Estimation of symbiotic dinitrogen fixation in alder forest by the method based on natural $^{15}$N abundance

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Abstract
Annual $N_2$-fixation in virgin forest ecosystems has been measured using a $^{15}$N natural abundance ($\delta^{15}$N) procedure. This method was compared to a $^{15}$N labelled fertilizer isotopic dilution method. For young alders (5-6 years old), $\delta^{15}$N of leaves gave results in good agreement with the isotopic dilution of fertilizer method. Since $\delta^{15}$N variability was expected according to plant physiology, for alder trees, leaves were collected at various heights after the end of the growing season, and, to take account of isotopic variations coming from derived inputs, $\delta^{15}$N of leaves of a large number of other plants in the same area were measured to give control values. Following this procedure, the $\delta^{15}$N method gave reliable evaluation of the nitrogen supply, by through $N_2$-fixation, to alders, which were found to maintain high nitrogen fixing capacity in a sequence ranging from first stage of establishment of climactic formation. Moreover, the same method is reported to discriminate various origins of $Alnus glutinosa$ grown in natural conditions, possibly in relation to the genetic diversity of this species.

Introduction

Nowadays, methods used to evaluate $N_2$-fixation under natural conditions are more limited for actinorhizal than for annual legume plants. The acetylene reduction assay and the $^{15}$N$_2$ incorporation cannot be used with trees except on excised nodules. Using the $^{15}$N labelled fertilizer method, the $N_2$-fixation determination is difficult because of soil depth and the problem of heterogeneity of labelling. Moreover, $^{15}$N input is diluted by different quantities of nitrogen previously present in fixing and non-fixing plants at the beginning of the experiment. Such a method is very expensive since it needs large quantities of labelled fertilizer (Feigenbaum applied 540 g of $^{15}$N-KNO$_3$ at 60% $^{15}$N atom to label only one twenty year old tree). However, this method can be used on young trees (Gauthier et al., 1986) and in limited investigations.

The method based on the difference in the $^{15}$N natural abundance ($\delta^{15}$N method) between atmospheric nitrogen and other available nitrogen sources should be a more suitable method to estimate $N_2$-fixation in natural ecosystems, particularly in forest ones. Few studies have been devoted to the use of this method with trees. Nevertheless, the results obtained by Shearer et al. (1983) on Prosopis and Acacia, and those by Coté and Camiré (1984) on $Alnus glutinosa$, offer hope of development. There are still many difficulties involved in the $\delta^{15}$N method to prove its validity and the main requirements for this need to be established as discussed below:

— to be valid, this method requires a significant difference between the $\delta^{15}$N of the atmospheric $N_2$ and the $\delta^{15}$N of the soil derived nitrogen which can limit the use of $\delta^{15}$N method in natural ecosystems because virgin soils can exhibit lower $^{15}$N abundance (closer to atmospheric $N_2$) than agricultural ones (Riga et al., 1971, Shearer et al., 1974)

— in order to determine the $\delta^{15}$N of the soil ni-
In tree species, it is necessary to use control plants which take up nitrogen from soil only. The non-fixing plants and the N\textsubscript{2}-fixing ones must be present at the same place and explore similar soil volumes. In natural ecosystems, the choice of reference plants is restricted to the trees present at the site. Also, numerous reference samples must be collected and analysed to allow the accurate estimation across the site, of the $\delta^{15}\text{N}$ from the soil-derived nitrogen (Shearer and Kohl, 1986).

The isotopic value of nitrogen originating from the N\textsubscript{2}-fixation process must be determined by nodulated plants growing on N-free medium. A significant error would be introduced in N\textsubscript{2}-fixing estimation if using atmospheric $^{15}\text{N}$ abundance only. The isotopic effect could be dependent on strains or plant species and change in $^{15}\text{N}$ abundance during N\textsubscript{2}-fixation (Steele et al., 1983, Yoneyama et al., 1986). In the case of A. incana and A. glutinosa shoots, $\delta^{15}\text{N}$ of fixed N\textsubscript{2} has been evaluated at $-2 \pm 0.5$. This value is stable with time and only slightly dependent on Frankia strains (Domenach et al., 1988).

For deciduous leaf trees, leaves are the most practicable tissues to collect to estimate the annual N\textsubscript{2} fixation. However, root nitrogen reserves participate in new leaf formation and 10% of the total nitrogen in the leaves at the end of the growing season originated from root storage, as determined by the $^{15}\text{N}$ labelled fertilizer method (Domenach and Kurdali, 1989). Then, to accept the appropriate sampling strategy dictates that the samples to be collected are the last appeared leaves to avoid the influence of the root nitrogen supply;

the $\delta^{15}\text{N}$ method may be incorrectly assessing an isotopic fractionation occurring during nitrogen assimilation or remobilisation within tree. The effect of this fractionation can be neglected when $^{15}\text{N}$ labelled fertilizer is applied.

Two cumulative methods, i.e. the application of $^{15}\text{N}$ labelled fertilizer (Fried and Middleboe, 1977) and the $^{15}\text{N}$ natural abundance measurements, based on the same isotopic dilution principle, can give similar values of N\textsubscript{2} fixation as has been found with annual legumes (Domenach and Chalamet, 1979; Ledgard, 1985). The present study was performed comparing these two methods on young alders in order to test the accuracy of the $\delta^{15}\text{N}$ method, and then, using it to assess N\textsubscript{2}-fixation in natural alder forest at different stages after establishment.

Materials and methods

Experiments

Experiment 1: Evaluation of fixed N\textsubscript{2} in alder using $^{15}\text{N}$ labelled fertilizer application compared to $\delta^{15}\text{N}$ method.

In Autumn 1985, a number of trees grown in natural ecosystems, Alnus glutinosa (L.) Gaernt, Alnus incana (L.) Moench and Populus alba, were transplanted into a nursery (pH 7.7, chalky clay soil, University of Lyon 1). These trees were 5–6 years old and about 1.50 meter high. In Spring 1986:

- 2 sets of 5 plants of Alnus glutinosa and 5 plants of Populus alba were labelled by adding 50 mg-N/tree of K$^{15}\text{NO}_3$ at 5% atom $^{15}\text{N}$. Each tree received 10 mg N/2 weeks of labelled fertilizer;
- 3 sets of 5 A. glutinosa, 5 A. incana and 2 P. alba were treated by adding 50 mg-N/tree of unlabelled KNO\textsubscript{3}.

In autumn 1986, all leaves, roots and shoots were collected. Their isotopic composition was determined by the method previously described by Domenach and Chalamet (1977).

Experiment 2: Estimation N\textsubscript{2}-fixation by native A. incana using the $\delta^{15}\text{N}$ method.

The experiment was carried out on an area of natural growing trees of Alnus incana (L) Moench (Col d'Ornon, altitude 1400 m., North Alps, France). Depending on the age of establishment, Four sites could be identified as:

- site 1: an early stage (pioneer site) where the trees grew on a lithosol formed on coarse carbonated gravels from a torrential deposit. Alders were less than one meter high;
- site 2: the secondary stage. The trees were located on a young undifferentiated soil and measured between 1 and 3 meters high;
- site 3: the mature state (climactic site): the soil exhibits an typical calcic mull A1 horizon and the trees were about 10 meters high;
- site 4: this zone was situated 500 meters below, the trees were 10–20 meters high, growing on a more developed soil (A1 and cambic B horizons).