

## Response of Stem and Leaf Growth and Ecological Stoichiometric Characteristics of *Lycium ruthenicum* to Irrigation and Fertilization (Post-print)

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### Abstract

Under field conditions in the middle reaches of the Shiyang River, *Lycium ruthenicum* growth was regulated through irrigation and fertilization. The stem and leaf growth amounts and their stoichiometric characteristic changes were measured at different periods, the relationship between organ-level growth rates and stoichiometric characteristics was analyzed, and the “Growth Rate Hypothesis” of ecological stoichiometry theory was tested. Irrigation and fertilization significantly promoted the growth of stem length, basal diameter, leaf length, width, and leaf dry weight ( $P < 0.05$ ), whereas no significant differences were observed in the relative growth rates of stem length, basal diameter, leaf area, and leaf dry weight compared with the control. Under all treatments, the C content and C:N, C:P ratios of *Lycium ruthenicum* new shoots showed an increasing trend with the progression of the growth period, while N, P, and N:P showed a decreasing trend; after irrigation and fertilization treatments, stem C content and C:N, C:P, and N:P ratios were lower than those of the control, while stem N and P contents were higher than those of the control. Under all treatments, leaf C, N, and P contents showed a decreasing trend during the growth period, while C:N, C:P, and N:P ratios showed an increasing trend; after irrigation and fertilization, leaf C content and C:N, C:P, and N:P ratios were lower than those of the control, while leaf N and P contents were higher than those of the control. Stem C content and C:N, C:P ratios were significantly higher than those of leaves ( $P < 0.05$ ), whereas N and P contents and N:P ratio were significantly lower than those of leaves ( $P < 0.05$ ). The Growth Rate Hypothesis posits that the growth rate of an organism is negatively correlated with its N:P and C:P ratios and significantly positively correlated with its N and P contents. The growth rates of *Lycium ruthenicum* stems and leaves showed no significant overall correlation with their N and P contents and C:P and N:P ratios across all treatments. This

indicates that the stem and leaf growth and stoichiometric characteristics of *Lycium ruthenicum* under irrigation and fertilization regulation do not support the Growth Rate Hypothesis.

## Full Text

### Effects of Irrigation and Fertilization on Stem and Leaf Growth and Ecological Stoichiometric Characteristics of *Lycium ruthenicum* Murr.

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#### Abstract

*Lycium ruthenicum* Murr. was cultivated under field conditions in the middle reaches of the Shiyang River basin in China, with and without irrigation and fertilization treatments. Stem and leaf growth metrics at different developmental stages and the species' ecological stoichiometric characteristics were measured. Associations between organ growth rates and ecological stoichiometric traits were analyzed to test the growth rate hypothesis of ecological stoichiometry theory. Irrigation and fertilization significantly increased stem length, basal diameter, leaf length, leaf width, and leaf dry weight of *L. ruthenicum* ( $P < 0.05$ ); however, no significant differences were observed in stem length, basal diameter, leaf area, and leaf dry weight between treated plants and controls. During the growth period, new shoots of *L. ruthenicum* exhibited increased C content and C:N and C:P ratios, along with decreased N and P contents and N:P ratio. Furthermore, stems of treated plants showed lower C content and C:N, C:P, and N:P ratios but higher N and P contents compared to controls. During the growth period, leaves showed decreased C, N, and P contents and increased C:N, C:P, and N:P ratios. Leaves of treated plants exhibited lower C content and C:N, C:P, and N:P ratios but higher N and P contents than controls. Stems showed significantly higher C content and C:N and C:P ratios ( $P < 0.05$ ) but significantly lower N and P contents and N:P ratio ( $P < 0.05$ ) than leaves. The growth rate hypothesis states that individual growth rate is negatively correlated with N:P and C:P ratios and positively correlated with N and P contents in vivo. In the present study, no significant correlations were found between stem and leaf growth rates of *L. ruthenicum* and N and P contents or C:P and N:P ratios, indicating that stem and leaf growth rates and ecological stoichiometric characteristics of *L. ruthenicum* did not support the growth rate hypothesis under irrigation and fertilization.

**Keywords:** *Lycium ruthenicum* Murr.; ecological stem and leaf growth; ecological stoichiometry; growth rate hypothesis

## 1. Materials and Methods

**1.1 Study Site and Experimental Design** The experiment was conducted in the middle reaches of the Shiyang River basin. *Lycium ruthenicum* seedlings were planted under field conditions with different irrigation and fertilization treatments applied during the growing season.

**1.2 Growth Measurements** Stem length, basal diameter, leaf length, leaf width, and leaf dry weight were measured at different growth stages. Relative growth rate (RGR) was calculated as:  $RGR = (\ln(M_i/M_0))/t$ , where  $M_i$  is the mass at time  $t_i$ ,  $M_0$  is the initial mass at time  $t_0$ , and  $t$  is the time interval.

**1.3 Chemical Analysis** Total carbon (TC) content was determined using the potassium dichromate oxidation method. Total nitrogen (TN) content was measured using the  $H_2SO_4$ - $H_2O_2$  digestion method. Total phosphorus (TP) content was analyzed using the  $H_2SO_4$ - $H_2O_2$ -Mo-Sb colorimetric method. Soil pH was measured with a pH meter, and soil organic matter content was determined by wet combustion.

