WELCOME!

Dear Conference Participant,

Wlcome to Braunschweig and the 2nd Conference on Application of Stable Isotope Techniques to Ecological Studies.
We are happy to have researchers and students from universities, governmental institutions and industry here who are going to take part in increasing our understanding of ecological processes. Presentations of works within the field of stable isotope research have always been embedded as workshops in larger meetings and focus has mostly been on tracer studies rather than natural isotope abundances.

The first conference of this series has been held in Saskatoon, Saskatchewan, Canada, two years ago and proved that the idea of bringing together researchers form a widespread area all having in common stable isotope research was fruitful. The number of participants as well as the number of oral and poster presentation increased tremendously and we had to ask several people who planned to give an oral presentation to change it into a poster as we did not want to have presentations of 15 min including discussion each. It was very hard for us to decide who would be the one to change to a poster, especially as the proposed presentations all sounded very interesting. It is not an expression of lack of quality at all.

We are looking forward to a very exciting meeting and are particularly happy to have Dr. Dan Yakir as our keynote speaker. Dr. Yakir’s research group applies and develops stable isotope methods to study plant, soil and ecosystem responses to climatic and environmental change.

As in 1998, we will have several theme sessions in our regular program and an extensive poster session. We hope that you’ll enjoy Braunschweig and have a productive and rewarding meeting.

Thank you for participating and helping us to make this meeting a success.

Anette Giesemann
Keith A. Hobson
Len Wassenaar

THE SCIENTIFIC PROGRAM

The program has 107 papers in total – 48 oral and 59 poster presentations.
Thanks to all who have contributed to make this meeting a success.

The presentations are organized in the following 8 sessions:

Session 1: Plant, soil and sediment ecology
Session 2: New methods and applications
Session 3: Dietary and trophic studies
Session 4: Applications of molecular isotopic analyses
Session 5: Archaelogical and paleological studies
Session 6: Contaminant pathways and impacts
Session 7: Wildlife research
Session 8: Variable scale ecosystem studies

Sessions 1 and 2 will be held on Monday followed by the poster session, sessions 3, 4 and 5 are planned for Tuesday and sessions 6 to 8 will be held on Wednesday.

Oral presentations will take place in the main lecture hall of the FORUM der FAL. Presentations will be 15 min plus 5 min discussion time.
Slide projectors and overhead projectors will be available.
Please label your carousels /slides with your surname and hand them to the person responsible for projection in the room back of the session hall.

Posters will be mounted in the SENATSSAAL. The poster session takes place Monday from 5 p.m. to 8 p.m. Snacks and drinks will be available. Transportation to the hotel downtown will be available from the FORUM.

Name badges will be handed out upon registration. Please wear it all times during the conference.

CONFERENCE PROCEEDINGS

Proceedings (optional to presenters) will be published in Stable Isotopes in Health and Environmental Studies. The manuscript deadline is June, 10th.
All manuscripts will undergo normal review procedures.
We will forward bulk submission to the journal. Be sure your paper adheres to the guide to authors for the journal (available at the registration desk).

STUDENT AWARDS

Awards will be given for the best student paper and the best student poster. They will consist both of a certificate and a donation.
Three attending scientists will form the jury.
Good luck!

SPONSORSHIP

The organisers thankfully acknowledge the financial support of the meeting through the following firms (in alphabetical order):

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We invite the participants to visit the exhibition presented in the Foyer and the PRÄSIDIUMSZIMMER

PROGRAM

Sunday, May 07, 2000

16.00	Pre-registration in the Courtyard by Mariott hotel
18.00	Pre-conference mixer in the Courtyard by Mariott hotel

Monday, May 08, 2000

08.30 - 10.00	Final Programm Pickup, Registration in the FORUM building of the FAL
10.00	Welcome and Introductions
	Dr. Klaus Sommer, Head of the FAL Anette Giesemann, local conference organizer Keith Hobson and Len Wassenaar co-organizers
10.30 - 11.15	Keynote Adress – Dr. Dan Yakir
11.15 - 15.20	***Session 1 - Plant, soil and sediment ecology***
11.15 - 11.35	Maya Jäggi: δ^{13}C and δ^{18}O ratios in Needles of Norway Spruce (*Picea abies*) as affected by environmental conditions in a dry (1998) and a wet (1999) year
11.35 - 11.55	Valery J. Terwilliger: Information about allocation of stored organic carbon to leaf growth and autotrophic resource use in tropical trees from natural abundances of stable isotopes
11.55 - 12.15	Rebecca Hood: The effect of soil temperature and moisture on organic matter decomposition and plant growth
12.15 - 14.00	Lunch break
14.00 - 14.20	Gerhard Gebauer: ^{15}N and ^{13}C natural abundance in orchid species and accompanying vegetation in Central Europe
14.20 - 14.40	James R. Ehleringer: Interpreting hydrogen and oxygen isotope ratios in tree rings: evaluation of a mechanistic model
14.40 - 15.00	Erik Hobbie: Estimating Carbon Allocation to Mycorrhizal Fungi from Natural Isotopic Measurements
15.00 - 15.20	Andrew Johnston: The use of stable isotope in the study of nitrogen cycling in agricultural soils

15.20 - 16.20	***Session 2 - New methods and applications***
15.20 - 15.40	Charles B. Douthitt: Measurement of δO and δD in biological tissues and compounds: TC/EA-IRMS and GC-TC-IRMS join EA-IRMS and GC-C-IRMS
15.40 - 16.00	John Morrison: Tracing animal migration patterns with online hydrogen isotope measurements - Butterfly, Whale and Bat studies
16.00 - 16.20	Matthias Kästner: Stable Isotopes in Environmental Biotechnology - Chances and Limitations
17.00 - 20.00	Poster Session - snacks and drinks available

Tuesday, May 09, 2000

08.30 - 12.10	***Session 3 - Dietary and trophic studies***
08.30 - 08.50	Joseph P. Shannon: Aquatic Food Web Construction of the Colorado River Through Grand Canyon Using Dual Stable Isotopes (^{13}C and ^{15}N)
08.50 - 09.10	Darryl Smith: Trophic position of southern California estuarine and island populations of the silverside fish *Atherinops affinis* (Teleostei: *Atherinopsidae*): Analyses of ^{15}N and ^{13}C stable isotopes and dietary items
09.10 - 09.30	Raymond Hesslein: An explanation of differences between δ^{13}C of inorganic carbon in lakes with different limnological characteristics and the relation of those signals to δ^{13}C in fish
09.30 - 09.50	Marilyn L. Fogel: Isotopic Tracers of Dynamic Food Web Changes in a Estuarine Ecosystem
09.50 - 10.10	Robyn E. Jamieson: The effect of lifetime shifts in trophic level on δ^{13}C: implications for otolith research
10.10 - 10.30	Susan Waldron: Stable isotope signatures of lotic invertebrates: Sources of variation and significant differences
10.30 - 10.50	Coffee break
10.50 - 11.10	Carolyn M. Kurle: Stable Isotope Assessment of Temporal and Geographic Differences in Feeding Ecology of Northern Fur Seals (*Callorhinus ursinus*) and Their Prey
11.10 - 11.30	Amanda Knoff: Stable Isotope Analysis of Intrapopulation, Spatial, and Temporal Variation of Laughing Gull (*Larus atricilla*) Diets in the Virginia Coast Reserve and Jamaica Bay, New York
11.30 - 11.50	Krishna Das: Stable Isotope and Cadmium Combined Analyses for Tracing Diet Among Top-Predators: A Case Study

11.50 - 12.10 Nancy Beavan Athfield: Stable isotope (^{15}N, ^{13}C) and radioisotope (^{14}C) determination of lifetime diet in Rattus exulans on offshore islands of New Zealand

12.10 - 14.00 Lunch break

14.00 - 17.00 Session 4 - Applications of molecular isotopic analyses

14.00 - 14.20 Roland Bol: Plant Functional Strategies and their Compound Specific Amino Acids $\delta^{15}N$ Values in Grasslands

14.20 - 14.40 H.T.S. Boschker: Compound specific isotope analysis in the microbial ecology of coastal ecosystems

14.40 - 15.00 Ian D. Bull: Detection and classification of atmospheric methane oxidising bacteria in soil

15.00 - 15.20 Paul M. Chamberlain: Detection of trophic preferences in collembola using compound-specific stable carbon isotopic analysis

15.20 - 15.40 Diane M. O'Brien: Nutrient allocation to reproduction in *Lepidoptera*: larval vs. adult origins of egg amino acids

15.40 - 16.00 Mark A. Teece: Corals are polytrophic and opportunistic feeders

16.00 - 16.20 Coffee break

16.20 - 16.40 Susan Ziegler: Seasonal and diel change in the sources of organic matter in a freshwater, tidal wetland

16.40 - 17.00 Jürgen Schleucher: Intramolecular deuterium distributions link biochemistry, physiology and climate research

17.00 - 18.00 Session 5 - Archaelogical and paleological studies

17.00 - 17.20 Robert K. Burton: The ecology of northern fur seals from the Holocene of California: evidence from stable isotopes and archaeofauna

17.20 - 17.40 Mark Clementz: Isotopic Analysis of Lipids Retrieved from 12 Ma Marine Mammal Bones

17.40 - 18.00 Bruce J. MacFadden: Isotopic evidence for the evolutionary paleoecology of fossil horses (Family *Equidae*) from North America

19.00 - 22.00 Conference dinner

Wednesday, May 10, 2000

08.50 - 10.30	***Session 6 - Contaminant pathways and impacts***
08.50 - 09.10	Stuart Bearhop: Variability in mercury concentrations of great skuas *Catharacta skua*: the influence of colony, diet and trophic status inferred from stable isotope signatures
09.10 - 09.30	Gilbert Cabana: Nitrogen isotope ratios: indicators of source of N or N transformations?
09.30 - 09.50	Karyne M. Rogers: A stable carbon and nitrogen isotopic study of biota recovery from a sewage contaminated near-shore marine site at Moa Point New Zealand
09.50 - 10.10	Mariusz Orion Jędrysek: S and O isotope ratios in spruce needles as a tracer of atmospheric pollution
10.10 - 10.30	Hosrt Schulz: Influence of reduced SO_2 input on natural isotope variations of sulfur and oxygen in various compartments of Scots pine stands in eastern Germany
10.30 - 10.50	Coffee break
10.50 - 12.10	***Session 7 - Wildlife research***
10.50 - 11.10	Sandra GarvieLok: Diet and Lifeway in Medieval Greece as Reflected in Human Bone Collagen $\delta^{13}C$ and $\delta^{15}N$
11.10 - 11.30	Sharon Z. Herzka: Insight into the settlement dynamics of fish larvae using $\delta^{13}C$ as a natural tracer
11.30 - 11.50	Noreen Tuross: Omnivory and Migration of the Whooping Crane
11.50 - 12.10	Cindy L. Sagers: Carbon and Nitrogen Isotopes Trace Nutrient Exchange in an Ant-Plant Mutualism
12.10 - 14.00	Lunch break
14.00 - 16.20	***Session 8 - Variable scale ecosystem studies***
14.00 - 14.20	Nina Buchmann: $^{13}CO_2$ fluxes above and within a spruce forest
14.20 - 14.40	Roy Neilson: Natural abundances of $\delta^{13}C$ and $\delta^{15}N$ reveal potential differences in feeding strategy among populations of *Xiphinema diversicaudatum* (Nematoda: *Longidoridae*)
14.40 - 15.00	Matthias Saurer: Canopy gradients in $\delta^{18}O$ of water vapour, leaf water and organic matter as ecophysiological tool

15.00 - 15.20	Matthew J. Wooler: An exploratory survey of $\delta^{13}C$ trends in *Acacia* wood from Western Australia to determine recent, human-influenced climate change
15.20 - 15.40	Donald M. Schell: Hindcasting Carrying Capacity of the Bering Sea: An Extended Record from Carbon Isotope Ratios in Sea Birds and Marine Mammals
15.40 - 16.00	Carol Kendall: Spatial Changes in Redox Conditions and Food Web Relations at Low and High Nutrient Sites in the Everglades
16.00 - 16.20	Jean Marc Guehl: Functional diversity in rainforests of French Guiana revealed by the $\delta^{13}C$ approach
16.20 - 16.40	Coffee break
16.40 - 17.40	Final remarks
	Best Student Awards - Presentation of the winners

Thursday, May 11, 2000

10.00 - 12.30	Field trip to the FACE experimental site - if requested

ABSTRACTS OF

ORAL PAPERS

Variability in mercury concentrations of great skuas *Catharacta skua*: the influence of colony, diet and trophic status inferred from stable isotope signatures

Stuart Bearhop[1], R.A. Phillips, D.R. Thompson, S. Waldron and R.W. Furness

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A range of parameters thought to contribute to intra-specific variation in mercury levels were investigated using the feather and blood of adult great skuas *Catharacta skua* from two northeast Atlantic colonies as sampling units. Different feather types and blood were taken to represent intake over different temporal scales. Mercury concentrations and stable isotope signatures of these tissues were determined. General linear models demonstrated that trophic status, as indicated by $\delta^{15}N$, had an influence on tissue mercury concentrations. However this effect was relatively minor compared to that of foraging area (indicated by colony and $\delta^{13}C$). Samples of the same feather types from the same individuals in consecutive years suggest that a factor other than dietary specialisation and foraging area is of major importance in determining intraspecific variability in mercury levels (possibly individual physiology). It was concluded that there are a number of interacting factors contributing to intra-specific variability in mercury levels and the relative importance of these factors varies both spatially and temporally.

Stable isotope (^{15}N, ^{13}C) and radioisotope (^{14}C) determination of lifetime diet in Rattus exulans on offshore islands of New Zealand

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We have developed a method of enhancing stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) information relative to diet, by adding "bomb pulse" radiocarbon ($\Delta^{14}C$) analysis to examine the isotopic values produced by cumulative feeding habits of *Rattus exulans* (*kiore*). In the preliminary stage of the project, we collected *kiore* on Kapiti Island and Taranga Island, New Zealand, and derived $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$ from *kiore* bone protein and a selection of foods available to the rats within the habitat in which they were trapped. The additional isotopic "clues" about diet from the radiocarbon analysis have provided a better understanding of the variation in diets of *kiore* in different habitats.

Plant Functional Strategies and their Compound Specific Amino Acids δ^{15}N Values in Grasslands

Roland Bol[1], N. Ostle[2], K.J. Petzke[3], J. Cockburn[4] and A. Watson[4]

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In this exploratory investigation, ion chromatography (IC) (MASON *et al.*, 1980) and gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) (METGES *et al.*, 1996) were used to characterise acid-hydrolysable amino acids in plant and soil samples from a permanent grazed grassland. The pasture had not received any inorganic N fertiliser since 1983.

Three plant species were collected with different functional strategies (GRIME *et al.*, 1988). *Lolium perenne*, *Juncus effusus*, and *Brachythecium rutabulum*. *L. perenne* is a perennial monocotyledon grass, with VA mychorrizha, classified as a competitive-ruderal by functional strategy. It is capable of rapid growth and tissue turnover as a result of efficient mineral N uptake (*L. perenne* capacity for uptake of organic N is not known). It is generally found in fertile habitats and is tolerant of frequent disturbance, in this instance grazing, trampling and camping. *J. effussus* is a perennial rush, without mychorrhiza, and is a competitor-stress tolerant plant. *Juncus* sp. are relatively slow growing and are found in habitats of moderate productivity and disturbance including waterlogged soils and riparian wetlands. Waterlogged soils tend to have low inorganic nutrient availability and soil pH, and therefore can only sustain plant species adapted to these conditions. Recent work has established that functionally related tundra sedges, *Eriophorum vaginatum* and *Carex aquatilis,* possess the capacity for organic N uptake (SCHIMEL & CHAPIN, 1996). *B. rutabulum* is a ruderal perennial species of bryophyte which initially occupies areas left bare due to poaching and trampling by cattle, but is also present within the sward. A characteristically shallow root systems suggest that B. rutabulum acquires most of its soil N from the uppermost part of the soil and from atmospheric N deposition (wet and dry).

The total C, N and amino acid N contents were at least three times higher in the plant samples than in the soil. Individual amino acids (IAAs) concentrations were up to ten times higher in plant tissues than in the soil. The highest total and individual concentrations of amino acids were found in the above ground shoots. IAAs concentrations in *J. effusus* were higher than in the other two plant species (Figure 1a), with the exception of proline (in *B. rutabulum*) and phenylalanine (in *J. effusus*). The relative contributions of glycine, alanine and arginine were highest in the soil, whereas glutamic acid and lysine were more prominent in plant samples. Relative contributions of IAA-N to plant N followed the same pattern.

Plant amino acid δ^{15}N‰ values were generally depleted by 2 to 7 δ^{15}N‰ units compared to the soil. Glycine, serine and glutamic acid were particularly ^{15}N depleted. The largest relative depletions of plant IAAs compared to soil were observed in *B. rutabulum*. In contrast, isoleucine and threonine were enriched in the plants by 3 to 4 δ^{15}N‰ units compared to soil

IAAs, with phenylalanine being generally enriched by 15 to 25 $\delta^{15}N‰$ units. Curiously, in the above ground plant parts of *J. effusus* this relative enrichment was only 3 $\delta^{15}N‰$ units compared to 25 units in the roots. Histidine values were also very high in shoot and roots of *J. effusus* (>20 $\delta^{15}N‰$ units) and thus enriched compared to the soil. The plant shoots generally had higher amino acid $\delta^{15}N‰$ values than their roots, however in most cases no more than 4 $\delta^{15}N‰$ units. Plant amino acid $\delta^{15}N‰$ values were generally higher in *J. effusus* than the other two plants. Specific features that could separate plant species, included (a) histidine showing *Juncus effusus* >> *Lolium perenne*, (b) threonine and phenylalanine, which separated the shoots of *Juncus effusus* > *Lolium perenne* > *B. rutabulum*, and c) isoleucine values in *Lolium perenne* > *Juncus effusus*.

Further work is needed to assess whether differences in IAA concentrations and $\delta^{15}N‰$ signatures observed between plants are the product of plant nutritive strategies and metabolism, or whether they are the product of localised variations in soil conditions and resource quality (e.g. soil N concentrations and quality, moisture content, temperature).

References

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Compound specific isotope analysis in the microbial ecology of coastal ecosystems

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Microorganisms play an important role in the cycling of carbon and nutrients in the coastal zone. The application of stable isotope analysis in studying the role of bacteria and other microbes has however been limited due to problems with isolating their biomass from water and sediment samples, and also from other organisms. Recently, we have started exploring the use of ^{13}C analysis of specific biomarkers by GC-c-IRMS to study the activities and population structure of microbial communities. Biomarkers studied so far are mainly polar lipid derived fatty acids (PLFA), which are a structural component of the microbial cell and are associated with recently formed biomass as they show a high turnover rate. There are a number of possible applications for this type of analysis in ecology:

i. Natural ^{13}C ratios of biomarkers can be used to trace the sources of organic matter utilized by heterotrophic bacteria,
ii. Labeling with ^{13}C-bicarbonate to study the coupling between autotrophic and heterotrophic processes for instance in benthic algal mats,
iii. Labeling with complex organic matter (e.g. algae or macrophyte material) to study the competition between bacteria and other organisms for these substrates,
iv. Addition of specific ^{13}C-labeled organic compounds to study their turnover and to directly link microbial populations to specific biogeochemical processes.

Recent examples of these fields of application will be presented.

$^{13}CO_2$ fluxes above and within a spruce forest

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Isotopic signatures of biological fluxes can give valuable insights into the origin of these fluxes and the partitioning into different components. Studying carbon and oxygen isotope ratios of carbon dioxide above and within terrestrial ecosystems can provide relevant information about the dominant flux component of the net ecosystem CO_2 exchange, i.e. assimilation or respiration. Furthermore, it allows to separate above- and below-ground origins of CO_2 within, leaving or entering the ecosystem. We used profile and eddy-covariance measurements to quantify CO_2 fluxes in combination with flask sampling and hyperbolic relaxed eddy accumulation (HREA) for ^{13}C analyses. $\delta^{13}C$ values of CO_2, measured by HREA for up- and downdraft CO_2 above the canopy, ranged between -8.29 and -7.51 ‰. The differences in $\delta^{13}C$ values between up- and downdrafts varied between -0.16 to 0.17 ‰, dominated by changes in updraft CO_2. At times with high net ecosystem assimilation (noon), the $\delta^{13}C$ values for updrafts were higher than those during the morning and the afternoon, when net ecosystem respiration is dominating the carbon isotopic signature of updraft CO_2. In contrast, downdraft $\delta^{13}C$ values were relatively constant during the day, indicating high turbulent mixing of the boundary layer. The ecosystem isotopic fractionation for our temperate spruce forest was lower compared to that of a deciduous temperate forest in Tennessee, probably indicating different water regimes.

Detection and classification of atmospheric methane oxidising bacteria in soil

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Well drained non-agricultural soils have been shown to support oxidation of methane directly from the atmosphere and this could contribute 5-10 % of the global methane sink (CICERONE & OREMLAND, 1988; CRUTZEN, 1991). This activity cannot be predicted from cultured laboratory strains of low affinity methanotrophs and it is speculated that the microbiology of methane at concentrations present in the atmosphere (high-affinity oxidation) is unknown (CONRAD, 1996; HANSEN & HANSEN, 1996). Here we present the results of an isotopic pulse-chase labelling experiment which, through the analysis of bacterially derived phospholipid fatty acids (PLFAs), provides unambiguous evidence for the bacterial assimilation of methane at true atmospheric concentrations (1.8-3.6 ppmv). The application of this technology is relatively facile and as such it is a powerful tool for investigating microbial populations in situations where molecular biological techniques and radiocarbon labelling have proven to be either deficient or inappropriate.

A long term experiment was set up to evaluate the assimilation of methane by methanotrophic bacteria in a terrestrial soil. Distilled water and 100 ppmv methane in air was pumped through two soils (buried organic and mineral horizons) at flow rates of 0.015 and 0.05 ml min^{-1} respectively for 6 months. After 6 months the original methane source was altered to contain 0.01 % isotopically heavy methane ($^{13}CH_4$) and the experiment was continued for an additional 3 weeks. An identical set of soils were similarly treated, however, in this case methane concentrations were kept between 1.8 and 3.6 ppmv. At the end of the experiment PLFAs were separated from each soil, hydrolysed, then analysed as fatty acid methyl esters (FAMEs) by GC, GC/MS and GCC/IRMS.

The PLFA derived FAME distributions obtained for each soil were virtually identical being dominated by C_{16} and C_{18} FAMES as well as various branched chain components at lower abundance. Soils exposed to methane levels of 100 ppmv exhibited the greatest incorporation of ^{13}C into bacterially derived PLFAs. The buried organic horizon exposed to 100 ppmv methane contained heavily labelled 16:0, 16:1ω8c, 16:1ω7c, br17:0, br18:0, 18:1ω9c and 18:1ω7c components indicative of low affinity methane oxidation being performed by both type I and type II methanotrophic bacteria. The buried organic soil exposed to atmospheric levels of methane (1.8-3.6 ppmv) only exhibited significant labelling of the br17:0 and 18:1ω7c PLFAs indicating that methane oxidation was being mediated predominantly by a novel methanotroph which expresses some similarity at the PLFA level to known type II methanotrophs (BULL *et al.*).

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The ecology of northern fur seals from the Holocene of California: evidence from stable isotopes and archaeofauna.

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Contemporary northern fur seals (*Callorhinus ursinus*) breed exclusively on offshore islands, primarily at high latitudes, and are otherwise highly pelagic. Unexpectedly, their remains are commonly found at Holocene archaeological sites in coastal California, Oregon, and Washington. Females and young from Alaskan rookeries migrate to forage over the slope/shelf break along California, but are rarely seen on land at middle latitudes with two notable exceptions of rookeries recently formed on islands off California. Carbon and nitrogen isotope compositions of ancient northern fur seals from central and northern California reveal they fed far offshore, as they do today, yet remained at middle latitudes throughout the year. We've identified the remains of numerous pups whose size and nitrogen isotope compositions indicate they had not been weaned and therefore were likely culled on nearby mainland rookeries. Evidence of rookeries at these sites suggests numerous other archaeological sites, where northern fur seal remains were found, may also have been near mainland rookeries.

The decline of mainland rookeries predates ~1000 BP and is most likely due to intensified human predation and not climate shifts as the isotope compositions of prehistoric harbor seals and California sea lions reveal that little has changed in their patterns of foraging and migration. With the cessation of historic commercial exploitation in the middle 1970's, the species appears to be re-colonizing portions of its former range at middle latitudes. The distribution of ancient rookeries can be used to identify locations where northern fur seals could successfully re-establish mainland rookeries in the future.

Nitrogen isotope ratios: indicators of source of N or N transformations?

