4. *APPLICATIONS*

The approach of using natural abundances of stable isotopes is solidly founded in the "hard sciences" of chemistry, physics and mathematics (see, for example, Hoefs, 1987; Fritz & Fontes, 1980; Hayes, 1983). The first scientific disciplines of the "natural sciences" to use stable isotopes at the natural abundance level were chemistry and the earth sciences. The differing behaviours of isotopes of the same elements revealed the mechanisms of many chemical reactions, especially those requiring catalysts (chemical helpers), and eventually the biological catalysts called enzymes (O'Leary, 1989). This basic work, evolving to studies on complex materials of both biochemical and inorganic origins, led to the realization that each chemical process, and the occurrence of each basic type of organism, throughout history, left certain types of characteristic isotope signatures in the rocks, in petroleum and sediments. The air we breathe and the water we drink (Raven, 1987) all contain isotope signatures which are the essence of their own history (Coleman & Fry, 1991).

More recently the natural abundances approach to using stable isotopes has been adopted in the life sciences. It has rapidly produced a new way of looking at the world, which is begining to permeate all of the life sciences. In the life sciences, this approach takes two major disciplinary forms: environmental and physiological studies. For environmental studies, large, statistically meaningful numbers of samples are needed; for this to be practical, automated analyses are required. This has only, within the last two to three years become practical – just barely, in the hands of a skilled and imaginative analytical chemist, using only the best of hand–built one–off instrumentation. As will become evident from the discussion, the usefulness and power of natural abundance methods expand greatly when a single investigator (or group) has easy use of several isotope pairs. This implies the need for a suite of instrumentation and scientific expertise not yet assembled at any one place anywhere in the world. The physiological work is mainly in the area of explaining mechanisms, using fractionations to identify and describe the importance of biochemical events in living organisms (O'Leary, 1989). The physiological studies are vital to the interpretation of the ecological work. The ecological work, in turn, tells us what processes are actually the most important in nature so that we can study them in the laboratory. We discuss, below, under two major headings, Environment and Physiology, the chief applications of this new research tool.

For the sake of clarity and ease of reading we have chosen to organize our review around a few seminal papers. These papers, however, contain large reference lists, and should be consulted if the reader requires more detailed and extensive information. Some of the major reviews or collected volumes which have appeared recently are: Coleman and Fry (1991); Rundel, Ehleringer and Nagy (1989); IAEA/FAO (1991); Pearcy, Ehleringer, Mooney and Rundel (1991); Griffiths (1991); Handley and Raven (1992); Peterson and Fry (1987).

4.1 *Environment*

Until recently, tracing the fates of important biological molecules in ecosystems relied solely on laborious and highly inaccurate budgets or alternately on trying to measure one or more important process (such as photosynthesis or nitrogen uptake); there was never a match

between the processes and the timing of the observed budgets. Peterson and Fry (1987) succinctly noted that the newly developing methods, using natural abundances of stable isotopes, combine the best of both approaches and eliminate some of the worst problems. These new isotope methods can be used in ecosystem source–sink studies for determining budgets (or fluxes) of major biogeochemical molecules (e.g., C, N, O, S, H, water, CO_2 chiefly as natural tracers) while simultaneously, the natural fractionations of the isotope pairs reveal the presence and magnitude of processes. This can be done simultaneously to get a match between processes and budgets. It is also usual in science to choose between a linear view of the world (eliminating changes and variations as "noise" which confuse the one true pattern) or to study the changes. With this new tool, both are useful; neither is rejected. The particular is integrated with the general.

For environmental studies, the envelope of expected theoretical fractionations for specific processes will have been determined in laboratory studies. Conversely, the description of isotope fractionations observed in nature suggest important lines of laboratory research into plant physiology and microbial processes. This new way of studying the natural world creates a particularly happy marriage of theory, experimentation and descriptive observation. Two oceanographic studies are now near–classics for their pioneering of this technique (Cifuentes *et al.*, 1988; Cifuentes *et al.*, 1989).

The major applications of the natural abundance levels of stable isotopes to terrestrial ecology and hydrology are in source–sink studies (including pollution studies) and in describing food webs (an elaboration of the source–sink concept, which allows us to describe complex interrelationships of feeding patterns in ecosystems). As will be seen, especially for $^{15/14}N$ ($\delta^{15}N$) and $^{2/1}H$ (δD), source–sink information can also be used to infer other information such as the rooting depth of trees, or the source of some nutrient element other than the one whose fractionation is being measured. While the study of one isotope pair often reveals all of the required information, in environmental studies, it is also frequently necessary to use a suite of isotope pairs simultaneously. This necessity arises because the fractionations we observe in nature are the net result of many complex processes. Some of these processes do not fractionate every isotope pair. When this occurs, we say that the process is transparent to the isotope pair which is not fractionated. However, most important events in the living world will fractionate some isotope pair. By tracking the fractionations of two or more pairs, simultaneously (frequently $^{13/12}C$, $^{15/14}N$, and $^{34/32}S$ in tandem) we can thread our way through the major complexities of natural systems.

The discussion below is organised by isotope pairs in the following order: $^{13/12}C$, $^{15/14}N$, $^{2/1}H$. Then the use of isotopic suites is discussed in relation to food web and pollution studies. Finally, some recent developments in hydrology ($^{2/1}H$, $^{18/16}O$, $^{15/14}N$) are discussed. There is some unavoidable repetition because of the inherent inter–relatedness of the isotope pairs, which indeed, comprises one of their chief utilitarian virtues. The superscript notation (e.g., $^{13/12}C$) represents the isotope pair; while notation of the form, $\delta^{13}C$, represents a value which measures the amount of change (or fractionation) in a sample relative to an internationally agreed standard) and is always given in units of parts in the thousand parts (written as, ‰). This is a logical extension of the familiar percent (%), which is parts in the hundred parts.

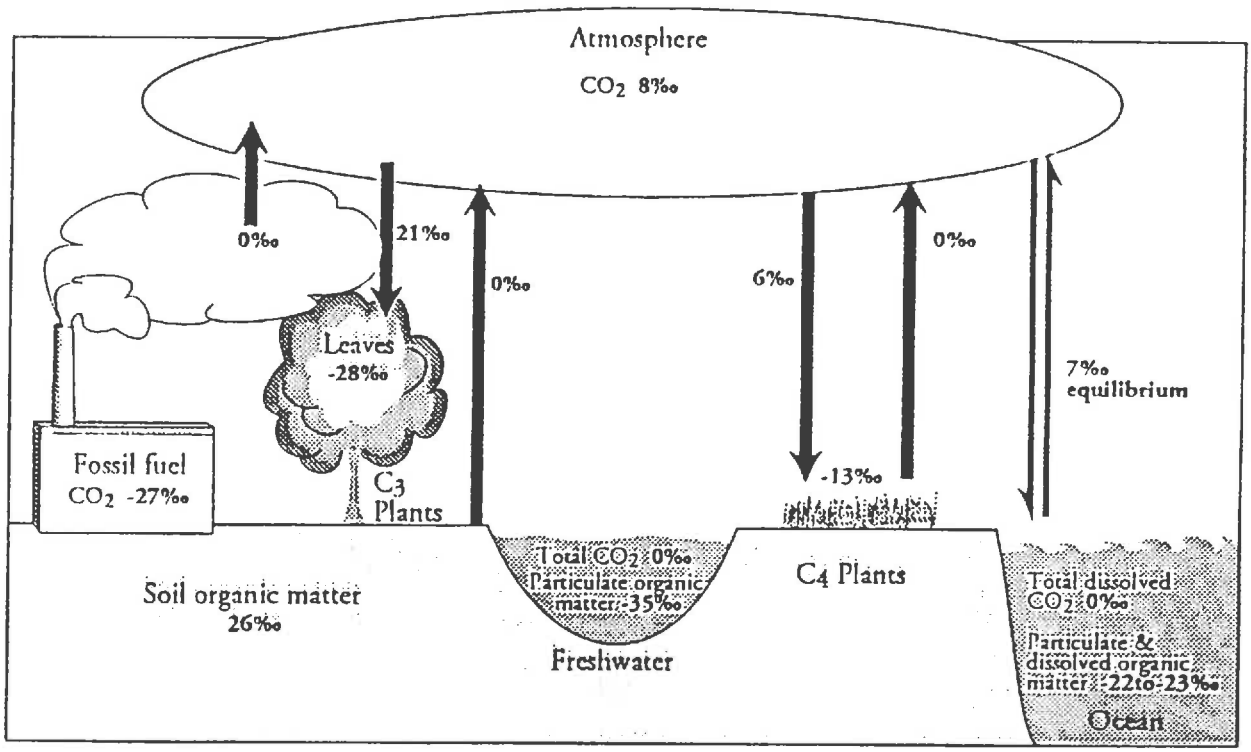

Figure 9. Our biosphere is carbon-based. Carbon occurs in large amounts and has relatively large fractionations. The diagram shows approximate ranges of many $\delta^{13}C$ values found in nature.

4.1.1 *Terrestrial Ecology*

Stable isotopes of carbon. Stable isotopes of carbon have been used at natural abundance levels more generally in ecological work than any other isotope. This follows perhaps from the relative ease with which this is measured, as much as from the biological importance of carbon. Almost as soon as it was discovered that there was more than one biochemical pathway for photosynthesis in plants (thanks to the new radio-active isotopes following WW II, it was also discovered almost simultaneously by two different research groups (Hatch and Slack, Australia & Kortshak, Honolulu) that plants having different photosynthetic pathways had different natural abundances of stable isotopes. One of the first ecological uses of $\delta^{13}C$ measurements was to determine whether plants exhibited C_3, C_4, C_3–C_4 intermediate, or CAM photosynthesis (see Section 4.2).

Much of the following was adapted from a particularly good review by Peterson and Fry (1987).

The main starch foods of the old and new world are wheat/rice and maize, respectively. Wheat and rice are C_3 plants ($\delta^{13}C$ = −32‰ to −28‰), and maize is a C_4 plant ($\delta^{13}C$ = −7‰ to −14‰). The different carbon isotope signatures of such food plants have proved a valuable tool to archaeologists in interpreting eating habits and times of introduction of new food plants. This also yields potentially valuable information for the paleo-ecologist, who is interested in past ecosystems as a way of determining how present ecosystems were derived.

The largest "ecosystem" which has been studied with $\delta^{13}C$ values is the atmosphere of the whole earth. $\delta^{13}C$ measurements have provided a valuable way of determining the past history of CO_2 concentrations in the earth's atmosphere (**an example of natural abundances used to determine mass balances**). The only other way of determining the past CO_2 concentrations of earth's air depends on estimating CO_2 mass amounts (budgets). We do not know enough about the past history of land-use and past vegetation to derive an accurate budget using this method.

Fortunately, $\delta^{13}C$ forms a part of the matter left behind in tree rings and in ice cores from glaciers, and since about 1950 we have been able to actually measure this value in the air. Figure 9 shows schematically the global carbon cycle with approximate $\delta^{13}C$ values for each major component. In the carbon cycle CO_2 exchanges with terrestrial ecosystems and with the oceans. As discussed above, plants discriminate against ^{13}C; plant material (organic matter) stored in the soil and in petroleum has negative $\delta^{13}C$ values (typically −26‰ to −27‰).

The different photosynthetic pathways mentioned above cause plants to have different $\delta^{13}C$ values because they use different enzyme systems to fix CO_2 from the air, with or without day/night variations (C_3 plants use the enzyme RUBISCO; C_4 plants use a "supercharger" enzyme called PEPc followed in series by RUBISCO; CAM plants are essentially C_4 plants with a day/night rhythm, fixing CO_2 at night as organic acids and releasing it inside the plant during the day). Soil organic matter contains about 7 times as much carbon as all of the living plants on earth today. Since there is little fractionation of respired soil CO_2, and the organic matter in the soil is derived from plants, mineralization and release of this organic matter as CO_2 leads to changes in the global $\delta^{13}CO_2$ values toward the value of the plant

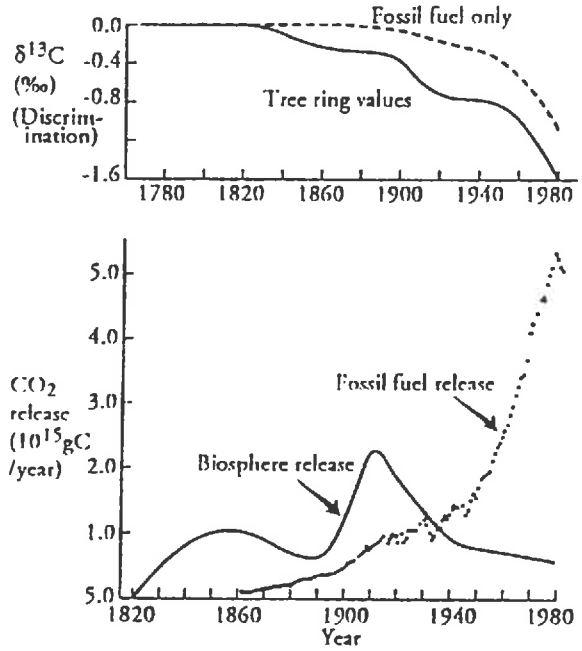

Figure 10. $\delta^{13}C$ measurements have revealed changes in atmospheric carbon. (a) shows what the atmospheric value of $\delta^{13}C$ would be (dashed line) if the signal were due solely to fossil fuel consumption and (solid line) the actual value. The difference between the two lines indicates a second source of carbon. (b) shows mass amounts of carbon released from different sources. The peaks after 1820 and around 1900 are due to forest clearing and new agriculture, largely in North America. Taken together, Figs. 10a show how $\delta^{13}C$ measurements have validated and fine-tuned the mass balance approach to global CO_2 modelling.

material.

