Respiratory Characteristics of Excised Bothriochloa ischaemum Roots under Different Water Supply Conditions: A Postprint Based on Stable Carbon Isotope Tracing

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Date: 2018-05-18T00:00:00+00:00

Abstract

Plant root respiration constitutes a major component of soil respiration, and investigating root respiration is of significant importance for ecosystem carbon budget and balance. Employing the 13C pulse labeling technique, we compared changes in excised root respiration rates and 13C isotopic ratios of Bothriochloa ischaemum across different root excision times (0, 6, 24, 48, 216, 360 h after labeling) under three water supply conditions, and analyzed the correlations between root parameters and excised root respiration. The results demonstrated that: 1) The temporal variation trend of excised root respiration rates was consistent across different excision times, with no significant differences observed among the three water supply conditions; all treatments exhibited a sharp decline within 0-20 min, with the decrease ranging from 32% to 39%. 2) Measuring the variation in 13C released by excised root respiration at different excision times provides a novel approach for real-time monitoring of the release process of 13CO2 transferred to Bothriochloa ischaemum roots; across different excision times, the mean 13C values released by root respiration within 2 h under the three water supply conditions followed the order: well-watered > mild stress > severe stress. As excision time progressed (0-360 h), the mean 13C released by root respiration first increased and then decreased, reaching a peak value of 31.46‰ at 216 h. 3) Excised root respiration rate and 13C released by root respiration were significantly influenced by root area, specific root area, nitrogen content, C/N ratio, and root tissue 13C. 4) Mild water stress could simultaneously promote root growth (C fixation) and root respiration (C metabolism).

Full Text

Excised Root Respiration Characteristics of *Bothriochloa ischaemum* Under Different Water Supply Conditions Measured Using Stable Carbon Isotope Techniques

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Abstract

Plant root respiration constitutes a primary component of soil respiration, and investigating root respiration is crucial for understanding ecosystem carbon budgets and balances. This study employed stable carbon isotope pulse-labeling technology to examine changes in excised root respiration rates and the ^{13}C isotopic ratios released from root respiration in *Bothriochloa ischaemum* under three water supply conditions at different excision times (0 h, 6 h, 24 h, 48 h, 216 h, and 360 h after labeling), and analyzed the relationships between root parameters and excised root respiration. The results showed that: (1) Excised root respiration did not differ significantly across the three water supply conditions at various excision times, but declined sharply by 32%–39% within the first 20 minutes. (2) Determining changes in ^{13}C isotopic ratios in excised roots after different excision times provided new insights into the release process of photosynthetic carbon transferred to the roots of *B. ischaemum*. The mean ^{13}C value was highest under well-watered conditions, intermediate under moderate drought stress, and lowest under severe drought stress. (3) Both excised root respiration rate and the ^{13}C isotopic ratio released from root respiration were significantly influenced by root area, specific root area, nitrogen concentration, C/N ratio, and root tissue ^{13}C. (4) Moderate drought stress contributed to a simultaneous increase in root growth rate (carbon fixation) and root respiration (carbon metabolic rate).

Keywords: *Bothriochloa ischaemum*; water supply condition; ^{13}C pulse labeling; excised root respiration rate; ^{13}C released from root respiration

Introduction

Soil respiration represents a critical component of ecosystem carbon cycling, serving as both an important source of atmospheric CO and a major output pathway for soil carbon pools. Global annual CO release from soils amounts to approximately 98 Pg, making it the second largest carbon flux in terrestrial ecosystems. Elevated soil respiration significantly increases atmospheric CO

concentrations, exacerbating the greenhouse effect. Plant roots are the primary source of soil respiration, with 40%–70% of carbon fixed through photosynthesis being released back to the atmosphere via root respiration. Research indicates that root respiration accounts for the majority of soil respiration, and carbon loss through root respiration constitutes a substantial proportion of total soil carbon efflux. Therefore, studying root respiration is essential for revealing ecosystem carbon budgets and biosphere carbon balance.

Environmental factors such as soil moisture and nutrients affect root respiration by influencing the formation of plant photosynthates and their allocation among leaves, stems, and roots, which subsequently impacts root morphology and respiration. Methods for measuring plant root respiration are categorized into direct and indirect approaches. Direct methods include excised root techniques and root chamber methods, while indirect methods involve root trenching and root exclusion techniques. These methods can determine root respiration rate variations and the contribution of root respiration to total soil respiration. However, different measurement methods yield different estimates; for instance, in temperate grassland studies, the contribution of root respiration to total soil respiration measured by isotope methods and root exclusion methods showed significant differences.

