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Meeting report

Stable isotope views on ecosystem function: challenging or challenged?

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Stable isotopes and their potential for detecting various and complex ecosystem processes are attracting an increasing number of scientists. Progress is challenging, particularly under global change scenarios, but some established views have been challenged. The IX meeting of the Spanish Association of Terrestrial Ecology (AAET, Úbeda, 18–22 October 2009) hosted a symposium on the ecology of stable isotopes where the linear mixing model approach of partitioning sinks and sources of carbon and water fluxes within an ecosystem was challenged, and new applications of stable isotopes for the study of plant interactions were evaluated. Discussion was also centred on the need for networks that monitor ecological processes using stable isotopes and key ideas for fostering future research with isotopes.

Keywords: carbon isotopes; mixing models; monitoring networks; oxygen isotopes; plant interactions; water isotopes

1. INTRODUCTION

Ecological systems are characterized by a high degree of complexity, where the interpretation of ecological patterns is hindered by the multiple processes co-occurring at different spatial and temporal scales. The study of ecosystems requires tools that indicate, integrate, record and trace fundamental ecological patterns and processes across these contrasting scales. In recent years, stable isotopes have become ideal candidates for these tools (Williams *et al.* 2007).

Stable isotope ecology has been challenging some established paradigms in ecology over the last decades, such as the paradigm of streamside trees using stream water (Dawson & Ehleringer 1991) or leaf temperatures varying across the globe (Helliker & Richter 2008), to name a few. However, interpretation of stable isotope data is intrinsically challenged by multiple co-occurring processes that constrain and affect isotopic footprints (Dawson & Siegwolf 2007). The use of stable isotopes in ecology is evolving, and an increasing number of scientists and disciplines around the world are benefitting from this tool. The increased application of stable isotopes reflects the relevance of the questions that can be addressed, particularly under the rapidly changing environmental conditions imposed by human activities.

Stable isotopes are often used to partition and disentangle complicated processes across the broad realm of ecology, ranging from biosphere–atmosphere interactions to plant community and demographic dynamics. However, this tool is notably limited by the multitude of end members and processes encountered in ecological field settings, which can hinder the interpretation of stable isotope data. The lack of continuous monitoring networks also hinders our ability to use isotopes to partition dynamic processes over a range of time scales. These problems, along with the new opportunities opened by the continuous exploration of stable isotopes, were addressed in a special symposium that was held during the IX meeting of the Spanish Ecological Association for Terrestrial Ecology (AAET, Úbeda, 22 October 2009). In this report, we exemplify how stable isotopes are concomitantly challenging and challenged, by focusing on a few case studies presented at the symposium. A summary of the main conclusions and insights is provided in the following.

2. UNVEILING COMPLEXITY IN PLANT AND ECOSYSTEM FUNCTION

One of the classical ecological applications of stable isotopes has been to decipher the water sources used by plants. Traditionally, this has been done by applying linear mixing models to the data based on the δ^2H and/or $\delta^{18}O$ of xylem and soil water. In short, after sampling the isotopic composition of the different end members (the potential sources of water), a linear mixing model is applied to calculate the proportion of each water source to stem water. This application was previously limited by the fact that when only two isotopes are measured (e.g. D and ^{18}O), the maximum number of possible end members (or sources) that can be estimated exactly is limited to three. Therefore, studies on water sources have been limited to a basic understanding of the relative proportion of water derived from three sources (e.g. deep, shallow and intermediate soil depths). Kiona Ogle described the application of hierarchical Bayesian deconvolution algorithms that overcome this limitation. Within the hierarchical Bayesian approach, multiple end members can be accommodated, whereby the relative contribution of each is partly constrained by biophysical knowledge (e.g. plant water

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uptake models) and potentially prior information (e.g. literature information on rooting distribution, hydraulic conductivities, etc.) (Ogle *et al.* 2004).

A similar problem arises for attributing sources and sinks of CO_2 in current efforts to down scale and partition night-time net ecosystem exchange (NEE), in combination, for instance, with eddy covariance measurements. To partition NEE, we need to integrate information from multiple end members (leaves and roots from different species, soil micro-organisms and in the karstic ecosystems addressed by Penélope Serrano-Ortiz, geochemical emissions).

Although this issue can now be tackled efficiently by hierarchical Bayesian approaches, Serrano-Ortiz also highlighted new problems to partitioning nocturnal NEE yet to be resolved. For instance, night-time NEE is often partitioned using Keeling plots, which regress the isotope of interest against the inverse of the concentration, yielding the isotopic signature of the source of CO_2 as the *y*-axis intercept. Keeling plots are based upon the assumption of a constant isotopic signature of the different sources of CO_2 overnight. This approach is further challenged by recent reports of large changes in the isotopic composition of leaf respiration during the night (Sun *et al.* 2009).

3. STABLE ISOTOPES AS AN EMERGING TOOL FOR UNDERSTANDING PLANT INTERACTIONS

The ecological applications of stable isotopes have, so far, been mostly confined to physiological and ecosystem level studies. Participants in the symposium discussed novel applications of stable isotope techniques to understand community processes, such as plant–plant interactions (Ramírez *et al.* 2009). Indeed, the use of stable isotopes to understand plant interactions, which allows for an examination of the mechanisms underlying plant competition and facilitation, may shed new light on the study of these key community processes. This is important due to the fact that a large number of studies in this field are based on empirical, pair-wise comparisons, where the underlying mechanisms have been largely overlooked (McGill *et al.* 2006). Can we move beyond this limitation through stable isotopes?

