RESEARCH OPPORTUNITIES IN PLANT ECOLOGY, PHYSIOLOGY AND BIOCHEMISTRY USING STABLE ISOTOPE TECHNIQUES AT THE NATURAL ABUNDANCE LEVEL

1. *BACKGROUND*

1.1. *What is a stable isotope?*

Stable isotopes are all around us, they leave useful signatures for all of the common, important processes shown in Fig. 1, as well as many others. We can use these signatures to identify and assess the importance of processes which we cannot see or measure in any other way. With the exception of a small amount of radioactive material, all matter is composed of stable isotopes.

Isotopes, in general, are different weights (masses) of the same element. An element, such as carbon is composed of atoms; atoms, in turn are composed of smaller basic units. The major basic units are protons, neutrons, and electrons. Protons have a positive electric charge; electrons have a negative electric charge; and neutrons are electrically neutral.

These different atomic building blocks have different masses. We can think of mass as weight, and we will use the term weight in this paper. Strictly speaking, however, weight is a unit we use to measure the influence of gravity upon mass.

When most people think of isotopes, they think of the radioactive type. Radioactive isotopes have nuclei (cores) which disintegrate to produce highly energetic, particles. It is well-known that radioactive isotopes are hazardous and must be handled carefully under strict controls.

Carbon-containing greenhouse gases are produced as methane (CH_4) and carbon dioxide (CO_2). In respiration an organism obtains energy by combining oxygen (0_2) with complex carbon molecules. The major chemical products are CO_2 and water.

Stable isotopes, on the other hand, do not emit charged particles; they are not dangerous and require no special safety controls or disposal techniques.

Stable isotopes of an element have the same numbers of electrons and protons, but different numbers of neutrons. Between two stable isotopes of the same element, the charge does not differ, but the weight (mass) does. Hydrogen, for instance, has two stable isotopes, denoted as ^1H and ^2H. The latter is frequently called deuterium and abbreviated, D. The major stable isotopes which are of interest in the study of biogeochemical processes (cellular, whole organisms, or natural systems) are the isotope pairs $^{2/1}$hydrogen (often referred to as D/H), $^{13/12}$carbon, $^{15/14}$nitrogen, $^{18/16}$oxygen, and $^{34/32}$sulfur; the last four of these elements are abbreviated C, N, O, and S. The larger number is always given first, because all changes in isotope ratios are referred to the heavier isotope. An isotope **ratio** is the ratio of the amounts of the heavy isotopes to the light isotopes; a **fractionation** is a change in this ratio and is due to some movement of molecules, containing the isotope pair, for example, a chemical reaction or a physical process, such as evaporation.

Because of the way the isotopes respond to this movement, one of the isotopes (usually the heavier one) tends to be left behind and the other isotope (usually the lighter one) more readily moves away toward the new site or toward the new chemical product. For example, when water evaporates from a pool, the molecules containing ^2H tend to remain behind, and the water molecules containing ^1H tend to evaporate into the air. The pool becomes enriched in the heavier form of hydrogen and the water vapour over the pool becomes diluted with the lighter hydrogen form. Hence the relative amounts of the ^2H and ^1H change in both the pool and in the air over the pool. This change in the ratios is called a fractionation. In this example, we can say that the process of evaporation has fractionated the water and that as a result, the water in the pool has become enriched (in the heavy isotope) and the water vapour has become depleted (in the heavy isotope).

The reactions of stable isotopes in nature can be classified according to the following scheme, as discussed below:

Physico–chemical reactions or **biochemical** reactions

both of which can be

equilibrium reactions or **kinetic** reactions

and can occur in

open systems or **closed** systems.

Physico–chemical reactions. The example given above of $^{2/1}H$ enrichment via evaporation is a physical reaction or process; a chemical process would be the exchange of ammonium to and from surfaces in soil particles.

Biochemical reactions. These involve actual chemical transformations and reassociations of elements into new molecules. They are usually done with the help of a catalyst (helper molecule) which remains unaltered by the reaction. In biological systems the catalysts are special molecules called **enzymes** (O'Leary, 1989).

Physico–chemical and biochemical reactions can be further subdivided into:

Equilibrium reactions. In equilibrium reactions, molecules continually exchange elements, but reach an "agreement" about the relative importance and amounts of each form after the reaction has proceeded for some time. One of the most important equilibrium reactions in nature is that of carbon dioxide gas dissolving in water. Dissolved CO_2 is in equilibrium with bicarbonate (HCO_3^-). As carbons are continually exchanged between CO_2 and HCO_3^- a δ value difference of about 7‰ to 11‰ develops between the two molecules.

Kinetic reactions. Kinetic reactions are more generally known about by non–scientists. In a very simple kinetic reaction starting substances A and B combine to form a product, AB (for example, sodium and chlorine combine to form sodium–chloride, which is also known as table salt). In biochemical systems, this kind of reaction is often mediated by an enzyme; enzymes have preferences for light isotopes as opposed to heavy isotopes (in general) and will

