

SIMSUG 2009, University of Glasgow 14th-15th January 2009

Organised by the Staff of the Stable Isotope Biochemistry Laboratory, SUERC



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SIMSUG 2009 Wednesday, 14th January 2009, Hunter Halls East, University of Glasgow

14:00 Welcome

Session 1: Compound-Specific Isotope Analysis

Chair: Tom Preston

- 14:15-14:45 Keynote 1: James McCullagh, Oxford *"LC-IRMS: Development and application of methods for physiological amino acid isotope analysis"*
- 14:45-14:50 Original Communications (15 + 5 mins):
- 14:50-15:10 OC S1 Andreas Hilkert *"Improvements in Compound Specific Isotope Analysis"*
- 15:10-15:30 OC S2 Daniel Abaye *"Determination of ¹³C abundance in underivatized amino acids by strong anion liquid chromatography coupled to IRMS"*
- 15:30-16:00 Tea with sponsors; posters
- 16:05-16:25 OC S3 Jennifer Dungait *"Analysis of lignin and carbohydrate components of soil organic matter using complementary TG-DSC-IRMS and GC-C-IRMS techniques"*
- 16:25-16:45 OC S4 Maanasa Raghavan *"Discerning Paleodiet through Compound-Specific Stable Isotope Analysis of Hair Keratin"*
- 16:45-17:05 OC S5 Rebeca Santamaria-Fernandez *"Precise and traceable absolute ¹³C/¹²C isotope amount ratios by multicollector ICP-MS"*

19:00 for 19:30 SIMSUG 2009 Dinner in the Ferguson Room, University of Glasgow

SIMSUG 2009 Thursday, 15th January 2009, Hunter Halls East, University of Glasgow

Session 2: Food, Drugs, Forensics and Environment

Chair:

- 09:00-09:30 Keynote 2: Simon Kelly, IFR
"The application of stable isotope analysis to detect the adulteration of food"
- 09:35-09:55 OC S6 Colin Snape *"Prospecting for drug cheats: a novel approach to carbon isotope ratio measurements of steroids"*
- 09:55-10:15 OC S7 Philippa Ascough *"Isotopic variations during formation of wood charcoal"*
- 10:15-10:35 OC S8 Tim Filley *"Productivity Driven Biopolymer Dynamics in Soil Organic Matter"*
- 10:35-11:05 Coffee with sponsors; posters
- 11:10-11:30 OC S9 Daniel Mayor *"Food quality affects carbon cycling in the deep sea"*
- 11:30-11:50 OC S10 Jens-Arne Subke *"Investigating carbon turnover in the Arctic tundra using a ¹³C pulse-chase approach"*
- 11:50-12:10 OC S11 Lorna Street *"Tracking the fate of carbon in Arctic moss communities using ¹³C isotope labelling"*
- 12:10-12:30 OC S12 Susan Waldron *"Delineating and quantifying dissolved inorganic carbon loss from peatlands"*
- 12:30- Buffet lunch in the Melville Room; posters

Session 3: Earth, Air and Water

Chair:

- 14:30-15:00 Keynote 3: Melanie Leng, BGS
"Combined oxygen and silicon isotope analysis of biogenic silica"

- 15:05-15:25 OC S13 David Large *"Influence of climate and hydrology on carbon in an early Miocene peatland"*
- 15:25-15:55 Tea with sponsors; posters
- 16:00-16:20 OC S14 George Swann *"A combined oxygen and silicon diatom isotope record of rapid palaeoenvironmental change in Lake El'gygytgyn, North East Siberia"*
- 16:20-16:40 OC S15 Catherine Jex *"Calibration of Speleothem $\delta^{18}O$ with Instrumental Climate Records from Turkey"*
- 16:40-17:00 Close

SIMSUG 2009 Posters

- PO B2 Schierbeek *"Simultaneous measurement of both concentration and ^{13}C enrichment of glutathione and glycine in one single run, using Liquid Chromatography Coupled to Isotope Ratio Mass Spectrometry (LC-IRMS)"*
- PO B3 Abaye *"Blood volume and red cell mass in children with moderate and severe malaria measured by chromium-53 dilution and GC/MS analysis"*
- PO B4 Moerdijk *"A versatile method for stable carbon-isotope (^{13}C) analysis of carbohydrates by high-performance liquid chromatography – isotope ratio mass spectrometry"*
- PO B5 Barclay *"Stable Isotope Incorporation into Faecal Bacterial RNA Reflects Predictable Changes in Short Chain Fatty Acids"*
- PO B7 Small *"Production of Complex Metaprobes: ^{13}C -Labelled Cereals"*
- PO B8 Preston *"Measuring the Liquid Phase Gastric Emptying Rate of Sip Feeds"*
- PO S1 Mander *"Estimation of Gastric Emptying parameters from the ^{13}C -octanoate breath test using Bayesian hierarchical methods"*
- PO S2 Gamboa Delgado *"Effects of dietary protein supply and quality on $\delta^{15}N$, $\delta^{13}C$ discrimination in penaeid shrimp and contribution of protein sources to growth"*
- PO S3 Thornton *"Tracing photosynthate carbon from the tree canopy to forest soil micro-organisms"*
- PO S4 Granger *"The hydrological response of heavy clay soils to rainfall as assessed using δ^2H "*
- PO S5 Murray *"Movement of newly assimilated carbon in the grass *Lolium perenne* and its incorporation into rhizosphere microbial DNA"*
- PO S6 Murray *"Temporal dynamics of a $^{13}CO_2$ pulse in grassland mesocosms"*
- PO S7 Barrett *"Configuring a Continuous Flow GC-IRMS (Multi-collector) System for isotopomer Analyses of Nitrous Oxide"*
- PO S8 Dixon *"Nitrification rates and nitric oxide production at two depths of two contrasting soils following the addition of artificial cattle urine"*
- PO S9 Dixon *"Field scale spatial distribution of soil $\delta^{13}C$ and $\delta^{15}N$ under permanent grassland"*
- PO S10 Bass *"Temporal and Spatial Variation in $\delta^{13}C$ -DIC and $\delta^{18}O$ -DO: a case study using Loch Lomond, Scotland"*
- PO S11 Mayor *"Food quality affects carbon cycling in the deep sea"*
- PO S12 Price *"Evaluation of a new pyrolysis method for analysis of compound specific stable oxygen isotopes"*

Keynote 1: LC-IRMS: Development and application of methods for physiological amino acid isotope analysis

James McCullagh

Department of Chemistry, University of Oxford, Mansfield Road, Oxford. OX1 3TA.

Liquid chromatography interfaced with isotope ratio mass spectrometry (LC-IRMS) has been of analytical interest for some time but it was not until 2004 that the first commercial instrumentation (LC-IsoLink™) become available. Since then a number of researchers have shown that this technique can provide high precision carbon isotope ratios for a range of underivatized compounds including biomolecules in complex matrices.

LC-RIMS therefore provides a new approach for natural abundance and tracer-enriched isotopic analysis. High $\delta^{13}\text{C}$ precision is achievable for mixtures of polyfunctional compounds, such as amino acids, sample preparation is straightforward and analytes that are not usually amenable to derivatization, such as sugars, can also be monitored. These advantages rely particularly on the effectiveness of online HPLC separation and focus will be given here to mixed-mode chromatography as a particularly suitable separation method. Two studies on physiological amino acids will be used as examples; the first focussing on human palaeodietary reconstruction and the second is a dietary tracer study on amino acid metabolism in fish.

LC-IRMS is a relatively new methodology and its applications are still being explored, however, its potential to deliver high precision isotopic information on amino acids, proteins, sugars and nucleic acids gives it exciting potential, particularly for biomolecular applications where conventional techniques remain limiting. With the development of chromatographic methods in particular it is anticipated LC-IRMS will become increasingly pertinent in a wide range of natural abundance and low enrichment isotopic studies.

