

## LIMITATIONS TO SYMBIOTIC NITROGEN FIXATION IN PRIMARY SUCCESSION ON THE TANANA RIVER FLOODPLAIN

DANIEL D. ULIASSI<sup>1</sup> AND ROGER W. RUESS<sup>1,2</sup>

<sup>1</sup>Department of Biology and Wildlife, University of Alaska, Fairbanks, Alaska 99775 USA

<sup>2</sup>Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99775 USA

**Abstract.** Constraints on nitrogen fixation are the ultimate causes of N limitation of primary production, but hypotheses concerning limitations to N<sub>2</sub> fixation remain largely untested in natural terrestrial ecosystems. We examined limitations to N<sub>2</sub> fixation by thinleaf alder (*Alnus tenuifolia*) in two stages of primary forest succession on the Tanana River floodplain (interior Alaska, USA) and focused on the hypothesis that N<sub>2</sub> fixation was limited by low soil P availability. Paired control and P fertilized plots were established at four replicate early successional alder stands and four later successional poplar (*Populus balsamifera*) stands (dense alder understories with mature poplar overstories) and N<sub>2</sub> fixation was estimated with an acetylene reduction assay. In alder stands, P fertilization increased total nodule dry biomass and increased total ecosystem N inputs, but it had little effect on nitrogenase activity per unit nodule dry mass (specific acetylene reduction activity, ARA). Specific ARA increased only in late July when soil temperature and ARA were at their maximum values. In contrast, fertilization had no effect on these measures in poplar stands where reduced soil moisture may have superseded limitation by P. We detected no differences in specific ARA, total nodule biomass, or N inputs, between alder and poplar stands but all of these measures were highly variable. Leaf area of the alder canopy emerged as the best predictor of ecosystem inputs of fixed N among control plots. Alders resorbed high amounts of P but little N (consistent with low P availability and a high P demand and a high N availability in alder), and P fertilization reduced P resorption but had no effect on N resorption. The timing of N<sub>2</sub> fixation and N resorption indicate that late-season increases in leaf N, following a midseason reduction in leaf N, were driven by N<sub>2</sub> fixation in excess of plant N demands as nodules continued fixing N while alder leaves senesced. These results have shown that P limits N<sub>2</sub> fixation in alder stands in this nitrogen-limited sere, but that factors limiting N<sub>2</sub> fixation can change over short successional time scales.

**Key words:** Alaska; alder; *Alnus*; allelopathy; nitrogen fixation; nitrogen retention; nodule biomass; phosphorus; *Populus*; primary succession; resorption; Tanana River floodplain.

### INTRODUCTION

Constraints on nitrogen fixation are the ultimate causes of N limitation of primary production (Vitousek and Howarth 1991), but hypotheses concerning limitations to N<sub>2</sub> fixation remain largely untested in natural terrestrial ecosystems. Primary succession frequently proceeds through early stages dominated by symbiotic N<sub>2</sub>-fixing plants (N fixers) (Walker 1993). Nitrogen inputs from these stages may determine both long term productivity in N-limited seres (e.g., Van Cleve et al. 1983, 1991, Vitousek and Walker 1989, Bormann and Sidle 1990) and the rate of succession (Chapin et al. 1994). Estimates of N<sub>2</sub> fixation during succession are generally inferred from measures of N accumulation across chronosequences (e.g., Crocker and Major 1955, Viereck 1966, Van Cleve et al. 1971, 1993a, Luken and Fonda 1983, Frenzen et al. 1988, Walker 1989). However, these provide only a measure of N retention (N inputs – N loss) and therefore are not a sensitive index

of N<sub>2</sub> fixation. We used acetylene reduction to examine the dynamics and regulation of N<sub>2</sub> fixation by thinleaf alder (*Alnus tenuifolia* Nutt.) in primary successional forests of the Tanana River floodplain (interior Alaska, USA) and focused on the hypothesis that N<sub>2</sub> fixation is limited by low phosphorus availability.

Cole and Heil (1981) argued that terrestrial N and P cycles should be tightly coupled because of the energy demands of microbial growth and correlations in the transformations and availability of these elements over long time periods. N<sub>2</sub> fixation is hypothesized to be a primary mechanism linking N and P availability in terrestrial ecosystems (Walker and Syers 1976, Cole and Heil 1981, Vitousek and Howarth 1991, Vitousek et al. 1993, Crews et al. 1995). Nitrogen fixers have a potentially higher P demand than non-N fixers (Ingstad 1981), and respond strongly to P fertilization in greenhouse experiments (e.g., Gates 1974, Israel 1987, Reddell et al. 1988, Sanginga et al. 1989, Ekblad and Huss-Danell 1995, Uliassi et al. 2000). Moreover, increasing P availability can reverse N inhibition of N<sub>2</sub> fixation, indicating that the ratio of the two nutrients is more important than their absolute quantity per se (Ekblad

and Huss-Danell 1995, Ekblad et al. 1995). Direct support for P limitation of N<sub>2</sub> fixation in ecosystems comes mainly from aquatic ecosystems (Schindler 1977, Vitousek and Howarth 1991), pasturelands and agro-ecosystems (e.g., Donald and Williams 1954, Crews 1993, Peoples et al. 1995), and young volcanic sites in Hawaii (Vitousek 2000), but there is additional support from nonsymbiotic fixation in a tallgrass prairie (Eisele et al. 1989), and correlations between P availability, or N:P ratios, and the distribution of N fixers (Högberg 1989).

Phosphorus availability is low throughout primary succession on the Tanana River floodplain (Marion et al. 1993) and may limit N<sub>2</sub> fixation by thinleaf alder. However, P availability alone is unlikely to explain the seasonal and successional dynamics of N<sub>2</sub> fixation. Canopy closure over symbiotic N fixers likely reduces their abundance and is often assumed to limit their ability to fix N<sub>2</sub> (e.g., Bormann and Gordon 1984) because of the high carbon cost of maintaining root symbionts (Gutschick 1981, Paul and Kucey 1981, Warmbourg and Roumet 1989). Carbon gain is positively correlated with fixation in young plants deriving most of their N from fixation (Huss-Danell and Sellstedt 1985, Huss-Danell 1990, Huss-Danell et al. 1992), but this relationship may not hold with increasing availability of external N, changes in carbon allocated to mycorrhizae, and limitations by other resources. Along the Tanana River floodplain, increasing dominance of balsam poplar (*Populus balsamifera*) may decrease light reaching the alder canopy but also increase competition for soil resources (Walker and Chapin 1986). Poplar litter leachates reduce N availability on the floodplain (Clein and Schimel 1995, Schimel et al. 1995) and they may also inhibit N<sub>2</sub> fixation either directly, or indirectly by complexing soil P or inhibiting mycorrhizal functioning (Rice 1974, Jobidan and Thibault 1982, Younger and Kapustka 1983, Schimel et al. 1998). Although arctic and taiga plants may be adapted to low soil temperatures (Chapin and Shaver 1985), seasonal and successional changes in microclimate could modify patterns of N<sub>2</sub> fixation (e.g., Harrington and Seiler 1988, Zitzer and Dawson 1989; for review see Dixon and Wheeler [1983]).

Nutrient resorption is a primary mechanism of nutrient conservation in plants (Vitousek 1982, Shaver and Chapin 1991) and may influence alder's demand for, and ability to fix, N<sub>2</sub>. A relationship between nutrient resorption and nutrient availability is sometimes (Shaver and Melillo 1984, Lajtha 1987), but not always (Birk and Vitousek 1986, Chapin and Moilanen 1991, Walbridge 1991, Aerts 1996), found in natural or fertilized systems. Across taxonomic groupings, N fixers are generally poor resorbers of N and would be expected to resorb disproportionately higher amounts of P because of the potentially high P demand associated with N<sub>2</sub> fixation (Dawson and Funk 1981, Côté et al. 1989, Killingbeck 1993, 1996). A relationship between

N<sub>2</sub> fixation and nutrient resorption within a single N<sub>2</sub> fixing species could exert a strong control on rates of ecosystem N accretion and cycling.

This field study examines symbiotic N<sub>2</sub> fixation by thinleaf alder in early- (alder dominated) and mid- (poplar dominated) stages of primary forest succession on the Tanana River floodplain in interior Alaska. Our four primary objectives were to: (1) test the hypothesis that P limits N<sub>2</sub> fixation with a field fertilization experiment, (2) characterize the seasonal patterns of nitrogenase activity and successional changes in N<sub>2</sub> fixation inputs using an acetylene reduction assay in conjunction with estimates of nodule dry biomass, (3) identify primary regulators of these patterns and, (4) evaluate the relationship between N<sub>2</sub> fixation and N and P resorption.

