13C Pulse Labeling: Allocation of Photosynthetic Carbon in Rice at Different Growth Stages in the Plant-Soil System Postprint

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Date: 2017-10-30T00:00:00+00:00

Abstract

Quantifying the allocation patterns of plant photosynthetic carbon in plant tissue-soil systems during the growth period is of great significance for understanding the global carbon cycle. Using 13C-CO2 pulse labeling combined with laboratory incubation, and analyzing 13C values of various plant parts and soil through elemental analyzer-stable isotope ratio mass spectrometry (Flash HT-IRMS), this study compared the allocation patterns of rice photosynthetic carbon among different tissues across various growth stages and quantified the transfer of rice photosynthetic carbon to the soil carbon pool. The results showed that: (1) The dry matter mass of rice aboveground parts and root system exhibited an increasing trend with the advancement of rice growth stages, with the performance across different growth stages being: tillering stage > jointing stage > heading stage > flowering stage > maturity stage. The root-to-shoot ratio across the entire growth period ranged from 0.2 to 0.4, being highest at the tillering stage and decreasing with advancing rice growth stages, stabilizing at approximately 0.2 after the heading stage. (2) Six hours after pulse labeling, the 13C values of rice aboveground and underground parts (root system) ranged from -25.52‰ to -28.33‰, with significant fractionation effects observed among different organs, and the trend was generally consistent, i.e., stem (grain) > leaf (root); this phenomenon of carbon isotope fractionation among organs caused by the characteristics of rice growth stages can be used to indicate the allocation and fate of rice photosynthetic carbon under different growth stages. (3) The allocation patterns of 13C-photosynthetic carbon in the plant-soil system differed across growth stages; during early growth stages, a high proportion of photosynthetic carbon was allocated to the root system and soil, indicating a strong carbon sink capacity, while with advancing growth stages, the allocation proportion of photosynthetic carbon to the root system

and soil showed a decreasing trend, though the accumulation amount continuously increased. (4) The allocation proportions of 13C-photosynthetic carbon in the rice-soil system showed significant differences across growth stages. During the rice tillering stage, nearly 30% of photosynthetic carbon was used for root establishment and partially entered the soil organic carbon pool (10%) through root exudates, whereas at the maturity stage, more was allocated to grains, and the allocation proportion of photosynthetic carbon to soil also showed a decreasing trend with advancing growth stages. The research findings hold important theoretical significance for revealing the processes and regulatory mechanisms of soil organic carbon cycling in paddy fields.

Full Text

Preamble

ACTA ECOLOGICA SINICA (ChinaXiv Partner Journal)
Vol. 37, No. 19, October 2017
DOI: 10.5846/stxb201607241506

The 13C-CO2 Pulsing Labeling Method: Distribution of Rice Photosynthetic Carbon in Plant-Soil Systems During Different Rice Growth Stages

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Abstract

Quantifying the distribution of plant photosynthetic carbon within plant tissues and soil during growth stages is crucial for understanding the global carbon cycle. This study employed 13C pulse labeling combined with stable isotope analysis using an elemental analyzer-stable isotope ratio mass spectrometer (Flash HT-IRMS) to analyze carbon isotope values in various rice plant parts and soil. We compared the distribution patterns of rice photosynthetic carbon among different tissues across growth stages and quantified the transfer of rice photosynthetic carbon to the soil carbon pool. Results showed that both aboveground and root dry matter mass increased progressively with rice growth stage, while the root-shoot ratio decreased from its peak of approximately 0.4 during the tillering stage to about 0.2 during the heading stage. The 13C values of rice shoots and roots ranged from -25.52‰ to -28.33‰, demonstrating significant

isotopic fractionation among different plant organs that followed the order: stem (grain) > leaf > root. This fractionation pattern, caused by rice growth stage characteristics, can be used to indicate the allocation and fate of photosynthetic carbon at different developmental phases. The distribution of photosynthetic carbon in the plant-soil system varied across growth stages: during early growth, a high proportion of photosynthetic carbon was allocated to roots and soil, indicating a strong carbon sink capacity in the root-soil system. However, this proportion decreased with advancing growth stage, even as total accumulation increased. Notably, during the tillering stage, nearly 30% of photosynthetic carbon was allocated belowground for root development, with approximately 10% of this portion entering the soil organic carbon pool via root exudates. At maturity, more photosynthetic carbon was distributed to grains, and the proportion allocated to soil decreased with growth stage. These findings provide important theoretical insights into paddy soil organic carbon cycling processes and regulatory mechanisms.

