

## Effects of Simulated Soil Erosion Stress on the Physiological and Growth Characteristics of *Artemisia ordosica* in Coal Mining Subsidence Areas (Postprint)

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### Abstract

To reveal the stress survival strategies of soil and water conservation plants in semi-arid coal mining subsidence areas, 2-3-year-old *Artemisia ordosica* was used as experimental material, and in-situ root fracture experiments were conducted on its roots to simulate soil erosion stress. Four treatments were established: severe stress (P1), moderate stress (P2), mild stress (P3), and control (CK), to investigate the response variations in growth indicators, photosynthetic characteristics, and physiological stress reactions of *Artemisia ordosica* to stress. The results demonstrated: (1) Soil erosion stress significantly inhibited the growth rate of *Artemisia ordosica*, with greater erosion damage leading to more pronounced growth suppression. Following severe stress, the growth rates of plant height, crown width, branch length, and branch diameter of *Artemisia ordosica* decreased by an average of 36.91%, 43.90%, 69.76%, and 66.76%, respectively, compared with the control group. (2) Soil erosion stress exerted a marked negative effect on photosynthesis in *Artemisia ordosica*, and the greater the erosion damage, the stronger the negative feedback. After severe stress, the leaf net photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration, transpiration rate, and chlorophyll content of *Artemisia ordosica* decreased by 39.86%, 59.26%, 7.82%, 51.55%, and 12.33%, respectively, compared with the control group. (3) Within 70 days of erosion damage occurrence, the SOD activity, POD activity, and CAT activity in leaves of *Artemisia ordosica* exhibited a trend of initial increase, followed by decrease, and subsequent stabilization compared with the control group, while MDA content fluctuated within a certain range. Redundancy analysis indicated that SOD activity had the most significant influence on the photosynthetic characteristics of *Artemisia ordosica*. Comprehensive analysis revealed that root fracture in *Artemisia ordosica* caused

by soil erosion damage in coal mining subsidence areas reduces its growth rate and inhibits leaf photosynthesis; however, *Artemisia ordosica* can maintain its basic growth by regulating its antioxidant enzyme activity, establishing it as an ecological restoration plant species with excellent stress resistance and adaptability in erosion areas.

## Full Text

### Effects of Simulated Soil Erosion Stress on Physiological and Growth Characteristics of *Artemisia ordosica* in Coal Mining Subsidence Areas

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## Abstract

To reveal the survival strategies of soil and water conservation plants in semi-arid coal mining subsidence areas under adverse conditions, 2-3-year-old *Artemisia ordosica* plants were used as experimental materials. In-situ root fracture experiments were conducted to simulate soil erosion stress, with four treatments: severe stress (P1), moderate stress (P2), mild stress (P3), and control (CK). The responses of growth indices, photosynthetic characteristics, and physiological stress reactions of *A. ordosica* to the stress were studied. The results showed that: (1) Soil erosion stress significantly inhibited the growth rate of *A. ordosica*, and the greater the degree of simulated damage, the more significant the growth inhibition. After severe stress, the growth rates of plant height, crown width, branch length, and branch diameter of *A. ordosica* decreased by an average of 36.91%, 43.90%, 69.76%, and 66.76%, respectively, compared to the control group. (2) Soil erosion stress had a significant negative effect on the photosynthesis of *A. ordosica*, and the greater the degree of damage, the stronger the negative feedback. After severe stress, the net photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration, transpiration rate, and chlorophyll content of *A. ordosica* leaves decreased by 39.86%, 59.26%, 7.82%, 51.55%, and 12.33%, respectively, compared to the control group. (3) Within 70 days after erosion damage, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in *A. ordosica* showed a trend of first increasing, then decreasing, and then stabilizing compared with the control group, while the malondialdehyde (MDA) content fluctuated within a certain range. Redundancy analysis showed that SOD activity had the most significant effect on the

photosynthetic characteristics of *A. ordosica*. Comprehensive analysis indicated that root fracture of *A. ordosica* caused by soil erosion damage in coal mining subsidence areas reduces its growth rate and inhibits leaf photosynthesis, but *A. ordosica* can maintain its basic growth by regulating its antioxidant enzyme activity, making it an ecological restoration plant species with excellent stress resistance and adaptability in erosion areas.