CO_2 also exchanges with the ocean. When CO_2 dissolves in water it forms an equilibrium reaction between dissolved CO_2 and a form of carbon called bicarbonate (HCO_3^-). The total carbon in surface ocean water has a $\delta^{13}C$ value of approximately 0‰. Carbonate sedimentation (the removal of carbon) involves very little fractionation. However, the uptake of carbon in planktonic photosynthesis involves a fractionation of about −19‰ to −24‰. The organic matter in the world's oceans mainly reflects a marine planktonic origin.

One cannot be so definite about freshwater carbon δ values. The $\delta^{13}C$ values vary widely depending on the source of the carbon. These could come from the weathering of carbonate rocks, from respiration of organisms, from mineral springs, from the atmosphere or from respired organic matter (mineralisation). When respiration is the main input, $\delta^{13}C$ values for dissolved inorganic carbon may be close to −20‰, and algae that further fractionate during carbon uptake can measure as low a value as −45%.

Because of the relationships explained above, it has been possible, mainly by using tree ring and glacial $\delta^{13}CO_2$ values, to calculate when large amounts of CO_2 were added to the atmosphere in the past, and what the general source for the added CO_2 was. Figure 10a shows that fossil fuels, alone, do not account for the additions of CO_2 to the atmosphere since about 1820. In this figure, the broken line represents the carbon isotope discriminations as recorded in tree rings up to 1980, if fossil fuels were the only source of new, added CO_2. The solid line shows the values actually recorded in tree rings. This value (since 1820) is considerably lower than can be explained by fossil fuels being the only source. The discrepancy is thought to arise because the main source of new atmospheric CO_2 between about 1820 and 1940 came from the mineralisation of soil organic matter and decaying plant biomass. This source was opened up by European exploration and settlements in various parts of the world when they cleared forests and ploughed grasslands. Figure 10b shows (solid line) the amount of new atmospheric CO_2 thought to arise from decaying organic matter and the amount thought to have arisen from the combustion of fossil fuels. The budget and the sources of CO_2 were calculated from $\delta^{13}C$ data, thus providing a second verifying method for understanding this now alarming phenomenon of increasing CO_2 levels. (The first method being measurement of amounts of CO_2 in the atmosphere)

$\delta^{13}C$ continues to be a valuable tool for global CO_2 modelling. Quay, Tilbrook & Wong (1992) recently used the $\delta^{13}C$ value of dissolved inorganic marine carbon to estimate the rate of oceanic uptake of anthropogenically and biospherically produced CO_2.

Because the different kinds of plants tend to occur due to different ecological conditions (especially temperature and water supply) $\delta^{13}C$ values can reveal a great deal about the ecology of a site, in both the past and the present. Ambrose and Sikes (1991) used $\delta^{13}C$ values to document the movement over recent geologic time of C_4 grasslands up and down the slopes of mountains in Kenya. This helps to reveal past climates and their effects on major ecosystems; this type of information, in turn, helps us to interpret the possible changes we can expect under future climate change.

The disappearance of Brazilian forests is a global concern related to climate change. As a part of documenting the actual change in area of one type of Brazilian forest, Dr John Proctor

(University of Stirling) and Dr Handley (SCRI) are preparing a proposal to study the movement of vegetation types in the Amazon basin using the technique of examining the $\delta^{13}C$ signatures left by C_3 *versus* C_4 plant roots in the soil. It is planned that this work will be backed up by radio-carbon dating of the soil layers by the SURRC (Dr Douglas Harkness) laboratory in East Kilbride and other investigators who are specialists in soil and in plant identifications in Brazil. Professor James Ehleringer of Utah recently expressed an interest in collaborating. Ecological work with stable isotopes is frequently multi-disciplinary, bringing scientists from many areas of expertise together to get a full picture of the problem.

Several studies (e.g., IAEA/FAO, 1990. pp. 247-269) have used the difference of $\delta^{13}C$ values in C_3 and C_4 plants to determine the rates of organic matter turnover in soils. The rates with which organic matter (dead plant, animal and bacterial material) in soils is of importance, *inter alia*, because organic matter is important in determining soil fertility. It helps to hold water and nutrients in a way that makes them available for plants; it improves soil texture and allows good drainage. Soil organic matter is a major storage mechanism for carbon. When this soil carbon is released, it is done so microbially as CO_2, influencing the greenhouse effect and climate change.

In 1984 Farquhar and Richards published a theoretical paper in which they described a relationship between the $\delta^{13}C$ value of a plant and its water cost of growth. Although this paper is frequently cited, the relationship between $\delta^{13}C$ and water cost was one that had been sought and investigated by several workers (e.g., O'Leary). As is often the case in science, what is historically viewed as the work of one group, is in fact the culmination of the efforts of many scientists. Water cost is of increasing interest under a future of climate change in which agronomic crops, especially, should be selected which can achieve the maximum amount of growth per amount of water used for growth. Temperatures may become warmer and water less abundant; present crops (including tree plantations) in northerly latitudes may become obsolete. In Scotland, for instance, Sitka spruce is unlikely to remain the best plantation treee in a warmer, drier climate. Replacement species and genotypes of crops must be found and tested for their ability to grow well under the stresses of increased temperature and limited amounts of water. Equally vital to our custodianship of our global home, is understanding the likely impacts of global climate change on natural or lightly managed ecosystems. Without this understanding our conservation efforts may be in totally unproductive (or even counter-productive) directions. A rise in temperature, a change in the amount of available sunlight, a change in the amount of rainfall--all of these factors will affect the success and distribution of natural vegetation and their soil and animal communities just as much as agricultural systems will be affected.

Although the basic relationship has been described; truly controlled experiments, documenting exactly the conditions under which the published relationship holds true, have not been done. Prof. James Ehleringer was the first major ecologist to apply the new relationship between $\delta^{13}C$ and water cost to desert ecosystems, in the Western USA. At first the relationships seemed to hold, in the field, under natural conditions. With more studies, at more sites, some of the relationships have appeared contradictory, the relationships less clear. Some relationships have held up in general (see, for example, Coleman & Fry, 1991; IAEA/FAO, 1991) such as the finding that long-lived desert plants appear to be more efficient in their use of water than short-lived plants. Dr Handley and Mr. David Odee have recently verified this relationship for acacia savannas in Kenya (unpublished data). Corollary work with $\delta^{2}H$ has

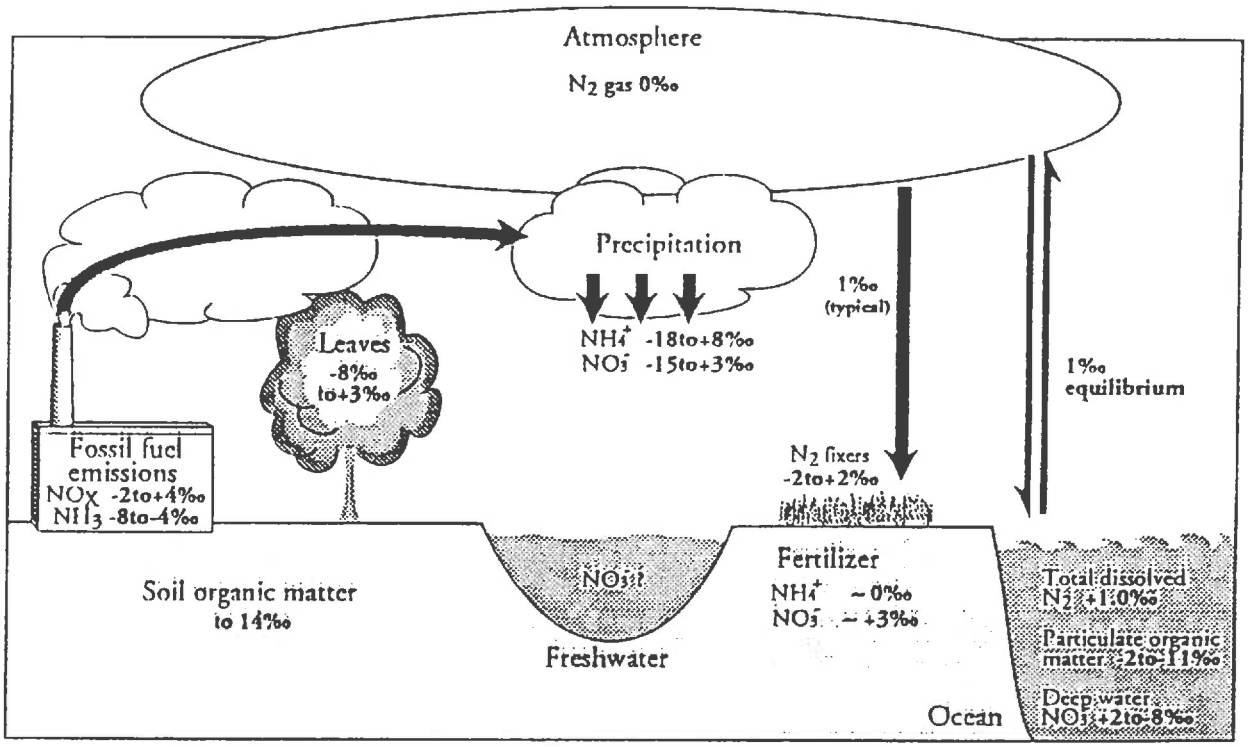

Figure 11. Nitrogen cycling is complex, making $\delta^{15}N$ more generally useful in its fractionations than as a direct tracer. This diagram gives approximate ranges of $\delta^{15}N$ values to be found in the natural world.

documented the importance to desert plants of deep rooting systems and made it possible to verify the sources of water that plants are using during the year.

Additionally, semi-controlled field experiments with crops have recently yielded contradictory relationships between water cost and $\delta^{13}C$ (for example, Austin *et al.*, 1990; Richards, 1991; Condon *et al.*, 1987; Read *et al.*, 1992). Since the $\delta^{13}C$ can be affected by latitude, altitude, relative humidity, water supply, plant type, temperature, nutrient supply (in short, by anything which affects plant carbon fixation), much more work is needed in order to determine which factors pre-dominate under which conditions with particular plants. On the other hand, the fact that all of these factors contribute to the $\delta^{13}C$ signal, mean that they can be experimental variables used in determining the way in which important plants function. To calibrate this new and potentially important $\delta^{13}C$ correlation with plant water cost of different plants, and to explore the further implications of this relationship for ecology (and agronomy) we need adequate growth facilities (including the ability to fully regulate light, temperature, relative humidity, air circulation, CO_2 concentrations and source $\delta^{13}C$ values) and at least one of these growth facilities must be plumbed to a dedicated mass spectrometer for measuring isotopic changes in $\delta^{18}O$ and $\delta^{13}C$. These controlled supporting studies, are needed for interpreting ecological data.

Stable isotopes of Nitrogen

In terrestrial ecosystems, the N-cycle provides a long-term loop of interdependent N fractionations, which includes both isotopic enrichments and depletions (Figure 11). These create a complicated situation in which to interpret $\delta^{15}N$ values. While the influence of vascular plants on the $\delta^{15}N$ of their environment is inevitable, a short-term sampling of plant N reveals that plant-$\delta^{15}N$ is a function of the $\delta^{15}N$-value of the source N. We shall, therefore, first discuss N-sources and then turn to a discussion of $\delta^{15}N$ of vascular plants themselves.

Soil is the major source of N for most terrestrial plants (excluding, until later discussion, some plants relying on varying amounts of symbiotic N_2-fixation). The major focus of soil $\delta^{15}N$ studies, to date, has been the variability of $\delta^{15}N$, both within sites and between sites of varying soil types, land uses, climates and origins. Some soils are highly enriched in ^{15}N (Shearer & Kohl, 1989) with notable exceptions being the depleted soils of conifer forests (Gebauer & Schulze, 1991) of chaparral vegetation, and some recent volcanic substrates (Vitousek *et al.*, 1989).

It has been observed that soils under N_2-fixing woody legumes are depleted in ^{15}N; this is attributed to the dilution effect of leaf litter. However, high rates of N_2-fixation can lead to acid soils with high nitrate concentrations. Subsequent leaching can remove the ^{15}N-depleted nitrate as well as exchangeable cations, leaving a ^{15}N-enriched, base-poor soil (Ugolini & Sletten, 1991; van Miegroet & Cole, 1985).

To understand the dispersal of $\delta^{15}N$ values in soils is to understand the vagaries of the terrestrial N-cycle and the nature of the long-observed patchiness of soil-N. In general, residual soil N is enriched in ^{15}N by the loss of depleted mineral N compounds due to nitrification, denitrification, ammonia volatilization, and leaching of depleted nitrate-N. Depletions, on the other hand, occur when the input of depleted ^{15}N exceeds the losses of

depleted N. There can be no enrichment or depletion of soil N without transfer of N into or out of the soil.

Variability of $\delta^{15}N$ across a site of soil appears to be less than the variability of soil N concentrations (Shearer & Kohl, 1989). Peoples *et al.* (1991) reported little horizontal variability of $\delta^{15}N$ at a number of temperate and tropical sites. Various workers have reported soil ^{15}N enrichment increasing with vertical depth in the profile (e.g., Natelhoffer & Fry, 1989). This relationship seems to hold, generally, for pasture, forest and shrubland, but not for cultivated soils. Peoples *et al.* (1991), however, found no evidence of this vertical enrichment in soil $\delta^{15}N$ in temperate or tropical pastures and cropped soils.

Högberg (1990a, 1991) found an enrichment of shallow soil ^{15}N in pine plantations receiving large amounts of fertilizer N over a period of 18 years; additionally there was a larger enrichment effect due to the application of urea than to the application of ammonium nitrate. He attributed the greater fractionation in urea plots to ammonia volatilization and high rates of nitrification. He actually measured the $\delta^{15}N$ of a grass, which served as a net integrator of soil $\delta^{15}N$, rather than measuring soil N directly. Högberg (1990a) also discussed the seemingly paradoxical enrichment of cultivated soils by the continuous addition of ^{15}N depleted fertilizers. This by no means explains anything. It is a valuable and interesting set of observations with suggested explanations. The suggested mechanisms have not yet been properly tested nor have other equally possible mechanisms been explored.

