Oecologia (2001) 126:94–103
DOI 10.1007/s004420000500

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Symbiotic N_2 fixation of *Alnus incana* ssp. *rugosa* in shrub wetlands of the Adirondack Mountains, New York, USA

Received: 23 November 1999 / Accepted: 17 July 2000 / Published online: 3 November 2000

Abstract Surface waters in forested watersheds in the Adirondack Mountains and northern New York State are susceptible to nitrogen (N) saturation. Atmospheric deposition of N to watersheds in this region has been measured but the extent of internal N inputs from symbiotic N_2 fixation in alder-dominated wetlands is not known. We estimated N_2 fixation by speckled alder in these wetlands by the ^{15}N natural abundance method and by acetylene reduction using a flow-through system. Foliar N derived from fixation ($\%N_{dfa}$) was estimated for five wetlands. The $\delta^{15}N$ of speckled alder foliage from four of the five sites did not differ significantly ($P \leq 0.05$) from that of nodulated speckled alders grown in N-free water culture (–1.2±0.1‰). Estimates from the ^{15}N natural abundance method indicated that alders at these sites derive 85–100% of their foliar N from N_2 fixation. At one of the sites, we also measured biomass and N content and estimated that the alder foliage contained 43 kg N ha^{-1} of fixed N in 1997. This estimate was based on a foliar N content of 55.4±7 kg N ha^{-1} (mean±SE), 86±4%N_{dfa}, and an assumption that 10% of foliar N was derived from reserves in woody tissues. At this site, we further estimated via acetylene reduction that 37±10 kg N ha^{-1} was fixed by speckled alders in 1998. This estimate used the theoretical 4:1 C_2H_2 reduction to N_2 fixation ratio and assumed no night-time fixation late in the season. Nitrogen inputs in wet and dry deposition at this site are approximately 8 kg N ha^{-1} $year^{-1}$. We conclude that speckled alder in wetlands of northern New York State relies heavily on N_2 fixation to meet N demands, and symbiotic N_2 fixation in speckled alders adds substantial amounts of N to alder-dominated wetlands in the Adirondack Mountains. These additions may be important for watershed N budgets, where alder-dominated wetlands occupy a large proportion of watershed area.

Keywords Actinorhizal plants · *Alnus* · Nitrogen fixation · Adirondack Mountains

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Introduction

The actinorhizal shrub *Alnus incana* ssp. *rugosa* (speckled alder) forms a root nodule symbiosis with N_2-fixing actinomycetes of the genus *Frankia*, and dominates some shrub and riparian wetlands of northeast North America (Furlow 1979). Speckled alder is the dominant species of the second largest wetland covertype [Scrub-Shrub 1 (Cowardin et al. 1979)] in the Adirondack Mountains (Roy et al. 1996), a region with surface waters that are susceptible to N saturation or excess N (Stoddard 1994). Nevertheless, N_2 fixation in speckled alder has not been quantified in Adirondack wetlands.

A wide range of N_2 fixation has been reported for speckled alder in other ecosystems. Voigt and Stuecek (1969) and Daly (1966) measured N accretion of 85–167 kg N ha^{-1} $year^{-1}$ in speckled alder stands, indicating high rates of N_2 fixation. In contrast, low rates of fixation have been estimated in speckled alder in pure stands (5 kg N ha^{-1} $year^{-1}$) or mixed stands with *Populus tremuloides* (1 kg N ha^{-1} $year^{-1}$) in northern Wisconsin (Younger and Kapustka 1983).

There are two practical ways of estimating N_2 fixation in woody plants in the field: ^{15}N natural abundance and acetylene reduction. Like all estimates of N_2 fixation in the field, they are accompanied by high levels of uncertainty (Winship and Tjepkema 1990; Binkley et al. 1994). The success of ^{15}N natural abundance to estimate

tissue N derived from fixation (Shearer and Kohl 1993) relies on significant differences in the ^{15}N of soil N, incorporated into tissues of non-fixing reference plants, and N_2 incorporated in tissues of fixing plants grown without other N sources. The method is also susceptible to error based on characteristics of non-N_2-fixing reference plants (Högberg 1997). The acetylene reduction assay estimates nitrogenase activity over short time periods, and so must be repeated to capture seasonal and diurnal patterns. Acetylene reduction involves reduction of C_2H_2 to C_2H_4 by nitrogenase, and so requires a conversion ratio for acetylene reduction to N_2 fixation. This ratio may be determined experimentally using $^{15}N_2$, but more often is assumed based on the minimum theoretical value of 4:1 (Schwintzer and Tjepkema 1994). Because speckled alder nodules exhibit a pronounced acetylene-induced decline, the initial peak rate is the only reliable measure of nitrogenase activity, and this can only be measured in an open flow-through system (Schwintzer and Tjepkema 1997).

Fixation of N_2 by alders, as well as by other actinorhizal plants such as *Myrica gale* (Schwintzer 1983), may be an important N input to wetlands and forested watersheds of the Adirondack Mountains and other watersheds of eastern North America. Accounting for natural N inputs in wetland areas is critical if we are to attempt to predict effects of anthropogenically derived N on sensitive ecosystems, particularly if riparian or wetland ecotones are considered control points for watershed N transport. Watershed-level predictions based on up-slope forest characteristics and downstream chemistry (e.g., time to or stage of N saturation, and wetland influence on watershed N retention) could be altered substantially by speckled alder wetlands juxtaposed between these points, where alders may add N through fixation, accelerate nitrification processes (Van Miegroet and Cole 1984), and use little soil-derived N (Mead and Preston 1992).

Symbiotic N_2 fixation in alders of areas such as the Adirondack Mountains, which receive elevated N in atmospheric deposition, may be reduced if inorganic N accumulates to high soil concentrations. Symbiotic N_2 fixation decreases in *Alnus* spp. subjected experimentally to high concentrations of N (Huss-Danell and Hahlin 1988). However, small, incremental N additions may maintain or even stimulate fixation in *Alnus* spp. (Stewart and Bond 1961; Ingestad 1980; Mackay et al. 1987), and the effects of inorganic N on fixation in *Alnus* in the field are less clear (Binkley et al. 1994).

