Scientific Report ClimMani Short Visit Grant 2012

Project Title:

Evaluating ^{15}N tracer field experiments in order to develop a framework for their analysis

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Purpose of the visit

The purpose of the visit was to study experiments with large scale ^{15}N applications in field situations, primarily forests. This concerned mainly experiments in the literature but also those carried out by the hosts in the past and present. Our focus was on the way the ^{15}N results are interpreted and what additional information, apart from the ^{15}N distribution, is used when interpreting these data. We have the intention to turn this into a review paper.

Description of the work

In Switzerland the papers published by Dr. Schleppi and co-workers on ^{15}N experiments were studied and discussed, and their long-term study site at Alptal was visited. This site was established in 1995 and contains a number of sub-catchments that have undergone different treatments and are intensively monitored. Various ^{15}N applications were carried out on this site. A large number of papers on ^{15}N experiments were studied and based on this literature a first outline of the planned review paper was made. During the visit discussions developed on how ^{15}N experiments could contribute to C sequestration estimates, and the role of the available N pool in the soil in the transformations of N in an ecosystem. A conceptual model was developed to assess how other factors than gross fluxes (such as the available soil N pool size) can affect the outcome of the ^{15}N recovery. It is the intention to use the results of this model in the planned review paper. A first example of the use of this model is presented below. Another activity concerned the visit of another field site, at Hofstetten. Here CO_2 enrichment experiments were carried out in a forest, and the effect on N dynamics and ^{15}N natural abundance was monitored.

In Denmark the outline of the planned review paper prepared in Switzerland was discussed. Subsequently, the main focus of the work was on the earliest known experiments with ^{15}N applications on forests. Many of these were of Scandinavian origin and concerned the effect of forest N fertilization on tree growth. These papers often mention the likelihood of errors in the estimation of this effect by means of ^{15}N, referring to background literature from agricultural disciplines. Therefore, this literature and recent literature citing it was compiled and studied as well. It made clear that these issues have a long history without being completely resolved, and it was concluded that it would be good to include this matter in the review. Some of it is presented below. Further activities included discussions on a PhD research project on ^{15}N: a paper was reviewed and experiments discussed on the application of ^{15}N in one or more forests in a region in China. In addition, a presentation was given on a ^{15}N experiment carried out in the Netherlands. Finally, a visit was paid to the Brandbjerg site of the Danish project CLIMAITE, in which the institute is participating. Here the effects of temperature, drought, and elevated CO_2 are studied on a heathland in a large, replicated experiment.

Some results

Effects of pool sizes on recovery results of ^{15}N

One of the questions that came up during the visit in Switzerland was how the size of certain N pools involved in the transformation of applied ^{15}N label would affect the distribution of the label. In order to investigate this a small conceptual model of N transformations was developed consisting of four N pools as shown in Fig.1. A constant influx of total N (i.e. ^{14}N plus ^{15}N) into the system takes place through the flux “supply”. The r1 to r4 are constant relative rates, and their values and the values of total N in pools A and B are chosen such that total N in pool A and B remain constant. As a result, the two other pools, C and D, increase in size at a constant rate. This model could be considered a much simplified representation of a

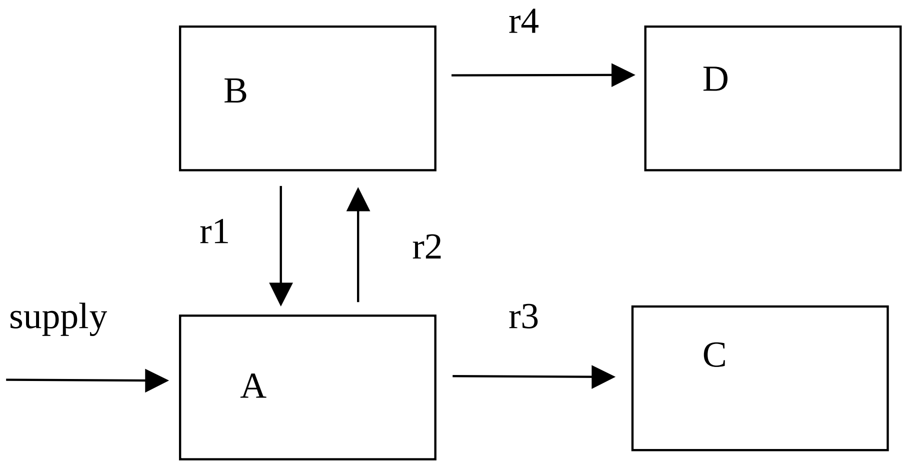

Fig. 1 Model of N transfers in soil and vegetation. For explanation of symbols see text.