Peoples *et al.* (1991) and Shearer and Kohl, 1989) reported that cropped soils tended to be more enriched in ^{15}N than pasture soils, which were more enriched than soils under woody legumes or tree plantations.

Disturbance appears to have a marked effect on soil $\delta^{15}N$. It has been observed (Peoples *et al.*, 1991) that tillage of soils under sod has the immediate effect of enriching the ^{15}N, presumably due to a flush of mineralisation of soil N. Singh, Raghubanshi & Singh (1991) showed that in tropical savannas of Utter Pradesh both burning and grazing enhanced soil N mineralisation and that of the two systems, the burned savannas experienced higher mineralisation rates and had greater pools of soil N. They also showed that the pool of soil N varied between wet and dry seasons by a factor of 15. Schulze *et al.* (1991) found in Namibia that the soils subjected to fire or heavy grazing had the greatest N values. Such flushes and cycles of mineralisation and nitrogen loss can be found in other natural systems with seasonal wetting and drying. Garcia–Mendez *et al.* (1991) found a strong pulse of N_2O loss from tropical dry forest soils in Mexico after the onset of seasonal rains. However, this rapid pulse accounted for less than 2% of annual N_2O loss from the soil pool and although they did not report soil $\delta^{15}N$ values, this small amount of the annual loss should not have had a dramatic effect on soil $\delta^{15}N$ for any one event. However, taken cumulatively, these wet–dry season fluctuations and mineralisation processes could account for large ^{15}N fractionations in soils. It is, in our opinion, enhancement of mineralisation, and not the type of disturbance (grazing, fire, rain) which drives the fractionation of soil N. Handley and co–workers are currently investigating several mechanisms which could be responsible for these empirical correlations.

Shearer and Kohl (1986) developed a technique using $\delta^{15}N$ for estimating *in situ* concentrations of plant N due to N_2-fixation, and hence by difference, as used by Handley, concentrations of plant N due to soil sources. This technique has been exhaustively documented and will not be reviewed in detail here. Other workers (Peoples *et al.*, 1991; Bergersen *et al.*, 1988) suggested minor, but important modifications for special circumstances. These appear to make the method more suited for natural systems. Handley (unpublished) recently used δD to verify major plant water sources and, thus, major sources of dissolved nitrate and ammonium.

During the development and acceptance of the $\delta^{15}N$ method much attention was focused on the choice of an appropriate reference plant (non-N_2-fixing plant) as an integrator of plant available soil $\delta^{15}N$. This may pose distinctly different problems in agricultural and natural settings. In agricultural settings, where species are homogenous, Bergersen *et al.* (1990) showed that rooting volumes and soil $\delta^{15}N$ variability were not serious considerations and further that $\delta^{15}N$ variations are not large between reference plants. In a natural stand of *Prosopis* (a desert tree) Shearer *et al.* (1983) showed that subsoil and groundwater N were not major N sources for these woody legumes. Bremer and van Kessel (1990) found, however, for a Saskatchewan agricultural soil that reference plants varied greatly across the site and also with time through the growing season. The literature is full of site-specific contradictory data. Handley (in preparation) found large variations of $\delta^{15}N$ in reference plants in East Africa at 13 sites. Because the research has not yet been done to explain the mechanisms causing the variations, no general explanations can be provided at this time.

Belsky *et al.* (1989) provided excellent baseline data, by traditional ecological methods for the apparent ecosystem effects of Kenyan acacias versus non-N_2-fixing boabab trees. Handley (recently re-funded by the EC to investigate Kenyan acacia savannas) will use natural abundances ($\delta^{15}N$, $\delta^{13}C$, δD) to test some of the relationships suggested by Belsky.

In natural settings, variability of $\delta^{15}N$ in non-fixing reference plants is common and can be large (e.g., Handley *et al.*, unpublished African data; Virginia & Delwiche, 1982). The reasons for this (still undiscovered) hold the key to some interesting information on N transfers in natural systems and will lead to an understanding of mechanisms. There is no fractionation of an isotope without material transfer of the isotope pair; material transfer requires a mechanism, of which the fractionation of the isotope pair is a distinct signature and a record. We do not examine the variations of these signatures for their own sakes, but as naturally occurring records of processes and fluxes.

In the context of estimating N_2-fixation in natural systems, it could imply that $\delta^{15}N$ of extractable soil N is a better indicator of soil $\delta^{15}N$ than non-fixing reference plants, because the reference plants may not be fulfilling the critical assumption that the ^{15}N fractionation upon uptake is the same in fixers and non-fixers. Alternately, it should be tested whether whole shoots of herbaceous annuals are not a better sampler of soil $\delta^{15}N$ than plant parts (usually leaves) of woody species.

Plant uptake of N involves a net fractionation, whether the source is N_2-fixation or soil N. It is clear that when the source is N_2-fixation (Peoples *et al.*, 1991) that the amount of fractionation is small, negative (depletion), and dependent upon the type of plant (taxon).

There is no one value which will account for symbiotic N_2–fixation in all plants. Additionally, growing conditions and type or strain of microsymbiont seem not to influence this value for the whole plant (Bergersen *et al.*, 1988, working with soybean), although such alteration may influence the relative $\delta^{15}N$ values of individual parts.

There is also a net fractionation of ^{15}N induced by plant uptake of soil N; different plants appear to express different fractionations; both enrichments and depletions are reported. This appears to be an additional fractionation effect which cannot be accounted for by the usual difference between $\delta^{15}N$ of total soil N and the $\delta^{15}N$ of plant–available N (inorganic N).

Peoples *et al.* (1991) and Bergersen *et al.* (1988) reported an enrichment of plant ^{15}N, relative to the nitrate source, when growing soybean in pots. Vitousek, Shearer & Kohl (1989) reported a consistent depletion of 4% relative to soil for a widely distributed tree in Hawaii. Yoneyama *et al.* (1991) found that plants examined in upland (drained) soils were depleted in ^{15}N relative to soil and that paddy–grown plants were enriched in ^{15}N relative to the soil. The upland and paddy soils had the same $\delta^{15}N$ value of +3.5‰. Gebauer and Schulze (1991) found for *Picea* that the tree roots were depleted relative to soil ^{15}N by 0.5‰ in the upper organic layer of the soil and that this depletion, relative to adjacent soil, increased with depth to a total of 4.2‰ at the lowest point measured. Virginia and Delwiche (1982) found that 90% of the non–N_2–fixing plants they examined in California (142 individuals) had $\delta^{15}N$ values lower than the soil in which they were growing (depletions). Unfortunately, the $\delta^{15}N$ soil values were not given.

Plant $\delta^{15}N$ has been related to life–form as well as to N_2–fixing status by two recent authors. Virginia and Delwiche (1982) found that in non–N_2–fixing plants, $\delta^{15}N$ declined with longevity and woodiness so that herbaceous annuals were most enriched, then in order of decreasing $\delta^{15}N$, were perennial herbs, shrubs and trees. They found no such relationship for N_2–fixing species. Yoneyama *et al.* (1990) found in Thailand that tree legumes had more positive $\delta^{15}N$ values than shrub legumes, suggesting that tree legumes relied less than shrubs did on N_2–fixation.

Högberg (1990b) proposed, based on field data, that in non–N_2–fixing trees in Tanzania, the type of mycorrhizal association (ecto *versus* Versicular arbuscular mycorrhiza) controlled the variation he observed in the $\delta^{15}N$ of Tanzanian trees. Handley & Daft (in preparation) have tested one aspect of this hypothesis. Enrichment ^{15}N has been used with mycorrhizal experiments, but we are unaware of any $\delta^{15}N$ experimental work. Högberg (1991), however, hypothesized that contamination of control plots with naturally depleted ^{15}N in a pine forest could be partially due to mycorrhizal transfer between trees in different treatment plots. Hamel & Smith (1991) documented and discussed the phenomenon of N–transfer between plants sharing a common mycorrhizal infection. This work was done with enrichment ^{15}N experiments.

It is unlikely that the widely observed variation of $\delta^{15}N$ in plants has a single cause, applicable at all sites for all plant taxa. It is possible that a multi–factorial description of $\delta^{15}N$ variability will emerge. Handley has formulated, as well as synthesized from other sources, several mutually consistent hypotheses related to possible sources of this observed variation (Handley & Raven, 1992). The testing of these hypotheses will be substantially facilitated by a focused programme at the SCRI studying natural abundances of stable

isotopes.

The same general relationships between N_2-fixing trees and other plants occur in northerly climates. In northern Europe (including Britain) alder (*Alnus* sp.) replaces acacias as the N_2-fixing tree; it also has a different microbial partner (actinorrhizas instead of rhizobia). Dr Handley is presently investigating actinorrhizal-type tree ecosystems in Spain.

Stable isotopes of hydrogen

Although δD (δ deuterium or δ^2H) will be discussed the Hydrology section below, it is also appropriate to say a few words about it here. It is not possible to deal with water entirely separately from the plants, because plants must use fresh water, be it from precipitation, surface waters, soil water, or subsurface groundwater (permanently saturated zone). Because of this relationship between plants and water, δD is equally useful to plant ecology and hydrological research.

White *et al.* (1985) determined that hydrogen in the water taken up by plants did not fractionate significantly until it reached a part of the plant which carried on evaporation. It had been well-known for many years that δD values were large and highly variable and relatively easy to measure. Whereas a significant difference in $\delta^{13}C$ might be 1‰, differences for δD were often 50‰ to 100‰ or more. Evaporation quickly changes the δD signature of water. In snow or rainfall, this signature changes with distance inland from a marine coast, with latitude, with altitude and with temperature. The amount of water condensed out of a given cloud mass also influences the value. The δD value of soil water in the non-saturated zone reflects an average value since the last major rain. The δD value of groundwater reflects a much longer term average of the source water, which may have travelled a great distance laterally. These two sources of water frequently enough have significantly different δD values, and these isotope signatures can be used to accurately determine the major source of a plant's water at any one time. This method of determining a plant's major source of water can be very accurate and show shifts in importance of sources within hours. White *et al.* (1985) showed for two pine sites that new rainfall was reflected in the tree-water on the first day after the rain and that daily changes in the δD of tree water could be detected until the effects of the rainfall disappeared. This analysis also showed which trees were using deep soil water and which ones were totally dependent on surface soil water. It also, coincidentally, establishes the depth of the tree roots, something very difficult to do by digging. And it measures the depth of the tree roots non-destructively.

Caldwell and Richards (1989) used δD to establish (in tandem with other verifying techniques) that a desert shrub was bringing groundwater to the surface soil horizons during the night and that nearby plants were parasitising this water. Being able to identify a process such as this can have important consequences for interpreting plant competitions and strategies in arid environments.

Handley is now using δD for verifying whether the major source of plant N is atmospheric N_2-fixation or soil N; if it is soil N, then the major strata of source N can be determined. This is because the inorganic soil N is dissolved in the water that the plants take up through their roots.

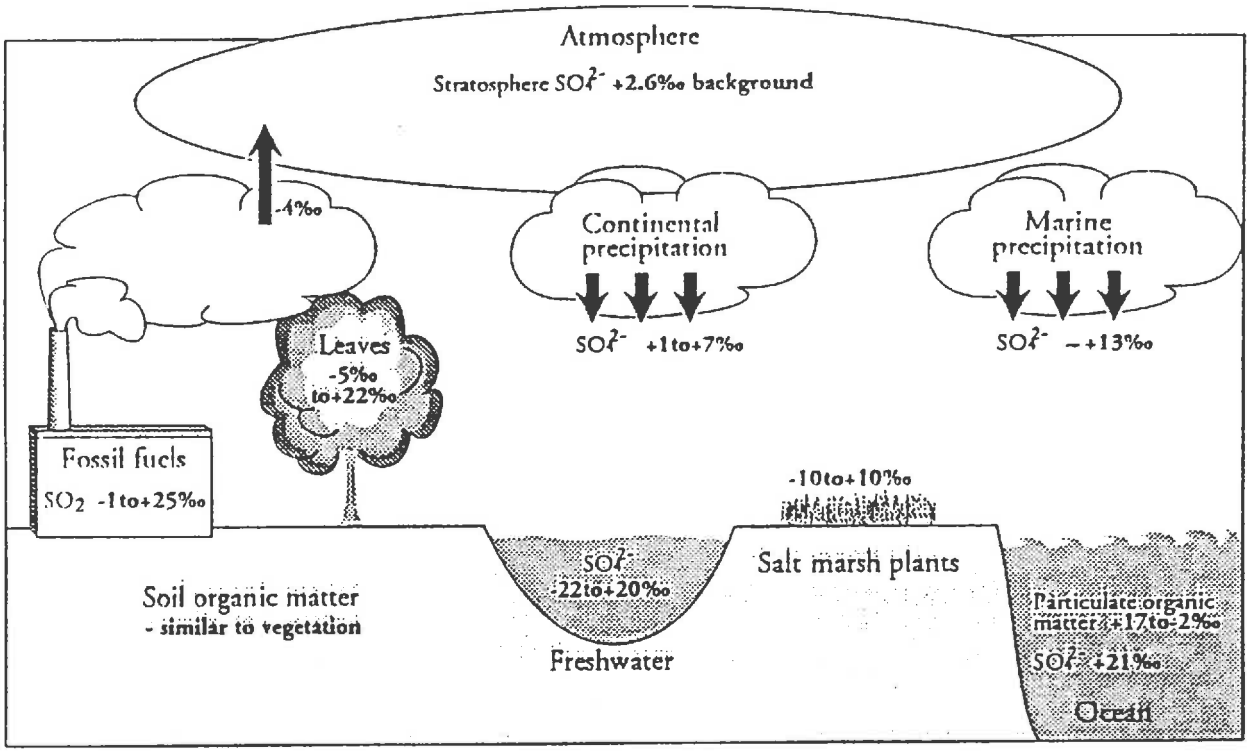

Figure 12. Sulfur is the best natural abundance tracer, in that few reactions fractionate it. This figure gives ranges of approximate values of $\delta^{34}S$ in the natural world.