This study provides the first field estimates for symbiotic N_2 fixation in speckled alder of the Adirondack Mountains. The objectives of this study were to estimate: (1) the fraction of alder foliage N derived from fixation in five alder scrub-shrub wetlands of northern New York State, a region where elevated N from atmospheric deposition might be expected to reduce the dependence of N_2-fixing plants on atmospheric N_2, and (2) rates of symbiotic N_2 fixation in a alder shrub wetland representative of the second largest wetland type in major drainage basins of the Adirondack Mountains (Roy et al. 1996).

Materials and methods

Site locations

Natural abundance ^{15}N dilution

Five alder wetlands were selected to estimate the contribution of atmospheric N_2 to foliar N in alder shrubs by the ^{15}N natural abundance method (Table 1). Three wetlands were on the Huntington Wildlife Forest in the central Adirondacks, one of which was used for the acetylene reduction assay (Table 1). The Huntington Wildlife Forest is a National Atmospheric Deposition Program and National Trends Network (NADP/NTN) monitoring site (NY20), and has been the locus of many biogeochemical studies (Raynal et al. 1985; Johnson and Lindberg 1992; Mitchell et al. 1994, 1996). Soils, surficial and bedrock geology at Huntington Wildlife Forest are typical of the Adirondack region and are described in Ohrui et al. (1999). Mean annual temperature is 4.4°C, with a dormant season mean of –2.8°C and a growing season mean of 14.3°C. Mean annual precipitation is 101 cm (Shepard et al. 1989). Vegetation on the upper slopes is mixed northern hardwood forest. The lower slopes are characterized by eastern hemlock [*Tsuga canadensis* (L.) Carr.], red spruce (*Picea rubens* Sarg.), balsam fir [*Abies balsamea* (L.) Miller] and yellow birch (*Betula alleghaniensis*). Nitrogen inputs in wet deposition at Huntington Wildlife Forest are approximately 5 kg N ha^{-1} $year^{-1}$ [National Atmospheric Deposition Program (NRSP-3)/National Trends Network 1999], with an additional 3 kg N ha^{-1} $year^{-1}$ added in dry deposition (Shepard et al. 1989).

Additional wetlands studied were located near Blue Mountain in the central Adirondacks, and near Altmar, Oswego County, N.Y., between Lake Ontario and the Tug Hill Plateau (Table 1). The latter site is within 8 km of the NADP/NTN Bennett Bridge monitoring site (NY52), which records the greatest N in wet deposition (approximately 10 kg N ha^{-1} $year^{-1}$) of any station in the national network [National Atmospheric Deposition Program (NRSP-3)/National Trends Network 1999].

Biomass estimation and the acetylene reduction assay

Biomass estimates for alder and the acetylene reduction assay were conducted in a 4-ha, riparian wetland dominated by speckled alder along Fishing Brook, at the Huntington Wildlife Forest (Table 1). This wetland is classified as Scrub-Shrub 1-Emergent 1 (SS1/EM1; Cowardin et al. 1979). The range in stem diameters of alders at Fishing Brook, measured 25 cm from the ground surface, was 0.5–12.1 cm, and the estimated stem density was 19,120±4,723 stems ha^{-1} (mean±SE, n=10) within the SS1 component of the wetland.

Biomass estimates

In July 1997, the biomass of above- and below-ground tissues of speckled alder was estimated at ten, randomly located points at Fishing Brook. At each point, stems >0.5 cm diameter were measured in 5×5 m plots. Foliar biomass, above-ground woody biomass, and annual increment were estimated from these measurements using allometric equations developed at Huntington Forest (Bischoff et al., in press; Hurd 1999). Roots and nodules were excavated along with soil to a depth of 25 cm in 0.5×0.5 m plots located 3 m away from large plots. No nodules or fine roots occurred below this depth due to the shallow depth of the watertable. Roots and nodules were washed, and hand sorted into root size classes (0–0.5, 0.5–3.0, >3.0 mm diameter), and living (firm, yellow) or dead (soft, brown) nodules, dried at 65°C, and weighed.

Table 1 Site locations (*HWF* Huntington Wildlife Forest) and descriptions for N_2 fixation study. *NADP/NTN* National Atmospheric Deposition Monitoring Program/National Trends Network for monitoring wet deposition chemistry: *NY20* site 20 (Huntington Wildlife Forest, Newcomb, N.Y.), *NY52* site 52 (Bennett Bridge, N.Y.). Methods: *ARA* acetylene reduction assay, using flow-through incubation of excised nodules with attached root at approximately 5 cm depth, 1998; *15N* ^{15}N natural abundance method to estimate the contribution of two N sources (N_2 and soil N) to alder foliage, 1997

Site (location)	Method	Coordinates	Elevation (m)	Distance from NADP/NTN site	Site description	Dominant reference species[a]
Fishing Brook (HWF)	ARA $^{15}N^3$	43°58′32″ N, 74°14′11″ W	485	1.1 km, 283° from NY20	Riparian	*Viburnum dentatum* var. *lucidum* (Caprifoliaceae)
Deer Pond (HWF)	^{15}N	44°02′33″ N, 74°14′40″ W	525	7.9 km, 347° from NY20	Riparian	*Corylus cornuta* (Betulaceae)
Deer Pond 10 (HWF)	^{15}N	44°01′42″ N, 74°13′57″ W	563	6.2 km, 352°, from NY20	Peatland, minimal surface flow	*Betula alleghaniensis* (Betulaceae)
Finch-Pruyn (Blue Mountain, N.Y.)	^{15}N	43°53′58″ N, 74°26′13″ W	562	19 km, 244° from NY20	Peatland, no surface flow[b]	*Betula papyrifera* (Betulaceae)
Kaine's (Altmar, N.Y.)	^{15}N	43°29′50″ N, 76°02′5″ W	183	7.8 km, 245° from NY52	Sandy, no surface flow	*Betula populifolia* (Betulaceae)

[a] Non-N_2-fixing reference species used in the natural abundance ^{15}N estimation of foliar N derived from fixation in speckled alders [b] Isolated from a low-order stream by a road

Percent N and C were determined in tissues from five plots with a Heraeus C-N analyzer.