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The high N isotopic ratio of sewage-derived N has been proposed as a useful tracer of the incorporation of N from human and animal wastes into aquatic food webs. However, other studies have shown that fractionation associated with gaseous losses (denitrification, volatilization) from high N-input systems can also lead to high $\delta^{15}N$. Thus $\delta^{15}N$ may be an indicator of nitrogen losses from overloaded systems rather than a tracer of the ultimate source of N. We investigated the relations among land use, total nitrogen concentration in water, and the $\delta^{15}N$ in 61 riverine locations within the U.S. using invertebrate-feeding fish collected as part of the National Contaminant Biomonitoring Program (NCBP) as indicators. Fish $\delta^{15}N$ (+4 to +18 ‰) was significantly ($P<0.01$) related to TN in water and the percent of the watershed exploited as urbanization and agriculture. However, high $\delta^{15}N$ values were observed in watersheds dominated by agriculture as well as ones dominated by urbanization. Thus $\delta^{15}N$ may not be an indicator of the source of nitrogen (fertilizer vs sewage), but instead an indicator of the extent of N-fractionating processes in ecosystems impacted by excesss nitrogen loadings.

Detection of trophic preferences in collembola using compound-specific stable carbon isotopic analysis

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Collembola are a class of ubiquitous microarthropods found in most biogeographical regions of the world and a wide range of habitats from soils to tree canopies. However remarkably little is known about the different feeding preferences of collembolan species and hence even broad trophic groupings are not well defined. Stable isotopic methods offer an ideal means of investigating collembolan trophic preferences, by monitoring the assimilation of isotopically different food sources into collembolan body tissues. Compound-specific analysis greatly facilitates collembolan studies since it utilises smaller sample sizes than bulk measurements, and enables the isotopic composition of individual metabolic products, rather than whole tissue, to be determined.

An isotopic diet-switching experiment was performed with two collembolan species *Folsomia candida* and *Proisotoma minuta* to characterise the changes in stable carbon isotopic composition of whole collembola, individual fatty acids (derived from triglycerides), individual amino acids (derived from proteins and peptides), and cholesterol. Animals were reared on a C_3 isotopically labelled yeast (*Saccharomyces cerevisiae*), then switched to yeast labelled with a C_4 isotopic signal. The C_3 and C_4 yeasts were cultured under identical conditions, with only the carbon source differing. Turnover rates of lipids reflected the differential rates of physiological utilisation and synthesis of the individual biochemical components.

Subsequently, a simple feeding preference test was performed using *F. candida*, the nematode *Panagrellus redivivus* and the fungus *Cladisporium cladisporides*; each with different stable carbon isotopic labels. By following the shift in $\delta^{13}C$ values of collembolan lipids, the feeding preference of *F. candida* for either the nematode or fungus was established.

Isotopic Analysis of Lipids Retrieved from 12 Ma Marine Mammal Bones

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Dietary interpretations of extinct animals (>1 Ma) based on stable isotope analysis have principally relied upon enamel, since it is less susceptible to diagenetic alteration. However, enamel is not always available, so other substrates must be used. Recently, specific lipids, such as cholesterol, from ~1 Ma fossil bones have been found to retain the original dietary carbon isotope signal. To determine if original lipids are retained in older fossils, we analyzed bone samples from middle Miocene (10 to 15 Ma) cetaceans and sirenians. The lipid compositions and carbon isotope values of fossil specimens were then compared with the compositions and carbon isotope values of associated sediments and modern marine mammals, to assess potential contamination and retention of original values.

Lipids extracted from fossil cetacean bone had carbon isotope values and compositions that were more similar to the surrounding sediment than to modern marine mammal bone, indicating external contamination. In contrast, sirenian bones yielded lipid concentrations and isotope values that were significantly different from the sediment and similar to values found in modern manatees. We conclude that in specific cases, original lipids can be preserved in fossil bone and provide a viable substrate for carbon isotope analysis.

Stable Isotope and Cadmium Combined Analyses for Tracing Diet Among Top-Predators: A Case Study

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In the North-East Atlantic, associations of tunas and dolphins are quite frequent events during the summer and the question arises how predators requiring similar diet in the same habitat share their environmental resources. As isotopic composition of an animal is related to that of its preys, stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) analyses were performed in three predator species from the Northeast Atlantic: the striped dolphin, *Stenella coeruleoalba*, the common dolphin *Delphinus delphis*, and the albacore tuna, *Thunnus alalunga*, and compared to their previously described stomach content (DAS *et al.*, 2000). Cd is mainly transferred through the diet and so, has been determined in the muscles and livers of the animals. Tunas muscles display higher $\delta^{15}N$ than in common and striped dolphins (mean: 11.4 ‰ *vs*. 10.3 ‰ and 10.4 ‰, respectively). Higher $\delta^{13}C$ are found in common (-18.4 ‰) and striped dolphin (-18.1 ‰) muscles than in albacore tuna (-19.3 ‰) These data presumably reflect a greater reliance on offshore food in relation with the tuna migration pattern. The most striking feature is the presence of two levels of cadmium concentrations in the livers of the tunas (32 mg kg^{-1} dry weight *vs.* 5 mg kg^{-1} dry weight). These two groups also differ by their $\delta^{15}N$ and $\delta^{13}C$ liver values. These results suggest that in the Bay of Biscay, tunas occupy two different ecological niches likely based on different squid input in their diet.

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Measurement of δO and δD in biological tissues and compounds: TC/EA-IRMS and GC-TC-IRMS join EA-IRMS and GC-C-IRMS

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Variations in the relative abundances of the stable isotopes of C, N, and S are increasingly being used in ecological studies as tracers of material transfer within an ecosystem, to define food webs, to aid in paleodietary reconstruction and to elucidate the details of parasitic and symbiotic relationships. A significant recent development has been the use of the ^{13}C content of phospholipid fatty acids for studying microbial ecology. These studies were made feasible by development of the continuous flow-IRMS methodologies of EA-IRMS and GC-IRMS. Other objectives include the definition of isotopic signatures which can be used to uniquely constrain "point of origin" and measurement of isotopic variations which can be used to reconstruct migratory patterns. Recent developments in CF-IRMS enable rapid, precise and accurate measurement of D and ^{18}O contents of organic molecules in both bulk samples (µg of O and H) and at the molecular level (ng of O and H). These new thermochemolytic (TC) techniques involve quantitative conversion of the ^{18}O and D in biomolecules into CO and H_2, respectively, at elevated temperatures (>1400 °C). It is expected the systematics of biological variation of δO and δD will be controlled by the large and well-understood variations in the isotopic composition of hydrogen and oxygen of meteoric waters. Work done in the MAT applications lab with the TC/EA and GC/TC establishes variations of δO and δD in a variety of natural products (coffee beans, honey, vegetable oils) which parallel the Meteoric Water Line. These new analytical capabilities hold considerable promise for definition of isotopic fingerprints and for tracking animal migration.

Interpreting hydrogen and oxygen isotope ratios in tree rings: evaluation of a mechanistic model

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A mechanistic model will be presented that quantifies both the physical and biochemical fractionation events associated with hydrogen and oxygen isotope ratios in tree-ring cellulose formation. The model predicts the isotope ratios of tree rings, incorporating both humidity and source water environmental information. Components of the model include (a) hydrogen and oxygen isotope effects associated with leaf water enrichment, (b) incorporation of leaf water isotope ratio values into photosynthetic carbohydrates along with the biochemical fractionation associated with autotrophic synthesis, (c) transport of exported carbohydrates (such as sucrose) from leaves to developing xylem in shoots and stems where cellulose is formed, (d) a partial exchange of oxygen and hydrogen isotopes in carbohydrates with xylem sap water during conversion into cellulose, and (e) a biochemical fractionation associated with cellulose synthesis. The leaf water model was robust over a wide range of leaf waters for both controlled experiments and field studies, far exceeding the range of values to be expected under natural conditions. The model prediction that tree-ring cellulose contains information on both environmental water source and atmospheric vapor pressure deficit (related to relative humidity) was tested with long-term observations under both field and greenhouse conditions. Our model resolves apparently conflicting interpretations of several previous cellulose stable isotope ratio studies.

Isotopic Tracers of Dynamic Food Web Changes in a Estuarine Ecosystem

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Throughout a seasonal cycle, food web dynamics change in response to photosynthetic and microbial processes (Bottom-Up effects) or life cycle changes and migration of animals (Top-Down effects). The interplay of these factors was investigated in the Rhode River Estuary, Chesapeake Bay, Maryland, a relatively pristine watershed that has been the subject of study by the Smithsonian for almost 20 years. Particulate organic carbon, zooplankton, benthic organisms, invertebrates, and all major fish species were collected from late spring through early fall for isotopic analysis. Allocthonous carbon entered the food chain via detritovores, which had $\delta^{13}C$ values influenced by either submerged aquatic vegetation at the mouth of the estuary or decaying tree leaves in the freshwater end. Nutrients (i.e., inorganic N) enter the estuary from the Chesapeake Bay via tidal flux. The $\delta^{15}N$ in all levels of the food webs were shifted 1-2 ‰ more positive at the two stations closest to the bay. Gelatinous plankton, ctenophores and jellyfish, exert a strong top-down influence on this estuarine food web in July and August. Predation by these organisms resulted in carbon and nitrogen isotopic shifts in zooplankton of 2-3 ‰, in addition to enrichments in benthic clams feeding primarily on zooplankton. Fish with restricted ranges had very distinct isotopic compositions at each station, even though their feeding strategies were identical, whereas larger fish that moved throughout the estuary were more homogenous isotopically. In this small watershed, stable isotopes were able to highlight the ecosystem controls that were important at an individual location in the estuary at a particular point in time.

Diet and Lifeway in Medieval Greece as Reflected in Human Bone Collagen $\delta^{13}C$ and $\delta^{15}N$

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While historical documents and food remains in archaeological sites allow the key items of past diets to be identified, it can be more difficult to establish their relative importance. This problem has led to debates over the importance of some resources – especially fish – to Greek diets in the past. The research described here uses human bone collagen $\delta^{13}C$ and $\delta^{15}N$ to investigate diet, particularly the consumption of marine foods, in medieval Greece. Bone from archaeological sites of the late Byzantine period was used to examine regional variation in resource use, while material from the times of the Frankish and Ottoman Turkish occupations was included to investigate the impact of ethnicity on diet. The results of the analyses suggest that marine resources played a smaller role in medieval Greek diets than has often been thought. Human bone collagen $\delta^{13}C$ and $\delta^{15}N$ values suggest diets largely depending on terrestrial resources. Clear differences in $\delta^{13}C$ and $\delta^{15}N$ are seen between the late Byzantine populations and some of the Frankish and Ottoman period remains, with the latter showing enriched mean values and greater dispersion. These differences may be partially due to changes in diet. However, especially in the case of the Ottomans, the increased dispersion is more likely due to residential mobility, with individuals moving into the area from elsewhere in the Mediterranean and retaining the local ecosystem $\delta^{15}N$ and $\delta^{13}C$ values of their point of origin. This hypothesis could be tested through stable oxygen or strontium isotope analysis.

^{15}N and ^{13}C natural abundance in orchid species and accompanying vegetation in Central Europe

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Most of the terrestrial plants from the boreal zone to the tropic region are associated with fungi forming different types of mycorrhizae. Mycorrhizal fungi are presumed to play an important role for the mineral nutrient and especially the nitrogen acquisition of plants. On the other hand, autotrophic plants provide mycorrhizal fungi with carbon compounds. This mutual interaction between plants and fungi is presumed to influence the natural nitrogen and carbon isotope signature of both types of organisms. Forest plants have already been found to be more depleted in ^{15}N and ^{13}C than fungal fruit bodies (see e.g. GEBAUER and TAYLOR, 1999; KOHZU *et al.*, 1999). The reasons underlying these findings, however, are not yet fully understood. Especially for the orchid family the interaction between plants and mycorrhizal fungi is rather close. Orchid seeds need fungi already for their germination. Orchids and fungi usually form endomycorrhizae. Some orchid species are even no longer autotrophic and have become mycoheterotrophic. These orchids digest fungal mycelium. Thus, orchids are presumed to be a suitable tool to improve our understanding of the mutualistic influence of plants and fungi on their isotopic signature and the underlying mechanisms.

This study provides comparative ^{15}N and ^{13}C data of orchid species and accompanying non-orchid vegetation forming different types of mycorrhizae (ectomycorrhiza, arbuscular mycorrhiza, ericoid mycorrhiza) from Central European forest and grassland ecosystems. The comparison indicates a pronounced isotopic distinction of some orchid species from non-orchids, while other orchid species have isotope signatures similar to the non-orchids. The isotopic distinction of some orchid species is apparently related to their ability to digest fungal mycelium. The mycelium-digesting orchids are more enriched in ^{15}N and ^{13}C than non-orchids, and thus have isotope signatures similar to fungi. Not only mycoheterotrophic orchids (e.g. *Neottia nidus-avis*), but also C-autotrophic orchid species (e.g. *Cephalanthera rubra*, *Ophrys insectifera*) were found in the group of mycelium digesting orchids.

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Functional diversity in rainforests of French Guiana revealed by the $\delta^{13}C$ approach

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Tropical rainforests are characterised by the co-existence of a multitude of tree species. More than 1300 tree species have been identified by botanists in French Guiana. Functional grouping of species based on ecophysiological traits is a necessary step to the understanding and modelling of the functioning (e.g. CO_2 and H_2O exchange) of complex ecosystems such as the tropical rainforest. The interspecific variability of sunlit leaf carbon isotope composition ($\delta^{13}C$), an indicator of leaf intrinsic water-use efficiency (WUE, CO_2 assimilation rate/leaf conductance for water vapour), was investigated in canopy trees of three lowland rainforest stands in French Guiana. We sampled leaves from trees of 102 different species. Mean species $\delta^{13}C$ varied over a 6.0 ‰ range within each stand, corresponding to WUE varying over about a threefold range. Such a range is much higher than the one found in other terrestrial ecosystems. We found a clear association between species $\delta^{13}C$ values and the different types of shade tolerance defined by features reflecting the plasticity of growth dynamics with respect to contrasting light conditions. Heliophilic species exhibited more negative $\delta^{13}C$ values (average $\delta^{13}C$ = -30.5 ‰) (i.e. lower WUE) than hemitolerant species (-29.3 ‰). However, tolerant species (-31.4 ‰) displayed even more negative $\delta^{13}C$ values than heliophilic ones. To our knowledge, this association constitutes a first validation, for rainforests, of the concept of functional types of species stating a unique grouping of species with respect to various functional traits.

Insight into the settlement dynamics of fish larvae using $\delta^{13}C$ as a natural tracer

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Many marine species exhibit a life cycle characterized by a pelagic larval stage followed by migration into estuaries and settlement into nursery habitat. Identifying the factors controlling the population regulation and recruitment of estuarine dependent species requires the characterization of inshore migration patterns over suitable temporal scales. Using red drum (*Sciaenops ocellatus*) as a model species, we have developed the use of $\delta^{13}C$ measurements to trace the settlement of individual larvae from coastal waters to nursery habitat. During the pelagic stage, the $\delta^{13}C$ values of red drum larvae reflect planktonic carbon sources. Following settlement, their isotopic composition shifts toward that of benthic carbon sources. The rate of isotopic shift is a function of the amount of biomass gain (growth) and metabolic turnover. To track the settlement history of individual larvae, we have developed a model that can be used to estimate size at settlement and time since settlement. The model is based on (1) characterization of the $\delta^{13}C$ of the larvae during the planktonic stage (2) the $\delta^{13}C$ of larvae collected in the nursery habitat, and (3) the contribution of growth and metabolic turnover to the rate of isotopic shift. At the population level, these results can be utilized to determine the timing of settlement pulses and to relate these events to physical forcing factors driving migration into estuaries, to examine the temporal coupling between the abundance of larvae in the plankton and settlement, and to differentiate settlement from movement within the nursery habitat.

An explanation of differences between $\delta^{13}C$ of inorganic carbon in lakes with different limnological characteristics and the relation of those signals to $\delta^{13}C$ in fish

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Because of the large discrimination between isotopes of carbon produced by the photosynthetic process and the relatively small discrimination by respiration, the $\delta^{13}C$ of inorganic carbon (DIC) in lakes can be used as an indicator of net fixation/respiration. This information can be correlated to physical, chemical and biological characteristics of the lakes to gain a better understanding of net metabolic characteristics. This approach is particularly sensitive in lakes of the Canadian Shield which have minimal inputs of DIC derived from geological carbonates. Detailed studies of the $\delta^{13}C$ of DIC in these lakes have shown it to have large seasonal variability. The $\delta^{13}C$ of aquatic organisms reflects the $\delta^{13}C$ of the DIC and integrates over time, depending on the carbon turnover of the organism. In a set of lakes ranging from 5 ha to 26000 km^2 average $\delta^{13}C$ of DIC ranged from -10 to +2 per mil and white sucker (*Catostomus commersoni*) from -20 to -33 per mil. We show that mature, relatively slow growing fish have $\delta^{13}C$ highly correlated to that of DIC and that their isotope signatures are simply measured indicators of the parameters influencing the net carbon metabolism of these lakes.

Estimating Carbon Allocation to Mycorrhizal Fungi from Natural Isotopic Measurements

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Determining carbon allocation below-ground and carbon allocation to mycorrhizal fungi is experimentally difficult except under laboratory conditions. Below-ground flux to mycorrhizal fungi in the field has been particularly difficult to estimate because of the rapid turnover of mycorrhizal root tips and hyphae, and the difficulties in translating any static measurement of hyphal mass into a carbon flux. The close coupling between N and C cycling in plants suggests that a theoretical treatment of plant-mycorrhizal C and N cycles may prove useful in determining carbon flux to mycorrhizal fungi. I start from two basic premises: (1) uptake of available soil N by fungi (and plants) is proportional to the growth of hyphae (and roots), and (2) isotopic fractionation of nitrogen between mycorrhizal fungi and plants means that isotopic measurements on plants, fungi, and soil available N can be used to estimate the fraction (T_r) of fungal nitrogen that is transferred to plants. From these premises I have derived theoretically a relationship between plant N demand, the fungal transfer ratio (T_r), and the flux of carbon to mycorrhizal fungi. Carbon demand with or without fungal translocation of N is also estimated. In agreement with field evidence, these equations predict decreased allocation of carbon to mycorrhizal fungi with increased N deposition along depositional gradients in Europe.

The effect of soil temperature and moisture on organic matter decomposition and plant growth

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The effects of soil temperature and moisture on organic matter decomposition and mineralisation have been studied in many experiments using soil core incubations. However, few attempts have been made to study both plant growth and organic matter mineralisation simultaneously under different temperature and moisture regimes. A better understanding of these processes and their interactions is required in light of the mounting evidence of global warming. The effect of soil temperature and moisture on both above- and below-ground plant growth and mineralisation of organic residues was investigated using ^{15}N-labelled soybean residues and temperature-controlled tanks in the glasshouse.

A factorial experiment with 24 treatments was set up: three soil temperatures (20, 26 and 30 °C); two soil moisture regimes (8 % and 12 %); residues added (100 mg N kg^{-1} soil in the form of ^{15}N-labelled soybean residues enriched at 1.82 atom % excess) or no residues; and either sown with ryegrass or not sown. There were four replicate of each treatment and the soil used was a sandy-loam pH 7.9, total N 1.1 mg N kg^{-1} soil and organic matter 20 mg kg^{-1} soil.

Pots were watered daily to the pre-determined weight. The concentration and ^{15}N-enrichment of soil inorganic-N were measured at 2, 4 and 6 weeks in the pots with no grass cover. Pots were harvested for above- and recoverable below-ground material six weeks after planting, for determination of biomass, N concentration, ^{15}N-enrichment and δ^{13}C.

The inorganic-N data suggested that available N increased significantly with addition of residues and decreased significantly with temperature. The moisture effect was not consistent across all treatments. Plant dry weight pot^{-1} decreased significantly with increase in soil temperature, and there was also a significant positive effect of increased moisture and addition of residues. Root-to-shoot ratio declined significantly with increase in temperature and moisture. Percentage nitrogen derived from residues (%Ndfr) increased linearly and significantly with increase in temperature and moisture. δ^{13}C decreased linearly and significantly with increase in temperature and decrease in moisture status. There was a significant correlation between transpiration and dry matter production, but there was no correlation between water use efficiency and δ^{13}C.

The results suggest that water and temperature play a significant role in N release and that microbial breakdown of roots with increasing temperature and N fertilisation may be an aspect which has been overlooked in climate change models.

δ^{13}C and δ^{18}O ratios in Needles of Norway Spruce (*Picea abies*) as affected by environmental conditions in a dry (1998) and a wet (1999) year

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The presented results are based on the studies that were carried out in the Research project HARWA – Optimum Nutrition and Recycling of Wood Ash in a Spruce and Beech Forest in Switzerland – in a 69 year old stand of *Picea abies*. The following different treatments were tested: woodash, irrigation, liquid-fertilization-irrigation and control.

Based on data of two consecutive years we found that the influence of the applied treatments showed no significant effect on the carbon and oxygen isotope ratio, irrespective of low (1998) or high (1999) precipitation amounts. However, we found a strong correlation between the sampling month, year and the δ^{13}C and δ^{18}O values of recent and one year old needle age classes. Especially in the recent needles we found a distinct seasonal course in both isotopes and both years. In recent needles of the dry year (1998) we found positive δ^{18}O values of organic matter in spring (May: 27.02 ‰ ± 0.98) and slightly higher values in summer (Aug: 27.92 ‰ ± 0.82). In the wet year (1999), however, the spring values were much more negative (May: 24.37 ‰ ± 0.54) and the δ^{18}O values in summer increased by more than 2 ‰ (July: 26.52 ‰ ± 0.32). A similar, but opposite seasonal trend could be seen for δ^{13}C in both years. In the dry year we observed positive δ^{13}C values in spring (May: -24.67 ‰ ± 0.66) that increased almost 3 ‰ towards summer (Aug: -27.40 ‰ ± 0.97). In the wet year, in contrast, we found less positive spring values (May: -25.89 ‰ ± 0.85) and an increase of about 1.5 ‰ towards summer (July: -27.26 ‰ ± 0.59).

Our results show, that the δ^{18}O of leaf organic matter in a wet year (1999) is mainly influenced by the isotopic composition of the soil water. In contrast, during the year with a low amount of precipitation (1998), we find a strong tendency that the δ^{18}O of leaf organic matter is more determined by the air humidity, but biochemical influence can’t be ruled out. For climate reconstruction, based on tree ring analysis, these findings might be very important.

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The effect of lifetime shifts in trophic level on δ^{13}C: implications for otolith research

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The isotopic trophic level effect has been established by numerous studies of marine food webs. These studies have mainly examined differences between species feeding at different levels of the food web, but feeding changes can also occur for individual species throughout development and maturation. In the case of Atlantic cod (*Gadus morhua*), size plays an important role in what they eat because they swallow their prey whole. Larval cod begin feeding on zooplankton, near the base of the food web, while young cod eat a diet rich in crustaceans such as copepods and amphipods. Fish becomes an increasingly important prey item as the cod gets larger. When they reach about 50 cm, fish is the dominant prey including capelin, sand lance, redfish, and herring as well as other types of fish. We expect that this change in diet would be reflected in the δ^{13}C of metabolic carbon and consequently affect δ^{13}C of otolith aragonite.

Muscle tissue from sixty-one cod have been analyzed for δ^{13}C and δ^{15}N. These cod were obtained from inshore areas of Newfoundland, Canada and range in age from less than one year (age 0) to 7 or 8 years. δ^{13}C increases by up to 2 ‰ in the first two years of life. Age 0 fish have an average δ^{13}C of -21 ‰ while adult values vary between -19 and -18 ‰. δ^{15}N also increases, from 11.7 ‰ in age 0 cod to as high as 15.8 ‰ in larger fish; this increase of about 4 ‰ corresponds to a trophic level increase of a little more than one level.

These data help to explain some of our observations regarding age-dependent ^{13}C variations in cod otoliths, aragonite structures found in the inner ears of teleost fish. They are deposited in seasonal layers which allow the reconstruction of isotopic time series spanning the life of the fish. The δ^{13}C of otoliths represents a mixture of DIC and metabolic carbon and has been shown to increase by up to 3 ‰ over the first 4-5 years of life. Part of this shift can now be understood as reflecting an increase in trophic level during this period. This will allow us to interpret the otolith results to obtain information on the migration, feeding and other life history details for cod which will be valuable to fisheries biologists who are attempting to explain the drastic decline in these stocks off the east coast of Canada.

