Water Use Efficiency of Pioneer Tree Species in Reclaimed Ecosystems of Open-pit Coal Mines in the Loess Hilly Region (Postprint)

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Abstract

Plant water use efficiency (WUE) reflects the balance between carbon fixation capacity and water consumption, revealing plant strategies for environmental adaptation. This study examined three pioneer tree species (Pinus tabuliformis, Ulmus pumila, and Robinia pseudoacacia) in reclaimed ecosystems of the Pingshuo opencast coal mining area in Shanxi Province—a typical opencast coal mine in the Loess Hilly Region of China—with different reclamation years (5, 15, 20, and 28 years). Based on measurements of leaf carbon (C), nitrogen (N) contents and C/N ratios, stable isotope techniques were used to analyze the temporal patterns of species WUE with reclamation years, and the correlation between leaf C/N and plant WUE was analyzed. The results showed: (1) Leaf C and N contents of Robinia pseudoacacia and Ulmus pumila increased with reclamation years, while the highest values of leaf C and N contents of Pinus tabuliformis appeared in 15-year and 20-year reclamation sites, respectively. Leaf C content and C/N ratio of Pinus tabuliformis were significantly higher than those of Ulmus pumila, while N content was lower than the other two species ($P<0.05$). Leaf N content of Robinia pseudoacacia was significantly higher than the other two species ($P<0.05$). (2) The patterns of WUE with reclamation years for the same species were as follows: Pinus tabuliformis had the highest WUE at 5-year reclamation and the lowest at 28-year reclamation; Ulmus pumila had the highest WUE at 28-year reclamation and the lowest at 5-year reclamation; Robinia pseudoacacia had the highest WUE at 15-year reclamation and the lowest at 5-year reclamation ($P<0.05$). Inter-specific differences in WUE showed that Pinus tabuliformis was higher than Ulmus pumila and Robinia pseudoacacia. (3) WUE of the three tree species showed significant positive correlations with leaf C content and C/N ratio, but no significant correlation with leaf N content. These results reveal the WUE characteristics of pioneer tree species in vegetation reconstruction of the Pingshuo mining area,

providing a theoretical basis for vegetation restoration in opencast coal mines in the Loess Hilly Region.

Full Text

Preamble

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Water Use Efficiency of Pioneer Tree Species in Reclamation Ecosystem of Open-Pit Coal Mine in Loess Hilly Region

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Abstract: Plant water-use efficiency (WUE), which measures the balance between carbon assimilation and water consumption, serves as a vital indicator of plant adaptation strategies to environmental constraints. This study investigated the dynamics of leaf WUE in pioneer tree species within the reclaimed ecosystem at the Pingshuo open-pit coal mine in Shanxi Province, a representative site in China's loess hilly region. Focusing on three dominant pioneer species—*Pinus tabuliformis*, *Ulmus pumila*, and *Robinia pseudoacacia*—across a chronosequence of reclamation ages (5, 15, 20, and 28 years), we determined leaf carbon (C) and nitrogen (N) concentrations, C/N ratios, and long-term WUE inferred from stable carbon isotope composition (δ^{13}C). The relationship between leaf C/N stoichiometry and WUE was also analyzed. The results revealed: (1) Leaf C and N concentrations in *R. pseudoacacia* and *U. pumila* generally increased with reclamation age, whereas *P. tabuliformis* peaked in C and N levels at 15 and 20 years, respectively. *P. tabuliformis* had significantly higher leaf C concentration and C/N ratio compared to *U. pumila* ($P<0.05$) but lower N concentration than the other two species ($P<0.05$). *R. pseudoacacia* maintained the highest leaf N concentration among the species ($P<0.05$). (2) Intra-specific WUE varied significantly ($P<0.05$) with reclamation age: *P. tabuliformis* had the highest WUE at five years and the lowest at 28 years; *U. pumila* peaked at 28 years and was at its minimum at five years; *R. pseudoacacia* reached its maximum WUE at 15 years and minimum at five years. Inter-specifically, *P. tabuliformis* consistently exhibited higher WUE than both *U. pumila* and *R. pseudoacacia*. (3) Across all three species, leaf WUE showed significant positive correlations with leaf C concentration and C/N ratio, whereas its correlation with leaf N concentration was insignificant. These findings elucidate the species-specific WUE characteristics and adaptive physiological adjustments of pioneer trees during vegetation succession in the Pingshuo reclaimed mine area, offering

a theoretical foundation for vegetation restoration efforts in open-pit coal mines within the loess hilly region.