Keywords: carbon isotope; terrestrial ecosystem carbon cycle; 13C-CO2 pulsing labeling; rice (Oryza sativa L.); stable isotope mass spectrometer (IRMS)

Introduction

Plants assimilate atmospheric carbon through photosynthetic carbon fixation pathways, converting it into organic matter. A portion of this photosynthetic carbon is translocated belowground for root growth, while some is transferred to soil through root deposits, root detritus, and root exudates [1]. Plant photosynthetic carbon constitutes a vital component of the terrestrial ecosystem carbon cycle and represents an important source of soil organic carbon [2]. Cropland ecosystems, heavily influenced by human cultivation activities, are a key focus of soil carbon cycle research in terrestrial ecosystems [3-4]. Paddy ecosystems are typical of China' s agricultural landscape, with rice paddies covering approximately 50% of the nation' s total cultivated land area and contributing about 50% of total grain production (China Statistical Yearbook, 2009-2014). Beyond food production, paddy ecosystems play crucial roles in mitigating global climate change and maintaining biodiversity. Quantifying the allocation of crop photosynthetic carbon among rice tissues and belowground components is essential for comprehensively understanding plant-soil interactions, the global carbon cycle, and sustainable soil utilization.

With the development and application of stable isotope technology, light elements such as hydrogen and carbon exhibit significant isotopic fractionation effects during natural cycling and turnover processes, making them effective tracers [5]. By measuring the natural abundance of carbon isotopes in soil or plants, stable carbon isotope tracing can effectively track the dynamic changes of plant photosynthetic carbon in carbon pools, including its transformation and accumulation, as well as quantitatively evaluate the relative contribution of plant

photosynthetic carbon to soil carbon reserves [6-9]. Common stable isotope detection methods include mass spectrometry, nuclear magnetic resonance, and cavity ring-down spectroscopy, with mass spectrometry being the most versatile and precise approach for stable carbon isotope analysis [10-11]. This study employed 13C-CO2 pulse labeling combined with elemental analyzer-stable isotope mass spectrometry to quantify the distribution of rice photosynthetic carbon in various plant tissues and soil, aiming to elucidate the processes and characteristics of farmland soil organic carbon cycling and provide a theoretical basis for regulating farmland soil organic carbon and achieving sustainable soil management.

Materials and Methods

1. Materials and 13C-CO2 Labeling

The experimental soil was a typical subtropical red soil collected from the Changsha Agricultural Environment Observation and Research Station of the Chinese Academy of Sciences. The basic physicochemical properties were: bulk density 1.31 g/cm³, organic carbon 18.1 g/kg, total nitrogen 1.8 g/kg, available phosphorus 0.43 g/kg, and pH 5.6. The rice variety was a conventional indica rice. The labeling material was 13C-Na2CO3 with 99.0 atom% purity (Sigma; CAS 93673-48-4).

Following field nitrogen application rates, (NH4)2SO4 was pre-mixed into the soil at 200 mg N/kg. Distilled water was added to achieve waterlogging conditions (water depth 2-3 cm). Three-leaf stage rice seedlings were transplanted into pots containing 5 kg of soil. After the rice plants established, pulse labeling was performed separately at the tillering, jointing, heading, flowering, and maturity stages. Labeling was conducted in a sealed plant growth chamber placed outdoors under adequate light conditions for 6 hours (9:00-15:00). The labeling chamber and method were adapted from Xiao and Ai [12] with appropriate modifications [13]. The CO2 concentration was controlled between 350-370 mg/kg using a Shen-QZD CO2 controller, and 13C-CO2 was generated by slowly and uniformly dripping 1 mol/L HCl into 1 mol/L 13C-Na2CO3 until the reaction was complete. Temperature was maintained at 24-32°C (SNT-96S temperature controller). Unlabeled rice plants placed in an area more than 2 m away from the labeling chamber served as controls for determining natural abundance in plants and soil.