#### STUDY SITE

This study was conducted in, and adjacent to, the Bonanza Creek Long Term Ecological Research Site (BNZ LTER) situated ~35 km southwest of Fairbanks, Alaska, on the active floodplain of the Tanana River. The Tanana River is a large, silt-laden, meandering river which drains the north slope of the Alaska range into the Yukon River. The climate is strongly continental and characterized by pronounced seasonal fluctuations in daylength, light intensity, and temperature, with mean daily temperatures ranging from 16.4°C in July to -24.9°C in January. Snow remains as a permanent cover for six to seven months of the year and the growing season is less than 100 d. Annual precipitation averages 269 mm, most of which falls in short duration thunderstorms. The climate and vegetation of this research site are described in detail by Viereck et al. (1993a, b).

Primary succession is initiated on newly deposited alluvium by several willow species (*Salix* spp.) and is dependent on species life history characteristics and their interactions with herbivory (Bryant 1987), the soil environment, and stochastic events including flooding and seed rain (Walker and Chapin 1986, Walker et al. 1986, Viereck et al. 1993a, Van Cleve et al. 1991). Willow are quickly replaced by alder within 5–10 yr, in part, because of selective herbivory by mammalian browsers (Bryant 1987). Balsam poplar eventually overtops alder and a mixed community develops with a mature poplar overstory and dense alder understory at 80 to 100 yr. Mature stands can persist for several decades but ultimately (100–150 yr) convert to white spruce (*Picea glauca*) and eventually black spruce (*Picea mariana*) (Viereck et al. 1993a, but see Mann et al. 1995).

New floodplain surfaces are extremely alkaline (pH > 7.5), resulting in low P availability throughout floodplain succession (Marion et al. 1993). Soil N and organic matter are low through the willow stage but rapidly increase during the transition to alder (Van Cleve et al. 1971, 1991, Walker 1989). Mass accumulation

estimates suggest that up to 70% of ecosystem N accumulated over this 200-yr sequence is fixed by alder within the first 30 yr of succession (Van Cleve et al. 1971, 1991). This is more rapid than in most other primary successional seres where N accumulation typically asymptotes within 50–200 yr (Reiners 1981, Walker 1993). This rapid N accretion is associated with increased mineralization and nitrification rates (Klingensmith and Van Cleve 1993), resulting in high N availability under alder thickets, which supports the rapid growth of poplar trees. Increasing poplar dominance reduces N availability by increasing N storage and reducing soil organic matter quality as the forest floor becomes dominated by recalcitrant poplar litter. Poplar litter contains high concentrations of tannins and phenolics of low molecular mass, which act in concert to reduce N availability by simultaneously inhibiting N mineralization and stimulating microbial immobilization (Clein and Schimel 1995, Schimel et al. 1995). Later in succession, the abundance of alder is further reduced with increasing spruce dominance. Nutrients are increasingly bound in slowly decomposing organic matter and soils cool and reduce rates of nutrient cycling (Van Cleve et al. 1991).

## METHODS

### *Experimental design*

In 1993 we selected four replicate stands in older alder communities (stands A1 through A4) and four in poplar communities with a mature poplar overstory and dense alder understory (stands P1 through P4) along a 10-km stretch of the Tanana River. These stands approximately correspond to stages IV–V and VI, respectively, in Viereck et al. (1993a). Names are assigned based on the dominant vegetation and sites will henceforth be called “alder” and “poplar” stands.

At each of the eight stands, we established a paired control and fertilized plot (each 15 × 15 m) for a total of 16 plots. Fertilized plots received a 2-yr total of 21.5 g P/m<sup>2</sup> applied by hand as commercially available granular superphosphate 0:45:0 fertilizer (Agrichem, Burlington, Washington, USA), in a series of two applications in 1993 and three applications in 1994. We initially fertilized plots in mid-June 1993, and then in mid-July 1993. In 1994 we fertilized in late May, mid-July, and early August. Fertilizer applications were always made following measurements for nitrogenase activity or the collection of soil cores so that measurements reflected changes from the previous fertilizer application. All measurements were made within a 14 × 14 m grid excluding a 1-m border around each plot to reduce potential edge effects.

### *Nitrogenase activity and nodule biomass*

We used an acetylene reduction assay in conjunction with measures of nodule dry biomass to estimate N<sub>2</sub> fixation (Lawrence and Tjepkema 1990, Uliassi et al.

2000). Nitrogenase activity was measured on plots twice in 1993 (25 July, and 4–6 September) and three times in 1994 (6–14 June, 5–11 July, and 30 July). Most measurements were taken at midday (between 1000 and 1600) Alaska standard time. Stands were randomly selected for measurement throughout a day, but control and fertilized plots at each stand were always measured consecutively. Acetylene reduction activity (ARA) was measured using a short term acetylene reduction assay, which was found to provide a linear production of ethylene with no apparent acetylene inhibition over the time course of our incubation.

We measured ARA three times (three sets of nodules collected from the perimeter of three haphazardly selected trees) within each plot at each sampling ( $n = 48$  sets of nodules), except in July 1993 when only three stands of each community were sampled ( $n = 36$  sets of nodules). For each incubation, nodule clusters with ~2 cm of subtending root were cut from the plant and gently cleaned of soil. These were immediately placed in a 60-mL plastic incubation syringe (Becton Dickinson, Franklin Lakes, New Jersey, USA) equipped with a stopcock and needle. Six milliliters of acetylene (C<sub>2</sub>H<sub>2</sub>), generated from the addition of CaC<sub>2</sub> to water, was drawn into the syringe (a time designated as time<sub>0</sub>) to produce a 10% volume/volume mixture of C<sub>2</sub>H<sub>2</sub> in air. Syringes were kept near ambient soil temperature before and during the incubation by placing them under the forest litter layer in contact with the soil surface. Gas samples (6 mL) were withdrawn for analysis at 30, 90, and 150 s following C<sub>2</sub>H<sub>2</sub> addition.

Gas samples were analyzed for ethylene (C<sub>2</sub>H<sub>4</sub>) using a Shimadzu (Shimadzu Scientific, e.g., Houston, Texas, USA) 14A gas chromatograph equipped with a 2-m Poropak N column and a back-flush valve to vent acetylene. To calculate acetylene reduction activity (ARA), the molar concentration of C<sub>2</sub>H<sub>4</sub> produced from time<sub>0</sub> through all sample collection times was regressed against field incubation time. The slope of this line was expressed as “specific ARA” (μmol C<sub>2</sub>H<sub>4</sub> [g dry nodule]<sup>-1</sup>·h<sup>-1</sup>) by dividing by the incubation dry mass of nodules. The mass of incubated nodules was determined in the laboratory after carefully washing nodules, removing them from the attached roots, and drying at 60°C.

Nodule biomass was sampled on each plot in October 1994. Twenty-five cores (15 cm in diameter × 15 cm deep) were systematically sampled from each of the 16 plots ( $n = 400$  cores) in an evenly spaced 14 × 14 m grid. Cores were brought to Fairbanks and kept frozen until processing. Roots and nodules were carefully washed free of soil and hand sorted. Nodules, defined as a contiguous clustered group of lobes, were removed from roots and sorted into seven diameter size classes. These size classes were: small single lobed nodules (sl), nodules <1 cm in diameter (excluding sl), and five additional classes increasing by 1-cm increments. We used the smallest two size classes as an approximate

surrogate for the age of nodules. We assume that both of these size classes were produced since the start of fertilization (Akkermans 1971, Schwintzer et al. 1982; D. D. Uliassi, *personal observation*) and that single lobed nodules are younger than nodules <1 cm. Total nodule dry biomass was measured in 394 cores, and nodules were sorted into size classes from 344 of these (distributed among all plots), therefore, the average total nodule biomass and the sum of the average nodule biomass components will not be identical.

#### *Vegetation parameters*

We estimated the age of the alder stands by determining the age of initial surface establishment and presence of visible vegetation from an intermittent chronology of aerial photographs dating from 1948 to 1971 or from estimates of the oldest poplar and spruce tree ages from nearby stands. Stem densities and diameters were measured in six (3 × 3 m) quadrats within each plot in 1994 ( $n = 96$  quadrats). We recorded the diameter of each alder stem >1 cm in diameter (measured ~1 m above the stem base) and each poplar stem >3 cm in diameter (measured at breast height). Trees smaller than this were counted but their diameters were not recorded. We calculated the leaf area index (LAI) of the alder canopy at each stand as an index of potential carbon gain and because we were interested in reporting N<sub>2</sub> fixation on a leaf area basis in order to appropriately scale N<sub>2</sub> fixation to estimates of resorption. We used the allometric equation predicting leaf area from stem diameters developed for these sites (Uliassi 1998): leaf area = 56.15 + 28.37[dbh], with leaf area in square centimeters, to calculate the LAI of each alder canopy.