S and O isotope ratios in spruce needles as a tracer of atmospheric pollution

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The biospheric part of the anthropogenic sulphur cycle was investigated in a heavily polluted area at the Northern slope of Karkonosze Mts., SW Poland. Spruce needles, from a 900 m vertical transect (400 to 1300 m asl), were collected on two consecutive days of spring 1998 and one day of winter 1999.

Concentrations (sulphate sulphur ($(SO_4^{2-})_n$) and organic sulphur ($(Sorg)_n$) and isotope ratios of organic sulphur and sulphate from spruce needles have been analysed. During spring $(SO_4^{2-})_n$ contents were lower (mean 0.066 wt %) and $(Sorg)_n$ contents were higher (mean 0.0836 wt %) compared to the winter sampling (mean 0.0147 and 0.0663 wt ‰ respectively). Both concentration values were rather constant with respect to altitude. However, in general, $\delta^{34}S(SO_4^{2-})_n$ values increased from 4.80 to 9.77 ‰ and $\delta^{34}Sorg_n$ from 0.03 to 7.19 ‰, with increasing altitude (Fig. 1). This is attributed to an increase in atmospheric SO_2 concentration and light intensity which leads to an increased extraction from needles of ^{34}S-depleted H_2S. The spring $\delta^{34}S(SO_4^{2-})_n$ value was on average about 1.9 ‰ higher than the winter $\delta^{34}S(SO_4^{2-})_n$ value, but the $\delta^{34}Sorg_n$ values were similar in both seasons. This relationship argues for an intensive reduction of sulphate stored in needles, which is the source of organic sulphur and H_2S. The $\delta^{18}O(SO_4^{2-})_n$ decreases with altitude due to the altitudinal ^{18}O-depletion of atmospheric precipitation and increased formation of needle sul-phate from atmospheric SO_2. The isotope effect related to a reduction process during spring can be also seen at higher altitudes by a negative correlation in the $\delta^{18}O(SO_4^{2-})_n$ - $(SO_4^{2-})_n$ and $\delta^{34}S(SO_4^{2-})_n$ - $(SO_4^{2-})_n$ of spring needles. Winter needles show an opposite trend of potential oxidation of the SO_2 assimilated (Fig. 2).

The $\Delta^{34}S(SO_4^{2-} - S_{org})_n$ value varied from –0.80 to 7.57 ‰ and shows a very good correlation to the abundance of dust on needles. Higher abundances of dust may limit foliar ventilation and, thus, higher $\Delta^{34}S(SO_4^{2-} - S_{org})_n$ values reflect conditions which are closer to sulphur isotope equilibrium in the sulphate-organic sulphur system, whereas lower $\Delta^{34}S(SO_4^{2-} - S_{org})_n$ values characterise a higher ventilation rate and more dynamic conditions for the sulphur system in needles.

H_2S extraction is the most likely mechanism to control variations in the observed δ-values, and dust abundance may control variations in the Δ-values.

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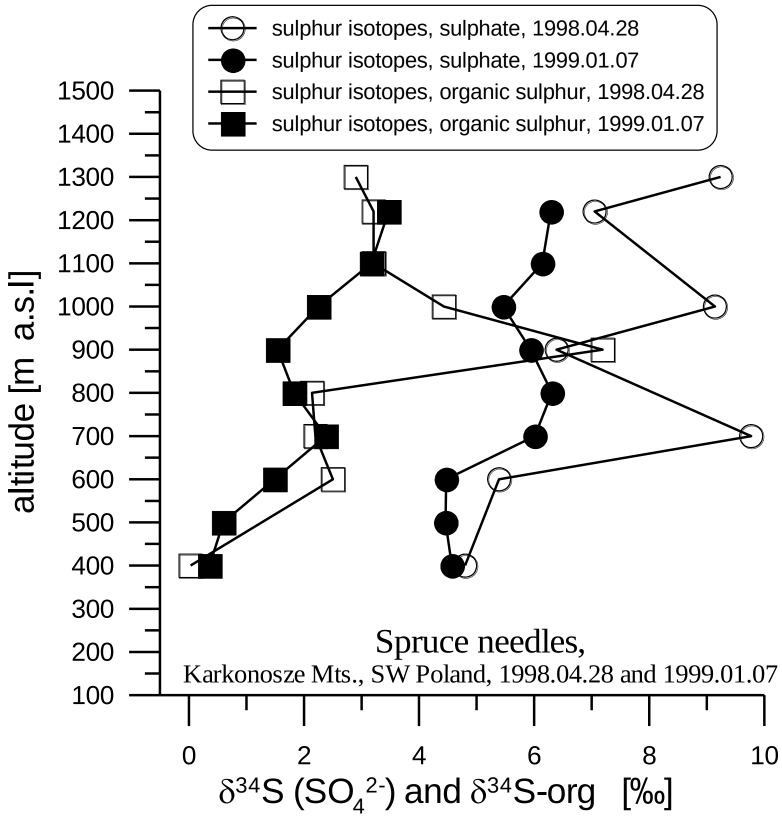
sulphur isotopes, sulphate, 1998.04.28
sulphur isotopes, sulphate, 1999.01.07
sulphur isotopes, organic sulphur, 1998.04.28
sulphur isotopes, organic sulphur, 1999.01.07
altitude [m a.s.l]
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
Spruce needles,
Karkonosze Mts., SW Poland, 1998.04.28 and 1999.01.07
0
2
4
6
8
10
δ34S (SO42-) and δ34S-org [‰]

Fig. 1:

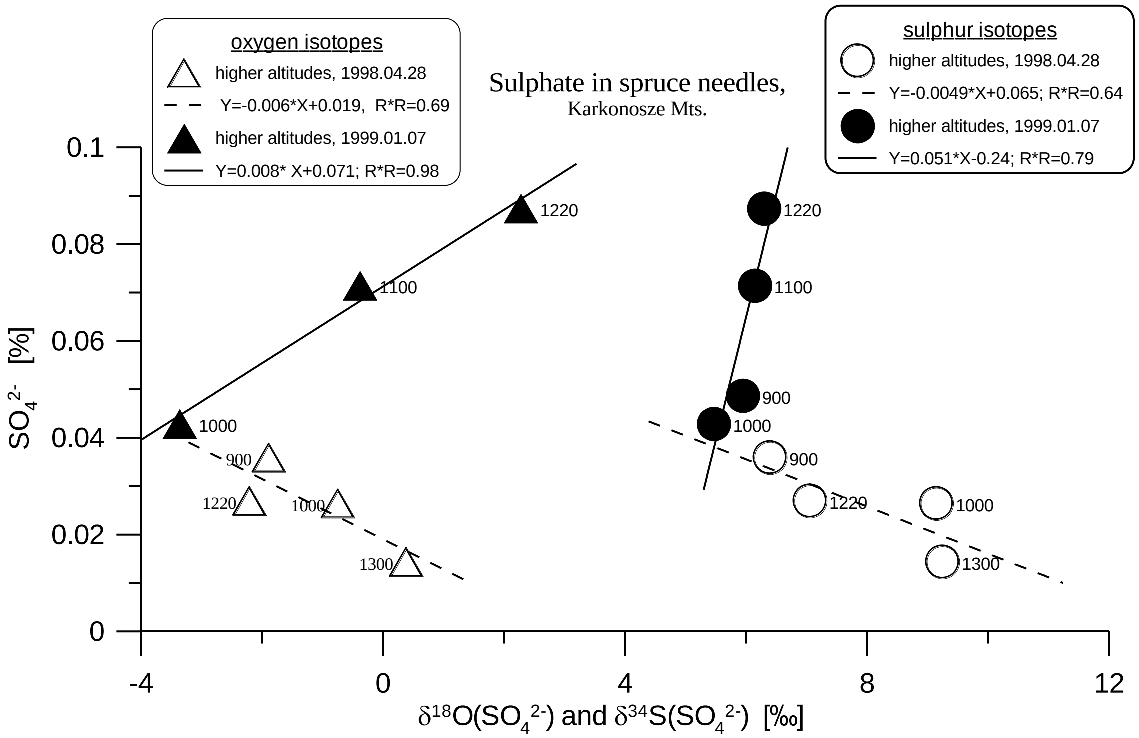
oxygen isotopes
higher altitudes, 1998.04.28
Y=-0.006*X+0.019, R*R=0.69
higher altitudes, 1999.01.07
Y=0.008* X+0.071; R*R=0.98
Sulphate in spruce needles,
Karkonosze Mts.
sulphur isotopes
higher altitudes, 1998.04.28
Y=-0.0049*X+0.065; R*R=0.64
higher altitudes, 1999.01.07
Y=0.051*X-0.24; R*R=0.79
1220
1100
1000
900
1220
1000
1300
1220
1100
900
1000
900
1220
1000
1300
SO42- [%]
0.1
0.08
0.06
0.04
0.02
0
-4
0
4
8
12
δ18O(SO42-) and δ34S(SO42-) [‰]

Fig. 2:

The use of stable isotope in the study of nitrogen cycling in agricultural soils

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The generation of nitrate from agricultural soils into surface waters is becoming an increasing problem as point sources of pollution are being reduced. It is tempting to think that reducing the amounts of fertiliser added to soils will have an immediate effect on surface water nitrate concentrations. There is evidence to suggest that this will not happen. What is required is an understanding of nitrogen cycling in soils so that changes in agricultural practise can be assessed in terms of the movement of nitrogen through the system. By measuring the δ^{15}N of NO_3^--N in parallel with the amounts of inorganic and total nitrogen of soil a great deal of information about the major processes in soil nitrogen cycling can be obtained. To be able to do this with any confidence requires that the appropriate analytical techniques be used. The methodology in measuring δ^{15}N values of NO_3^--N will be discussed in the light of recent developments, with examples of their application to agricultural soils.

Stable Isotopes in Environmental Biotechnology - Chances and Limitations

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In the last decade stable isotope chemistry have received increasing attention in environmental science. The stable isotope signature of organic substances yield useful information to decipher the formation, transport, transformation and final fate of organic substances in the environment. The isotopic composition of mayor elements of organic substances i.e carbon, hydrogen and nitrogen are of interest to solve crucial questions (needs) of modern environmental and biological research. The recent developments in IRM-GC-MS enables us to explore the large potential isotope chemistry in environmental biotechnology. Moreover, stable isotopes labelled tracers may be applied in structural studies with conventional GC-MS and Nuclear Magnetic Resonance spectroscopy. The aim this work is to discuss the potential of new stable isotope approaches in the context of environmental biotechnology.

1) Analysis of isotopic fractionation processes to characterise the biological turnover

This approach is used for the determination mass fluxes and partitioning of organic compounds during biochemical reactions and for the identification of sources and substrates. The degradation of organic substances by micro-organisms often leads to the relative enrichment of the heavier carbon isotopes in the residual substrate fraction. Well know are the large fractionation effects during methane oxidation. Recently, a concept was developed to use isotopic fractionation of dissolved xenobiotics in quasi closed systems to quantify the biodegradation. This concept was applied to characterise the microbial in situ biodegradation within contaminated aquifers using empirically obtained biogenic fractionation factors. The degradation of contaminants can be calculated from the differences of the isotopic signature in the centre of contamination to the margin of the plume. Isotopic fractionation may be a promising tool to estimate the degradation process in the subsurface or in biotechnological processes in comparison to other concentration diminishing processes such as adsorption and dilution. With the improvement of sensitivity of IRM-GC-MS systems, studies of microbial degradation of specific organic compounds in environmental systems became feasible.

2) Application of stable isotope labels in mass balance to trace the fate of organic chemicals

This approach is used for the determination of the fate of a specific compound in environmental compartments and organisms. Stable carbon isotopic measurements have been employed to characterise the transformation of a ^{13}C-labeled polycyclic aromatic hydrocarbon (PAH), anthracene, in closed soil bioreactors. Beside the use of the label for structural studies with GC-MS, the ^{13}C-label was used to calculate the carbon mass balance including the mineralization, the formation of biomass and the formation of non-extractable soil bound residues. The comparison of the mass balance with ^{13}C- and ^{14}C- labelled anthracene in separate batch experiments revealed similar results. The application of stable isotope labels to obtain of mass balance enabled us to trace the speciation of the xenobiotic carbon into specific fractions such as metabolites and biomass quantitatively. This include the incorporation of the xenobiotic carbon into bacterial fatty acid or amino acid fractions. Stable isotopes labelled substrates can be applied to open systems without the typical safety requirements of radio-labelled tracers and thus may be an interesting substitute of radioactive tracers in many biotechnological studies.

Spatial Changes in Redox Conditions and Food Web Relations at Low and High Nutrient Sites in the Everglades

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The isotopic compositions of sediment, plant, insect, and fish samples collected at several hundred sites in the Everglades (USA) show strong spatial patterns on a landscape scale, with little change with species or season. We speculate that biogeochemical processes (such as denitrification, sulfate reduction, respiration, and methane production/oxidation) control the isotopic compositions of dissolved nutrients, and that the local isotopic compositions of biota then reflect those of the nutrients utilized, as modified by trophic fractionations and other factors. In particular, areas dominated by sulfate reduction, which often correlate with high methyl mercury contents, appear to be "labeled" by the C, N, and especially the S isotopic compositions of organisms. The temporal and spatial isotopic patterns caused by environmental conditions must be "subtracted" from the biota isotopic compositions before spatial and temporal changes in trophic relations can be determined.

The isotopic compositions of organisms from areas of high and low nutrient concentrations are very different. We observe the expected increases in $\delta^{13}C$ and $\delta^{15}N$ with increasing trophic level at low-nutrient marsh sites. However, at high-nutrient marsh and canal sites, the $\delta^{13}C$ values consistently decrease with increasing trophic level, producing food web structures that are not consistent with theory. In general, organisms collected in high-nutrient sites near the Everglades Agricultural Area have higher $\delta^{15}N$ values than ones collected in more pristine areas to the south. Near the agricultural areas, organisms in the canals generally have higher $\delta^{15}N$ values than samples from adjacent marshes, and the $\delta^{15}N$ values decrease with distance from the canals. This difference probably reflects denitrification and ammonium uptake in anoxic waters and sediments in stagnant parts of the canals. While is not yet clear what causes the striking isotopic difference between nutrient-impacted sites and more pristine sites, isotopes appear to provide a quick and easy method for determining if high-nutrient areas might be causing significant changes in food web relations.

Stable Isotope Analysis of Intrapopulation, Spatial, and Temporal Variation of Laughing Gull (*Larus atricilla*) Diets in the Virginia Coast Reserve and Jamaica Bay, New York

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In this study, the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values for blood, muscle, feather, and egg membranes were compared to examine temporal, interpopulation, and intrapopulation variation in the diets of Laughing Gulls (*Larus atricilla*) nesting in coastal Virginia and Jamaica Bay, New York. Stable isotopes are a powerful tool for the study of trophic linkages in dynamic coastal estuarine systems. The results reveal that there was a significant amount of intrapopulation variation in the diets of Laughing Gulls from both colonies. In addition, young Virginia Laughing Gulls consumed more foods of marine origin than did young from the New York colony. Results also indicate that young from the Virginia colony consumed proportionately more marine food items than did adult females during the period prior to egg laying. The stable isotopic compositions of blood and muscle from young Virginia Laughing Gulls indicate that there were two distinct feeding strategies employed by the parental gulls. Some young were fed a diet consisting of more estuarine species whereas other young were fed more marine food items. Based on sampling different portions of feathers from prefledged young, a temporal shift to a diet consisting of higher trophic level foods (higher $\delta^{15}N$ and $\delta^{13}C$ values) over time was observed for the young in the Virginia colony. No such temporal diet shift was apparent from feather analyses of the New York colony young. This study provides further insights into how stable isotope analysis can be applied to the study of avian feeding ecology.

Stable Isotope Assessment of Temporal and Geographic Differences in Feeding Ecology of Northern Fur Seals (*Callorhinus ursinus*) and Their Prey

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We investigated the feeding ecology and foraging location of migrating and breeding northern fur seal (*Callorhinus ursinus*) adult females and juvenile males from the Pribilof Islands, Alaska using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope compositions of fur seal skin and whole prey. Pregnant, migratory females had $\delta^{15}N$ values ~0.6 ‰ higher, and $\delta^{13}C$ values ~1.5 ‰ higher than migratory juvenile males. Post-parturient and lactating breeding season females had $\delta^{15}N$ values ~0.8 ‰ higher than pregnant, migratory females, and $\delta^{13}C$ values that were not significantly different. Two opportunistically collected, migrating, nulliparous females had $\delta^{13}C$ values ~1.0 ‰ lower than migrating, pregnant females, and $\delta^{15}N$ values that were not substantially different. Reported $\delta^{15}N$ enrichment values between trophic levels indicate that migrating males, migrating females, and breeding females consumed prey with $\delta^{15}N$ values between ~11.5 ‰ and ~13.5 ‰, ~12.3 ‰ and ~14.3 ‰, and ~13.10 ‰ and ~15.10 ‰, respectively. Isotopic composition of prey suggests that migrating juvenile males, migrating females and breeding season females were foraging on 1-2 year-old walleye pollock (*Theragra chalcogramma*), Pacific herring (*Clupea harengus pallasi*), and squid (*Gonatopsis borealis* and *Barryteuthis magister*); 1-2 year-old walleye pollock, Pacific herring, and eulachon (*Thaleichthys pacificus*); and 1-3 year-old walleye pollock, Pacific herring, and eulachon, respectively. Walleye pollock showed progressive ^{15}N and ^{13}C-enrichments with age, with 3-4 year-old $\delta^{15}N$ values 5.44 ‰, and $\delta^{13}C$ values 1.15 ‰ higher than 0 age pollock. Atka mackerel also showed isotopic enrichment with age. The large fish $\delta^{15}N$ values were 0.76 ‰ higher, and the $\delta^{13}C$ values were 0.33 ‰ higher than those of the small fish. The $\delta^{15}N$ values of the pregnant females indicate they were feeding on more ^{15}N-enriched prey than juvenile males during migration. The higher $\delta^{13}C$ values for the pregnant females further suggest they were feeding in different locations than the males and the nulliparous females during migration, which is likely a result of regional differences in $\delta^{13}C$ organic carbon values forming the base of the different food webs. The higher $\delta^{15}N$ values for post-parturient, lactating females over migrating, pregnant females point to either a prey ^{15}N-enrichment over time, or a more likely ^{15}N-enrichment due to the nutritional stress of lactation and the feeding/fasting regime of breeding females. The similar $\delta^{13}C$ values for migrating and breeding season females indicate that migration and breeding season feeding locations have organic carbon with similar $\delta^{13}C$ values at the base of the food webs in each location. $\delta^{15}N$ values observed to be similar for nulliparous and pregnant females imply that they are feeding at similar trophic levels despite indications of feeding in separate ecosystems during migration.

Isotopic evidence for the evolutionary paleoecology of fossil horses (Family *Equidae*) from North America

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The 55-million-year fossil record of horses (Family *Equidae*) is a classic example of macroevolution and paleoecology. Since the late 19th century the evolution of fossil horse teeth from primitive short-crowned browsers to advanced high-crowned grazers during the Miocene has been interpreted as an adaptive dietary shift related to the spread of grasslands. This classic hypothesis can be tested using carbon isotopes because of the different photosynthetic pathways comprising C_3 browse versus C_4 grasses incorporated into extinct herbivore diets. Carbon isotopic values of tooth enamel indicate that grazing horses during the middle Miocene were feeding principally on C_3 grasses (tooth $\delta^{13}C < -10$ per mil = plant $\delta^{13}C$ of ca. -24 per mil). The shift to grazing principally on C_4 grasses (tooth $\delta^{13}C$ of ca. 0 per mil = plant $\delta^{13}C$ of ca. -14 per mil) occurred during the late Miocene ca. 7 million years ago.

Six sympatric latest Miocene (5 million year old) horse species from Florida all had high-crowned teeth suggesting grazing, yet ecological theory would predict interspecific dietary differences. Carbon isotopes and enamel microwear together indicate that these Florida horses ranged from browsers, to mixed feeders, to almost pure C_4 grazers depending upon the species. Despite some browsing and/or mixed diets in these Florida horses, the presence of high-crowned teeth seems related to the grazing morphotype that evolved during the middle Miocene, ca. 10 million years earlier (ca. 15 million years ago).

In conjunction with morphological data, stable isotopes offer extraordinary potential to understand the evolutionary paleoecology of fossil groups. Up to now, most of the combined isotopic and paleontological studies of fossil mammals have centered on macroevolutionary problems, i.e., between or among species. In future studies new techniques, such as serial analyses of teeth that reveal monthly isotopic archives, have the potential to provide valuable new insights on microevolution, i.e., the population biology within extinct species.

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Tracing animal migration patterns with online hydrogen isotope measurements - Butterfly, Whale and Bat studies

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At the inaugural Ecology 1998 conference in Saskatoon a new method for the online measurement of hydrogen isotopes in biological samples was introduced to the isotope community by MICROMASS. The method involved the pyrolysis of biological samples at a temperature of 1070 °C in a helium stream. The product gases, hydrogen and carbon monoxide were separated by a packed GC column prior to the analysis of hydrogen isotopes. A MICROMASS ISOPRIME IRMS with a unique electrostatic filter incorporated into the detector array was used to perform the measurements.

The technique was applied successfully to the monitoring of monarch butterfly migration patterns by measurement of the D/H ratio of the butterfly wing. Samples were taken from three specific sites in North America, Florida, Indianapolis and Manitoba. These sites are clearly separated in latitude. The results showed that the D/H ratio of the butterfly wing is strongly correlated to the H/D ratio of the local precipitation at the latitude of the larval growth site.

The work presented here extends the application of on line pyrolysis -IRMS into two further animal migration studies. The Mexican long nosed bat (*Leptonycteris nivalis*) and the bowhead whale, (*Balaena mysticetus*).

The range of the Mexican long-nosed bat extends from the latitude of Mexico City, north to the border with the USA. It is also bounded by the Sierra Madre Oriental to the east and the Sierra Madre Occidental to the west. Rapid expansion of the human population in that region is destroying its habitat, and it is currently an endangered species. $\delta^{13}C$ and $\delta^{15}N$ values have shown that this species feeds almost exclusively on nectar and pollen from CAM Agave species. We took monthly hair samples from a bat population that utilizes a cave in the northern portion of its migratory range (near Monterrey) from April through October and analysed them for δD. Mean monthly δD values for the population (approx. -44 ‰) varied little from the time the bats arrived at the cave in April until they departed for the southern portion of their range in late October. Because isolines for rainfall δD are oriented largely north-south in Mexico, we suggest tentatively that this population of *L. nivalis* migrates north-south along a relatively narrow longitudinal range within which rainfall δD varies little.

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This migratory pattern is consistent with the distribution of the Agave species they depend on for their diet.

Previous measurements of naturally occurring stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) along the baleen plates of both western Arctic and eastern Arctic bowhead whales have been used to establish the nutritional ecology and growth rates of these animals as they migrate annually between isotopically different foodwebs. The use of naturally occurring hydrogen isotopes (δD) were investigated as a potential tool to independently evaluate the carbon and nitrogen measurements. Samples taken at regular intervals (2.5 cm) along the baleen were analysed for δD. The results show clear cyclical behaviour as the western animal migrates between waters influenced by freshwater flow from the Mackenzie Delta and more marine waters of the Bering Sea. The eastern Arctic animal similarly showed cycles in δD and $\delta^{15}N$ but not in $\delta^{13}C$ measurements. We suggest that seasonal fasting, in addition to geographical movements, may be responsible for observed isotopic patterns in baleen of bowhead whales.

Aspects of the analysis such as the type and make up of the pyrolysis reactor, the reactor temperature , analytical characteristics, the results of the δD. analysis and how they relate to the migration characteristics of all 3 animals, will be discussed.

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Natural abundances of $\delta^{13}C$ and $\delta^{15}N$ reveal potential differences in feeding strategy among populations of *Xiphinema diversicaudatum* (Nematoda: Longidoridae)

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$^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) natural abundances from four geographically disparate populations (Germany, New Zealand, Scotland and Switzerland) of the longidorid nematode, Xiphinema diversicaudatum, were analysed to determine whether whole body $\delta^{13}C$ and $\delta^{15}N$ would alter when transferred from one plant host to another. A priori assumptions were that the populations of *X. diversicaudatum* would be isotopically similar based on $\delta^{13}C$ (similar ^{13}C sources from new plant host) and $\delta^{15}N$ (identical feeding strategy) values.

With the exception of the population from Germany, all *X. diversicaudatum* populations, after feeding for 28 days on a new plant host, *Petunia hybrida*, were *c.* 1.5 ‰ more ^{13}C-depleted compared with nematodes from the original plant hosts. As with previously published data for different *Xiphinema* species, whole body $\delta^{15}N$ values were more ^{15}N-enriched compared with those taken from the original plant host. However, nematodes from the German population were *c.* 1.5 ‰ less ^{15}N-enriched than specimens from the other three populations.