ORIGINAL COMMUNICATIONS

OC S1: Improvements in Compound Specific Isotope Analysis

Hilkert A., Juchelka D., Krummen M. and Schwieters J.B.

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About 30 years ago D. E. Matthews and J.M. Hayes introduced compound specific isotope analysis (CSIA) by isotope ratio monitoring GC/MS (irm-GC/MS).

At the beginning the challenge was the development of a suitable reaction interface that provides quantitative conversion of compounds while maintaining chromatographic integrity.

Today continuous flow techniques can be found in all fields of application with improved performance on sample size, throughput, multiple isotope methods, overall precision and ease of use. Multi-element and multi-component analyses are performed to deduce unambiguous isotope fingerprints on ^{13}C , ^{15}N , ^{18}O and ^2H .

The growing interest and appreciation in CSIA requires new features and functionalities of the instrumentation.

A new concept for an automated multi-element irm-GC/MS will be discussed. It includes automated switching between the combustion reactor and the high temperature conversion reactor. The combustion mode has been redesigned to determine C and N isotope ratios using identical reactor conditions. The concept incorporates the ConFlo IV as an universal interface to the IRMS. All ConFlo IV capabilities are available for irm-GC/MS, e.g. injection of up to five reference gases, reference gas dilution and automatic H_3^+ factor determination.

The principle of the devices will be discussed with respect to dynamic range, precision, accuracy and sample size. Examples for multi-element and multi-component isotope analysis will be shown.

OC S2: Determination of ^{13}C abundance in underivatised amino acids by strong anion liquid chromatography coupled to IRMS.

Abaye DA, Morrison DJ, Preston T

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Introduction: LC-IRMS interfaces have enabled precise determination of ^{13}C at natural abundance without the need for prior derivatisation^{1,2}. Baseline resolution of analytes is key in achieving high precision. Once separated, amino acid (AA) C is oxidized to CO_2 with on-line introduction into the IRMS. So far cation exchange (CX: 2-dimensional³; weak CX; strong CX⁴; mixed mode⁵) have been reported for separation and analysis of ^{13}C by LC-IRMS. Here, we show an alternative approach, strong anion exchange (SAX) chromatography, can also be used to advantage.

Methods: We present our preliminary findings whereby 10 μl of an underivatised AA mixture (0.1 – 0.5mM), and hydrolysates of representative proteins (prawns and bovine serum albumin; BSA), were resolved using a SAX column (AminoPac PA10; DionexTM) and simple inorganic eluents (NaOH, NaNO_3 ⁶). Background inorganic C content was minimised through careful preparation of alkaline reagents and use of an upstream CO_2 removal device (CR-ATC; DionexTM). The interface (Liquiface; GVI, UK) uses persulfate oxidation at 90 $^\circ\text{C}$ to convert AA-C to CO_2 , which is then introduced into the IRMS (IsoPrime; GVI, UK) in a helium carrier after passage through a membrane separator and nafion dryer.

Results: The column resolved (resolution $R_s > 1.5$) 14 of the 16 protein amino acids (R_s range 2.4–12.9; ISD cyclo-Leu). Basic and neutral AAs (Arg, Lys, Ala, Thr, Gly, Val, Ile, Leu, Met; co-elution Ser/Pro) were resolved with 35mM NaOH, then His, Phe, Glu, Asp, Tyr were resolved with 45mM NaOH in an increasing gradient of a nitrate counter ion (10-45mM NaNO_3). IRMS results showed good reproducibility of peak areas. Precision of $\delta^{13}\text{C}$ analysis (SD‰) has improved for most of the amino acids as the procedure is optimised: Prawns (range), Arg 0.73‰ to Ile 1.67‰; BSA, Arg 0.40‰ to Ile 2.05‰.

Conclusion: SAX resolved 14 of the 16 expected protein amino acids following acid hydrolysis in underivatised form. SAX is a viable alternative to CX, especially when analysis of basic AA is important. However, a column with higher resolving power is necessary for analysis of all protein AA. The technology shows promise for applications in ecology, archaeology, forensics and health.

References: ¹Krummen M. et al., RCM, 2004, 18: 2260; ²Godin J-P et al. RCM, 2005, 19: 2689; ³Tripp JA. et al., J. Sep. Sci., 2006, 29: 41; ⁴Godin J-P, 2005; 19:2689; ⁵McCullagh J. et al., RCM, 2008, 22:1817; ⁶Boschker HTS. et al. RCM, 2008; 22: 3902.

OC S3: Analysis of lignin and carbohydrate components of soil organic matter using complementary TG-DSC-IRMS and GC-C-IRMS techniques

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Bulk stable carbon isotope analyses are now used routinely to explore organic matter cycling in soils. However, whole soil $\delta^{13}\text{C}$ determinations comprise the average $\delta^{13}\text{C}$ value of a wide range of organic components with contrasting $\delta^{13}\text{C}$ values and mean residence times in soils. Plant cell wall materials are the major inputs to soil organic matter in grassland systems, but their qualification and quantification pose analytical challenges due to the chemically intractable nature of lignin and polysaccharide polymers, and their ubiquitous nature in edaphic systems.

Several stable isotope ratio mass spectrometric methods have been developed to qualify and quantify different fractions of organic matter in soils and other complex matrices. In this study, thermogravimetry-differential scanning calorimetry-isotope ratio mass spectrometry (TG-DSC-IRMS) and gas chromatography-combustion-IRMS (GC-C-IRMS) analyses were applied to determine the $\Delta^{13}\text{C}$ between C_3 and C_4 organic material (plant material, silage and dung). Subsequent field experiments explored the incorporation and turnover of lignin and carbohydrates from natural abundance ^{13}C -labelled C_4 cattle dung applied to a C_3 UK grassland pasture soil over time (Bol *et al.*, 2000; Dungait *et al.*, 2005).

Both thermogravimetric and compound-specific stable ^{13}C isotope approaches showed that fluxes of carbon derived from polysaccharides, i.e. cellulose and monosaccharide components, were more similar to the behaviour of bulk carbon in soil than lignin (Lopez-Capel *et al.*, 2005; Dungait *et al.*, 2008), but that lignin and its 4-hydroxypropanoid monomers were unexpectedly dynamic in soil (Lopez-Capel *et al.*, 2005; Dungait *et al.*, submitted).

References

- Bol R, Amelung W., Friedrich C, Ostle NJ. *Soil Biol. Biochem.* 2000; 32:1337.
- Dungait JAJ, Bol R, Evershed RP. *Isotopes Environ. Health Stud.* 2005; 41:3.
- Lopez-Capel E, Bol R, Manning DAC. *Rapid Commun. Mass Spectrom.* 2005, 19:3192.
- Dungait JAJ, Stear NA, van Dongen BE, Bol R, Evershed RP. *Rapid Commun. Mass Spectrom.* 2008; 22:1631.
- Dungait JAJ, Bol R, Bull ID, Evershed RP. *Eur. J. Soil Sci.* Submitted.

OC S4: Discerning Paleodiet through Compound-Specific Stable Isotope Analysis of Hair Keratin

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Compound-specific stable isotope analysis of biological tissues is rapidly establishing itself in the field of bioarchaeology because of improved discriminatory abilities when determining paleodiet. Hair keratin is used in this context because of its ease and low destructive potential while sampling, and, its ability to provide segmental-based temporal resolution due to post-keratinization metabolic inertness.

We have developed a new method for the chromatographic separation and measurement of the stable carbon isotope ratios of individual amino acids in hair keratin using the LC IsoLink system, which interfaces liquid chromatography with IRMS. This paper reports the testing of a wide range of chromatographic conditions including gradient length, gradient start time and flow rates. The final protocol yields maximum baseline separation of 17 of the 18 amino acids in keratin. Key parameters that affect the efficacy of the method are demonstrated, including precision and accuracy, sample size and concentration.

