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Effects of Nitrogen Addition on Leaf and Shoot Size of Xinjiang Wild Apple Seedlings: Postprint

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Date: 2022-01-26T00:00:00+00:00

Abstract

Malus sieversii (Xinjiang wild apple) is one of the progenitors of cultivated apples worldwide and constitutes an important strategic plant germplasm resource in China. Currently, its populations are experiencing severe degradation, and understanding its response to nitrogen addition is essential for conservation efforts. This study performed one-way ANOVA and allometric analysis on leaf and twig size traits of Xinjiang wild apple seedlings under different nitrogen addition treatments to elucidate the species' response to nitrogen addition and provide a theoretical basis for population restoration. The results demonstrated that: (1) Medium nitrogen treatment significantly promoted twig stem elongation and markedly reduced leaf area ratio and leaf-to-stem mass ratio; high nitrogen treatment significantly decreased leafing intensity. (2) The effects of nitrogen addition on growth rates and allocation patterns between leaves and twigs of Xinjiang wild apple were manifested not in biomass but in leaf and twig morphology. The growth rates of twig length-total leaf area and twig length-leaf number relationships showed significant increases under low and medium nitrogen treatments. The low and medium nitrogen treatment groups tended to produce more numerous, smaller, and lighter leaves, whereas the trend under high nitrogen treatment differed from that under low and medium nitrogen treatments. (3) Appropriate nitrogen addition can improve twig growth status and the relationships among leaf and twig functional traits. It is recommended that during in-situ transplantation of wild apple seedlings, habitat-specific nutrient differences should be considered and targeted nitrogen addition be implemented.

Full Text

Effects of Different Levels of Nitrogen Addition on Leaf-Stem Sizes of *Malus sieversii* Seedlings

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Abstract

Malus sieversii is one of the ancestors of cultivated apple worldwide and represents an important strategic plant germplasm resource in China. However, its populations are currently experiencing severe degradation. Understanding the response of *M. sieversii* to nitrogen addition is essential for effective conservation. We conducted one-way ANOVA and allometric analysis on leaf and stem size traits of current-year twigs from *M. sieversii* seedlings under different nitrogen addition treatments to elucidate their responses and provide a theoretical basis for population restoration. The results showed that: (1) Nitrogen treatment significantly promoted stem elongation and significantly reduced leaf area ratio (LAR) and leaf-to-stem mass ratio (LAMR) in *M. sieversii*; high nitrogen treatment significantly reduced leafing intensity (LI). (2) The effects of nitrogen addition on growth rate and allocation patterns between leaves and stems were manifested not in biomass but in morphological traits, with significant increases in total leaf area and twig length under low and medium nitrogen treatments. Low and medium nitrogen treatments favored the production of more numerous, smaller, and lighter leaves, whereas the response pattern under high nitrogen differed from that under low and medium nitrogen. (3) Appropriate nitrogen addition can improve twig growth status and the functional relationships between leaves and stems. We recommend considering nutrient differences between habitats and implementing targeted nitrogen fertilization during transplantation of *M. sieversii* seedlings into their native environment.

Keywords: *Malus sieversii*; nitrogen addition; leaf-stem relationship; allometric scaling relationship; functional traits

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1. Materials and Methods

1.1 Study Area Overview

The experimental site was located at the Wild Fruit Forest Improvement Farm in Xinyuan County, Ili Kazakh Autonomous Prefecture, Xinjiang (43°25 11 N, 83°34 17 E). The area has an average annual temperature of 7–9°C, average minimum temperature of -7.4°C, annual precipitation of 260–500 mm, average frost-free period of 169 days, and annual sunshine duration of 2500 hours. The elevation is approximately 1000 m.

1.2 Experimental Design and Methods

The field experiment utilized one-year-old *M. sieversii* seedlings from a consistent seed source. Referencing nitrogen application rates and methods from nearby orchards, we established four treatment groups: N0 (0 $g \cdot m^{-2}$) as the control, N10 (10 $g \cdot m^{-2}$) as low nitrogen, N20 (20 $g \cdot m^{-2}$) as medium nitrogen, and N40 (40 $g \cdot m^{-2}$) as high nitrogen. Each treatment comprised 15 replicate plots, with 10 seedlings per plot (150 seedlings per treatment). A completely randomized design was employed, with each plot measuring 1.5 m × 1.5 m and separated by 0.3 m buffer zones to prevent cross-contamination. Fertilization points were established 0.6 m from each corner along the diagonal. One-year-old seedlings were planted at these points and watered to ensure survival. Nitrogen fertilizer (urea, CH_4N_2O) was buried in 20 cm deep pits at each fertilization point to facilitate deep root nutrient uptake. Fertilization was applied twice annually (spring and autumn) for three consecutive years, with routine field management maintained throughout.