Keywords: Pingshuo open-pit coalmine; water use efficiency; stable isotope technology; nutrient content

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1.1 Study Area

The Pingshuo open-pit mining area is located in the Jin-Shaan-Mongolia border region of the Loess Plateau (112°10 ~113°30 E, 39°23 ~39°37 N), characterized by a temperate semi-arid continental monsoon climate. The region has an average annual temperature of 7.8 °C and average annual precipitation ranging from 428.20 to 449.00 mm. The area contains four major aquifers, with the Cambrian-Ordovician limestone karst fissure aquifer being the only water-rich layer; the remaining aquifers have low water content, resulting in generally scarce surface and groundwater resources. Soil types are primarily chestnut and cinnamon soils with organic matter content of 3–10 $g \cdot kg^{-1}$. Vegetation coverage is low and dominated by drought-tolerant species. Land reclamation began in 1985, forming three major waste dumps (West, South, and Inner). After more than 30 years of vegetation reconstruction, the reclaimed dump areas have achieved over 80% vegetation coverage, establishing a multi-layered plant community structure dominated by *Hippophae rhamnoides*, *Robinia pseudoacacia*, *Pinus tabuliformis*, and *Ulmus pumila*.

1.2 Sample Collection and Measurement

In August 2023, we selected sample plots of *P. tabuliformis*, *U. pumila*, and *R. pseudoacacia* at different reclamation ages (5, 15, 20, and 28 years) in the West and South waste dumps of the Pingshuo mining area. Five trees were randomly selected in each plot, and 50 healthy, pest-free leaves were collected from each tree. Leaves were placed in self-sealing bags, labeled with species and growth year information, and transported to the laboratory. After washing with pure water to remove surface soil and dust, samples were oven-dried at 105 °C for 30 minutes to deactivate enzymes, then dried at 65 °C to constant weight. Dried leaves were ground into powder using a mortar and pestle, sieved through a 0.15 mm mesh, and stored for analysis.

Leaf C and N concentrations were measured using an Elementa Vario Micro Cube carbon-nitrogen elemental analyzer. For $\delta^{13}C$ analysis, samples were combusted at high temperature in an elemental analyzer to produce CO_2, which was purified and introduced into a Picarro G2131-i CO_2 isotope analyzer. Sample $\delta^{13}C$ values were calculated by comparing the $^{13}C/^{12}C$ ratio against international standard materials (Vienna Pee Dee Belemnite, VPDB). Each sample was measured three times, with the mean value used for analysis. Measurement precision was <0.3‰.

1.3 Plant Water Use Efficiency Calculation

Water use efficiency (WUE) was calculated using the formula:

$$WUE = \frac{C_a \times (b - d - \phi \times (t - d))}{1.6 \times (b - \delta^{13}C_p)}$$

where C_a represents atmospheric CO_2 concentration (387.91 $\mathrm{mol \cdot mol^{-1}}$), b is the isotopic fractionation coefficient during carboxylation (27‰), d is the diffusion fractionation coefficient (4.4‰), t is the fractionation coefficient during CO_2 dissolution (0.7‰), ϕ is the ratio of CO_2 leakage from bundle sheath cells to ambient air (0.3), and $\delta^{13}C_p$ is the plant carbon isotope ratio.

Carbon isotope discrimination (Δ) was calculated as:

$$\Delta = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p/1000}$$

where $\delta^{13}C_a$ is the atmospheric carbon isotope ratio (‰) and $\delta^{13}C_p$ is the plant carbon isotope ratio (‰).

1.4 Data Statistics and Analysis

Statistical analyses were performed using IBM SPSS Statistics software. One-way ANOVA was used to compare differences in leaf C, N, C/N ratios, δ^{13}C values, and WUE among different reclamation ages for the same species, as well as differences among species at the same reclamation age. The significance level was set at $\alpha = 0.05$. Data visualization was conducted using Origin 2022 software.