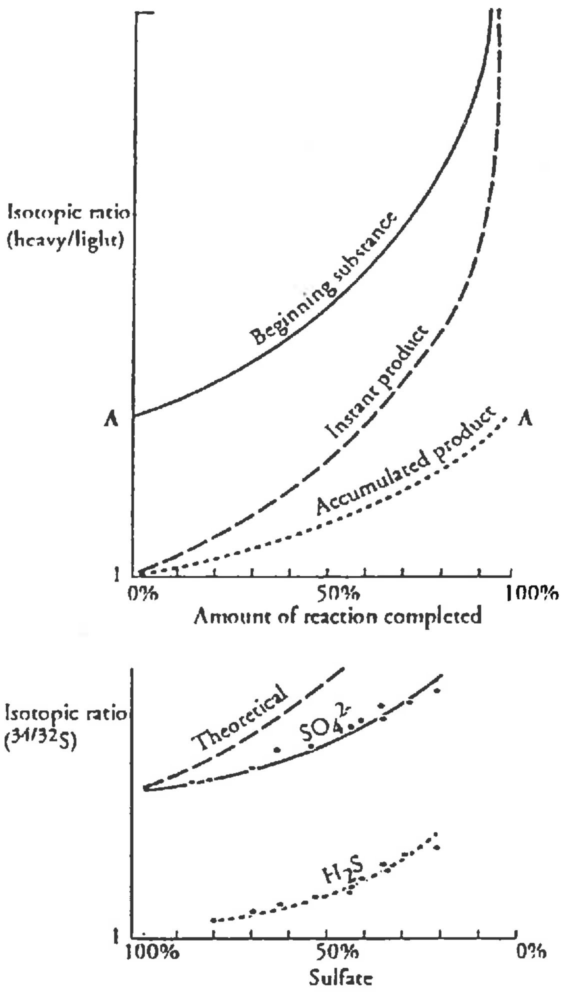

Figure 2. (a) Isotopic changes in a closed system, where the dashed line is the instantaneous product and the dotted line is the accumulated product of a kinetic chemical reaction. At first the begining substance has isotopic ratio, "A". The instantaneous product is lighter in isotopic ratio (dashed line). As the reaction proceeds, the begining substance becomes more and more enriched, as does the instantaneous product, albeit more slowly. In the final phase of the reaction, when all of the begining substance is converted to product, the isotopic ratio of the accumulated product is A, the same as the begining value. (b) A semi-closed system is illustrated, the conversion in sediments of sulfate (SO_4^{2-}) to hydrogen sulfide (H_2S). SO_4^{2-} is the begining substance (solid line), and H_2S is the cumulative product (dotted line). The dashed line at the top represents the theoretical enrichment of SO_4^{2-}, if this were a fully closed system. Reaction 2b never goes to completion or collapses to its original isotope ratio because of small, external inputs.

mostly "choose" the light isotopes. So the product, AB, tends to contain more of the lighter isotope than the starting substances. This is only true if some of the begining substances have not been utilised. If all of the substances react completely, then there is no net change in isotopic composition.

Kinetic reactions are also divided into two types:

Open reactions. In open reactions, some of the starting substances are constantly being replaced from a very large storage pool. These are the most common reactions in nature. For example, green plants "fix" carbon dioxide (CO_2) into starches and sugars from the atmosphere. But the atmospheric storage reservoir of CO_2 is very large compared to the needs of the plants. So the plants bear a fractionation signature chiefly because their enzymatic "helper" prefers the light ^{12}C isotope; but the air is relatively unaffected by the process (except in the very long cumulative and global sense). Because there is a nearly infinite source of CO_2 the plants are free to discriminate (choose) the mass of isotope they "prefer" at a rate which is specific to the enzyme.

Closed reactions. In actual fact, there are no good examples of completely closed reactions in nature. Completely closed reactions happen only in vessels in laboratories. However, there are a number of semi-closed reactions in nature; these are semi-closed because the replenishment rate of the starting material is extremely slow relative to the speed of the reaction. An example which we will explore further (Section 4.1.1) is the semi-closed reaction of microbial reduction of sulfate to hydrogen sulfide in muds which have very little oxygen. Closed reactions do not show a steady amount of fractionation. Instead (Figure 2), the product is more depleted in the heavy isotope than the reactants; as the reaction proceeds the difference between the isotope ratios of the reactants and the products decreases. When the reaction is 100% complete, the product has the same isotope ratio as the reactants did in the beginning. In a semi-closed system, such as the mud and sulfur mentioned above, total conversion never happens, and the resulting fractionations can be large.

The reactions described above are all simple single reactions. It is more common in nature that fractionating reactions are complex and multiple and coupled (linked together). These can be thought of in three ways, as the most common cases (although these are certainly not all of the possible schemes): (a) tightly linked reactions with several enzymatic steps, (b) a complex reaction with a reversible first step, and (c) a branched reaction with more than one possible product. There are other models which we could discuss, but these will serve to illustrate the general schema which commonly occur.

In many tightly linked biological reactions, there is a starting substance and a series of intermediate substances which are completely converted to a product. In this case, where all of the intermediate substances are completely converted to some new chemical product, there can be no fractionation in the intermediate steps. If we see a fractionation in looking at the starting and ending substances, then that fractionation must have happened in the first step.

Photosynthetic carbon fixation is a good example of the second kind of reaction with a reversible first step. The movement of CO_2 from the atmosphere into internal leaf air spaces is reversible, since the gas can move back into the atmosphere. The fractionation we observe in plant parts is dependent firstly on this backward movement and secondly on the chemical combination of the carbon into simple sugars in the plant.

Thirdly, we come to branched reactions. The photosynthesis reaction discussed above is a special form of a branched reaction, in which one of the possible pathways is for the material (CO_2) to flow backward. In branched reactions the reacting substances have the "choice" to go in more than one direction, combining with more than one set of reactants to form more than one set of products. The isotopic composition of the products will depend on the fractionation factor of the enzymes involved **and** it will also depend on how much of the materials branch in which direction and how rapidly. Many biological reactions are branched reactions, such as the formation of amino acids, lipids and carbohydrates. Melzer and O'Leary (1991), for example, recently showed for a natural organic acid, malic acid, that the isotopic composition of the carbons in the acid molecule was determined by the relative proportions of two pathways available to form the acid.

In summarizing the above discussion, isotopic fractionation in most biochemical reactions happens when molecules of slightly different weights react at different rates. Chemical and biochemical research conceptually divides reactions into a series of individual steps that, when combined in kinetic models, yield the appropriate net fractionation for the overall reaction. These models can be expanded and applied at an ecosystems level to understand fluxes of materials, but have not yet been widely used by biologists to do so. Stable isotope budgets must balance in ecosystems as do budgets of total element fluxes.

Much of the foregoing text has been adapted from an excellent review article by Peterson and Fry (1987). Although a few years old now, nothing else has replaced this review for its comprehensiveness or its clear readability and logic. It would be impossible to describe this new field of research without substantially using this review.