**Keywords:** *Artemisia ordosica*; coal mining subsidence area; root fracture; soil erosion stress; physiological and growth characteristics

## Introduction

The Shendong Mining Area is a typical ecologically fragile zone located in the southern Ordos Plateau, characterized by harsh natural environments, low vegetation coverage, and frequent multi-force erosion events [1]. For many years, coal mining activities have caused varying degrees of surface displacement and deformation, triggering large-scale ground subsidence and development of ground fissures [2], which has intensified soil erosion, destroyed vegetation, and consequently affected natural vegetation growth and community composition. These changes have also altered vegetation community structure and ecosystem stability at local scales [3]. Plant roots play a crucial role in both anchoring soil to resist erosion and in absorbing and transporting water and nutrients. Leaf photosynthesis serves as the primary source of materials and energy required for plant growth and development [4], and can also be used to evaluate plant growth vigor and stress resistance [5]. Combining leaf and root studies provides a better understanding of plant adaptation mechanisms to erosive stress environments [6]. Plants produce stress responses in erosive environments by regulating antioxidant enzyme activities and osmotic substances to maintain cell structural integrity and normal physiological function [7]. Currently, most systematic research has focused on the effects of root cutting on morphological characteristics [8] and biomass accumulation [9] in fruit trees or grassland plants, while studies on how soil erosion damage causing root fracture affects leaf photosynthetic physiological characteristics remain limited.

*Artemisia ordosica* is a shrub species widely distributed in semi-arid mining areas and plays an important role in ecological restoration and vegetation recovery efforts. This study used *A. ordosica* from coal mining subsidence areas as experimental material to simulate the destructive process of soil erosion on *A. ordosica* roots through in-situ root fracture experiments. The dynamic response characteristics of photosynthetic physiology and growth traits to soil erosion stress were analyzed to elucidate the stress adaptation mechanisms of *A. ordosica*, providing a scientific basis for vegetation restoration and conservation in semi-arid coal mining subsidence areas.

## 1. Materials and Methods

### 1.1 Study Area

The study area is located in the Shendong Mining Area in the southern Yijinhuoluo Banner of Ordos City, Inner Mongolia Autonomous Region. Situated on the southern Ordos Plateau, northern Loess Plateau, and southeastern edge of the Mu Us Sandy Land, the geographic coordinates are 110°05'51" - 110°30'12" E, 39°27'45" - 39°15'34" N. The region has a temperate semi-arid continental monsoon climate, with a minimum temperature of -28.1°C and maximum temperature reaching 38.9°C. Annual evaporation is 1636-2535 mm, while annual precipitation is 300-500 mm, mostly concentrated in summer and autumn. The area experiences strong winds and frequent sandstorms, with severe wind erosion. Prevailing winds are westerly and north-westerly, with an average annual wind speed of  $3.6 \text{ m} \cdot \text{s}^{-1}$  and maximum wind speed reaching  $22.0 \text{ m} \cdot \text{s}^{-1}$ . The soil is primarily aeolian sandy soil with loose surface layers. Extensive coal mining has created large goaf areas, and gravity erosion has caused plant tilting and death. Vegetation consists mainly of xerophytic and semi-xerophytic shrubs and herbs, with dominant species including *Artemisia ordosica*, *Caragana microphylla*, *Salix psammophila*, and *Corethrodedron fruticosum*.

### 1.2 Experimental Materials and Design

The soil erosion stress experiment was conducted in July 2021 during the vigorous growth period of *A. ordosica*. In the study area, 2-3-year-old *A. ordosica* plants were randomly selected, and their plant height, crown width, and ground diameter were measured to calculate average values as standard plant indices. Four experimental plots (20 m × 30 m each) were established in areas with good plant growth. Within each plot, 12 *A. ordosica* plants meeting the standard indices were selected as test plants, for a total of 48 samples. The basic growth status of standard *A. ordosica* plants is shown in .

To simulate root damage caused by soil erosion in coal mining subsidence areas, this study designed an in-situ root fracture experiment. Using the plant base as the center, a circle with a radius of 50 cm was drawn on the ground surface and divided into four sectors. Each sector was excavated sequentially with a shovel to a depth of 40 cm (since *A. ordosica* root systems are concentrated horizontally within 0-40 cm and vertically within 0-60 cm). When roots were encountered during excavation, they were cut with pruning shears. After cutting, the original soil was backfilled. The experiment included four erosion stress gradients: P1 (severe stress: excavating sector A, cutting approximately 23.86% of roots); P2 (moderate stress: excavating sectors A+B, cutting approximately 49.20% of roots); P3 (mild stress: excavating sectors A+B+C, cutting approximately 75.54% of roots); and CK (control: same soil excavation disturbance but without root cutting). Each treatment had 12 replicate plants. The erosion stress treatment method is illustrated in [Figure 1: see original paper].