The only known biological difference between each of the four *X. diversicaudatum* populations, apart from their geographic location, was the cropping of the source sites. The German population originated from an ancient Roman viticulture site where grapevines were grown on shale to the virtual exclusion of other plants. The other populations originated from arable, soft fruit or ornamental sites, at each of which a range of weed species were present. Exposure to a wide range of alternative host plants may have led these populations to evolve an adaptive polyphagous feeding strategy, in contrast to a more selective strategy adopted by the German population.

Nutrient allocation to reproduction in *Lepidoptera*: larval vs. adult origins of egg amino acids

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Reproductive resource allocation is complex and poorly understood. Here we describe a simple method that separates larval from adult dietary contributions to reproductive output in holometabolous insects. We fed female *Lepidoptera* nectar diets distinct in $\delta^{13}C$ from larval hostplant, and traced the allocation of dietary carbon into eggs. From the resultant pattern of carbon incorporation we propose a general model of nutrient allocation in *Lepidoptera*. The model identifies two classes of allocated nutrients: a class that exhibits isotopic turnover with adult diet (I), and a class that retains a larval signature throughout the adult lifetime (II). A fixed contribution of class II nutrients reflects constraints upon the availability or synthesis of specific nutrients from the adult nectar diet. We use compound-specific stable isotope analysis (GC - IRMS) to determine whether amino acids follow the predictions of the model. Dispensable amino acids showed substantial synthesis from the nectar diet (class I), whereas indispensable (i.e. essential) amino acids derived entirely from the larval diet (class II). The proportion of dispensable amino acid carbon deriving from the nectar diet varied consistently among species from 30% to 100%, suggesting that the processes determining nutrient allocation are very general. Our study highlights the power of using stable isotopes in concert with mathematical models to elucidate basic mechanisms in physiology.

A stable carbon and nitrogen isotopic study of biota recovery from a sewage contaminated near-shore marine site at Moa Point, New Zealand

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Stable carbon and nitrogen isotopes have been used to monitor the recovery of some flora and fauna after closure of a sewage outfall, discharging milli-screened effluent into a near-shore site at Moa Point Bay, New Zealand. An initial study characterising the extent of the discharge and the effects on seaweed, blue mussels and limpets. The immediate area surrounding the discharge area was found to contain limited biodiversity, with an abundance of *Ulva lactuca*, a bright green lettuce-like seaweed, typically found in areas with high nutrient input. The nitrogen ($^{15}N/^{14}N$) ratio is shown to be a good tracer of sewage pollution in seaweed and associated grazers (*Cellana denticulata*) as a result of the increased contribution of urea and ammonia to the surrounding marine environment from the sewage outfall. The carbon ($^{13}C/^{12}C$) ratio is suggested as a more appropriate sewage tracer for mussels (*Mytilus galloprovinialis*), which filter feed the particulate organic matter from the effluent. Lower carbon:nitrogen ratios were found in *Ulva lactuca* sampled from the outfall region, compared to uncontaminated control sites, however carbon:nitrogen ratios do not vary significantly amongst shellfish species.

After closure, monitoring continued for 9 months and showed that the carbon and nitrogen isotopic signatures of algae (*Ulva lactuca* L.) returned to similar control site levels within 3-6 months. Limpet and blue mussels (*Cellana denticulata* and *Mytilus galloprovinialis*) showed slower recovery times, with the isotopic signature remaining in the animal's tissue for up to 9 months.

Carbon and Nitrogen Isotopes Trace Nutrient Exchange in an Ant-Plant Mutualism

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Mutualisms, biological interactions from which each species benefits, are thought to be evolutionarily stable only under a limited set of circumstances. Underlying all mutualisms is an intrinsic conflict between the parties, in that each is under selection to further exploit the other. Conflict between mutualists will lead to instability unless each species receives a net benefit from the interaction. To understand how mutualisms persist, then, it is essential to document the exact nature of the interaction. The relationship between Cecropia trees and Azteca ants has been a model for the study of mutualism since its description more than 100 years ago. Ants live in Cecropia's hollow stems and harvest specialized food bodies produced by the host. In return, ants defend the plant from leaf-feeding herbivores and encroaching vegetation. Central to modeling this relationship is understanding the exchange of nutrients between symbionts. Here we present evidence that the flow of nutrients has been misjudged. Although ants consume plant products, they provide more nutrients than they receive. Using stable isotope analysis, we calculate that only about 18% of worker ant carbon is derived from Cecropia, whereas 93% of the nitrogen in ant-occupied host plants is derived from debris deposited by ants. Ants rely on sources other than their host for food and, in doing so, make a rich source of nitrogen available to the host.

Canopy gradients in $\delta^{18}O$ of water vapour, leaf water and organic matter as ecophysiological tool

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The microclimatic conditions in forests and in grasslands differ at different heights above ground, caused on the one hand by the structure of the vegetation, which influences light regime and turbulence, and on the other hand by biological activity, namely photosynthesis and transpiration. Understanding these gradients is important for understanding plant productivity and ecosystem function. Isotopes can be an important tool in this respect because different sources of water vapour and CO_2 are isotopically distinct, in particular transpiration and evaporation as sources of water vapour and plant and soil respiration as sources of CO_2. Therefore, isotope studies might help to disentangle the complex mixing processes within the canopy.

In this study, we present oxygen isotope data of different compartments of a forest (a *Fagus* stand) and a grassland ecosystem (Mt. Bondone, Italy, 1520 m a.s.l.). The $\delta^{18}O$ of water vapour is increasing with height in the forest, whereby the values range from -14 ‰ near the soil to -12 ‰ in the upper canopy. In contrast, we find a decreasing trend with height (0-2 m above ground) for a grassland in the same area. Accordingly, the slopes of the "Keeling plot" (relating $\delta^{18}O$ of water vapour to the inverse of the vapour concentration) have a different sign for the two ecosystem types. A positive slope is found for the forest, indicating soil evaporation as the dominant source for ambient water vapour [1], whereas the slope is negative for the grassland, indicating transpiration as the dominant source. Further, $\delta^{18}O$ gradients with height for leaf water and organic matter were determined. The oxygen isotope composition of organic matter, measured with an on-line pyrolysis method [2], is proposed as a potential new tool for determining the long-term microclimatic conditions within the canopy.

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Hindcasting Carrying Capacity of the Bering Sea: An Extended Record from Carbon Isotope Ratios in Sea Birds and Marine Mammals

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Recent work on the interrelationships between carbon isotope ratios in marine phytoplankton and cell growth rates has shown that a close direct relationship exists that is consistent over many sites in the world oceans. This implies that if sequential measurements of isotope ratios from a region can be compared, the relative rates of primary production can be inferred. Since carbon isotope ratios are essentially conservative in consumers, animal tissues can be used as a proxy. This study compares the long-term isotope records from sea birds, primarily Thick-billed Murres, (*Uria lomvia*) with those from bowhead whales (*Balaena mysticetus*). By using baleen plates from 37 whales archived at the Los Angeles County Museum and recently taken by native hunters, an isotopic record was constructed that extends from 1947 to 1998. From this, we infer that seasonal primary productivity in the Bering Sea was at a higher rate over the period 1947-1966 and then underwent a general decline that continues to the most recent samples (1998). We have compared feather and muscle tissues from murres with the whale isotope ratios and find a very close correlation that will allow extending the record further back in time. Bird tissues, as expected, are more variable due to greater mobility and flexibility in diet but average isotope ratios are remarkably similar to those of the whales. Assuming a close similarity to the published relationships between primary productivity and carbon isotope ratios, the whale data suggest a loss of 35-40 percent of the seasonal primary productivity and hence carrying capacity of the northern Bering Sea that existed 35 years ago. Whale baleen from archaeological sites and current work on archived birds in museum collections will extend the record back into the 1800s.

Intramolecular deuterium distributions link biochemistry, physiology and climate research

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How do organisms (e.g. C_3 plants) and environmental conditions (e.g. increasing CO_2 concentrations, changing climates) interact? In palaeoclimatology, δD(cellulose) is used to estimate δD(precipitation); this assumes that biochemical isotope discriminations are constant under all environmental conditions. How can we improve on this?

Biochemical reactions influence the isotope abundance of individual intramolecular positions of metabolites. As a result, intramolecular isotope distributions are non-random. For D, the intramolecular variation can be ten-fold larger than the variation in δD(precipitation), which carries climatic information. Biochemical information is encoded in, and can be extracted from, intramolecular deuterium distributions (IDDs), independent of δD(precipitation). δD(metabolite) is influenced by δD(precipitation) and by biochemical D discriminations, and must therefore be regarded as an average of the IDD. Because of the influence of biochemical D discriminations, δD(metabolite) and the IDD are needed to unambiguously extract climatic information from δD(metabolite).

Intramolecular isotope distributions can be measured using IRMS, but that in general requires cumbersome breakdown reactions to measure the isotope abundance of each position separately. In contrast, the complete IDD of a metabolite can be measured on one sample using nuclear magnetic resonance.

Metabolite molecules synthesized in different metabolic pathways encounter enzymes with different isotope discriminations. If the difference is substantial, the observed averaged IDDs can be used to derive the ratio of the metabolic fluxes through the pathways: (i) Glucose formed by C_3 and C_4 metabolisms differs in IDD, which therefore is a potential measure of C_3/C_4 species composition. (ii) We interpret a dependence of the IDD of glucose of C_3 plants on CO_2 concentration as a measure of the ratio of photorespiration to photosynthesis. This may be used to examine climate/plant interactions, in this case the "CO_2 fertilization effect" or long-term adaptations of plants to increasing atmospheric CO_2 concentrations.

Influence of reduced SO_2 input on natural isotope variations of sulfur and oxygen in various compartments of Scots pine stands in eastern Germany

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The natural isotope composition ($\delta^{34}S$ and $\delta^{18}O$ values) and the concentrations of total sulfur (S_t), organic sulfur (S_{org}) and sulfate sulfur (SO_4^{2-}-S) were determined between 1991 and 1999 in needles and humus layers from adult Scots pine stands at three sites (Neuglobsow, Taura, Rösa). Isotope measurements on aqueous SO_4^{2-}-S were complemented by $\delta^{34}S$ and $\delta^{18}O$ analyses on atmospheric SO_2 and SO_4^{2-}-S in bark, which reflects atmospheric inputs of throughfall, in order to identify S sources. The sites differed markedly in both, the mean SO_2 input (Neuglobsow < Taura < Rösa) and the annual mean throughfall rates of SO_4^{2-}-S. Accordingly, the SO_4^{2-}-S concentrations in needles and humus layers showed significant site-dependent differences and temporal variations in 1993 and 1997 became visible. With decreasing atmospheric inputs the concentrations of inorganic SO_4^{2-}-S significantly decreased in current year needles, while the concentrations of S_{org} increased slightly, but significantly. The achieved results indicate that the observed changes in inorganic and organic S compounds in pine needles are caused by a shift from oxidative to reductive S detoxification, depending on stomatal SO_2 uptake and/or SO_4^{2-}-S uptake via the roots. Still, the observed correlation between SO_4^{2-}-S in the needles and in the humus layer left two questions open: (1) What proportion of the accumulated SO_4^{2-}-S in the needles derived from stomatal S uptake, and (2) Which is the major source of SO_4^{2-}-S in the soil?

The natural isotope variations of S and O in both compartments were analysed to aim a better understanding of the fate of atmospheric deposition and to obtain further information on the physiological processes in Scots pines as well as on the mineralization process in the humus layer. In all three areas $\delta^{34}S$ values for SO_4^{2-}-S in the humus layer were generally found to be similar to the mean $\delta^{34}S$ values of bark SO_4^{2-}-S (range of +3.6 to +6.0 ‰) indicating, that the atmospheric deposition rather than mineral weathering is the major source of this S. However Taura seems to be subjected to a S source different from the ones participating in S supply at Neuglobsow and Rösa, as in both years (1993 and 1997) the mean $\delta^{34}S$ value of aqueous SO_4^{2-}-S in bark from Taura was about 1.5 ‰ higher than at the other locations.

Comparison of the $\delta^{18}O$ values of humus layer SO_4^{2-}-S and SO_4^{2-}-S from atmospheric deposition showed that SO_4^{2-}-S in the humus layer was depleted by several per mil (+4.5 to +8.6 ‰ resp.). These results clearly indicate that SO_4^{2-}-S in the humus layer does not only originate from S deposition, but a considerable proportion also derived from mineralization processes.

Pine needles showed between +3.5 to +9.3 ‰ for S_t, +7.0 to +12.3 ‰ for SO_4^{2-}-S and +2.8 to +8.7 ‰ for S_{org}, and $\delta^{18}O$ values between -1.0 to +2.5 ‰ were observed. Together with the knowledge that both $\delta^{18}O$ and $\delta^{34}S$ values of SO_4^{2-}-S in pine needles are increasing since 1993, these results allow the conclusion that changes in the S metabolism of the needles as well as in the SO_4^{2-}-S uptake via roots are present. Based on these results, S uptake rates are calculated and time dependent changes in S concentrations and S and O isotope composition will be discussed.

Aquatic Food Web Construction of the Colorado River Through Grand Canyon Using Dual Stable Isotopes (^{13}C and ^{15}N)

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We collected benthic primary producers, consumers, and fish samples from pool, cobble and drift habitats seasonally for three years within a 370 km reach of the Colorado River below Glen Canyon Dam (GCD). The aquatic community is structured by consistently cool (10 °C) and clear releases from GCD, while tributaries create seasonally turbid conditions. Tributary or watershed influence was detected by a 5.4 ‰ enrichment of δ^{13}C algal values between GCD and down river sites. A similar pattern was observed in δ^{15}N algal values that were enrich 2.9 ‰ with distance from GCD. This isotopic gradient in δ^{13}C was carried through the macroinvertebrates and fish, but the δ^{15}N data was more variable for these consumers. Juvenile fish, both native and alien, consumed aquatic macroinvertebrates whereas adult fish were piscivorous. Predacious alien brown trout had δ^{15}N values of 15.9 ‰, while the average macroinvertebrate sample had δ^{15}N values of 9.1 ‰, indicating too large of a trophic step to be feeding primarily on macroinvertebrates. Terrestrial insects were routinely found in the gut contents of fish but did not provide a significant amount of carbon (~16 %) or nitrogen (~7 %) according to mixing models. Stable isotope techniques provides information about the Colorado River recovery from the impoundment of GCD through influence of tributaries and their effects on the food web. This data set also indicates the importance of maintaining adequate benthic biomass for young fish, both endangered native and alien sport fish, through moderate operations of GCD.

Trophic position of southern California estuarine and island populations of the silverside fish *Atherinops affinis* (Teleostei: *Atherinopsidae*): Analyses of ^{15}N and ^{13}C stable isotopes and dietary items.

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The silverside fish *Atherinops affinis* (Teleostei: *Atherinopsidae*) is an abundant fish species of the eastern north Pacific and an important component of coastal and estuarine food webs. The feeding strategy of *A. affinis* appears to be one of an opportunistic herbivore; prior dietary studies have noted differences in diet based principally on habitat but also on season and fish age. We sought to clarify exact role of *A. affinis* in the southern California marine food web during one summer by comparing the trophic position of estuarine and island populations though analyses of stable isotopes (^{15}N and ^{13}C) and dietary items. Dietary analysis has traditionally been used to determine an animal's trophic level but has limitations due to differential digestibility of food items. We combined traditional dietary analysis with stable isotope analysis to obtain a more complete picture of *A. affinis* feeding. ^{15}N indicates trophic position, and ^{13}C can be traced to dietary origins. By examining these stable isotopes in both muscle and liver tissues, which have different turnover rates, we were able to gain a temporal insight into feeding by first-year topsmelt during one summer season. During summer months, *A. affinis* comprise a large portion of various sea bird diets, and, therefore, play an important role in the flow of energy, nutrients, and contaminants through marine communities.

Corals are polytrophic and opportunistic feeders

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Coral reefs thrive in nutrient-poor tropical waters through the continual and efficient recycling of essential nutrients by the many species that inhabit this complex and diverse ecosystem. Although corals are simple, bottom-dwelling animals, the cells of reef-building corals contain symbiotic algae (= zooxanthellae). These zooxanthellae produce organic matter through photosynthesis and transport much of this material to their coral host animal. The success of corals in low-nutrient waters is due largely to the variety of modes that corals utilize to obtain nutrition. The coral animal has two primary feeding modes: capture of zooplankton by polyps (heterotrophic process) and receipt of translocated photosynthetic products (autotrophic process) from the symbiotic zooxanthellae.

Stable isotope (C and N) analysis of corals and their symbiotic zooxanthellae indicate the different feeding strategies of particular species. The two reef-building coral species, *Acropora palmata* and *Montastrea annularis*, rely on resident zooxanthellae for providing photosynthetically produced organic material for growth. Compound specific isotope analysis of these species suggests the direct transport of fatty acids between the zooxanthellae and the coral host. In contrast, the feeding habits of a neighboring hermatypic species, *Porites asteroides,* differ substantially. The coral animal obtains most of its nutrition through holozoic feeding and obtains little organic matter from its symbiont. Stable isotope analyses of corals provides much needed information on the role that symbiotic zooxanthellae play in the nutrition and overall health of coral species.

Information about allocation of stored organic carbon to leaf growth and autotrophic resource use in tropical trees from natural abundances of stable isotopes

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Allocation patterns of photosynthates are of fundamental interest in ecology but have been difficult to ascertain in the field. Organic reserves contribute greatly to early leaf growth. Studies where ends of growing seasons are dry suggest that $\delta^{13}C$ values of early-season leaves reflect p_i/p_a (ratio of internal and atmospheric CO_2 pressures) and WUE (water-use efficiency) during the previous growing season, rather those of the leaves during their expansion. We obtained $\delta^{13}C$ values of newly emerging to senescing leaves produced during the middle of rainy season of four tree species in Panamá. New leaves that were produced when p_i/p_a was highest exhibited ^{13}C enrichment indicative of low p_i/p_a during the dry season yet it is unlikely that their stored organic substrate for growth was produced then. Furthermore, stored carbon alone did not account for the ^{13}C enrichment during early leaf construction, because $\delta^{13}C$ values of newly emerging leaves exceeded those of starch and mobile organic fractions in storage. A coarse, mass balance approach can estimate the proportional investment of organic reserves for leaf growth and improve estimates of p_i/p_a during more autotrophic developmental stages. Finally, these early effects were not diluted by later, more autotrophic growth as suggested by a tendency for p_i/p_a values to be underestimated in all leaves, regardless of age. δD values of leaf cellulose nitrate showed similar age trends. Quantification of isotopic effects associated with heterotrophic leaf growth has powerful ecological potential and may also permit more accurate standard inferences from isotopic analyses.

Omnivory and Migration of the Whooping Crane

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In the middle of the twentieth century the whooping crane (*Grus americana*) had declined to one population of thirty-four individuals that migrated between the Aransas refuge in Texas, USA and Wood Buffalo National Park in the Northwest Territories of Canada. The estimates of whooping crane numbers in the previous century range from hundreds to thousands of individuals from several migratory and nonmigratory populations. Utilizing contour feathers of whooping cranes from twelve museum collections we have documented the range of carbon and nitrogen isotopes in historic populations. The $\delta^{13}C$ and $\delta^{15}N$ of historic whooping crane contour feathers span nearly the entire range of isotopic values observed in terrestrial ecosystems, and document the prevalence of C-4 breeding environments prior to 1900. Both herbivorous and omnivorous trophic strategies are recorded at the historic breeding grounds in the isotopic values in feathers. Individual amino acid $\delta^{13}C$ values reflect the bulk isotopic values, but differ in the isotopic enrichment in glycine as a function of trophic level. Finally, the isotopic values from these historic museum collections will be compared to data from both sandhill cranes and whooping cranes that are part of the captive breeding program at the Patuxent Wildlife Research Center in Laurel, Maryland, USA.

Stable isotope signatures of lotic invertebrates: Sources of variation and significant differences

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SIA has usually been applied to freshwater food webs to characterise the mean isotopic enrichment or signature of different populations or taxa in a particular system at a particular time; quantitative and statistical analyses for hypothesis testing are less common, and little attention has focused on the ecological significance of the within-population variation in isotope signature. Nevertheless, numerical analyses are required in order to fully exploit this valuable tool. The success of such analyses will be restricted, in part by error and variation in the data, thus sources and magnitude of variation need to be identified and careful consideration given to the number of replicates required to test hypotheses.

Through measurement of the carbon and nitrogen isotopic signatures for eight populations of lotic invertebrate in a subset of a stream food web, we consider the two main sources of variation associated with estimates of the mean isotope signature: variation among individuals within the population, and variation that arises from analysis by CF-IRMS. We examine how sample replicates number offsets each of these sources of variation, and through quantifying the minimum detectable difference among pairs of species, examine how our ability to detect statistically significant differences among species changes with replication and the number of individual animals combined in each replicate. Such an exercise can provide guidance on how to make the most efficient use of resources in future SIA studies of trophic interactions.

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An exploratory survey of $\delta^{13}C$ trends in *Acacia* wood from Western Australia to determine recent, human-influenced climate change

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Shifts in land use have occurred during the last century in the Outback of Central Australia, with Aboriginal land use until circa 1930s, succeeded by European cattle ranching until circa 1985. Many stations formerly managed by Europeans have subsequently returned to Aboriginal ownership. To examine whether these recent environmental changes influenced the ecophysiology of the region we examined cross sections from isolated trunks of Acacia that surround Lake Gregory, Western Australia. Carbon isotopic discrimination ($\delta^{13}C$) in wood can be used to study past variations in the ecophysiology of species in reaction to environmental variations, in addition to providing a proxy of past environments. Major factors influencing recent $\delta^{13}C$ in plants include i) changes in water availability, ii) increasing atmospheric CO_2 as a result of anthropogenic input, iii) changes in soil salinity and iv) ontogeny. Low frequency $\delta^{13}C$ trends from 7 trunks of *Acacia* (~5 - 12 cm) show significant enrichments (3 - 5 ‰) in ^{13}C during tree growth, which is common between trees from a similar locality and between trees from different localities. Precipitation records for the region do not support a climate-related influence to account for the low frequency ^{13}C enrichment in *Acacia*. Enrichment in ^{13}C of Acacia could be the result of ontogenetically-related mechanisms, although not related to canopy effects given the isolated nature of the *Acacia* specimens examined. Higher frequency excursions of 1-2 ‰, both positive and negative, from this low frequency trend are also observed. In age-matched wood samples, these excursions may be related to monsoon-effected flooding of Lake Greogry (e.g., 1993-1994). Stochastic flooding of Late Gregory, a briny terminal lake, is considered as a source of both salt and water to *Acacia* roots and soils surrounding the lake. Increased precipitation during strong monsoon periods should result in depleted ^{13}C (i.e. water availability), whereas increased soil salinity would lead to subsequent ^{13}C enrichment. Tree ring data will be presented with respect to these two balancing physiological mechanisms. Physiological changes in *Acacia*, as evident from long and short term variations in the $\delta^{13}C$ of wood samples, will be related to shifts in land use that occurred in the Lake Gregory area in addition to regional climatic trends.

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Ecosystem gas exchange: the isotopic perspective

Dan Yakir - Keynote Speaker

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Dan Yakir is currently at the Department of Environmental Sciences and Energy Research, Weizmann Institute of Science (http://www.weizmann.ac.il/ESER/), Rehovot, Israel. His research group applies and develops stable isotope methods to study plant, soil and ecosystem responses to climatic and environmental change. Current research focuses on the ^{18}O signal in CO_2 as a tracer of biophysical processes at cellular through the organism to ecosystem and global scales. Dan will be giving an overview of the current potential of stable isotopes measurements together with concentration and flux measurements of CO_2 and water vapor. For such applications it is critical to know the isotopic identity of specific ecosystem components, such as the isotopic composition of CO_2, organic matter, water and water vapor, as well as the associated isotopic fractionations in the soil-plant-atmosphere system. This knowledge, combined with stable isotope and concentration measurements, allows estimation of the contribution of specific ecosystem components to exchange fluxes with the atmosphere. This approach was used to separate net CO_2 exchange into photosynthetic and soil respiration components and evapotranspiration flux into soil evaporation and leaf transpiration.