Stable carbon isotope (^{13}C) pulse-labeling technology offers a novel approach for studying organic carbon input and output in root respiration, with advantages including high sensitivity, minimal disturbance, and theoretical robustness. This technique can quantitatively investigate carbon input to roots and rhizosphere respiration, but cannot reflect the metabolic processes of photosynthetic carbon after transport from shoots to roots. Few studies have examined the temporal respiration release process of carbon in roots under different water conditions, which is critical for understanding carbon balance.

Bothriochloa ischaemum is a perennial grass species (Poaceae) typical of arid environments, characterized by rapid reproductive capacity, rainfall interception, water conservation, soil retention, and strong regional adaptability. It has become a highly productive grass species in the loess hilly region of northern Shaanxi Province. With its well-developed reticulate root system, *B. ischaemum* is an important plant for carbon storage in arid and semi-arid regions and plays a significant role in degraded grassland restoration on the Loess Plateau. Water stress is the most common environmental constraint in these regions, and roots serve as the exchange organ for material transport between soil and plants. Studying root responses under different water supplies is therefore crucial for revealing the essential nature of plant drought resistance.

While measuring ^{13}C released from excised root respiration can reveal patterns in respiration rate changes, it cannot explain the transport and release processes of photosynthetic carbon after it reaches the roots. Since soil moisture, root morphology, and root tissue nutrient content can all influence excised root respiration, this study employed stable carbon isotope pulse-labeling to investigate excised root respiration and its influencing factors under three water supply con-

ditions: high water (80% field capacity), moderate water (60% field capacity), and low water (40% field capacity). This approach provides a new perspective for quantitatively studying the respiration release process of photosynthetic carbon transported to roots and for understanding root carbon sequestration.

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1. Experimental Materials

The soil used in this experiment was loess collected from 0–30 cm depth in northern Shaanxi in May 2015. Surface humus and litter were removed, and the soil was uniformly mixed, air-dried, and sieved. *Bothriochloa ischaemum* seeds were collected from undisturbed grasslands in northern Shaanxi in October 2015, stored in paper bags under natural conditions until use.

The experiment was conducted as a pot-controlled study using custom-made plexiglass containers. Each container held 2.5 kg of soil with a bulk density matching that of the sampling site (1.2 g/cm^3). The containers measured 19 cm × 4 cm × 27 cm. A total of 3880 g of soil was used across all treatments.

2. Experimental Design

Figure 1 [Figure 1: see original paper] shows the double-layer sealed plexiglass labeling chamber used for ^{13}C pulse labeling of *B. ischaemum* seedlings. The inner chamber dimensions were 50 cm × 50 cm × 80 cm, with gas-tight lids and 8 mm diameter inlet/outlet ports on opposite sides for gas exchange. All connections were sealed with Vaseline.

Each plexiglass container was sown with *B. ischaemum* seeds. After germination, only the most vigorous seedling per container was retained, with replanting performed for non-germinated spots. Water control treatments began on June 15, 2016, with three soil moisture gradients maintained by weighing: - High water (HW): 80% field capacity - Moderate water (MW): 60% field capacity - Low water (LW): 40% field capacity

Blank soil samples were established for each treatment. Soil water content was controlled using the weighing method, with regular weighing and watering. The experiment concluded on August 25, 2016. The initial biomass of seedlings was recorded before water control treatments. Since the difference between initial and final biomass accounted for only 0.1% of the total pot weight and the control period was relatively short, the effect of biomass increase on water treatment control was considered negligible.

3. Experimental Measurement Methods

3.1 ^{13}C Pulse Labeling Labeling was performed in the double-layer sealed plexiglass chamber between 9:00–12:00. Each water treatment randomly selected 12 *B. ischaemum* seedlings. A beaker containing approximately 2 g of

Na CO (^{13}C = -25‰) was placed inside, connected to a liquid delivery tube and a gas delivery tube extending outside the chamber. The labeling chamber was monitored using a CO isotope mass spectrometer (Los Gatos Research, USA). Initial CO concentration was 450 mol/mol. ^{13}C-labeled Na CO (^{13}C = 5000‰) was injected via syringe to maintain CO at 400–450 mol/mol. If ^{13}C fell below 4000‰, additional ^{13}C-labeled Na CO was added. Labeling lasted 120 minutes, with chamber temperature maintained at 27–28°C.