1.2 *What are natural abundances of stable isotopes?*

Stable isotope research techniques fall into two categories: enrichment method and natural abundance method. When an isotope pair, which is enriched in the heavy form, is introduced into a system in order to trace a process (i.e., as a strong signal which accompanies a process), this is called the enrichment method. It is conceptually the same as placing dye in a river to trace a current or flow. An agronomist, for example, may give plants fertilizer in which the 99% of the nitrogen is the heavy isotope (^{15}N) and only 1% of the nitrogen is the normally abundant ^{14}N. He might, for instance, do this to determine how much fertilizer nitrogen a plant is using by measuring how much of the heavy nitrogen is then found in the plant. The unit of measurement is atom percent (atom %).

The enrichment method has been used for many years. It will continue to be valuable, alongside the use of natural abundances. Most of the problems with the enrichment method arise from changing the system under study by adding isotopes to it. These additions may also change the system in unpredictable ways, producing results which are false products (artifacts) of the method, rather than being real characteristics of the system we are studying. These artifacts can severely interfere with our attempts to interpret an experiment, both agricultural and ecological.

When naturally occurring levels of isotopes are used as an experimental tool, when no extra amounts of heavy isotope are added, this is called the natural abundance method. The natural abundance method is the focus of our discussion. Natural abundances are measured in δ, units which are parts per thousand (‰) difference from a standard. The alternative unit, atom %, is too large to describe the usual fractionations found in nature. For example, a one–δ–unit change of naturally occurring N isotopes is only 0.0004 atom %; a number this small would be very awkward to use. A typical notation for the natural abundance of N is:

$\delta^{15}N = +6.8‰$, relative to atmospheric nitrogen as the universal standard.

We sometimes use natural abundances, in much the same way as enrichment isotopes, to trace processes, seeking to find the isotope ratio essentially unchanged as it is carried along with the process which we are studying. Properly speaking, this is also a tracer method, although not enriched over the natural levels of isotope ratios (sometimes called **signatures**). However, there is a much more important use for natural abundances. There is a unique feature of natural abundances which will reveal valuable information in a way which no other technique has ever been able to do. Natural abundances can be used as natural markers, revealing through their fractionations (changing values) the occurrence and importance of natural processes, which could not be detected in any other way. Fractionations, which are unwanted in the enrichment method, become natural markers and signposts to processes which may be biochemical, enzymatic, microbial, physiological, or physico–chemical. Nothing is added to the system, and only small samples are taken. The risks of measuring experimental artifacts (and coming to false conclusions, thereby) is lessened, and the risk of damaging the environment is completely eliminated. No radioactivity is involved. Nothing is added, and only very small samples are taken.

With naturally occurring stable isotope fractionations, we can sometimes identify signatures which we can interpret. We know what the signatures of these isotopes mean, and we can use them immediately to identify a process and assess its importance. More frequently, however, this is not possible. We know very little about the ranges of δ–values produced by specific biogeochemical processes under a given set of conditions, and this is an exciting new area of research. Meanwhile, we can still use natural abundances as an iterative approach, moving back and forth between laboratory and nature, between laboratory and glasshouse. The more we know about the system under study (by using supporting alternative methods), the better we can interpret the isotope signatures; the more we know about isotope signatures from laboratory studies of fractionation in model systems, the better we can use them to interpret the natural systems which we wish to understand.

With naturally occurring stable isotope signatures, the important information may be in the variability, not in a linear (tracer) signal as frequently sought by traditional statistics or mathematics. What may be traditionally discarded as noise, contains the patterns (both in space and time) of fractionations (changes) which reveal details of the processes. To deal with this problem, we need a new mathematics, the mathematics of chaos and fractals. This new mathematics, along with statistics, can unravel the complex patterns which are a feature of the non–linear systems frequently found in nature. We need to use this new mathematical tool to understand first simple, then increasingly complex, systems in the laboratory, testing formal hypotheses, in order to explain the ranges of isotope signatures associated with specific processes and sets of processes found in nature.

The use of stable isotopes is often greatly enhanced by the simultaneous use of two or more isotopes. Not every process fractionates every isotope. When a given process does not change the value of an isotope, it is said to be transparent to that isotope. It is unlikely, however, that major processes will fail to fractionate two or three important isotopes simultaneously. Nitrogen, which is important in biological systems, has the additional problem that it has very complex processes associated with it, and very small fractionations (signals) associated with some of these processes. While a particular source of N may have a strong isotope signature, this signature is quickly smudged and averaged–out. However, N, C, O, and S are often closely coupled in nature; the source or fate of N can be traced through following N alongside two or more of the other isotopes.

The simultaneous use of suites of isotopes is frequently a tool in ecological food web studies. These studies are source–sink descriptions; the same technique is also applied to pollution studies, where stable isotope signatures are used to trace pollution back to its source or to estimate its ultimate extent of influence.

The ease with which the various isotopes can be used, as well as their general usefulness, differs among isotopes. In Section 4, we discuss the major isotopes useful for the life sciences at natural abundance levels as well as the instrumentation and techniques required. We also provide case study examples of all the major uses of natural abundances of stable isotopes in looking at biogeochemical systems: **mass balances** (budgets; (δ^{13}C), **fluxes** (or **process rates**; δ^{15}N, and δ^{34}S), and as **natural tracers** to determine sources and sinks (δ^{13}C, δ^{15}N, and δ^{34}S *en suite*) of nutrients and pollutants.

2. *POLITICAL RELEVANCE*

2.1 *Global relevance*

The groundswell of international opinion has turned toward truly effective custodianship of natural resources. Agriculture must provide more food with less environmental impact than ever before. Pollution must be combated and the integrity of natural systems maintained. There is a public clamour for statutory bodies to ensure a greatly enhanced environmental quality of life. Into this context, a new technology and corresponding methodology are emerging. This new approach provides a completely benign and totally new way of studying natural and environmental problems; it gives us non-intrusive ways to trace pollution, identify contaminations, and study any natural system, including agronomic, ecological, cellular, microbial, aquatic, freshwater or terrestrial. This new technology has already revealed important subtleties of nature which were previously unsuspected. Although in its infancy in the natural and life sciences, this emerging approach promises a wealth of new understanding of the natural world.