#### *N and P resorption*

We collected alder leaves from five trees at all control and fertilized plots five times throughout the 1994 growing season ( $n = 400$  leaf samples) to ensure that we measured peak nutrient concentrations for accurate resorption calculations. For each plot, 15 leaves were removed from throughout the canopy of each of five trees until the final sampling in September, when newly fallen leaf litter was collected from beneath trees. Leaves from within or beneath a tree were composited as one sample. The leaf area of each sample was measured on a LI-COR 3000A leaf area meter (LI-COR, Lincoln, Nebraska, USA). Leaves were oven dried at 60°C for 48 h, ground in a Wiley mill (850- $\mu$ m mesh), and analyzed for N and P.

We present resorption as resorption efficiency (percentage of nutrient resorbed based on mass per unit leaf area), and resorptive pool size (mass of nutrient resorbed per unit leaf area). Resorption efficiencies and resorptive pool sizes were calculated as the maximum nutrient concentration minus senescent nutrient concentration on a leaf area basis. Resorption was defined as zero in the few cases where the nutrient concentra-

tion in senescent tissue exceeded the maximum green leaf nutrient concentration.

#### *Soil measurements and preparation*

To assess soil nutrients, five soil cores (5 cm in diameter by 20 cm deep) were collected from each plot following measurements of nitrogenase activity at each sampling period in 1994 ( $n = 240$  cores). The 20-cm depth was chosen because it encompassed the main rooting zone of alder. Recently deposited litter was removed prior to sampling. Cores were stored in portable coolers, brought to Fairbanks, and frozen until analysis. Because of the frequent deposition of alluvium during flooding events, the cores comprised multiple layers of buried organic and mineral soils. We separated each organic and mineral layer, grouped them into an organic and mineral fraction, and then oven dried each to constant mass at 60°C.

Bulk densities of the organic and mineral fractions were calculated on a subset of intact cores based on wet volume and dry mass with coarse roots removed. Except for the driest sites, cores remained intact during sampling, and it was possible to clearly separate buried organic layers from each core. Total masses of organic and mineral soils per unit area were determined from our bulk density measurements and the volume of the soil fraction in each core. Total N and P were calculated for organic and mineral fractions on all soil cores collected throughout the year. Resin- and acid-extractable phosphorus were measured on the 80 cores collected in early July 1994.

At each sampling period, soil temperature (at 15 cm depth) and moisture were recorded at each plot. Soil moisture (percentage of volume) was measured using a time domain reflectometer (Soil Moisture, Santa Barbara, California, USA) with 30 cm waveguides inserted vertically into the soil at each measurement.

#### *Chemical analysis*

We measured soil P availability using two separate indices: (1) a modified dilute acid-fluoride extraction (Olsen and Summers 1982), and (2) a laboratory resin extraction. Both extractions were performed on 3 g of unground soil with large pieces of organic material (i.e., twigs, coarse roots, and bark; all >2 mm) removed. For the resin extracts, 3 g of soil was rehydrated 12 h before analysis and placed in 200-mL specimen cups. Approximately 4 g of Dowex 40 mesh anion exchange resin (Bio-Rad, Richmond, California, USA) was placed in nylon mesh cylinders (7 × 1.5 cm). One cylinder was placed in each specimen cup with the hydrated sample and 75 mL of distilled/deionized water was added. The cups were then shaken for 2 h. The resin was removed from each cylinder and eluted with 0.1 mol/L HCl using a drip-through system (method from T. Quintel, *personal communication*). Solution PO<sub>4</sub> was then analyzed colorimetrically on a modified

TABLE 1. Selected soil chemical characteristics of alder and poplar stands on the Tanana River floodplain, Alaska, USA.

Soil parameter	Alder control	Alder fertilized	Poplar control	Poplar fertilized
REP ( $\mu\text{g/g}$ organic)	7.12 $\pm$ 3.79	27.9 $\pm$ 7.9	19.91 $\pm$ 4.94	64.5 $\pm$ 26.9
REP ( $\mu\text{g/g}$ mineral)	1.35 $\pm$ 0.38	2.02 $\pm$ 0.7	0.84 $\pm$ 0.29	3.66 $\pm$ 0.90
REP ( $\text{mg/m}^2$ )	288 $\pm$ 81	656 $\pm$ 166	434 $\pm$ 43	1444 $\pm$ 332
AEP ( $\text{mg/m}^2$ )	372 $\pm$ 66	2634 $\pm$ 816	586 $\pm$ 75	3707 $\pm$ 574
Total P ( $\text{g/m}^2$ )	79 $\pm$ 13 <sup>a</sup>	74 $\pm$ 0 <sup>a</sup>	65 $\pm$ 8 <sup>a</sup>	95 $\pm$ 19 <sup>b</sup>
Total C ( $\text{g/m}^2$ )	3287 $\pm$ 221	3714 $\pm$ 187	5721 $\pm$ 545	5515 $\pm$ 301
Total N ( $\text{g/m}^2$ )	233 $\pm$ 47	227 $\pm$ 17	315 $\pm$ 30	280 $\pm$ 22
Organic soil				
P (%)	0.04 $\pm$ 0.01	0.08 $\pm$ 0.02	0.07 $\pm$ 0.0	0.11 $\pm$ 0.01
N (%)	0.83 $\pm$ 0.2 <sup>a</sup>	0.99 $\pm$ 0.23 <sup>b†</sup>	1.24 $\pm$ 0.2 <sup>a</sup>	1.04 $\pm$ 0.16 <sup>b*</sup>
C (%)	12.51 $\pm$ 2.97	14.33 $\pm$ 3.18	23.05 $\pm$ 3.53 <sup>a</sup>	20.53 $\pm$ 3.95 <sup>b*</sup>

Notes: REP = resin extractable P; AEP = acid extractable P. Concentrations per square meter include all buried organic and mineral layers to a depth of 20 cm, adjusted for differences in bulk densities. Percentages P, N, and C reported for organic soil are from a composite of all buried organic layers to 20 cm. All values are means  $\pm$  1 SE ( $n = 4$  plots). Significance of  $F$  values is reported as follows: †  $P < 0.1$ ; \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ . When interactions are present (community  $\times$  fertilization), significance of alder vs. poplar is for control sites only, and differences between control and fertilized are indicated by different superscript letters within each community (alder or poplar).

Technicon (Tarrytown, New York, USA) autoanalyzer (Whitledge et al. 1981).

Total N and C concentrations of soil and leaf samples were determined on a LECO CNS 2000 autoanalyzer (LECO, St. Joseph, Michigan, USA). Total phosphorus concentrations of soil and leaf samples were determined colorimetrically following perchloric acid digestions.

#### Statistical analysis

Main treatment effects (community type and P fertilization) were tested using ANOVA with the mean of samples within each plot as the true replicate, blocked by stand. Significant interactions were followed by paired comparisons. Treatment effects on specific ARA and nodule biomass components were analyzed using MANOVA followed by univariate ANOVAs when effects were significant (Scheiner 1993) because (1) specific ARA at each sampling period was not independent of other sampling periods and (2) nodule biomass values of individual size classes were not independent of total nodule biomass. Differences between senescent leaf N concentrations and midseason green leaf N concentrations at control plots were analyzed using a paired  $t$  test.

Multiple stepwise regression models were used to select from variables to explain variation in (1) specific ARA across the 1994 growing season and among plots at each sampling period, and (2) ecosystem N inputs at control plots in 1994. Soil moisture and soil temperature were entered as potential explanatory variables of specific ARA. Seasonal averages of soil moisture and soil temperature and resin-extractable P and LAI were entered as potential explanatory variables of ecosystem N inputs at control plots.

SAS was used for all statistical analyses (SAS Institute 1995). Some data were log- or square-root transformed to minimize the influence of outliers and meet

parametric model assumptions. Data in figures and tables are untransformed values and are presented as means  $\pm$  1 standard error.

## RESULTS

### Soil physical and chemical characteristics

*Phosphorus availability.*—Indices of P availability from resin and acid extractions were positively correlated in both alder and poplar soils ( $r = 0.85$  and  $0.95$ ) but on average, acid extractions yielded values  $3.4\times$  higher than resin extracts. Acid extractions are sensitive to soil pH and may under- or overestimate P availability in highly alkaline soils depending on the degree of P precipitated with calcium carbonate (Binkley and Vitousek 1991). Because of the high and variable pH of the floodplain soils (Marion et al. 1993), we have chosen resin-extractable P as our index of P availability in all analyses. We present both only for direct comparisons with other studies (Table 1). Poplar soils had higher resin-extractable P concentrations than alder soils, and fertilization increased total resin-extractable P per unit area of the top 20 cm of soil in both alder and poplar soils (Table 1). Despite much higher ( $F_{1,18} = 62.41$ ,  $P < 0.001$ ) extractable P concentrations in organic horizons relative to mineral horizons on a mass basis ( $\mu\text{g P/g}$  soil; Table 1), the total amount of extractable P per unit area was similar between the organic and mineral fractions because of the high bulk density of the mineral component (Table 2). Details of soil N and C are provided in Table 1. Fertilization increased the N concentration in organic soil in alder stands but decreased concentrations of both N and C in organic poplar soil (Table 1).