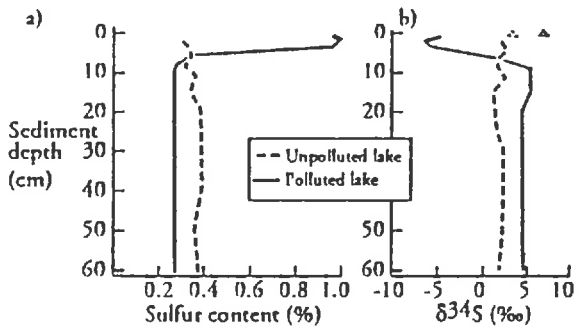

Figure 13. (a) Shows depth of lake sediments on the left-hand axis and the content of sulfur (% dry weight) on the horizontal axis. These values are plotted for a lake near the copper smelter discussed in the text and for a similar but distant lake. At the sediment depth corresponding to establishment of the smelter, sulfur content of the sediment rises sharply in the polluted lake. The distant lake sediments show no change. (b) Shows the variation in fractionation ($\delta^{34}S$) with sediment depth (vertical). It can be seen that in the same horizon with sulfur increase, $\delta^{34}S$ values drop sharply. Hence, we have two verifying markers of smelter pollution.

Dawson and Ehleringer (1991) showed that in a desert community in North America, stream and surface waters were important for certain trees only in the early establishment phase of their lives. When these trees reached a certain age and size, they avoided competing with their neighbours for the scarce and unreliable soil and stream water by using deep soil and groundwater, instead. They were streamside trees that did not use stream water.

Stable isotopes of sulfur

Sulfur is difficult to work with because it contaminates equipment, causes "memory effects" in mass spectrometers and is caustic. Therefore, the amount of ecological work done with sulfur has been small. Sulfur, however, faithfully retains the source signal under many circumstances, making it a useful, natural tracer. Figure 12 shows approximate values for typical sulfur sources. The work which has been done showed how powerful a tool $\delta^{34}S$ can be in unravelling ecological processes. Our example for $\delta^{34}S$ is one of **natural abundance level stable isotopes being used to calculate rates or processes (or fluxes) in nature.** Most of the sulfur emitted into the atmosphere appears to be man-generated. Industrialisation has increased these emissions enormously. We describe below a study by Jerome Nriagu and colleagues (see Peterson & Fry, 1987) documenting the effects of a copper smelter on sulfur accumulation and microbial sulfur transformations in a nearby lake.

In the sedimentary layers at the bottom of the contaminated lake, there is a record of sulfur accumulated, its chemical form, and its isotopic form (Figure 13a and b). The offending smelter was started in 1889, and sharp increases in the amounts of sulfur in the lake sediments were found in the strata laid down between 1880 and 1890. However, the $\delta^{34}S$ has changed since that time.

Samples were collected of other sulfur sources (air, water, chimney stacks, lake water) and they all averaged about 4‰ to 5‰. The $\delta^{34}S$ in the ores used at the smelter were about 2‰ and should have only slightly depressed the $\delta^{34}S$ value of the lake sediments. Bearing in mind that the deeper sediments are older, Figure 13b shows that after 1889 the lake sediments contained more sulfur in total (% of sediment) and that the $\delta^{34}S$ value was depressed as low as about −7‰. Controlled examination of more recent sediments and their sulfur processes has led to an explanation.

Increased sulfate stimulates sulfate reduction (sulfate is reduced to sulfide by removal of the oxygen atoms); the resulting sulfides are isotopically lighter (more depleted) than the begining sulfate. The sulfides are trapped in the sediments by chemically combining with iron. Indeed, large amounts of the lake sediment sulfur was in the form of iron sulfides. Hence the peculiar $\delta^{34}S$ signature in the lake is not due to a change of sulfur source, but to the enhancement of a microbial process due to adding amounts of sulfur to the lake.

There are several natural sources of sulfur apart from human produced sulfur "pollution." The amounts of background natural sulfur can often be distinguished from the "pollution" sources by using $\delta^{34}S$.

This is a good example of how **natural abundance levels of stable isotopes can be used to measure processes and how controlled studies, remote from the natural site can provide the needed interpretation of the *δ*-values measured in nature.**

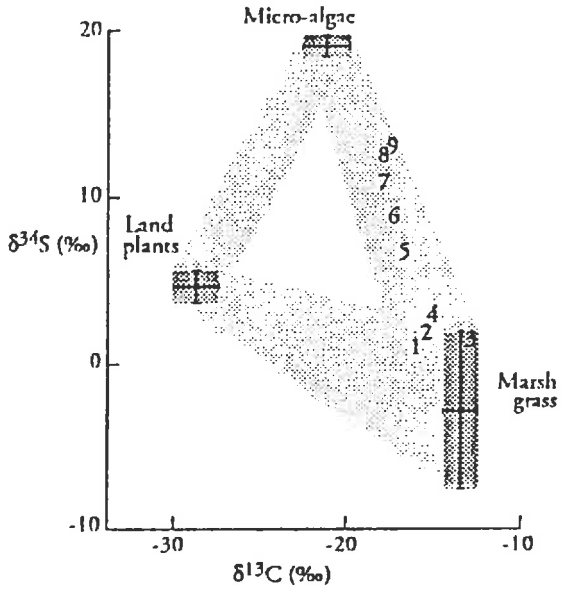

Figure 14. The graph shows the values measured of $\delta^{34}S$ (vertical axis) *versus* $\delta^{13}C$ (horizontal axis) for four types of samples: upland plants, saltmarsh grass, oceanic microalgae, and mussels (numbers 1 through 9, which represent samples along a transect from an inland creek (1) seaward (7–8) to the ocean (9). Analyses of mussel body tissues reveal that all of their food was derived from saltmarsh grass and microalgae, none from land plants. The feeding preference for saltmarsh grass or microalgae appears to depend on position on the transect, rather than population differences in the mussels.

A new machine now exists (one only) for automatically measuring $\delta^{34}S$ (Haystead, 1990); we are looking forward to acquiring another one of these and developing it to a usefully high standard in the same way that we were able to develop the automated $\delta^{13}C$ and $\delta^{15}N$.

Food webs and pollution

In ecosystems it is frequently important to determine sources and sinks. Where does a substance come from; where does it go? Sometimes the substance is food for organisms. In this case we model the sources and the sinks as a "food web", because the interrelationships among organisms are complicated and more like a web than it is like a chain or straight line. In the case of pollution (or nutrient fluxes) it important to know the source, but also to know the extent of the spread and the ultimate destination.

Because not every process fractionates every isotope, and because ecosystem processes tend to be complex, and because what we actually measure is the net result of these complicated processes, it is necessary to use more than one isotope simultaneously. For whole ecosystem studies, a suite of stable isotopes is more likely to reveal a story than any one isotope used alone.

One of the now-classic examples documented by Peterson and Fry (1987) is the study by Peterson *et al.* (1985). It became desirable to know what the food source was for ribbed mussels. There were three possible sources for the detritus used for food by this filter feeder: (1) terrestrial plants far inland, (2) saltmarsh grasses which lined the creeks in which some of the mussels lived, or (3) oceanic single-celled algae and animals (phytoplankton). Figure 14 shows the results of analyzing the tissues of the mussels, which were sampled along a line from the ocean into the land (inland up a creek). Analysis of both $\delta^{34}S$ and $\delta^{13}C$ showed that none of the mussels ate the detritus from dead terrestrial plants. Further it showed that the mussels living in the salt marsh creeks ate the salt marsh grass detritus and the ones living along the coast tended to eat both salt marsh grass detritus which flowed down the creeks to the sea and oceanic phytoplankton. This revealed the further information that feeding habits were a matter of location and not related to sub-speciation of the mussels or any other intrinsic population factors. Their original data set also included $\delta^{15}N$. However, $\delta^{34}S$ was a better messenger of food-source information.

A more recent example of suites of isotopes resolving an ecological/conservation question came from papers published by van de Merwe *et al.* (1990) and Vogel *et al.* (1990). The question of the source of elephant ivory became a major political issue. Some ivory was legal and from legitimate culling of legal sources; other ivory was from illegal poaching. The above-mentioned workers showed that natural abundance levels of stable isotopes ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{87}Sr$) could identify exactly, to the very game park, where the elephant ivory came from. This arises because animals are what they eat (isotopically) plus a few parts per thousand (DeNiro & Epstein in Ehleringer *et al.*, 1986).

Hamilton, Lewis and Sippel (1992) recently showed for the first time, using $\delta^{13}C$ and $\delta^{15}N$, that aquatic invertebrates and fishes in a tropical river floodplain fed almost exclusively on algae, not utilizing at all various aquatic weeds which are abundant there.

Fry (1991) recently reported on $\delta^{15}N$ and $\delta^{13}C$ used simultaneously for 17 long term Ecological Research Sites in North America and Puerto Rico. These measurements revealed a wealth of information about food web relationships and nutrient cycling.

An animal's body retains the signal of the food. In general, $\delta^{13}C$ is not very much enriched in the animal; $\delta^{15}N$ is enriched by about 2‰ to 4‰ in the animal's body. Sulfur is not enriched at all. By knowing the amounts of enrichment expected at every level of the food web (trophic level) it can be determined how (relative to its neighbours) an animal is feeding. We can determine, for instance, whether an animal feeds primarily on plants, on other animals, or on a particular food source with a unique isotope signature.

As a human perspective on the food web and pollution aspects of natural abundances, these isotopes can be used in determining certain types of food contamination. Fruit and cane sugar all have slightly different $\delta^{13}C$ signatures. By examining the $\delta^{13}C$ of wine, for instance, one can tell if it is actually sweet by nature or by the addition of extra sugars after the vintner's skills are finished. δD can tell us if fruit juices are actually reconstituted from local water rather than freshly squeezed.

Hydrology

The utility of δD and $\delta^{18}O$ for tracing water sources and sinks and in determining plant water use was discussed above. δD combined with $\delta^{18}O$ may in the long run be a better measure of the amount of water used by plants than $\delta^{13}C$, because it actually measures changes in the water which his being evaporated through the plant leaves. (see, for example, Flanagan & Ehleringer, 1991; Walker & Lance, 1991).

The latest Journal of Hydrology (1992) contains four articles by Calder and colleagues on using deuterium enriched water to directly measure the absolute amount of water lost by trees. This method is still in the development stage. Once a mathematical model has been agreed, it should be theoretically possible to use it with natural abundance levels of deuterium. This is very likely to be a good example of pioneering a technique by using the easier, direct method of clear enrichment tracing, and then being able to transfer the technique to the subtler, less intrusive natural abundance techniques. Dye *et al.* (1992) showed that the deuterium and heat-flow method gives comparable estimates for transpiration in trees.

Simpson and Herczeg (1991) used δD to model the water loss due to evaporation from the Murray River. The Murray River is an important source of water for irrigation in drought-prone Australia.

Groundwater pollution by nitrates has come to public attention on health grounds. There is now an EC limit on nitrate in drinking water. One of the difficulties in attacking the problem is identifying the polluters. Natural abundances are ideally suited for tracing the source of the nitrate back to its origins and describing the processes it undergoes. Three papers come to mind.

$\delta^{15}N$ of the NO_3^- molecule undergoes many processes which may change the isotope signature substantially from the source-N; it is frequently impossible to tell what the source of the N is. Amberger and Schmidt (1987), however, were able to show that the $\delta^{18}O$ of the nitrate

molecule was much more faithful to the source signal and that by using $\delta^{18}O$ they could discriminate between two possible source of nitrate pollution.

In a sandy soil, where the pathway to groundwater was straightforward and rapid, Flipse *et al.* (1985) were able to trace the source of groundwater nitrate pollution using $\delta^{15}N$, alone.

In a beautifully logical paper, Mariotti *et al.* (1988) used $\delta^{15}N$ to model the natural denitrification of nitrate–N from the groundwater in French limestone formation.

The stable isotopes of chloride are also used by hydrologists. This stable isotope pair is of less general use to other disciplines than the ones discussed above.

4.1.2 *Agroecology*

Nearly all the applications of natural abundances of stable isotopes in agricultural and environmental studies have relied on the differences in ^{13}C discrimination between C_3 and C_4 species (see Section 4.2). A few others have used ^{15}N discrimination in studies of N_2 fixation. Very few have used the relative stability of ^{18}O to trace the sources of water or atmospheric gases.

Rooting studies

If a mixture of C_3 and C_4 species is growing together, a common occurrence in the tropics in which many voracious weeds are C_4, samples of soil will contain a mixture of their roots. If this mixture of roots is separated from soil, homogenised and its $\delta^{13}C$ determined, it will have a value intermediate between that of the C_4 plants (approx. −10‰) and that of the C_3 (approx. −28‰), both of which can be ascertained by sampling and analysing the plants' leaves. The closeness with which the root mixture's $\delta^{13}C$ signal approaches that of the C_3 plant is proportional to the mass of C_3 root in the soil. The technique has been used by Ludlow, Troughton and Jones (1976), Svejcar and Boutton (1985) and Wong and Osmond (1991).

Svejcar and Boutton extracted from soil roots known to be attached to C_3 or C_4 species. They then recombined ground root material in various proportions, and determined the mixtures $\delta^{13}C$. The $\delta^{13}C$ value of *Sorghum bicolor* (the C_4 species) roots was −13.2‰, that of *Helianthus annuus* (C_3) was −26.2‰. A 75:25 mixture of C_4:C_3 roots ought to have yielded a $\delta^{13}C$ signal between these 'pure' signals, −16.45‰. In fact, this mixture had a $\delta^{13}C$ of −16.5‰.