3.2 Sample Collection and Excised Root Respiration Measurement After pulse labeling, samples were collected and excised root respiration was measured at six time points: 0 h, 6 h, 24 h, 48 h, 216 h, and 360 h. For each collection, roots were manually separated from soil, rinsed with distilled water, blotted dry, and immediately placed in a custom root respiration chamber connected to a CO isotope mass spectrometer. Measurements were taken in high-speed mode, recording data every 5 minutes for 0–2 h to determine root respiration rates. After scanning, roots were oven-dried at 105°C for 30 minutes, then at 75°C to constant weight, and finally ground for analysis.

3.3 Stable Carbon Isotope and Chemical Component Analysis Root morphology parameters were determined immediately after collection using a scanner (Expression 1680, ETSON) and root image analysis software (WinRHIZO Tron 2005a, Regent Instruments, Canada). Specific root length was calculated as the ratio of root length to corresponding root dry weight.

Root tissue ^{13}C was measured using an elemental analyzer (MultiN/C 3100, Analytik Jena AG, Germany). Approximately 0.005–0.006 g of ground root sample was combusted at 1050°C in a solid combustion chamber, and the generated CO was analyzed by isotope mass spectrometer. ^{13}C values were expressed relative to the PDB (Pee Dee Belemnite) standard using the formula: ^{13}C (‰) = [(^{13}C/^{12}C)sample/(^{13}C/^{12}C)PDB - 1] × 1000.

Root nitrogen concentration was determined using a Kjeldahl nitrogen analyzer (Kjeltec 2300, Foss Tecator AB, Sweden), and C/N ratio was calculated.

4. Data Analysis

All experimental data were analyzed using Microsoft Excel 2013 and SPSS 21.0. Linear regression and one-way ANOVA were used to analyze relationships between root parameters and root respiration.

Results

1. Characteristics of Excised Root Respiration Rates Under Different Water Supply Conditions Under all three water conditions, excised root respiration rates showed consistent trends across different excision times:

a sharp initial decrease within 0–20 min, followed by a slight increase, then a decrease, and finally stabilization. Within the first 20 minutes after pulse labeling, root respiration declined by 32%–39% (32.80%, 35.18%, and 33.82% for HW, MW, and LW, respectively), with no significant differences among water treatments. Respiration rates reached their lowest values at approximately 60 minutes, after which changes stabilized.

Over the entire 0–360 h period, excised root respiration rates showed a bimodal pattern under HW and MW treatments, with the first peak occurring at 0–48 h (356 g g ^{1} h ^{1} for HW, 338 g g ^{1} h ^{1} for MW) and the second peak at 216 h (419 g g ^{1} h ^{1} for HW, 413 g g ^{1} h ^{1} for MW). Under LW treatment, respiration showed a single peak at 216 h (431 g g ^{1} h ^{1}), followed by fluctuating changes.

Figure 2 [**Figure 2: see original paper**] illustrates changes in excised root respiration rates with respiration time under different water supply conditions.

2. ^{13}C Values Released from Root Respiration Under Different Water Supply Conditions Environmental conditions during measurement were stable, with chamber temperature at 27–28°C and CO concentration at 300–450 mol/mol. Under all three water conditions, ^{13}C values released from root respiration showed an initial increase followed by a decrease over time.

Under HW treatment, ^{13}C values increased gradually from 0–70 min post-labeling, peaked at 80 min, then declined slowly. Under MW treatment, values rose slowly from 0–45 min, peaked at 80 min, then decreased. Under LW treatment, values increased rapidly from 0–50 min, peaked at 90 min, then declined slowly. The mean ^{13}C values ranked as: HW > MW > LW.

Over the 0–360 h period, ^{13}C values released from root respiration increased initially then decreased, with the highest value of 31.46‰ occurring at 216 h under HW treatment. The increase magnitude was greatest under HW (417.78%), intermediate under MW (213.40%), and smallest under LW (375.67% increase to 19.64‰ at 216 h, then decreasing to 16.60‰ at 360 h).

Figure 3 [**Figure 3: see original paper**] and **Figure 4** [**Figure 4: see original paper**] show changes in ^{13}C values with root excision time and respiration time, respectively.

3. Root Parameter Characteristics Under Different Water Supply Conditions Root parameters varied significantly among water treatments (**Table 1**). Under HW treatment, total root length and root area increased significantly, being 2.7–3.0 times greater than under LW treatment. Specific root length was significantly greater under LW treatment compared to the other treatments, while specific root area showed no significant differences. Root nitrogen concentration increased with water supply, while C/N ratio decreased with increasing water availability. Root carbon concentration showed no significant differences among treatments.