Seasonal and diel change in the sources of organic matter in a freshwater, tidal wetland

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A study of the isotopic composition of organic matter was conducted in a tidal freshwater marsh over seasonal and diel time scales to determine the sources of dissolved organic matter (DOM) and the processes leading to its formation. Bulk carbon isotopic composition of particulate organic matter (POM; 0.7 - 10 µm) ranged from -35 ‰ in winter to -25 ‰ in spring. Changes in the carbon isotopic composition of DOM were more significant over daily time scales where values ranged from -28 to -22 ‰ following changes in chlorophyll a concentrations. The carbon isotopic composition of individual amino acids in DOM and its potential sources revealed diel and seasonal change in the contribution of phytoplankton and bacteria to the DOM pool. Diel changes in the carbon isotopic composition of phenylalanine, synthesized by common pathways in phytoplankton and bacteria, were similar in both DOM and POM. The diel change in carbon isotopic composition of isoleucine, synthesized through different pathways in phytoplankton and bacteria, were distinctly different in DOM versus POM. This disparity indicated a decoupling of the POM and DOM pools which suggests a larger source of bacterial organic matter at night. The isotopic composition of individual amino acids in DOM were more closely linked to both phytoplankton and bacteria during the summer. In both fall and spring the isotopic composition of the amino acid pool of DOM suggested it was not a direct result of either bacterial or phytoplankton production but more likely the result of diagenetic processes.

ABSTRACTS OF

POSTERS

Using isotope ratio mass spectrometry to identify the degraders of toxic oleic acid in an anaerobic digester

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Triglycerides comprise up to 65 % of meat industry wastes and are problematic in anaerobic digesters. With its more than 80 million sheep this problem is very important in New Zealand which is a large producer of sheep tallow. Anaerobic degradation of triglycerides is achieved by the concerted action of hydrolytic fermentative, syntrophic acetogenic and methanogenic bacteria. High levels of free fatty acids can destabilise anaerobic digesters due to inhibition of syntrophic acetogenic and methanogenic bacteria. Especially, the unsaturated fatty acid oleic acid is bacteriotoxic. Now a microbial consortium was found which can deal with oleic acid. To identify the bacteria responsible for the degradation of oleic acid, ^{13}C-labelled triolein, tripalmitin, palmitic acid and glycerol were added in parallel to the consortium and the incorporation of ^{13}C of the substrate into the fatty acids of the phospholipids was determined by isotope ratio mass spectrometry. The label of ^{13}C-oleic acid from triolein reached its maximum after 8 h and returned to background level after 48 h. Here palmitic acid was labelled but not stearic acid giving clues for the degradation pathway of oleic acid. Essential the same was found with tripalmitin. With glycerol, however odd-numbered and branched fatty acids were labelled but ^{13}C was not enriched in stearic acid. Furthermore, the label remained more or less constant for 48 h. The results showed that different types of bacteria are involved in the degradation of oleic acid, stearic acid and glycerol. The transient labelling with the fatty acids can best be explained by their degradation by a group of *Clostridium* species forming spores after the consumption of the substrate. The pattern of fatty acids labelled by ^{13}C-glycerol is characteristic for Gram positive bacteria which did not change much during the experiment. With these results in hand a strategy can be developed for monitoring and optimising anaerobic digesters.

Food resources and niche separation in Lumbricidae, Diplopoda and Collembola of an arable field: evidence from stable isotope analysis (delta ^{13}C, delta ^{15}N)

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To analyse food resources and niche separation between different saprophagous/microphytophagous soil invertebrate species the natural abundances of the stable isotopes of nitrogen (delta ^{15}N) and carbon (delta ^{13}C) were measured in an agricultural food web. Three earthworm (*Lumbricidae*) species, three diplopod (*Diplopoda*) species, two collembolan (*Collembola*) species and enchytraeids from an unploughed winter rye field (precrop winter wheat) near Göttingen (Germany) were analysed.

Soil fauna was extracted from five replicate soil cores (diameter 21 cm) taken at each of five plots (2 m^2) in April 1999. In addition, fresh rye plants (roots and shoots) and winter wheat litter were collected from each plot. ^{13}C and ^{15}N content of the animal and plant materials were determined by a gas isotope mass spectrometer.

Rye plants and winter wheat litter had a delta ^{15}N value of 4,3 ‰ and -2,6 ‰, respectively. Delta ^{15}N values of the earthworm species decreased in the order *Aporrectodea rosea* (6,5 ‰ ± 0,4) > *Aporrectodea caliginosa* (5,2 ‰ ± 0,2) > *Lumbricus terrestris* (3,1‰ ± 0,5). The three earthworm species were separated clearly by their delta ^{15}N and delta ^{13}C values indicating utilization of different food resources. In contrast, *Aporrectodea caliginosa* and the enchytraeids, sharing nearly the same delta values, presumably used very similiar food resources.

The diplopod species were also separated by their delta ^{13}C and delta ^{15}N values with those of *Brachyiulus pusillus* being significantly different from those of *Blaniulus guttulatus* and *Brachydesmus superus*. Delta ^{15}N values decreased in the order *Blaniulus guttulatus* (5,1 ‰ ± 0,9) > *Brachydesmus superus* (3,9 ‰ ± 0,2) > *Brachyiulus pusillus* (2,6 ‰ ± 0,4).

Delta ^{15}N and delta ^{13}C values of the epedaphic collembolan species *Isotoma viridis* and *Orchesella villosa* differed significantly indicating different food resource utilization. In addition, a significant difference between juvenile and adult stages of *Orchesella villosa* suggests switching of food resources during ontogenesis.

In conclusion, the analysis indicate the use of different food resources by different species of saprophagous/microphythophagous invertebrate groups although we assume that in the arable system studied theses species are not limited by food resources (plant/litter, microbial biomass).

Modelling Methane Concentration and Isotopic Composition in Soil

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Concern about global change has prompted considerable interest in cycles of atmospheric trace gasses. Much research is being devoted to studying fluxes across the ecosystem-atmosphere boundary. Upland forest systems are important sinks of atmospheric methane (CH_4), which is one very significant greenhouse gas. Our knowledge on the turnover of CH_4 in these soils, however, is still incomplete.

A mathematical model including both sink and source layers is used to fit concentrations and $\delta^{13}C$ values of soil gas CH_4. Fitting data from a forest soil revealed a high correlation index ($R^2 = 0.960$) when the model includes both source and sink characteristics. In comparison, a pure consumption model reproduced the data with a much lower correlation index ($R^2 = 0.820$). The model shows, that a beech (*Fagus sylvatica*) forest with a net consumption of atmospheric CH_4 having a $\delta^{13}C$ of - 48 ‰, concomitantly produces CH_4 with a $\delta^{13}C$ of about - 60 ‰ as expected for biogenic methane.

Bomb Carbon as a Tracer of Dietary Carbon Sources in Omnivorous Feeders

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Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and radiocarbon ($\Delta^{14}C$) data is used to investigate the influence of dietary carbon on isotope ratios in the bone protein of an omnivore, *Rattus exulans* / *kiore*. The $\Delta^{14}C$ signal appeared to be transferred through trophic levels in the various dietary components, and ultimately to the rat end-member, which we assumed to be a function of essential amino acids. We then analysed a selection of amino acid separations from a hydrolysate of each bone and found that essential and non-essential amino acids produced widely varying $\delta^{13}C$ and $\Delta^{14}C$, relative to the hydrolysate and to the original whole bone protein. The continuing work focuses on understanding the ways in which essential amino acids may transfer their $\Delta^{14}C$ through a food chain.

The stable isotope biogeochemistry of the sulfur cycle in tidal flats of the North Sea

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The stable isotope biogeochemistry of the sulfur cycle was studied in tidal flats of the southern and eastern North Sea. Sampling sites differed signifcantly in their sedimentological and geochemical properties (grain sizes; organic matter and metal contents; permeability/porosity). Measurements were carried out to investigate the seasonal dynamics of the biogeochemical reactions anf the relationship between the sulfur, organic carbon, manganese, and iron cycles. Special attention has been paid to the influence of temperature and the organic matter load as a process-controlling variables.

Besides major, minor and several trace elements, sediment cores were analyzed down to 20 cm depth for the grain size distribution, mineral composition, TOC, and water contents. Pore waters were analyzed for pH, $\sum CO_2$, NH_4^+, salinity, dissolved major and minor ions, and selected trace elements (e.g., Mn^{2+}; Fe^{2+}). Leaching with Na-dithionite gave an estimate of the solid (reactive) iron and manganese pools. The sulfur pools (sulfate, hydrogen sulfide, AVS, pyrite, S°, organic sulfur) were quantified by different analytical methods and isotopically ($^{34}S/^{32}S$) characterized (C-irmMS). $^{18}O/^{16}O$ ratios of H_2O and SO_4^{2-} were considered, too. Microbial sulfate reduction rates were measured by the $^{35}SO_4^{2-}$ tracer technique. The type of organic matter available for microbial degradation was characterized by the carbon isotope and C/N ratios.

The results from different flat types (mud, mixed and sand flats) will be discussed in the context of typical sedimentary conditions and compared to experimental results. Additionally, the question how a disturbance of the ecosystem (e.g., "anoxic event") is preserved in the isotope record of the sediments will be considered.

Stable carbon isotopes reveal selective feeding by zooplankton under natural conditions in an estuarine mangrove ecosystem

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Between January 1995 and August 1996, suspended matter and zooplankton were sampled at different locations in a mangrove ecosystem located in the Gautami Godavari estuary and adjacent Kakinada Bay (Andhra Pradesh, India). Suspended matter was sampled at 13 different stations, and was found to have a highly variable carbon stable isotopic composition, with $\delta^{13}C$ values ranging overall between -30.94 and -19.18 ‰, and a highly variable elemental (C:N) composition. Our data suggest that the phytoplankton component has a seasonally and spatially variable $\delta^{13}C$ signature which is surpressed by the terrestrial signal, but may at times fall in the same range as the $\delta^{13}C$ of the allochtonous matter. Our data suggest that phytoplankton exhibits lower $\delta^{13}C$ values shortly after the onset of the monsoon rainfall, when large amounts of terrestrial detritus are carried to the area. At each of the four sites selected for concurrent zooplankton sampling, the zooplankton showed a much wider range of $\delta^{13}C$ than did the suspended matter, with overall $\delta^{13}C$ values between -30.14 and -16.45 ‰. In addition, spatial differences in average $\delta^{13}C$ were much more pronounced for zooplankton than for total suspended matter. These data indicate that zooplankton feed on a component of the suspended matter pool which has more pronounced seasonal and spatial $\delta^{13}C$ variations than the total suspended matter. Thus, despite the large amounts of terrestrial and mangrove detritus present in the water column, the locally produced phytoplankton appears to be a more important carbon source for the zooplankton.

Genetic determinism of $\delta^{13}C$ in maritime pine

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Maritime pine (*Pinus pinaster* AIT.) is one of the major conifer species in south-western Europe. This species is often submitted to drought in summer, limiting the growth capacity. A major aim of breeding programmes for this species is to improve the adaptation to drought. This can be done by using molecular markers in combination with physiological traits, such as $\delta^{13}C$. It has been shown that $\delta^{13}C$ of plant material is a quantitative trait for water use efficiency and for adaptation to drought. Measurements of $\delta^{13}C$ were conducted in a progeny trial (600 14-year-old trees belonging to a 12 x 12 half diallele) to assess genetic parameters. This trait was found to be under genetic control with a narrow-sense heritability of 0.17 and broad-sense heritability of 0.3. A genetic dissection was also performed to determine the location, number and effect of genomic regions (QTLs, quantitative trait loci) involved in the control of $\delta^{13}C$. Significant QTLs were detected in two independent pedigrees, confirming that this trait is under genetic control. In the first pedigree, $\delta^{13}C$ was measured on needles of 200 2-year-old F2 seedlings. In the second pedigree, $\delta^{13}C$ was determined from stem-wood extracted cellulose of 200 14-year-old F1 trees. Genetic linkage maps were established to precisely locate these QTLs.

These results are very promising for both conventional selection based on phenotypic evaluation of water use efficiency ($\delta^{13}C$) and/or marker aided selection.

Water-use along a hydrological gradient in central Florida: A tale of two *Pinus* species

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Although central Florida is relatively flat, the distribution of species on the landscape is controlled by subtle changes in elevation. Along a four-meter elevation gradient, xeric sandhill vegetation dominated by *Pinus palustris* (Longleaf pine) gives way to mesic pine flatwoods dominated by *Pinus elliottii* (Slash pine). I examined the water-use and gas-exchange of these two species to understand what regulates their different distributions on the landscape. In the xeric sandhill, the water table depth was approximately 4 meters below the surface whereas in the mesic flatwoods the water table was less that one meter below the surface. Using δD analysis, both species use groundwater most of the time. Due to the mesic nature of the flatwoods, Pinus elliottii had lower water-use efficiency as indicated by δ^{13}C analysis of the foliage. However, during an extreme drought where the water table depth dropped over a meter below its average in both ecosystems, *Pinus palustris* was able to utilize surface rain events whereas *Pinus elliotti* did not. As a result, *Pinus palustris* was able to maintain gas-exchange activity during the drought, but *Pinus elliottii* shut down. In conclusion, both species utilize groundwater sources under normal conditions, but in the xeric sandhill, *Pinus palustris* can also utilize surface rain events to offset doughtier conditions, whereas in the more mesic environment, *Pinus elliottii* relies strictly on groundwater.

Sources of errors in stable isotope studies: the effects of displacement and pool substitution in nitrogen fertiliser studies

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The ecology of nitrogen turnover in soil and also the efficiency of fertiliser use by cultivated plants are frequently studied by using ^{15}N labeled fertilisers. Interpretation of data from such investigations is often hampered by added nitrogen interactions 'ANI', that may contribute to random or systematic error in these studies. Besides real added nitrogen effects or real priming effects, which signify the additional release of nitrogen from soil pools upon fertiliser addition, there are apparent ANI, mainly displacement and pool substitution effects. They may cause false estimations of the process rates in focus. With regard to displacement, two processes have to be considered. The first is the exchange of added ammonium with the so-called fixed ammonium pool, which denotes the ammonium fraction that is fixed in the protected interlayers of clay minerals. The second concerns the exchange of nitrogen with the soil microbial biomass. Both were studied in laboratory and field experiments.

Under controlled conditions, displacement with the clay bound ammonium was immediate and did not decrease upon nitrification of the free soil ammonium. It accounted for 1.0 to 1.4 % of the applied fertiliser N regardless of application rate. In the field, with changing conditions, displacement of the fixed ammonium was also immediate and showed an additional clear increase over winter and later on a clear decrease until summer. Finally, depending on rate and especially timing of fertiliser application, 2 to 4 % of the applied fertiliser remained irreversibly fixed in clay interlayers until harvest. The displacement with microbial biomass was generally shorter lived in the field as compared to fixed ammonium, but soil microorganisms have to be suspected to mediate displacement and pool substitution with more stable soil organic matter fractions such as the non-hydrolysable and hydrolysable unkown soil organic nitrogen.

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Assessing the predictive power of a two-source isotope mixing model in aquatic ecology using a statistical resampling technique

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The ability of a quantitative two-source isotope mixing model to predict the relative importance of allochthonous and autochthonous inputs to an aquatic food web leading to juvenile Atlantic salmon (*Salmo salar*) was assessed using a statistical resampling approach and stable-isotope data ($\delta^{13}C$ and $\delta^{15}N$) obtained from Catamaran Brook, New Brunswick, in August, 1997. When the difference between the 2 primary food sources was small (e.g., 2-5 ‰), the model performed poorly and assigned 95 % confidence limits in the range of ±20 % to 70 %. Performance was enhanced when the difference between the food sources was increased (e.g., 6-10 ‰), but the predictive power of the model was invariably dependent on sample size. Altering the degree of trophic fractionation showed only minor effects on the predictive capacity of the model. These results suggest that, at natural abundance levels, stable-isotope data should be used as qualitative estimates of diet, and that only robust datasets are amenable to quantitative interpretation. Statistical power curves are presented to aid ecologists in future attempts to design successful stable-isotope studies.

D/H, ^{13}C, ^{15}N, ^{37}Cl Isotopic Content of Commonly Used Herbicides - A Possible Tool for Fingerprinting Non-Source Point Agricultural Constituents

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The isotopic signatures of commercially available herbicides, including several varieties of atrazine found in southern Ontario were analyzed to determine their respective D/H, ^{13}C, ^{15}N, ^{37}Cl signatures. Herbicides were analyzed for their isotopic signatures in an attempt to develop a discrete tool for tracing and identifying non-point source contaminants in agricultural watersheds. Findings demonstrate that of the agrochemicals evaluated, ^{37}Cl signatures range between δ^{37}Cl -4.6 and +4.1, whereas most naturally occurring δ^{37}Cl signatures range is -1.0 to +1.0. Nitrogen, carbon and D/H isotopes of the agrochemicals were also measured. ^{15}N values ranged for, -10.9 to + 1.4, showing large variations. Also, a broad range in ^{13}C values and D/H were observed (-37.1 to -21.4 and -169.6 to -44.6 respectively). Each agro-herbicide investigated sustained a high repeatability throughout the analytical methods. The distinct ensemble of isotopic signatures of each off the agro-herbicides investigated suggests that the tracing of these, or similar types of non-point source constituents may assist in characterizing contamination sources or for use in regional basin scale groundwater/surface water flow calibrations.

Effect of elevated [CO_2] on uptake and translocation of C and N in young beech as affected by internal N stocks - A dual ^{13}C and ^{15}N long term labelling experiment

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In the present study, dynamics of uptake and translocation of C and N in three year old beech as affected by altered atmospheric [CO_2] and the N-nutrition status were investigated. The indoor experiment is based on the labelling of the external nutrient pools with ^{13}C and ^{15}N, respectively. The soil mineral N is labelled using $^{15}NH_4{}^{15}NO_3$ in the nutrient solution, the atmospheric CO_2 is labelled by its depletion in ^{13}C.

The beech grew at atmospheric CO_2 concentrations of 350 ppm (ambient) and 700 ppm (elevated), respectively. For each CO_2 concentration, two N-treatments were investigated in which the tree experienced different pre-treatments in the year prior to the experiment: (i) beech were sufficiently supplied with (unlabelled) nitrogen and (ii) beech were grown on sand with no N-addition.

During the experiment, (labelled) N supply was not limited in all treatments.

Our results show that reduced internal N stocks and elevated [CO_2] result in a slight increase of belowground partitioning of new carbon assimilates. Approximately 70 % of the total C uptake after 24 weeks were translocated to the root system. For beech with well filled N stocks growing at ambient [CO_2] belowground allocation was 67 %. The increase of belowground partitioning was even stronger when both effects (increased [CO_2] and reduced internal N stocks) were combined (79 % of the new C translocated belowground).

38 % of the total N uptake after 24 weeks were found in the shoot for beech with well filled N stocks growing under ambient [CO_2]. The partitioning of the N uptake to the shoot was increased under elevated [CO_2] (50 %) and for beech with reduced N stocks (47 %). However, the partitioning was more basipetal when both effects were combined (33 % of the new N translocated aboveground).

No discrimination against ^{13}C during microbial respiration of added C_3-, C_4- and ^{13}C-labelled sugars to a C_3-soil

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The purpose of this study was to test if the changes in δ^{13}C of emitted CO_2 that follow the addition of C_4-sucrose, as a microbial substrate, to the soil of a C_3-ecosystem can partly be explained by a discrimination against ^{13}C. This study is a part of an experimental series designed to assess whether or not the contributions from C_3-respiration (root and microbial) and C_4-respiration (microbial) to total soil respiration can be calculated from such changes in δ^{13}C of emitted CO_2. We approached this problem by adding C_3-glucose ($\delta^{13}C = -23.4$ ‰), C_4-sucrose (-10.8 ‰) or ^{13}C-labelled glucose (103.7 ‰) to the intact mor-layer, the upper organic soil (-26.5 ‰), of a boreal *Pinus sylvestris* L. forest. We sampled the stationary gas phase under soil covers before and 1 h after additions. Results from analyses of δ^{13}C of the CO_2 in the samples confirm that any discrimination, either during microbial respiration of the C_4-sucrose, or during CO_2-diffusion in the soil or into the soil covers (used to create a head space), should be minor.

Deuterium Pattern in Karstic Waters Study
Cas Study for Danube Delta Vicinity

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As the sensitive and nonreactiv tracer of the water movement in karstic areas, deuterium was been used to determine the natural flow of groundwater in the karst vicinal of Danube Delta-Romania. The sets of isoconcentration lines for δD values correlated with the water type and its origin were the analytical tools for groundwater's analysis of the flow paths, recharge-discharge areas in the study karst.

The intrusion of light isotopic water from high altitude (δD<-85 ‰) in the karstic lowland occurs by fractures (conduits) and the limestone drainage occurs by diffuse flow towards the DanubeDelta and by conduits in southern part of area studied, in the northern part of Razim Lake, below the level of the lake's water.

From the isotopic pattern maps and isolines configuration it can be delineated four hydrologic substructures.

1. The North part acts as a discharge area for both the isotopically light water and the local infiltration water (δD>-75 ‰).

2. Southwestern part has local character of flow paths and distinct seasonal effects for deuterium variability.

3. The phreatic system recharged from the Danube River has flow paths controlled by river flow direction and geological conditions.

4. Conduit aquifer and mixing aquifer with the waters originated in mixing processes of water with lower isotopic content and the water tributary to Danube River.

The deuterium content can provide control and direct evidence of transport processes that induce its distribution and have a direct bearing on the preservation of the dynamic balance of groundwater

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The origin of soil organic C, dissolved organic C and soil respiration in a long-term maize cropping system, determined by ^{13}C natural abundance

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For a quantitative analysis of SOC dynamics it is necessary to trace the origins of the soil organic compounds and the pathways of their transformations. We used the ^{13}C isotope to determine the incorporation of maize residues into the soil organic carbon (SOC), to trace the origin of the dissolved organic carbon (DOC), and to quantify the fraction of the maize C in the soil respiration. The maize-derived SOC was quantified in soil samples collected to a depth of 65 cm from two plots, one 'continuous maize' and the other 'continuous rye'(reference site) from the long-term field experiment 'Ewiger Roggen' in Halle, Germany. This field trial was established in 1878 and was partly changed to a continuous maize cropping system in 1961. Production rates and δ^{13}C of DOC and CO_2 were determined for the Ap horizon in incubation experiments with undisturbed soil columns.

After 37 years of continuous maize cropping, 15 % of the total SOC in the topsoil originated from maize C. The fraction of the maize-derived C below the ploughed horizon was only 5 to 3 %. The total amount of maize C stored in the profile was 9080 kg ha^{-1} which was equal to about 31 % of the estimated total C input via maize residues (roots and stubble). Total leaching of DOC during the incubation period of 16 weeks was 1.1 g m^{-2} and one third of the DOC derived from maize C. The specific DOC production rate from the maize-derived SOC was 2.5 times higher than that from the older humus formed by C_3 plants. The total CO_2-C emission for 16 weeks was 18 g m^{-2}. Fifty eight percent of the soil respiration originated from maize C. The specific CO_2 formation from maize-derived SOC was 8 times higher than that from the older SOC formed by C_3 plants. The ratio of DOC production to CO_2-C production was three times smaller for the young, maize-derived SOC than for the older humus formed by C_3 plants.

Natural isotopic composition of fertilizer nitrogen as a tracer for the partitioning in the soil and the biomass of vineyards

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Nitrogen fertilisation of vineyards is considered as an environmental problem for the resulting excess of nitrate is easily leached off into the local groundwater. Vines start their growth and consequently their uptake of nitrogen from the soil pool late in the year about May and stop it completely after grape harvest. During winter, our season of long-lasting precipitations and of low evaporation too, the soil pore volume is saturated and leaches down water rich in nitrate. Therefore nitrate concentration in the groundwater supplied from wine growing areas tends towards nitrate levels above European community regulations. Otherwise the need of vines competes with other sinks of nitrogen. The bare soil is covered with other plants as well as to store the nitrogen as to protect the soil from the heavy harvesting machines. But this vegetation cover is in competetion to nitrogen and water too. An additional input of nitrogen was expected by growing legumes, especially clover.

Clover, a nitrogen-fixing plant has an isotopic composition of nitrogen close to air (about 0 ‰). Usually the nitrogen pool of the soil, which is large compared to the standing crop and the annually added portion of the fertilizer added, is enriched in ^{15}N (about 7-10 ‰ related to air nitrogen, fertilizer in our case ammonia 1 ‰, nitrate 4 ‰). The light nitrogen of clover will disappear quickly either by rapid turnover or (as we think) mainly by dilution into the big well-buffered soil nitrogen pool. For the vines which insert their roots at a depth of about 40 cm below the ground level this freshly produced nitrogen will not be available immediately. Mostly calcareous ammonnitrate ("Kalkammonsalpeter") is used. The ammonium usually originates from the HABER-BOSCH-procedure fixing air nitrogen chemically without larger isotopic fractionation. In contrast the nitrate portion often originates from organic material which is enriched in ^{15}N/^{14}N. Both nitrogen portions differ not only in their isotopic composition but in their physiological and ecological behaviour also. Ammonium is bound tightly to the soil matrix whereas nitrate remains mobile. Nitrate is prefently taken up by plants.