This new technique, which has exploded into the world of natural sciences, is the use of non-radioactive, natural abundance levels of stable isotopes. Other types of isotopes have been used for a long time; and natural abundances of stable isotopes have been used in earth sciences since the decade of the 1950's. Slowly, over the last 15 years, this technique has begun to permeate every research, conservation and monitoring area of the life sciences, from genetics and molecular research to whole ecosystems. Until recently, it was retarded in its progress by the lack of suitably automated methods. The methods development aspect is now advanced to the stage that meaningful research can be done easily and cheaply on a large number of samples, as more methods development takes place simultaneously in pace with research needs as they arise.

Although this new technology has most effectively taken hold in the USA and Australia, it is fragmented. There is no one centre of excellence, in the overall field, anywhere in the world. Particularly, there is no effective coordinated centre in Europe. As we discuss below, the Scottish Crop Research Institute is uniquely well-poised to create a world-class centre of excellence in Britain, at a cost which will soon pay for itself and with an initial expenditure which is modest. Indeed, there is already substantial investment in complementary facilities for NMR, GPR, HPLC, GCMS and LCMS. In the global context, such a centre would have far-reaching implications for boosting British industrial hi-tech.

In the research and intellectual context, this methodology has been recognised as crucial to research progress. The number of scientific papers appearing every year in this field for plant sciences has increased from 5 in 1984 to more than 60 in 1990, when our computerised data base ends*. The International Atomic Energy Agency and FAO, in Vienna, are attempting to take the lead. So far they have fallen at the gate of expertise and excellence. There is a group in Germany begining to use some stable isotopes in a serious way, but not all. If Scotland does not seize the initiative someone else soon will.

Footnote: This is a very conservative estimate for the plant sciences, since the real explosion in this literature has occurred in the last 18 months.

2.2 *UK Position*

A centre of excellence for natural abundance research will answer a number of growing practical needs. It will provide state-of-the art techniques for, *inter alia,* complying with EC environmental legislation; for identifying and containing sources of pollution; food contamination has been a major concern in the last few years. This technique is extremely useful in this regard. With global change, the need is increasingly urgent to understand the responses of food and timber crops to changed climate and CO_2 levels. Natural abundances of stable isotopes are possibly as near as we can get to directly asking plants, soil, and animals exactly how they feel and what they are doing.

Although an increasing number of British scientists are becoming interested in using this new tool, there are very few scientists in the UK who have experience in the field of biological uses of natural abundances, and the few that exist are scattered geographically and in their research interests. For example, one scientist in Newcastle only works with isotopes of carbon and mainly land plants; one in Plymouth mainly works on isotopes of nitrogen, but in the ocean. The same could be said of all of European science. No one group in Europe has established a clear reputation as a centre, nor has any group yet made a serious attempt to do so. In the UK, only at the SCRI is there both the intellectual expertise to cover the entire spectrum of knowledge (both of the useful isotopes and the entire spectrum of research fields in which this new methodology could make the best impact) combined with the vision and will to create a centre of excellence.

There is, globally, a growing awareness of the need to use this new research tool; and in the UK the desire to use it is very evident to us. Many researchers approach us with questions about the method and requests for collaboration or analytical services. These requests are so recent, however, that many of these are still in the proposal-pending or even proposal-preparation stage. The need clearly exists, and a properly rationalised centre would be well-used.

Britain has long excelled in the life sciences, and in the so-called "hard sciences" of chemistry, physics, and mathematics. Developing and applying the natural abundances technique is in this same tradition of British excellence. It is founded in the hard sciences and used to advance our knowledge of complex living systems. Unlike some other cutting edge developments, it is relatively inexpensive. The costs are extraordinarily modest compared with research in oceanography, astronomy or high energy physics. We can become the world leader, while this window of opportunity exists, for less money than it costs to keep a very modest oceanographic research vessel at quay-side for a week.

The instrumentation which is presently used to best advantage is British; the active methods development is now British. This is a crucial opportunity to combine British intellectual capability, research expertise, and British commercial interests into one solid advance.

2.3 *Tayside, Scotland*

Here in Tayside there is a unique combination of (1) wide expertise in the use of natural abundance levels of stable isotopes, (2) enough existing instrumentation base for building a larger programme including complementary NMR facilities, (3) a strong baseline of experience in the subject, (4) existing local commitment and large external funding, together with both (5) the will and the clarity of vision to apply this new approach to many areas of the life sciences simultaneously. At SCRI there is the coordination of many areas of natural sciences. In addition to this strong base, there is also a close cooperation with scientists at the nearby University of Dundee, many of whom share appointments at SCRI.

Between the two institutions, and freely shared, there are now 7 mass spectrometers of various kinds and ages, including a newly purchased state–of–the–art fully Automated Nitrogen and Carbon Analyser (ANCA), which is based on the principle of Continuous Flow – Isotope Ratio Mass Spectrometry (CF–IRMS) and developed by the British company, Europa Scientific, for use with natural abundances at SCRI. For higher precision slower analyses of CO_2, there is a new VG–Isogas instrument in the University of Dundee Mass Spectroscopy Resource. For medical research, The Mass Spectroscopy Resource is already one of the finest centres in Europe for the use of stable isotopes (mostly very low level enrichment, but within the natural abundance range). It is, however, used at almost full capacity and would benefit very soon from equipment replacements, additions and updates. Only three of the machines in Tayside are suitable for use with the natural abundance levels of stable isotopes; they are almost fully used now. The newly acquired machine at SCRI was fully committed for the next three years use before its recent purchase.

