Nitrogen fixation in Sitka alder by ^{15}N isotope dilution after eight growing seasons in a lodgepole pine site

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Owing to high costs and limitations of the method, there have been no field studies using ^{15}N isotope dilution to measure the amount of N fixed by alder in the field. As part of a long-term (8-year) study of the fate of ^{15}N-labelled fertilizer applied to lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) in the field in interior British Columbia, we observed much lower ^{15}N enrichment in Sitka alder (*Alnus sinuata* (Reg.) Rydb.) than in lodgepole pine or other understory species. Calculations using lodgepole pine, herbs, and shrubs as nonfixing reference crops all indicated that the percent of N derived from the atmosphere in Sitka alder at this site was very high, 94–99%. The long period for stabilization of residual ^{15}N-labelled fertilizer in organic form in the soil and for establishment of plant root systems alleviated many of the problems associated with the ^{15}N isotope dilution method. By contrast, calculations of the percent of N derived from the atmosphere at the end of the first growing season gave erratic and unreliable results.

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En raison des coûts élevée et des limites de la méthode, aucune étude sur le terrain par la méthode de dilution de l'isotope ^{15}N n'a été réalisée afin de mesurer la quantité d'azote fixée par l'aulne. Dans une étude à long terme (8 ans) du devenir du fertilisant ^{15}N appliqué sur le terrain au pin lodgepole (*Pinus contorta* Dougl. var. *latifolia* Engelm.) à l'intérieur de la Colombie-Britannique, nous avons observé des enrichissements beaucoup moindres en ^{15}N chez l'aulne de Sitka (*Alnus sinuata* (Reg.) Rydb.) que chez le pin lodgepole ou les autres espèces de sous-étage. Des calcules dans lesquels on utilisait comme plantes de référence non fixatrices le pin lodgepole, les herbes et les arbrisseaux ont montré qu'à ce site, le pourcentage d'azote dérivé de l'atmosphère et retrouvé chez l'aulne de Sitka était très élevé et atteignait 94–99%. La longue période nécessaire à la stabilisation du fertilisant ^{15}N résiduel sous forme organique dans le sol et à l'éstablissement de systèmes racinaires a atténué bon nombre des difficultés liées à la méthode de dilution de l'isotope ^{15}N. Par contre, les calculs du pourcentage d'azote dérivé de l'atmosphère effectués à la fin de la première saison de croissance ont donné des résultats irréguliers et peu sûrs.

Introduction

Under natural conditions, lodgepole pine depends on fire for regeneration, and alder is a common understory component in some regions. Fire results in losses of organic matter and N, and alder has the potential to contribute significantly to the N economy through biological N_2-fixation (Binkley 1983; Bormann and DeBell 1981; Tarant and Miller 1963). The relative proportions of fixed and soil-derived N in a plant can be assessed using isotope dilution of ^{15}N following application of ^{15}N-enriched fertilizer (Chalk 1985; Witty 1983), or in certain cases, by exploiting the variation in natural abundance of ^{15}N (Shearer and Kohl 1986, 1988). Four studies used the ^{15}N natural abundance method to investigate N_2-fixation by alder in the field (Beaupied *et al.* 1990; Binkley *et al.* 1985; Coté and Camire 1984; Domenach *et al.* 1989), while Chatarpaul and Lachance (1989) and Kurdali *et al.* (1990) used the ^{15}N isotope dilution method in pot studies. There have been no reported applications of the ^{15}N isotope dilution method to alder in the field.

The problems and limitations of the ^{15}N isotope dilution technique include difficulties in the choice of the nonfixing reference plant and decrease during the growing season in the ^{15}N enrichment of N derived from ^{15}N-labelled fertilizer (Chalk 1985; Witty 1983). It has been suggested that the uncertainty associated with the latter can be lessened by using ^{15}N in more stable forms in the soil, such as using slow-release fertilizer, by incorporation of ^{15}N-labelled plant residues, or by addition of ^{15}N-labelled fertilizer followed by a waiting period of 1 year or more (Chalk 1985; Fried *et al.* 1983; Giller and Witty 1987; Rennie 1986). Boddey *et al.* (1990) recommend using the mean of several reference crops in the calculation of N_2-fixation.