For commercial vineyards around Neustadt/Palatinate we have shown that the vines preferently use the nitrogen pool of the soil instead of the fertilizer added directly. During late season the fertilzer may also reach the root zone of the plants and may be taken up too. Combining the isotopic measurements and simple soil chemical results enables one to assess the effect of fertilizer nitrogen in the agroecosystem vineyard and to propose realistic measures for the application of nitrogen fertilizers. But the movement and storage of nitrogen from fertilizer into differnt pools necessitates some years of observation. Fertilization studies mostly suffer from the limited area under observation and therefore are away from practice. Applying fertilizer of different isotopic composition offers an easy, non-invasive and cheap method to study the fate of nitrogen in the fertilzer-soil-plant-fruit-consumer chain under realistic conditions without artificial impacts and enables optimizing the use of nitrogen fertilizers under realistic conditions. We offer cooperation and experience in this kind of application.

Oxygen-Deuterium Isotopic measurements of Water using a variety of EA - Pyrolysis - IRMS Continuous Flow techniques

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The technique of interfacing an elemental analyser to a stable isotope ratio mass spectrometer has since its inception in 1983 (PRESTON and OWENS) proved a remarkably versatile analytical tool for the measurement of carbon and nitrogen isotopes across a wide application base. By 1994 Sulphur isotopes had been added to the list and more recently those of oxygen and hydrogen. The analysis of oxygen and hydrogen involved a departure from the normal flash combustion mode of operation to one of pyrolytic thermal decomposition of the sample.

This work describes the application of the technique to the measurement of hydrogen and oxygen isotopes across an extensive range of sample types. Samples such as chlorinated hydrocarbons, water, simple organic such as cellulose, biotite; phosphates, nitrates etc were measured using a variety of pyrolysis reactor configurations and temperatures. Results will be presented and, where appropriate, comparisons made of the results from the same samples but using different pyrolysis techniques.

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Isotope fractionation of aromatic hydrocarbons - A perspective to characterise microbial *in situ* degradation

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The direct measurement of the *in situ* biodegradation of pollutants is needed for a precise prediction of the fate of contaminants in aquifers. A decrease of pollutants downstream a contamination plume could have many reasons like dilution, adsorption to soil or biological degradation by the indigenous microflora. To characterise in situ microbial degradation of aromatic hydrocarbons we have developed a concept based on isotope fractionation of contaminants during biodegradation. Laboratory derived compound specific isotope fractionation factors were applied to quantify the microbial degradation of contaminants in aquifers independent of abiotic factors like dilution or adsorption to aquifer material.

Toluene-degrading bacterial strains were cultured under aerobic and anaerobic conditions (nitrate-, iron- and sulfate-reduction) and the concentration and isotopic composition of toluene was monitored. The residual toluene fraction was significantly enriched in $\delta^{13}C$. The individual $^{13}C/^{12}C$ kinetic isotope fractionation factors αC were 1.0027 for *P. putida*, 1.0017 for *T. aromatica*, 1.0017 for *G. metallireducens*, and 1.0017 for *Desulfotomaculum* strain TRM1, respectively. Similar experiments were performed to measure the isotopic fractionation of hydrogen isotopes during the microbial degradation of toluene. In case of the toluene degradation the isotopic fractionation factor of hydrogen αD 0.2723 was some orders of magnitude higher than of αC. Therefore, also hydrogen isotopes may be well used to characterise *in situ* biodegradation processes.

In a field study, a contaminated, anoxic aquifer was analyzed for BTEX contaminants. These compounds revealed a significant concentration gradient along an 800 m contamination plume. A distinct increase of the $\delta^{13}C$ values was observed for the residual non-degraded toluene (7.2 ‰), and o-xylene fraction (8.2 ‰,). The contribution of microbial degradation to the total contaminant removal was calculated with laboratory-derived $^{13}C/^{12}C$ isotope fractionation factors suggesting that more than 98 % of both compounds were removed by biodegradation.

The finding that organic compounds with a molecular mass of aromatic hydrocarbons are subject to $^{13}C/^{12}C$ isotope fractionation by microbial degradation offers new perspectives in microbial ecology, bioremediation and biogeochemistry, including the opportunity to monitor biological degradation processes in the environment.

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Stable C and N isotope ratio changes associated with early diagenesis and bioturbation of clay deposition in two contrasting estuaries

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To evaluate the potential impact of soil erosion on the biodiversity of benthic communities and the subsequent recovery macrobenthic mechanisms, an experiment was conducted which introduced terrigenous clay slurries in patches on the inter-tidal flats of two contrasting estuaries. To determine the long-term impacts of these terrigenous sediment inputs, we used stable C and N isotope ratios to trace the sediment particulate mixing. Early diagenesis was also observed resulting in enrichment of ^{15}N and depletion of carbon immediately below the clay-sediment interface. Colonisation of the clay patches by crabs produced bioturbation that stopped just above this depth thus leaving the interface between clay and sediment still well defined after 12 months. However, crab burrows allowed surface transport mechanisms to move external sediment down into the patches thus offering a form of mitigation for the clay deposit in the longer term. In the absence of crabs, polychaetes were able to colonise the clay patches and their bioturbation was observed to pass through the clay-sediment interface. In these areas the clay was more evenly mixed into the original sediments and the boundary was more diffuse. Burial of the clay occurred at the exposed sites leaving the clay intact as a discrete layer well below the sediment surface.

Soil respired $\delta^{13}C$ signatures reflect root exudate or root turnover signatures in an elevated CO_2 and ozone mesocosm experiment

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Bulk tissue and root and soil respired□ $\delta^{13}C$ signatures were measured throughout the soil profile in a Ponderosa Pine mesocosm experiment exposed to ambient and elevated CO_2 concentrations. For the ambient treatment, root (0-1mm, 1-2mm, and >2mm) and soil $\delta^{13}C$ signatures were -24.9 ± .2 ‰ for both bulk and respired CO_2 for six soil horizons (Oa, Oi, A, AC, C1 and C2) spanning 1 m in depth. For the elevated CO_2 treatments, soil signatures did not match the signatures of soil respired CO_2. Rather, soil signatures remained constant at 24.9 ± .2 ‰ throughout the soil profile, whereas soil respired CO_2 decreased with depth (-27.2 ± .2 ‰ for the Oa horizon through -30.8 ± .2 ‰ in the C2 horizon). Since root respired CO_2 signatures for the elevated CO_2 treatment were quite negative (-39.7 ± .2 ‰), we suggest that root exudates or root turnover had a large effect on soil respiration deeper in the profile where root biomass was greater in the mesocosms. CO_2 from root decomposition and rapid metabolism of root exudates appeared to be respired first leaving more recalcitrant C pools in the soil horizons. In addition to introducing a new technique for measuring respired CO_2 signatures, we also provide preliminary results of a survey comparison of tissue and respired CO_2 signatures for a range of plant species.

Natural variation of $\delta^{13}C/^{12}C$ and $\delta^{15}N/^{14}N$ ratios in Scots pine needles from forest stands with varying anthropogenic impact

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The $\delta^{15}N$ and $\delta^{13}C$ values and the nitrogen content of pine needles (*Pinus sylvestris* L.) were determined (FINNIGAN MAT 252) between 1995 and 1997 at 19 sites in the forests north-east of the former industrial region of Leipzig-Halle. Significant differences ($p<0.001$) in the $\delta^{15}N$ values were found between the sites of up to 7 δ‰. At the sites where at least 5 individual trees were analysed, the standard deviation of the mean values was in the range of ±0.5 δ‰; only at a few sites did it range between 0.5 and 1 δ‰

Very low variation within the sites was also measured for the $\delta^{13}C$ values (standard deviation < ±0.5 δ‰). However, surprisingly significant differences of up to 2.8 δ‰ were ascertained between sites. The extreme sites with positive $\delta^{15}N$ values also exhibited more positive $\delta^{13}C$ values. Similarly, those sites with more negative $\delta^{15}N$ values also turned out to have the most negative $\delta^{13}C$ values, too.

Also significant differences in the total nitrogen content of the needles at the various sites were determined. Individual sites with especially positive $\delta^{15}N$ and $\delta^{13}C$ values have a very high nitrogen content as well.

At three different sites, the trees were investigated over a three-year period (1995–97). Significant differences in the $\delta^{15}N$ values were not observed during this time among either the current year’s or one-year-old needles. Moreover, no differences were found between the needles from different years. This indicates that the differences between the sites represent a long-term state and must be attributed to different site effects.

Moreover, the soil was also investigated at these three sites. $\delta^{15}N$ values in the range between 0 and –2.2 δ‰ were found in the mineral soil, and between -3.6 and -6.9 δ‰ in the organic layer. The needles at these sites were always more negative.

The results of these investigations indicate that the different δ values of nitrogen at the sites must be attributed to different anthropogenic impact – especially as far as the nitrogen load is concerned, which comprises compounds from agricultural or combustion sources. It is well known that nitrous compounds emitted from combustion processes have more positive δ values, whereas those from agricultural sources are more negative. Analysing the natural variation of N- and C-stable isotopes in pine needles seems to be a suitable technique for obtaining more information about the anthropogenic impact suffered by a specific site.

Intraannual tree-ring carbon isotope variations: Endogenous and/or exogenous signals?

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Stable carbon and oxygen isotopes in tree rings are frequently used as proxies to investigate the environmental conditions under which the corresponding trees grew. This approach is based on the assumption that the isotope fractionations and therewith the corresponding isotope ratios of plant organic matter are controlled by environmental conditions, primarily via the aperture of the stomates. Trees are, however, rather complex systems and many different effects, not only covering e. g. meteorological variables, have to be considered. The causes of isotope patterns and trends in tree rings are as yet not fully understood, especially with regard to the combined effects of several climatic or environmental quantities.

The process by which a certain isotope ratio is laid down in plant organic matter is considered as a step by step fractionation, comprising diffusion, dissolution and the initial carboxylation reaction. An expression for the ratio of carbon isotopes in chemicals produced at leaf level has been developed in 1982. However, carbon isotope ratios produced at leaf level do not necessarily show up in the same configuration within the corresponding tree-rings. Rings, when analyzed intraannually show a non-uniform ^{13}C content in their radial direction, indicating a clear-cut and annually recurring pattern. Some previous investigations already pointed in this direction. Up to now there is no clear evidence to what extent this radial isotope pattern within a ring mirrors the seasonal development around a tree or points to special weather events. In many cases the relative trend seems to be independent of weather conditions, presumably featuring specific biochemical reactions. Tropical trees with no signs of tree rings show the same isotope pattern possibly pointing to some 'internal clock'.

The intraannual time resolution of the isotope curves that will be presented is partly less than a day and should e. g. be able to mirror strong and abrupt weather events if the corresponding isotope signals that form at leaf level were directly transfered into the tree-rings. This is, however, rarely the case. Presumably carbohydrates with different residence times in different carbon reservoirs which undergo various isotope fractionations play an important, yet unknown role.

Carbon Isotope Fractionation in Different Biomolecules Used in Microbial Ecology. A Kinetik Study to Monitor Substrate Metabolisation

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In microbial ecology stable isotope measurement is a powerful tool to identify new metabolic pathways and to elucidate the role of different microorganisms in a complex microbial community. By isotope ratio mass spectrometry very small changes of the natural abundance of ^{13}C in the substrate and the bacterial biomass can be indicated and the application of ^{13}C-marked substrates allows to monitor the flux of carbon into the bacterial cell and its different compounds. Additionally, the rate of substrate metabolization and carbon incorporation into microbial biomass can be determined by this technique. To evaluate the rate of substrate degradation and discrimination of ^{13}C in the biosynthesis of interesting macromolecules, calibration studies had to be done.

In our approach we used two strains, isolated from polluted environment and identified as *Pseudomonas* sp. and *Alcaligenes* sp., and grew them with the substrates glucose and acetate as sole carbon source. The ^{13}C-isotopic value of these substrates, the bacterial biomass and compounds like RNA and DNA, 12 amino acids and the fatty acids derived from the phospho- and glycolipids were analysed.

Despite of strain specific variations, the correspondence of the isotopic ratio of the substrate, the biomass and the nucleic acids could generally not be confirmed. After addition of ^{13}C-marked substrate during the exponential growth phase, the carbon flux into these cell compounds and its kinetic parameters were determined. In general the nucleic acids showed a slight ^{13}C-depletion compared to the biomass. The different metabolic pathways of the 12 amino acids were mirrored by their different ^{13}C-enrichments. Very fast responses and strong enrichments were measured in the amino acid alanine, which makes it an excellent isotopic marker molecule. Exceptional was the finding that the fatty acids of the glycolipids displayed no ^{13}C-enrichments at all, contrary to the fatty acids of the phospholipids, which reflected the usage of ^{13}C-marked substrates very well. This study allowed us to assess the practical application of different bacterial cell compounds for substrate usage with stable isotope analysis in microbial ecology.

Universal on-line gas preparation and introduction system for IRMS: the GasBench II

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The GasBench II achieves the precision and accuracy of the dual viscous flow inlet system using modern continuous flow techniques. Implementation of existing GC technology for headspace sampling has allowed full automation of sample preparation for wide variety of substrates, including H_2O, $CaCO_3$, and DIC. We have evaluated the performance of this system for a wide variety of tasks, including analysis of atmospheric CO_2, N_2O, CH_4 and other trace gases.

The interface allows the isotope ratio determination of CO_2 over a concentration range of 100 to 800 ppm, which corresponds to 30-100 µg $CaCO_3$. The use of the built in Autodiluter extends the accessible dynamic range by an order of magnitude, allowing automated analysis of up >1000 µg of $CaCO_3$ with continuous flow techniques. Sample analysis using the technique of multiple loop-injection results in multiple GC pulses of the same sample gas within one acquisition providing a precision for carbonates of <0.08 ‰. The use of reference gas pulses at the beginning and the end of the acquisition completes the analogy to the dual inlet system, and provides the link to the international standards. In PostCon mode, the GasBench II can be used to analyze CO_2 (and other condensible gases) at concentrations between 10-100 ppm. Automated cryogenic traps replace the sample loop, and allow cryotrapping and cryofocussing of samples e.g. laser ablation (CO_2, O_2), laser-aided combustion (CO_2). Further lower sample concentrations (down to 1 ppm) can be demonstrated by combining the GasBench II with a preconcentration device (PreCon)

In addition to the widely used 10 ml Exetainer and Vacutainer serum vials, the object oriented programming of the CTC CombiPAL autosampler allows sampling from almost any septum-sealed vial or container, including those of custom design. Important aspects of sample preparation (He flushing, addition of equilibration gases, acid dosing) is handled by the autosampler under control of the IRMS software (ISODAT and ISODAT-NT).

Using stable isotopes to delineate catchment areas for avian migration monitoring stations in North America

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Migration monitoring stations are used to provide information on population trends of North American migrant birds. However, apart from inferring general origins of birds there has been no way to delineate geographic catchment areas sampled by these stations. The ability to resolve catchment areas being sampled would greatly enhance our ability to link and constrain population trend information to specific geographic areas and better focus conservation efforts. We used stable-hydrogen (δD) and carbon ($\delta^{13}C$) isotope values of tail feathers of fall hatch-year (HY) and spring after-hatch-year adult (AHY) Swainson's Thrush (*Catharus ustulatus*) moving through two migration monitoring stations in Canada to determine natal and previous breeding catchment area for those stations. The $\delta^{13}C$ of feathers provided no information on geographic origins of birds, but was solely indicative of dietary carbon inputs. Conversely, δD values of the feathers provided key geographic information of where feathers were grown. The δD values of fall HY birds moving through Delta Marsh Bird Observatory in Manitoba were more negative than those moving through Long Point Bird Observatory in southern Ontario, indicating more northerly origins. Interpolation of the data revealed that both stations sampled birds from a broad region of the Canadian boreal forest. The Delta station catchment area encompassed northwest Manitoba as far as northwestern Alberta and southeast Yukon. The Long Point station catchment area extended from north and central Ontario and Quebec. We show that δD measurements can be used to augment conventional observational techniques for population monitoring, and provides a new means of delineating geographic regions contributing the most to annual production of young. A significant advantage of the isotope technique is the ability to extrapolate quantitative information from remote regions where observational techniques are not possible.

Stable-Carbon, Nitrogen and Sulfur Isotope Ratios in Riparian Food Webs on Rivers Receiving Sewage and Pulp-mill Effluents

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We examined spatial trends in stable-nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) isotope ratios in riparian food webs leading to a riparian, insectivorous bird species, the tree swallow (*Tachycineta bicolor*), on rivers receiving pulp mill and sewage effluents and compared them to ratios of these isotopes in components of the effluents themselves. The study was conducted at sites upstream and downstream from sewage and pulp-mill effluents on two rivers in western Canada. Sewage effluents contained materials with $\delta^{15}N$ or $\delta^{13}C$ values that were distinct from background conditions in the receiving environment. Pulp-mill effluents contained materials with $\delta^{34}S$ values that were distinct from those at upstream sites on the rivers. At both locations, effluent-derived N and S were observed in algae and suspended sediments at sites downstream from the effluents. This was also observed in adult aquatic insects, the one exception being that uptake of effluent-derived N was detected isotopically only at one location. In tree swallows, use of sewage-derived N was evident only at one location, while use of pulp mill-derived S was evident only at the other location. Our study demonstrates the usefulness and limitations of stable isotope measurements for tracing movement of sewage and pulp mill-derived nutrients in freshwater ecosystems and further indicates that such tracing may be extended to riparian species feeding on aquatic prey.

Temporal course of isotope signature in C and N fractions of soil and its gaseous emissions after incorporation of ^{13}C and ^{15}N labelled mustard residues

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One of the most important factors affecting soil fertility is N mineralization. Studies including ^{15}N dilution techniques suggest that gross mineralization of organic N in arable soils is in the same order of magnitude as the N supplied via conventional N-fertilisation practices or crop N-uptake and is therefore an important source of plant available N. N and C turnover in soils is linked through several processes like denitrification, microbial immobilisation or net-mineralization. In order to elucidate these poorly understood links and to gain an understanding about the processes and site characteristics that govern soil fertility and eventually result in different crop yields, a field experiment was set up in autumn 1999. In the context of the introduction of precision farming techniques, areas of high and low yield were identified in an agricultural field. Residues of white mustard which had been ^{13}C and ^{15}N-labelled in a greenhouse were incorporated in the upper horizon of soils located in the high and the low yield area, respectively. Control plots were included that were treated equally except for the addition of residues.

The addition of mustard residues corresponded to an amount of 250 g m^{-2} dry mass, 5 g m^{-2} N (15.7 at% ^{15}N) and 100 g m^{-2} C (δ 101 %0 PDB). Isotopic labelling (^{13}C and ^{15}N) was monitored in total soil C and N, soil extractable organic C and mineral N, soil CO_2 and N_2O-emissions, soil microbial biomass C and N. During the initial mineralisation period (up to 3-4 weeks after incorporation) a discrepancy between ^{13}C enrichments in mineralized C (CO_2-Emission) compared to the extractable organic C or soil microbial biomass C was detected.

More detailed results will be presented at the conference.

Stable Isotope Studies in a Neotropical Rainforest and Savanna Area

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Over the past few years, study of the natural ratios of the stable isotopes has attracted increasing attention as a way of characterising ecological and ecotoxicological processes.

The goal of our investigations was to improve our understanding of the N cycle in neotropical rainforest ecosystems by determining the natural isotope abundances of nitrogen of different plant species and growth forms, as well as of plant (leaves, roots, bark) and ecosystem (mineral soil, humus layer) compartments.

Significant differences among the δ^{15}N value (but less so among the □δ^{13}C values) occur in the leaves of the representatives of the various families. Whereas *Annonaccae*, *Euphorbiaceae*, *Vochysiaceae* and *Myristicaceae* appear isotopically lighter with respect to nitrogen, the representatives of *Caesalpiniaceae*, *Moraceae*, *Arecaceae* and *Rubiaceae* at the same sites are isotopically heavier. Mistletoes (*Loranthaceae*) occupies a position between the two groups.

Detailed studies of plant compartments of representatives of different families (*Celastracaea*, *Vochysiacaea*, *Annonacaea*, *Caesalpiniacaea*) were performed at the same location. Although relatively minor differences were found in the δ^{15}N values in old and young leaves and bark within the same species, larger differences were observed between the species of individual families. Root material exhibited the most positive δ^{15}N values.

When studying the N fractions of the site soil of either the total fraction (organic soil horizon, mineral soil) or the potentially available nitrogen from these fractions (HWS-organic layer, HWS-mineral soil), the δ^{15}N values of the organic soil horizon produced results comparable with the plant sample material, whereas by contrast the nitrogen of the mineral soil showed more positive δ^{15}N values.

Considerable site-dependent differences in the natural nitrogen isotope ratios were detected in the representatives of *Melastomataceae*, *Annonaceae* and *Rubiaceae* found both in the rainforest and the savanna locations of the study area. The plants from the savanna locations were mainly all characterised by negative ^{15}N/^{14}N isotope ratios and differed significantly from the rainforest species, which generally displayed positive δ^{15}N values.

One reason for this could be the different species composition and activity of the soil microorganisms in the two ecosystems, resulting in N fractionation and transformation processes occurring which are site-specific, and which also lead to different N-isotope ratios within the same plant family.

Cormorant-Derived Nutrients from Fresh Water Ecosystem and it's Utilization by Terrestrial Plants

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Interaction between aquatic and terrestrial ecosystem is considered as an important factor to determine each ecosystem structure. Considering the multi-ecosystem use of water birds, they should have an important role in the interaction between aquatic and terrestrial ecosystem. The Great Cormorant *Phalacrocorax carbo hanedae* feeds on fish in lakes and rivers and returns to the colony in the forest. This behaviour creates material flow from fresh water to forest. We examined the flux of nutrients from fresh water to forest by the cormorant and plant nutrient sources in the forest by nitrogen stable isotope ratio. We collected the guano and the leaves of dominant plants in the cormorant's colony near Lake Biwa, Japan, and their nitrogen stable isotope ratio and the amount of nitrogen and phosphorous of the guano were measured. Nutrient flux to the forest was estimated to 8.0 drywt.kg/ha/day in nitrogen and 4.9 drywt.kg/ha/day in phosphorous at high-density area. Nitrogen stable isotope ratio of guano was 12.5 ± 2.0 ‰ (n=36), which was similar to leaves in the colony. Compared with the ratio of rain-derived nitrogen, it was clear that the plants use the cormorant-derived nitrogen. Nitrogen isotope ratio of the leaves was still high in the area where the cormorant had left a few years ago and no guano was found. This indicates that cormorant-derived nutrients remain for a long time after the abandonment of the colony.

Isotopic Compositions of Biota as Indicators of Nutrient Sources, Land Use, and Biogeochemical Reactions in Wetland and Riverine Systems

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We are attempting to use the N, C and S stable isotopic compositions of biota in marshes and riverine systems as indicators of local environmental conditions that may impact water quality. Although the isotopic compositions of dissolved species such as nitrate, bicarbonate, and sulfate have commonly been used as indicators of the nutrient sources, land uses, and biogeochemical reactions that affect the local environment, the concentrations and isotopic compositions of these species may be strongly affected by seasonal changes in hydrology and anthropogenic inputs. The theoretical basis of our study is that the isotopic compositions of plankton and non-fixing plants should reflect to a large extent the isotopic compositions of the dissolved N, C, and S in the environment that are being utilized by these plants (as modified by various possible fractionating mechanisms in the plants), and that these isotopic signatures should be reflected up the foodwebs. Hence, under favorable conditions, the isotopic compositions of biota can reflect (and integrate) the extent of changes in nutrient sources and land uses - and the effects of processes such as denitrification, sulfate reduction, and methane production/oxidation in the riparian zone, hyporheic zone, and water column - that affect the isotopic compositions of the dissolved species. Several major pilot studies are underway to test this hypothesis: in the Everglades, in the Mississippi River Basin, and at the national scale. One main focus of the study has been to determine at what scale source and land-use isotopic signatures become "overprinted" by post-depositional biogeochemical recycling.