Estimating % foliar N derived from fixation using natural abundance ^{15}N

Natural ^{15}N abundance was expressed in δ units, or parts per thousand (‰) deviation from the ^{15}N:^{14}N ratio of atmospheric N_2 (0.0036765) (Shearer and Kohl 1993). A two end-member mixing model was used to estimate percent N derived from the atmosphere ($\%N_{dfa}$) in alder foliage, using the $\delta^{15}N$ values of assimilated soil N, measured in non-fixing reference plant foliage in the field, of alder foliage in the field, and of assimilated N_2 measured in foliage of nodulated alders grown in an N-free medium (Shearer and Kohl 1993). The mean and standard error of $\%N_{dfa}$ were estimated for each of the five field sites, based on pair-wise sampling of fixing and reference plants (Shearer and Kohl 1993).

Foliage from ten, paired alder shrubs and non-N_2-fixing reference plants was collected from throughout the canopy using pole pruners, at each site in late August 1997. Mature but recently formed leaves were sampled, to avoid leaves formed early in the season that may have derived N from woody storage tissues (Domenach and Kurdali 1989). Non-fixing reference plants were selected based on relatedness, similarity of phenology and growth form, and proximity (stems within 1 m) to alder. The nomenclature for reference species follows Gleason and Cronquist (1991); for *Alnus*, Furlow (1979). At four of the five sites, reference plants belonged to the same family as alder, i.e., Betulaceae (Table 1). At the fifth site (Fishing Brook), *Viburnum dentatum* var. *lucidum* was used. We assumed that alders and reference plants were rooted in, and taking up the same form of N from the same soil N pool, and that isotopic fractionation of N during uptake was the same in alders and reference plants. A preliminary sample of buds and twigs was taken at one of the sites in May 1997, and of foliage at two of the sites in June 1997, to assess seasonal and species differences in ^{15}N. Tissues were dried at 65°C, and petioles removed from foliage to facilitate comparison of field plants and similarly processed reference plants with smaller leaf:petiole mass ratio. All dried tissues were ground twice in a Wiley mill to pass a 1-mm mesh screen, then further ground to a homogenous powder using a ball-and-capsule vibrating mill.

Speckled alder plants were grown from seed without N to measure the $\delta^{15}N$ of N fixed by alder. Seeds were collected at the Fishing Brook field site, pre-chilled for 14 days, surface sterilized in 30% v/v H_2O_2 for 10 min, and germinated in the light. Plants were inoculated with 20 µl ground nodule suspension (25 mg fresh weight ml^{-1}) from field nodules, then grown on glass beads for 2 weeks with N-free, 1/4-strength Hoagland's solution (Crocker and Schwintzer 1993), with P added at 1 mg l^{-1} as KH_2PO_4. Plants were transferred individually to 60-ml water culture vials with the same solution, and grown for 11 weeks in a growth chamber with a 16-h photoperiod (140–260 µmol m^{-2} s^{-1} PPF, 25°C light and 20°C dark). The solution was changed every 2 weeks, and brought to volume with deionized H_2O between changes. Leaves, stems, roots, and nodules were dried at 65°C, weighed, and ground to a homogenous powder in a vibrating mill.

Field samples from alder and reference plants were analyzed for $\delta^{15}N$ and %N at the stable isotope laboratory of Boston University (Finnigan Delta-S ratio mass spectrometer, Heraeus C-N analyzer). Samples from nodulated alders grown in –N culture were analyzed for $\delta^{15}N$ and %N at Cornell University (Europa Scientific GEO 20-20 isotope ratio mass spectrometer, Europa Scientific ANCA SL solid-liquid elemental analyzer). The $\delta^{15}N$ of NIST 1547 (peach leaves) was determined at both laboratories. Inter- and intralaboratory precision for $\delta^{15}N$ was ≤0.2‰. The $\delta^{15}N$ and N contents of component tissues of nodulated alders grown in –N solution were used to account for seed contribution to $\delta^{15}N$ (Shearer and Kohl 1993), using bulked samples for stem, root, and nodule tissue %N and $\delta^{15}N$. Tissues from alders at Fishing Brook were analyzed for %N to estimate N storage and above-ground increment. For the Fishing Brook site, fixation per unit area was estimated from $\%N_{dfa}$ and N content in alder foliage in 1997, assum-

ing that 10% foliar N was derived from root reserves (Domenach and Kurdali 1989). Mean differences between foliar $\delta^{15}N$ of field plants and that of nodulated alders grown in N-free culture were tested using Student *t*-tests (α=0.05) in SAS (1996).

