2 mL of glacial acetic acid, and 2 mL of acidic ninhydrin were mixed together, according to the assigned tube number. Water was boiled in an induction cooker. Several test tubes were tied together and heated in boiling water for 30 min. After cooling, 4 mL toluene was added to each tube with a pipette. The stopper was replaced, and then tubes were shaken for 30 s. The supernatant solution was carefully removed with a pipette, and toluene solution was used as a blank. Colorimetry was carried out at a wavelength of 520 nm, and standard curves were drawn in Excel after data collection.

For the free proline content determination, 0.5 g of the cut sample was added to the test tube. Tubes were boiled in water and cooled for 10 min. The samples were filtered, and 2 mL Pro extract was added, followed by 2 mL glacial acetic acid and 2 mL acid indanone. Tubes were again heated in a boiling water bath for 30 min. After cooling, 4 mL toluene was added. The supernatant was centrifuged at 3000 r/min for 5 min. The supernatant solution was removed with a pipette. The absorbance value (520 nm) was determined, and the calculation formula was as follows:

$$Proline\ content\ per\ unit\ fresh\ weight\ (\mu g/g) = \frac{X*5}{2*sample\ weight},$$

where X is the concentration of free proline of 2 mL of determination solution in the standard curve.

4) Determination of malondialdehyde (MDA) content

Thiobarbituric acid (TBA) was added to 5 mL 5% TCA and 0.3 g of the sample, which was previously ground into a homogenate [30]. The samples were centrifuged at 3000 r/min for 10 min. Two milliliters of 0.67% solution associates were added to 2 mL of the centrifugal supernatant. Samples were sealed with plastic wrap and placed in a 100°C water bath for 30 min. After cooling, samples were centrifuged at 3000 r/min for 1 min. The absorbance of the supernatant fluid was measured at 600, 532, and 450 nm. The formula was as follows:

$$C\ (\mu mol/L) = [6.45 \times (A_{532} - A_{600})] - (0.56 \times A450).$$

Statistical Analysis

There was a total of 20 treatment groups (T1–T20), with three replicates per group; therefore, each index was composed of 60 samples. Normal processing and plotting were done using Microsoft Excel 2007, and using SPSS 19.0, a one-way ANOVA was performed. SAS9.0 was used for regression model analysis and a regression coefficient test, which is generally used for experiments with many variables. In this study, the following regression model was used:

$$Y = a0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{11}X_1{}^2 + a_{22}X_2{}^2 + a_{33}X_3{}^2,$$

where Y represents the response variable; X1, X2, and X3 represent the level-coded values of soil water holding capacity (W), nitrogen fertilizer application (N), and phosphorus fertilizer application (P), respectively; and the parameter a denotes the regression coefficient of the model. The insignificant factors were removed through the significance test of regression coefficients.

Results

Soluble Protein

Overall, the soluble protein in seedling roots was higher than that in the leaves (Fig. 1), and the soluble protein content in roots and leaves of seedlings was significantly different under different water and fertilizer treatments (P<0.05, Table 4). The differences in soluble protein content in leaves are shown in Fig. 2a). There was no significant difference between T9 and T12, but the soluble protein content in these groups was significantly higher than that in the other groups. T12 had the highest soluble content at 2.006 mg/g, which was 8.29 times that of T1 (0.242 mg/g), the lowest value. Further analysis showed that T11, T12, and T13 had unimodal trends, indicating that the application of nitrogen and phosphorus fertilizer had a greater effect on the soluble protein content of seedling leaves. Although the content of soluble protein tended to increase with greater soil water content and nitrogen and phosphorus applications, excessive P application inhibited the content of soluble protein, such as in T8. The difference in root soluble protein content among all groups is shown in Fig. 2b), in which
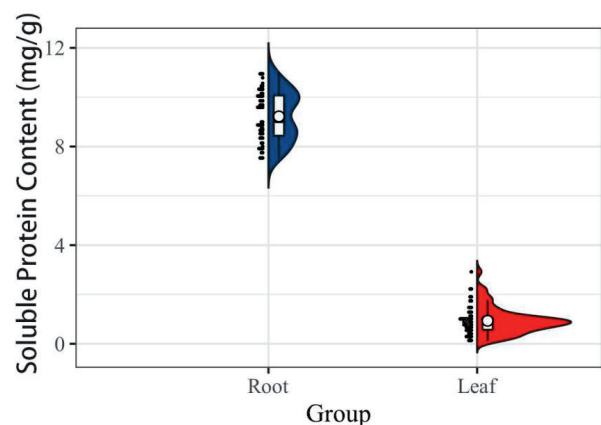