### Nitrogenase activity and nodule biomass

We overlaid data from 1993 and 1994 to discern a more complete seasonal pattern of ARA because our earliest measurements within a season are from 10 June

TABLE 1. Extended.

Control vs. fertilized	Alder vs. poplar	Community × fertilized
$F_{1,6} = 7.59†$	$F_{1,6} = 4.33†$	
$F_{1,6} = 5.83†$	$F_{1,6} = 0.61$	
$F_{1,6} = 11.51*$	$F_{1,6} = 5.29†$	
$F_{1,6} = 27.17*$	$F_{1,6} = 1.55$	
	$F_{1,12} = 0.52$	$F_{1,6} = 4.48†$
$F_{1,6} = 0.1$	$F_{1,6} = 118.74***$	
$F_{1,6} = 0.92$	$F_{1,6} = 10.24*$	
$F_{1,6} = 9.78*$	$F_{1,6} = 4.72†$	
	$F_{1,12} = 2.19$	$F_{1,6} = 12.61*$
	$F_{1,12} = 5.95$	$F_{1,6} = 9.38*$

1994 and our latest from the first week of September 1993. We assume that alders were of similar phenological stages within a growing season between the two years because leaf emergence date differed by only 2 d between 1993 and 1994, and specific ARA in control plots did not differ between years ( $P > 0.1$ ) at similar sampling periods in late July. However, 1993 was a year of severe drought in interior Alaska and precipitation was 42% lower than 1994 (Ruess et al. 1998).

Specific nitrogenase activity (ARA, expressed as micromoles ethylene per gram nodule dry mass per hour) exhibited distinct seasonal patterns in each community (Fig. 1). Specific ARA was low ( $<7.5 \mu\text{mol C}_2\text{H}_4\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ) early and late in the season. In control plots, fixation rates in poplar stands peaked ( $17.5 \pm 3.1 \mu\text{mol C}_2\text{H}_4\cdot[\text{g dry nodule}]^{-1}\cdot\text{h}^{-1}$ ) in early July, while specific ARA continued to increase in alder stands at least until late July when the highest rates were recorded ( $25.0 \pm 8.0 \mu\text{mol C}_2\text{H}_4\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ) (Fig. 1). We entered specific ARA at each sampling period as the dependent variable in separate MANOVAs for 1993 and 1994. These revealed no fertilization or community (alder vs. poplar) effects, but we had predicted that differences would exist in midsummer when plant N demands may be highest. Therefore, we analyzed midsummer rates in both years using ANOVA. In late July 1993, fertilization significantly increased specific ARA in alder stands ( $F_{1,3} = 19.17$ ,  $P = 0.022$ ;  $n =$  three control and two fertilized plots in both alder and poplar stands at this sampling). In late July 1994, alder stands exhibited 1.5× higher rates than poplar stands ( $F_{1,6} = 11.88$ ,  $P = 0.014$ ), but fertilization had no significant effect on specific ARA.

Across all control plots (alder and poplar stands) and sampling periods in 1994 (early June to 30 July), soil temperature emerged as an important explanatory variable of specific ARA (Fig. 2A). Soil temperature increased through early August but was relatively invariant across plots within a sampling period (Fig. 2B) and did not explain variation in specific ARA among plots at any given sampling period. Soil moisture did not enter into the regression explaining specific ARA

in control plots across the 1994 growing season or within any sampling period in 1994 ( $P > 0.1$ ). However, soil moisture in poplar control plots decreased to half that of alder stands by midsummer ( $F_{1,12} = 7.73$ ,  $P = 0.017$ ), and fertilization further decreased soil moisture in poplar stands to half that of poplar controls (10.1 to 4.2 percent dry mass;  $F_{1,6} = 12.11$ ,  $P = 0.013$ ) (Fig. 3A). When all control and fertilized plots ( $n = 16$  plots) were included in the regression, specific ARA in late July 1994 was positively correlated with soil moisture (Fig. 3B).

We detected no C<sub>2</sub>H<sub>4</sub> production in any soil samples collected from any plot at the time of symbiotic fixation measurements. This suggests that nonsymbiotic fixation was low, or nonexistent, or that a different sampling approach (i.e., more soil or longer incubations) would be required to detect this flux.

We found no significant difference ( $P > 0.1$ ) in total nodule dry biomass between alder control ( $27.3 \pm 5.3 \text{ g/m}^2$ ) and poplar control plots ( $17.7 \pm 4.5 \text{ g/m}^2$ ). Fertilization increased total nodule biomass in alder stands to  $42.3 \pm 9.0 \text{ g/m}^2$  ( $F_{1,6} = 6.53$ ,  $P = 0.043$ ) but had no effect on total nodule biomass in poplar stands ( $P > 0.1$ ), which averaged  $14.7 \pm 1.9 \text{ g/m}^2$  in fertilized plots. Fertilization increased the biomass of single-lobed nodules in alder and poplar stands by 203% ( $F_{1,6} = 11.59$ ,  $P = 0.014$ ) and increased the biomass of nodules  $<1$  cm in alder stands by 81% ( $F_{1,6} = 8.76$ ,  $P = 0.025$ ) but had no effect in poplar stands (Fig. 4). There were no significant differences ( $P > 0.1$ ) in nodule biomass of these two size classes between alder and poplar stands. There were also no statistically significant treatment effects in the nodule biomass of any other size class despite the apparent increase in the biomass of 5–6-cm nodules in fertilized alder stands.

#### Ecosystem nitrogen inputs

We used the seasonal pattern of ARA per unit area to generate individual seasonal curves for each control plot. The area under each curve was used to calculate total nitrogen inputs by using the theoretical C<sub>2</sub>H<sub>2</sub> reduction to N<sub>2</sub> fixation conversion ratio of 3:1 (molar ratio of ethylene to NH<sub>3</sub> = 1.5; Stewart et al. 1967). We also assumed that our measured rates represent the

TABLE 2. Selected soil physical characteristics of alder and poplar stands on the Tanana River floodplain, Alaska.

Soil parameter	Alder	Poplar
Organic BD (g/cm <sup>3</sup> )	$0.34 \pm 0.09$	$0.21 \pm 0.05$
Mineral BD (g/cm <sup>3</sup> )	$1.04 \pm 0.04$	$1.02 \pm 0.06$
Organic (kg/m <sup>2</sup> )	$12.1 \pm 1.9$	$17.6 \pm 1.5$
Mineral (kg/m <sup>2</sup> )	$158.7 \pm 20.0$	$116.7 \pm 10.0$
Buried organic (%)	$25.5 \pm 14.0$	$49.1 \pm 17.8$

Notes: BD = bulk density; mass of organic and mineral horizons is calculated to a depth of 20 cm. "Buried organic" is the percentage of organic soil overlain by alluvial silt deposits. Values are means  $\pm 1$  SE ( $n = 4$  stands; control and fertilized plots combined).