### 1.3 Measurements

**1.3.1 Growth Measurements** Growth changes of *A. ordosica* were observed and recorded on days 1, 10, 21, 35, 50, and 70 after root fracture treatment (or until plant death). For each test plant, three healthy branches were selected and marked. Branch length was measured on each observation day, and the base diameter of marked branches was measured using electronic calipers (0.01 mm precision) with a cross method. Plant height and crown width were also measured precisely.

**1.3.2 Photosynthetic Parameter Measurements** On days 1, 10, 21, 35, 50, and 70 after root fracture treatment, a Li-Cor Li-6800 portable photosynthesis system was used to measure net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO<sub>2</sub> concentration (Ci) of test plant leaves. Measurements were taken between 10:00–11:00, with three healthy leaves from the top of each test plant branch measured repeatedly and instantaneous values recorded. Leaf area was determined by scanning leaves fixed to graph paper with transparent tape and importing the images into AutoCAD 2007 to calculate actual leaf area. During photosynthetic measurements, approximately 0.5 g of leaves from the base of sun-exposed branches were collected. Chlorophyll content was measured using the dimethyl sulfoxide method: leaves were placed in centrifuge tubes with dimethyl sulfoxide, sealed and kept in darkness for three days, then absorbance was measured at 645 nm and 663 nm using a UV spectrophotometer.

**1.3.3 Physiological Index Measurements** On days 1, 10, 21, 35, 50, and 70 after root fracture treatment, healthy leaves were collected from each test plant to determine physiological indices, with three replicates for each index. Peroxidase (POD) activity was measured using the guaiacol method; superoxide dismutase (SOD) activity using the nitroblue tetrazolium method; catalase (CAT) activity using the UV absorption method; and malondialdehyde (MDA) content using the thiobarbituric acid method [10].

**1.3.4 Statistical Analysis** Excel was used for data processing and statistical testing, SPSS 20.0 for one-way ANOVA and least significant difference (LSD) multiple comparisons to test differences in physiological and growth indices of *A. ordosica* under different soil erosion stress levels ( $P < 0.05$ ). Origin 2018 was used for graphing. Pearson correlation analysis was used to analyze the relationship between photosynthetic characteristics and physiological stress responses after erosion stress, and Canoco 5 was used for redundancy analysis to determine the influence of various factors, with correlation analysis plots drawn using R language.

## 2. Results

### 2.1 Effects of Erosion Stress on Growth Characteristics of *A. ordosica*

As shown in [Figure 2: see original paper], during the entire experimental period (1-70 days after stress), plant height, crown width, branch length, and branch diameter of *A. ordosica* under different erosion stresses were significantly lower than those of the control group. After severe stress, these indices decreased by 36.91%, 43.90%, 69.76%, and 66.76% respectively compared to the control; after moderate stress, they decreased by 25.69%, 19.58%, 45.59%, and 44.01%; and after mild stress, they decreased by 17.24%, 8.40%, 26.81%, and 23.48%. Overall, root fracture caused by soil erosion stress significantly inhibited the growth of *A. ordosica*, with significant differences in growth indices among different stress levels ( $P < 0.05$ ), indicating that erosion stress limited the growth and development of *A. ordosica*.

### 2.2 Effects of Erosion Stress on Photosynthetic Characteristics of *A. ordosica*

As shown in [Figure 3: see original paper], during the entire experimental period (1-70 days after stress), Pn, Gs, Ci, Tr, and chlorophyll content of *A. ordosica* leaves under erosion stress were significantly lower than those of the control group. After severe stress, these indices decreased by 39.86%, 59.26%, 7.82%, 51.55%, and 12.33% respectively; after moderate stress, they decreased by 27.31%, 47.46%, 4.75%, 31.89%, and 6.23%; and after mild stress, they decreased by 14.50%, 18.99%, 5.31%, 12.75%, and 2.34%. Overall, as erosion stress intensity increased, root fracture caused by soil erosion stress significantly inhibited the photosynthetic characteristics of *A. ordosica*, with significant differences among treatments ( $P < 0.05$ ), indicating that photosynthesis in *A. ordosica* was suppressed by erosion stress.