Fig. 1. Soluble protein content in roots and leaves of *Lonicera caerulea* seedlings.

Table 4. Regression model and coefficient test of soluble protein content in *Lonicera caerulea* seedlings.

Variate	y (leaf)	y (root)
x_1	0.040	0.013
x_2	0.041	0.089
x_3	0.040	0.068
x_1^2	0.071	0.001
x_2^2	0.026	0.001
x_3^2	0.077	0.090
x_1x_2	0.054	0.023
x_1x_3	0.021	0.091
x_2x_3	0.05	0.053
R^2	0.659	0.506
P	0.001	0.015

$$y_{(Leaf)} = 0.083 + 0.747X_1 + 0.650X_2 + 0.154X_3 + 0.0185X_1X_2 + 0.0745X_2^2,$$

$$y_{(Root)} = 4.988 + 0.013X_1 + 0.023X_1X_2 + 0.002X_1^2 + 0.009X_2^2,$$

In the leaf model, the single factor regression coefficients were all positive, and the order of absolute values was $X_1>X_2>X_3$. Soil moisture and nitrogen and phosphorus application had significant positive effects on leaf soluble protein content, and the effect sizes were as follows: soil moisture>nitrogen application> phosphorus application. The combination of soil water × nitrogen application had a significant positive coupling effect on leaf soluble protein content. In the root model, the X_1 regression coefficient was positive, indicating that more soil moisture promoted higher root soluble protein content, and the combination of soil moisture × nitrogen application rate had a significant positive coupling effect on root soluble protein content.

Soluble Sugar

The soluble protein of seedling leaves was higher than that of the roots (Fig. 3). Soluble protein content in the roots and leaves of seedlings was significantly different among the water and fertilizer treatments (P<0.05, Table 5). The difference in soluble sugar content in leaves among groups is shown in Fig. 4a). The highest content was 701.366 μg/g in the T1 treatment group, which was 2.66 times higher than that in the T11 treatment group (263.463 μg/g). Further analysis showed that the content of T12 was 59.48% higher than that of T11, indicating that the soluble sugar content of seedling leaves was greatly affected by the amount of nitrogen applied. T13-T15 decreased at first and then increased, indicating that the amount of phosphorus application also influenced the soluble sugar content. Too much phosphorus inhibited the soluble sugar content. The difference in root soluble

the highest content of T7 was 10.259 mg/g, being 1.33 times that of T5 (7.739 mg/g) in the lowest group. Further analysis of T6, T7, and T8 showed that under the same water content, proper application of nitrogen and phosphorus fertilizer promoted an increase in soluble protein, but excessive application of P fertilizer inhibited the content of soluble protein.

