Stable isotopes and biological processes

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In the last century, Kjeldahl's new chemical methods made it possible to measure the amounts of N fixed as NO_3^--N, NH_4^+-N and bulk organic N. Following World War II, the newly available enrichment techniques, using the stable isotopes of N, contributed greatly to knowledge about the movements of N in organisms and their environments. For some types of N studies, the use of highly ^{15}N-enriched tracers is still the preferred method. However, N cycling is complex, and many unresolved questions still exist. The only new way forward consists of using the natural abundance levels of $^{15}N/^{14}N$ ($\delta^{15}N$). At SCRI, we are pioneering new understandings arising from this novel tool while, simultaneously, investigating the mechanisms underlying the repeatable patterns of $\delta^{15}N$ found in plants, animals, soils and waters.

Work done at SCRI has established that types of soil N cycling, chiefly related to soil moisture availability and globally related to mean annual rainfall[1], explain a large proportion of the observed variation in the $\delta^{15}N$ of plants and soils. Field-based observations[2] suggest that, within sites, there may be a rank order of plant $\delta^{15}N$ which can be related to types of mycorrhizal association and, because of the role of these associations, to the chemical composition of the major soil N source. Even more recent field-based research[3] suggests that the within-site variation of plant $\delta^{15}N$ may be related to the interaction of mycorrhizal association with the availability of major plant nutrients such as N and P. Where nutrients are deficient, plants with ecto- and ericoid mycorrhizas appear to have much lower $\delta^{15}N$ values than those occurring in conditions of better supply. Controlled experiments with arbuscular mycorrhizal (AM) associations[4,5,6] have suggested that the species of AM-forming fungus interacts with external N concentration to increase plant $\delta^{15}N$. These data also suggest the possibility of using $\delta^{15}N$ to assess the effectiveness of mycorrhization in important commercial crops such as orchids and plantation forests.

The plants, alone, constitute another source of variation for plant $\delta^{15}N$. The extent to which plants fractionate the isotopes of source N upon assimilation of N and the extent to which plant N, having a non-average $\delta^{15}N$ value, is lost from the plant constitute other important lines of research within the Stable Isotopes Unit. In collaboration with the Cellular and Molecular Genetics Department, we have demonstrated, for the first time, an association between whole-plant $\delta^{15}N$ and plant growth. In a glasshouse environment, genotypes of wild barley (*Hordeum spontaneum* C. Koch) were exposed to stresses in the form of drought or nitrogen starvation. The potentially most productive genotypes (those which were most stress tolerant – in terms of growth and nitrogen content) had the most negative whole-plant $\delta^{15}N$ values. Under the conditions of this experiment, a more negative whole-plant $\delta^{15}N$ was equivalent to a greater discrimination against ^{15}N relative to the original source $\delta^{15}N$. Discrimination at a whole-plant level can occur only if some nitrogen is lost from the plant after being isotopically altered by metabolic processes. When measured in such experiments, whole-plant $\delta^{15}N$ may, therefore, reflect the extent to which nitrogen can be *retained* within tissues when plants are stressed, a feature of clear agronomic and ecological importance.

N transformations in soils affect plant $\delta^{15}N$ and generate N pools of interest to ecologists and environmentalists. In the Stable Isotopes Unit, we are using $\delta^{15}N$ of soil N and soil N pools to describe the spatial and temporal processes which generate NO_3^- in the soils and waters of the Ythan River Catchment, north of the city of Aberdeen. While the Ythan River is not

a source of potable water, it does exhibit seasonal blooms of the green alga, *Enteromorpha*. The interaction of river water chemistry and algal blooms is being investigated jointly with Professor John A. Raven (Dundee University).

It is conventional wisdom that excess amounts of NO_3^- (arbitrarily and by statute more than 50 mg/l) are responsible for nuisance algal blooms such as those found during the summer in the Ythan Estuary. This assumption is enshrined in EU legislation, requiring the designation of 'Nitrate-vulnerable zones'. Recently, this 50 mg-limit was extended to cover all surface waters, and an environmental monitoring network is being erected to measure the concentrations of NO_3^- in Scotland, England and Wales[7]. The new interpretations and techniques already being developed at SCRI constitute the only UK skill-base for complementing this monitoring with information on the sources of observed NO_3^- and for monitoring the extent to which the various, mandatory land management strategies ameliorate such NO_3^- generation.