The new method is demonstrated in the archaeological context by an investigation of hair recovered from the remains of four individuals from Uummannaq in Greenland, dating back to sixteenth-seventeenth century. Analyses of both bone and hair from the same individual are reported in order to address possible differential routing of amino acids.

This methodological study attempts to highlight the potential of compound-specific analysis using LC/IRMS for the study of hair keratin.

OC S5: Precise and traceable absolute $^{13}\text{C}/^{12}\text{C}$ isotope amount ratios by multicollector ICP-MS

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Carbon $^{13}\text{C}/^{12}\text{C}$ isotope amount ratios have been measured for four reference materials with carbon isotope amount ratios ranging from 0.010659 ($\delta^{13}\text{C}_{\text{VPDB}} = -46.6\text{‰}$) to 0.011601 ($\delta^{13}\text{C}_{\text{VPDB}} = +37\text{‰}$). Internal normalisation by measuring boron $^{11}\text{B}/^{10}\text{B}$ isotope amount ratios has been used to correct for the effects of instrumental mass bias. Absolute $^{13}\text{C}/^{12}\text{C}$ ratios have been measured in liquid samples and corrected for instrumental mass bias and full uncertainty budgets have been calculated using the Kragten approach.

Excellent linear correlation ($R = 0.9997$) was obtained between corrected carbon isotope amount ratios and expected carbon isotope amount ratios of the four chosen NIST RMs in solution. The method is suitable for the measurement of carbon isotope amount ratios within the natural range of variation of organic carbon compounds, carbonates, elemental carbon, carbon monoxide and carbon dioxide. In addition excellent agreement has been found between the carbon isotope amount ratio value measured by MC-ICP-MS and the IRMS measurements. The novel methodology opens new avenues for the measurement of absolute carbon isotope amount ratios in a wide range of samples as well as the coupling of an LC system for the measurement of compound specific absolute ratios.

Sulfur isotope ratio measurements by MC-ICP-MS will also be discussed and the application of stable isotope measurements to counterfeit detection will be presented.

Keynote 2: The application of stable isotope analysis to detect the adulteration of food

Simon Kelly

Institute of Food Research, Norwich Research Park, Colney, Norwich, UK, NR4 7UA.

Food adulteration describes the process of extending or substituting a premium food product with cheaper ingredients, usually for financial gain. This process defrauds the consumer, places the honest trader at an economic disadvantage, contravenes the 1990 UK Food Safety Act and at worst can pose a serious risk to human health.

This presentation gives an overview of the historical development of the application of stable isotope analysis to food authenticity studies; from the $^2\text{H}/^1\text{H}$ measurements of orange juice water by Bricout and Merlivat in 1971¹ (to distinguish between fresh and tap-water reconstituted concentrate), through significant stable isotope methodologies that have been incorporated into European legislation (e.g. SNIF-NMR technique²) to the focus of current research into determining geographical and production origin (e.g. 'Organic' foods³).

¹ J Bricout and L Merlivat (1971) "Deuterium content of orange juice" *Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences Serie D* 273 (12) 1021

² Commission Regulation (EEC) No. 2676/90 determining Community methods for the analysis of wines

³ A S Bateman, S D Kelly and M Woolfe (2007) "Nitrogen isotope composition of organically and conventionally grown crops" *Journal of Agricultural and Food Chemistry* , 55, 2664-2670

OC S6: Prospecting for drug cheats: a novel approach to carbon isotope ratio measurements of steroids

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The use of anabolic steroids in sport has been banned since the 1960s and anabolic androgenic steroids are named on the World Anti Doping Agency (WADA) 2008 prohibited list. Yet, the analysis of endogenous steroids and their metabolites can be challenging for doping laboratories. Steroid androgens and their metabolites in urine are conjugated with bulky glucuronide or sulphate groups attached to them making them water soluble. These added parts must be removed prior to gas chromatography analysis, a step called deconjugation. Subsequently, the steroid still has other chemical groups attached directly to its carbon skeleton which hinder isotope analysis and thus require a further treatment step called derivatisation to make the steroids amenable for analysis. Current deconjugation and derivatisation procedures suffer from severe limitations which can result in incomplete deconjugation or derivatisation, partial steroid conversion, production of multiple derivatives, and corruption of the carbon isotope ratio.

Determining whether the steroid is endogenous or exogenous in origin is of particular importance in the anti-doping arena. Current WADA regulations allow a difference of 3‰ between a suspected exogenous steroid and an endogenous reference compound that reflects the carbon in an athlete's diet. However, the precision of modern to gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) allows the measurement of differences of just 0.1‰. Clearly, the conservative range given by WADA does not exploit the precision of modern analytical equipment.

Whilst GC-C-IRMS offers the ability to discern whether the origin of the steroid is endogenous, the aforementioned issues with deconjugation and derivatisation hamper steroid analysis. Therefore, a suitable preparative alternative able to cleave both sulphate and glucuronide moieties and avoid the need for derivatisation would be particularly advantageous. Hydropyrolysis is an approach traditionally applied to the field of geochemistry, and more recently to the study of fatty acids and sterols. This hydrogenation method involves the catalytic addition of hydrogen to the carbon skeleton under high hydrogen gas pressure and temperatures. The functional groups are delicately stripped, retaining the carbon skeleton and stereochemistry.

This paper demonstrates the potential of hydropyrolysis to eliminate the current deconjugation and derivatisation steps and enable GC/C/IRMS analysis of any steroid conjugate, irrespective of the conjugate moiety. In particular, a hydropyrolysis protocol is described for conjugated steroids that defunctionalizes the parent steroid and simultaneously removes glucuronide and sulfate conjugate moieties. It is also demonstrated how the process efficiently renders 5 α -androstane-3 α , 17 β -diol-17-glucuronide and testosterone-17-sulfate suitable for subsequent GC/C/IRMS determination with no isotopic fractionation.

OC S7: Isotopic variations during formation of wood charcoal

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Charcoal is formed via the incomplete combustion of biomass in reducing conditions, resulting in a material with high environmental recalcitrance, and typically high (60-90%) carbon content. Consequently, charcoal forms part of the global “black carbon” continuum, with some material not re-oxidized to CO₂ over even geological timescales, and showing high abundance; for example, comprising ~30% of the total organic carbon in some Australian and US soils. These features make charcoal a crucial source of palaeoenvironmental and archaeological proxy data, used to reconstruct records of fire history, human societal and climate change.

In living plants, distinct differences in stable carbon isotopic composition ($\delta^{13}\text{C}$) are apparent between the Calvin-Benson (or C₃) and Hatch-Slack (or C₄) photosynthetic pathway and $\delta^{13}\text{C}$ is also strongly influenced by factors of soil moisture status, relative humidity, irradiance and temperature. Charcoal $\delta^{13}\text{C}$ measurements are therefore used to infer variations in climatic conditions during plant tissue formation, and in quantitative evaluation of temporal changes in vegetation cover. Such measurements are commonly made under the assumption that isotopic fractionation induced in the plant tissue prior to and following charcoal production is absent. However, growing evidence suggests an offset in $\delta^{13}\text{C}$ can exist between the initial plant tissues and charcoal in a range of species, although the sign and magnitude of fractionation effects is highly variable between studies.

We present results of a systematic study on the effect of production variables (gas composition, temperature, particle size and heating duration upon the molecular and stable isotope composition of two wood species (mangrove and pine). In both species, charcoal was depleted in $\delta^{13}\text{C}$ relative to the starting wood by up to 1.6‰ in pine and 0.8‰ in mangrove. This effect is predominantly attributed to the progressive loss of isotopically heavier polysaccharides, and kinetic effects of aromatization during heating. However, it is also apparent that $\delta^{13}\text{C}$ variations were modulated by both starting species and atmosphere, with different molecular structural changes during heating associated each wood type revealed by solid state ¹³C nuclear magnetic resonance spectroscopy. These are particularly evident at lower temperatures, where variation in the oxygen content of the production atmosphere results in differences in the thermal degradation of cellulose and lignin. It is concluded that production of charcoal from separate species in identical conditions, or from a single sample exposed to different production variables, can result in significantly different $\delta^{13}\text{C}$ variations during heating. These effects have significant implications within the use of charcoal isotope composition in palaeoenvironmental and archaeological reconstructions.