While investigating the long-term fate of ^{15}N-labelled fertilizer eight growing seasons after application to lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) near Spillimacheen in the British Columbia interior, we observed that enrichments of ^{15}N above natural abundance (at.% $^{15}N_{ex}$)

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TABLE 1. Source of Sitka alder N eight growing seasons after ^{15}N-labelled fertilizer application: means and ANOVA summary for $\%N_{dff}$ and $\%N_{dfa}$ estimated using three different nonfixing reference plants for Sitka alder

(A) Means of $\%N_{dff}$ and $\%N_{dfa}$

	Source of Alder N			
		$\%N_{dfa}$		
Treatment	$\%N_{dff}$	Herbs	Shrubs	Pine
[^{15}N]urea	0.04	99.4	99.2	98.4
$^{15}NH_4NO_3$	0.08	98.5	98.6	98.0
$NH_4{}^{15}NO_3$	0.11	98.6	98.8	94.0

(B) ANOVA[a]

	$\%N_{dff}$			$\%N_{dfa}$		
Source	df	p	SE	df	p	SE
Treatment	2	0.2606		2	0.1588	
Plant type	—	—		2	0.0008	
Treatment × plant type	—	—		4	0.0090	
			0.034			0.42

[a] $\%N_{dfa}$ analysed by split-plot model with treatments as main plots and plants as subplots.

for Sitka alder (*Alnus sinuata* (Reg.) Rydb.) growing with the lodgepole pine were only slightly above values of alder from the control plots and much lower than those for herbs, woody shrubs, or lodgepole pine. It appeared that this site presented an opportunity for a long-term investigation of ^{15}N isotope dilution with alder in the field. While site heterogeneity and choice of reference plant could not be manipulated, and the sampling scheme had not been designed for this purpose, we had the unique advantages of a field situation with large well-established plants, a choice of reference crops with different rooting habits, and an unusually long time scale for stabilization of the ^{15}N in soil organic matter. Furthermore, we could compare the results with those obtained from samples collected in 1981, one growing season after the fertilization.

Material and methods

The study site, experimental design, and 1-year results have been reported previously (Preston *et al.* 1990), although N_2-fixation in alder was not addressed. Briefly, the study was established in 1981 near Spillimacheen (50°53′N, 116°51′W) in the British Columbia interior. The stand of 11-year-old lodgepole pine regenerated following a fire in 1967 and was thinned to 3.6 × 3.6 m (770 stems/ha) in 1976. Understory species included Sitka alder, willows (*Salix* spp.), birch leaf spirea (*Spirea betulifolia* Pall.), fireweed (*Epilobium angustifolium* L.), and bunch berry (*Cornus canadensis* L.). The soil is a Dystric Brunisol with a gravelly clay loam texture.

Fertilizer was applied at 100 kg $N \cdot ha^{-1}$ in January 1981 as part of a study originally designed to test the efficacy of putting fertilizer on the snow during the winter. There were 16 single-tree plots of 2-m radius, with four replicates of each treatment: [^{15}N]urea, $^{15}NH_4NO_3$, $NH_4{}^{15}NO_3$, and control (no fertilizer addition). The minimum distance between plots was 6 m; the average distance between nearest neighbours was 17 m. The fertilizer at.%$^{15}N_{ex}$ was 2.705% for [^{15}N]urea, 2.606% for $^{15}NH_4NO_3$, and 1.894% for $NH_4{}^{15}NO_3$ (enrichments reported on total N basis, not for individually labelled ions).

Half of the plots were harvested in October 1981 to determine the distribution of ^{15}N-labelled fertilizer in trees, understory, and soil after one growing season (Preston *et al.* 1990). The remaining eight plots were harvested in August 1988 to determine the long-term distribution of ^{15}N in the ecosystem after eight growing seasons. At this time, plot trees ranged from 7.5 to 9.4 m in height, while alders were 2–2.5 m in height and in variable amounts from plot to plot. The canopy was open, with pines having green branches to their base. The alders were in good light conditions.

Procedures for the 1988 field sampling and for N and ^{15}N analysis of plant samples were similar to those used in the 1981 sampling (Preston *et al.* 1990) and are described in detail elsewhere (Preston *et al.* 1992). Briefly, in 1988 there was complete harvesting of aboveground parts and partial harvesting of roots of lodgepole pine. For the understory species, only aboveground parts were harvested and separated into herbs, shrubs (mainly willow), and alder, but not further separated into plant components. In 1981, understory was harvested by species.

After oven-drying at 70°C for 48 h, samples were analysed for total N by the semimicro Kjeldahl method with a pretreatment with permanganate and reduced iron to include nitrate and nitrite. The distillates were collected in boric acid – ethanol, dried at 70°C, converted to dinitrogen gas using the Rittenberg reaction with alkaline sodium hypobromite, and analysed for ^{15}N enrichment using a Vacuum Generators Sira 9 mass spectrometer (Preston *et al.* 1990).