Acetylene reduction

On eight dates between 12 May and 6 October 1998, nitrogenase activity was measured at Fishing Brook using a flow-through acetylene reduction system modified from Schwintzer and Tjepkema (1997). Seven groups of six- to ten-nodule clusters were excavated along with ≥2 cm attached, subtending roots, and adhering soil, in random locations excluding biomass plots. All assays were done between 11 a.m. and 4 p.m., immediately after collecting the nodules. Additionally, nitrogenase activity was measured at 7 p.m., 12 midnight, and 7 a.m. on 22–23 July, and at dawn on 17 September. A 60-ml syringe barrel was used as a cuvette, and inserted into the soil at a depth of 5–10 cm where nodules were typically found. A dual-probe thermistor thermometer (Cole-Parmer model 8402-20) was used to monitor temperature inside and outside the cuvette. A portable, peristaltic pump operated by an internal lithium battery (Cole/Parmer Masterflex L/S) was used to pump (1) humified air for a 10-min preincubation period, then (2) a 10% C_2H_2/humified air mixture through the cuvette. The C_2H_2 was generated from CaC_2 in the field. Gas bags were kept in a cooler and gas lines kept shaded. Flow rates were measured with a bubble flowmeter, and varied between samples from 270 to 350 ml min^{-1}, but were kept constant within samples. Gas samples were withdrawn at the outlet of the cuvette at 1, 2, 3, and 4 min after introduction of C_2H_2 with Hamilton Gastight no. 1002, 2.5-cm^3 glass sample-lock syringes in order to determine the initial peak rate of nitrogenase activity. These sampling times were selected because speckled alder growing in the field in Maine had its initial peak of nitrogenase activity 2–3 min after addition of acetylene (Schwintzer and Tjepkema 1997). Minus-nodule controls were included at each sample date, by incubating alder roots without nodules to detect other sources of ethylene. Small amounts of ethylene were detected in minus-nodule controls, and were subtracted from sample concentrations. Controls for loss of ethylene from syringes were included on four sampling dates, using a 10 ppm ethylene standard at the time of sampling and of analysis, an interval of approximately 20 h. Percent ethylene loss per 20 h from syringes was 0.5–2%, except for the 6 October sample date (17%, *n*=4). A Varian Star 3400 cx gas chromatograph (183 cm×0.64 cm outer diameter glass column, packed with Porapak N 80/100 mesh) with flame ionization detector was used to analyze gas samples for C_2H_4, calibrated with 10 or 100 (±5%) ppm C_2H_4 standards (Scott Specialty Gasses). A sample volume of 0.5 cm^3 was injected onto the column.

Assayed nodules were transported from the field in ice chests, washed, sorted into live (firm, yellow) and dead (soft, brown) categories, and dried to constant mass at 65°C. Specific nitrogenase activities per gram of live nodule dry mass were extrapolated to 24-h periods until midway between the 26 August and 17 September sampling dates, because night-time nitrogenase activity on 22–23 July was similar to day-time rates. After this time, rates were extrapolated to 12-h periods because little dawn nitrogenase activity was detected on 17 September. We assumed fixation commenced with bud break in early May, and ended with final leaf fall in October (Huss-Danell 1990), and that the acetylene reduction to N_2 fixation ratio for nitrogenase was the theoretical minimum of 4:1 (Simpson and Burris 1984; Bergersen 1991; Miller 1991; Huss-Danell et al. 1992; Schwintzer and Tjepkema 1994).

Peak rates of specific nitrogenase activities were multiplied by nodule mass per unit area to obtain N_2 fixation per unit area. Error estimates for each sample date were calculated by estimating the variance of the product of two independent random samples (Mood et al. 1974) from means and variances of nitrogenase activity on each sample date, and of nodule mass. Ninety-five percent confidence intervals for each sample date were then constructed from standard errors and *t*-values, and seasonal fixation was calculated by integrating under the seasonal curve.

Results

Alder biomass

Nitrogen concentrations and contents, and C:N ratios of live alder nodules, roots, foliage, and above-ground woody tissues at Fishing Brook are given in Table 2. Nitrogen content of roots and nodules was 16% of above-ground tissues. The annual, above-ground N increment was estimated as 75 kg N ha^{-1} $year^{-1}$, 74% of which was in foliar production (Table 2). Rates of below-ground N increment were not measured.

Estimating % foliar N from fixation using natural abundance ^{15}N

The $\delta^{15}N$ value of alder foliage in the field did not differ statistically from that of –N-grown alders, except at one site (Fishing Brook), and there was little or no variability in $\delta^{15}N$ of alder foliage between sites (Fig. 1). The $\delta^{15}N$ value of reference foliage at all sites was significantly different from that of –N-grown alders (Fig. 1). Reference foliage at three sites had greater foliar $\delta^{15}N$ than field alders: *Corylus cornuta* at Deer Pond; *V. dentatum* var. *lucidum* at Fishing Brook, and, *B. populifolia* at Kaine's. Reference foliage at two sites was more depleted in ^{15}N: *B. alleghaniensis* and *B. papyrifera* at Deer Pond 10, and at Finch-Pruyn.

The $\delta^{15}N$ value of alder buds collected at Kaine's in May 1997 was –1.1±0.1‰, identical to that of foliage in August. The foliar sampling conducted in June 1997 showed that *B. alleghaniensis*, *B. papyrifera*, *Acer rubrum*,

Table 2 Percent nitrogen, carbon to nitrogen ratio, N content, and annual N increment per hectare for speckled alder tissues at Fishing Brook, Huntington Wildlife Forest, N.Y., in 1997. Values are the mean±SE. Sample *n*=5 for %N and C:N, and 10 for the biomass component of N content and increment (*n.m.* not measured)

	Foliage	Wood	Roots (0–0.5 mm)	Roots (0.5–3.0 mm)	Roots (>3.0 mm)	Nodules
%N	2.69±0.06	1.02±0.03	1.70±0.08	1.00±0.03	0.80±0.02	3.28±0.16
C:N	18.68±0.37	49.61±1.68	27.95±0.76	49.97±1.73	60.91±1.56	15.98±0.70
kg N ha^{-1}	55.4±7.0	198.2±29.4	4.2±0.6	11.8±1.7	21.9±8.4	2.1±0.6
kg N ha^{-1} $year^{-1}$	55.4±7.0	19.4±2.6	n.m.	n.m.	n.m.	n.m.