OC S8: Productivity Driven Biopolymer Dynamics in Soil Organic Matter

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Plant responses to atmospheric CO₂ enrichment, particularly above- vs. belowground allocation of net primary production (NPP), will affect soil organic matter (SOM) dynamics and stocks. Here we address mechanisms responsible for increased belowground C storage at the sweetgum FACE experiment at Oak Ridge National Laboratory. Plant C fixed under elevated CO₂ was traced into free and microaggregated particulate organic matter (POM) and silt plus clay associated C using stable isotopes and alkaline CuO-extractable biopolymers (i.e. lignin phenols and aliphatic biopolymer substituted fatty acids-SFA). Ratios of syringyl to vanillyl and cinnamyl to vanillyl lignin phenols indicated that root-derived lignin was quantitatively more important than foliar-derived lignin, and that lignin in all fractions has become more root-like after 5 y of CO₂ enrichment. This increase in root lignin was also associated with ¹³C depletion of all soil fractions, as a result of inputs of C fixed under the ¹³C-depleted elevated CO₂, as well as C accrual in all soil fractions but microaggregated silt and clay. Sweetgum leaf litter and roots had distinct molecular signatures in their relative abundances of octadecyl and hexadecyl-SFA, allowing us to track their relative contributions to SOM. In contrast with lignin analyses, the SFA content of both coarse and fine POM was apparently dominated by leaf cutin rather than root suberin. However, consistent with lignin analyses, both fractions showed progressive enrichment with root input (suberin) as a result of increased root growth in the elevated-CO₂ treatment. Biomarker as well as bulk soil and compound-specific carbon isotope analysis of earthworm and beetle larvae indicate their roles in processing pre fumigation SOM and recent root and leaf litter. These results documenting enhanced root biopolymer retention lend further support to the hypothesis that allocation patterns under elevated CO₂ have shifted towards greater delivery of C directly to the soil matrix where the potential for soil C accrual is increased, and confirm that changes in plant allocation patterns can have significant impacts on SOM dynamics and stabilization.

OC S9: Food quality affects carbon cycling in the deep sea

Daniel J Mayor¹, Barry Thornton², Steve Hay³, Ursula Witte¹

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Deep sea (>1000 m) sediments represent an enormous habitat, covering >50 % of the Earth's surface. They store vast quantities of carbon and therefore play a major role in the global carbon cycle. Our knowledge of organic carbon mineralization rates in this habitat has largely been inferred from *in situ* oxygen uptake rates. Such an approach provides little information on how deep sea sediment communities respond to the discrete pulses of diatoms (unicellular algae) and zooplankton faecal pellets that are deposited en masse at the end of the spring bloom. Diatoms contain large quantities of labile compounds such as omega-3 polyunsaturated fatty acids (ω -3 PUFAs) and represent a nutritious, 'high-quality' food source for deep sea organisms. Conversely, zooplankton faecal pellets are considered to be a 'low-quality' food source because they are essentially devoid of ω -3 PUFAs. This raises the question: Does 'food quality' affected rates of carbon mineralization in deep sea sediments?

Stable isotope pulse-chase experiments were conducted on sediment communities retrieved from 1070 m in the Faroe-Shetland Channel, NE Atlantic in May and October 2008. Highly replicated pulses of ¹³C-labelled diatoms and zooplankton faecal pellets were used to examine how food quality affects carbon cycling. Preliminary analysis of the data indicates that food quality does not affect rates of oxygen consumption. In contrast, it is apparent that food quality has a clear and highly significant effect on the rate with which ¹³CO₂ is produced.

OC S10: Investigating carbon turnover in the Arctic tundra using a ^{13}C pulse-chase approach

Jens-Arne Subke, Vincenzo Leronni, Harry W. Vallack, Andreas Heinemeyer, Phil Ineson

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Climatic models predict significant warming in the Arctic, with profound impacts on the carbon (C) balance of tundra ecosystems. A significant increase in respiration from vegetation and particularly soils is anticipated, but equally an increase in C assimilation by tundra vegetation in response to warmer temperatures is likely. Our ability to predict likely changes in the C balance in tundra ecosystems relies on the use of C exchange models between plants, soils, and the atmosphere, but at the moment, data on critical processes such as C assimilation and release are scarce. A key question in this context relates to the fractions of assimilated C retained in plant biomass, and those allocated to respiratory processes, which determine the rate of C turnover in the plant-soil system.

We present data from a ^{13}C pulse-chase experiment on 4 contrasting plant and lichen communities in the Swedish tundra: (1) *Betula* dominated communities, (2) *Empetrum* heath communities, (3) *Carex* (sedge) communities, and (4) exposed ridges (lichen communities). We assess the C turnover following a 3-hour pulse using isotopically highly enriched (95 atom% ^{13}C) CO_2 by tracing the label through leaf biomass and return of excess ^{13}C in respiration from plants and soil over an 8-day period. The results indicate a fast turnover of assimilated C in leaves, with a mean residence time (MRT) of about 1 day, and no differences between communities. For the ecosystem respiration flux, the isotopic label diminished at an even faster rate, with an MRT of less than 0.5 days. The ^{13}C content in fine roots showed only a slight and not significant enrichment 6 days after pulse labelling, while total soil (including fine roots) indicated a significant increase for only one of the communities (*Betula*) from 6 days after the labelling. The significance of these findings and possibilities of how they can be used in tundra ecosystem models are discussed.

OC S11: Tracking the fate of carbon in Arctic moss communities using ^{13}C isotope labelling

Street L.E.,¹ **Subke J.A.**,² **Ineson P.**,² **Heinemeyer A.**,² **Sommerkorn M.**,³ **Williams. M.**¹

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Arctic surface temperatures are predicted to rise 4-7°C by 2050. Warming of this magnitude is likely to have important feedback effects on the global climate system. For example, Arctic soils contain large amounts of carbon which may be released as CO₂ if rates of decomposition increase with temperature. Conversely, carbon sequestration may increase if plant growth is stimulated under milder conditions. It is imperative that we understand the processes of carbon uptake and storage in vegetation, if we wish to predict the future carbon balance of Arctic ecosystems. Mosses are a ubiquitous component of Arctic vegetation yet their contribution to carbon dynamics has not been well quantified. In this study we test the hypothesis that both allocation to growth, and rates of carbon turnover, differ significantly between moss community types.

We use ^{13}C isotope labelling to follow the fate of recently assimilated carbon in moss communities in Swedish sub-Arctic tundra. We track assimilated ^{13}C in *Sphagnum fuscum* and *Polytrichum strictum*, two contrasting moss species, following a 3-hour fumigation under 95% atom enriched $^{13}\text{CO}_2$. We present data on the isotopic enrichment in moss tissue as well as respired CO₂ (measured by a field-deployed mass spectrometer) through time following pulse labelling. Our results show clear differences between species in the relative amounts of assimilated C returned to the atmosphere through respiration versus that stored as biomass through growth. Around 75% of gross carbon uptake remained in *S. fuscum* tissues 5 days after labelling, whereas in *P. strictum* tissues only 30% remained. The mean residence time of assimilated carbon in *S. fuscum* was around 5 days compared to around 3 days for *P. strictum*. Our results emphasise the importance of moss species composition in Arctic carbon dynamics.