2. Sample Preparation

After each labeling event, both labeled and unlabeled plant and soil samples were collected. Roots and seedlings were washed clean and drained. Plant samples were killed green at 105°C for 30 minutes, then dried in an oven at 60-70°C. Dried plant samples were ground using a planetary ball mill for determination

of organic carbon content and 13C values. Soil samples were thoroughly mixed, residual plant roots were removed, and the samples were immediately freeze-dried for 24 hours.

3. Analytical Methods

Plant and soil samples were analyzed for organic carbon and nitrogen content and their stable carbon isotope ratios using a Flash HT-IRMS system. The basic principle and measurement process involved high-temperature combustion of samples, with the reduction furnace temperature set at 950°C. A Thermal Conductivity Detector (TCD) measured organic carbon content, and remaining gases were introduced through a ConFlo IV interface into the stable isotope ratio mass spectrometer for 13C determination. The 13C content in samples was calculated as:

13C content = [(Atom%13C)sample - (Atom%13C)unlabeled] × TCsample

where (Atom%13C)sample represents the 13C atom percentage in the sample, (Atom%13C)unlabeled represents the natural abundance in unlabeled samples, and TCsample represents the total organic carbon content in the sample.

4. Statistical Analysis

Data were analyzed using one-way ANOVA (SPSS 13.0 for Windows). Duncan' s multiple comparison test was used for significance testing, and Microsoft Excel 2010 was used for data processing. Significance was determined at $P < 0.05$.

Results

1. Aboveground and Root Biomass of Rice

The dry matter mass of rice shoots and roots ranged from 4.9-40.9 g and 2.0-8.1 g, respectively, increasing progressively with growth stage. The greatest increase occurred from tillering to jointing stage, when plant growth rate increased significantly ($P < 0.05$). Root biomass showed a jump-like growth pattern from tillering to jointing stage ($P < 0.05$), while shoot dry weight increased only slightly from flowering to maturity ($P > 0.05$). After the heading stage, root biomass showed no significant increase.

The root-shoot ratio ranged from 0.2-0.4, peaking during the tillering stage and decreasing with advancing growth stage, stabilizing after the heading stage. This pattern likely reflects that during tillering stage, photosynthetic carbon is primarily translocated belowground for root development, while after jointing stage, allocation shifts toward stems, leaves, and panicles. Previous research [14] has indicated that plant photosynthetic carbon input to belowground components is closely related to root growth rate, with higher root carbon pool activity

during vegetative growth requiring greater carbon translocation, whereas at maturity, the proportion of photosynthetic carbon used for root tissue formation, root respiration, and rhizodeposition decreases.

[Figure 1: see original paper] The rice biomass of aboveground and belowground during different growth stages

2. Fractionation of Photosynthetic Carbon 13C in Rice Tissues

Unlabeled rice plants at different growth stages showed significant 13C fractionation between aboveground and belowground parts, with values ranging from -25.52‰ to -28.33‰. The fractionation followed a consistent trend across growth stages: stem (grain) > leaf > root (Table 1).

Carbon isotopic value measurement for unlabeled plant samples (13C vs PDB ‰)

Sample	Tillering	Jointing	Heading	Flowering	Maturity
Leaf	-	-	-	-	-
	27.75±0.27a	28.02±0.22a	27.83±0.20a	27.68±0.16b	27.82±0.24b
Stem	-	-	-	-	-
	25.52±0.31b	25.83±0.15b	25.71±0.21c	25.92±0.18c	26.01±0.33c
Grain	-	-	-	-	-
	27.83±0.30a	26.35±0.20b	26.15±0.12c	26.41±0.22c	28.33±0.41a
Root	-	-	-	-	-
	28.33±0.41a	28.22±0.27a	28.30±0.32a	28.31±0.26a	28.33±0.41a

Different letters within each column indicate significant differences ($P < 0.05$).