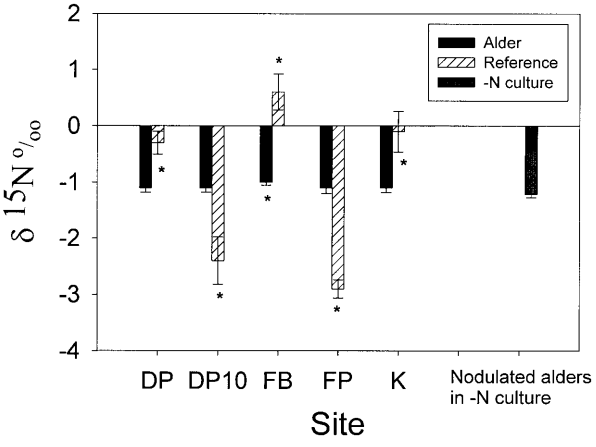

Fig. 1 The $\delta^{15}N$ of speckled alder foliage in the field (n=10 each site), non-fixing reference plant foliage in the field (n=10), and of alder foliage from nodulated speckled alders grown in N-free water culture (n=5). Values are means and SEs. *Asterisks* denote mean foliar $\delta^{15}N$ significantly different ($P \leq 0.05$) from that of nodulated alders grown in –N culture (*DP* Deer Pond, *DP10* Deer Pond 10, *FB* Fishing Brook, *FP* Finch-Pruyn, *K* Kaine's)

Table 3 Foliar $\delta^{15}N$ at Deer Pond 10 and Kaine's in June 1997. Values are the mean±SE, sample n=1–3

Species	$\delta^{15}N$‰
Deer Pond 10	
Betula alleghaniensis	–3.05±0.26
Betula papyrifera	–2.10
Acer rubrum	–4.40±1.40
Viburnum nudum var. *cassinoides*	–3.25±0.75
Kaine's	
Prunus pennsylvanica	–0.10
Betula populifolia	–0.13±0.50
Alnus incana ssp. *rugosa*	–0.93±0.40

and *Viburnum nudum* var. *cassinoides* were all relatively depleted in ^{15}N at Deer Pond 10, that *B. populifolia* and *Prunus pensylvanicum* $\delta^{15}N$ values were similar to one another at Kaine's, and that alder at Kaine's had foliar $\delta^{15}N$ values close to –1 early in the growing season (Table 3).

Alders at these sites derived 85–100% of foliar N from fixation (Fig. 2). At Fishing Brook, an estimated 43±20 kg foliar N ha^{-1} $year^{-1}$ (mean±SE) was derived from fixation, based on the product of foliar N content, corrected for 10% N from root reserves (Domenach and Kurdali 1989), and $\%N_{dfa}$, with the variance estimated following Mood et al. (1974).

Acetylene reduction

The peak rate of nitrogenase activity was observed within the initial 4 min after introduction of acetylene on all dates except in May and June, when the average time course of acetylene reduction had a low, upward slope, indicating that the value measured at 4 min was near the peak. Low levels of nitrogenase activity were detected in early May (Fig. 3C), when alder leaves had partially

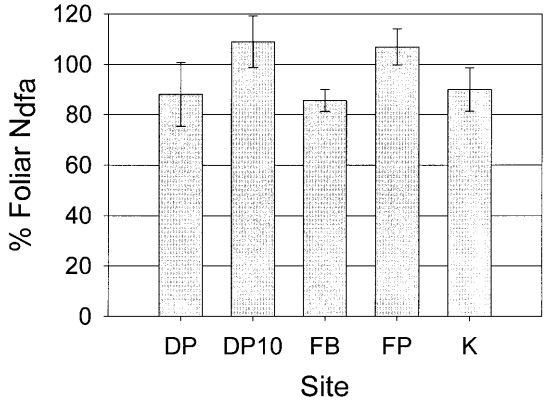

Fig. 2 Estimated %N derived from fixation ($\%N_{dfa}$) in foliage of alders from five field sites in northern New York State in 1997. Values are estimates and SEs determined from the ^{15}N natural abundance method (Shearer and Kohl 1993). Sample n=10 at each site (*DP* Deer Pond, *DP10* Deer Pond 10, *FB* Fishing Brook, *FP* Finch-Pruyn, *K* Kaine's)

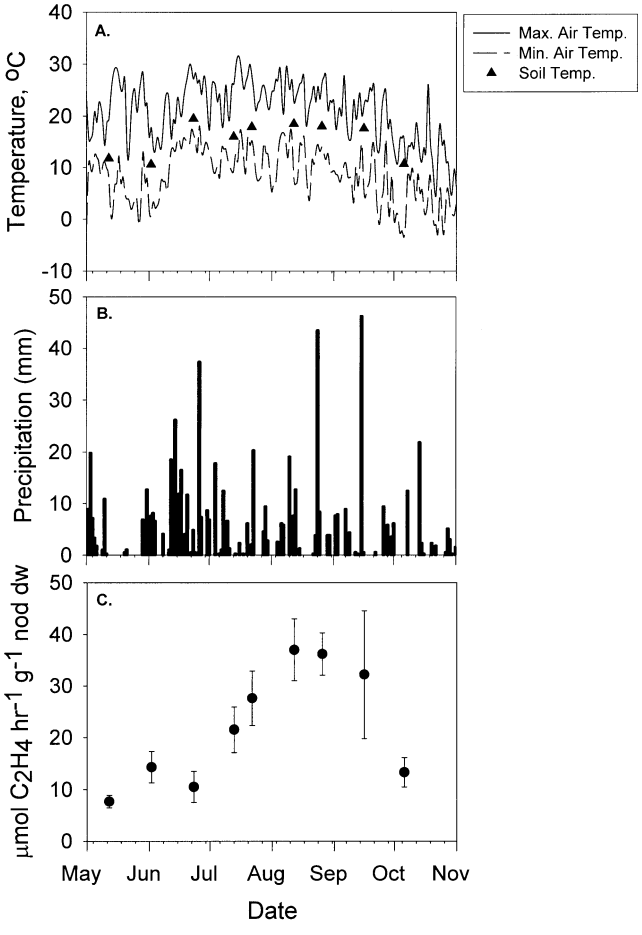

Fig. 3 Air and soil temperature (**A**), precipitation (**B**), and specific activity of nitrogenase (C_2H_4 production) (**C**) during the growing season of 1998 at Fishing Brook, HWF. Air temperature and precipitation data are from a meteorological station <1 km from the study site. Soil temperatures are means during the acetylene reduction assay. Values are means and SEs (n=7) for mid-day rates, except for 22–23 July, which is the mean night-time value (n=9) of samples taken at 7 p.m., 12 midnight, and 7 a.m.