OC S12: Delineating and quantifying dissolved inorganic carbon loss from peatlands

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Carbon can be lost from peatland soils to the drainage system as dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and particulate organic carbon (POC). Linking loss of soil-derived inorganic carbon with the fluvial load presents a challenge – how to separate the inorganic carbon contribution from groundwater or minerogenic soils from that from the peatland. We have overcome this by using stable carbon isotope measurements as a tracer of each end-member. Our study area is a small order river system draining a relatively pristine peatland in NE Scotland. In our preliminary characterisation of DIC systematics we sampled diurnally, using a nested catchment sampling matrix (1km sq., 42km sq. and 90 km sq.) over a 14 month period. We will present data showing how we separate groundwater from soil water and additionally consider how our understanding of dissolved inorganic carbon dynamics in fluvial systems changes with increasing scale and hydrological conditions. We will show that concentration, [DIC], and carbon isotopic composition can be described well by the continuously-logged parameters, discharge and pH respectively, and thus provide potential in assessing whether our observations are generic to other catchments at the terrestrial-freshwater interface and in forming the linkages between wetlands, streams and groundwaters.

Keynote 3: Combined oxygen and silicon isotope analysis of biogenic silica

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There is increasing interest in the use of biogenic O and Si isotope ratios to understand climate and environmental processes. All of the literature deals with either oxygen or silicon. This is partly because measurement of the oxygen isotope compositions are done using either vacuum dehydration, isotope exchange or stepped fluorination techniques, while increasingly researchers are turning to multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) for Si isotope analysis even though Si isotope analysis can be done using fluorination methods. The procedure for simultaneous determination of isotopic abundances of oxygen and silicon from the same biogenic silica and mineral quartz will be described. Pure minerals are disassociated into O and Si compounds using a fluorination technique, whereby Bromine Pentafluoride (BrF_5) and heat produce oxygen (O_2), silicon tetrafluoride (SiF_4) and other fluorine by-products (eg. BrF_3). These compounds are cryogenically separated using cold traps. Yields for oxygen and silicon recovery are 70-80% for biogenic silica depending on the nature of the hydrous layer and 97-99% for pure quartz. Reproducibility of the oxygen isotopic composition ranges between 0.2-0.4‰ and silicon between 0.05-0.12‰.

The analysis of the more commonly used isotopes of oxygen together with silicon is useful because of the growing interest in the global biogeochemical cycle of silicon and its coupling with the carbon cycle. A wide range of disciplines including plant physiology, hydrology, oceanography and palaeolimnology have begun to focus on processes involving biogenic silica in the modern environment. Some recent work undertaken will be presented.

See: Isotopes in Biogenic Silica (IBiS), a special issue of Journal of Quaternary Science, 2008, Volume 23, Issue 4.

OC S13: Influence of climate and hydrology on carbon in an early Miocene peatland.

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Our understanding of the hydrodynamic response of peatland to climate change is restricted to the Holocene, which confines our knowledge of the fundamental controls on this important carbon reservoir to recent sedimentary successions. To understand the interaction of peatland hydrodynamics, climate and the carbon cycle on longer time scales, a 95.4 m record from lower Miocene lignite from the Gippsland Basin, Australia is considered. $\delta^{13}\text{C}$ and colour records for the lignite were created by analysing samples every 0.1 m. Solid-state ^{13}C NMR results indicate that lignite colour is related to the relative abundance of aliphatic carbon. The lack of a direct correlation between colour and $\delta^{13}\text{C}$ demonstrates that the $\delta^{13}\text{C}$ signal has not been significantly influenced by the diagenetic processes that produce the colour. An offset correlation occurs between $\delta^{13}\text{C}$ and colour with the degree of offset decreasing from 4.5 m at the base to about 0 m at the top. This offset is considered to represent a zone of surface influence that extends up to 20 m below the peat surface. Using numerical modelling we demonstrate that this zone of surface influence and its gradual decline in thickness could arise as a consequence of enhanced fluid flow in regions of high tensile stress within the unconfined peat body. The removal of lignin and its derivatives from the zone of surface influence will be favoured by cooler drier periods, with lower sea level and high hydraulic gradients across the peatland. Therefore in the early Miocene this peatland acted as a carbon source during global cooling.

OC S14: A combined oxygen and silicon diatom isotope record of rapid palaeoenvironmental change in Lake El'gygytgyn, North East Siberia.

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Understanding the long-term climatic changes of sites within the Arctic circle remains an essential pre-requisite for assessing the vulnerability of these regions to future climate change. To date, existing records from North East Russia have demonstrated significant spatial variability across the region. Diatom $\delta^{18}\text{O}$ and $\delta^{30}\text{Si}$ result here from Lake El'gygytgyn, Russia, highlight the presence of several abrupt climatic events across West Beringia from the last glacial maximum through to the modern day. In combination with other changes, the results raise the potential for strong climatic teleconnections to exist between the region and sites in the North Atlantic and tropical Pacific Ocean. Superimposed on a long-term cooling trend through the Holocene is a significant decrease in regional air temperatures over the last 700 years up to the modern day. With these changes possibly related to an increase in El Niño event frequency and strength, further high-resolution work is required to better understand the climate processes operating at these remote Arctic locations.

OC S15: Calibration of Speleothem $\delta^{18}\text{O}$ with Instrumental Climate Records from Turkey

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Stalagmite records of oxygen ($\delta^{18}\text{O}$) isotopes, sampled at sub-annual resolution by micro-mill techniques are correlated with climate parameters over the instrumental period (1961 to 2005 AD). The strongest correlations were found between $\delta^{18}\text{O}$ and total winter precipitation (October to January) smoothed by 6 years. Two smoothing options were chosen to account for variability in mixing and residence times of stored water in the karst aquifer prior to entering the cave: 1) An average of the last 6 years of winter precipitation which yielded a product correlation of -0.71 ; and 2) a mixing model of 10% short term/event water (<1 year) and 90% water of a longer residence time in the karst aquifer (2 to 6 years) which gave a product correlation of -0.72 . Winter precipitation is reconstructed back to AD 1948 based on linear regression of $\delta^{18}\text{O}$ with observed winter precipitation using both smoothing methods with an uncertainty of ± 31 mm of observed total winter precipitation (ONDJ).

This is the first speleothem calibration and reconstruction of its kind in Turkey.

POSTER PRESENTATIONS

PO S1: Estimation of Gastric Emptying parameters from the ^{13}C -octanoate breath test using Bayesian hierarchical methods

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The ^{13}C -octanoate breath test for the measurement of gastric emptying is a convenient methodology since it imposes no radiation burden, is safe and non-invasive. However in some instances (particularly if gastric emptying is delayed) the modelling of the breath-test profile produces physiologically implausible results. We sought to investigate whether these modelling failures could be avoided by using Bayesian hierarchical techniques rather than non-linear least squares for the fitting process.

Bayesian methods allow prior beliefs to be used to guide the fitting process. At a minimum for the breath test our prior knowledge is that the parameters of the bell-shaped curve used to describe the breath test output cannot be zero or negative, and the estimated recovery of the labelled substrate at infinite time cannot be greater than the dose given. Hierarchical analysis allows each breath test analysed to borrow information from the remainder in the set, resulting in a more coherent picture of the data as a whole. The Bayesian hierarchical analysis was implemented using the freely available WinBUGS* software. This uses the Metropolis-Hastings algorithm to perform Markov chain Monte-Carlo simulations. The time taken for the analysis was acceptable – for a single chain with 20,000 iterations 116 breath tests were fitted in 21.7 minutes on a medium specification workstation.

In general there was little difference in the gastric emptying parameters obtained from non-linear least squares and Bayesian methods when the envelope of the bell-shaped curve representing the breath-test data is well-defined. However when gastric emptying was delayed the Bayesian method gave much more plausible results. For example in the re-analysis of data from a study of the effects of test meal size on gastric emptying parameters a number of the results previously obtained for the largest meal size had to be excluded from the analysis since the half-time was implausibly long. However this was completely resolved by using the Bayesian method.