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2 Results and Analysis

2.1 Variation Characteristics of Leaf C, N, and C/N Ratio with Reclamation Age

Leaf C concentration in *P. tabuliformis* ranged from 474.0–509.4 $\mathrm{mg \cdot g^{-1}}$, peaking at 15 years and showing a trend of initial increase followed by decrease. Leaf N concentration varied from 7.1–25.6 $\mathrm{mg \cdot g^{-1}}$, with the highest value at 20 years and the lowest at 5 years. The C/N ratio ranged from 19.35–71.68, peaking at 20 years and reaching its minimum at 15 years. For *U. pumila*, leaf C concentration ranged from 377.2–448.2 $mg \cdot g^{-1}$, increasing with reclamation age and peaking at 28 years. Leaf N concentration varied from 20.4–38.3 $mg \cdot g^{-1}$, with the highest value at 28 years and lowest at 15 years. The C/N ratio ranged from 11.48–21.56, peaking at 5 years and reaching its minimum at 28 years. In *R. pseudoacacia*, leaf C concentration ranged from 433.6–481.9 $mg \cdot g^{-1}$, increasing with reclamation age and peaking at 28 years. Leaf N concentration varied from 38.2–49.2 $mg \cdot g^{-1}$, with the highest value at 28 years and lowest at 5 years. The C/N ratio ranged from 9.72–11.35, decreasing initially then increasing, with the maximum at 5 years and minimum at 20 years.

Inter-specific comparisons revealed that *P. tabuliformis* had significantly higher leaf C concentration and C/N ratio than *U. pumila* ($P<0.05$), but significantly lower leaf N concentration than the other two species ($P<0.05$). *R. pseudoacacia* maintained significantly higher leaf N concentration than both *P. tabuliformis* and *U. pumila* ($P<0.05$).

[Figure 2: see original paper]

2.2 Variation Characteristics of Leaf $\delta^{13}C$ and WUE with Reclamation Age

Leaf $\delta^{13}C$ values in *P. tabuliformis* ranged from –27.79‰ to –25.99‰, peaking at 5 years and declining with reclamation age. In *U. pumila*, $\delta^{13}C$ varied from –29.52‰ to –26.89‰, increasing gradually with reclamation age and peaking at 28 years. For *R. pseudoacacia*, $\delta^{13}C$ ranged from –29.33‰ to –28.31‰, showing a slight decreasing trend with reclamation age.

Mean WUE values were 98.12 ± 9.67 $mol \cdot mol^{-1}$ for *P. tabuliformis*, 82.93 ± 15.23 $mol \cdot mol^{-1}$ for *U. pumila*, and 76.91 ± 2.01 $mol \cdot mol^{-1}$ for *R. pseudoacacia*. *P. tabuliformis* exhibited significantly higher WUE than both *U. pumila* and *R. pseudoacacia* ($P<0.05$). Intra-specific WUE varied significantly with reclamation age ($P<0.05$): *P. tabuliformis* peaked at 5 years and was lowest at 28 years; *U. pumila* peaked at 28 years and was lowest at 5 years; *R. pseudoacacia* reached maximum WUE at 15 years and minimum at 5 years.

2.3 Relationship Between Leaf WUE and C, N, and C/N Ratio

Across all three species, leaf WUE showed significant positive correlations with leaf C concentration ($R^2 = 0.32$, $P<0.05$) and C/N ratio ($R^2 = 0.22$, $P<0.05$), but no significant correlation with leaf N concentration ($R^2 = 0.03$, $P>0.05$).

[Figure 3: see original paper]

3 Discussion

3.1 Characteristics of WUE Across Tree Species and Reclamation Ages

Water use efficiency is a complex eco-physiological trait influenced by multiple factors including climatic conditions, soil properties, plant growth stage, and physiological regulation mechanisms. These factors collectively affect photosynthetic capacity and stomatal conductance, thereby influencing leaf WUE. Our results demonstrate distinct patterns among species across reclamation ages: *P. tabuliformis* showed highest WUE at the early reclamation stage (5 years), declining thereafter; *U. pumila* exhibited continuously increasing WUE with reclamation age, peaking at 28 years; *R. pseudoacacia* showed highest WUE at 15 years, with a slight decline thereafter but an overall increasing trend with reclamation age. These divergent patterns reflect unique adaptive strategies among species during ecosystem succession in the reclaimed mine area.

As a pioneer species, *P. tabuliformis* exhibited high WUE during early reclamation stages, likely associated with its rapid adaptation to the harsh environment of newly reclaimed sites. During initial vegetation restoration, reclaimed sites are characterized by high temperature, high compaction, low nutrients, and low moisture. *P. tabuliformis* can enhance photosynthetic efficiency while reducing transpiration water loss to improve WUE, ensuring survival and growth under harsh conditions, which explains its high survival rate during early restoration. As reclamation age increased, soil moisture conditions gradually improved and vegetation competition intensified, potentially shifting *P. tabuliformis* growth strategies toward biomass accumulation rather than water conservation, leading to decreased WUE. This "early water-saving, later growth" strategy offers clear ecological advantages in resource-limited environments.