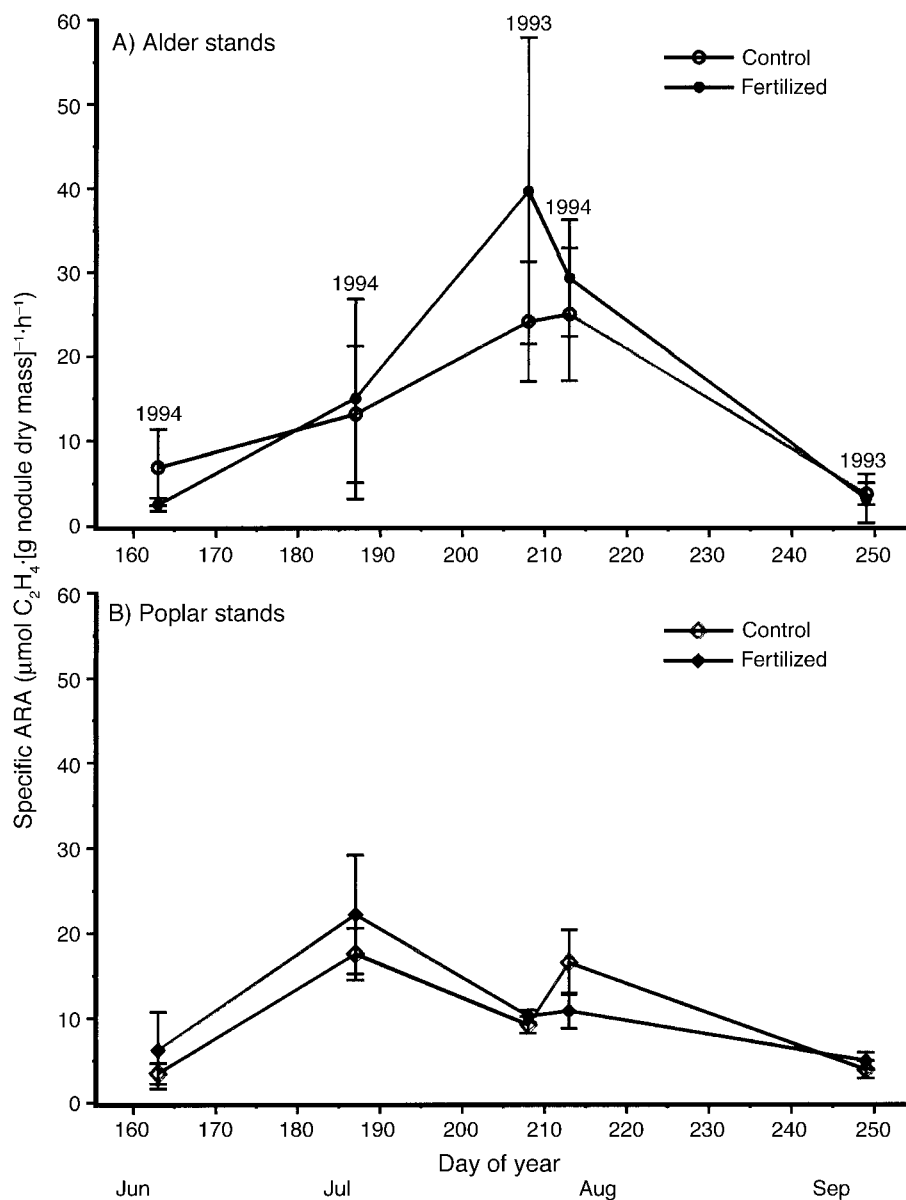


FIG. 1. Seasonal patterns of specific acetylene reduction activity (ARA) for each treatment. This seasonal pattern pools measurements from 1993 and 1994. All values are means  $\pm$  1 SE ( $n = 4$  plots, except  $n = 3$  plots in July 1993).

rates for a 24-h period as has been seen in other high-latitude N fixers (Weisz and Sinclair 1988, Huss-Danell et al. 1992). Total annual  $\text{N}_2$  fixation inputs at control plots averaged  $59 \pm 11 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in alder stands (range: 39–88  $\text{kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ ), and only  $38 \pm 11 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in poplar stands (range: 15–65  $\text{kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ ), but we could not detect a significant difference ( $P > 0.1$ ). Using  $^{15}\text{N}_2$ , K. M. Klingensmith (*unpublished data*) calculated a mean acetylene reduction to  $\text{N}_2$  fixation ratio of 6.4 (molar ratio ethylene to  $\text{NH}_3 = 3.2$ ) from four seedlings of *Alnus tenuifolia* with a range in ratio values from 3.7 to 8.7. Using this mean ratio of 6.4 would reduce our estimates of seasonal  $\text{N}_2$

fixation inputs by 53% (28  $\text{kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in alder stands and 18  $\text{kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  in poplar stands). Phosphorus fertilization increased total nitrogen inputs to  $140 \pm 41 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  (+138%) in alder stands ( $F_{1,9} = 8.02$ ,  $P < 0.019$ ) but had no significant effect in poplar stands, where N inputs were  $33 \pm 3 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ . In late July 1994 (the nearest measurement of specific ARA compared to our estimate of nodule biomass), ARA per unit area was higher in alder control plots than in poplar control plots ( $F_{1,12} = 4.62$ ,  $P < 0.053$ ) and fertilization nearly doubled ARA per unit area in alder stands from 629.8 to 1101.0  $\mu\text{mol C}_2\text{H}_4 \cdot \text{m}^{-2} \cdot \text{h}^{-1}$  ( $F_{1,6} = 22.95$ ,  $P = 0.003$ ; Fig. 5).

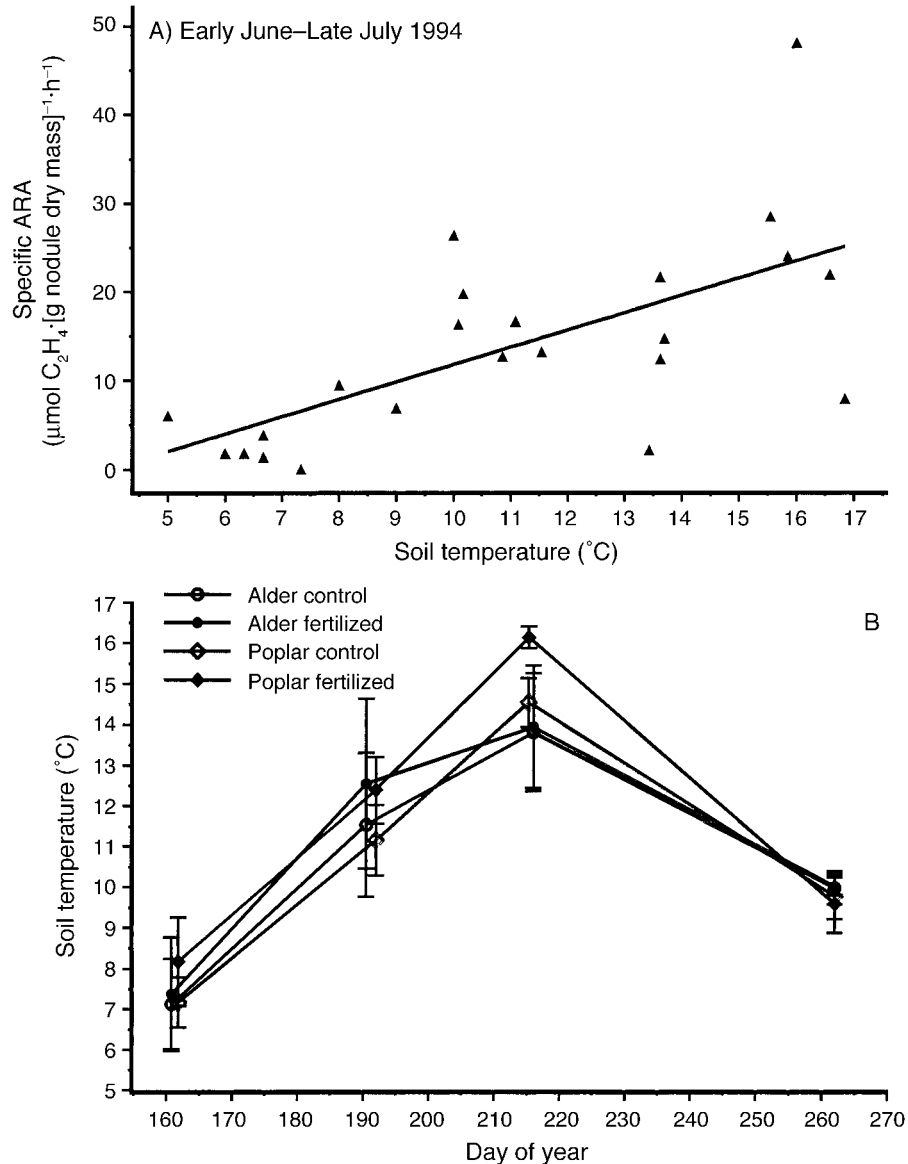


FIG. 2. Soil temperature and relationships to specific acetylene reduction activity (ARA) in 1994. (A) Relationship between soil temperature and specific ARA across control plots from early June to late July 1994 ( $n = 23$  plots); specific ARA =  $-7.76 + 1.95(\text{temperature})$ ,  $R^2 = 0.39$ ,  $P < 0.001$ . (B) Seasonal pattern of soil temperature at all plots in 1994. Values are means  $\pm 1$  SE ( $n = 4$  plots).

We used our estimates of LAI of the alder canopy, resin-extractable P per square meter, and seasonal averages of soil temperature and soil moisture as independent explanatory variables to model ecosystem N inputs from fixation at control plots. These variables were chosen prior to analysis because they represent potential long term influences on alder growth, nodule biomass, and specific ARA (Dixon and Wheeler 1983). Only LAI emerged from the model as an important explanatory variable and was positively correlated with ecosystem N inputs (Fig. 6). If the extreme LAI of 4.8 is removed from the analysis, the slope remains positive but the relationship is no longer significant.

We estimated net annual inputs ( $I$ ) for our stand ages ( $t$ ) using the N mass balance equation developed by Van Cleve et al. (1993a) for mineral soil within the BNZ LTER:

$$I = 5511.7e^{-12.19/t/p^2}$$

This equation yields an estimated net annual N increment of 43.0 kg·ha<sup>-1</sup>·yr<sup>-1</sup> in our alder stands (29 yr old) and 9.7 kg·ha<sup>-1</sup>·yr<sup>-1</sup> in poplar stands (68 yr old).