A regression model was established with the soluble protein content of *L. lonicera* seedlings as the response variable and X_1, X_2, and X_3 as the independent variables. Both regression models were significant (P<0.05), with R^2 values of 0.659 and 0.50, indicating that soil water and fertilizer factors were positively correlated with soluble protein content in roots and leaves. After eliminating the insignificant factors, the regression model of response variables and soil water and fertilizer factors was as follows:
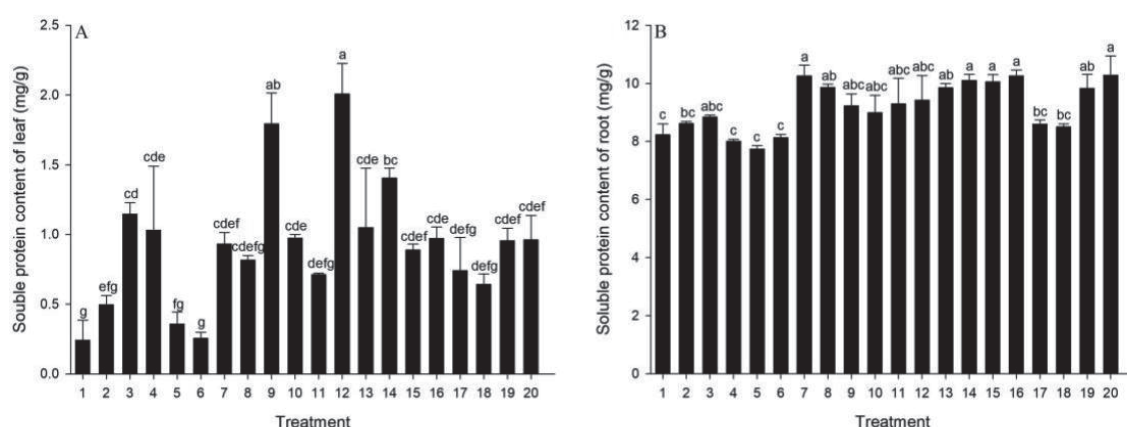

Fig. 2. Changes in soluble protein content in leaves and roots of *Lonicera caerulea* seedlings under different treatments.
Note: The fertilization of each treatment group in the figure 1 is shown in supplementary Table S2 (All bar charts have the same abscissa).
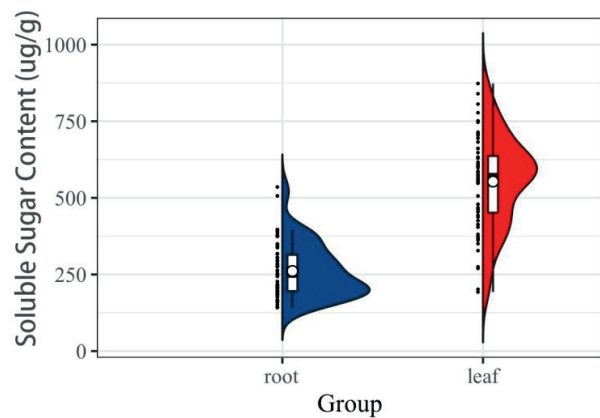
Fig. 3. Soluble sugar content in roots and leaves of *Lonicera caerulea* seedlings.

Table 5. Regression model and coefficient test of soluble sugar content in *Lonicera caerulea* seedlings.

Variate	y (leaf)	y (root)
x_1	0.025	0.000
x_2	0.094	0.052
x_3	0.041	0.033
x_1^2	0.086	0.000
x_2^2	0.000	0.070
x_3^2	0.004	0.001
$x_1 x_2$	0.028	0.038
$x_1 x_3$	0.014	0.069
$x_2 x_3$	0.048	0.069
R^2	0.576	0.605
P	0.004	0.001

sugar content among groups is shown in Fig. 4b). The highest content in the T9 treatment group was 400.263 μg/g, which was 2.33 times that in the lowest treatment group (T15; 172.018 μg/g). Further analysis showed that there were significant differences between T9 and T10. The amounts of nitrogen and phosphorus applied in those two treatments were the same, and the difference in soil water was relatively large (Supplementary Table S2). Soil water content that was too low inhibited the increase in soluble sugar content of the root system. T5, T7, and T8 showed a monotonically increasing trend, indicating that under the same water content, the root soluble sugar content increased as greater amounts of nitrogen and phosphorus were applied. The application of phosphorus also had an effect on the soluble sugar content of seedlings.