soil vegetation system, where pool A is the soil available N pool and B some vegetation pool, while the other two pools are inert soil N (C) and inert vegetation N (D). Alternatively C or D could even stand for N lost from the system, e.g. by leaching. Simulations were carried out in which early in the simulation a pulse of ^{15}N label was applied to the system through the flux "supply". Two different simulations were carried out, which only differed in the size of pool A. In the second simulation the size of this pool was half the size it had in the first simulation, while the relative rates r2 and r3 were doubled to keep the absolute fluxes the same in both simulations. The resulting ^{15}N dynamics in both simulations are shown in Fig. 2. It shows that

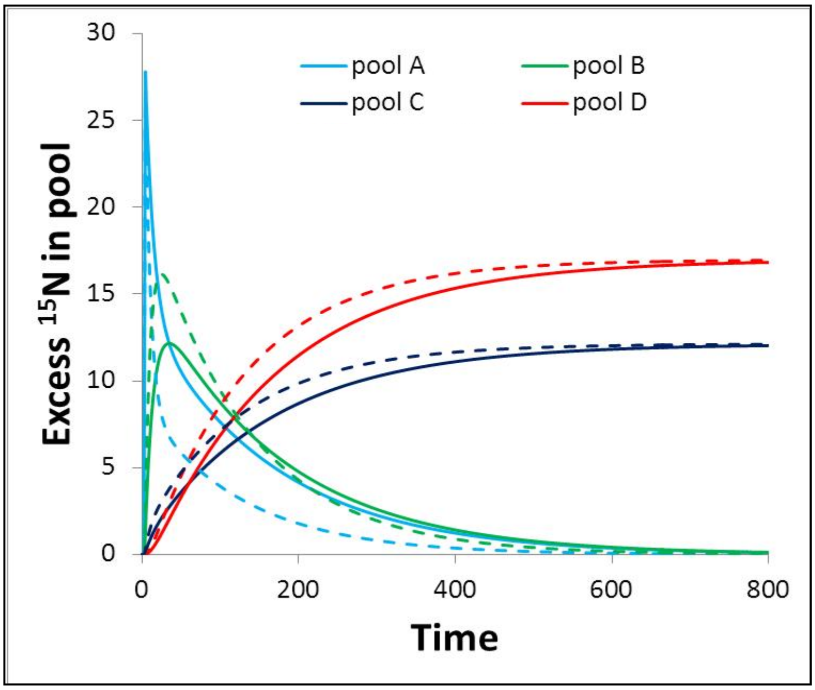

Fig.2 Simulation of a ^{15}N tracer of the model of Fig.1; solid lines simulation 1; dashed lines simulation 2 with total N in pool A halved

during the time label is present in pools A and B there are clear differences in the amount of label in pools C and D. However, in the end after all label has disappeared from pools A and B, the same amount of ^{15}N is present in pools C and D and the size of pool A does no longer affect the presence of labeled ^{15}N in pools C and D. This model should be further investigated. However, in its present form the simulations show that the measurement of gross N transformation rates by means of ^{15}N labeling can give varying results depending on the properties of the system, at least during some time after addition of the label. A better assessment of these phenomena would be possible if the parameter values used are replaced by more realistic values from actual forest experiments. These are available from the hosts.

History of use of ^{15}N in N research and its interpretation

According to Hauck and Bremner (1976) the use of ^{15}N in biological research started around 1940, not long after it became possible to produce substances with increased ^{15}N abundance. In the following decades there were hundreds of publications on agricultural research involving ^{15}N, but only in the 1970s ^{15}N started to be used for large scale field research, mostly in agricultural systems (Hauck and Bremner 1976; Nadelhoffer and Fry 1994). In a meta-analysis of ^{15}N field experiments in natural ecosystems by Templer et al. (2012) the oldest experiment in a forest they present is one from 1967 (Björkman et al. 1967). Nômmik states in a publication related to this experiment that at that time there had been no other forest ^{15}N experiments published (Nômmik 1966). According to Nadelhoffer and Fry (1994) large scale ^{15}N tracer experiments were a new development, as on large scales only ^{15}N natural abundance techniques had been used while ^{15}N tracers had been used only in small scale laboratorium situations.
Nômmik (1966) already mentioned that there may be difficulties in interpreting these experiments. He ascribed this to the microbial N immobilization-remineralization cycle in the soil. Others have also mentioned this interpretation problem (Gardner and Drinkwater 2009; Hamid and Ahmad 1995; Kumar and Goh 2002; Molina et al. 1990; Nômmik 1990; Powlson et al. 1992; Stevenson et al. 1998; Woods et al. 1987). Mostly they refer for a more detailed analysis to other papers (Hauck and Bremner 1976; Jansson 1958; Jenkinson et al. 1985), but these do not present any clear solutions how to handle this problem.
A discussion (Edwards 1978a,b; Hauck and Bremner 1978) incited by the paper by Hauck and Bremner (1976) makes it clear that the problem does not simply concern an error in the measurements, but rather concerns a lack of correspondence between the research question and the measurement. It makes a difference whether you want to know where the labeled N is going to or whether you want to quantify how much N is taken up as a result of fertilization with labeled N. Especially in agriculture and fertilizer research the latter is often the question posed. In such a case one should realize that “how much N is taken up” is not necessarily the same as “how much labeled N is taken up”. To illustrate this, an example that probably is not the most relevant, but hopefully the easiest to understand, is the situation where the N uptake of the vegetation does not respond at all to an addition of N. If in such a situation labeled N is used as fertilizer it might well be that some label ends up in the vegetation, not because the vegetation takes up extra N but because some of the label has taken the place of the unlabeled N in the soil. In such a situation just considering the labeled N taken up would result in the erroneous conclusion that the uptake of N by the crop has increased as a result of fertilization. Therefore, the primary question in this matter should be whether the research question matches the measurements, and not whether certain processes occur or do not occur in an ecosystem, as in some publications is the argument in this matter. This aspect should be considered when assessing the interpretation of ^{15}N experiments in the literature.