4. Correlations Between Excised Root Respiration and Root Parameters Under Different Water Supply Conditions Correlation analysis revealed that under HW conditions, excised root respiration rate was not significantly affected by root morphological or nutrient factors. However, under MW conditions, root respiration rate showed significant positive correlations with root area, specific root area, and root tissue ^{13}C. Under LW conditions, root respiration rate was significantly positively correlated with root area and specific root area.

Root-respired ^{13}C showed significant positive correlations with root area and specific root area under MW conditions, and significant positive correlations with C/N ratio under LW conditions. Root tissue ^{13}C was significantly negatively correlated with root nitrogen concentration under MW conditions.

Table 2 presents the correlation matrices between root respiration and root parameters under different water supply conditions.

Discussion

1. Real-Time Monitoring of ^{13}C Changes for Accurate Description of Excised Root Respiration Excised root methods and ^{13}C isotope techniques are primary approaches for measuring root respiration, which constitutes a significant contribution to soil respiration. The release of recently photosynthesized carbon represents plant carbon transformation and utilization. In this study, excised root respiration rates declined rapidly by 32%–39% within the first 20 minutes, then stabilized—a pattern consistent with previous studies on various species including pine, maple, oak, and barley.

This rapid initial decline occurs because after root excision, easily decomposable organic matter is quickly metabolized, while transport of photosynthates from shoots cannot meet the immediate carbon demand. Subsequently, as easily decomposable substrates are exhausted, respiration stabilizes. The short-term wave-like variations likely result from root damage stimulating rhizosphere microbial activity, but limited carbon availability constrains sustained microbial activity.

While excised root respiration rates describe overall post-excision changes, they cannot reflect the temporal allocation process of photosynthetic carbon from shoots to roots, particularly the time-dependent release of photosynthates after reaching the roots. Using ^{13}C pulse labeling to trace photosynthetic carbon transferred to roots and measuring ^{13}C in root-respired CO addresses this limitation. Our results show that ^{13}C values peaked at 216 h post-labeling, consistent with studies on maize and Inner Mongolian grasslands indicating that the majority of root-respired carbon appears 240 h after labeling. This demonstrates that our approach effectively tracks the transport and respiratory release of photosynthetic carbon to roots.

Furthermore, ^{13}C values were influenced by water stress, with greater water availability delaying and increasing the ^{13}C peak (HW > MW > LW). This aligns with findings that root respiration rates increase with soil moisture up to a threshold, and that drought reduces ^{13}C values in plant tissues due to decreased photosynthetic capacity and ^{13}C discrimination.

2. Effects of Root Parameters on Carbon Fixation and Respiration Under Different Water Conditions Under HW conditions, root-respired ^{13}C showed a highly significant negative correlation with root tissue ^{13}C. When water is abundant, plants grow well and fix more ^{13}C-depleted carbon, but enhanced root respiration releases more ^{13}C-enriched CO , creating a competitive relationship that results in negative correlation.

Under MW conditions, root respiration rate was significantly positively correlated with both root-respired ^{13}C and root tissue ^{13}C. Moderate water stress promotes root growth and increases root area, enhancing absorption capacity. The simultaneous increase in photosynthetic carbon fixation and root respiration creates positive correlations among these parameters.

Under LW conditions, root respiration rate was significantly positively correlated with root area and specific root area. Severe water stress causes roots to proliferate in search of water, increasing root length and area. Since respiration occurs primarily through root surfaces, larger root area directly enhances respiration. The increased allocation of photosynthates to roots under stress also contributes to positive correlations between root tissue ^{13}C and respiration parameters.

Root-respired ^{13}C was significantly positively correlated with C/N ratio under LW conditions. Severe water stress reduces nitrogen absorption capacity, prompting plants to allocate more carbon to roots to enhance nitrogen uptake, which increases C/N ratio and respiration rate—a pattern consistent with cost-benefit theories of root function.

Conclusion

1. Excised root respiration rates of *B. ischaemum* under three water supply conditions showed no significant differences across excision times but declined consistently by 32%–39% within the first 20 minutes, reflecting the intrinsic respiration rate level.
2. Using ^{13}C released from root respiration after ^{13}C pulse labeling enables real-time monitoring of photosynthetic carbon transport to and respiratory release from roots. Over 0–360 h post-labeling, ^{13}C values showed an initial increase followed by decrease, peaking at 216 h with a maximum of 31.46‰.

3. Both excised root respiration rate and root-respired ^{13}C were significantly affected by root area, specific root area, C/N ratio, and root tissue ^{13}C across water treatments.

4. Moderate water stress can promote root growth and enhance root respiration, providing new insights for quantitatively estimating the transport and metabolic relationships of photosynthetic products to belowground tissues.

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