A regression model with the soluble sugar content of *L. sinensis* seedlings as the response variable and with X_1, X_2, and X_3 as the independent variables was established and tested. Both regression models were significant (P<0.05) (T5). The R^2 values were 0.576 and 0.605, indicating that soil water and fertilizer factors were correlated with the soluble sugar content in roots and leaves. After eliminating the insignificant factors, the regression models of the response variables and soil water and fertilizer factors were as follows:

$$y \text{ (leaf)} = 1223.269 + 16.048X_1 + 7.014X_3 + 4.165X_1X_2 + 1.317X_1X_3 + 19.330X_2X_3 - 60.534X_2^2 - 2.290X_3^2,$$

$$y \text{ (root)} = 1403.129 + 30.257X_1 + 29.065X_3 + 1.750X_1X_2 + 0.186X_1^2 + 1.450X_3^2.$$

In the leaf model, the X_1 and X_3 regression coefficients were all positive, and the absolute value of the size was in the order $X_1 > X_3$, showing that the soil moisture content and P application were the main factors. Soil moisture × N, soil moisture × combination,
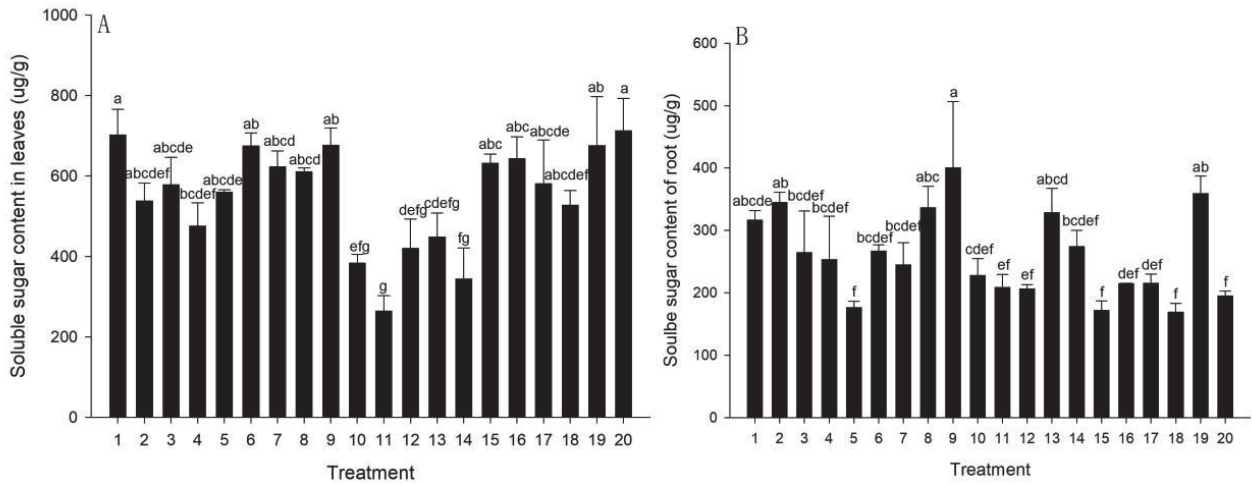

Fig. 4. Changes in soluble sugar content in leaves and roots of *Lonicera caerulea* seedlings under different treatments.
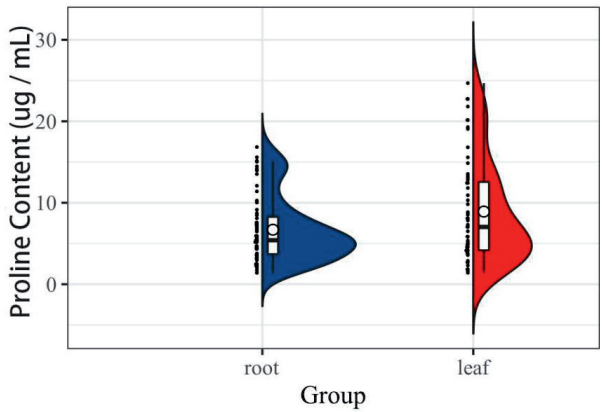
Fig. 5. Proline content in roots and leaves of *Lonicera caerulea* seedlings.

Table 6 .Regression model and coefficient test of proline content in *Lonicera caerulea* seedlings.