Wong and Osmond (1991) grew wheat (C_3) with a C_4 weed, *Echinochloa frumentacea*, in order to untangle their competitive interactions under fertile or infertile, illuminated or shaded and ambient or enriched CO_2 concentrations. This study could not have been done without measurements of the roots' $\delta^{13}C$. They found that an enriched CO_2 concentration stimulated the growth of wheat roots, as reflected by the root mixture's $\delta^{13}C$ shifting closer to the 'pure' C_3 signal. In infertile soil, the growth of C_4 roots was inhibited, adding fertilizer caused the mixture's $\delta^{13}C$ to shift closer to the 'pure' C_4 signal.

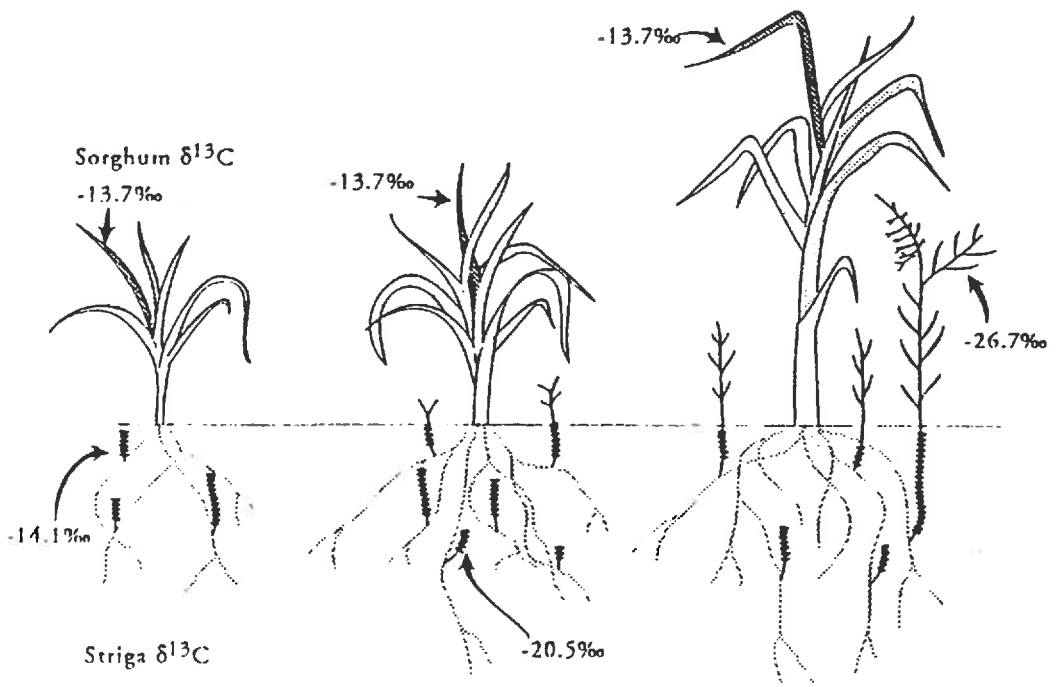

Figure 15. The development of *striga* plants on *sorghum* and their $\delta^{13}C$ values; (a) before emergence, (b) juvenile, (c) mature plants (after Press *et al.*, 1987).

The $\delta^{15}N$ of foliage may be related to the depth to which the root system has grown. Virginia and Delwiche (1982) suggested that the ^{15}N abundances of leaves of a range of species was related inversely to their presumed rooting depth, in both N_2–fixing plants and non–fixers. Why this should be so is not clear. If such strong relations be found between these variables, it would be a very valuable tool with which to study root–system growth relatively non–invasively.

Another way of doing this has not been tried, but merits some investigation. If gas sampling probes are installed at known depths in the soil, it is possible to collect samples of soil atmosphere. The CO_2 in the sample will be a mixture of CO_2 respired from roots, from microbes growing on living root products, and those growing on dead materials. But if the plant was a C_4 species, and the residues of plants in the soil were derived from only C_3 species (or vice versa), the root–derived CO_2 could be detected and quantified at any depth by measuring the $\delta^{13}C$ of the CO_2 sample. This is possible because the $\delta^{13}C$ of CO_2 derived from residues of previous crops will have a C_3 signal, and so can be distinguished from that emanating from living roots. At depths containing no living roots, the $\delta^{13}C$ signal of the CO_2 will be close to the C_3 end of the spectrum; in soil packed with living roots, it will be closer to the C_4 end.

Plant–to–plant transfer of C

Some parasitic plants derive C not only from atmospheric sources but from other plants to which they are attached. The problem is to quantify the parasite's dependence on its host for C. Press *et al.* (1987) devised ingenious solution to this problem by measuring the $\delta^{13}C$ of a C_3 parasite (*Striga hermonthica*) growing on a C_4 host (*Sorghum bicolor*). *Striga* grows on the roots of its host, and is a serious cause of yield loss in tropical cereal crops.

The $\delta^{13}C$ of *Sorghum* leaves was measured by Press *et al.*(1987) to be – 13.7‰. That of *Striga* plants growing on it changed as it grew (Figure 15). Juvenile *Striga* plants lacked chlorophyll and had a $\delta^{13}C$ close to that of its host –14.1‰. Emergent *Striga* plants had a few green leaves and their $\delta^{13}C$ was –20.5‰, indicating that an increasing proportion of its C being fixed from its own photosynthesis. By the time *Striga* matured, its $\delta^{13}C$ was –26.7‰, well in the range of autotrophic C_3 species. But because *Striga* cannot be grown independently of its C_4 host, a 'pure C_3' $\delta^{13}C$ value cannot be obtained directly. Instead, Press *et al.* (1987) used a model to calculate what it would have been had it been able to grow throughout its life on atmospheric CO_2: the value was –31.8‰, significantly different from that measured. This implies that in the leaves of mature *Striga* plants there is a mixture of C derived from its autotrophic fixation of CO_2, and from its heterotrophic dependence on its host's C. Integrating over the whole life cycle of *Striga*, Press *et al.* (1987) were able to calculate from the $\delta^{13}C$ values that at least a quarter of the parasite's C was derived from its host.

This technique suggests itself in other applications that have hitherto defied conventional approaches. For example, the possibility that C can be transported between plants via a common hyphal network of mycorrhizal fungi has been studied by feeding the leaves of one plant with CO_2 labelled with the radioactive isotope ^{14}C. The appearance of ^{14}C in the leaves of another plant to which it is attached mycorrhizally has been taken as evidence that a net

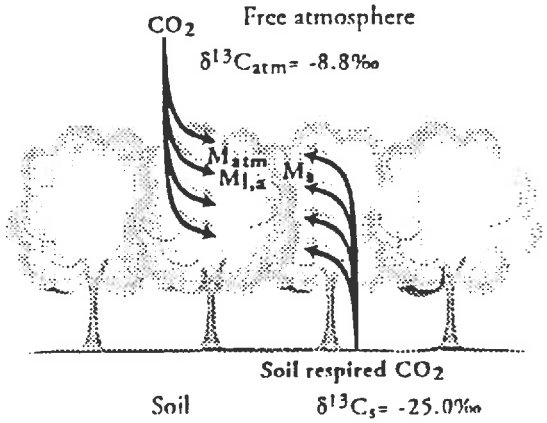

Figure 16. Schematic flux distribution of CO_2 in a forest. M_{atm} and M_s represent the contributions of CO_2 from the free atmosphere and the soil respectively. $M_{l,a}$ stands for the actual CO_2 concentration in the vicinity of leaves, i.e. the mixture of CO_2 from the free atmosphere and the soil (after Schleser and Jayasekera, 1985).

transfer of C has taken place. But this technique cannot detect any isotopic exchange – ^{12}C for ^{14}C – which might occur in the absence of any *net* transfer, and it cannot quantify any transfer (Newman, 1988). But if one plant was a C_4 species, and the other a C_3, their $\delta^{13}C$ values would show whether isotopic exchange occurs (if it did, both their $\delta^{13}C$ values would shift from control values of non-mycorrhizal plants, as C_4-derived C, and vice versa. $\delta^{13}C$ determinations would allow any net transfer to be quantified, as Press *et al.* (1987) did. $\delta^{13}C$ measurements offer what is probably the only way of approaching this controversial problem experimentally.

Reassimilation of soil-derived CO_2

Some of the CO_2 that reaches a plant's leaves comes not from the atmosphere, but from CO_2 derived from the respiration of roots and microbes in the soil (Figure 16). The extent to which leaves assimilate this soil-derived CO_2 depends largely on the turbulence of air flow through the canopy. The more closely packed plants are, and the slower the air flow, the less mixing will occur between soil-derived CO_2 and CO_2 in the atmosphere above the canopy.

Atmospheric CO_2 has a $\delta^{13}C$ of about –8‰; that derived from soil organic matter is around –25‰, *if* the extant plants and the residues in the soil are C_3 species. This difference makes it possible to use ^{13}C measurements to quantify reassimilation of soil-derived CO_2 by the canopy. Schleser and Jayasekera (1985) did this for a single beech (*Fagus sylvatica*) tree growing in a forest. They measured differences in the $\delta^{13}C$ of leaves between the bottom of its canopy and the top. Those at the bottom had values of –30‰; those at the top, –25‰. It was concluded that lower leaves obtained 22% of their fixed carbon from soil-derived CO_2; at the top of the canopy this figure was only 5%.

Partitioning whether the soil-derived CO_2 comes from the decomposition of dead residues or from living roots and their products is possible if, again, the living plant is a C_4 species growing in soil that has only grown C_3 species (Schönwitz, Stichler & Zeigler, 1986). Such approaches offer a means of making detailed measurements of local C budgets from year-to-year.

Soil organic matter turnover

One of the least understood parts of natural nutrient cycles is the role of soil organic matter (SOM) in the soil (SOM) as a source of labile nutrients and potential pollutants, and as a sink for them. This uncertainty arises from the heterogeneous chemical nature of SOM, and from the inadequacy of conventional means of studying it. Martin *et al.* (1990) pointed out that long-term (>1000 year) dynamics of SOM can be studied using ^{14}C dating. The mineralization of ^{14}C-labelled plant material can be studied over 1–2 years. Between these extremes, only natural abundances of stable isotopes can provide any information.

Martin *et al.* studied SOM turnover in a West African savannah. The site had been dominated by C_4 grassland vegetation, but 25 years before the study, it had started to become colonized by C_3 woody species. They found that the total C content of the site's SOM had not changed significantly over that period, 52–70% of the original C_4 SOM had been turned over since the vegetation change began.

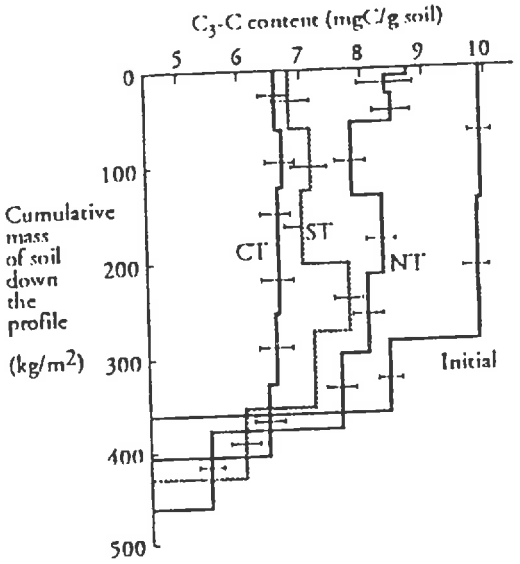

Figure 17. Profiles of initial organic C and C remaining from the initial carbon after 17 years of continuous main cultivation with different tillage practices at Boigneville (CT: conventional tillage: NT: no tillage: ST: superficial tillage). The *y*-axis is the cumulative mass of soil down the profile. Error bars were calculated on a 95% confidence interval and C and $\delta^{13}C$ measurements of soil samples.

Balesdent, Mariotti and Boisgontier (1990) found that different tillage practices can lead to differences in the rates at which crop residues are mineralized. Conventionally–tilled soil resulted in twice the rate of SOM mineralization of that in untilled soil (Figure 17). this conclusion was reached by comparing how much C_3–derived C remained in the SOM of soil that had grown maize (C_4) continuously for 17 years. Only half as much C_3–derived SOM remained in the tilled soil, compared with the untilled control.

Nadelhoffer and Fry (1988) used a combination of ^{13}C and ^{15}N measurements to study soil turnover in soil beneath an oak (*Quercus* spp.) forest. To do this, they used the differences in $\delta^{15}N$ and $\delta^{13}C$ between SOM already in the soil, and those of fresh leaf and root litter. From their data they were able to postulate the existence of two pools of SOM. One, in the upper layers of the soil involved mixing fresh litter with native SOM, a process that decreases $\delta^{15}N$ and $\delta^{13}C$. Another, in deeper layers, involved decomposition of the mixed SOM, which increased ^{15}N and ^{13}C. Similar results for ^{15}N with soil depth have been reported by Ledgard, Freney and Simpson (1984) for pasture soils and by Tiessen *et al.* (1984) for uncultivated prairie and arable field soils.

The great potential of using multiple stable isotopes to probe the trophic interactions in soil, similar to the work of Peterson, Howarth and Garritt (1985) at the ecosystem level, has yet to be realised.

Pollutant tracking

Modern agriculture is as concerned with environmental protection as it is with crop production. For effective monitoring of environmental risks and for policing any violations of legal limits as (for example, nitrate loading in groundwater) it is important that the ultimate source of the pollutant can be determined reliably. To do this stable isotopes that show *little* discrimination as they pass through biological nutrient cycles and are transported physically are needed. Neither ^{15}N nor ^{13}C are suitable, but ^{18}O is.

Yoshinari and Whalen (1985) measured emissions of the 'greenhouse' gas nitrous oxide (N_20) from a municipal waste water treatment plant. They measured its $\delta^{18}0$ and found that its value, 23‰, was significantly different from that in the atmosphere, 45‰. N_20 was produced by nitrification in the waste water plant. The conclusion, was, therefore, that the N_20 in the atmosphere derives from sources other than nitrification. And the $\delta^{18}0$ values resulting from these other processes must be collectively greater than 45‰. This must limit the number of possible processes which could contribute to the N_20 balance of the atmosphere.