1.3 Sample Collection and Measurement

In October 2019, current-year twig samples were collected from *M. sieversii* seedlings. Sun-exposed twigs at 1.5 m height were selected, with lateral branches adjacent to main branches cut at the base using pruning shears. Samples were placed in envelopes and transported in ice-filled coolers to the laboratory. Leaves were removed from twigs and scanned, with total leaf area per twig (TLA) and leaf number (LN) determined using Adobe Photoshop CC 2018. Twig basal diameter (TBD) was measured with digital calipers, twig length (TL) with a ruler, and stem dry weight (SM) and total leaf dry weight (TLM) were obtained after oven-drying at 75°C to constant weight using an analytical balance (precision 0.001 g).

1.4 Data Processing

Based on measured twig traits, we calculated specific leaf mass (SLM), individual leaf dry weight (ILM), leaf area ratio (LAR), leafing intensity (LI), specific leaf area (SLA), and leaf-to-stem mass ratio (LAMR) using the following formulas:

- $LA = TLA/LN$ (individual leaf area)
- $SLM = TLM/LN$ (specific leaf mass)
- $LAR = TLA/TLM$ (leaf area ratio)
- $LI = LN/SM$ (leafing intensity)
- $SLA = TLA/TLM$ (specific leaf area)
- $LAMR = TLM/SM$ (leaf-to-stem mass ratio)

Statistical analysis was performed using SPSS 23.0 for descriptive statistics and Pearson correlation analysis, and GraphPad Prism 7 for one-way ANOVA with least significant difference (LSD) tests for multiple comparisons. Allometric relationships were expressed as $Y = \beta X^{\alpha}$, where Y and X represent functional traits, β is the normalization constant, and α is the allometric scaling exponent. Data were log-transformed: $\log Y = \log \beta + \alpha \log X$. Isometric growth occurs when $\alpha = 1$, and allometric growth when $\alpha \neq 1$. Standardized major axis (SMA) estimation was used for parameter estimation and multiple comparisons of allometric relationships using SMATR software.

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2. Results and Analysis

2.1 Effects of Nitrogen Treatment on *Malus sieversii* Twig Traits

Nitrogen application promoted twig stem elongation, with significant promotion observed in the medium nitrogen treatment (Fig. 1 [Figure 1: see original paper]). Compared with the control, twig length increased by 27.11%, 43.35%, and 21.68% under low, medium, and high nitrogen treatments, respectively. No significant differences were observed among treatments for other morphological or biomass indices. At the stem level, nitrogen addition promoted biomass accumulation through both length increase and thickening. At the leaf level, TLM showed an increasing trend, while SM exhibited a pattern of decrease under medium nitrogen and increase under low and high nitrogen treatments.

Among ratio indices, LI showed a significant decline, decreasing by 9.94%, 3.34%, and 21.62% under low, medium, and high nitrogen treatments, respectively, compared with the control. LAR and LAMR decreased significantly under medium nitrogen treatment, with decreasing trends under low nitrogen and increases under high nitrogen treatment. SLA showed a pattern of initial decrease followed by increase with rising nitrogen levels, with significant reductions observed in the medium nitrogen treatment compared with the control and high nitrogen treatment. Overall, nitrogen addition affected various growth characteristics, with ratio indices being more sensitive to nitrogen application than morphological and biomass indices.

2.2 Correlations Among Twig Traits

Correlation analysis revealed strong relationships among most traits (Table 1). Twig traits showed extremely significant positive correlations with leaf traits ($P < 0.01$), while all twig traits were significantly negatively correlated with ratio traits ($P < 0.01$). Among leaf traits, all were extremely significantly positively correlated ($P < 0.01$) except for the correlation between SLA and LAR. Leaf traits were also significantly negatively correlated with ratio traits ($P < 0.01$), except for the correlation between SLA and LAR. Overall, correlations among twig traits were stronger than those among leaf traits, with all twig-leaf trait pairs showing positive correlations, while ratio traits were mostly negatively correlated with twig and leaf traits.

2.3 Allometric Relationships Between Leaf and Stem Sizes

Allometric equation parameters were estimated for all leaf-stem size index pairs, revealing 15 pairs with significant allometric relationships (Table 2). The allometric scaling exponents of 5 trait pairs differed significantly among nitrogen treatments: TL-TBD, TL-LN, TL-TLA, TLM-TLA, and SM-TLM. Except for the SM-TLM pair, which had a negative exponent, all other pairs had positive exponents.

The stem biomass-total leaf biomass relationship showed isometric growth (common slope = 1.0023) with no significant effect of nitrogen treatment on the allometric relationship. In contrast, the twig length-twig basal diameter relationship showed a significantly increased scaling exponent under low and medium nitrogen ($\alpha = 1.162$) compared with the control ($\alpha = 0.909$), but decreased under high nitrogen ($\alpha = 0.939$). The twig length-leaf number relationship exhibited a significantly reduced exponent under high nitrogen ($\alpha = 0.686$) compared with the control ($\alpha = 1.287$).