Outline of the Review Paper

Comparing the interpretations of large-scale ^{15}N-labeling experiments in forests and other terrestrial ecosystems can clarify the general limitations of these experiments in understanding N transformations. Researchers have tried to overcome these limitations in various ways depending on the specific circumstances of their sites and experiments.

Not every ^{15}N labeling experiment has the same objective and the type of objective affects the way the results should be analyzed and the problems involved. Often research is aimed at the distribution of the labeled N through the ecosystem, but there is also research that aims at the effect a labeled N addition may have upon a certain flux or pool. Ignoring these differences has led to several controversies in the literature (see section *'History of use of ^{15}N in N research and its interpretation'* above). In particular there have been a number of discussions on whether pool substitution effects should be taken into account. Problematic in this respect is that if this effect indeed plays a role, there is, at present, no clear-cut correction method available for it.

The simplest interpretations of ^{15}N experiments are those that focus on the presence or absence of ^{15}N label in a certain pool. Such experiments are used to prove that the labeled N component applied is or is not transferred to the pool investigated (e.g. presence in tissues already present before the start of the labeling). This is fairly straightforward and does not give many problems. The same is true for experiments that present results of pools that contain label without any dilution. This shows that the labeled N component is transferred directly to this pool (e.g. the presence of labeled NO_3^- in bypass leaching).

More difficult cases are those with pools with diluted label. Dilution means that the label has mixed with some other N and this requires extra information in addition to the abundance of the label (^{15}N deltas) in order to be able to interpret the results. In the first place the pattern of the N transformations has to be known, and for N this can be difficult as N transformations are many and especially in soil systems they are difficult to identify. E.g. the labeled N may flow directly to two different pools, but it may also be that these pools form a sequence and the N flows from the first to the next. Such arrangements have of course important consequences on the label dynamics, and their interpretation. Models of ^{15}N dynamics in ecosystems are often mixing models making explicit all N transformations that can occur, but the exact nature of the set of transformations remain to some extent hypothetical. Under controlled lab conditions with only a limited number of N transformations the degree of dilution has been used successfully as a measure of the gross N transformation rates in so called pool dilution experiments. In field experiments, however, the uncertainty is much larger. Researchers have tried to reduce this uncertainty in a number of ways. For example, they studied the short-term dynamics of the pools by carrying out more frequent measurements. Others have looked in much more detail to which organic substance the tracer was bound e.g. by fractionating organic soil pools and measuring the ^{15}N in the resulting fractions.

But even if the transformation paths of the labeled N are known, interpretation of ^{15}N abundances may be difficult. For example, dilution of the label may be affected by the varying size of intermediate N pools. This can be illustrated by outcomes of a simple conceptual model (see section *'Effects of pool sizes on recovery results of ^{15}N'* above). Such models can help with identifying the uncertainties and their sizes, when parameterized with data from the experiment considered. With some modifications this model can be applied to other experiments as well.

One of the most promising approaches to reduce these uncertainties appears to be the use several parallel treatments and drawing conclusions not directly from the individual treatments but rather from the differences between the treatments. There are examples of this, such as different levels of N addition, application in different seasons, and different CO_2 levels. The, often implicit, basic assumption is then that circumstances such as the N transformation pattern and N pool sizes are similar in both treatments.

Planning of the paper

In the next couple of months the review paper will be completed in collaboration with the hosts according to the above outline. A large number of research papers has been compiled and will be used to illustrate and expand the points presented. The above-presented model will be made more realistic by adapting the parameter values to existing experiments, so more precise conclusions can be drawn. The paper will be submitted to a peer-reviewed journal.

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