The research proposed here fits into a larger background of facilities and expertise already existing in Tayside and complements on–going work at the other SARIs. In the past four years, there has been major investment in magnetic resonance facilities by the SCRI and Dundee University. The Tayside region is now well–equipped for NMR spectroscopy; It has the only NMR micro–imaging facility, the only fully computer–controlled EPR spectrometer and the only ENDOR equipment in Scotland. These make use of stable isotopes in investigations of chemical and biochemical reactions, either through the use of natural abundance ratios, or by selective enrichment at specifried chemical positions in reactants. By the use of this approach, significant progress has been made in the elucidation of biochemical pathways in plants and plant parasitic nematodes, the latter work involving *in vivo* NMR spectroscopy.

NMR and the proposed natural abundances of stable isotopes centre are mutually supportive. NMR requires large sample sizes and is slow; it is used for precise determinations of fine levels of chemical detail on a very few samples and is relatively expensive to do. Natural abundances of stable isotopes by mass spectroscopy will produce larger–view answers to problems addressed on many levels of detail, with the massive amounts of sample replication required for whole organism and ecological research, and at a modest price. Questions which cannot be completely answered with mass spectroscopy will be further investigated, on a more limited scale with NMR.

The present proposal aims to extend our ability to approach ecological and biogeochemical research problems through the use of stable isotopes. In so doing, we will create within Tayside a multidisciplinary world–class centre which represents a focus for all aspects of biological research. The main focus of this centre's work will, however, complement other existing facilities within Scotland (e.g. the substantial programme in using mainly enrichment–levels of stable isotopes in animal research at the Rowett Institute). Our major focus will be in developing to a high standard and scope the use of natural abundances of stable isotopes, both single pairs and *en suite*, for ecological studies involving soils, water, plants and microbiology, with the investigation of woody species also beginning to obtain some emphasis. In so far as animals are part of ecosystems and food webs, they will form a part of our research (e.g. insects, plant pathogens, grazers and their nutrient interactions with the agricultural and natural ecosystems under study).

A totally new centre of expansion and rationalisation of resources is required if the natural abundance methodology is to span all of the natural sciences at SCRI. Mass spectroscopy chiefly requires high–quality, expert personnel, once machines are in place. Personnel quality is critical. The precise and exact use and maintenance of the machines is crucial to obtaining reliable results. Additionally, sample preparation methods remain a challenge. By no means can we automate all sample analyses. Many types of samples require exact, and frequently imaginative, chemistry for appropriate pre–treatment. Sample preparation remains an active area of development. Finally, all of this must have added to it imaginative scientists with experience in using the natural abundance levels of stable isotopes in their own well–developed specialties of research; scientists who can ask important questions and test important hypotheses. The crucial nucleus of all these required elements already exists at the SCRI. The creation of a centralized, regional mass spectroscopy centre serving all of the research needs of Tayside is a rational approach.

We discuss below (a) the human resourcing and expertise existing to this initiative; first at SCRI, then locally but closely allied to SCRI, then collegial links internationally. Many of the key figures hold posts dependent on competitive grant fundings, putting the future of this research in doubt. The urgency to rationalise stable isotope facilities and resources has been recognised by our colleagues in the European Medical Research Council, who are meeting soon to discuss plans. The need in the other non–medical natural sciences is equally urgent and timely.

(b) the locally and externally funded research already existing for natural abundance work, demonstrating the existing commitment, and

(c) projected future demand. These projections are quite conservative; they include only the already proposed research or interest already expressed by other researchers, now, at a time when the SCRI is not yet generally known for natural abundance research outside a small group of specialists. New enquiries and expressions of interest occur with increasing frequency. This strong demand reflects both the commitment and expertise of the local scientific community to natural abundance research and a very strong and growing demand in the global scientific community for this type of support to be made available for many lines of research. Scientists the world–over recognize the need and are actively seeking natural abundance assistance from a centre which does not yet exist. We could gain substantially by providing a home for this work.

2.3.1 *Human resources and expertise*

At SCRI contract full–time personnel already working with natural abundance levels of stable isotopes include:

Dr Linda L Handley, plant community ecologist, specializing in the functional plant ecology of nitrogen, water and carbon use. She took her Ph.D. and post–doctoral training at the University of Hawaii, and also worked at the East–West Centre. She later worked for three years at the University of Florida's Miami campus, where she still holds a non–salaried Professorship. Dr Handley has especially strong experience in international collaborative studies. She was instrumental in developing the capability for rapid, high–precision $\delta^{15}N$ and $\delta^{13}C$ analyses at the SCRI. She maintains a long–standing and close collaboration with the Mass Spectrometry Resource, was one of the key figures in the recent consolidation of the mass spectrometry of Dundee University's Department of Biological Sciences with the single Resource (see below), and has many long–standing ties with countries in the developing world. She maintains close links and communications with natural abundance workers worldwide. Dr Handley draws her salary from the research contracts she, herself, obtains.

At SCRI permanent, full–time personnel with research programmes commissioned by the Scottish Office Agriculture and Fisheries Department include:

Professor John R Hillman, FRSE and Director of SCRI, for many years used stable isotope standards and analyses for examining plant growth substances and related compounds, utilising for this work combined gas chromatography–mass spectroscopy.

Dr Bernard A Goodman is the Head of the Spectroscopy Section in the Director's Group of the SCRI. He has wide experience in the use of magnetic resonance techniques (EPR and NMR) and Mössbauer Spectroscopy in the study of the bonding and chemical environments of selected isotopes in both natural abundances and enriched situations.

Dr Bruce Marshall Head of Soil–Plant Dynamics Group, Deputy Director of Dundee Centre for Non–Linear Systems in Biology and coordinator of the AFRC Soil–Plant–Microbial Interactions Working Party. Stable isotopes at natural and low levels of enrichment provide a major tool for advancement of science in his areas of interest and provide a major challenge for the development and application of new mathematical theories to assist biological understanding of the processes involved.