For the total leaf mass-total leaf area pair, scaling exponents showed no significant differences among treatments (common slope = 1.637), but intercepts differed significantly (N10 > N20 > CK > N40). The stem mass-total leaf mass pair also showed no difference in scaling exponents (common slope = 1.906), but significant intercept shifts occurred (N40 > CK > N20 > N10). The twig length-total leaf area relationship demonstrated increased scaling exponents under low and medium nitrogen, but the exponent decreased significantly under high nitrogen, returning to control levels, indicating that high nitrogen reduced investment in leaf area expansion.

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3. Discussion

Current-year twig traits reflect plant growth status and adaptation to environmental changes. Under nitrogen addition, only twig length showed significant differences among all twig traits in *M. sieversii* seedlings, indicating that twig length is the most responsive trait, increasing initially then decreasing with nitrogen concentration—a pattern consistent with results for *Moringa oleifera* tertiary branch length under nitrogen addition. Plant phenotypic plasticity is primarily manifested in allocation and trade-offs among organs. Ratio indices in *M. sieversii* showed more pronounced responses to nitrogen addition than biomass indices. Specific leaf area (SLA) reflects leaf resource acquisition capacity, and its stability in *M. sieversii* suggests that nitrogen addition did not significantly alter photosynthetic capacity. Studies show that enhanced photosynthetic capacity from nitrogen addition is primarily expressed at the physiological level (e.g., increased chlorophyll content, enzyme activity), which may not be captured by morphological traits alone.

Leafing intensity (LI), representing leaf number per unit stem mass, reflects plant lushness. High nitrogen significantly reduced LI in *M. sieversii*, consistent with allometric analysis showing that plants decreased LI while increasing investment in individual leaves under high nitrogen. Leaf area ratio (LAR) and leaf-to-stem mass ratio (LAMR) reflect allocation proportions between leaves and stems. Significant reductions in both indices under medium nitrogen indicate that low and medium nitrogen treatments increased stem investment while leaf morphology and biomass were relatively insensitive to nitrogen addition. This does not necessarily indicate unchanged photosynthetic capacity but rather that *M. sieversii* maintains stable leaf functional traits under varying nitrogen conditions.

The isometric relationship between stem and leaf biomass across all treatments contrasts with studies on *Arabidopsis thaliana*, where nitrogen addition altered biomass allocation patterns. This discrepancy may reflect different response strategies between woody and herbaceous plants. Woody plants generally maintain fixed growth rates between leaf and stem biomass across environmental gradients, as observed in temperate species along altitudinal gradients. The allometric results for *M. sieversii* show that at equal twig length growth rates, low and medium nitrogen treatments increased total leaf area growth rates, indicating greater investment in photosynthesis and relief from nitrogen limitation. At high nitrogen levels, this increased leaf area growth rate declined to control levels, suggesting reduced investment in leaves and photosynthesis under high nitrogen.

Overall, *M. sieversii* exhibited promoted photosynthesis and growth under low and medium nitrogen but inhibition under high nitrogen. Species vary in their sensitivity to nitrogen addition: some studies show no photosynthetic inhibition at high nitrogen concentrations, while others demonstrate clear suppression. Conifers appear more sensitive to nitrogen addition than deciduous broadleaf trees, which have higher nitrogen tolerance thresholds. Most experiments based on nitrogen deposition rates may not reach the threshold for broadleaf trees, whereas our study, using orchard application rates, exceeded the tolerance threshold of *M. sieversii*, producing negative effects. Unbalanced nutrient addition can also negatively affect photosynthesis and yield, suggesting high nitrogen inhibition may reflect nutrient imbalance.

Nitrogen addition caused limited variation in leaf traits of *M. sieversii*, with changes occurring primarily at the individual leaf level. The allometric relationships among treatment groups showed no significant differences in slopes, indicating consistent proportional growth among indices. However, intercept shifts occurred in several trait pairs, reflecting altered allocation patterns. For example, the low and medium nitrogen treatment curves for the twig length-total leaf area relationship shifted upward significantly, while the high nitrogen treatment curve shifted downward. At a given basal diameter, low nitrogen treatment produced the highest LI with smallest, lightest leaves, while high nitrogen treatment showed lower LI with fewer but larger, heavier leaves. This trade-off between leaf size and leafing intensity under different nitrogen concentrations aligns with previous research.

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4. Conclusion

Under low and medium nitrogen treatments, sensitive twig traits in *M. sieversii* seedlings showed increasing trends, particularly for stem investment. High nitrogen treatment produced no such increases, and allometric relationships differed from those under low and medium nitrogen, indicating negative effects on growth. Appropriate nitrogen addition can improve twig growth and functional relationships between leaves and stems. We recommend avoiding excessive nitrogen fertilization during artificial seedling cultivation while implementing targeted fertilization based on habitat differences during native habitat transplantation. This approach will maintain optimal seedling growth and reduce mortality of young individuals. Investigating seedling responses to nitrogen addition provides crucial theoretical support for *M. sieversii* population restoration and conservation. Further research is needed to elucidate the internal response mechanisms of leaf-stem size relationships to different nitrogen doses and to develop scientifically sound fertilization protocols.

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