Variate	y (leaf)	y (root)
x_1	0.000	0.092
x_2	0.043	0.032
x_3	0.010	0.007
x_1^2	<0.0001	0.023
x_2^2	0.029	0.019
x_3^2	0.054	0.074
x_1x_2	0.016	0.079
x_1x_3	0.028	0.009
x_2x_3	0.004	0.000
R^2	0.659	0.674
P	0.000	<0.0001

and P fertilizer application × combined application rate had a significant coupling effect on leaf soluble sugar content. The effect degree was N application amount × P application amount>soil moisture × N application amount>soil moisture × P application amount. In the root model, the regression coefficients of X_1 and X_3 were all positive, and the order of their absolute values was X_1>X_3. The combination of soil moisture × nitrogen application rate had a significant positive effect on the root soluble sugar content.

Free Proline

Overall, the proline content of seedling roots was stable, while the range of its variation in leaves was large (Fig. 5). Under different water and fertilizer treatments, the proline content of seedling leaves and roots was significantly different (P<0.01, Table 6). The differences in proline content in the leaves among all groups are shown in Fig. 5a). The highest content in the T5 treatment group was 18.668 μg/g, which was 4.43 times that in T18 (4.214 μg/g), the lowest value. Further analysis showed that the T6 and T7 treatment groups were significantly higher than T8, indicating that under the premise of constant water content, with an increase or decrease in the amount of P application, the proline content of leaves was reduced. The difference in root proline content among all groups is shown in Fig. 6b) The content of proline in T4, T5, and T6 was significantly higher than that in the other treatment groups. The content of the T6 treatment group was 15.442 μg/g, which was 6.116 times that of T2 (2.525 μg/g), the lowest treatment group. Further analysis showed that the T2, T3, and T4 treatment groups showed a monotonically increasing trend, indicating that under water stress, with the rapid increase of phosphorus application, the content of proline increased rapidly and then inhibited the stable growth of seedlings. There were significant differences among the T6, T7, and T8 treatment groups, indicating
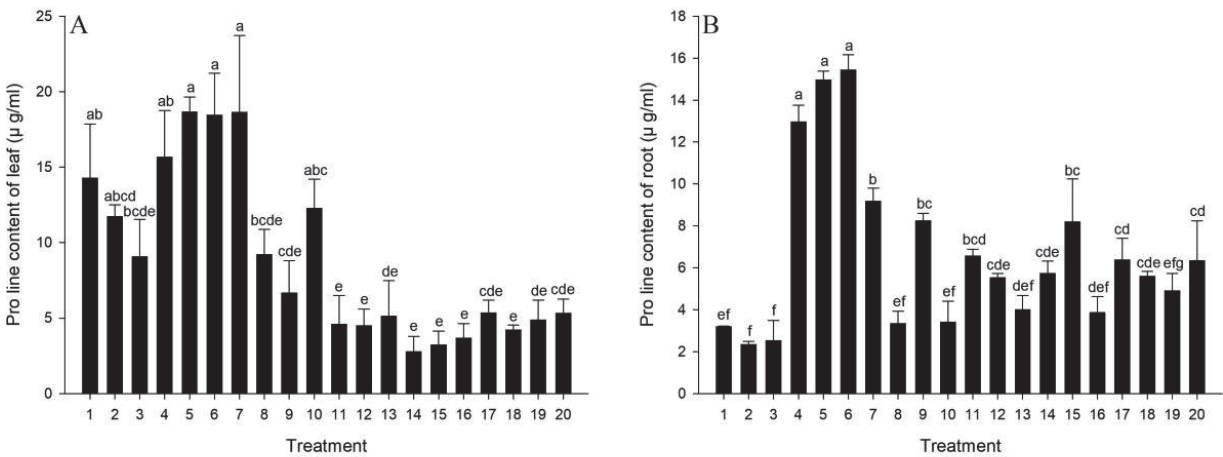

Fig. 6. Changes in proline content in leaves and roots of *Lonicera caerulea* seedlings under different treatments.

that under sufficient nitrogen and soil moisture applications and moderate phosphorus application, the root proline content was lower, while the root proline content was increased when phosphorus application was too high.