The percent of N derived from atmosphere ($\%N_{dfa}$) was calculated as follows (Witty 1983):

$$\%N_{dfa} = \left\{ 1 - \frac{\text{at.}\%^{15}N_{ex}(\text{fs})}{\text{at.}\%^{15}N_{ex}(\text{nfs})} \right\} \times 100$$

where fs and nfs stand for the fixing and nonfixing plant systems, respectively. Values for at.%$^{15}N_{ex}$ were calculated by subtracting at.%^{15}N values for corresponding samples from the control plots. The percent of N derived from fertilizer ($\%N_{dff}$) was calculated as follows:

$$\%N_{dff} = \left\{ \frac{\text{at.}\%^{15}N_{ex} \text{ in sample}}{\text{at.}\%^{15}N_{ex} \text{ in fertilizer applied}} \right\} \times 100$$

Results and discussion

Despite the low enrichments used in the original ^{15}N application, and the long interval before the 1988 sampling, the ^{15}N enrichments in plant biomass were completely adequate for analytical purposes. Values of at.%$^{15}N_{ex}$ were up to 0.12% in lodgepole pine, and up to 0.2% in understory herbs and shrubs. By contrast, at.%$^{15}N_{ex}$ for alder was in the range of 0.001–0.003%.

Table 1 shows means and ANOVA summary for $\%N_{dff}$ and $\%N_{dfa}$ for alder for the three fertilizer treatments. Whether herbs, shrubs, or lodgepole pine were used as the nonfixing reference plant, the values of $\%N_{dfa}$ indicate that almost all of the N in the alder was derived from atmospheric N. There was no overall effect of treatment (source of ^{15}N) and no difference between herbs and shrubs; with these plants as the reference, some 98–99% of the N in aboveground Sitka alder was derived from N_2-fixation. The values of $\%N_{dfa}$ derived by using lodgepole pine as the reference plant were slightly lower, particularly for those plots treated with $NH_4{}^{15}NO_3$, where the $\%N_{dfa}$ was 94%. This may be due to the different rooting pattern of lodgepole pine; it may have been obtaining more of its N deeper in the soil profile, with lower at.%$^{15}N_{ex}$ derived from ^{15}N-labelled fertilizer. These differences are small, however, and there can be no doubt that almost all of the aboveground alder N was derived from atmospheric N in this low-fertility site.

TABLE 2. Source of Sitka alder N (mean and range of $\%N_{dff}$ and $\%N_{dfa}$) one growing season after ^{15}N application

		$\%N_{dfa}$[b]			
	$\%N_{dff}$[a]	*Pinus contorta*	*Salix* spp.	*Cornus canadensis*	*Epilobium angustifolium*
Mean	18.9	62.6	74.7	74.3	91.1
Range	0.9–44.4	52–83	34–98	15–98	88–95
Alder > ref.[c]		2	1	1	2

NOTE: Five of the six plots had alder. The last line gives the number of plots in which alder had a higher enrichment than the reference plants.

[a] Treatment differences not significant (p = 0.7669).

[b] Mean and range for plots where reference plant is more enriched than the alder.

[c] The number of plots in which alder had a higher enrichment than the reference plants.

Using the ^{15}N natural abundance method, Domenach *et al.* (1989) and Beaupied *et al.* (1990) also found very high values for $\%N_{dfa}$ for black alder (*Alnus glutinosa* (L.) Gaertn.), while Coté and Camire (1984) found a lower value of 68%, possibly due to higher fertility at their site. As an initial approximation we estimate from plot biomass figures that on these plots 50 kg $N \cdot ha^{-1}$ has been accumulated into aboveground alder biomass over the eight growing seasons. Of this, we estimate that less than 2% has come from the soil rather than atmospheric N_2-fixation. This integrated value (Kohl and Shearer 1981) reflects only N that is currently in aboveground biomass and does not include the contribution of N to the ecosystem through decomposing alder litter (Kurdali *et al.* 1990) or via the roots.

By contrast, in 1981 when more of the fertilizer N was available in mineral form, there was considerable uptake by alder, consistent with reports of dominance of N assimilation over fixation when mineral N is available (Huss-Danell *et al.* 1982). In 1981, the ^{15}N uptake was quite variable, with the alder deriving from 1 to 44% of its N from the fertilizer (Table 2). Values calculated for $\%N_{dfa}$ also varied considerably between treatments and reference plants. In some cases the alder was more enriched than the reference plant; this was most likely due to changes in the ^{15}N enrichment in mineral N during the first growing season and differences in plant uptake patterns (Chalk 1985; Rennie 1986; Witty 1983). These data clearly illustrate problems that can occur in estimating N_2-fixation in the 1st year after application of ^{15}N.

Conclusions

While this study was not originally designed to investigate N_2-fixation, it has proved useful in this regard. First, there is little doubt that the alder in this site is deriving a large part of its N from atmospheric rather than soil sources. Second, non N_2 fixing trees and understory species can provide a range of reference crops on which to base the calculation of N_2-fixation (Boddey *et al.* 1990), when there has been an adequate time period for stabilization of the ^{15}N in soil and establishment of reference plants. Third, isotope dilution using ^{15}N fertilizer immobilized in organic form in the soil is a viable approach for long-term investigation of N_2-fixation by alder in the field. One distinct advantage of this use of long-term ^{15}N dilution is that it is well suited to the kinds of successional changes that occur in forestry settings.

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