In contrast, WUE in both *U. pumila* and *R. pseudoacacia* increased with reclamation age, likely related to enhanced root development and environmental adaptability. As reclamation time and tree age increased, root systems of these species gradually extended deeper and expanded, enabling more effective acquisition of water and nutrients from deeper soil layers, thereby alleviating water stress and improving WUE. Additionally, as drought-tolerant species, *U. pumila* and *R. pseudoacacia* gradually adapted to water-deficient conditions during long-term growth in arid regions, developing more efficient water use mechanisms such as finer stomatal regulation and stronger osmotic adjustment capacity. The significant increase in *U. pumila* WUE with reclamation age demonstrates strong physiological plasticity and adaptive potential, suggesting it could play increasingly important ecological functions during later reclamation stages.

The inter-specific comparison (P. tabuliformis > U. pumila > R. pseudoacacia) reflects fundamental differences in water use strategies among plant functional groups. In our study, *P. tabuliformis* leaf WUE was significantly higher than both *U. pumila* and *R. pseudoacacia*, which may be attributed to growth strategies, environmental adaptability, and inherent biological characteristics. As an evergreen conifer, *P. tabuliformis* typically exhibits higher WUE than deciduous broadleaf species. Evergreen species have longer leaf lifespan and extended photosynthetic periods, while conifer needles have smaller surface area, effectively reducing water transpiration and achieving higher WUE under equivalent water conditions. Furthermore, *P. tabuliformis* has relatively low soil moisture requirements, demonstrating strong adaptability in arid and semi-arid regions through precise stomatal conductance regulation to reduce water consumption and improve WUE. These characteristics collectively contribute to the superior WUE performance of *P. tabuliformis* in the Pingshuo reclaimed ecosystem.

3.2 Relationship Between Leaf WUE and C, N, and C/N Ratio

Leaf nutrient content serves as a crucial indicator of plant growth status, photosynthetic capacity, and environmental adaptability, influenced by soil conditions, climate factors, and management practices. Carbon is the product of photosynthesis and forms the basis of plant growth and metabolism, while nitrogen is an essential macronutrient for photosynthesis and chlorophyll synthesis. The relationship between C and N during plant development involves complex trade-offs, and adequate N supply typically enhances photosynthetic efficiency and plant WUE.

Our findings show significant positive correlations between leaf WUE and both C concentration and C/N ratio across all three species, but no significant correlation with N concentration. This suggests that in the resource-limited environment of the Pingshuo reclaimed mine area, *P. tabuliformis* may adopt a growth strategy prioritizing C accumulation and efficient water use. Specifically, high leaf C concentration indicates strong C storage capacity, while high C/N ratio suggests relatively low N demand or more efficient utilization of limited N. *P. tabuliformis* may compensate for relatively low N by enhancing leaf C fixation capacity and allocating more C to structural biomass (e.g., cellulose, lignin) to improve drought resistance and structural stability. This resource allocation strategy is particularly advantageous in environments with limited nutrients and water.

In contrast, although *U. pumila* and *R. pseudoacacia* had relatively lower C concentrations, *U. pumila* still exhibited relatively high WUE, suggesting different resource utilization strategies and adaptive mechanisms. The C/N ratio not only reflects plant nutrient status but also indicates soil nutrient conditions. In the Pingshuo reclaimed mine area, *P. tabuliformis* had relatively high C/N ratio, indicating lower N demand during growth or more efficient use of limited N. *R. pseudoacacia*, as a nitrogen-fixing species, can enhance soil N content through biological nitrogen fixation, promoting nutrient cycling and soil fertility improvement. Therefore, reclamation planning should consider mixed forest designs that leverage complementary effects among different species to ensure efficient water resource utilization while promoting nutrient cycling and soil improvement, thereby accelerating ecosystem function recovery and environmental improvement in mining areas.

4 Conclusion

This study investigated the water use efficiency characteristics of three pioneer tree species (*Pinus tabuliformis*, *Ulmus pumila*, and *Robinia pseudoacacia*) in the reclaimed ecosystem of the Pingshuo open-pit coal mine using stable isotope technology. The results demonstrate significant inter- and intra-specific variations in leaf WUE across reclamation ages. *P. tabuliformis* exhibited the highest WUE among the three species, with significant positive correlations between WUE and leaf C concentration and C/N ratio. These findings provide scientific evidence for optimizing species selection and vegetation management strategies in the ecological restoration of open-pit coal mines in the loess hilly region.

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Note: Figure translations are in progress. See original paper for figures.