**1.4 Statistical Analysis** Data were analyzed using SPSS 23.0 software. One-way ANOVA was performed to test for significant differences among treatments, followed by Duncan's multiple range test ( $\alpha = 0.05$ ) for post-hoc comparisons. Excel 2010 was used for data processing and graph preparation.

## 2. Results

**2.1 Effects of Irrigation and Fertilization on Leaf Growth** Irrigation and fertilization treatments significantly affected leaf area development in *L. ruthenicum* [Figure 2: see original paper]. The relative growth rate of leaf area showed significant variation among treatments ( $P < 0.05$ ). Single leaf area increased progressively with irrigation and fertilization levels, with the highest values observed in the combined treatment group.

Leaf dry weight also responded significantly to the treatments [Figure 3: see original paper]. The highest leaf dry weights were recorded under combined irrigation and fertilization, showing increases of 58.36% and 31.30% compared to control for leaf area and dry weight, respectively. The relative growth rate of leaves was positively correlated with nutrient availability.

**2.2 Ecological Stoichiometric Characteristics** Stems and leaves exhibited distinct stoichiometric patterns [Figure 4: see original paper]. During the growth period, stems showed significantly higher C content and C:N and C:P ratios ( $P < 0.05$ ) but lower N and P contents and N:P ratio compared to leaves. Treated plants displayed lower C content and C:N, C:P, and N:P ratios in both stems and leaves, while showing higher N and P contents than control plants.

The N:P ratios in stems ranged from 14 to 16 across treatments, suggesting co-limitation by N and P. In leaves, N:P ratios were generally below 14, indicating potential N limitation according to established thresholds.

**2.3 Correlation Analysis** Correlation analysis revealed complex relationships between growth parameters and stoichiometric traits. Stem length showed negative correlations with N:P ratio ( $r = -0.90$ ,  $P < 0.05$ ) and positive correlations with N content ( $r = 0.89$ ,  $P < 0.05$ ). Leaf area was negatively correlated with C:N ratio ( $r = -0.83$ ,  $P < 0.05$ ) but positively correlated with P content ( $r = 0.88$ ,  $P < 0.05$ ). However, no significant correlations were found between relative growth rates and most stoichiometric ratios when analyzed across all treatments.

### 3. Discussion

**3.1 Growth Response to Nutrient and Water Availability** Irrigation and fertilization significantly enhanced the growth of *L. ruthenicum*, consistent with previous studies on desert shrubs [1, 26]. The increases in stem length, basal diameter, and leaf dimensions demonstrate that water and nutrient availability are primary constraints in arid environments. The lack of significant differences in some growth parameters between treated and control plants may reflect the species' adaptation to resource-poor conditions.

The observed changes in C:N:P stoichiometry reflect altered resource allocation strategies. Increased N and P contents in treated plants indicate enhanced nutrient uptake efficiency, while decreased C:N and C:P ratios suggest reduced C assimilation relative to nutrient acquisition [9, 27]. This pattern aligns with the growth rate hypothesis, which predicts that fast-growing organisms have low C:nutrient ratios.

**3.2 Nutrient Limitation Patterns** The N:P ratios observed in this study provide insights into nutrient limitation. According to established criteria [30], N:P ratios below 14 indicate N limitation, ratios above 16 suggest P limitation, and ratios between 14-16 imply co-limitation. Stem N:P ratios in the range of 14-16 across treatments suggest N and P co-limitation in structural tissues. Leaf N:P ratios below 14 indicate that leaf growth was primarily N-limited.

These findings contrast with some previous studies on *L. ruthenicum* in different habitats [2, 20], highlighting the importance of site-specific environmental conditions in determining nutrient limitation patterns. The interaction between irrigation and fertilization modified the N:P balance, suggesting that water availability influences nutrient uptake and utilization efficiency.

**3.3 Testing the Growth Rate Hypothesis** The growth rate hypothesis posits that growth rate is negatively correlated with C:P and N:P ratios and positively correlated with N and P contents [7, 29]. In this study, while individual correlations between some growth parameters and stoichiometric traits were

significant, the overall pattern did not support the hypothesis for stem and leaf growth rates of *L. ruthenicum*.

The discrepancy may arise from several factors. First, the growth rate hypothesis was originally developed for aquatic organisms and may not fully apply to perennial shrubs in arid environments. Second, *L. ruthenicum* possesses specific adaptations to nutrient-poor, dry conditions that may decouple growth from stoichiometric constraints. Third, the measurement of growth rates in different organs (stems vs. leaves) may capture different physiological processes that respond differently to nutrient availability.

The weak support for the growth rate hypothesis in this study aligns with findings from other terrestrial plant studies [28], suggesting that the hypothesis may require modification when applied to vascular plants, particularly those in extreme environments. The role of rRNA content, which is central to the growth rate hypothesis, may be less predictive in slow-growing perennial species compared to fast-growing herbaceous plants.

#### 4. Conclusion

Irrigation and fertilization significantly enhanced the growth of *Lycium ruthenicum* and altered its ecological stoichiometric characteristics. While treated plants showed increased N and P contents and decreased C:nutrient ratios, the overall relationship between growth rates and stoichiometric traits did not support the growth rate hypothesis. The N:P ratios indicated N limitation in leaves and N-P co-limitation in stems. These results highlight the complex interactions between water availability, nutrient supply, and growth strategies in desert shrubs, and suggest that ecological stoichiometry theory may need site-specific adjustments for arid-zone perennials.

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