Biotic stresses can cause plant $\delta^{15}N$ variations, which may become useful in understanding host-pathogen interactions. These interactions are inherently complex, and investigations are usually biased towards the effects of pathogens on plant hosts. Research within the Stable Isotopes Unit demonstrated that $\delta^{15}N$ could be used to detect physiological response(s) of a plant host to pathogen infection[8,9]. We followed-up this initial insight by investigating the effect of host on pathogen (using $\delta^{15}N$ and $\delta^{13}C$). Five species of plant-parasitic nematode from the family Longidoridae were transferred from their original host plants to seedlings of *Petunia hybrida* cv. Blue Picotee. After feeding on the new *Petunia* host for 28 days, three *Xiphinema* nematode species were ^{15}N-enriched and ^{13}C-depleted, compared with nematode populations that had fed solely on the original host plants. In contrast, no such changes were detected for the single species of *Longidorus* and *Paralongidorus* studied. Changes in whole body $\delta^{13}C$ are considered to be indicative of the new plant host (*P. hybrida*), whereas, differences in whole body $\delta^{15}N$ are probably related to the different feeding strategies used by the longidorid nematodes in this study. The techniques developed for this study could be applied to other soil microfauna, thus allowing investigation into the relationships that exist within the decomposer food web.

Underpinning all isotopic research at SCRI is state-of-the-art chemistry, new sample preparation techniques and development of new instrumentation.

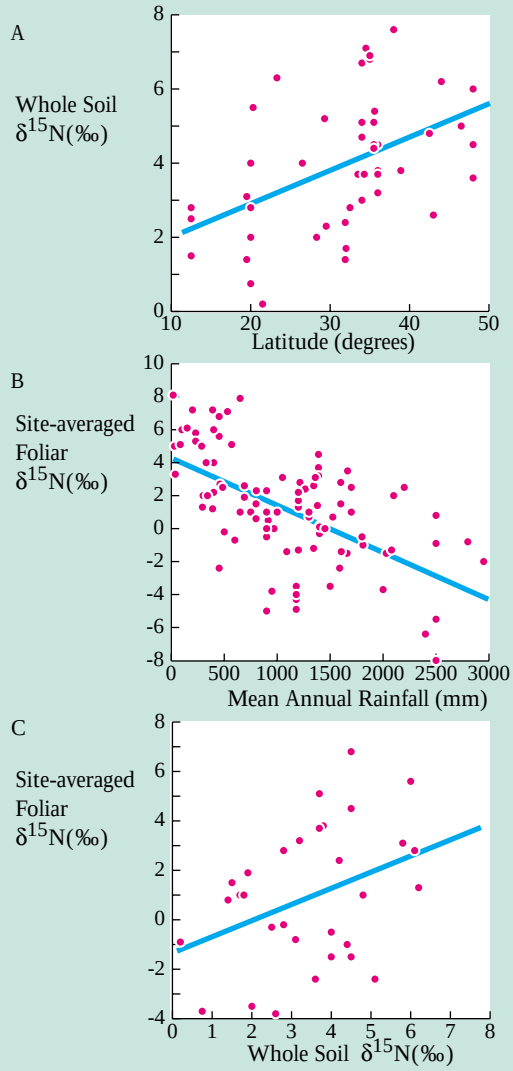

Figure 1 Globally, the $\delta^{15}N$ of soils and plants are correlated with mean annual rainfall or with latitude, for which rainfall is a major component. The relationship with site moisture reflects the variations of isotopically fractionating biological processes which are moisture-dependent.

The substantial increase in our understanding of natural abundance patterns of $\delta^{15}N$ [10] has been achieved by examining large data sets obtained by rapid, automated isotope analyses using state-of-the-art continuous-flow mass spectrometers. These instruments couple an elemental analyser to the isotope ratio mass spectrometer and operate under computer control. We have developed a number of robust analytical protocols for plant and soil samples using this instrumentation. Data from landscape-scale studies or from large sets of genotypes can be obtained readily and subjected to rigorous statistical analysis.

However, data from whole plant or soil samples do not tell the whole story, and increasingly we need information on particular chemical species within these samples. Separating individual compounds from a complex matrix such as soil is much more taxing than the simple drying, grinding and weighing of whole samples. Any separation must avoid loss or contamination which will alter the isotopic composition.

We are pursuing this problem for soil nitrogen species on two fronts. The first is the conversion to cleanly extractable organic derivatives, which can then be analysed using our high-throughput instruments. This approach is the most practical for studies requiring large data sets. The second approach is to use on-line separation, where components eluting from a gas chromatograph are analysed for isotopic composition. However, this approach will always be demanding of both operator and instrument time, and is best suited to detailed analysis of model systems which complement large field studies.

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