#### *Alder leaf area and carbon allocation*

Alder stands had higher LAIs than poplar stands ( $F_{1,6} = 8.37$ ,  $P = 0.023$ ) but we found no statistically sig-

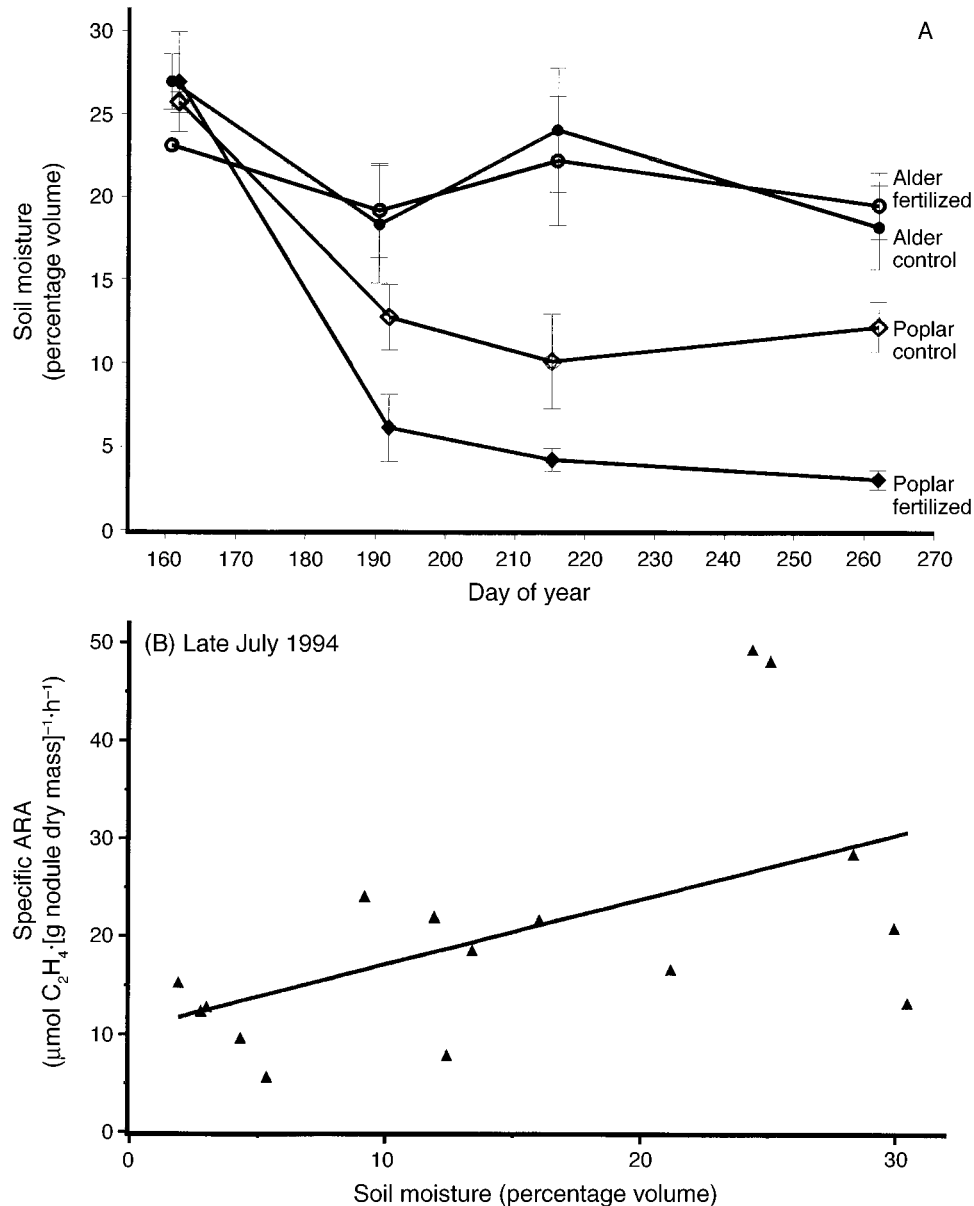


FIG. 3. (A) Seasonal patterns of soil moisture in alder and poplar stands (control and fertilized plots) in 1994. Data are means  $\pm$  1 SE ( $n = 4$  plots). (B) Relationship between soil moisture and specific ARA across all plots in late July 1994 ( $n = 16$ ): specific ARA =  $10.52 + 0.66(\text{soil moisture})$ ,  $R^2 = 0.29$ ,  $P = 0.031$ .

nificant differences between control and fertilized plots (Table 3). On average, specific leaf area (SLA = leaf area/leaf mass) was higher in poplar than in alder stands, and fertilization slightly increased SLA in alder stands. Details of vegetation parameters are provided in Table 3.

Overall, fertilization increased total nodule biomass per unit area of leaf in alder and poplar stands ( $F_{1,7} = 4.15$ ,  $P = 0.081$ ). Nodule biomass per unit area of leaf increased from  $10.3 \pm 2.4$  to  $17.2 \pm 5.9$  g nodule/m<sup>2</sup> leaf in alder stands (+68%), and from  $7.6 \pm 1.8$  to  $11.1 \pm 2.4$  g dry nodule/m<sup>2</sup> leaf in poplar stands

(+46%), but there were no differences ( $P > 0.1$ ) between alder and poplar stands in the nodule biomass to leaf area ratio. We also found no difference in nitrogen fixed annually per unit leaf area between alder and poplar control stands ( $2.0 \pm 0.1$  and  $1.7 \pm 0.5$  g N·(m<sup>2</sup> leaf)<sup>-1</sup>·yr<sup>-1</sup>, respectively).

#### *N and P resorption*

Maximum leaf N and P concentrations (g/m<sup>2</sup> leaf) in alder leaves were lower in poplar stands than in alder stands (Table 4). Nitrogen and P resorption efficiencies were not different ( $P > 0.1$ ) between alder and poplar

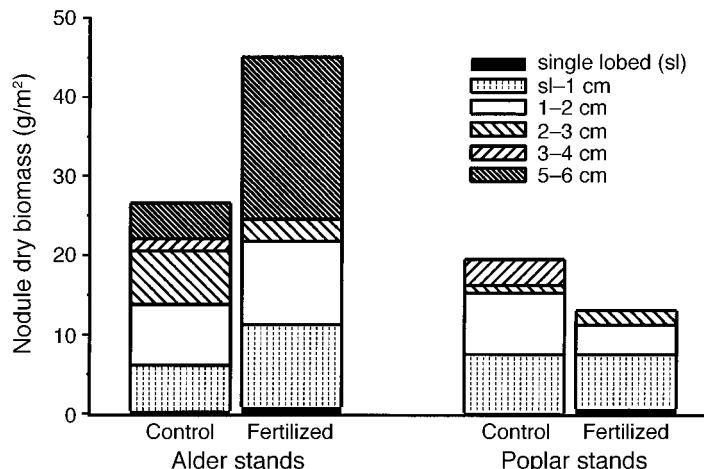


FIG. 4. Living nodule biomass in six size classes (increasing by 1-cm increments) at alder and poplar stands (control and P fertilized plots) in October 1994 ( $n = 4$  plots).

stands. P resorptive pool sizes were higher in alder stands (Table 4) and positively correlated with maximum P concentrations ( $r = 0.84$ ) across control plots because P concentrations of senescent leaves were relatively invariant. Among control plots, leaf N increased until early July but decreased by late July. However, leaves gradually recovered N throughout the remainder of the season ( $F_{1,5} = 15.47$ ,  $P = 0.011$ ) in control and fertilized plots (Fig. 7). Fertilization had no effect on N resorption efficiencies, resorptive pool sizes, or maximum and senescent N concentrations (Table 4). P resorption efficiencies and resorptive pool sizes decreased with fertilization because fertilization increased maximum and senescent-leaf P concentrations (Table 4). N<sub>2</sub> fixation per unit area of leaf was not correlated with N or P resorption efficiencies or resorptive pool sizes. Plant N status (maximum leaf N concentration) did not explain N resorption efficiencies or resorptive pool sizes. Likewise, maximum P concentrations did not explain P resorption efficiencies.