A regression model with proline content of *L. lonicera* seedlings as a response variable and X_1, X_2, and X_3 as independent variables was established and tested. The results are shown in Table 6. As shown in Tables 3 and 4, both regression models were highly significant (P<0.001). The R^2 values were 0.659 and 0.673, indicating that there was a strong correlation between soil water and fertilizer factors and the proline content of roots and leaves. After eliminating the insignificant factors, the regression models of the response variables and soil water and fertilizer factors were as follows:

$$y \text{ (leaf)} = 75.750 - 1.967X_1 - 18.017X_2 - 3.345X_3$$
$$- 0.193X_1X_2 - 0.034X_1X_3 - 2.068X_2X_3$$
$$+ 0.016 X_1^2 + 1.634X_2^2,$$

$$y \text{ (root)} = 3.222 - 4.680X_2 - 1.967X_3 - 0.029X_1X_3$$
$$- 2.122X_2X_3 + 0.002X_1^2 + 0.519X_2^2.$$

In the leaf model, the regression coefficients of X_1, X_2, and X_3 were all negative, indicating that the single factor had a negative effect on the proline content of the leaves, while the combination of soil moisture × nitrogen application amount, soil moisture × phosphorus application amount, and nitrogen application amount × phosphorus application amount had a significant negative coupling effect on the proline content of the leaves, and the effect degree was N × P>soil moisture × N>soil moisture × P. In the root model, the single factor also had a negative effect on the root proline content, and the combination of soil moisture × P and N × P had a significant negative coupling effect on the root proline content.

Malondialdehyde

The content of malondialdehyde in leaves was higher than that in roots, and the variation range was larger (Fig. 7). The content of malondialdehyde in leaves and roots of seedlings under different water and fertilizer treatments was significantly different (P<0.05, Table 7). The content of malondialdehyde in the leaves of each group is shown in Fig. 8a). The content of the T18 assistant group was the highest (95.011 μmol/g), which was 1.62 times that of T6 (58.796 μmol/g). Further analysis showed that the content of malondialdehyde increased among the T8, T9, T10, and T11 treatment groups, indicating that water stress and nitrogen deficiency were not conducive to the maintenance of leaf membrane stability. The malondialdehyde content of the leaves in each group is shown in Fig. 8b). The content of the T8 treatment
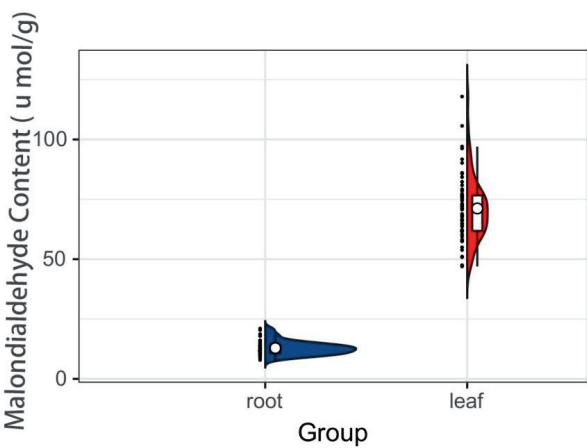

Fig. 7. Malondialdehyde content in roots and leaves of *Lonicera caerulea* seedlings.

Table 7. Regression model and coefficient test of malondialdehyde content in *Lonicera caerulea* seedlings.

Variate	y (leaf)	y (root)
x_1	0.011	0.005
x_2	0.056	0.070
x_3	0.031	0.020
x_1^2	0.099	0.004
x_2^2	0.062	0.018
x_3^2	0.034	0.055
x_1x_2	0.070	0.091
x_1x_3	0.046	0.033
x_2x_3	0.061	0.086
R^2	0.507	0.514
P	0.040	0.031

group was 17.29 μmol/g, which was 1.9 times that of T2 (9.118 μmol/g), the lowest value. Seven treatment groups from T4 to T10 showed a bimodal trend, indicating that the interaction of water, nitrogen, and phosphorus affected the content of root malondialdehyde, thus affecting the growth and development of *L. caeruleae* plants.