$\delta^{18}0$ has been used to track nitrate in hydrological catchments. $\delta^{18}0$ of agricultural fertilizer will be different from that of nitrate mineralized from native organic matter and from that deposited as wet or dry deposition. Measurements of the $^{18}0$ of river water, in nitrate–sensitive areas for example, would then reveal how much of it was derived from agricultural usage of fertilizer and how much from other processes. This is a potentially valuable application of stable isotope technology but it has yet to be exploited, generally.

Plant breeding

One of the most potentially useful applications of stable isotope technology is in screening

genotypes for certain phenotypic trials. Because measurements integrate over many processes, they can serve as selection criteria, provided that they correlate consistently with the phenotype. The most widely used application to date has been to use $\delta^{13}C$ measurements of leaves as a screen for water cost of growth in C_3 crops.

Farquhar and Richards (1984) reported that variations in ^{13}C were correlated with variations in dry matter production per unit water transpired by wheat genotypes. The correlation is strengthened if plants are subjected to drought, which, in any case, is the condition under which improved water cost is likely to be an important feature of crop production. An example of the relation is given in Figure 18 for crested wheatgrass (*Agropyron desertorum*), a valuable forage species in semi-arid regions of North America.

It is important to emphasise that the relation between water cost and ^{13}C is only empirical. It arises through the mutual relations between C fractionation and stomatal conductance, and stomatal conductance and transpiration. The relation is not strong for C_4 species, so precluding its application to selection among maize, sorghum or sugar cane genotypes.

A major area worthy of further investigation is the search for reliable correlations between known environmental factors (nutritional, physical, biological) and stable isotope fractionations of leaves. If, for example, a certain nutritional deficiency was manifested by a shift in isotopic composition (and this is not unlikely, given the theoretical relation between N nutrition and ^{13}C (Raven and Farquhar, 1991), screening plants' isotopic compositions could reveal this deficiency without having to measure soil parameters. It is likely that for this to be successful, suites of stable isotopes will have to be used in order to maximise the 'axes' for discrimination, and improving the sensitivity of the screen. Such applications are in their infancy, but offer exciting possibilities for the future.

4.1.3 *Aquatic Ecology*

The major use of measurements of the natural abundance of stable isotopes in aquatic ecology, both freshwater and marine, is in investigating food chains (and webs) and, more generally, biogeochemical cycling. The major isotopes used are $^{13/12}C$ and $^{15/14}N$, with $^{34/32}S$ called in mainly as a "tie-breaker" when the conjoined use of C and N isotopes fails to resolve a problem (Peterson & Fry, 1987; Fry, 1989).

Starting at the first step in the photosynthesiser-herbivore-carnivore(s) food chain, $^{13/12}C$ measurements on plants (including microalgae, the main photosynthesisers in large water bodies) can yield important evidence about the form of carbon (carbon dioxide or bicarbonate) which the plant uses (MacFarlane & Raven, 1990; Raven & Johnston, 1991). The inability to use bicarbonate could be important in some environments and could help to explain the results of competition between organisms. While the $^{13/12}C$ technique does not always yield unequivocal results, it does permit conclusions to be drawn, or independent testing of conclusions based on other techniques, which would otherwise be very difficult. An example is the adequacy of CO_2 as carbon source for deep water red algae in their light-limited environment; most seaweeds use bicarbonate (Johnson *et al.*, and Maberly *et al.* in preparation).

Analogous to photosynthesis for carbon is assimilation of inorganic forms of nitrogen by plants as a way of making inorganic forms of essential elements available to plants and hence to other organisms. Atmospheric N_2, and N_2 dissolved in natural waters, is depleted in ^{15}N relative to other nitrogen forms (inorganic, such as ammonium and nitrate, or organic). This is a result of the discrimination against ^{15}N in the denitrification process, carried out by some bacteria which returns "combined" nitrogen to the atmosphere. Biological nitrogen fixation discriminates very little between ^{15}N and ^{14}N, so organisms relying on N_2–fixation (e.g., many cyanobacteria) have $^{15/14}N$ values similar to those in atmospheric N_2 (Handley & Raven, 1992). While assimilation of combined nitrogen by aquatic plants can, when the combined nitrogen is present at high concentrations, show significant discrimination against ^{15}N, the combined nitrogen levels in many natural waters (e.g., with no upwelling or pollution) is low enough to give minimal discrimination during assimilation. This means that, in the aquatic environments with low combined nitrogen levels in which N_2–fixation is likely to occur, the organic nitrogen in photosynthetic organisms reflects the $^{15/14}N$ ratios of the nitrogen sources, so that N_2–fixers have lower $^{15/14}N$ than do non–fixers using combined nitrogen. $^{15/14}N$ isotope studies have shown that **potentially** N_2–fixing cyanobacteria in both freshwater and in the sea actually **do** fix N_2 under natural conditions (Handley & Raven, 1992).

A larger fraction of at least the microscopic photosynthesisers in aquatic habitats is consumed by herbivores than is the case for land plants under natural conditions. Tracking which animal consumes which plant in a relatively non–invasive way is facilitated by $^{13/12}C$ and $^{15/14}N$ studies. The $^{13/12}C$ ratio of herbivores is close to (often slightly higher than) that of their food source, the discrepancy apparently relating to a slight ^{13}C–depletion in carbon dioxide respired by the herbivores (Peterson & Fry, 1987). For nitrogen, the ^{15}N enrichment in the animal reactive to its plant food source is larger (Peterson & Fry, 1991). While ^{15}N depletion in excreted nitrogen is (as with the smaller carbon effect) the most likely explanation, complete nitrogen balance sheets which prove this are not yet available (Handley & Raven, 1992). However, the $^{15/14}N$ of herbivores in habitats in which N_2–fixing and non–N_2–fixing photosynthesisers both occur shows which category of photosynthesiser they are consuming, or if they are eating both. For ^{13}C the situation is more complex, since there is usually a greater range of $^{13/12}C$ ratios in aquatic plants in a given habitat than is the case of $^{15/14}N$ ratios. Nevertheless, extreme values of $^{13/12}C$ in herbivores clearly targets the extremes of the range in photosynthesisers as their food source although intermediate values could result from a mixture of the extremes as well as eating solely plants with an intermediate value of $^{13/12}C$. A combination of $^{13/12}C$ and $^{15/14}N$ studies can help to resolve plant–herbivore interactions which are intractable with either isotope alone; we shall see later that $^{34/32}S$ can help further in resolving problems, of attribution of plant food sources for animals (Peterson & Fry, 1987; Fry, 1990).

Primary carnivores (animals eating herbivores) and secondary (or even tertiary) carnivores (animals eating primary or secondary carnivores) obey the same rules of discrimination during assimilation and excretion of their food sources as do the herbivores. Thus, a primary carnivore is usually slightly enriched in ^{13}C than is its herbivore food source(s), while it is substantially enriched in ^{15}N relative to its food; similar isotope ratio differences occur at the higher levels of carnivory. This consistency of relationships between isotope ratios of consumers and consumed along a food chain enables an organism of unknown feeding habits to be aligned to a trophic level when the $^{15/14}N$ of all of the primary producers in the system is the same. Alternatively, an organism known to operate at a given trophic level in a system

with inputs of both N_2 and combined N can be shown to rely predominantly on N_2–fixing, or on non–N_2–fixers, or a combination, at the primary producer level (Peterson & Fry, 1987; Handley & Raven, 1992).

A component of the aquatic food chain which has thus far not been dealt with is the detritus food chain which uses organic material which has not been consumed as live organisms, or which is the product of defecation or excretion by animals. While by no means negligible in the open ocean or large lakes, the significance of detritus consumption is greater in coastal waters, estuaries, rivers and smaller bodies of freshwater. In addition to products of photosynthesis within the water body, detritus comes from allochthanous material entering the water body **naturally** from terrestrial communities, and as a result of man's activities as sewage and related waste. Detritus–consuming animals can themselves be consumed by carnivores, providing a parallel input to the upper trophic levels from that provided by the photosynthesiser–herbivore route. A number of studies in rivers and estuaries have used stable isotope natural abundance to determine the reliance of higher trophic levels on various autochthonous and allochthanous sources of organic matter. In the most complex food webs, with organic inputs from C_3 and C_4 terrestrial plants and large and small aquatic plants, the position of consumers in the two dimensional space defined by the axes of $^{13/12}C$ and $^{15/14}N$ may not define dependence on a unique group of primary producers. A substantial increase in resolution is introduced *via* a third pair of stable isotopes, i.e., $^{34/32}S$ (Peterson *et al.,* 1985).

The rationale for use of sulfur as a tracer in stable isotope analysis of food webs lies, as with nitrogen, more in the different $^{34/32}S$ in the inorganic source material available to various primary producers than to the fractionation of isotopes during their assimilation by the primary producers. Again,, as with nitrogen, microbial dissimilatory reduction of a fraction of the available pool of the oxidized form, sulfate (nitrate) to sulfide (nitrogen or nitrous oxide) yields a ^{34}S (^{15}N)–enriched pool of the oxidized substrate (Peterson & Fry, 1987). Superimposed on these major differences are "natural" variations in the $^{34/32}S$ of sulfate and sulfide as a function of habitat, and inputs of anthropogenic sulfur dioxide derived from essentially complete oxidation of reduced sulfur in fossil fuels. Most of the inputs of sulfur to primary producers involve sulfate assimilated with little isotopic discrimination, although some rooted aquatic plants living on anoxic sediments assimilate sulfur with the low $^{34/32}S$ typical of the sulfide which is the dominant inorganic sulfur form in these environments (Peterson & Fry, 1987; Raven, 1987). The $^{34/32}S$ in primary producers shows variations which do not parallel variations in $^{15/14}N$, and in turn neither of them parallels variations in $^{13/12}C$. These unique combinations of the ratios of the three isotope pairs in different primary producers, and the reproductive changes in ratio along a food chain (small for carbon and sulfur, larger for nitrogen), form the basis of their use in determining the source of the organic material used by various members of the food web.

Stable isotope natural abundance studies of sulfur could help in the quantitative determination of the sources of atmospheric oxidized sulfur which not only contributes to acid rain but also from cloud condensation nuclei, thus affecting albedo and precipitation. The two major sources are the above mentioned combustion of fossil fuels and the oxidation in the atmosphere of dimethylsufide derived from dimethylsufoniumpropionate, a substantial component of many marine microalgae. While technically difficult, $^{34/32}S$ measurements on sulfur dioxide produced by combustion of fossil fuels have been compared with the (likely) $^{34/32}S$ of sulfur dioxide derived from dimethylsulfide in interpreting the $^{34/32}S$ of atmospheric

sulfur dioxide remote from either source (Nriagu *et al.,* 1991).

4.2 *Physiology*

4.2.1 *Plant physiology*

Besides its intrinsic interest as the discipline which attempts to find "how a plant works," plant physiology forms an essential link between the study of individual chemical and physical processes in plants and their performance in managed nor natural plant assemblages. A number of recent advances in plant physiology have depended crucially on measurements of the natural abundance of stable isotopes; many of these have involved analysis of the carbon dioxide fixation mechanisms in photosynthesis.

Most terrestrial plants use the C_3 pathway of photosynthesis, in which atmospheric carbon dioxide diffuses to the chloroplasts, where it is fixed by the enzyme ribulose 1,5–bisphosphate carboxylase–oxygenase (RUBISCO). The diffusion of CO_2 is initially in the gas phase, from bulk atmosphere through leaf diffusion boundary layers, stomata and intercellular gas spaces up to the (wet) cell walls of photosynthetic cells. The CO_2 dissolves in the cell wall water, and then diffuses in the aqueous and lipid phases through the wall and the cell interior, including the membranes encountered along this pathway. Considerations of the area over which diffusion occurs and the distance involved, suggest that most of the restriction of the rate of photosynthesis which is related to transport of carbon dioxide can be attributed to the gas phase, despite the ten thousand–fold lower rate of diffusion of carbon dioxide in water (and lipid) than in air. However, diffusion of carbon dioxide is not generally the major limitation on the rate of photosynthesis in C_3 plants supplied with adequate light and water; the order of fractional limitation of photosynthesis is: biochemistry (about two–thirds), gas phase carbon dioxide diffusion (about one–quarter) and finally liquid phase diffusion (about one–twelfth or less) (Raven, 1981; Woodrow *et al.*, 1986; Woodrow & Berry, 1988). The fraction of the total limitation which resides in the various parts of the pathway has implications for the inputs of other resources which are needed for photosynthetic CO_2 fixation. Thus, a smaller fractional limitation by diffusion in the gas phase would involve a greater water loss per unit of carbon dioxide fixed, while a smaller fractional limitation by biochemical reactions would involve a greater investment in catalytic (enzymic) protein and thus a larger nitrogen cost (Quick *et al.,* 1991; Stitt *et al.,* 1991). The ecological and agricultural implications of these consequences are clear.