This work indicates that the WinBUGS implementation of a Bayesian hierarchical method is an attractive proposition for the fitting of gastric emptying breath-test curves. The only major drawback is that since in this method all the breath tests under consideration are solved simultaneously any small change to a single datum requires that the whole analysis be re-run. It is important to ensure that data input is accurate and checked carefully. The great advantage of the method is that it can avoid the modelling failures encountered when the kinetics of label processing are slow when compared with the experimental timescale. This usually occurs when gastric emptying is delayed, for example in the obese or with a large test meal.

*Lunn, D.J., A. Thomas, N. Best, and D. Spiegelhalter, *WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility*. *Statistics and Computing*, 2000. **10**(4): p. 325-337

PO S2: Effects of dietary protein supply and quality on $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ discrimination in penaeid shrimp and contribution of protein sources to growth.

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The penaeid shrimp present an attractive invertebrate model for experiments investigating dynamics of stable isotope incorporation, dietary effects on discrimination factors and isotopic routing. They are easily cultured in the laboratory, exhibit very rapid growth rates of three orders of magnitude within a few weeks and their omnivorous feeding habit enables flexibility in devising experiments using formulated compound diets. Results may have application in interpretation of ecological studies; penaeid shrimp have commonly been used in isotopic studies of tropical coastal food webs, for example ecosystem function of mangroves and habitat connectivity. Furthermore, there are direct practical applications in aquaculture nutrition; replacement of fishmeal in feeds with plant ingredients is a critical element of long term sustainable expansion of shrimp aquaculture.

A series of experiments have taken advantage of the differing natural stable isotopic ratios in plant- and fish-sourced dietary components in compound diets, fed to two shrimp species *Litopenaeus vannamei* and *Penaeus semisulcatus*. Isonitrogenous and isocaloric diets were formulated, with stable isotopic compositions manipulated by changing inclusion levels of protein and carbohydrate sources. Dietary carbohydrate was found to have a negligible effect on either $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values in shrimp muscle tissue, thus experiments have focused on using diets of differing isotopic composition to estimate protein turnover and the effect of protein source on diet-consumer isotopic discrimination. The contrasting isotopic values ($\delta^{15}\text{N}/^{14}\text{N}$ and $\delta^{13}\text{C}/^{12}\text{C}$) of fishmeal and soya allow estimation of relative contribution of each ingredient to muscle tissue growth using an isotopic mixing model.

There was a high degree of variability in diet-consumer discrimination ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$) depending on protein source and size of animals. In juvenile *P. semisulcatus* fed a mixture of soya and wheat gluten, discrimination values were as high as 7.9 and 6.8‰ for $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ respectively, with values of 5.3 and 3.5‰ in earlier post-larval stages. This compared to values of 2.1 and 2.4 and -0.9 and -1.3‰ for $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ respectively, in the same groups of animals fed fishmeal as the only protein source, and 1.9 and 2.8‰ for $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ respectively when fed live natural diets (mussel). A similar trend was seen in *L. vannamei* where diet-consumer isotopic discrimination ranged from 0.8 – 7.8‰ $\Delta^{15}\text{N}$ and 2.3 – 6.5‰ for $\Delta^{13}\text{C}$, both discrimination factors were strongly correlated with the level of soya protein in diets. Discrimination was also significantly, though to a lesser extent, affected by overall dietary protein level (being greater where protein supply was limited) and on the age of the animals (being greater in juveniles than in postlarvae). In all cases, analysis of muscle tissue indicated a biased contribution of C & N from fishmeal compared to soya; in shrimp fed a compound diet containing a 50:50 ratio of the two ingredients, 69% of N and 59% of C in muscle tissue apparently originated from fishmeal, while in animals a 10:90 ratio of the two ingredients the relative contributions of C & N from fishmeal were 32% and 24% respectively, despite soya having higher nitrogen digestibility than fishmeal in shrimp.

The results demonstrate the potential for application of bulk natural stable isotope analysis in evaluation of plant-source ingredients and the optimisation of their use in diets, while the tissue turnover rates and discrimination factors usefully inform interpretation of ecological studies using these species. Looking ahead, amino acid compound-specific stable isotopic analysis offers the potential for investigation of the mechanisms underlying these observations, with penaeid shrimp as a useful model with large and rapid tissue isotopic signature responses to diet.

PO S3: Tracing photosynthate carbon from the tree canopy to forest soil micro-organisms

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The total respiration of forest soils comprises of that derived from the breakdown of soil organic matter and litter (heterotrophic respiration) and that derived from carbon recently fixed by plants (autotrophic respiration). In forest ecosystems, the detailed fate of recently fixed photosynthates transported down into tree roots and subsequently into mycorrhizal fungi and other soil micro-organisms is unknown. Neither do we know the rate at which carbon fixed by the trees reaches and supports the activity of the various soil microbe groups.

Fifty m² patches of 4 m tall boreal *Pinus sylvestris* L. (Scots pine) forest, were enclosed within 200 m³ chambers. Different areas of forest were pulse labelled for 1.5 h with ¹³CO₂ in both June and August 2007. Soil beneath the trees was sampled prior to the ¹³CO₂ labelling and on several occasions for up to 700 h after labelling. Soils were immediately frozen and kept in this state until freeze dried. Phospholipid fatty acids (PLFA) were extracted from the freeze dried soil, derivatised to their fatty acid methyl esters (FAMES) and taken up in isohexane. The ¹³C/¹²C ratios of the individual FAMES were determined using a GC Trace Ultra with combustion column attached via a GC Combustion II interface to a Delta^{Plus} XP isotope ratio mass spectrometer (All Thermo Finigan, Bremen, Germany).

Two of the PLFAs which became enriched with ¹³C, 18:2ω6,9 and 18:1ω9, were indicative of fungi. Of these fungal markers 18:2ω6,9 was the most strongly labelled and is thus a promising marker for ectomycorrhizae, the type of mycorrhizae associated with trees. Both 18:2ω6,9 and 18:1ω9 showed maximum ¹³C enrichment 1-2 weeks after pulse labelling. In contrast, no PLFAs associated with gram positive bacteria and only one PLFA, 18:1ω7, associated with gram negative bacteria, became ¹³C labelled. Generally the PLFAs became more enriched with ¹³C following the labelling period in August compared with labelling in June, this suggests the pattern of allocation of recently fixed carbon in *P. sylvestris* is seasonal.

PO S4: The hydrological response of heavy clay soils to rainfall as assessed using $\delta^2\text{H}$

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Clay soils are generally considered to be retentive of water due to the small pore size and poor connectivity. Consequently any precipitation falling on this type of heavy soil generally leaves the system quickly either as surface run-off or through macropores and, where installed, agronomic drainage leading to characteristic hydrographs which are typically 'flashy' in nature. The assumption therefore is that this storm event drainage represents storm event water, with soil water conserved within the soil matrix i.e. the $\delta^2\text{H}$ of water leaving this soil system would have a $\delta^2\text{H}$ similar to that of the precipitation causing the drainage.

A small study was set-up at the Rowden Experimental Research Platform in Devon to observe the ^2H in water leaving two agronomically drained 1ha lysimeter plots during a storm event. Bulk rainwater samples were collected throughout the duration of three storm events, while manual high temporal resolution sampling of surface runoff and drainage water was undertaken from both lysimeter plots during each of these events. Samples were filtered (0.45 μm) and sub-samples were kept refrigerated in filled sealed vials prior to analysis. Samples were analysed for $^2\text{H}/^1\text{H}$ isotope ratio using a Delta^{Plus}-XP High Temperature Conversion/Elemental Analyser with data processed using the proprietary Isodat NT software (version 2.0). Measured $^2\text{H}/^1\text{H}$ and isotope ratios are expressed as δ values in [‰] relative to VSMOW. Measured $\delta^2\text{H}$ -values were scale corrected according established methods, with stretch factors typically being of the order of 1.048.