A regression model for the content of malondialdehyde in seedlings as the response variable and X1, X2, and X3 as the independent variables was established and tested. The results are shown in Table 7. Both regression models were significant (P<0.05). Their R^2 values were 0.507 and 0.514, indicating that soil water and fertilizer factors were correlated with the malondialdehyde content of roots and leaves. After eliminating the insignificant factors, the regression models of response variables and soil water and fertilizer factors were as follows:
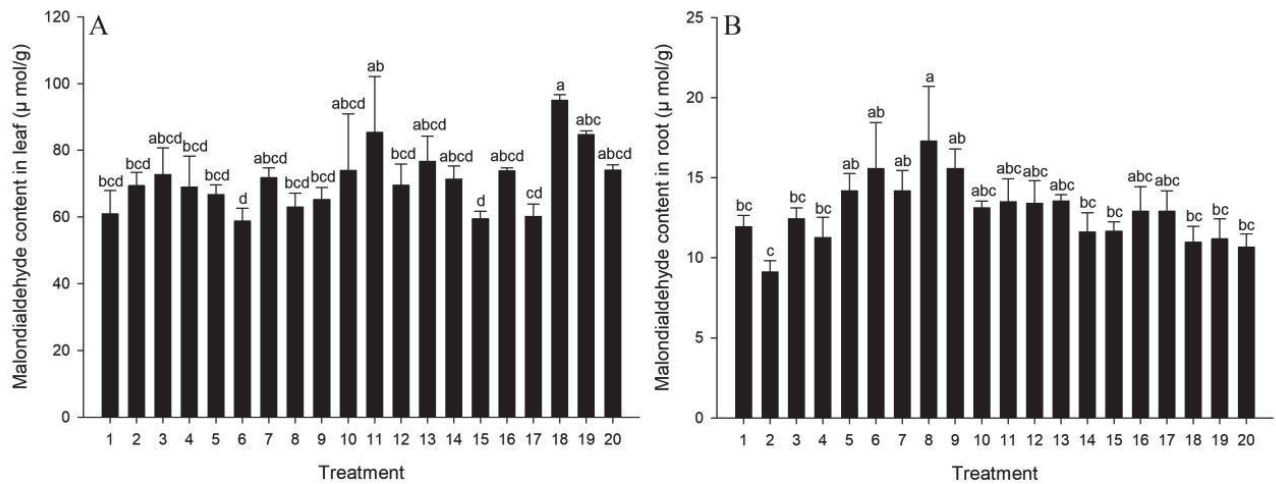
Fig. 8. Changes of malondialdehyde in leaves and roots of *Lonicera caerulea* seedlings under different treatments.

$$y \text{ (leaf)} = 37.513 - 4.409X_1 - 3.848X_3 - 0.078X_1X_3 - 0.126X_3^2,$$

$$y \text{ (root)} = 31.927 - 1.516X_1 - 1.358X_3 - 0.016X_1X_3 + 0.004X_1^2 + 0.522X_2^2.$$

In the leaf model, the single factor had a negative effect on the content of leaf malondialdehyde, and the absolute value order was $X_1 > X_3$. The combination of soil moisture × P had a significant negative coupling effect on the content of the leaves. In the root model, the regression coefficients X_1 and X_3 were both negative, indicating that soil moisture and phosphorus application had a negative effect on root malondialdehyde content, and the order of absolute value was $X_1 > X_3$, while the combination of soil moisture × P amount had a significant negative coupling effect on roots.

Discussion

Soil water is the main source of water uptake by plants [31]; it can positively affect the content of soluble protein and soluble sugar in plants [32]. Under suitable soil moisture conditions, nitrogen can significantly increase the soluble sugar content of *L. caeruleae* seedlings, but the soluble sugar content of seedlings is very low without nitrogen fertilizer, which suggests the application of nitrogen fertilizer may be beneficial to maintain the stability of this shrub's tissues and cells. The soluble sugar content of seedlings is positively related to soil moisture and fertilizer application, and the combination of too little soil moisture and fertilizer application is not conducive to the stable growth and development of seedlings. Malondialdehyde accumulation causes damage to the structure of plant cell membranes, and its increased content in plants is a response to the degree of environmental stress they experience [33, 34]. Under stressful soil moisture

conditions, the malondialdehyde content in plants will also increase, which is the protection system developed by plants to resist changes in the external environment [35]. In this study, under mild water stress, the malondialdehyde and proline contents in seedlings also increased; the cells were evidently stressed, but the seedlings could resist the effects of this adverse environment. However, when the stress reached the threshold of plant tolerance, that is, when the maximum damage that the plant could withstand was exceeded, the stress environment caused irreversible damage to the seedlings' tissues.