The natural abundance of stable isotopes of carbon can be used to provide data on the fractional limitations of photosynthesis by these various processes, based on the much smaller discrimination between ^{13}C and ^{12}C which occurs in diffusion in the gas or liquid phases than in the action of RUBISCO in catalysing carboxylation (Farquhar *et al.,* 1989). Since any carbon losses from the plant in respiration (as CO_2) or as organic material reflect, over substantial time periods, the $^{13/12}C$ of the plant as a whole,, the $^{13/12}C$ of organic material in the plant, relative to that of the CO_2 from which it was derived, can be used to indicate discrimination between ^{13}C and ^{12}C which was achieved during CO_2 fixation reactions. This **achieved** discrimination can then be used in a model which uses the known discriminations which would be achieved by diffusion alone and by enzymatic CO_2 fixation alone to compute the fractional limitation to photosynthesis by these two component processes. The results of

such computations yield results which compare favourably with measurements of leaf carbon dioxide uptake and water vapour loss as a means of estimating fractional limitations or, indeed, of long term dry weight gain and water loss determinations under controlled environment conditions (Farquhar *et al.*, 1989; Raven & Farquhar, 1990). Such ^{13}C and ^{12}C measurements can yield valuable information, obtained in a relatively non-invasive way, on water costs of growth and fractional limitations on photosynthesis by transport processes and by biochemistry. These measurements do **not** give data on **rates** of processes, including the effectiveness of nitrogen use in photosynthesis; unlike water costs, nitrogen costs are per unit time. The analysis also needs correction for any changes (as a function of N source, for example) in the fraction of plant carbon which is derived from carboxylation reactions other than RUBISCO (Raven & Farquhar, 1990). However, the variations in $^{13/12}C$ which result from different degrees of augmentation of RUBISCO catalysed CO_2 fixation by other carboxylases with lower $^{13/12}C$ discrimination are themselves of interest. Granted that such effects are corrected for, the $^{13/12}C$ ratio of C_3 plants in the field may be helpful in indicating the water cost of growth (see Terrestrial Ecology Section).

A recent technical innovation is the measurement of "instantaneous fractionation" during net CO_2 fixation. Here, the rate of net CO_2 fixation is measured, as in conventional leaf gas exchange, by adjusting the rate of gas flow through a leaf chamber such that the draw down of CO_2 is measurable but does not cause undesirable gradients of CO_2 across the leaf surface. Furthermore, the $^{13/12}C$ of the inflowing and the outflowing CO_2 is measured, thus permitting determination of the C isotope discrimination during CO_2 fixation (Evans *et al.*, 1986). Among the important contributions this technique has already made is that of providing an estimate of the fractional limitation on photosynthesis caused by CO_2 diffusion in the aqueous (and membrane) phases. Simultaneous measurement of water vapour loss and carbon dioxide uptake permits estimation of fractional limitation of photosynthesis by CO_2 flux through the part of the pathway which is common to carbon dioxide and water vapour, while $^{13/12}C$ measurements permits estimation of the limitation of photosynthesis by gas phase **and** liquid phase transport of carbon dioxide (Evans *et al.*, 1986).

A relatively under-exploited area of research is the use of $^{13/12}C$ measurements in investigation of the response of C_3 plants to long-term exposure to CO_2 partial pressures of twice the present atmospheric values, i.e., the 700 Pa expected within the next 100 years. A particularly potent experimental combination would be $^{13/12}C$ analysis of transgenic plants which have over- or under-expression of key catalysts or structures which could alter fractional limitation of photosynthesis by the various parts of the CO_2 acquisition pathways (Woodrow & Berry, 1989; Quick *et al.*, 1991; Stitt *et al.*, 1991; Hudson *et al.*, 1992).

A small fraction of the species of terrestrial plants possess a C_4 pathway of CO_2 acquisition. Despite the small number of C_4 species, they are important components of natural and managed herbaceous vegetation in climates with growing season temperatures warmer than those found now in Britain. It is likely that Global Climate Change will alter the British climate such that C_4 plants will perform better than they do at the moment, at least as far as temperature goes, although increased CO_2 partial pressure could decrease the advantages that C_4 plants have over C_3 competitors in warmer environments. $^{13/12}C$ discrimination studies have made very important contributions to our understanding of the mechanism of C_4 photosynthesis, and further $^{13/12}C$ studies could help in making additional progress (Farquhar, 1983; Farquhar *et al.*, 1989; Raven & Farquhar (1990).

The essence of C_4 photosynthesis is the maintenance of a CO_2 concentration at the site of RUBISCO activity which exceeds that in water in diffusive equilibrium with air. This higher CO_2 concentration in C_4 plants needs an energy input to a special mechanism based on novel anatomy and novel, and additional, expression of common, enzymes, in particular sites. An initial carboxylation by phosphoenolpyruvate carboxylase (PEPc) is followed by transport of carboxylic acids to the site of RUBISCO where CO_2 is regenerated to form the CO_2 pool at a high steady state concentration. Much of this CO_2 is refixed by RUBISCO but, as might be expected from the small and lipid-soluble nature of CO_2 and the distance between RUBISCO and the nearest intercellular gas spaces, some leaks out.

The low discrimination between ^{13}C and ^{12}C in CO_2 diffusion,and in fixation (actually HCO_3^-) by PEPc, means that a totally "leak-proof" C_4 plant would have a $^{13/12}C$ in organic material which was little lower than that in atmospheric CO_2, i.e., none of the potential discrimination by RUBISCO could be expressed. This contrasts with C_3 plants where at least half of the intrinsic discrimination capacity of RUBISCO is expressed. While the $^{13/12}C$ ratio of organic C in C_4 plants is indeed higher than that in C_3 plants, it is not as low as would be expected for a "leak-proof" C_4 mechanism. The fraction of CO_2 leakage can, with certain assumptions, be used to predict $^{13/12}C$ in the plant, and *vice versa*. Predictions of leakage as a fraction of CO_2 pumped into the compartment containing RUBISCO from the $^{13/12}C$ ratio is in agreement with anatomy and enzymology of the various metabolic subtypes. Furthermore, the predicted energy costs of C_4 photosynthesis, including the costs associated with additional pumping of CO_2 in plants with a larger fractional leakage, agree with the measured energy costs of net CO_2 fixation (mol photons absorbed per mol C fixed) (Farquhar *et al.*, 1989). It is, however, clear that more work is needed on the *in vivo* $^{13/12}C$ discrimination achieved by PEPc.

The smaller $^{13/12}C$ discrimination by C_4 than by C_3 terrestrial plants means that $^{13/12}C$ ratios of plants, including herbarium specimens and many fossils, can be used to decide if a plant shows C_3 or C_4 metabolism. A caveat must be heeded, in that CAM (Crassulacean Acid Metabolism) plants have similarly high $^{13/12}C$ values to those of C_4 plants (Farquhar *et al.*, 1989). Accordingly, anatomical investigations are needed in parallel with $^{13/12}C$ measurements to distinguish C_4 from CAM plants. A further use for $^{13/12}C$ measurements in distinguishing metabolic pathways in no-longer-metabolising (dead) plants is that of determining if a dead plant with a chloroplast-containing bundle sheath cells (the cells which, in C_4 plants, are the sole location of RUBISCO) was a true C_4 plant or a C_3-C_4 intermediate. The latter plants have certain gas exchange characteristics resembling those of C_4 plants, yet lack a full operational CO_2 pump. They rely at least in part on refixation of CO_2 released in light-dependent metabolism (photorespiration) which, by allowing two opportunities for RUBISCO to discriminate between ^{13}C and ^{13}C in producing at least some of the plant organic C, can yield $^{13/12}C$ ratios even lower than in typical C_3 plants. Accordingly, a high $^{13/12}C$ in a plant with a chloroplast-containing bundle sheath indicates C_4 metabolism, while a low $^{13/12}C$ ratio in a plant with this sort of anatomy indicates C_3-C_4 intermediate metabolism

This combination of anatomical and $^{13/12}C$ studies was vital in characterising the oldest known C_4 plant, a fossil grass from sediment laid down some ten million years ago. This plant had a typical C_4 (or C_3-C_4 intermediate) bundle sheath, thus ruling out its being a C_4 metabolism rather than C_3-C_4 intermediate behaviour (Tidwell & Nambudiri, 1989). Subsequent work showed a closely related fossil grass from the same sediment was a C_3 plant since it did not have chloroplast-containing bundle sheath but did have a low $^{13/12}C$ (Tidwell & Nambudiri,

1990). This finding of closely related plants with the two different pathways (C_3 and C_4) has many analogues in present-day plants. Furthermore, the finding of a C_4 plant ten million years old is interpretable in terms of the climate (warmer than today's) and the trend of atmospheric CO_2 concentration (falling), which would increasingly favour C_4 relative to C_3 plants although C_4 plants are probably older than this (Raven, 1991). Such fossil studies are important in considering projected future climate change.

A final use of $^{13/12}C$ ratios to which we shall refer in the context of plant physiology is that of determining the pathway of synthesis of major metabolites for which two or more plausible alternative pathways are possible. An example is the synthesis of oxalic acid which, as oxalate salts, is a widespread and abundant component of acid-base regulation mechanisms as well as a potential anti-biophage. Measurements of $^{13/12}C$ ratios of oxalate and of bulk plant organic carbon can distinguish biosynthetic pathways which involve PEPc from those (e.g., the oxidation of glycolate produced by the oxygenase activity of RUBISCO) which do not (Raven *et al.*, 1982). The biosynthetic pathway, especially in view of the exchangeability of the O atoms in oxalate, needs further investigation.

Studies on the natural abundance of nitrogen isotopes have so far had less impact on plant physiology than has corresponding work with carbon isotopes. However, there are prospects for important contributions to plant physiology, as well as for extensions of the use of physiologically-based interpretations of nitrogen isotopes in ecological studies.

Nitrogen assimilation for N_2 (nitrogen fixation) shows very little fractionation of ^{14}N from ^{15}N; this assertion is based on work on whole nitrogen-fixing bacteria, cyanobacteria or symbiotic plants, but it seems to represent accurately the properties of the enzyme nitrogenase. The enzymes assimilating "combined" nitrogen (ammonium, nitrate) do show discrimination, but this is not expressed to any great extent when the external supply of combined nitrogen is growth-limiting. Since (see sections on Ecology) the combined nitrogen available to plants in the field has a higher $^{15/14}N$ than does atmospheric N_2, plants growing on N_2 as nitrogen source will be depleted in ^{15}N relative to plants growing on combined nitrogen **unless** the combined nitrogen concentration is high enough to permit fractionation during assimilation (Handley & Raven, 1992).

A potential contribution of nitrogen isotope natural abundance studies to plant physiology is in more detailed investigations of the variation in $^{15/14}N$ among plant organs and among individual nitrogen compounds. The patterns which are emerging will help in analysing the location of interconversions and, in some cases, which of alternative mechanisms are being used (Handley & Raven, 1992).

Other isotopes which have contributed to plant physiology, with the potential for contribution to ecological analyses, are oxygen ($^{18/16}O$) and hydrogen ($^{2/1}H$).

Photosynthetic oxygen evolution shows no $^{18/16}O$ discrimination, with implications for the mechanism of water dehydrogenation (Guy *et al.*, 1987; Raven, 1990). Respiratory oxygen uptake discriminates against $^{18}O_2$, and differences in this discrimination for different oxidases (e.g., the "alternate oxidase" relative to cytochrome oxidase) holds out great promise for a non-invasive estimation of the role of the alternate oxidase in plant respiration (Guy *et al.*, 1987, 1989; Raven, 1990). The technology is not easy, but the rewards are potentially great.

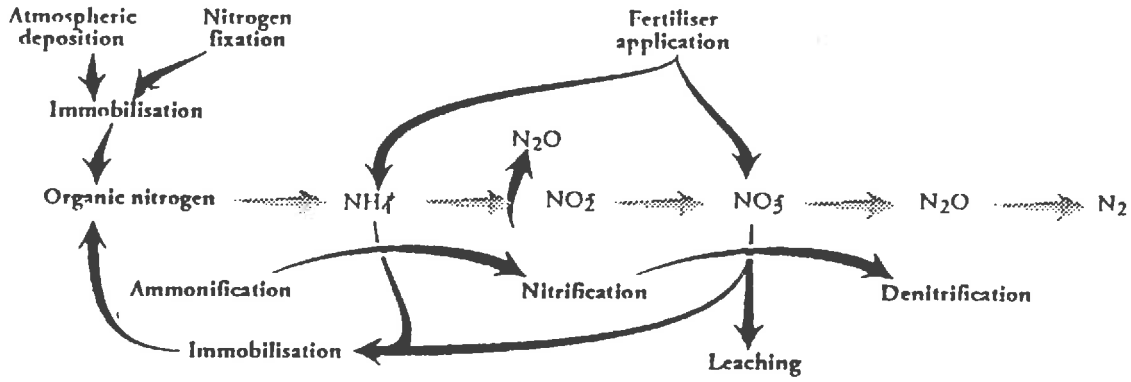

Figure 19. A simplified segment of the nitrogen cycle.

The combination of non–discriminatory water dehydrogenation to produce O_2, and a discriminatory (against $^{18}O_2$) O_2 uptake in respiration, explain the "Dole Effect", i.e., the higher $^{18/16}O$ ratio in atmospheric O_2 than in water in the sea or freshwater. Quantitatively, the "Dole Effect" also involves consideration of the $^{18/16}O$ ratio in the water molecules which are the substrate for water dehydrogenation. For the sea, which accounts for about one–third of global gross O_2 production, the water consumed in photosynthesis has a very similar $^{18/16}O$ ratio to that in seawater. For the terrestrial vascular plants, which account for most of the remaining two–thirds of global gross O_2 production, the $^{18/16}O$ of the water which is substrate for O_2 evolution in these plants is higher than that in seawater. This results from the fractionation of oxygen and hydrogen isotopes during transpiration, with the water vapour passing to the atmosphere having less ^{18}O and ^{2}H than soil water, leaving leaf water which is enriched in ^{18}O and ^{2}H relative to ground water and (usually) the seawater "standard" for ^{18}O and ^{2}H.

The enrichment in ^{18}O and ^{2}H in the water in leaves is a complex phenomenon; leaf water is not homogeneous with respect to ^{18}O and ^{2}H. Knowledge of this compartmentation is crucial in interpreting the quantitative aspects of the Dole Effect, and is being actively studied in several laboratories (Yakir *et al.*, 1990). The relationship between the varying enrichment in ^{18}O and ^{2}H of different leaf water compartments and the $^{18/16}O$ and $^{2/1}H$ ratios of (non–exchangeable) organic O and H is also in need of further investigation, since a reliable relationship between his more transportable organic O and H and the leaf water would be very useful in ecological studies of the use of $^{18/16}O$ and $^{2/1}H$ in the estimation of transpiration rates and of water source (provided the alternative sources have different $^{18/16}O$ and/or $^{2/1}H$ ratios) (Luo & Sternberg, 1992). Such knowledge would also have significance in the analysis of the isotope ratios of fossils in terms of their environment.