Sulfur isotope analysis of organic material by direct on-line combustion: Preliminary results and potential applications

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We are investigating the measurement of sulfur isotope ratios in organic material by direct on-line combustion for ecological studies which include defining the diets of bear populations, examining hydroperiod fluctuations between freshwater and brackish environments using peat cores, and identifying pollution chronologies from tree ring analysis. Sulfur isotope compositions can vary widely as a result of geologic processes and such data could be a powerful tool for examining the connection between earth and life sciences in the study of ecosystems. Sulfur isotopes have received little attention in ecology studies because plant and animal materials typically have relatively low amounts of sulfur (<1 wt.%) and sulfur must be extracted prior to analysis. Since ecology studies often involve hundreds of samples, labor-intensive sulfur extraction methods are not feasible. To address the potential of on-line methods, we have compared results obtained by direct on-line combustion of organic material using a CARLO ERBA NC2500 elemental analyzer connected to a FINNIGAN DELTA PLUS XL mass spectrometer to results obtained from combustion of sulfur extracted from organic material. On-line analysis of peat and animal tissue gave reproducibility of 0.5 ‰ and values are systematically shifted by +0.8 ‰ relative to values obtained by Eschka sulfur extraction. Successful direct on-line combustion of organic material appears to be a function of the sample matrix as well as the concentration of sulfur and initial results indicate that on-line combustion of organic material for sulfur isotope analysis provides fast, reliable results with minimal sample preparation.

Carbon and Oxyden Isotopic Composition of Bird Eggshells from Belarus

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This work is dedicated to studying of the relationship of carbon and oxygen isotope composition of eggshells poultry and wild birds with characteristic features of their habitat, feeding, and interspecific differences.

1. The variations of carbon isotope composition within separate types are little.
2. It is necessary to note, that average isotope composition of carbon of hen eggshells from poultry farms is ~2,5 ‰ heavier than that of home hens, and ~8,5 ‰ heavier than that of wild birds ($\delta^{13}C_m$= -16,8 ‰). This fact, firstly, is probably connected with the difference of feeding, as isotope composition of carbon eggshells is mainly defined by the isotope composition of the food, consumed by birds. And secondly, we cannot fail taking into consideration the fractionation of isotopes in the bios stems within the organism.
3. There exists a single-line dependency between the color of hen eggshells and isotope composition of carbon. Yellow eggshells have intermediate value $\delta^{13}C$ between brown ($\delta^{13}C_m$= -10,5 ‰) and white ($\delta^{13}C_m$= -9,3 ‰), which is probably connected with the influence of chemical composition of pigment on distribution of isotopes; differences in isotope composition of oxygen practically are not observed.
4. A small difference in oxygen isotope composition has been revealed, which depends on habitats (feeding). So, the lightest oxygen isotope composition is that of hen eggshells from poultry farms ($\delta^{18}O_m$= +21,3 ‰), the heaviest -of home hens ($\delta^{18}O_m$= +23,9 ‰), though if compare average isotope oxygen composition between types (hens - wild birds) this difference practically disappears.
5. There exists a dependency between oxygen isotope composition of eggshells and the temperature of the surrounding ambience. Eggshells accumulate more ^{16}O in winter, than in summer. This can be connected, firstly, with the fact that $\delta^{18}O$ CO_2 atmosphere in winter is easier, than in summer, secondly, that in summer the ration of feeding of birds is rich of vegetation, which accumulates ^{18}O. Oxygen isotope composition of water and atmospheric CO_2, is reflected on the distribution of the oxygen isotopes of the shell that allows using it, as an ecological situation indicator of the environment dwelling of the birds.

The established particularities of the distribution $\delta^{13}C$ and $\delta^{18}O$ in the bird eggshells can be used for studying of the ecological aspects of the habitats of the birds and influences of economic activity on the environment.

Isotope analysis of pyrolysis products from Sphagnum peat and dissolved organic matter from bog water

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Elemental analyses (H, C, N, O, S) and bulk isotope analyses (^{13}C, ^{15}N) of Sphagnum moss, underlying peat from a bog and the bog water (Hohlohsee, Black Forest, Germany) were performed to identify humification processes in the early diagenesis of peat formation and to determine the origin of dissolved organic matter (DOM) in the bog lake. Atomic ratios of bulk elemental analysis suggest a compositional shift from carbohydrate dominated structures to phenolic polymers. Observed variations of bulk $\delta^{15}N$ and total nitrogen are likely due to microbial production.

Changes in the isotopic signature (^{13}C) of pyrolysis products from the moss, peat and DOM were applied to identify molecular processes in the formation of DOM. For this purpose we coupled a pyrolysis-gas chromatography system to a mass-spectrometer and an isotope-ratio mass-spectrometer (Py-GC/MS-IRMS). With this combination of structural and isotopic information of individual pyrolysis products we identified different molecular processes: 1.) biological degradation of source material, 2.) selective preservation of individual compounds 3.) formation of microbial biomass (e.g. trophic level effect).

The comparison of pyrolysis products from peat and DOM from the bog lake indicates different sources. $\delta^{13}C$-values of most pyrolysates were more depleted than in the moss. This strongly suggests that DOM of the lake is formed in-situ by microbial production and not simply released from the peat profile. The uptake of ^{13}C depleted carbon, e.g. respired CO_2, CO_2 from the oxidation of methane or acetic acid is proposed as an important factor in the formation of dissolved organic matter.

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Nutrient leaf uptake by the seagrass *Posidonia oceanica* : A ^{15}N tracer study

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The marine phanerogams are widespread in many coastal waters of temperate and tropical zones. They often grow in oligotrophic conditions, and, therefore, should develop capacities to uptake and/or retains efficiently nutrient to ensure their growth. The uptake of nutrient, particularly nitrogen, by seagrass leaves is one of the possibility to meet the nutrient requirement of the plants.

The aim of this study was to measure the *in situ* uptake rates of nitrate and ammonium by the *P. oceanica* leaves and to estimate the relative contribution of this process in the nitrogen budget of this species.

Experiments were carried out from 03 March 1999 to 08 June at 10m depth in the Revallata Bay (Gulf of Calvi, Corsica, France). One shoot of *P. oceanica* was enclosed in a plexiglass cylinder. Roots were isolated from leaf compartment by a rubber membrane. Tracer solutions ($(^{15}NH_4)_2SO_4$ or $^{15}NO_3Na$) were added in leaf compartment at low concentration, i.e. 0.05 µM. Nutrient concentrations in the cylinder were measured before and just after tracer additions and at the end of experiments (1 hour). The ^{15}N abundance was measured in the different types of tissues with a VG Optima mass spectrometer (Micromass) coupled with an elemental analyser (Carlo Erba).

The measured uptake rates were very low for both nutrients and very inferior to the uptake rates measured for other benthic producers and particulate suspended matter. No correlation was observed between ambient concentrations of nutrient and leaf uptake rates. We calculate that this process represents less than 25 % of the nitrogen required to ensure the annual primary production of this species. Annual N requirement is probably met mainly by two other process: root uptake and internal N recycling.

Seasonal and diurnal cycles in $^{13}C/^{12}C$ and D/H of methane emissions from rice fields

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In the framework of the EU project RICEOTOPES, high precision measurements of the $^{13}C/^{12}C$ and D/H ratios of methane emissions from rice fields in Italy were carried out by Mass Spectrometry and Tunable Diode Laser Absorption Spectrometry during two vegetation periods of rice. Significant seasonal variations were found for both $\delta^{13}C$ and δD. The results confirm earlier observations by BERGAMASCHI [1997] in finding a seasonal cycle with isotopically depleted methane in the main growing season and higher values at the beginning and the end of season during drainage of the field. The measured diurnal cycles show a strong correlation between the methane emission rate and the isotopic composition of methane, which depends on the season and can be explained by variations of the different pathways for methane production, oxidation and release into the atmosphere. A box model for simulation and interpretation of these measurements is also presented here.

Millipedes and earthworms increase the decomposition of ^{15}N-labelled winter rape litter in an arable field

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Effects of *Diplopoda* and *Lumbricidae* on the decomposition of ^{15}N-labelled litter of winter oilseed rape were investigated in a microcosm field experiment on an oat field near Göttingen managed by integrated farming over a period of 264 days. A total of 32 experimental containers (ι10 cm, height 20 cm) were filled with defaunated soil (sieved <4 mm, incubation at -80 °C for 14 days). Rape plants grown in hydroculture with the addition of $^{15}NH_4{}^{15}NO_3$ were dried and cut into pieces of 2-4 cm. Three gramm dry weight of the ^{15}N-labelled rape litter were placed either on top of the soil or burrowed into the soil simulating mulching and ploughing, respectively. The microcosms were equipped with a lysimeter at the bottom to collect the leaching water. An earthworm an a diplopod species which were abundant at the study site were collected and 9 adult individuals of *Blaniulus guttulatus* (Fabricius 1798, *Diplopoda*) and 2 of *Aporrectodea caliginosa* (Savigny 1826, *Lumbricidae*) were added to the microcosms separately or in combination following a factorial design. In general, presence of the animals accelerated the decomposition of the litter material. The effects were most pronounced in presence of *Aporrectodea caliginosa*. The total amount of nitrate, ammonium and the amount of ^{15}N leached from the microcosms was increased in presence of earthworms or of both earthworms and diplopods. ^{15}N-labelled nitrogen in the microbial biomass was increased when either earthworms or millipedes were present but was decreased in presence of both species. As expected, the effect of earthworms was more pronounced than that of the diplopods but both species proved to be important members of the detritus food web of the agricultural system studied.

Measurement of seagrass production using the stable ^{13}C carbon isotope

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Despite of the relevance of seagrass productivity in littoral ecosystems, production quantification has been, but until recently, seldom adequately addressed. The methods currently employed include biomass, marking and metabolic techniques. The major inconveniences of such methods are the disregarding of grazing and leaf detachment, the need of a long period of time to appreciate growth or the inconveniences and restrictions of the use of radioactive isotopes. With this work, we propose a method based on the use of the stable ^{13}C isotope. The method has been compared to the classical O_2 - evolution method in microcosm experiments with the seagrass *Zostera marina*. Overall agreement including experiments performed different days and under different environmental conditions was excellent ($^{13}C = 0.99 + 0.89 \times O_2$; $R^2 = 0.780$, $P < 0.01$, $N = 40$). This result suggests that, in our experiments, the carbon incorporation rates derived from the ^{13}C - method approximated net production. Carbon enrichment due to the isotopic addition to the incubation medium, did not affect carbon incorporation rates at enrichments up to 9 % of the natural DIC concentration. Incorporation rates were also found to be incubation time-independent at least for incubation periods up to 6 h. We conclude that the ^{13}C - method is a valid alternative and a promising tool for field and laboratory seagrass ecological and ecophysiological research.

Which equation is superior for the calculation of daily energy expenditure in doubly-labelled water studies? A validation study with nectar-feeding bats

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The doubly labelled water (DLW) method provides a unique basis for estimating energy expenditure (DEE) and water flux in free-ranging animals (N. LIFSON & R. MCCLINTOCK 1966: J. Theoret. Biol. 12:46). This method relies on the fact that the turnover of oxygen in a body depends on the loss of CO_2 and H_2O over time, whereas the turnover of hydrogen depends only on the loss of H_2O. Hence, by measuring the difference in washout rates of hydrogen and oxygen with heavy isotopes, it is possible to estimate the rate of CO_2 production. Previous validation studies have shown that the extent of isotopic fractionation - the different evaporation rate of light and heavy isotopes - greatly affects the outcome of DLW experiments (J.R. SPEAKMAN 1997: The DLW Method). In their initial paper, LIFSON & MCCLINTOCK published an equation to control for fractionation assuming that 50 % of the daily water loss evaporates. This assumption may be inappropriate for many animals.

The goal of this study was to investigate whether the DLW estimates can be improved, by controlling for the correct ratio of fractionated (= evaporated) water loss to total water loss. We used the DLW method on captive 10 g nectar-feeding *Glossophaga soricina* and compared the DLW estimates with measurements based on an energy balance method. For each bat we determined the rate of evaporation at rest during a DLW experiment and derived an estimate of the ratio of evaporative water loss to total water loss. We modified LIFSON & MCCLINTOCK's equation according to this ratio and then determined which of all available equations was superior in predicting the DEE of our study animals. Our results show that the DLW estimate was most accurate when calculated with our modified equation: 0.6 % deviation compared to 9.7 and -4.6 % when calculated with LIFSON & MCCLINTOCK's equations, assuming either no fractionation or 50 % fractionated water loss. We conclude that the DLW method is improved by controlling for the actual ratio of fractionated water loss to total water flux of a study species.

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Isotopic study of the assimilation of sulfur in the proteins of wheat seeds

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Changes in fertilizer practices and a decrease of atmospheric SO_2 have led to a deficiency of available sulfur, which affects cereal production. For winter wheat (*Triticum aestivum*) this deficiency not only affects the grain yield but also the quality of the flour used for making bread . Sulfur can be supplied as fertilizer but, in order for this to be done in an efficient and environmentally acceptable manner, it is necessary to have a better knowledge of the assmilation of sulfur by roots and/or leaves. Furthermore, both its transfer to the seed proteins and how the fertilizer affects the baking quality of the flour must be established.

In this study, winter wheat will be fertilized by foliar application of assimilable ^{34}S and the assimilation will be followed by EA-IRMS (Elemental Analyser-Isotopic Ratio Mass Spectrometry). EA-IRMS has been developed for the accurate measurement of the sulfur content and the isotopic deviation of each part of the plant. When the sulfur content is high enough, the measurements can be obtained directly. When the sulfur content is to low, however, a preliminary mineralisation must be done in order to convert all the sulfur into sulfate prior to its precipitation as $BaSO_4$.

Preliminary results for the sulfur content and the isotopic deviation of organic and inorganic fractions of the different parts of the plant (roots, stems, leaves, seeds) will be presented. Special attention will be paid to the main storage proteins (gliadins and glutenins) which are important for determining the quality of the wheat flour.

The overall objective of this work is to optimise the yield and the quality of wheat flour by determining the right quantity of sulfur to be applied and the best period for this treatment, while at the same time avoiding any negative impact on the environment.

Above-ground grazing alters below-ground soil trophic interactions

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Natural abundances of $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) of plant, soil invertebrate and whole soil samples taken from four contrasting grazing regimes were analysed to determine the effect(s) of above-ground grazing and/or fertiliser application on below-ground soil trophic interactions.

$\delta^{13}C$ and $\delta^{15}N$ values clearly separated primary producers, herbivores, detritivores, whole soil and carnivores. Application of N fertiliser had no measurable isotopic effect on below-ground soil trophic interactions. In contrast, earthworms were ^{15}N-enriched by *c.* 2 ‰ relative to slugs in grazed treatments but were similar to slugs in ungrazed treatments suggesting that above-ground grazing by sheep either directly and/or indirectly affected below-ground putative N sources for earthworms. Furthermore, it is possible that above-ground grazing may have increased the length of the below-ground food chain as demonstrated by a larger difference in $\delta^{15}N$ values (6 ‰ vs 4 ‰) between putative carnivore and primary producer in grazed and ungrazed treatments.

Unlike $\delta^{15}N$, both earthworm and slug $\delta^{13}C$ differed between grazing treatments. This is consistent with the difference in plant $\delta^{13}C$ between treatments and is a direct consequence of the removal of sheep-grazing, leading to the predominance of *Ranunculus repens* L. (creeping buttercup), the foliage of which was the least ^{13}C-depleted of all plant species analysed. There is also evidence to suggest that both slugs and earthworms have an average 2 ‰ ^{13}C-enrichment per trophic level as opposed to the often quoted 1 ‰ ^{13}C-enrichment for reasons that as yet are unknown.

Determination of trophic status of seabirds using carbon and nitrogen isotopes

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The North Sea represents one of the world's major wintering site for numerous sea, shore and waterbirds species.

For two winters (1996-1997 and 1997-1998), carbon and nitrogen stable isotope analysis (δ^{13}C and δ^{15}N) have been performed in the pectoral muscle of common guillemots *Uria aalge*, razorbills *Alca torda*, dovekies *Alle alle*, herring gulls *Larus argentatus*, one black-legged kittiwake *Rissa tridactyla* and one black scoter *Melanitta nigra*, all beached on the Belgian coast.

The black scoter displays the lowest isotopic values (δ^{13}C = -32.5 ‰; δ^{15}N = 5.9 ‰) while the highest values are found in common guillemots (δ^{13}C: mean = -17.0 ‰; δ^{15}N: mean = 16.0 ‰). The data are consistent with the species known diets: dovekies which feed mainly on planktonic invertebrates show the second lowest trophic position, both *Laridae* species present intermediate isotopic values in agreement with their mixed diets while razorbills and guillemots occupy the top of the trophic web, which fits with their piscivorous feeding habits. The lowest position of the black scoter may be attributed to its more vegetarian diet. The guillemot isotopic values significantly differ between the two considered periods (δ^{15}N = 16 ± 1.2 during 1997-1998 winter; δ^{15}N = 15.3 ± 0.9 during 1996-1997 winter). This isotopic increase, also noted for the δ^{13}C values, suggests either change of the preys origin or a shift in the guillemot diet to preys of higher trophic levels.

On the certification of the sulphur isotopic spike reference materials IRMM-643, IRMM-644, IRMM-645 and IRMM-646

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The aim of this certification process was to establish several isotopic spike reference materials for sulphur, which are both certified for their isotopic composition as well as for the amount content of sulphur. As starting material Ag_2S were chosen, because this compound is known to be chemically stable, stoichiometric and non-hygroscopic and can therefore be weighed with a small uncertainty. Four different materials, already certified for isotopic composition were gravimetrically prepared. As precursor materials for the preparation of IRMM-643, IRMM-644 and IRMM-645 the reference materials IAEA-S-1, IAEA-S-2 and IAEA-S-3 distributed by the IAEA (Vienna) were chosen. As starting material for the preparation of IRMM-646 an Ag_2S material was taken, which is enriched in ^{34}S and from which the isotopic composition is well known.

A small amount of substance (Ag_2S) was metrologically weighed and transferred to a 1 l quartz flask and dissolved in concentrated HNO_3 followed by the addition of Br_2 solution on a hot plate until all Ag_2S had disappeared. Heating was continued to remove the excess of Br_2. When the solution was completely transparent, distilled water was added to the solution to bring it up to the desired volume. The weight of the final solution was afterwards determined metrologically. The results for each reference material in this certification process are displayed in the following table.

The isotopic composition and the sulphur amount content of the spike standard reference materials IRMM-643, IRMM-644, IRMM-645 and IRMM-646. The indicated uncertainties are valid for the last digits (k=1).

	IRMM-643	**IRMM-644**	**IRMM-645**	**IRMM-646**
molar abundance ^{32}S	0.9504076 (44)	0.9493945 (80)	0.9518005 (38)	0.0368837 (15)
molar abundance ^{33}S	0.0074869 (28)	0.0075665 (33)	0.0073742 (18)	0.000453289 (72)
molar abundance ^{34}S	0.0419597 (36)	0.0428775 (37)	0.0406872 (28)	0.9626456 (23)
molar abundance ^{36}S	0.00014579 (450)	0.0001614 (27)	0.0001381 (20)	0.0000174 (18)
$n^{32}S/n^{33}S$	126.942 (47)	125.473 (55)	129.072 (32)	81.369 (13)
$n^{32}S/n^{34}S$	22.6505 (20)	22.1420 (20)	23.3931 (17)	0.0383149 (16)
$n^{32}S/n^{36}S$	6519 (20)	5880 (100)	6890 (100)	2120 (220)
sulphur amount content [mmol kg^{-1}]	0.35212 (41)	0.34400 (39)	0.39118 (23)	4.9336 (70)

Isotopic composition of nitrogen phases in modern and ancient sediments

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During the last decade the widespread usage of stable nitrogen isotopes in ecosystem studies seduced different authors into applying established interpretations to varying $\delta^{15}N$ values of total sediments. Several reports have been published dealing with the nitrogen isotopic composition of ancient sedimentary rocks. One interesting feature were the overall depleted $\delta^{15}N$ values ranging from -3 to +2 ‰ – which in nowadays environments would be interpreted as indicative of a high percentage of nitrogen fixing organisms within the primary producers association. But several uncertainties as fractionation during diagenesis, differing organic matter composition, importance of inorganic nitrogen species and others may invalidate this interpretation of bulk nitrogen isotopes in ancient sediments.

Therefore this study attempted to characterize the proportions and nitrogen isotopic composition of different nitrogen phases (porewater ammonium and nitrate, adsorbed ammonium, absorbed amino acids, with organic solvents extractable nitrogen containing compounds, hydrolysable amino acids, and isolated amorphous and bodily preserved organic remains) in modern and ancient sediments.

Stable Nitrogen Isotopes in a Forested Watershed in Taiwan

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A number of differences in rates and patterns of nitrogen cycling have been observed between forest ecosystems in tropical and temperate regions. We conducted an inventory of ^{15}N natural abundance of soils and vegetation in a forested watershed in Taiwan to examine patterns in isotopic composition along a hillslope gradient in a subtropical forest. Soil δ^{15}N values in the forest floor ranged from -1.8 to 1.8 ‰. Mineral soils had higher δ^{15}N values (4.1 to 6.0 ‰). Foliage δ^{15}N values for overstory trees ranged from -6.6 to -2.0 ‰ and were generally lower at the upper elevation site. The smaller difference between plant and soil δ^{15}N values at the lower elevation site is probably related to differences in net nitrification rates. Foliar %N and δ^{15}N values were positively correlated (r=.563, p=0.004), a similar pattern to that recently reported from boreal forests. A presumed non-mycorrhizal species of the Proteaceae had both a low foliar %N and high δ^{15}N value, a pattern previously reported for a shrub ecosystem in Australia. These results indicate that fundamentally similar processes may control plant N isotopic composition in subtropical forests as in other more-well studied biomes. Futhermore, our results highlight the need for more information regarding the relationships between N stable isotopes and processes affecting rates of N cycling, especially as related to wider(-)scale patterns in forest ecosystems within the East Asian region.

Carbon isotope signatures of leaf carbon fractions: A new approach for studying short-term water deficits of plants

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Since 20 years natural abundance levels of carbon isotopes are used to assess water-use efficiency of plants based on the effects of stomatal limitation on carbon isotope discrimination by Rubisco. The approach was used, with varying success, in screening crops for enhanced biomass production and water-use efficiency. However, this method can neither be used to assess the response of plants to short-term water deficit situations nor to study stress recovery, although these parameters are probably more relevant for the selection of drought tolerant and highly productive crops.

Therefore the present study aimed at developing a method for assaying short-term stress responses by fractionating bulk leaf carbon into lipids, soluble sugars, starch and cellulose and analysing their $\delta^{13}C$ values. The method relies on the non-equilibrium of carbon isotope abundance in carbon fractions of different turn-over time. Cellulose and cell wall carbon shows the slowest turn-over and (due to the fact that it constitutes most of the leaf carbon) often masks short-term changes in carbon carbon fractions, such as soluble sugars and starch, that undergo rapid synthesis and breakdown. The time-course of $\delta^{13}C$ values of different leaf carbon fractions of adzuki bean during a 7-day drought period will be presented.

Beside this a simple "field"-method based on the comparison of carbon isotope signatures of bulk leaf carbon and tissue sap, the latter representing a more active metabolic pool (i.e. soluble carbohydrates), was developed. The method was applied to a study of the stress response of a drought and salt tolerant tomatoe (*Lycopersicon esculentum*) cultivar. After three days of withholding water (drought treatment) or exposing the plants to 250 mM NaCl (salinity treatment) the $\delta^{13}C$ value of the bulk leaf material was not significantly different from that of control plants. However, the difference between bulk leaf and tissue sap carbon isotope composition ($\Delta\delta^{13}C = \delta^{13}C_{bulk} - \delta^{13}C_{sap}$) was only 0.40 ‰ in controls, while it was -0.95 ‰ and -2.24 ‰ in salt and drought stressed leaves, respectively. The differences in $\Delta\delta^{13}C$ between controls and both treatments were highly signifi-cant ($P<0.001$), thus proofing the suitability and sensitivity of the method for assessing short-term stress responses.

$\delta^{13}C$ and $\delta^{15}N$ Signatures of Different Size Fractions in the Pom Pool. A Study from the Atlantic Sector of the Southern Ocean

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We present data on the $\delta^{13}C$ and $\delta^{15}N$ signal of different size fractions in the POM pool in surface waters from the South Atlantic near the Polar Frontal Zone, extending to the Antarctic continent.

Samples were collected during the expedition ANT XVI/3 with RV "Polarstern" as part of the international Southern Ocean Joint Global Ocean Flux Study (SO-JGOFS) from March 18, 1999 to May 10, 1999.

The filtration systems for the size fractionated POM sampling consisted of normal filter holders for sceen filters with net poresizes of 100 and 20 µm, followed by a cascade of magnetic stirred filtration units equiped with membrane filters in pore sizes of 8, 3, 1 and 0.4 µm.

The membran filtration system allowed larger volume filtration (40 to 60 L), with reduced effects of filter clogging. This filtration technique enabled us to collect sufficient amounts of particulate material for isotopic measurements in each size fraction on a wide range of natural plankton abundances.

For both blooming and regenerating plankton communities a consistent gradient towards lighter

δ values with decreasing particle size down to 1 µm was observed. Within a single POM sample the measurements of the different size fractions ranging from netplankton to picoplankton show relatively high differences up to 4 ‰ in the $\delta^{13}C$ and the $\delta^{15}N$ values.