Fertilization can effectively improve the nutritional status of seedlings, increase the content of small molecular organic matter in plants, and strengthen their adaptability to adverse conditions [36, 37]. Fertilization has been shown to alleviate the effects of stress on plant growth under water stress [38, 39]. For example, the higher the relative membrane permeability of plant material, the higher the content of malondialdehyde, and the more serious the damage to the plant. An appropriate amount of soil moisture and fertilizer application can therefore foster low malondialdehyde content in seedlings, which is conducive to their cell membrane stability [40]. In this study, with more fertilizer applied, the malondialdehyde content in *L. caeruleae* seedlings decreased, as did their proline content. These results suggest fertilization could also significantly improve the survival ability of seedlings and alleviate water stress.

In terms of the coupling effect of water and fertilizer, under the condition of 60%-75% soil moisture content, the soluble protein content of seedlings was higher under high nitrogen application and a suitable phosphorus ratio. Under the condition of moderate soil moisture content, the soluble protein content of seedlings was increased when more nitrogen fertilizer was applied [41]. Evidently, appropriate increases in soil moisture and fertilizer promote seedling growth, but a combination of soil moisture and nitrogen application that is too high risks impairing the stable,

normal development of seedlings, and the absence of either nitrogen or phosphorus greatly limits seedling growth [42]. Proline is highly water-soluble, which is conducive to maintaining water retention in plant cells and preventing their desiccation. The proline content in plants is very low and often exists in a free state, being mainly distributed in roots, stems, leaves, and other organs [43]. In this study, the proper combination of soil moisture and fertilizer promoted the physiological activities of seedlings. The soluble protein content in the seedling roots was relatively high and may be the cause of the osmotic regulation mechanism itself.

Conclusion

In conclusion, soil moisture and fertilizer application significantly affected the growth and physiological characteristics of *L. caeruleae* seedlings, and there was a significant coupling response between the two. The appropriateness of soil fertilizers could effectively increase soil nutrients. It was found that a reasonable combination of water and fertilizer (soil moisture at 71.3% of field water capacity, nitrogen fertilizer at 7.516 g/plant, and phosphorus fertilizer at 6.573 g/plant) could effectively increase soil nutrients and improve the growth of roots and leaves of seedlings.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Material

Table S1. Quadratic regression general rotation combination design.

Treatment	W	N	P
1	−1	−1	−1
2	−1	−1	1
3	−1	1	−1
4	−1	1	1
5	1	−1	−1
6	1	−1	1
7	1	1	−1
8	1	1	1
9	−1.682	0	0
10	1.682	0	0
11	0	−1.682	0
12	0	1.682	0
13	0	0	−1.682
14	0	0	1.682
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Table S2 .Weight quality of each treatment and each application of fertilizer.

Code	Pot weight (kg)	Carbamide (g/per)	Superphosphate (g/per)
1	11.273	0.723	3.125
2	11.273	0.723	11.875
3	11.273	2.753	3.125
4	11.273	2.753	11.875
5	12.121	0.723	3.125
6	12.121	0.723	11.875
7	12.121	2.753	3.125
8	12.121	2.753	11.875
9	10.990	1.740	7.500
10	12.404	1.740	7.500
11	11.697	0.000	7.500
12	11.697	3.477	7.500
13	11.697	1.740	0
14	11.697	1.740	15.000
15	11.697	1.740	7.500
16	11.697	1.740	7.500
17	11.697	1.740	7.500
18	11.697	1.740	7.500
19	11.697	1.740	7.500
20	11.697	1.740	7.500