4.2.2 *Microbial Physiology*

The main areas of interest, relating to microbial physiology, will involve the transformations of elemental species within biogeochemical cycles in the environment. Such cycles would include the transformations of species of carbon, nitrogen, oxygen, sulfur and hydrogen, primarily, but the list could be extended to include microbial activities in, the mobilisation and immobilisation of other elements.

A detailed explanation of one of these biogeochemical cycles, the nitrogen cycle, illustrates the general applicability of isotope ratio mass–spectrometry to the study of the many steps involved in the biological, and abiotic, transformations of these elements in the biosphere.

Essentially the nitrogen cycle is an interconnected series of transformations, under biological control (Figure 19). These processes are in no way interdependent, and there is no orderly progression from one compound to the next, as occurs in a genuine biochemical cycle, such as the citric acid cycle.

The nitrogen cycle, because of its great complexity, is variable in its operation in different types of ecosystem, in different regions, and in different localities. Indeed it shows substantial variability, at all scales investigated, both spatially and temporally.

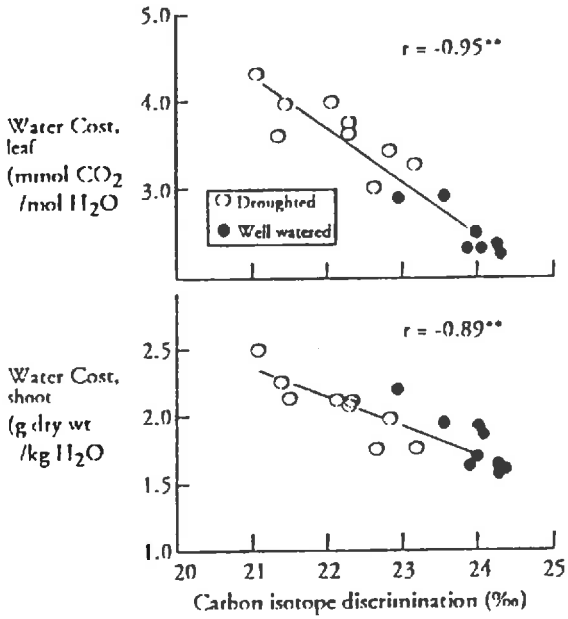

Figure 18a and b. Relationship between C isotope discrimination (Δ) and on a single leaf (water cost leaf = 16.87 – 0.60Δ) or shoot (water cost shoot = 7.12 – 0.23Δ) versus water cost of growth, based on nine clones of crested wheatgrass from low, medium, and high Δ classes grown at two soil moisture levels. Values for water cost leaf represent means of 30 determinations averaged across five sampling dates. Values for water costs represent means of six determinations. (Read *et al.*, 1991).

The processes important in the soil are: nitrogen–fixation by free living organisms, ammonification or mineralisation of the organic matter, nitrification, denitrification and immobilisation. These processes operate within a system having additional inputs and outputs. The inputs include, organic nitrogen from plant and animal residues, atmospheric fixed nitrogen, and fertiliser nitrogen. The outputs include, ammonia volatilisation, nitrous oxide produced during both nitrification and denitrification, leaching, mainly of nitrate, and uptake by plants, and microorganisms, of both NH_4^+ and NO_3^- –N.

Many microbially mediated biochemical reactions are involved in these processes, some of which have been more thoroughly investigated than others.

Important reactions are:

1] $-CNH_2^- \rightarrow NH_4^+$

2] $NH_4^+ + [O_2] \rightarrow NH_2OH + OH$

3] $NH_2OH + [O_2] \rightarrow NO_2^- + H_2O$

4] $NH_2OH + H_2O \rightarrow N_2O + H_2O$

5] $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e$

6] $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

Reaction 1] represents ammonification, or mineralisation, of soil organic matter and has been rather poorly investigated. Reactions 2], 3], and 5] represent the main steps in nitrification, and reaction 4] shows the production of nitrous oxide, during this process. Reaction 6] indicates the several steps in the process of denitrification. Isotopic fractionation can, and does occur during the operation of many steps in both nitrification and denitrification, and probably during ammonification also. Such fractionation explains the different δN signatures of the various components of the soil nitrogen system, and why the overall δN value for soil is generally significantly above unity. The observed isotopic fraction (ß) for several of the above steps has been determined, both in pure culture systems, and in several cases in soil. However even if the ß observed for a particular reaction were constant, and it is not, the degree to which the $\delta^{15}N$ of a particular compound in a complex system, such as soil, would be altered by isotopic fractionation would be difficult to predict without extremely meticulous laboratory and field based investigations of the system.

Despite the difficulty of interpretation the use of stable–isotope ratio analyses to quantify the rates, and routes, of these various processes of conversion, and the factors that control them is attractive for several reasons. For instance, compared to isotope–enrichment, and depletion techniques, the use of natural abundance ratios does not involve perturbation of the system, and no methodological artifacts are produced. The technique can also be used over a wide range of agricultural and natural systems, without cost constraints, on multiple sample points and is the only technique that allows for the discrimination of the source of the nitrogen species analysed.

As discussed above every process, whether occurring at the molecular, cellular, organism or ecosystem level, has the potential to discriminate between the different isotopes of the elements involved. By considering a suite of stable isotopes, such as ^{15}N, ^{13}C and $^{18}0$, processes that do not discriminate between isotopes of one element can be detected, because of the discrimination of another isotope pair in the compound. For example the use of oxygen signatures can be revealing in the study of the nitrogen cycle. $\delta^{18}0$ of atmospheric oxygen is +23‰, whilst that of rain water and other natural fresh waters is near to −7‰. It is apparent that N_20 resulting from denitrification will have a $\delta^{18}0$, similar to that of the precursor nitrate resulting from reactions 2] to 5]. Because reaction 5] involves oxygen derived from water it's overall signature will be depressed. In contrast, N_20 resulting from reaction 4] will have a higher $\delta^{18}0$ because it's oxygen is derived entirely from the atmosphere, a relatively enriched source.

The potential for this methodology can be validated by reference to previously published work.

The natural concentrations of ^{15}N are usually given as parts per thousand (‰) differences from atmospheric molecular N_2 and written in δ notation.

Fresh organic matter is normally slightly depleted in ^{15}N, with respect to the nitrogen source. This depletion is small when it is obtained by direct assimilation of N_2 by the plants via symbiotic bacteria, or free living organisms. However if the nitrogen source is nitrate or ammonium nitrogen, with variable ^{15}N contents, resulting from different assimilation pathways, a full spectrum of $\delta^{15}N$ values can be expected. However during decay, i.e. mineralisation of the organic matter to NH_4^+, when smaller molecules are being extracted from the more complex organic matter, the process involved may lead to enrichment, in ^{15}N, of the remaining soil organic matter. Studies of soils of different origins have shown a large scatter of values, between −4.5 and +17‰. Differences were found between soils, and between the different horizons in the same soil. This results from a difference in the state of decay of the soil organic matter, and also from differences in the quantity and quality of the vegetative, and animal inputs, on the different soils. Since decay enriches the remaining organic matter, in ^{15}N, the more evolved the remaining organic matter is, the greater the ^{15}N content. Farming practices enhance bacterial activity, so influencing the sequence of events in the soils, and as a result cultivated areas have a higher ^{15}N content than uncultivated areas. Studies of the $\delta^{15}N$ values of nitrate have produced contradictory results. Some studies have shown no systematic differences in ^{15}N between the soil organic matter and the evolved nitrate. However other studies have shown that the first nitrate produced has a low $\delta^{15}N$, which subsequently increases towards a steady–state value. This may well be due to the state of the organic matter from which it is derived. As freshly incorporated organic matter is decomposed, favouring of the lighter isotope will result in the nitrate produced having a relatively low $\delta^{15}N$. However, as a direct result of this the $\delta^{15}N$ in the remaining organic matter will be increased, and this in turn will result in an increase in the $\delta^{15}N$ value of any nitrate produced later in the decay cycle. Other processes, such as denitrification will also increase the soil nitrate $\delta^{15}N$ value, as the lighter isotope will again be preferentially used in the reduction reactions to nitrous oxide and dinitrogen. Immobilisation of the nitrate, into the biomass tissue will also have the same effect, on the residual soil nitrate.

Two other factors require consideration in the interpretation of ^{15}N data relative to soil

nitrates. Firstly, the limiting effect of one of the sequence of successive reactions where each possesses its own fractionation effect for nitrogen. The reactions in the nitrogen cycle will be affected in a similar way and so differences in the rates and means of production, of for example nitrate, will be reflected in variations of the $\delta^{15}N$ values, because of the fractionation effects. Although the loss of nitrate via leaching into the groundwaters does not fractionate isotopes, it does modify the balance of the nitrogen isotopes in the system, because the quantity of nitrate has been decreased. So nitrate production, denitrification, leaching and reassimilation by the microorganisms affect the quantity of the available nitrate and its isotopic composition. For instance, an area of poor drainage will produce different $\delta^{15}N$ values from a well drained one, all other factors being equal.

Stable isotope signatures can be used to discern the processes involved in the nitrogen cycle. Isotopes make excellent tracers of sources and sinks, and can identify contributing processes involved in e.g. N_20 formation and evolution. Discrimination of the sources of N_20, an important greenhouse gas, will be very important, for environmental monitoring and management. Use of this fractionation signature is the only way of attributing the product, N_20, to the responsible process, nitrification or denitrification. As the reactive sequences that result in N_20 formation are very different in the two processes, the isotopic signatures should also be very different. The depletion, of ^{15}N, in the N_20 produced during nitrification has been reported to be as much as – 60‰, and for denitrification between −10 and −30‰. Which of these reactions is occurring can also be discerned from changes in the source material signature. The use of the isotopic fractionation of $^{18}0$ would greatly enhance such studies, as discussed above.

It is also possible to follow the fate of nitrates in ground waters, and aquifers. Determination of the isotopic signature will show whether reductions in the nitrate concentrations are due to dilution with nitrate–free water, or a result of denitrification. The rates of denitrification can also be assessed, as the isotopic fractionation factor has been shown to be rate dependent, with a value of −33‰ during highly active periods, and falling to −5‰ when activities are low.

As the isotopic ratio of nitrogen differs between pools within ecosystems, measurements of changes in these ratios allows for the transfer between pools to be traced. This approach can be useful in studies of the effects of nitrogen–fixation on systems. There is no variation in the ^{15}N abundance of atmospheric N_2 collected from broadly dispersed locations. Atmospheric nitrogen, fixed by microorganisms, that are either free–living or in symbiotic association, will produce nitrogenous compounds with a δN value of 0. The mean δN value for the surrounding soil will be positive, to varying degrees, and so any movement, or transformation of the fixed–nitrogen can be followed by changes in the background δN value of the soil.

Thus careful experimentation, following changes in the stable isotope signatures, in both the source materials and products, is a powerful analytical technique that will produce data that will greatly assist in the understanding of the dynamics of, and the processes involved in, transformations in many of the biogeochemical cycles in the environment.

4.2.3 *Animal physiology*

There are very few studies of physiological processes in animals which exploit natural variations in stable isotope abundance. A notable exception is the attempt to model isotope distribution in the body water of animals. Particular examples are ^{18}O variation in bats (Speakman and Racey 1987), and of both deuterium and ^{18}O in birds (Tatner 1988). The isotopic composition of the body water is influenced by a number of factors, including ingested water and food and evaporative water loss. In addition to these factors, which affect both hydrogen and oxygen, the oxygen composition is also influenced by carbon dioxide production and the incorporation of atmospheric oxygen. These models have been used to interpret observed differences in body water composition between species (carnivorous and herbivorous birds) and between different physiological states (pregnant and lactating bats). A most interesting application of this method is in the prediction of the respiratory quotient for free living animals, but unfortunately it appears that the sensitivity to changes in respiratory quotient is not sufficiently high for general application.

While natural abundance methods are so far largely unexploited in animal physiology, it should be noted that many methods for both chemical isolation and isotope analysis used in stable isotope **tracer** studies are well developed. This has been driven by the need to use stable isotopes for safe tracer studies in humans, and methods are available for the study of protein, fat and carbohydrate metabolism both in the whole body and specific tissues. Of particular relevance to natural abundance studies is the fact that the end products from such tracer studies are often enriched by only the same order as natural abundance variation, and some knowledge of natural baseline variation has accrued from these studies. For example the baseline variation in plasma and muscle protein has been reported (Hays *et al.*, 1990) Recent development work on ^{15}N tracer studies of protein synthesis has shown marked inter-amino acid and inter-organ differences in ^{15}N abundance (Watt and Scrimgeour, unpublished). These differences are qualitatively interpretable in terms of differences in amino acid transamination. Such differences can be readily quantified from combined $^{15}N/^{13}C$ isotope dilution for specific amino acids, but no attempt has been made so far to systematise or interpret the natural variations in a quantitative fashion.

The use of 'natural tracers' has been applied to the study of carbohydrate metabolism. Carbohydrate (usually glucose) derived from C_4 plant species such as maize has a sufficient excess of ^{13}C to be detected above the normal baseline ^{13}C level derived from the whole diet. Thus a bolus of maize derived carbohydrate results in an increase in the expired $^{13}CO_2$ from which the proportion oxidised can be calculated (Pirnay *et al.*, 1977). Boutton (1991) discussed the use of natural and enhanced ^{13}C diets as tracers in humans and large animals. Hare *et al.* (1991) fed C_3 and C_4 diets to pigs as natural tracers of carbon metabolism. They determined that non-essential amino acids were enriched in ^{13}C relative to diet and that a non-essential amino acid was depleted. Sutoh, Koyama and Yoneyama (1987) described the ^{15}N fractionations associated with nitrogen metabolism in cattle and pigs.