Preliminary data indicates that the $\delta^2\text{H}$ precipitation varies greatly between individual storm events at this site with values of -28.3, -68.3 and -91.7‰, well within the range of $\delta^2\text{H}$ observed in precipitation at this site over a 4 year study (Leng, pers comm.). However, initial results of $\delta^2\text{H}$ from the surface runoff and drainage water leaving these plots indicate that this water is more enriched compared to that of precipitation. Values of $\delta^2\text{H}$ measured water leaving the lysimeter plots via installed drainage during the second storm (with a precipitation $\delta^2\text{H}$ of -68.3‰) ranged between -33.4 to -38.9‰ and showed no difference between the two lysimeters or a significant change over time. From the limited amount of surface flow generated only a small number of samples were collected from this pathway however, $\delta^2\text{H}$ of the runoff water was more depleted than in the drainage water with values ranging between -41.5 to -55.1‰. While the surface runoff might have been expected to have had a $\delta^2\text{H}$ more similar to that of the antecedent rainfall, the values observed were still more enriched than the rainfall.

This preliminary data suggest that the nature of the hydrological response of heavy clay soils to rainfall is potentially more complex than initially believed and that old soil water may be being mobilised by rainfall.

PO S5: Movement of newly assimilated carbon in the grass *Lolium perenne* and its incorporation into rhizosphere microbial DNA.

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One of the key processes that drives rhizosphere microbial activity is the exudation of soluble organic carbon (C) by plant roots. This paper describes an experiment designed to determine the impact of defoliation on the partitioning and flux of C in grass (*Lolium perenne* L.)-soil and grass-sterile sand microcosms, using ¹³CO₂ pulse labelling methods. The atom% ¹³C ratio in the shoots declined over time, but that of the roots remained stable throughout the experiment. There were peaks in atom% ¹³C of rhizosphere CO₂ in the first few hours after labelling probably due to root respiration, and again at around 100 h. The second peak was only seen in the soil microcosms and not in those with sterilised sand as the growth medium, indicating possible microbial activity. Incorporation of the ¹³C label into the microbial biomass was immediate and increased at 100h when incorporation into replicating cells, as indicated by the amounts of the label in the microbial DNA, started to increase. These results indicate that the rhizosphere environment is conducive to bacterial growth and replication. The results also show that defoliation had no impact on the pattern of movement of ¹³C from plant roots into the microbial population in the rhizosphere.

PO S6: Temporal dynamics of a $^{13}\text{CO}_2$ pulse in grassland mesocosms

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Temperate grassland ecosystems have a stable and permanent plant cover, which provides a habitat for a large and diverse invertebrate soil fauna. These fauna constitute the decomposer food web. The primary role of this food web is in the cycling of organic matter which is initially derived from the plants. The use of stable isotope tracing is a powerful tool in helping us to better understand the trophic linkages in the soil communities and the fluxes of C through them. In this study we investigated the dissipation of a $^{13}\text{CO}_2$ pulse in a grassland sward. A total of 44 monoliths (40 cm diameter x 10 cm depth) were taken from an upland grassland sward and transferred into plastic boxes which were kept outside under ambient conditions. The monoliths were subjected to a 6.5 h pulse of $^{13}\text{CO}_2$ delivered via a stable isotope delivery system (Ostle et al. 2000). The monoliths were then covered with a mesh to prevent ingress and egress of invertebrates. The monoliths were destructively sampled on a log time basis (i.e. at 0, 2, 3, 6, 10, 18, 32, 56, 100, 178 and 316 days after pulsing), three replicate boxes were sampled on each occasion. The mesofauna were extracted using Tullgren funnels from a single 8cm diameter core taken from each monolith, the collected fauna were identified to group. The foliage of the plants was cut to ground level, separated into grasses and forbs, dried and ground. The remaining material was washed through a series of sieves and the different fractions (litter, organic material and mineral) were collected separately. Any macro-invertebrates were also collected at this time.

Results show the rapid uptake of the pulse by the plants coupled with a logarithmic decline in the stable isotope signature over the experimental period. This was mirrored by the pulse signal in the collembola which also showed a rapid uptake and a decline which tracked that of the plants. The slug community also showed an initial enrichment, but this was maintained to a greater extent than the collembola. Other groups, such as the mites, showed a slow increase throughout the experimental period. Overall the results from this study allow us to start to infer the trophic interactions and the extent to which plant C is partitioned within the soil.

Ostle et al. (2000) Carbon assimilation and turnover in grassland vegetation using an in situ $^{13}\text{CO}_2$ pulse labelling system. Rapid Communications in Mass Spectrometry 16, 2179-2183

PO S7: Configuring a Continuous Flow GC-IRMS (Multi-collector) System for isotopomer Analyses of Nitrous Oxide.

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The positioning of a ^{15}N atom within the nitrous oxide (N_2O) molecule at natural abundance levels has been shown to have potential as an indicator of N_2O sources. A measure of the proportion of ^{15}N in each position can be used to compare different processes emitting N_2O , and on a larger scale indicate which processes are primarily responsible for emissions at a site. This has particular implications for tracing biological processes in soils that cause N_2O emission (nitrification, denitrification, nitrifier-denitrification, nitrate ammonification, for example). These processes are currently hard to measure in the natural environment because of problems with using enrichment techniques on a large scale (leaching of substrates, dilution by rainfall, expense, etc.).

Current systems analysing N_2O samples for isotopomer (positional ^{15}N labelling) ratios have been either dual-inlet or requiring two separate sample injections. We discuss the set up and calibration, along with initial isotopomer ratio measurements, of a continuous flow GC-IRMS for positional N_2O analysis. The advantages of this system include speed of analysis (necessary for large experiments), lower sample volumes required, and less interference from ions of similar masses. A continuous flow isotopomer analysis method will allow data collection on positional labelling of N_2O from a variety of processes, using laboratory and environmental samples, and will enable this technique to be properly tested as a method for source apportioning of N_2O , which in turn will help to inform mitigation strategies for this greenhouse gas.

PO S8: Nitrification rates and nitric oxide production at two depths of two contrasting soils following the addition of artificial cattle urine

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Nitrogenous materials can be transferred out of the top soil, either vertically to a greater depth, or in lateral pathways to surface waters and may also become transformed with the potential of generating environmentally active agents. Both microbial process of denitrification and nitrification are able to produce nitrous oxide (N₂O), nitrite (NO₂⁻) and nitric oxide (NO) which may escape into the atmosphere or to waters.

We compared two soils, a poorly drained soil of the Hallsworth series and a freely drained soil of the Crediton series. Samples of the soils were collected from 0 to 20cm and from 70 to 90cm and passed through a 6 mm. sieve. Sieved soil was sub-sampled (150g on an oven dry basis) into 168 acid washed 1L Kilner jars. Labelled KN¹⁵O₃ (to supply NO₃⁻ at 0.68 μmol N g⁻¹ oven dry soil at 49.3 at% 15N) was added to each jar with either artificial cattle urine (133.3 μg N and 176 μg C g⁻¹ oven dry soil) or deionised water. Additional water was added to each jar to attain a water filled pore space of 70%. Three replicates of each treatment were arranged randomly in an incubator at 15°C. The jars were sealed with restricted aeration (via a needle) and incubated for 0.16, 1, 2, 4, 8, 16, and 31 days after application of treatments. At each sampling, the jars were opened and fully vented, the needles removed then sealed for 4 h. The headspace was then sampled and analysed for N₂O and CO₂ by GC and for ¹⁵N enrichment of N₂O and N₂ by IRMS. Also the soil was extracted with 2M KCl at pH 8.0 and analysed for NO₂⁻, NO₃⁻ and NH₄⁺. The ¹⁵N enrichment of the extracts was also measured by IRMS after conversion to N₂O and N₂. We calculated rates of gross nitrification and nitrate consumption from the isotopic dilution of the enriched NO₃ pool.