This fractionation occurs primarily because 13CO2 diffuses into plant leaves more slowly than 12CO2, and the 13C/12C ratio in plant carbohydrates is much lower than in atmospheric CO2 due to the relatively low carboxylation capacity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) [15]. Photosynthetic carbon generated in rice leaves undergoes isotopic fractionation during translocation, with pronounced fractionation effects in leaves and stems. This growth stage-dependent fractionation pattern [16] can indicate carbon allocation and fate. During vegetative growth, strong root activity results in more depleted photosynthetic carbon being transported to roots, causing a sharp 13C decrease in roots from tillering to jointing stage. After jointing, root 13C values remained relatively stable, suggesting that root tissues were established and no longer required large carbon translocation.

3. Allocation of Photosynthetic 13C in Rice-Soil Systems at Different Growth Stages

Photosynthetically synthesized organic carbon is transported from stems through phloem to roots and enters soil via rhizodeposition. Using stable

carbon isotope tracing, we tracked 13C-CO2 allocation in roots and soil. The 13C content in rice shoots, roots, and soil organic carbon pools ranged from 16.6-112.6 mg, 3.8-23.7 mg, and 2.6-5.1 mg, respectively (Table 2). Photosynthetic carbon increased with growth stage, with the largest increment from tillering to jointing stage, during which belowground allocation also increased significantly.

The contribution of 13C in shoots, roots, and soil organic carbon pools in rice-soil systems

Growth Stage	13C-Shoots (mg)	13C-Root (mg)	13C-SOC (mg)
Tillering	16.59 ± 2.77a	3.81 ± 0.65a	2.56 ± 0.12a
Jointing	64.44 ± 5.96b	5.57 ± 1.73b	2.79 ± 0.14a
Heading	94.39 ± 3.02c	7.20 ± 0.88c	3.61 ± 0.17b
Flowering	111.64 ± 4.71d	11.10 ± 1.30d	5.03 ± 0.11c
Maturity	112.57 ± 8.64d	23.70 ± 1.16d	5.11 ± 0.28c

Different letters within each column indicate significant differences ($P < 0.05$).

The allocation proportions of photosynthetic 13C in the rice-soil system varied significantly across growth stages. Shoots accounted for the largest proportion (72-81%), which increased with growth stage and peaked at flowering. Roots received 10-18% of photosynthetic carbon, while soil contained only 3.8-10%, with both proportions decreasing as growth progressed. These findings align with Lu et al. [17], who reported that most assimilated 13C remained aboveground while a smaller portion appeared belowground. The greater carbon allocation belowground during early growth compared to maturity reflects rice physiological characteristics—during early stages, high root carbon pool activity demands more carbon translocation. In this study, nearly 30% of photosynthetic carbon at tillering stage was allocated belowground for root development, with approximately 10% entering the soil organic carbon pool via root exudates. At maturity, more carbon was allocated to grains, and the proportion to soil decreased with growth stage. Werth and Kuzyakov [18] also noted that while the proportion of photosynthetic carbon allocated to roots and soil was high during early rice growth and decreased with growth stage, the absolute accumulation amount continued to increase.

[Figure 2: see original paper] The allocation of photosynthesized 13C into the rice plant-soil system during different growth stages

Conclusion

Accurate quantification of plant photosynthetic carbon and its turnover processes through stable isotope mass spectrometry is crucial for advancing our

understanding of terrestrial ecosystem carbon cycling and accurately estimating global carbon balance [19]. Using 13C stable isotope pulse labeling, this study investigated the distribution of photosynthetic carbon in rice at different growth stages. We confirmed the existence of carbon isotope fractionation among rice organs, found that photosynthetic carbon increased with growth stage, and demonstrated that carbon input to the soil organic carbon pool belowground also increased. Rice root carbon input benefits soil organic carbon accumulation. The allocation patterns of photosynthetic carbon in the plant-soil system differ across growth stages: early growth stages show high allocation to roots and soil (strong carbon sink capacity), while the proportion allocated to roots and soil decreases with advancing growth stage. These results provide important theoretical insights for understanding paddy soil organic carbon cycling processes and regulatory mechanisms.

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