Dr David Robinson is a plant physiologist studying the functioning of plant roots and root systems in water and nutrient uptake, and in nutrient cycling. He combines both theoretical and experimental approaches in his work. He has used ^{15}N–enriched materials to measure rates of N uptake by roots of different species. In 1984 he was appointed to study N cycling in arable crops and soils at the Macaulay Institute in Aberdeen. He used ^{15}N tracers to follow the fate of N, and was involved in setting–up and, for a while running, the Macaulay's stable isotope mass spectrometer. Since 1987 he has worked in SCRI's Soil–Plant Dynamics Group, and has been an Honorary Lecturer in the Department of Biological Sciences at the University of Dundee. He has recently co–authored a substantial review article on the use of ^{15}N in plant and soil science, and on its analysis.

Mr Ron Wheatley has studied nitrogen cycling in mineral and organic soils for many years, with a particular interest in elucidating the factors involved in controlling the processes of nitrogen–mineralisation, nitrification and denitrification. $\delta^{15}N$ has been used to study the nitrogen–species source and transformation processes involved in the formation of N_20. He is involved in a study of soil–nitrogen transformations in sustainable ecosystems in Kenya, an EC funded project, in collaboration with Spain. He is presently involved in a joint submission, with colleagues in Holland and Spain, for EC funding of work on nitrogen immobilisation/mineralisation, that will involve the use of $\delta^{15}N$ methodology.

Dr Roberto Viola studies the regulation of carbohydrate metabolism in plants. He has developed experimental methodology for the use of ^{13}C–enriched substrates in NMR–studies of plant biochemistry. He is presently involved in a joint submission with Germany, Belgium and Holland for EC funding of work to study genetic manipulation of the pathways of starch/sucrose synthesis in plants; this work involves the use of ^{13}C methodology.

Dr Richard A Jefferies studies water relations in potatoes and has recently begun using $\delta^{13}C$ to assess the water cost of growth in different varieties of potatoes. With much of Britain facing increasingly frequent summer droughts, this line of research will become very important.

Dr John W Crawford heads the mathematical modelling group. This group provides us with the latest theoretical backup in rigorous mathematical analysis and experimental design and provides the "hard science" theoretical framework for data interpretation at all levels of systems, from molecules to whole plants to whole ecosystems.

Dr James W McNicol heads the SCRI branch of the Scottish Agricultural Statistics Service and provides advanced consulting and advice in statistical design of experiments, data sampling and interpretation of data amenable to statistical methods.

Technical support staff with experience in the required analytical methods exist and can provide training and supervision of new staff appointed to this institute.

The following co–initiators of this proposal are located at nearby Dundee University:

Professor Michael J Rennie of the Dundee University Mass Spectroscopy Resource.

Professor Rennie heads the Mass Spectroscopy Resource at Dundee University, which is operated and managed by Dr Charles Scrimgeour, an analytical chemist, who specializes in mass spectroscopy. At the moment this laboratory leads in European experimentation, instrumentation, and methods development in mainly enrichment (but also to some extent natural abundance–level) research with stable isotopes for animal research. Their own research specialty lies in fuel and amino acid metabolism and body composition measurements. They have one of the few centres in Europe which has instruments capable of being used to measure the tracer metabolism of fats, carbohydrates, and amino acids, as well as measuring the incorporation of tracers into biological macromolecules and use of the double–labelled water method for determining energy turnover. This group consisting of 5 scientific staff, substantially support and assist, at the moment, all of the other biological and ecological stable isotope work in Tayside, including plant and ecological sciences. Until very recently they had all but one of the machines in the area capable of natural abundance mass spectroscopy. Major state–of–the–art equipment now in the Mass Spectroscopy Resource will support an expanded effort in natural abundances located at SCRI. These include: Finigan Delta IRMS, VG SIRA II IRMS, Europa Scientific Tracer Mass ANCA, Finigan 1020 GCMS, and Hewlett–Packard 5971 GCMS.

Professor John A Raven, F.R.S., University of Dundee Department of Biological Sciences, is a member of the governing board of SCRI, long time collaborator on research and graduate student training at SCRI. He is also the husband of Dr Handley. Professor Raven has many publications (over 200) which include studies of plant pH regulation, nutrient assimilation, nutrient cycling, photosynthesis, carbon uptake and allocation, enrichment and natural abundance work with stable isotopes of carbon, plant growth analysis, carbon chemistry and isotope geochemistry in relation to plant productivity of freshwater, marine and terrestrial systems, and evolutionary plant studies. He is a close and long–time collaborator with the Mass Spectrometry Resource and was, with Professor Rennie and Dr Handley, one of persons chiefly responsible for consolidating the University's mass spectrometry into one resource. He was recently the chief author of a proposal (co–authored by Professor J I Sprent) which obtained for the Mass Spectrometry Resource a VG SIRA II IRMS with in–line Carlo–Erba. This machine is now used by investigators at both the University and the SCRI. However, because of the insecure financial basis for key personnel at the Resource, this shared use is on a cost per sample basis.

Geographically more distant, but tightly linked to SCRI and still within Britain are:

Dr Howard Griffiths, Newcastle University, and former student and Post–Doctoral employee of Professor Raven and now close colleague of both Professor Raven and Dr Handley. Dr Griffiths specializes in studying the discrimination of $^{13/12}C$ in forests and has published extensively on his work in Trinidad. He also does pioneering work on real–time carbon discrimination by plants with a dedicated instrument in his laboratory. Dr Griffiths and Dr Handley share stable isotopes research with colleagues in the University of Lisbon, where they jointly gave seminars on the subject last year. Dr Griffiths continues to share work and publications with Professor Raven; their latest joint effort (with a student from Lisbon University) concerns $^{13/12}C$ discrimination in lichens.

Dr C T Wheeler, Glasgow University. His work on N_2–fixation in plantation forestry will increasingly depend upon Dr Handley's stable isotope skills at SCRI and the continuing availability of analytical services. Dr Wheeler and Dr Handley have submitted, together, a proposal to do natural abundances of stable isotopes research in India as a part of assessing the utility of prior treatment of tree seedling before planting. He has also published and worked with Professor Hillman.

