^{2}H NMR IN BIOGEOCHEMISTRY

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Introduction

Climate is changing due to human activity, causing more frequent climatic extremes [1].

Future climate depends on *current* fluxes of greenhouse gases, and on *long-term* interactions between greenhouse gases, atmosphere, and biosphere.

Current fluxes of greenhouses gases must be understood for climate prediction and to implement international treaties.

Long-term interactions between the global vegetation and the atmosphere must be understood because greenhouse gases build up in the atmosphere on a *time scale of centuries*.

Conclusions

^{2}H NMR makes a unique contribution to the increasing use of molecular information to study biogeochemical processes.

Current biogeochemical fluxes:

^{2}H NMR is superior to ^{1}H NMR for quantifying unfrozen water in frozen soil for several reasons:

- lower sensitivity to field inhomogeneity and paramagnetic impurities
- bigger line shape difference between the ice and liquid signal
- sharper response to water fusion
- no possibility of hydrogen in the organic material interfering with the measurement

Long-time interactions:

^{2}H NMR can identify and separate climate and physiological signals in tree ring cellulose:

- C(2)-^{2}H isotopomer carries climate signal
- C(6)-^{2}H isotopomers carry physiological signals

Combining *climate* and *physiological* signals allows us to study long-term interactions between vegetation and climate, to forecast how plants will respond to climate change.

Quantifying Unfrozen Water in Frozen Soil by High-Field ^{2}H NMR

N_2O is a greenhouse gas, part of the biogeochemical N cycle, and involved in atmospheric chemistry. The *current N_2O flux* has natural and anthropogenic parts, among the former *biological activity in frozen soils* in high northern latitudes. To assess biological activity in frozen soils, *the fraction of liquid water in microscopic soil pores must be known.* We show that solid-state ^{2}H NMR, using a quadrupole echo sequence, is a reliable method to measure this fraction, and that humus-rich soils can contain more than 10% of their dry weight of liquid water at -5°C [2].

At high magnetic field strength, liquid and frozen water have similar ^{1}H NMR line widths, which makes it very difficult to accurately quantify the liquid and solid water components (Fig. 1 b).

In contrast, *liquid and solid water signals are easily separated in the ^{2}H spectra*, since the liquid water exhibits a narrow peak, while the ice signal is a much broader doublet (Fig. 1 d). In ^{2}H spectra, *the liquid water content is simply determined by integrating the narrow central signal.*

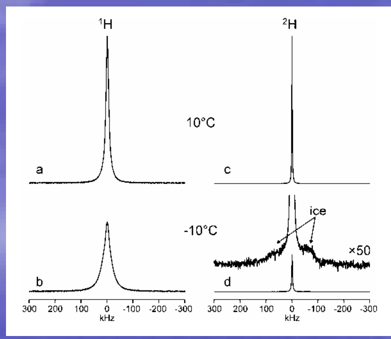

Fig. 1: *^{1}H and ^{2}H NMR spectra* at 400 MHz magnetic field strength *of a humic soil* containing 40% (w/w) water, *above and below the bulk water freezing point.*

a) ^{1}H at +10°C, a peak with a full line width at half height of 14 kHz.
b) ^{1}H at –10°C, what appears as a single peak is actually composed of two components, which can hardly be separated. Deconvolution yields approximate line widths of 15 kHz (liquid) and 40 kHz (ice).
c) ^{2}H at +10°C, a peak with 2.2 kHz line width.
d) ^{2}H at –10°C, a peak with 3.6 kHz line width (liquid) and a barely visible doublet of frozen H_2O with roughly 140 kHz splitting (expanded insert).

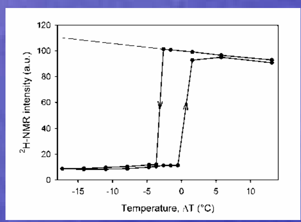

Fig. 2: A *soil freezing curve* for a humus-based soil containing 51%(w/w) water. The diagram illustrates the ^{2}H NMR intensity of unfrozen water as a function of cooling and heating. The dotted line is a fit to Curie's law and represents the expected full intensity if all water where unfrozen. The temperature scale ΔT is defined as the sample temperature minus the bulk water freezing point, which is 0.4°C at 10 mol% ^2H_2O.

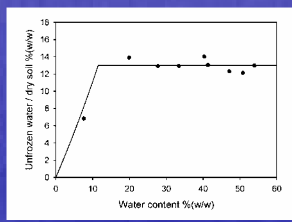

Fig. 3: *The absolute amount of unfrozen water in the investigated soil is not affected by the total water content.* The mass ratio of unfrozen water/dry soil at ΔT = -4.7°C is *constant at 13%* except at very low water contents, where all the available water remains unfrozen. The surface-associated water in small pores remains unfrozen, while the bulk water freezes.