emerged. The specific activity of nitrogenase peaked at 37±6 µmol C_2H_4 h^{-1} g^{-1} in early August (Fig. 3C). Low rates in late June corresponded with frequent precipitation, and depressed air temperatures (Fig. 3). Specific activity remained elevated through August and early September, then decreased sharply through leaf senescence (Fig. 3C). Rates measured during the night and at dawn on 22–23 July were 29±10 (7 p.m.), 29±7 (12 midnight), and 25±11 (dawn) µmol C_2H_4 h^{-1} g^{-1} (mean±SE, *n*=3), and were in line with the seasonal increase of day-time rates between June and August (Fig. 3C). Dawn rates on 17 September were very low: 2 µmol C_2H_4 h^{-1} g^{-1} (soil temperature 13.4°C) vs 32 µmol C_2H_4 h^{-1} g^{-1} (soil temperature 17.6°C) measured during the previous day.

Based on an active season of 170 days and the assumptions stated above, we estimate that symbiotic N fixation in this system resulted in inputs of 37±10 kg N ha^{-1} in 1998 (integration of mean and 95% confidence interval seasonal curves).

Discussion

Alder biomass

Estimates of alder biomass are based on area of Scrub-Shrub 1 cover type only, and do not include emergent marsh and open water areas of the wetland. Large stems (>5 cm diameter) contributed large fractions of above-ground biomass and N content to overall totals. Nodule biomass of 65 kg ha^{-1} was lower than that of *Alnus viridis* ssp. *sinuata* shrubs and European *A. incana* ssp. *incana*, but similar to that of *A. incana* ssp. *rugosa* in mineral clay soils of northern Wisconsin (Table 4).

Total above-ground N increment was estimated as 75 kg ha^{-1} $year^{-1}$ (Table 2). The biomass increment of reproductive structures in a similar speckled alder wetland was estimated as 98 kg ha^{-1} $year^{-1}$ (Tilton and Bernard 1975), which would add an additional 2.4 kg N ha^{-1} $year^{-1}$ based on %N of 2.4, measured in seeds.

Estimating % foliar N from fixation using natural abundance ^{15}N

Alder foliage from the five wetlands had very similar $\delta^{15}N$ values, and the shrubs appeared to be deriving 85–100% foliar N from fixation (Figs. 1, 2), despite elevated atmospheric N deposition in the region. Soil N apparently does not accumulate to sufficiently high levels in these systems to decrease the dependence of alders on N_2. Concentrations of combined NO_3^- and NH_4^+ in 25 cm groundwater in an alder wetland at Huntington Wildlife Forest was approximately 40–60 µmol l^{-1} during the growing season (Hurd 1999).

This estimate of 85–100%N_{dfa} matches that for *A. incana* ssp. *incana* from four sites along a successional sequence in France (Domenach et al. 1989). Calculation of fixation per unit area (43 kg N ha^{-1} $year^{-1}$) based on

Table 4 Nitrogenase activity, estimated N_2 fixation, and nodule mass of several shrubby species of *Alnus*

Alder species	Nitrogenase activty (µmol C_2H_4 h^{-1} g^{-1} nodule dry weight)	Adjusted estimate of N_2 fixation (kg N ha^{-1} $year^{-1}$)[a]	Nodule mass (kg ha^{-1})	Assay system	Site description	Geographic location	Reference
A. incana ssp. *rugosa*	37[c]	37	65	Open	Riparian wetland	New York, USA	This study
A. incana ssp. *rugosa*	<10[c]	1–4	58	Closed	Clay soil, alder, or poplar/alder	Wisconsin, USA	Younger and Kapustka 1983
A. incana ssp. *tenuifolia*	38[d]			Closed	River floodplain willow/alder	Alaska	Schimel et al. 1998
A. incana ssp. *incana*[b]	<15[d]	32	150	Closed	Mineral soil, 30-year-old stand	Southern Norway	Johnsrud 1978
A. incana ssp. *incana*[b]	43[c]	20	44	Closed[e]	Sandy experimental plot, 2-year-old stand	Umeå University, Sweden	Huss-Danell and Ohlsson 1992; Huss-Danell et al. 1992
A. viridis ssp. *sinuata*	36[c]			Closed	4- to 8-year-old clear cut	Vancouver Island, B.C.	Binkley 1981
A. viridis ssp. *sinuata*	20[c]	15	110	Closed	15-to 20-year-old Douglas fir/sitka alder/red alder	Vancouver Island, B.C.	Binkley 1981
A. viridis ssp. *viridis*	<7[c]	6	43	Closed	High altitude (1,800 m)	France	Moiroud and Capellano 1979

[a] Adjusted for a 4:1 ratio of acetylene reduction to N_2 fixation
[b] *A. incana* ssp. *incana* is a tree species
[c] Seasonal peak of nitrogenase activity
[d] Mean rate of nitrogenase activity
[e] Intact plants grown in large-diameter PVC cuvettes

foliage alone for Fishing Brook in 1997 was similar to the estimate of 37 kg N ha^{-1} $year^{-1}$ from the acetylene reduction assay in 1998. Root reserves were estimated to contribute 10% of foliar N to *A. glutinosa* in France by the end of the growing period, with the highest contribution to leaves formed early in the season (Domenach and Kurdali 1989). We sampled mature, recently formed leaves at the end of the growing season, and our estimate assumes a constant $\%N_{dfa}$ throughout the season and 10% of annual foliar N from root reserves. The $\delta^{15}N$ value of alder buds collected in May at Kaine's was identical (1.1±0.1‰, mean±SE) to August foliar values, and that of alder foliage in June at Kaine's was similar to August values and values for N fixed in foliage (Fig. 1, Table 3). Moreover, N was not retranslocated from foliage in a similar *A. incana* ssp. *rugosa* stand at Huntington Wildlife Forest (Bischoff et al., in press), hence there is probably little reliance on N reserves in these plants. We did not include fixed N in stems and roots, thus our estimate of 43 kg N ha^{-1} $year^{-1}$ probably underestimates fixation.