Dr N P Owens–Plymouth former student and honorary staff member of Dundee University uses stable isotopes in marine research.

Dr T Preston, former student at Dundee University and now colleague continues to use stable isotopes in medical nutrition research.

Finally, abroad there are good and long–standing links with:

Dr Graham Farquhar, CSIRO, Canberra, Australia whose brilliant theoretical work has led to a major breakthrough in using $\delta^{13}C$ to study plant water relations. He and Professor Raven have co–authored major publications, including major works on $\delta^{13}C$ in plant biology.

Dr Marion O'Leary who has been the major worker in using $\delta^{13}C$ to explain the biochemistry of plants, is a colleague well-known to both Dr Handley and Professor Raven.

Dr Leonel Sternberg, University of Miami, who contributes to our knowledge of the isotopes of oxygen and hydrogen in plants and is a long-standing colleague of Dr Handley, who was for several years at a Miami University.

Dr Marilyn Fogel, Washington, D.C., whose pioneering research work with natural abundances is internationally acknowledged. She and Dr Handley and Professor Raven frequently correspond about isotope research.

Dr Brian Fry, Woods Hole, Massachusetts, who is best known for his ecological work (largely aquatic) with the natural abundances of $^{13/12}C$ and $^{15/14}N$. He and Dr Handley have known each other since 1987; he arranged for her invitation to the stable isotopes meeting in Tokyo this September to be the sole invited British speaker.

2.3.2 *Local and External Funding to Initiators for Research Using Natural Abundances of Stable Isotopes*

AFRC. Influence of ammonium, nitrate and gaseous ammonia on acid-base regulation gas exchange and $^{13/12}C$ ratio in a C_3 and a C_4 grass. Prof J A Raven. £87,000.

NERC. Interaction of mechanisms of inorganic C acquisition with light supply to benthic red algae. Prof J A Raven. £117,586.

EC. Study of N distributions in plants and soils, C and water relations in Kenya and Spain using $\delta^{15}N$, $\delta^{13}C$ and $\delta^{21}H$. Dr Linda Handley. £500K.

UK. Spanish Bilateral Treaty. Travel grant to collaborate with Spanish group on $\delta^{15}N$ on $\delta^{13}C$ in *Casuarina* bioenergy plantations. Dr Linda Handley. £4K.

Sweden Energy Forestry Project. Feasibility study. $\delta^{13}C$ for selecting willow clones and the influence of N-source on $\delta^{13}C$. Dr Linda Handley. £2K.

SCRI Core Funding (ROAME'd). Quantify the effects of water and nutrient stresses on physiology of growth in crops (using potato and field bean). Dr Donald MacKerron. £219,231.

SCRI Core Funding (ROAME'd). Quantify effects of environment on growth and vegetative developmental processes in potato and woody crop species. Dr Donald MacKerron. £161,298.

Present equipment existing worth (£200K)

SCRI and Mylnefield Research Services (£125K)

2.3.3. *Projection of Immediate Demand*

The following proposals are pending or in preparation. They all depend upon natural abundances of stable isotopes.

Dr Amelia Martins–Lucao, University of Lisbon, with Dr Handley. Work on carob trees using $\delta^{13}C$, $\delta^{15}N$, δD. EC. Pending.

Dr C T Wheeler, University of Glasgow, with Dr Handley. Work on N_2–fixation in *Casuarina* plantations in India. EC. Pending.

Professor M J Rennie, Dundee University and the SCRI, with Dr Handley. Study of nitrogen relations in dairy cattle and pasture lands in Scotland. AFRC. Pending.

Dr John Proctor, Stirling University with Dr Handley. $\delta^{13}C$ and δD to investigate the retreat or advance of secondary forests in the Amazon. NERC. In preparation.

Dr Amelia Martins–Lucao, Lisbon University, Dr Joffre, Montpellier, Dr Handley and Professor Dieter Mueller–Dombois. Stable isotope approaches to investigating the physiology of cork oaks in Spanish, Portuguese and French dehesas. EC. In preparation.

Professor John Raven, Dundee University with Dr Christian Wilhelm, Allgemeine Botanik, Germany. UVB increase and dimethyl sulfide production by marine phytoplankton. EC. Pending.

Professor John Raven, Dundee University with Dr I Kaplan, Israel. $\delta^{13}C$ as a measure of fossil fuel use. EC. Pending.

Dr Roberto Viola with Professor Mark Stitt, Heidelberg, Germany. The effect of genetic manipulation of glycolytic enzymes on the metabolism of ^{13}C–enriched sugars in plants. British Coucil/EC. Pending.

Dr D Robinson with Dr A H Fitter, York University. Measurement of C transport via mycorrhizas using C_3 and C_4 plants. NERC/TIGER. Pending.

Dr D Robinson, Professor J A Raven with Dr A H Fitter, York University. Use of $\delta^{18}0$, $\delta^{15}N$, and $\delta^{13}C$ to gauge the effects of elevated CO_2 on soil nutrient cycling. AFRC. Pending.

Mr R E Wheatley. Effects of potato cultivation on soil nitrogen immobilisation, mineralisation and transformations. Part of a larger proposal. Potato Marketing Board/Ministry of Agriculture Food and Fisheries. Pending.

Mr R E Wheatley. Study of soil–nitrogen transformations, as affected by cultivation and crop. Large multi–investigation collaboration with Holland and Spain. EC. Pending.

Dr Richard Jefferies and Dr Donald MacKerron. δ^{13} and δD with heat flow techniques to determine drought tolerance in potato. Collaborative with Italian investigators. EC AIR Programme. Pending.

Dr Richard Jefferies and Dr Donald MacKerron. Calibration of $\delta^{13}C$ and water costs of growth in potato cultivars.

In addition, the following groups have expressed an interest in research collaboration, analytical services or both.