Cristina Moreno-Gutiérrez and José I Querejeta showed that bulk leaf $\delta^{18}O$ reflects the intensity of interspecific competition for water between the woody shrub *Rhamnus lycioides* and neighbouring pine trees. Analyses of $\delta^{18}O$ and $\delta^{2}H$ in xylem water indicated that *Pinus halepensis* and *R. lycioides* rely on soil water stored at similar depth in a pine plantation in semiarid southeast Spain. Interestingly, *R. lycioides* shrubs growing in the close vicinity (less than 100 cm) of adult pine trees showed lower stem water contents and more enriched leaf $\delta^{18}O$ values (which indicates a reduction in stomatal conductance and transpiration) than those located further away from the nearest tree. In contrast, *Anthyllis cytisoides* shrubs using isotopically enriched water stored in surface soil layers showed less-enriched bulk leaf $\delta^{18}O$ values (suggesting increased stomatal conductance and transpiration) when growing within the canopy edge of pine trees. This pattern of decreasing leaf $\delta^{18}O$ enrichment in the close proximity of pine trees may indicate potential facilitative effects of tree canopy shading on *A. cytisoides* physiology.

This new application of stable isotopes is not exempt from new problems. For instance, it is yet to be clarified how mesophyll hydraulic conductance might affect the oxygen isotopic enrichment of leaf water and organic matter. Ferrio *et al.* (2009) have shown that the rapid response of mesophyll hydraulic conductance (g_m) to changes in water availability may influence leaf water ^{18}O enrichment. This change in g_m may complicate the interpretation of $\delta^{18}O$ data as indicators of changes in stomatal conductance and transpiration.

Juan-Carlos Linares showed that $\delta^{13}C$ in tree rings may also record past changes in the intensity of plant competition (Linares *et al.* 2009), and Jordi Voltas warned us about the difficulties in using tree rings as proxies of growth conditions. There are strong interspecific differences in physiological strategies under drought, which develop in a species-specific, intra-annual pattern of $\delta^{13}C$ in tree rings. Whereas seasonal $\delta^{13}C$ changes in tree rings of some species will largely reflect fluctuations in photosynthetic gas exchange (Klein *et al.* 2005), in other instances $\delta^{13}C$ variations may not coincide temporally with an increase or relief of water stress, but rather may represent conditions at a time earlier than when the differentiation of xylem cells occurs (Drew *et al.* 2009).

4. ISOTOPE NETWORKS

Maria-Teresa Sebastià presented the network SIBAE (Stable Isotopes in Biospheric–Atmospheric-Earth System Research; http://www.cost-sibae.ethz.ch), a multidisciplinary network aimed at organizing scientific activities, knowledge transfer and training. Similar networks exist in other parts of the world (e.g. Biosphere–Atmosphere Stable Isotope Network; http://basinisotopes.org). In general, networks aimed at monitoring ecological processes through stable isotopes are rare. One of the most compelling reasons to establish a monitoring network is that it can act as an early alarm system, which could warn about significant changes or losses in ecosystem functions (Williams *et al.* 2007). The development of novel ecological applications of stable isotopes and the continuous progress in overcoming old difficulties, along with technological advances that facilitate the continuous measurement of stable isotopes at high frequency, will facilitate the establishment of such networks and improve the usefulness of the information collected.

In order for these multidisciplinary networks to succeed, they must be rewarding to researchers. In a wide survey among participants in integrative landscape projects, Tress *et al.* (2005) found that researchers have a more positive experience if (i) there is a high degree of integration of the project across disciplines, (ii) a large component of the project is focused in basic science research, (iii) there are multiple types of products generated by the project, and (iv) the project is highly productive in terms of education and training.

5. SYNTHESIS AND OUTLOOK

When stable isotopes emerged as a powerful tool for ecological studies in the early 1980s, they were received with enthusiasm. Relatively simple analyses of the isotopic composition of xylem water, leaves and tree rings seemed to provide insightful information on fundamental plant processes, such as water sources, water-use efficiency or past growth conditions, to name a few. Complications in how to interpret the stable isotope composition of plants and ecosystem components have increased in recent years as the scientific problems become more complicate and as the underlying assumptions about key physiological processes are challenged.

This may give the impression that stable isotope research has entered into a vicious circle: in order to interpret the stable isotope composition of plants, we need to constantly evaluate underlying assumptions and revise the methods of computation, such that an important part of stable isotope research deals with processes underlying fractionation and discrimination, aside from ecological questions. However, the insights provided by past and current limitations in stable isotope research have opened the door in terms of advancements in isotope technologies, experimental methods and data synthesis approaches. We have highlighted some of these advancements in this report.

There is no doubt that stable isotopes can indicate, integrate, record and trace ecological processes across different temporal and spatial scales. The use of stable isotopes within networks that monitor ecological patterns and processes over broad spatial and temporal scales may reveal great insights into the changes in the goods and services provided by ecosystems, and how they are being altered by human activities and global change. As we deepen our understanding of biological processes affecting the stable isotope composition of plants and ecosystems, we will certainly unveil biological processes affecting ecosystems in a way that could not be anticipated with other experimental approaches.

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