DISCUSSION

*Limitations to nitrogen fixation*

Our results support the hypothesis that N and P cycles are coupled through N<sub>2</sub> fixation on the Tanana

River floodplain. In alder stands, P fertilization increased total nodule biomass, nitrogenase activity per unit area (late July 1994) and potentially increased annual N inputs by as much as 138%, but it had only a limited effect on specific ARA. In contrast, P fertilization in poplar stands had no detectable effect on total nodule biomass, specific ARA, or annual N inputs, and it tended to reduce (not significantly) nitrogenase activity per unit area by late July 1994. Although a greenhouse study showed that P limited alder growth and N<sub>2</sub> fixation in poplar soil (Uliassi et al. 2000), fertilization in the field appears to have altered competitive interactions between alder and poplar trees. Poplar stands were farther from subsurface water, and fertilization likely increased water limitation to alder by accelerating the growth of overstory poplar trees. Phosphorus had a much smaller effect on N<sub>2</sub> fixation in alder stands than in a fertilization study on young volcanic sites in Hawaii (Vitousek 2000). The difference in response between these studies emphasize a need for multiple studies in a range of environments before general con-

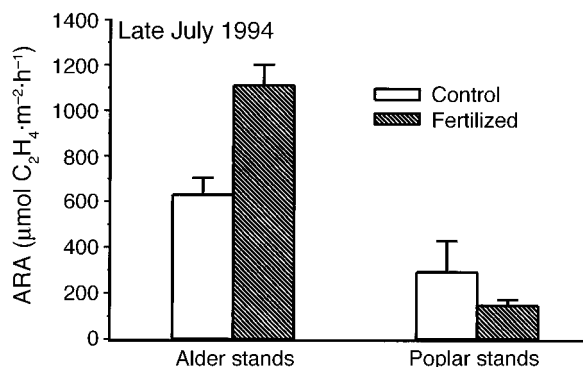


FIG. 5. Nitrogenase activity per unit area in alder and poplar stands (control and P fertilized plots) in late July 1994. Values are means and 1 SE ( $n = 4$  plots).

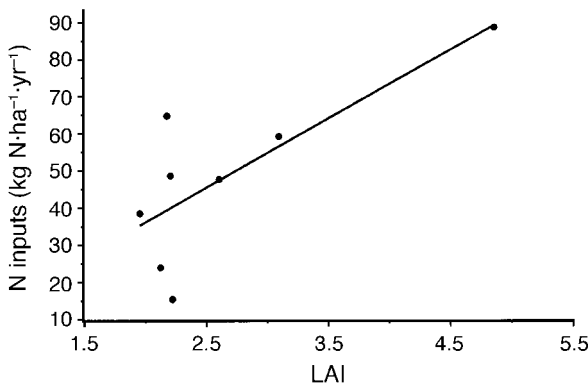


FIG. 6. Relationship between leaf area index (LAI) and annual nitrogen inputs from fixation at each of the eight control plots:  $N \text{ inputs} = -0.92 + 18.59(\text{LAI})$ ,  $R^2 = 0.59$ ,  $P < 0.027$ .

TABLE 3. Selected vegetation parameters of alder and poplar stands on the Tanana River floodplain.

Parameter	Alder control	Alder fertilized	Poplar control	Poplar fertilized
Alder trees				
Stems/ha	7361 ± 1935	4768 ± 1319	7129 ± 902	3981 ± 652
Diam/stem (cm)	4.58 ± 0.44 <sup>a</sup>	7.02 ± 0.54 <sup>b</sup>	3.77 ± 0.45	3.83 ± 0.41
LAI	3.02 ± 0.66	3.76 ± 1.45	2.28 ± 0.11	1.51 ± 0.36
SLA (cm/g)	173.21 ± 10.46	185.67 ± 10.77	219.63 ± 4.78	221.67 ± 2.77
Poplar trees (>3 cm dbh)				
Stems/ha			1018 ± 342	1018 ± 342
Dbh/stem			25.57 ± 4.54	23.10 ± 3.86

Notes: LAI = leaf area index; SLA = specific leaf area (leaf area/leaf mass). All values are means ± 1 SE ( $n = 4$  plots). Significance of  $F$  values is reported as follows: †  $P < 0.1$ ; \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ . When interactions are present (community × fertilization), significance of alder vs. poplar is for control sites only, and differences between control and fertilized are represented by different superscript letters within each community (alder or poplar).

clusions can be drawn about the effects of P on  $N_2$  fixation in natural terrestrial ecosystems.

The strongest effects of P fertilization were on nodule biomass. Results from a companion greenhouse study (Uliassi et al. 2000) showed that thinleaf alder seedlings grown in P fertilized soil at field temperatures increased host plant growth and biomass allocation to nodules but did not increase specific ARA. This pattern is further supported by other studies of *Alnus* in northern Sweden (Ekblad and Huss-Danell 1995), but is contrary to many studies that report marked increases of specific ARA with P fertilization in both actinorhizal and leguminous species (e.g., Israel 1987, Russo 1989, Fraga-Beddiar and Tacon 1990). These alders, and perhaps other high-latitude N fixers, may adjust  $N_2$  fixation more strongly through changes in nodule biomass rather than changes in nitrogenase activity because further increases in nitrogenase activity may be hindered at low soil temperatures. Stimulation of new nodule initiation in fertilized alder and poplar stands suggests that ecosystem inputs of fixed N could be larger (at least in the alder stands) in subsequent years as these younger nodule cohorts develop in fertilized alder and poplar plots. However, higher P availability may not necessarily increase total ecosystem inputs of fixed N over long time periods because of competitive and facilitative interactions through the course of succession (Walker and Chapin 1986, Chapin et al. 1994, Fastie 1995).

Fertilization increased specific ARA in alder stands at a time when soil moisture and soil temperature were near their highest seasonal values. This suggests that specific ARA was limited by P only following release from limitation by other factors or when N demands were highest during the period of maximum plant growth. Taiga forests of interior Alaska are dominated by cold soils, which limit rates of nutrient cycling and productivity (Van Cleve et al. 1983, 1991, 1993b), and cold soils also appear to be a primary regulator of N fixation. Greenhouse studies on temperate N fixers have shown that nitrogenase activity is often positively correlated with soil temperatures up to 20–25°C, which

may be an optimum in several species (Akkermans 1971, Dixon and Wheeler 1983, Weisz and Sinclair 1988, Huss-Danell et al. 1989, Zitzer and Dawson 1989). Specific ARA was positively related to soil temperature until late July when soils reached their maximum temperatures. Soil moisture in poplar stands declined to half that of alder stands by late July and we found that specific ARA was positively related to soil moisture at this time period if all plots (control and fertilized) were included in a regression. Taken together, this suggests that specific ARA is limited by soil temperature early in the year, but then soil P becomes limiting in alder stands and soil moisture becomes limiting in poplar stands.

The acetylene reduction assay should provide a sensitive index of relative changes in nitrogenase activity, but extrapolations to actual amounts of N fixed must be interpreted with caution. There are many errors and assumptions associated with measurements of ARA (e.g., Giller 1987, Winship and Tjepkema 1990). We believe we have minimized these by using a short-term incubation, which should minimize the influence of acetylene on nitrogenase, producing seasonal curves of specific ARA in all plots, and by thoroughly sampling nodule biomass. However, to calculate ecosystem inputs of fixed N we assumed that nodule biomass, which was sampled only at the end of the experiment (to avoid large disturbances in plots), was stable over the time course of the experiment, and we used the theoretical 3:1 conversion ratio of acetylene reduction to  $N_2$  fixation (Stewart et al. 1967). Nodule biomass was likely more stable in control plots than fertilized plots and this approach should provide at least a relative index of ecosystem inputs of fixed N among control stands, but estimates of N inputs in fertilized stands must be interpreted more cautiously.

#### *N<sub>2</sub> fixation and N retention*

Successional patterns of N accumulation and soil N mineralization and nitrification (Klingensmith and Van Cleve 1993b, Van Cleve et al. 1993b, Clein and Schimmel 1995) have suggested that the N cycle tightens

TABLE 3. Extended.

Control vs. fertilized	Alder vs. poplar	Community × fertilization
$F_{1,7} = 9.16^*$	$F_{1,7} = 0.29$	$F_{1,6} = 10.85^*$
$F_{1,7} = 0$	$F_{1,12} = 1.56$	
	$F_{1,7} = 8.37^*$	$F_{1,6} = 4.06^\dagger$
	$F_{1,12} = 16.84^{***}$	
$F_{1,3} = 0$		
$F_{1,3} = 6.2^\dagger$		

from alder to poplar stages, with decreased amounts of N inputs and outputs and greater internal recycling of N with ecosystem age. A comparison of our estimates of N<sub>2</sub> fixation with predicted estimates of soil N accumulation (Van Cleve et al. 1993a) suggests that N losses through denitrification or leaching are 28% and 75% of N fixed in alder and poplar stands, respectively. The potential for nitrification and nitrate accumulation should be large in both alder and poplar stands because of continued N inputs through fixation. Reported declines in nitrification and mineralization with increasing poplar dominance (Van Cleve 1993, Clein and Schimel 1995) may, in part, represent differences in site selection and the seasonal timing of measurements. Nitrogen losses through denitrification may be episodic but high in wet anaerobic soils or rhizospheres early in the year following flushes of nitrate production before plant uptake has begun, or in hot spots where active heterotrophs have depleted soil O<sub>2</sub> (Groffman and Tiedje 1989, Groffman and Turner 1995). Alternatively, inorganic and organic N leaching could represent a large pathway for N loss, particularly during spring runoff following snowmelt or during periodic flooding. Even if we use the higher conversion ratio of ethylene to NH<sub>3</sub> developed by K. M. Klingensmith (*personal communication*), our estimates of N<sub>2</sub> fixation are reduced by 53% but still indicate substantial N loss in the poplar stage of succession.

