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Advances in the Application of Stable Carbon Isotope Technology in Soil Organic Carbon Research: Postprint

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Date: 2021-03-02T00:00:00+00:00

Abstract

Soil carbon turnover represents a critical process of carbon migration and transformation among the atmosphere, biosphere, and lithosphere. Its subtle variations influence atmospheric CO₂ concentrations and alter carbon dynamics and partitioning between aboveground and belowground plant components. Although stable carbon isotope technology is widely employed in investigating carbon biogeochemical cycles across various temporal and spatial scales, comprehensive reviews specifically addressing its application to soil organic carbon research remain scarce. This study, based on a systematic compilation of current literature utilizing stable carbon isotope techniques to examine soil carbon origin, dynamics, and turnover, briefly summarizes the patterns of stable carbon isotope ($\delta^{13}\text{C}$) variation along terrestrial ecosystem plant leaf-litter-soil continua, leaf-soil continua, and within soils. The paper emphasizes the primary factors influencing soil carbon cycling and their corresponding adaptive mechanisms, while concurrently comparing natural ^{13}C abundance and artificial ^{13}C labeling methodologies. Furthermore, it identifies critical aspects requiring enhancement and outlines future research priorities and trends in applying stable carbon isotope approaches to soil carbon dynamic studies. Elucidating these processes and mechanisms will establish a foundation for predicting ecosystem source/sink functionality, strengthen quantitative investigations of terrestrial ecosystem carbon cycling, and advance understanding of soil carbon source/sink potential and the underlying mechanisms governing soil organic carbon turnover.

Full Text

Application of Stable Carbon Isotope Technique in Soil Organic Carbon Research: A Literature Review

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Abstract: Soil carbon turnover represents a critical process in carbon transfer among the atmosphere, biosphere, and lithosphere. Even minor changes in the soil carbon pool can significantly influence atmospheric CO₂ concentrations and the dynamics of above- and belowground carbon allocation. Stable carbon isotope technology is now widely applied in carbon biogeochemical cycle research across various temporal and spatial scales, yet comprehensive reviews of its application to soil organic carbon studies remain limited. This paper synthesizes current research on the use of stable carbon isotope techniques to investigate soil carbon origins, dynamics, and turnover. We summarize variation patterns of stable carbon isotopes ($\delta^{13}\text{C}$) in terrestrial ecosystem plant-litter-soil continuums, leaf-soil continuums, and soils. The review focuses on: (1) characteristics of stable carbon isotope technology in soil carbon cycling, (2) primary influencing factors and their adaptive patterns, (3) comparisons between natural and artificial ^{13}C labeling methods, and (4) key aspects requiring strengthening and future research directions. Clarifying these processes and mechanisms will provide a foundation for predicting ecosystem source-sink effects, enhance quantitative research on terrestrial ecosystem carbon cycling, and improve understanding of soil carbon source-sink potential and soil organic carbon turnover mechanisms.

Keywords: stable carbon isotope; $\delta^{13}\text{C}$; soil organic carbon; $\Delta\delta^{13}\text{C}$; influence factors

1 Principles of Stable Carbon Isotope Technology

Isotopes are different forms of the same chemical element with identical proton numbers but different neutron numbers (mass numbers). Isotopes are classified as either radioactive or stable. Stable carbon isotopes include ^{12}C and ^{13}C , with natural abundances of 98.89% and 1.11%, respectively. Isotopic abundance refers to the ratio of atoms of a specific isotope to the total atoms of that element in an isotopic mixture. The abundance ratio of heavy to light isotopes of the same element is commonly expressed as the isotope ratio (R), such as $^{13}\text{C}/^{12}\text{C}$. Since light isotopes are far more abundant than heavy isotopes in nature, the isotope ratio is very small. For convenience, the isotopic composition of samples is typically expressed using the δ notation, which represents the per mil (‰) difference between the sample's R value and that of a standard:

$$\delta(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where R_{standard} varies depending on the reference material, resulting in different δ values. The carbon isotope standard is Pee Dee Belemnite (PDB) from the Cretaceous Pee Dee Formation in South Carolina, USA, with an R value of 1.124×10^{-4} . Although this standard is now exhausted, artificially prepared standards with an R value of 1.11797×10^{-4} are commonly used. Different photosynthetic pathways (C₃, C₄, CAM) exhibit varying degrees of fractionation due to differences in carboxylase enzymes, leading to distinct carbon isotopic compositions among plants. Because of these differences in plant $\delta^{13}\text{C}$, the resulting surface humus and rhizodeposits also differ, ultimately affecting soil organic carbon isotopic composition.

2 Factors Influencing Natural Abundance of Soil Stable Carbon Isotopes

2.1 Temperature

Temperature directly and indirectly affects carbon decomposition processes by influencing soil microbial and enzyme activities and substrate availability, thereby altering soil organic carbon input and abundance. Although numerous studies have analyzed temperature effects on soil organic carbon, contradictory results exist and require further investigation. For example, under ^{13}C pulse labeling, control experiments show that unit temperature change affects C_{soil} . However, research on the Tibetan Plateau found negative correlations between C_{leaf} and temperature, and between C_{soil} and temperature. This may be because low temperatures in alpine regions limit soil organic matter decomposition and respiration, increasing soil organic carbon turnover time. Thus, lower temperatures correspond to higher $\delta^{13}\text{C}$ values. Additionally, temperature effects on soil nutrient mineralization and moisture may directly

or indirectly influence $\delta^{13}\text{C}$ values. Overall, temperature's influence on stable carbon isotopes remains inconclusive and warrants further study.

2.2 Precipitation

Water is a primary driver of soil carbon dynamics. Insufficient soil moisture inhibits microbial decomposition of organic carbon, while excessive moisture reduces soil oxygen content, decreasing microbial activity. Current research generally reveals a negative correlation between soil organic carbon $\delta^{13}\text{C}$ and moisture, though some studies report positive relationships. Under drought conditions, plants reduce leaf stomatal conductance, decreasing internal CO_2 concentration, which leads to higher $\delta^{13}\text{C}$ values. Conversely, soil substrates become enriched in heavy carbon (^{13}C). Microbial decomposition of plant debris preferentially selects lighter isotopes, further enriching the remaining substrate in ^{13}C . Consequently, vegetation with different carbon isotopic compositions significantly influences soil organic carbon $\delta^{13}\text{C}$.

Research across precipitation gradients shows that $\delta^{13}\text{C}$ values decrease with increasing precipitation, with a “turning point” occurring at approximately 3000 mm annual precipitation. This pattern may arise because extremely wet environments limit soil enzyme and aerobic microbial activity, slowing organic carbon decomposition rates. While study directions and magnitudes vary, most confirm that moisture significantly affects soil organic carbon $\delta^{13}\text{C}$.

2.3 Vegetation Type

Vegetation litter constitutes the primary source of soil carbon, with approximately 60 Pg of carbon annually input to soils. Vegetation type largely determines soil carbon pool quality. Studies show that $\delta^{13}\text{C}$ values are significantly enriched in surface soil layers, independent of soil physicochemical properties, and mainly influenced by isotopic fractionation during litter decomposition. $\delta^{13}\text{C}$ values are significantly negatively correlated with fine sand content and positively correlated with sand content. These patterns reflect that surface soil layers rich in organic matter have higher sand content, which decreases with depth, while deeper soils often exhibit greater $\delta^{13}\text{C}$ values. Soil element content directly affects the application and results of stable isotope technology in soil carbon turnover studies, with $\delta^{13}\text{C}$ serving as an indicator of substrate quality. Since microbial decomposition is limited by substrate quality, soils with high $\delta^{13}\text{C}$ values have lower decomposition rates. However, rapid decomposition in high $\delta^{13}\text{C}$ soils causes loss of light carbon (^{12}C) and enrichment of heavy carbon (^{13}C).

Most studies focus on plant responses to specific factors, with fewer investigating direct relationships between leaf and soil $\delta^{13}\text{C}$. Leaf morphology and thickness affect microbial decomposition rates, influencing soil carbon input and ultimately causing $\delta^{13}\text{C}$ variation. In alpine, plateau, and coastal ecosystems, litter type and surface vegetation significantly affect soil $\delta^{13}\text{C}$. Therefore,

studying individual soil or climate factors without considering vegetation may over- or underestimate their effects.

2.4 Soil Properties

At large scales, soil organic carbon $\delta^{13}\text{C}$ distribution is primarily influenced by climate and vegetation type. Due to high spatial heterogeneity and long turnover times, $\delta^{13}\text{C}$ distribution is also affected by soil factors such as texture, pH, and electrical conductivity. Generally, $\delta^{13}\text{C}$ decreases with increasing particle size. Larger soil particles are less favorable for microbial decomposition and contain less organic carbon. Soil pH and electrical conductivity are major factors affecting $\delta^{13}\text{C}$ changes, yet research results are contradictory. These conflicting findings warrant further investigation to better understand soil properties' role in stable isotope abundance studies and identify dominant factors controlling abundance changes.

3 Application of Stable Carbon Isotope Technology in Soil Carbon Cycle Research

3.1 Carbon Input and Release

Soil carbon primarily originates from litter decomposition and root exudate release. Plant litter is decomposed by soil microorganisms into organic carbon, while root exudates provide additional carbon inputs. Stable carbon isotope technology can distinguish carbon sources from different vegetation types (grassland, forest, shrubland) within the same region and characterize organic carbon sources and their vertical distribution, which reflects the mixing degree of new and old carbon. Due to different plant allocation ratios, soil stable isotopic composition varies, potentially causing carbon source differences at different soil depths. In mixed-stage soils, surface $\delta^{13}\text{C}$ values are significantly higher than other layers. The $\delta^{13}\text{C}$ of plant organic carbon ranges between -27‰ and -25‰ , higher than that of C plants (-15‰ to -13‰). Root distribution in the 0-10 cm layer also affects soil organic carbon $\delta^{13}\text{C}$ through rhizodeposition. When C plants appear in C communities, surface soil $\delta^{13}\text{C}$ is first affected, becoming more enriched as C plants increase.

Soil carbon release is a key component of the atmospheric CO_2 concentration and global climate change. Soil respiration constitutes the second-largest carbon emission source in most ecosystems, accounting for 60%-90% of total ecosystem respiration. Stable carbon isotope technology provides a new method for studying soil carbon release. Generally, soil respiration $\delta^{13}\text{C}$ begins depleted, becomes enriched, and finally stabilizes. However, because stable carbon isotope abundance changes cannot be measured directly, most studies conduct laboratory incubation experiments. This technology can also assess residence times of different components and their contributions to soil respiration.

3.2 Carbon Transformation

Natural Labeling: Researchers have used stable carbon isotope methods to study soil organic carbon decomposition and transformation rates. Zhao et al. investigated carbon decomposition in Tibetan Plateau marsh meadows, grassland meadows, and degraded meadows, finding that $\delta^{13}\text{C}$ increased with depth and that degraded meadows had higher decomposition rates. In Acton and Garten's research, the linear regression slope between soil $\delta^{13}\text{C}$ and the logarithm of soil organic carbon content was defined as the β value, which reflects organic carbon turnover rate. More negative slopes indicate faster microbial decomposition and organic carbon transformation. Since each carbon pool has different turnover rates, carbon movement from plants to soil exhibits time-lag effects. The $\delta^{13}\text{C}$ variation pattern effectively clarifies carbon changes from plant to soil pools, accurately recording climate information during plant growth and comprehensively reflecting plant carbon assimilation and soil carbon dynamics. Conducting $\delta^{13}\text{C}$ studies at different scales can fully explain carbon allocation characteristics in plant-soil systems.

Artificial Labeling: Artificial labeling involves continuous ^{13}C labeling throughout the plant growing season to quantitatively measure carbon input to soil at different times and study soil carbon turnover. Studies using ^{13}C pulse labeling on ryegrass found that ^{13}C concentration was highest in roots within 24-48 hours, likely because soil respiration rapidly loses most carbon while only a small portion of root deposition enters soil. Research on wheat in semi-arid regions showed that elevated CO_2 concentration (550 ppm) increased aboveground biomass by 13.5% and root biomass by 16.7%. Soil $\delta^{13}\text{C}$ increased significantly, especially when labeling during the grain-filling stage, indicating that soil carbon transfer occurs mainly during this period. Studies on temperate desert *Nitraria tangutorum* showed that newly fixed carbon concentrated in aboveground parts within 24-48 hours, gradually turning over to belowground parts, with approximately 31.9% stored in roots, 35.59% returned to atmosphere through respiration, and 15.8% stabilized in soil carbon pools.

Artificial labeling can directly calculate different proportions and quantities of soil carbon transformation, effectively clarifying carbon migration and conversion processes. However, high costs and precision limitations restrict wider application, and uneven ^{13}C distribution in plants and soils introduces sampling errors.

4 Conclusion

Stable carbon isotope technology can elucidate organic carbon sources, release, and transformation processes in terrestrial ecosystems. Integrating vegetation type, climate factors, and soil properties in stable carbon isotope studies will facilitate research on dominant factors controlling soil carbon turnover. With increasing instrument variety and automation of stable carbon isotope mass spec-

trometers and laser isotope analyzers, this technology is being widely applied in carbon cycle research. Overall, stable isotopes offer advantages in studying ecosystem carbon turnover, but limitations remain, including requirements for sample carbon content, relatively simple vegetation types under natural conditions, and varying times for organic matter return and decomposition.

Future research should focus on: (1) Standardizing methodologies, including sampling, measurement, and mathematical modeling procedures. Current solid samples require aluminum foil packaging and cold storage for laboratory analysis. (2) Improving studies on factors affecting carbon turnover, including anthropogenic activities (forest management, fire, clear-cutting, grazing) that directly or indirectly alter $\delta^{13}\text{C}$ values. (3) Strengthening molecular-level research, as stable carbon isotope ratios differ significantly among substances, enabling tracing of carbon flow in ecosystems. Current applications focus on individual plants, ecosystems, communities, and regional scales, but molecular-scale research, such as microbial decomposition effects on soil stable carbon isotope composition, remains limited. Future studies should enhance exploration at the soil organic carbon molecular level to deepen understanding of soil carbon turnover mechanisms.

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