### 2.3 Effects of Erosion Stress on Physiological Characteristics of *A. ordosica*

As shown in [Figure 4: see original paper], during the entire experimental period (1-70 days after stress), SOD, POD, and CAT activities in *A. ordosica* leaves showed a trend of first increasing, then decreasing, and gradually stabilizing compared with the control group, while MDA content fluctuated within a certain range. SOD, POD, and CAT activities peaked at 10 days after stress. Throughout the experiment, after severe stress, SOD, POD, and CAT activities decreased by an average of 31.41%, 12.17%, and 1.93% respectively, while MDA content increased by an average of 28.33%; after moderate stress, SOD, POD, and CAT activities decreased by an average of 54.82%, 36.83%, and 20.37%, while MDA content increased by an average of 16.87%; and after mild stress, SOD, POD, and CAT activities decreased by an average of 4.57%, 10.63%, and 15.47%, while MDA content increased by an average of 36.83%. Overall, with increasing erosion stress intensity, the physiological indices of *A. ordosica* showed

significant differences ( $P < 0.05$ ).

## 2.4 Correlation Analysis of Photosynthetic and Physiological Indices

As shown in [Figure 5: see original paper], after erosion stress, Pn in *A. ordosica* leaves was extremely significantly positively correlated with Gs ( $P < 0.01$ ); Tr was significantly positively correlated with Ci ( $P < 0.05$ ); SOD, POD, and CAT activities were extremely significantly positively correlated with each other ( $P < 0.01$ ); and MDA content was extremely significantly negatively correlated with SOD, POD, and CAT activities ( $P < 0.01$ ). Redundancy analysis ([Figure 6: see original paper]) was used to further analyze the influence of physiological stress responses on photosynthetic characteristics after erosion stress. The first and second constraint axes explained 21.45% and 12.9% of the variation in photosynthetic characteristics, respectively. The results showed that SOD activity was the most important factor affecting photosynthetic characteristic changes, while POD activity was a secondary factor.

## 3. Discussion

### 3.1 Effects of Soil Erosion Stress on Growth Characteristics of *A. ordosica*

Roots are important contributors to material and energy exchange between soil and aboveground plant parts. When roots are subjected to stress damage, whole-plant growth becomes retarded or even stagnates [11]. In this study, the growth rates of plant height, crown width, branch length, and branch diameter of *A. ordosica* showed severe retardation with increasing root fracture intensity. Andersen's study [12] showed that fruit tree height, crown width, branch number, and branch length were inhibited after root cutting, and Sun's research [13] found that both aboveground and underground biomass of maize gradually decreased with increasing root damage severity. Zhang et al. [14] also confirmed that root damage significantly inhibited plant height, crown width, and branch length of *Caragana microphylla*. This inhibition occurs because root fracture caused by frequent gravity erosion in semi-arid mining areas weakens plants. In this study, root fracture reduced plant water acquisition from soil, decreased root hydraulic conductivity and water transport rates within roots to the stem base [15], blocked water and nutrient transport functions, reduced photosynthates, and lowered the growth rate of *A. ordosica*. Ground subsidence and fissure development in coal mining subsidence areas caused *A. ordosica* root fracture and reduced the number of living roots, leading to decreased water and nutrient absorption capacity and inhibited normal growth and development. Even after root cutting treatment, *A. ordosica* did not die during the experimental period, possibly because it adapted to erosion stress by reducing aboveground functional traits to ensure individual survival.