1. Forest Energy Project, Sweden. Preproposal feasibility experiments begun.
2. W H Knight, large vegetable farm in England. Preproposal feasibility experiments begun.
3. British group researching mycorrhizal inter–relations with tropical forestry have lost their analytical services in New Zealand; the demand in Britain for analytical services and help with stable isotope experimentation and interpretation is great. The lack is made conspicuous by the fact that this group had to send their samples to New Zealand. This same analytical lab in New Zealand has been serving a noted researcher's lab in Umea, Sweden.
4. Scottish researchers doing plant biology in Brazil would like analytical and interpretive help with stable isotopes.
5. Tanzanian investigator recently inquired about setting up research project in parallel with Dr Handley's recently funded Kenyan–EC project on $\delta^{15}N$ patterns in tropical savannas.
6. Measurement of $\delta^{13}C$ CO_2 evolved from soil derived from living roots and dead ones. Dr D Robinson.
7. Dr Handley invited to collaborate on $\delta^{13}C$ research with regards to major forests of Hawaiian Islands through the University of Hawaii. Also work planned on thornlessness in *Prosopis versus* ability to fix N_2 in Hawaii, where a good model system already exists.

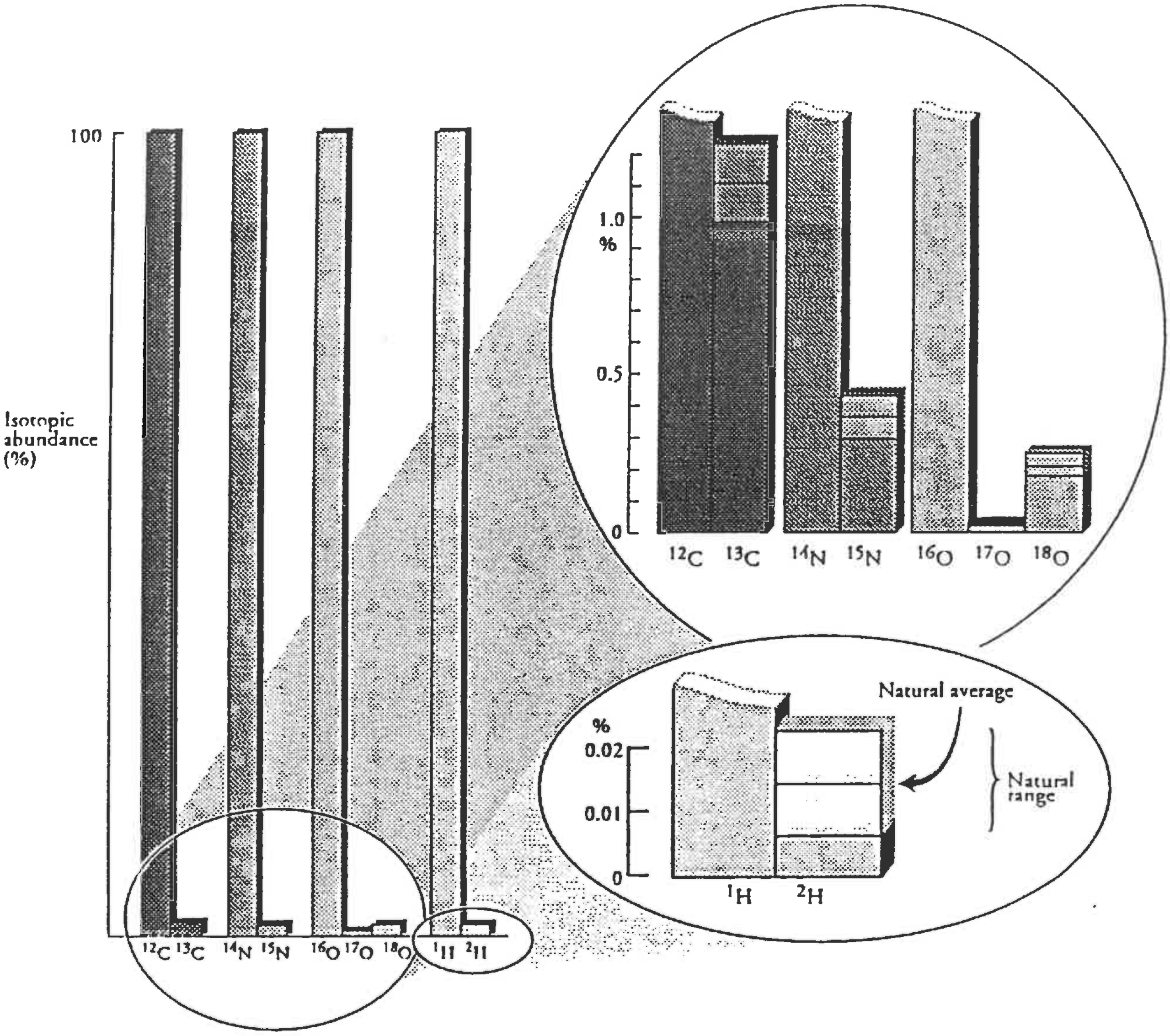

Figure 3. Histograms showing the relative abundances (amounts) of the light and heavy isotopes of hydrogen (H), carbon (C), nitrogen (N) and oxygen (O). The scale expanded histograms show the average natural abundance and the range of natural variation of the heavier isotope(s) of each element more clearly .

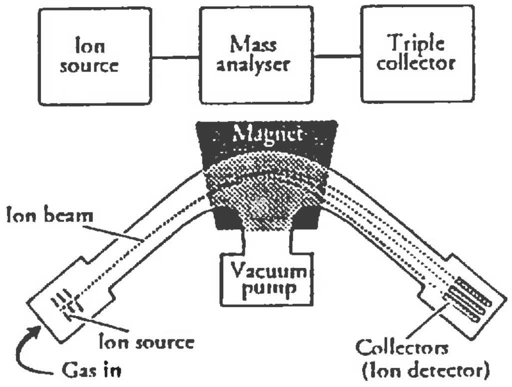

Figure 4. Layout of Magnetic Sector Mass Spectrometer, showing the basic features of ion source, mass analyser and ion detector common to all mass spectrometers.