The $\delta^{15}N$ value of fixed N in foliage was –1.2±0.1‰, similar to the value of approximately –1.4‰ in *A. incana* ssp. *incana* (Domenach et al. 1989). The $\delta^{15}N$ value of alder foliage in four of five field sites did not differ significantly from that of plants which obtained all N from fixation, and averaged –1.1‰ at each of these sites (Fig. 1). At the fifth site, the difference between the $\delta^{15}N$ value of alder foliage in the field and in plants which obtained all N from fixation was only 0.2‰ (Fig. 1). These results suggest strongly that alders in these systems rely heavily on N_2 fixation for foliar N. Field studies in France with *A. glutinosa* (Domenach et al. 1992) and in subarctic forest of northern Sweden with *A. incana* ssp. *incana* (Michelsen et al. 1998) also found mean foliar $\delta^{15}N$ near –1.1‰, with low variance, suggesting strong dependence on N_2 for growth in these sites. The foliar $\delta^{15}N$ of *Alnus rubra* seedlings inoculated with familiar or unfamiliar *Frankia* strains, and planted in sites of varied elevation and soil N, was also –1.1 to –1.3‰ (Markham and Chanway 1999). The foliar $\delta^{15}N$ of *A. incana* ssp. *incana* along a successional sequence in France was –1.4 to –2‰ (Domenach et al. 1989). In contrast, foliar $\delta^{15}N$ of older *A. rubra* at four sites, with varying total and inorganic ^{15}N of soil, varied by site between –2.8 and 4.5‰ (Binkley et al. 1985).

The two-component model using natural abundance ^{15}N dilution to estimate $\%N_{dfa}$ must be applied cautiously due to the assumptions that fixing plants and non-fixing reference plants in the field are taking up N (1) from the same soil N pool, (2) in the same form, and (3) without differential isotopic fractionation. It has been suggested that in order to apply the method quantitatively, the $\delta^{15}N$ of reference plants must differ from that of N_2-fixing reference plants by ≥5‰, and variability in $\delta^{15}N$ of reference species within sites must be low or explainable (Högberg 1997). These criteria are recommended because smaller differences can be caused by differences in rooting patterns and in uptake preferences for different N species. Presence or type of mycorrhizal association is also correlated with species foliar $\delta^{15}N$, with foliar $\delta^{15}N$ of non-mycorrhizal plants >ectomycorrhizal >ericoid mycorrhizal plants (Michelsen et al. 1998). Therefore mycorrhizal differences between fixing and non-fixing reference plants could result in differential fractionation of sequestered soil N, thus violating assumption 3 above.

The greatest difference in this study between the two end-member $\delta^{15}N$ values was approximately 2‰; however, the estimates were not greatly sensitive to changes in the non-fixing, reference plant value. Our estimate was much more sensitive to the $\delta^{15}N$ value of fixed N than to $\delta^{15}N$ of non-fixing reference plants in the field, due particularly to the small difference between end-member values.

Only one species of reference plant was used at Deer Pond, Fishing Brook, and Kaine's (Table 1). At Deer Pond 10, reference species were *B. alleghaniensis*, *B. papyrifera* and *C. cornuta* (Betulaceae). At Finch-Pruyn, the reference species were *B. papyrifera* and *B. alleghaniensis*. Sampling a broader diversity of reference plants was not possible due to the low diversity of woody plants in the alder-dominated systems. We believe that the differences in $\delta^{15}N$ of reference plants between sites reflect site differences in ^{15}N of soil, because preliminary sampling showed similar $\delta^{15}N$ of different species within sites (Table 3). Site differences in reference plant $\delta^{15}N$ may have also resulted from the uptake of different species of N. Nevertheless, $\delta^{15}N$ of alder tissues across sites did not respond to such differences if they occurred.

The Betulaceae commonly form ectomycorrhizae, and similar Hartig nets confined to the epidermal layer have been observed in *A. incana* ssp. *rugosa*, and in the genera *Betula*, *Carpinus*, and *Corylus* (Godbout and Fortin 1983). Hence, we suspect that modification of the soil ^{15}N signature by mycorrhizae was similar between alders and reference plants of the Betulacaeae. Ectomycorrhizae are associated with depleted $\delta^{15}N$ values in associated plants (Michelsen et al. 1998), and ectomycorrhizae were commonly observed on alder root systems at Fishing Brook during the acetylene reduction assay. However, $\delta^{15}N$ of alder foliage was still very similar to alder-fixed N at this site (Fig. 1).

The rooting depth of alder and reference plants was similar at all of these wetland sites, because the low depth to the watertable confined roots to shallow soils. Indeed, pairs of alder and reference plants were often rooted on the same microtopographic hummocks. Above-ground phenology was also similar between alder and reference plants, suggesting similar periods of N uptake.