### 3.2 Effects of Soil Erosion Stress on Photosynthetic Characteristics of *A. ordosica*

Photosynthesis produces large amounts of dry matter that provide essential basic materials for biochemical reactions within plants [16]. In this study, as root fracture intensity increased, Pn, Gs, Ci, Tr, and chlorophyll content of *A. ordosica* significantly decreased. After root damage, the water transport function of xylem vessels was partially lost, inhibiting stem sap flow and causing a substantial decrease in leaf transpiration rate, which weakened leaf photosynthetic capacity. These results are consistent with Zhao' s findings [17] of photosynthetic inhibition in *Robinia pseudoacacia* and *Ulmus pumila* after root cutting, and Feng' s conclusion [18] that root cutting significantly reduced photosynthetic and transpiration rates in *Platyclusus orientalis* seedlings. Stomatal and non-stomatal limitations are the main factors affecting photosynthesis. Ci values can be used to identify limiting factors: when Pn, Gs, and Ci all decrease, stomatal factors limit photosynthesis; when Pn decreases while Ci increases, non-stomatal factors are limiting [19]. In this study, during the initial (1–10 days) and intermediate (11–21 days) stages after erosion stress, both Pn and Gs of *A. ordosica* decreased significantly as damage increased, while Ci also decreased, indicating that photosynthetic rate decline during this period was mainly due to Gs limitation preventing sufficient CO<sub>2</sub> from entering stomata [20], resulting in Ci levels that could not meet photosynthetic demands. However, during the later stage (22–70 days), although Pn and Gs decreased substantially, Ci showed a slight increase, indicating that non-stomatal limitations became dominant. This shift occurred because long-term erosion stress adversely affected photosynthetic organs and decreased photosystem II activity [21], weakening leaf photosynthesis. Under severe erosion stress, chlorophyll content in *A. ordosica* decreased significantly because extensive root fracture destroyed the root system, interrupted water transport, caused leaf functional loss, and resulted in reactive oxygen species accumulation that damaged chloroplast structure, thereby hindering chlorophyll synthesis and photosynthetic efficiency. This is consistent with Liu et al.' s [22] findings of decreased chlorophyll content in *Sophora moorcroftiana* seedlings after root cutting. In this study, the inhibitory effects on photosynthetic characteristics of *A. ordosica* persisted for a long time after stress, because soil erosion stress accelerated surface water evaporation, created unfavorable environments such as soil drought, caused water limitations, damaged cell membranes, and affected the photosynthetic system [23], leading to metabolic disorders.

### 3.3 Effects of Soil Erosion Stress on Physiological Characteristics of *A. ordosica*

Plant physiological indices are sensitive indicators of life cycles and environmental changes [24] that reflect the ability to resist external adverse conditions [25]. This study showed that under severe erosion stress, *A. ordosica* could maintain relatively high antioxidant enzyme activities. SOD activity was the most im-

portant factor affecting photosynthetic characteristic changes, possibly because *A. ordosica* rapidly produced large amounts of reactive oxygen species after root fracture damage. By increasing antioxidant enzyme activities to eliminate excess hydrogen peroxide and reactive oxygen, the plant could activate its emergency antioxidant system promptly. Higher SOD activity indicates stronger resistance, as it can counteract and block damage to cells from oxygen free radicals [26]. This demonstrates that within certain erosion stress levels, *A. ordosica* can maintain normal physiological metabolism and reduce cell membrane lipid peroxidation by regulating its protective enzyme system activity, thereby improving its ability to adapt to erosion stress.

MDA is a product of membrane lipid peroxidation that directly reflects cell damage degree [27]. In this study, during the initial and intermediate stages after severe stress, MDA content increased significantly compared with the control, indicating substantial damage to the membrane system. This occurred because extensive root fracture and other external stimuli generated large amounts of free radicals and induced significant membrane lipid peroxidation, producing peroxides that destroyed the cell membrane system and could lead to cell death. Similar results were reported by Yang et al. [28]. However, during the later stage of severe stress, MDA content decreased significantly compared with the control, indicating that reactive oxygen species production and elimination in *A. ordosica* reached a relatively balanced state, and the effect of root fracture on MDA content gradually decreased, demonstrating good adaptability to adverse conditions.

#### 4. Conclusion

Soil erosion in coal mining subsidence areas significantly affects plant growth and physiological processes. Ground subsidence or fissure development causes *A. ordosica* root fracture, significantly inhibiting growth rates of plant height, crown width, and branches, with more severe erosion damage showing more obvious growth inhibition. This type of soil erosion also significantly inhibits leaf photosynthesis in *A. ordosica*, with Pn, Gs, Ci, Tr, and chlorophyll content decreasing significantly as erosion stress intensity increases, gradually reaching dynamic equilibrium with prolonged stress time. After erosion stress, *A. ordosica* shows obvious physiological stress responses to regulate adverse feedback on photosynthetic characteristics. Changes in SOD activity are the main factor affecting photosynthetic characteristics. Over a relatively long period after this type of soil erosion occurs, the growth, photosynthetic, and physiological characteristics of *A. ordosica* can gradually stabilize, indicating that under soil erosion stress dominated by ground subsidence and fissure development in semi-arid coal mining subsidence areas, *A. ordosica* can still maintain normal growth and photosynthetic physiological metabolism by regulating its antioxidant enzyme system activity. This species exhibits excellent stress resistance and adaptability, making it a valuable ecological restoration plant for erosion areas.

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