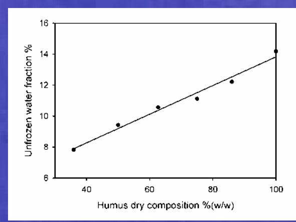

Fig. 4: *The soil antifreeze capacity increases approximately linearly with increasing humus content.* Humus-rich soils provide a larger hydrophilic surface area, so a larger fraction of water is surface-associated and less prone to freezing. The samples were mixtures of humus and sand, and had a water content of 44% (w/w). The data were acquired at ΔT = -4.7°C.

Separating Climate and Physiological Signals in Tree Ring Cellulose

Long-term interactions between vegetation and atmosphere cannot be studied with manipulative experiments. Our solution is to study ^{2}H in tree rings, which store information over centuries.

The ^{2}H abundance of tree ring cellulose is influenced by several physical and biochemical processes. From the ^{2}H abundance we can draw conclusions about these processes, which means we can extract *climate and physiological signals*.

The quantification of the deuterated isotopomers of tree ring glucose by ^{2}H NMR is the key to extract *signals on four processes* (Fig. 5):

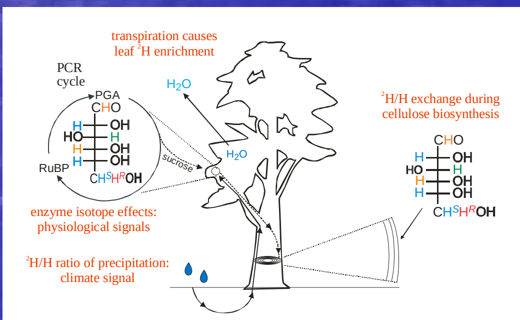

Fig. 5. Hydrogen isotope fractionation processes in a tree. Colours of hydrogen atoms symbolise ^{2}H/^{1}H ratios of individual C-H positions.

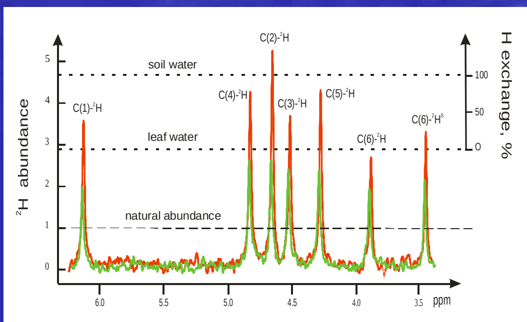

Fig. 6: ^{2}H/^{1}H exchange during cellulose biosynthesis. Deuterium-NMR spectra of leaves glucose (green) and of tree ring cellulose (red)

1. The ^{2}H/^{1}H ratio of tree ring cellulose depends on the ^{2}H/^{1}H ratio of soil water, which contains a *climate signal* [3]. This signal would allow climate reconstruction during centuries, but it is distorted by physiological ^{2}H discriminations.
2. Transpiration driven by the air humidity causes a ^{2}H enrichment of leaf water. This fractionation represents a *microenvironment signal* (humidity). These two processes influence the ^{2}H abundance in all C-H groups of glucose.
3. Isotope effects during photosynthesis discriminate against individual deuterated isotopomers [4,5]. These discriminations represent *physiological signals*.
4. Exchange of H in the C-H groups with H of stem water during cellulose biosynthesis [6,7] can modify the abundance of deuterated isotopomers of tree ring cellulose. This exchange restores the undistorted *climate signal* in the C(2)-^{2}H isotopomer, as shown in figure 6.

We created a ^{2}H gradient in trees, by labelling soil water with ^{2}H and growing them in high air humidity. Then most ^{2}H gets washed out of the leaves, while stem water keeps the ^{2}H label of soil water. In figure 6, we compare ^{2}H spectra of a glucose derivative from leaf glucose and tree ring cellulose. The increased ^{2}H enrichment of tree ring cellulose is due to exchange of C-H groups with ^{2}H-enriched stem water. Not all C-H of cellulose exchange to the same degree, C(2) shows almost full exchange, C(6) shows little exchange.

- ^{2}H/^{1}H exchange restores the *climate signal* from soil water in C(2)-^{2}H.
- C(6) remembers what happened in the leaf, that is it carries *physiological signals*.

Literature

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