We found little evidence to support the hypothesis that poplar leachates inhibit N<sub>2</sub> fixation under poplar stands (Rice 1974, Jobidan and Thibault 1982, Younger and Kapustka 1983, Schimel et al. 1998). There was no consistent decrease in specific ARA, nodule biomass, or ecosystem inputs of fixed N between the alder and poplar stages of succession, but values were highly variable and a larger sample size may be needed to detect any potential differences. In addition, a greenhouse experiment using intact soil cores from these stands found that alder seedlings grew to a similar mass, had similar foliar N concentrations, and had similar whole plant nitrogenase activity when grown in alder and poplar soils (Uliassi et al. 2000). Poplar leachates did reduce rates of specific ARA when directly applied to alder in a separate greenhouse experiment (Schimel et al. 1998), but in the field, at natural levels of P, the effects of leachates appear to be superseded by other limiting factors. It is possible that leachates did inhibit a P fertilization response in poplar stands but this was not tested in our study.

#### Carbon gain and allocation

Of four potential explanatory variables entered in a multiple regression, only the LAI of the alder canopy explained a significant portion of the variation in ecosystem inputs of fixed N at our control stands. There are often tight relationships between plant leaf area or leaf mass and N<sub>2</sub> fixation in seedlings and young nitrogen fixing plants (Dixon and Wheeler 1983, Bormann and Gordon 1984, Huss-Danell 1990, Huss-Danell et al. 1992). Our data show a strong relationship between N<sub>2</sub> fixation and leaf area at the ecosystem level in mature stands of alder. However, this relationship may not hold for other successional stages if large changes in alder densities affect canopy structure and carbon gain (Bormann and Gordon 1984). We also have strong evidence that P fertilization altered patterns of C allocation, because P increased total nodule biomass per unit of leaf area in alder and poplar stands. With

TABLE 4. Nitrogen and phosphorus resorption from senescing alder leaves (1994) in alder and poplar stands (control and fertilized plots) on the Tanana River floodplain.

Parameter	Alder control	Alder fertilized	Poplar control	Poplar fertilized	Control vs. fertilized	Alder vs. poplar
<b>Nitrogen</b>						
Maximum (g/m <sup>2</sup> )	2.13 ± 0.06	2.09 ± 0.16	1.64 ± 0.06	1.52 ± 0.06	$F_{1,6} = 0.58$	$F_{1,6} = 24.95^*$
Senescent (g/m <sup>2</sup> )	1.81 ± 0.15	1.91 ± 0.23	1.53 ± 0.04	1.42 ± 0.05	$F_{1,6} = 0.01$	$F_{1,6} = 32.99^{***}$
Resorption pool (g/m <sup>2</sup> )	0.33 ± 0.18	0.19 ± 0.13	0.11 ± 0.03	0.10 ± 0.07	$F_{1,6} = 0.90$	$F_{1,6} = 0.01$
Resorption efficiency (%)	13.9 ± 7.2	11.3 ± 6.7	6.8 ± 1.4	9.4 ± 4.4	$F_{1,6} = 0.11$	$F_{1,6} = 0.01$
<b>Phosphorus</b>						
Maximum (g/m <sup>2</sup> )	0.14 ± 0.01	0.18 ± 0.01	0.09 ± 0.003	0.12 ± 0.01	$F_{1,6} = 6.99^*$	$F_{1,6} = 12.4^*$
Senescent (g/m <sup>2</sup> )	0.07 ± 0.01	0.16 ± 0.03	0.05 ± 0.01	0.11 ± 0.02	$F_{1,6} = 14.94^*$	$F_{1,6} = 3.16$
Resorption pool (g/m <sup>2</sup> )	0.07 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	$F_{1,6} = 13.24^*$	$F_{1,6} = 4.05^\dagger$
Resorption efficiency (%)	51.4 ± 5.9	10.5 ± 4.2	38.9 ± 6.5	15.5 ± 8.4	$F_{1,6} = 14.67^*$	$F_{1,6} = 0.02$

Notes: Maximum = maximum nutrient concentration measured in alder leaves within the season; senescent = senescent leaf nutrient concentration. Resorptive pool sizes and resorption efficiencies are calculated from the difference between maximum and senescent leaf nutrient concentrations. All values are means ± 1 SE ( $n = 4$  plots). Significance of  $F$  values is reported as follows:  $^\dagger P < 0.1$ ;  $* P < 0.05$ ;  $*** P < 0.001$ .

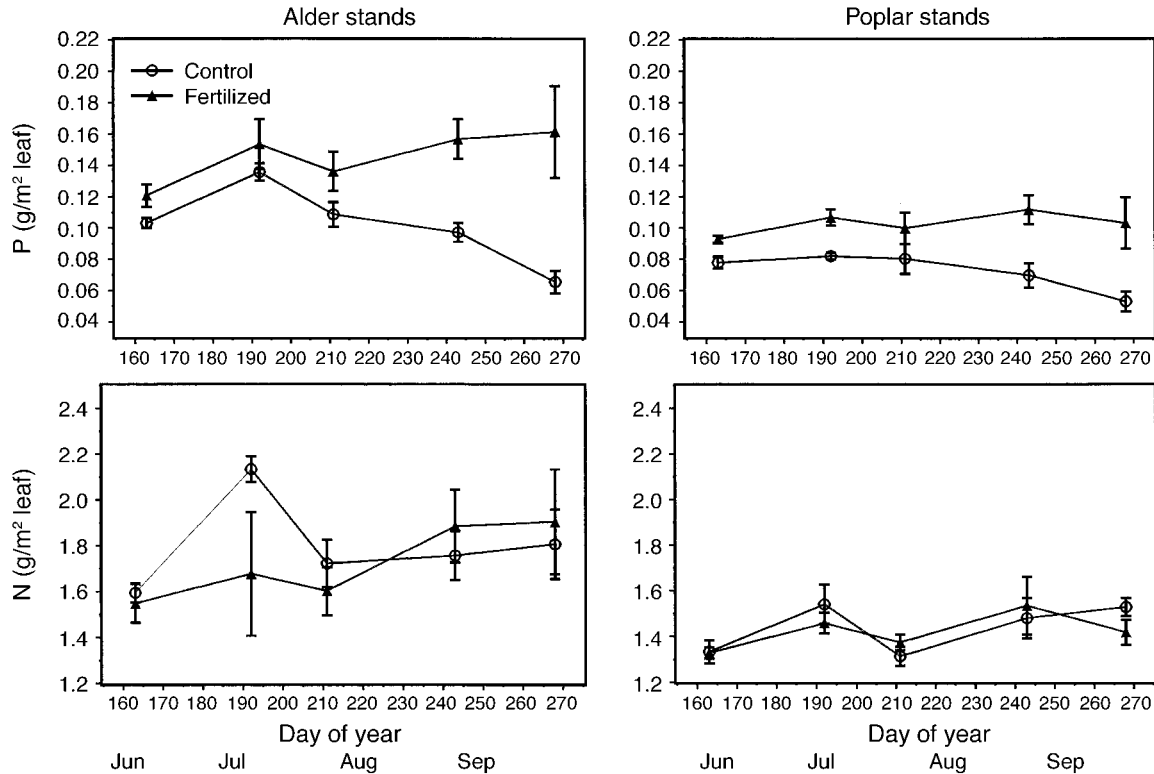


FIG. 7. Seasonal patterns of alder leaf N and P concentrations ( $\text{g}/\text{m}^2$ ) for each treatment in 1994. Values are means  $\pm$  1 SE ( $n = 4$  plots).

increasing P availability, alder may have reduced C allocation to roots (Uliassi et al. 2000), mycorrhizae (Paul and Kucey 1981, Ekblad and Huss-Danell 1995, Ekblad et al. 1995), and secondary metabolites (Reichardt et al. 1991), and preferentially allocated C to support  $\text{N}_2$  fixation.

#### N and P resorption

Our resorption data support growing evidence that nutrient resorption responds to nutrient extremes, such as fertilization and  $\text{N}_2$  fixation, but not to subtle variations in nutrient availability (Shaver and Melillo 1984, