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The application of isotope ratio mass spectrometry to the study of the ecophysiology of plant seeds

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Background

The isotope ratios of plant and animal tissues represent a temporal integration of significant physiological and ecological processes. The timescale of this integration depends on the element turnover rate in the tissue or pool in question.[1] In addition, isotopes indicate the presence and magnitude of key ecological processes.[2,3] The presence or absence of such processes, and even their magnitude in relation to other processes, are indicated by the stable isotope ratio value relative to known background levels. Many ecological processes produce a unique isotope fingerprint and, for that reason, it becomes possible to discriminate between the geographical origin of the plant or animal tissues.[4–6] The use of this physical parameter allows the discrimination between samples that may be identical from a chemical point of view. In cases where substances or residues accumulate in an incremental fashion, such as tree rings, animal hair and ice cores, isotope ratios can be used as a record of system response to changing environmental conditions or a proxy record for environmental change.

The $\delta^{13}C$ {$[R(^{13}C/^{12}C)_{sample}/R(^{13}C/^{12}C)_{standard} - 1] \times 1000$; R = absolute ratio} of organic and gas samples varies during the processing of carbon in the biogeosphere. The basis for much of the observed variation rests on the two major metabolic processes, photosynthesis and respiration,[7] with additional variation being expressed during biosynthetic, anabolic or catabolic reactions that rely on carbon-based substrates.[8] Plants contain less ^{13}C than the atmospheric CO_2 on which they rely for photosynthesis. They are therefore "depleted" of ^{13}C relative to the atmosphere. This depletion is caused by enzymatic and physical processes that discriminate against ^{13}C in favour of ^{12}C.

In plants, water availability can be important in inducing changes in stomatal physiology and/or biochemical discrimination that, in turn, are expressed in the $\delta^{13}C$ of photosynthetic products as well as tissues and compounds synthesised from these products.[9] Thus, when external factors exert a direct influence on these controls, the plant will integrate such influences, which become apparent in photosynthesis and are ultimately expressed as a change in the $\delta^{13}C$ of the resulting products.[10] When the sugars are used in the synthesis of the different components of the plant, additional fractionations occur in various compounds in the plant, so that cellulose and lignin exhibit lower $\delta^{13}C$ than the sugars formed in the leaf.[4] The nature of these fractionation processes, which have been modelled at leaf level,[11] is not yet fully characterised for other plant tissues (for example, seeds). The wide range of $\delta^{13}C$ values within biological and geological materials suggests that multiple and very different types of processes can lead to the observed variation.[12] As environments change in both space and time, it is therefore no surprise that we would expect changes in the $\delta^{13}C$ of many different types of materials to also occur. As such, $\delta^{13}C$ can serve as an important indicator of change.[13]

In addition, isotopes in water and other substances have been extensively used for almost five decades to improve understanding of hydrologic, climatologic and oceanographic processes as well as other environmental processes involving several geochemical cycles. At plant root level, there is no observable fractionation of O and H isotopes during water uptake.[14] The isotopic composition of water in roots and stems, therefore, reflects the isotopic composition of water available to the plant. There are two potential sources of water for plant roots. Water may be taken up from the deep, ground-water reservoir, or may be obtained from recent precipitation. While there is no fractionation of isotopes during water uptake by plants, fractionation does occur during transpiration. The isotopic composition of the source water is overprinted by the evaporative–transpirative signal in the leaf, which is largely influenced by vapour pressure deficit (relative humidity).[10] As a consequence, the biosynthetic compounds that incorporate H or O may or may not also have associated fractionations that are "recorded" in the

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organic molecules that contain these elements.[13] Many of these fractionation effects are among the largest known in biological systems, leading to highly enriched organic matter. In recent years, the processes that lead to enrichment in O and H of organic matter are becoming better understood and such materials are becoming increasingly more valuable as "biomarkers" of ecological change, largely because they are known to record temperatures, water sources and even levels of relative humidity that were present at the time of synthesis.

Another intensively studied biogeochemical cycle, yet still poorly understood from a stable isotope perspective, is the nitrogen cycle.[15] Nitrogen is the element that most often limits plant growth in many terrestrial ecosystems.[16] As studies using $\delta^{15}N$ data increased in the areas of physiology, ecology and biogeochemistry, it became clear that new challenges had to be faced in applying N isotopes to trace, integrate, or record certain process.[15] Of upmost importance is the challenge of characterising the many and varied fractionation factors associated with the transformation, utilisation and immobilisation of N substances as they move through the N cycle. Several authors state that plant $\delta^{15}N$ is not a tracer for nitrogen source; instead, it provides a synthesis of the $\delta^{15}N$ of the nitrogen source, of the fractionation events that occur during nitrogen absorption, of fractionations originating from different mycorrhizal associations and during assimilation, allocation and loss of nitrogen from the plant.[17]

Recently, it has also become apparent that S isotopes have great potential for detecting and, therefore, understanding the nature and magnitude of ecological change. The $\delta^{34}S$ values of major contributors to the atmosphere can be broadly subdivided into marine and continental sources, having both anthropogenic and natural components.[18] Sulfur is not just an atmospheric pollutant but also an essential nutrient for all vegetation. For most plants, the normal source of sulfur is sulfate, taken up from roots. The plant's assimilatory sulfate reduction proceeds without important sulfur isotope fractionations.[19] According to general experience, the bulk plant sulfur is depleted by only 1–2‰ relative to its primary sources, soil and sea spray sulfate or SO_2 from the atmosphere.[19] This element exhibits natural variations in the abundance of its stable isotopes due to fractionations that occur during chemical, physical and certain biological processes. Those variations impart a characteristic "isotopic signature" that can be used to trace the origins of sulfur-bearing compounds.[20]

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