This isotopic trend with particle size occurred independent from the regional shifts in planktonic biomass distribution. The latter show a gradient from the high abundance of large diatom cells dominant under blooming conditions in the Polar Frontal Zone, towards higher abundances of smaller cells in the regenerating community to the south. Possible reasons for these isotopic distribution in the marine POM pool are discussed.

Reflect stable isotopes the foodweb development in regenerating ecosystems?

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30 years phosphorus fertilizer production completely disturbed a shrubrich limestone dry meadow ecosystem with heavy metal enriched dusts. Since the production stopped in 1990 the influence of pollution decreases continuously and organisms slowly recolonize this site.

Reconstruction of the trophic web and the increase of biodiversity should be investigated using stable isotopes.

Therefore a model food chain was established using cultivated woodlice (*Porcellio dilatatus*) supplied with similar food of different δ^{15}N-values and identical δ^{13}C-values. In both cases food consisted of cellulose paper and the cellulolytic fungus *Chaetomium globosum* (Ascomycetes) grown on this paper. After 8 weeks under controlled temperature and light regime in a growth chamber, continuously collected faeces and exuviae, remaining food and woodlice were investigated.

It is expected that after a certain time of adaptation isotopic ratios shift within the woodlice to higher δ-values. This shift should reflect the differences of the supplied food.

Results confirm that δ^{15}N- and δ^{13}C-values of the woodlice increase in comparison to their food. However, observed trophic level shifts for δ^{15}N were not equal but 5,71 ‰ and 2,48 ‰, respectively.

This paper will focus on factors controlling the observed trophic relation in soil food webs. Of particular interest are turnover rates, mass balances and isotopic fractionations.

Application of ^{13}C Tracer Method to the study on effects of gamma-ray irradiation to primary production processes of *Euglena gracilis* Z

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Phytomastigophorea *Euglena gracilis* Z (hereafter *Euglena*) is a kind of freshwater phytoplankton endowed with photosynthesis activity. Several authors reported that *Euglena* is highly resistant against gamma-ray irradiation up to several hundreds Gy, based on the population-based studies. The effects of gamma-ray irradiation on the photosynthetic activity of *Euglena*, however, still remain uncertain. In this paper, discussions are mainly focused on the effects of gamma radiation to primary production processes of *Euglena*. To estimate a primary production rate, ^{13}C tracer method was applied.

Euglena was cultured at 25 °C in polycarbonate bottles with a half-strength modified #36 TAUB & DOLLAR'S salt solution containing Ammonium Chloride of 20.2 mg/l. The solution including the incubated *Euglena* was irradiated with ^{60}Co gamma rays of total dose of 100 Gy. Immediately after the gamma rays irradiation, a certain amount of $NaH^{13}CO_3$ was added into the solution. After 12 hours incubation under the existence of light, the solution was filtered by precombusted filters (WHATMAN GF/F). The isotopic ratio of carbon in *Euglena* on the filter was analyzed using a mass spectrometer with an elemental analyzer (FINNIGAN TRACERMAT). In addition, to confirm viability of *Euglena*, an abundance of *Euglena* was counted by colony forming unit.

A decrease in the primary production rate during 12-hour incubation under the existence of light, compared to the control, was observed in 100 Gy gamma-irradiated cells as shown in Figure 1. No significant difference in viability was observed between the controls and 100 Gy irradiated samples. Thus, it implies that the function of the enzyme related to photosynthesis might be suppressed by gamma irradiation. Further work is needed to determine a dose response and to investigate a mechanism of an effect of gamma-ray irradiation to photosynthesis activity.

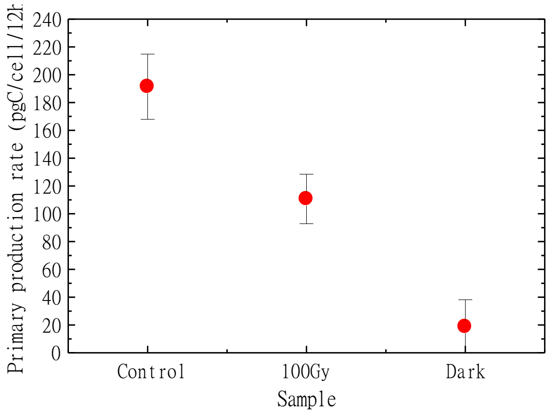

Figure 1: Primary production rate of each sample.

A simple urea leaf-feeding method for the production of ^{13}C and ^{15}N labelled plant material

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Techniques using ^{13}C enriched materials are becoming increasingly popular in soil biology and terrestrial ecology. However, their wider use has been restricted by the technical requirements and high costs associated with the production of ^{13}C labelled plant material by $^{13}CO_2$ release in purpose-built labelling chambers. We describe a simple and inexpensive method not requiring any special equipment or facilities for the small-scale production of ^{13}C and ^{15}N labelled plant material. The method is based on foliar feeding of maize with a 0.2 % urea solution (97 atom% ^{13}C, 2 atom% ^{15}N) by daily misting. Maize was grown in the greenhouse in a compost–soil mixture and enclosed in clear polythene bags between urea applications. Final enrichment in 4 week old maize shoots was 211 ‰ $\delta^{13}C$ (~1.34 atom% ^{13}C) and 434 ‰ $\delta^{15}N$ (~0.52 atom% ^{15}N). The enrichment of hot-water extractable fractions (289 ‰ $\delta^{13}C$, 469 ‰ $\delta^{15}N$) were only slightly higher than those observed in plant bulk material, which suggests that daily urea applications ensured fairly uniform labelling of different biochemical fractions and plant tissues. The total dry weight of labelled shoot biomass was 46 g, and 1 g labelled urea was used to produce this. Recovery of applied ^{13}C in plant shoots was about 20 %. Enrichment of maize roots was lower (21 ‰ $\delta^{13}C$ and 277 ‰ $\delta^{15}N$) and no attempts were made in this study to recover roots quantitatively.

Nitrate Source and Transport in Seepage Water Along a Loess-Profile

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The aim of the study carried out on a 300 m to 600 m hill site catchment area was to investigate the lateral and horizontal transport of nitrate in seepage water along a layered loess profile.

The loess region of the eastern Harz foreland (Germany) provides very good conditions for agriculture. But subsurface waterflow caused groundwater pollution and drinking water problems in the study area, esp. by carrying out nitrate from the arable land. In the study area periglacial sediments (loess and solifluidal debris) were accumulated in large areas of the plateaus during the Weichselian glacial (7 m on average). The loess was covered with chernozems but today, however, the area is strongly eroded with large amounts of calcaric Regosols and of colluvial soils.

The test site was equipped with suction lysimeter in different depths to cover the two loess layers and the solifluidal debris layer beneath a fossil humus zone (1-4 m [loess sediment], 8 m [solifluidal debris]). A well located down hill was used to describe the groundwater of the catchment area.

Soil water analyses point to a considerable lateral seeping water flow down to a depth of 3-4 m. The soil water samples from the loess profile show a varied nitrate concentration. Below the humus zone the flow dynamic and the nitrate content is complete different to the loess sediments.

For better understanding of this flow dynamic we analyzed the ^{15}N and ^{18}O signature of nitrate to assess source and transport of nitrate. Several tritium analyses of seeping water were carried out to record the residence time of soil and/or seeping water.Large δ^{15}N variations between 0.8 ‰ and 9 ‰ are observed in the loess layers with nitrate contents from 15 to 70 mg/l. Tritium values point to present precipitation. Below the loess sediments nitrate is slightly enriched in ^{15}N up to 8.3 ‰ varying of about 2.5 ‰ with nitrate content up to 250 mg/l. From the slightly enriched δ^{15}N, and the diminished saisonal variations a long term wash out effect from the catchment area is assumed for the seeping water of the solifluidal debris. The isotope data do not support a direct admixing of nitrate from the loess sediment.

In the down stream of the catchment area the δ^{15}N up to 14.2 ‰ and the δ^{18}O of about 6.8 ‰ to 10.6 ‰ let assume another source of nitrate.

Stable isotope ratios and uric acid preservation of termites belonging to three feeding habits in Thailand

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Termites are abundant soil animals, which consume large amount of dead organic matter in the tropics. Previous studies have shown that carbon and nitrogen stable isotope ratios can be used as an indicator of the feeding habits. We determined carbon and nitrogen isotope ratios of termites individually across three kinds of feeding habits, mainly sampled from a dry evergreen forest (DEF) in Sakaerat, Thailand.

Microcerotermes crassus, which is an abundant wood-feeder, and *Dicuspiditermes makhamensis*, a soil-feeding termite, were confirmed to be plotted in separate on the δ^{13}C-δ^{15}N plain. In contrast, lichen feeding termites: *Hospitalitermes birmanicus*, *H. ataramensis* and *H. bicolor* were characterized by the low δ^{15}N values, suggesting that they consumed the nitrogen deposited through the atmosphere.

Termites preserve uric acid as a metabolite. Uric acid concentration among three feeding habits was also determined individually. Uric acid was low in *D. makhamensis*, while high in *Hospitalitermes* species, and intermediate in *M. crassus*. δ^{15}N values of workers in the same caste were more varied in *Hospitalitermes* species, and their δ^{15}N values were correlated with carbon/nitrogen ratios of individuals. We suggest that preservation of uric acid can affect on trophic enrichment of δ^{15}N in termites although heterogeneity of diet among individuals might be considered as well.

Wide range of δ^{15}N in termites suggests that termites are widely adapted to nutrient cycling in a tropical forest ecosystem.

Early diagenesis of mammal bone - a multi isotopic approach (S, O, Sr and Nd) - on fossilized material

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Elemental and isotopic composition of mammalian bone apatite may reflect average paleoclimatic conditions and paleodiet prior to the death of the animal. Hence, stable isotopes of O and C in bone phosphate are widely used as proxies for paleoecological and paleoclimatic reconstructions. All paleoenvironmental interpretations rely on original isotopic and trace element compositions in bone. Diagenesis may, however, obscure or completely destroy the primary biologic information and lead to wrong conclusions.

During early (weeks to months after death) diagenesis rapid microbiological decay of the collageneous matrix weakens the bone structure and allows for chemical and isotopic exchange with ambient fluids. Diagenetic influence in most bones is to be expected because completely fossilized bone consists of close to 100 % apatite, whereas fresh bone contains nearly 50 Vol.% collagen. Furthermore, the small and thermodynamically unstable bone apatite crystallites tend to recrystallise and increase in size while replacing the collagen. Both processes result in formation of diagenetic apatite, which may have a different elemental and isotopic composition compared to the primary biogenic apatite.

CF-IRMS (S, O, C, N), TIMS (Sr, Nd) isotope measurements and AAS trace element analyses are applied to a wide range of fossil bone samples and sediments from different diagenetic environments in an attempt to characterise relevant processes of early bone diagenesis. First CF-IRMS S-isotope measurements of Tertiary to Pleistocene marine and terrestrial fossil bones give δ^{34}S values of -34.3 to +1 ‰ and S-concentrations of 0.01 to 3 %. Modern bone samples analyzed have δ^{34}S values of -23 to +14.3 ‰ and lower S-concentrations of 0.01 to 0.3 wt%, indicating diagenetic addition of sulphur (dominantly in the form of pyrite) with lower δ^{34}S values compared to the original bone. This change appears to be the same for marine as well as terrestrial environments of fossilization. Oxygen isotope profiles across collagen-free recrystallized and collagen-bearing parts of the same Pleistocene bones from fluvial deposits have the same isotopic ratios indicating no change in isotopic composition with recrystallization. In contrast, ^{18}O/^{16}O ratios of Miocene whale bone (22.4 ‰), sea cow bone (22.3 ‰) and shark teeth (22.8 ‰) of a marine fossil site are similar and do not display the expected difference between homeothermic mammal and heterothermic fish remains. This indicates possible diagenetic alteration of the phosphate oxygen isotope composition in this site. The Sr isotope composition of these bones and of the above mentioned bone profiles show significant differences indicating diagenetic addition of Sr from ambient fluids during bone fossilization. In contrast, Nd isotope composition of these bone samples is the same within analytical error. This indicates an early diagenetic incorporation of REE, absent in fresh bone samples, into the bone apatite. Therefore, bones used for paleoecological purposes have to be carefully selected to exclude diagenetic changes.

A new method for measuring stable isotopes in water, using laser spectrometry

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In the past years we have developed a new method for measuring the stable isotopes in water (^{2}H, ^{17}O and ^{18}O). This new method will be explained and recent results presented.

The traditional method for isotope ratio measurements, using mass spectrometers (IRMS), is not suitable for water because of its "sticky" behaviour. Prior to the measurement, chemical conversions are necessary. For deuterium these conversions involve reduction of the water sample over hot zinc or, for better results, hot uranium. For $H_2{}^{18}O$ equilibrium with CO_2 (with known isotope ratio) has to be established at well-controlled temperatures. Both conversions are very time-consuming and a possible source of errors. Moreover it is virtually impossible to measure $H_2{}^{17}O$ with traditional methods.

At the Centre for Isotope Research (CIO) in Groningen we have developed a new technique for measuring isotopes in water. In this technique the difference in the absorption of infrared laser light by the isotopomers is used as a measure for their abundance. We inject 10 µl water samples in evacuated multi-pass cells and, as soon as the water is evaporated, measure the absorption spectrum.

The new technique is now ready to be used in a variety of interesting applications. Among the first experiments we carried out is the measurement of a series of biomedical doubly labelled water (DLW) samples. We validated the determination of energy expenditure in quails with the DLW method against the direct method, using respiration chambers. The DLW samples where measured both with IRMS after traditional sample handling and with our laser-based method. The next application will be the measurement of an ice-core. With our technique we have a high sample throughput and this offers new opportunities in climate research.

See also: E.R.Th Kerstel, R. van Trigt, N. Dam, J. Reuss, and H.A.J. Meijer, Anal. Chem. 71 (1999), 5297-5303

Patterns in nature: A fun look at stable isotope fractionation

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A young (?) scientist with a wild imagination,
Ignoring her predilection for procrastination,
Searched the recesses of her mind,
Crusading to find,
A deeper understanding of stable isotope fractionation.

We rely on differences between sources and fractionation within food webs to reveal information and establish links that is missed using more conventional techniques, such as gut content analysis. But what exactly do these isotopic signatures represent, and why do we see such differences in isotopic fractionation between carbon, nitrogen, hydrogen and sulphur, our isotopic building blocks? This poster considers, in light of our current knowledge, a new perspective on stable isotope fractionation.

And did the young (?) scientist succeed? No, far from it, but visit this poster and perhaps some interesting discussion will ensue that can extend both our understanding.

Adaptations during early development of primary hemiepiphytes of the genus *Clusia* in a tropical lowland rain forest, Costa Rica

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The term primary hemiepiphytes refers to woody plants which germinate in tree canopies (epiphytic phase) and during their juvenile phase send aerial roots to the ground soil, thus becoming terrestrially rooted plants (hemiepiphytic phase).

In this study we examined physiological alterations in carbon acquisition and nitrogen use during the transition from epiphytic habit to terrestrial life of *Clusia* species. Plant material was collected in the Esquinas National Park near La Gamba, Costa Rica, from February 1998 to March 1999. The 8 sympatric species of *Clusia* exhibited varying degrees of nocturnal CO_2 fixation (Crassulacean Acid Metabolism, CAM) as shown by $\delta^{13}C$ values and diurnal acid fluctuations with *C. osaensis* having the highest proportion of CO_2 fixation by PEP carboxylase (ΔH^+ up to 580 µmol L^{-1}) and the highest leaf area based water content (600 g H_2O m^{-2} leaf area). Two species, namely *C. osaensis* and *C. valerii*, were studied in more detail. Both species showed considerable diurnal acid fluctuations in early seedling stages. CAM activity increased in both species with age and was al-ways higher in youngest fully expanded leaves than in expanding or older leaves. *C. osaensis* exhibited a particularly high plasticity in CAM expression with $\delta^{13}C$ values ranging from -18.4 to -29.7 ‰ vs. V-PDB. Nitrogen concentrations of *C. osaensis* and *C. valerii* seedlings decreased from 1.25 to 0.68 % (w/w DW) during the first year of development. When plants established soil contact nitrogen contents increased to 1.12 %. Nitrogen isotope values initially declined from 1.3 ‰ (seeds) to -6.0 ‰ vs. at-air (saplings) indicating enhanced use of atmospheric nitrogenous compounds (being highly depleted in ^{15}N). Increased utilisation of ground soil nitrogen resulted in elevated $\delta^{15}N$ values during the hemiepiphytic phase.

Thus, CAM activity as a water conserving strategy during the epiphytic phase facilitates the establishment of *Clusia* spp. in a highly exposed environment with frequent and severe water deficits. Furthermore, a high nitrogen use efficiency during the epiphytic stage reflects the limited amount of available nitrogen in canopy soils although concentrations are high in this compartment. The switch in nitrogen acquisition from precipitation and suspended soil to ground soil during the transition from epiphytes to terrestrially rooted hemiepiphytes was parallleled by a change in the N isotope composition of leaves.

A Rapid Online Technique to Determine $\delta^{18}O$ and $\delta^{17}O$ of Dissolved and Gaseous Oxygen

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Oxygen is a critical element for the viability of life in terrestrial and aquatic systems, yet few studies have used the isotopic composition of O_2 as an indicator of biogeochemical processes. Part of the reason is that conventional offline methods are laborious and costly, and many analysts are reluctant to introduce O^2 in their IRMS systems. Here we demonstrate that $\delta^{18}O$ and $\delta^{17}O$ values of molecular oxygen in air, soil gas, and of dissolved oxygen (DO) are easily and quickly measured by GC-IRMS. Air or gas samples were collected in gas tight syringes or pre-evacuated bottles. For DO, water samples were drawn into a pre-evacuated 120 ml flasks and the O_2 was extracted into a 5 ml pure helium headspace by shaking for 1 hour. Samples of headspace or gas samples were injected directly into a helium stream, passed through a CO_2 and H_2O trap, and the remaining N_2 was resolved from O_2 by passing the sample through a 1 m Type 5A packed GC column. The O_2 was introduced into the IRMS source via an open split. $\delta^{18}O$ and $\delta^{17}O$ values of O_2 were measured using a MICROMASS OPTIMA triple collecting IRMS in CF-mode, using 99.99 % tank O_2 as the reference gas. Air oxygen (+23.5 ‰, VSMOW) was used as the standard. Air contamination of samples was eliminated through the use of gas-tight syringes equipped with 3-way valves with He flushing the needle. Instrument source linearity was excellent, exhibiting a +0.5 permil shift over an air injection range size of 5000 µl to 200 µl (r^2=0.7, n=23). Repeated measurements of air yielded a long-term reproducibility of ± 0.2 per mil for $\delta^{18}O$ and ± 0.5 per mil for $\delta^{17}O$. Total analysis run-time per sample is less than 2 minutes, and as little as 1 µmole of O_2 may be measured using standard source tuning. There are 4 significant advantages of this method over offline techniques: (1) this method does not require conversion of O_2 to CO_2, thereby reducing the possibilities of oxygen isotope fractionation, especially with small samples, (2) samples are processed directly and rapidly in contrast to laborious offline methods, (3) use of O_2 as the analysis gas allows measurement of $\delta^{17}O$ values, and (4) this technique does not require the construction and use of expensive vacuum and cryogenic manifolds for sample processing. Results from several field studies (rivers and soils) will be presented to show the range of $\delta^{18}O$ values encountered in nature.

The Braunschweig Carbon Project: Atmospheric Flux Monitoring and Free Air Carbon Dioxide Enrichment (FACE)

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With respect to the predicted climate changes and the rise in the concentration of atmospheric CO_2 it has become evident that the role of terrestrial ecosystems in the global carbon (C) turnover is not yet fully understood. This is also true for C fluxes in agricultural ecosystems. As rising atmospheric CO_2 concentrations will directly affect plant photosynthesis, plant biomass production and plant water relations and fluxes of other elements, it is of particular importance to predict the dynamics of C fluxes between the atmosphere and agricultural canopies and soils, respectively, and the potential consequences for agroecosystems under future CO_2 conditions. Currently there are hardly any experimental approaches under real field conditions in crop rotation systems to measure C fluxes at larger spatial scales and to simulate future CO_2 scenarios. The Braunschweig Carbon Project is a combination of micrometerological flux measurements of atmospheric trace gases and other air constituents and of a large scale free air carbon dioxide enrichment (FACE) experiment (ca. 510 m^2 enriched with CO_2 to 550 ppm) applied to an arable crop rotation system. C turnover in the plant-soil system is specifically addressed by permanently applying a stable carbon isotope signature to the enriched plots using CO_2 with a certified $^{12}C/^{13}C$ ratio. The objectives of the project are (i) to investigate the C turnover at different scales in time and space, (ii) to validate existent and develop new plant growth, soil, soil water and ecosystem models by creating coherent data sets, (iii) to assess and quantify the atmosphere-plant-soil exchange of relevant C species (and species of other elements) and water under current and future CO_2 concentrations, (iii) to link soil biological processes of C turnover to the CO_2 exchange at the atmosphere-plant interface and (iv) to improve the assessment of effects of changes in atmospheric CO_2 concentrations on crop growth and C turnover.

The project which is unique in the the combination of these two approaches is especially designed to meet the requirements of process (canopy, soil) and landscape models and contribute to the improvement and validation of models to predict the consequences of climate changes for agricultural ecosystems. The rationale of the project and a description of the structure and design of the experimental approach will be presented.

Can we constrain estimates of nocturnal ecosystem respiration using stable isotope techniques?

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Knowledge of respiration is essential for understanding and predicting forest ecosystem carbon dynamics because (i) respiration returns a large fraction of gross assimilation to the atmosphere and (ii) respiratory fluxes are sensitive to climatic variability [1,2].

Nocturnal ecosystem respiration can be measured directly, by collecting measurements of CO_2 fluxes at night using the eddy covariance method or indirectly, using 'scaled-up' chamber based respiration measurements and a knowledge of biomass within the forest stand. However, comparison studies do not give close agreement between ecosystem respiration values made by the two methods in forest ecosystems [1,4,5].

Here, I present a strategy for partitioning ecosystem respiration and constraining nocturnal ecosystem respiration estimates in a young (19 years) Sitka spruce (*Picea sitchensis* BONG. CARR.) plantation in Scotland, UK. Net ecosystem exchange of CO_2 and H_2O is monitored continuously at this site using the eddy covariance technique [6].

The strategy has been designed to-

(i) quantify how ecosystem respiration is partitioned between the ecosystem components of foliage, wood and soil respiration at night,

(ii) investigate how closely estimates of respiratory contributions made by different components measured using chamber techniques and natural abundant stable isotope ratios of $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{18}O/^{16}O$ ($\delta^{18}O$) agree,

(iii) and investigate whether seasonal changes in the contributions of different components are reflected in both methods.

A Keeling plot of CO_2 concentrations and $\delta^{13}C$ and $\delta^{18}O$ in gas samples collected within and outside the canopy will identify the main isotopic source of respired CO_2 [3]. $\delta^{18}O$ of foliage and soil water samples collected from the canopy and gas samples collected within the chambers will provide two independent sets of end members for a mixing model which will partition the proportional contribution of respiration sources. The merits of the stable isotope technique in constraining chamber based estimates of nocturnal ecosystem respiration will be explored with respect to these results.

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Pathways of formation of N_2O in acidic forest soil

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The beech forest in the Solling area in Lower Saxony is characterised by increasing emission of N_2O with increasing soil temperature in spring. In summer the emission reaches a maximum depending on temperature and water content. This emission type and the controlling parameters are known. However the pathways of the formation of N_2O from forest soils are not well-known. Therefore a field experiment was carried out from May to October 1998. ^{15}N was used as tracer for the determination of the different formation pools of N_2O. The tracer was given separately as [^{15}N]ammonium or [^{15}N]nitrate. The traced compounds were injected by syringes in the organic layer. Plots without treatments and with water in the organic layer were used as control. The emission of N_2O from the Solling site showed a seasonal course as expected. The course of the emission rates of N_2O followed the temperature of the organic layer. The emission of N_2O did not depend on the content of ammonium and nitrate in the soil. The comparisons of the N_2O emission rates and the ^{15}N abundances of the N_2O showed in spring and early summer a share of denitrification on the formation of N_2O between 20 and 60 %. In summer the N_2O evolved only from the nitrate mainly contained in the organic layer. These mechanism of the formation of N_2O in the field can be explained by an increasing microbial O_2 consumption. Until summer more anaerobic sites were produced by microbial activity in the soil. In the summer there was a deficiency of oxygen in the soil and the N_2O was produced only by denitrification in the organic layer.

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