Gross nitrification rates generally increased with time until day 16 in the top layer of both soils when artificial urine was added, with the highest rate (6.6 μg N g⁻¹ dry soil d⁻¹) found in the freely drained soil. The cumulative production rates of N₂O and CO₂ were both similar in the 0-20 cm and the 70-90 cm layers of the freely drained soil in both sub-treatments, but in the poorly drained soil, N₂O production was approximately 10 times higher in the 0-20 cm soil layer compared with the 70-90 cm layer in both sub-treatments. Production of CO₂ was approximately four times higher in the 0-20cm layer compared with the 70-90 cm layer, and in this layer, CO₂ production in the urine sub-treatment was approximately twice that of the water control. Production of NO₂⁻ and NO was highest in the 70-90cm layer of the freely drained soil which received the urine treatment, with maximum production rates of 3.5 μg NO₂-N g⁻¹ dry soil on day 16.0 and 17 μg NO-N g⁻¹ dry soil on day 31. By the end of the experiment the 70-90cm layer of the freely drained soil had a cumulative nitrification rate approximately 10-fold higher than the same layer of the poorly drained soil. The results show the possibility for nitrogenous species to be transformed into potential environmental pollutants at depth in some grassland soils.

PO S9: Field scale spatial distribution of soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ under permanent grassland

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The objective of this experiment was to investigate the spatial distribution of soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ under long-term managed grassland and to examine the efficacy of two different soil sampling strategies. The simple null hypothesis that was tested was that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values do not vary spatially across a 1 ha managed, grassland field. An undrained field at North Wyke Research in Devon, UK was sampled in the summer of 2007. This field is mainly comprised of perennial ryegrass (*Lolium perenne* L.), has been under pasture for at least 50 years, receives regular inorganic fertiliser ($200 \text{ kg N ha}^{-1} \text{ y}^{-1}$) and is grazed by beef cattle. The soil is a poorly drained, silty clay loam of the Hallsworth soil series. Initially, a simple Latin square sampling +1 design was used to generate 26 random samples. Briefly, the field was divided into a 25 x 25 grid and in each column a cell representing a row was selected using random numbers, such that no row was selected more than once. Finally, one column was randomly selected again, in which one row was randomly selected for a second time, this representing the +1. A further 144 samples were collected from a 12 by 12 grid across the whole plot. The soil was sampled to a depth of 10 cm on both occasions; the large roots and above-ground vegetation was removed and then the soil was dried at 30°C before being ground in a mechanical grinder. The ground soil was weighed into tin capsules and analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The 26 samples from the Latin square sampling +1 approach were analysed at SCRI and the 144 samples from the 12 x 12 grid were analysed at Rothamsted Research, both using an automated nitrogen-carbon analyser (ANCA) coupled to a 20/20 isotope ratio mass spectrometer (SerCon Ltd, Crewe, UK).

The $\delta^{13}\text{C}$ from the 144 samples were in the range of -31.4 to -29.3 (per mil) with a standard deviation of 0.3 and a mean of -30.1. The $\delta^{15}\text{N}$ values showed greater variation and ranged from 2.8 to 8.5 (per mil) with a standard deviation of 0.9 and a mean of 4.6. Percentages of carbon and nitrogen in the soil were 0.27 to 0.76 and 2.54 to 8.65 respectively. The statistical relevance and implications for sampling strategies for soils in grassland systems based on results using this method as compared to the Latin square sampling + 1 approach will be discussed.

PO S10: Temporal and Spatial Variation in $\delta^{13}\text{C}$ -DIC and $\delta^{18}\text{O}$ -DO: A Case Study Using Loch Lomond, Scotland

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Variability in ^{13}C -dissolved inorganic carbon and ^{18}O -dissolved oxygen reflect the balance between pelagic photosynthesis and respiration in Loch Lomond. Thus spatial heterogeneity was estimated using a combined natural abundance isotope approach. Concurrent measurement of $\delta^{13}\text{C}$ -DIC and $\delta^{18}\text{O}$ -DO four times between November 2004 and September 2005, including variation over small and large spatial scales, both horizontal and vertical was undertaken. Both $\delta^{13}\text{C}$ -DIC and $\delta^{18}\text{O}$ -DO changed as observed in earlier work over a seasonal cycle, becoming concurrently more enriched in the summer months likely responding to increased photosynthetic and respiratory rates respectively. The level of enrichment depended on the location of the sample site, specifically if in the oligotrophic north or mesotrophic south. With increasing depth $\delta^{13}\text{C}$ -DIC became more depleted and $\delta^{18}\text{O}$ -DO more enriched, reflecting the shift from photosynthetic to respiratory dominance. The horizontal distribution of $\delta^{13}\text{C}$ -DIC and $\delta^{18}\text{O}$ -DO in the epilimnion showed significant heterogeneity. In general the mesotrophic south basin had the most enriched $\delta^{13}\text{C}$ -DIC, becoming more depleted with increasing latitude, except in winter when the opposite pattern was observed. Local areas of enrichment or depletion were often observed near significant inflows. Loch Lomond is a hydrologically and bathymetrically complex system and thus has the potential for significant pelagic spatial heterogeneity and is thus a suitable site to assess the effectiveness of single point sampling. Using the still uncommon paired isotope approach we have revealed spot sampling to be of little value in a complex system and a more thorough approach is recommended. We strongly recommend consideration of spatial variation in others future work.

PO S11: Food quality affects carbon cycling in the deep sea

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Deep sea (>1000 m) sediments represent an enormous habitat, covering >50 % of the Earth's surface. They store vast quantities of carbon and therefore play a major role in the global carbon cycle. Our knowledge of organic carbon mineralization rates in this habitat has largely been inferred from *in situ* oxygen uptake rates. Such an approach provides little information on how deep sea sediment communities respond to the discrete pulses of diatoms (unicellular algae) and zooplankton faecal pellets that are deposited en masse at the end of the spring bloom. Diatoms contain large quantities of labile compounds such as omega-3 polyunsaturated fatty acids (ω -3 PUFAs) and represent a nutritious, 'high-quality' food source for deep sea organisms. Conversely, zooplankton faecal pellets are considered to be a 'low-quality' food source because they are essentially devoid of ω -3 PUFAs. This raises the question: Does 'food quality' affected rates of carbon mineralization in deep sea sediments?

Stable isotope pulse-chase experiments were conducted on sediment communities retrieved from 1070 m in the Faroe-Shetland Channel, NE Atlantic in May and October 2008. Highly replicated pulses of ¹³C-labelled diatoms and zooplankton faecal pellets were used to examine how food quality affects carbon cycling. Preliminary analysis of the data indicates that food quality does not affect rates of oxygen consumption. In contrast, it is apparent that food quality has a clear and highly significant effect on the rate with which ¹³CO₂ is produced.

PO S12: Evaluation of a new pyrolysis method for analysis of compound specific stable oxygen isotopes

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The compound specific analysis of stable isotopes of oxygen provides information about sources of oxygen in an extensive range of applications such as paleoclimatological and food adulteration studies. Here, we describe a low temperature pyrolysis method for compound specific oxygen isotopes. The precision and linearity is evaluated extensively using vanillin, which is of great interest in food authentication studies. An Isoprime GC V interface combined with an Agilent 6890 GC with a 30m HP-5 capillary column was configured for oxygen analysis. The reactor configuration included an Al₂O₃ outer furnace tube with an inner nickel tube packed with black carbon and a 1% hydrogen in helium sample line gas. The reactor temperature used in this study is 1250°C for quantitative conversion. The advantage of the inner nickel tube is to prohibit oxygen exchange with Al₂O₃ at high temperatures. Low temperature pyrolysis with this configuration (nickel tube with black carbon) provides excellent reproducibility (0.3 ‰), minimal linearity effects and accurate results. In addition to the superb analytical results, nickel has an economic advantage over the commonly used platinum catalyst because it is approximately 1/10th the price of a platinum tube.