We did not account for any transfers of fixed N that may have occurred between alder and reference plants through decomposition of litter (van Kessel et al. 1994; Baker et al. 1995), or mycorrhizal transfer, which might be expected to occur if the plants shared common ectomycorrhizal mycelia (Arnebrant et al. 1993). Such trans-

fer could reduce the estimate of $\%N_{dfa}$, by convergence of $\delta^{15}N$ values between the fixing and reference plants through time. However, such a transfer by mycorrhizae has shown to be of no biological importance in experimental studies (Ekblad and Huss-Danell 1995). Most N fixed in the previous growing season is likely lost through leaching and flooding during the dormant season in alder-dominated riparian systems. The $\delta^{15}N$ of reference plants was possibly reflecting N enrichment of soil N in riparian (Fishing Brook, Deer Pond) or sandy (Kaine's) sites where N losses may have been greater than in organic sites with little surface water drainage (Finch-Pruyn, Deer Pond 10).

The acetylene reduction assay

Nitrogenase activity at Fishing Brook peaked in early August in 1998, following a cool, wet spring and early summer. In contrast, speckled alder in Maine had much higher specific activities in late June 1994 (74 µmol C_2H_4 g^{-1} h^{-1}) than in August (21 µmol C_2H_4 g^{-1} h^{-1}) (Schwintzer and Tjepkema 1997). Acetylene reduction activity in *Alnus* nodules responds both to current and previous environmental conditions (Huss-Danell 1997), and low rates in late June followed a period of low temperatures and frequent rainfall (Fig. 3). Nevertheless, seasonal patterns in nitrogenase activity may have reflected factors that were not considered in this work. Peak rates for *A. incana* ssp. *rugosa* in *Populus*/*Alnus* stands of northern Wisconsin were very low (Table 4) and were measured using 12-h incubations in a closed system demonstrating high C_2H_4 loss. Rates for early successional *A. incana* ssp. *tenuifolia* and young *A. incana* ssp. *incana* were very similar to our peak (Table 4). Peak rates for 4- to 8-year-old *A. viridis* ssp. *sinuata* were also very similar to those of our study, but those of older plants were lower (Table 4). Nodule age explained variability in specific activity of nitrogenase in nodules of *Alnus nepalensis* in plantations in the eastern Himalayas (Sharma and Ambasht 1984), with young nodules exhibiting peak rates >1,800 µmol C_2H_4 g^{-1} h^{-1}, but oldest nodules producing <50 µmol C_2H_4 g^{-1} h^{-1}. We also observed highest rates in sample units comprised of younger nodule clusters; however, this effect was averaged in our study by including randomly encountered, multiple-nodule clusters of different size classes in each assay.

The error estimates for seasonal fixation incorporate only the sample errors in the assay and nodule biomass estimate. There is additional unmeasured uncertainty due to diurnal variation in nitrogenase activity, day-to-day variation due to temperature and possibly cloudiness, poor resolution of peak rates in the spring, seasonal change in active nodule mass, and the assumption of the 4:1 conversion ratio for nitrogenase. The 43 kg ha^{-1} $year^{-1}$ estimate found by Johnsrud (1978) for *A. incana* ssp. *incana* in Norway, when adjusted based on a 4:1 conversion ratio of acetylene reduction to N_2 fixation, was very similar to our estimate of 37 kg ha^{-1} $year^{-1}$ (Table 4). Our estimate was much greater than those of speckled alder estimated in northern Wisconsin (Table 4), but less than N accretion estimated for speckled alder stands by Voigt and Stuecek (1969) (85 kg ha^{-1} $year^{-1}$) and especially Daly (1966) (170 kg ha^{-1} $year^{-1}$). The low estimates in northern Wisconsin may have been a result of nitrogenase inhibition by associated poplar (Younger and Kapustka 1983; Schimel et al. 1998). Closed assay systems likely decreased estimates in previous studies because of the C_2H_2-induced decline (Schwintzer and Tjepkema 1997). Acetylene reduction probably underestimated N_2 fixation at Fishing Brook, because 37 kg N ha^{-1} $year^{-1}$ is only 67% of annual foliar N production and 49% of the estimated total aboveground N increment, yet ^{15}N results suggest that most foliar N is derived from fixation. Our estimates of N_2 fixation via ^{15}N natural abundance and acetylene reduction are roughly similar. However, they cannot be directly compared because they were measured in different years, and year-to-year variations in N_2 fixation and biomass production may have occurred.

Conclusions

We conclude that *A. incana* ssp. *rugosa* shrubs of northern New York State obtain large fractions of foliar N from fixation, and estimate that at least 37–43 kg N ha^{-1} $year^{-1}$ is fixed by this species in a riparian shrub wetland of the central Adirondack Mountains. Fixation per unit total wetland area is probably less, because the extent of other vegetation cover types and open water within wetland complexes varies. Nevertheless, N inputs from actinorhizal fixation can contribute large amounts of N to watersheds if alder-dominated wetlands occupy a large area. Studies using surface water N chemistry to understand time to or stage of N saturation, and wetland influence on watershed N retention should account for this large internal N source in wetland ecotones dominated by actinorhizal plants. On-going studies are estimating the abundance of alder-dominated wetlands in the Adirondack region, determining whether alders increase N in wetland and surface waters, and evaluating the effects of increased available N on N_2 fixation in speckled alder.

Acknowledgements This research was funded by a USDA-CSRS McIntire-Stennis grant for research in forest science and by contract with Niagara Mohawk Power Corporation Inc. (NMPC). We are grateful to Drs. Ed Neuhauser and Martin Smith of NMPC for their interest and support of this project. We thank Amanda Elliott for assistance in the field and laboratory; John Tjepkema and Greg Boyer for assisting T.M.H. with the acetylene reduction assay; Robert Michener and staff of the Stable Isotope Laboratory of Boston University, and James W. Burdett, Christopher Alpha, and staff of the Cornell Laboratory for Stable Isotope Analysis; the staff of Huntington Wildlife Forest for weather data and field support; Finch-Pruyn Inc. and Lilian Kaine for access to field sites; and Drs. Myron Mitchell, Donald Leopold, Christopher Cirmo, Russell Briggs, and two anonymous reviewers for comments on an earlier draft of the manuscript.

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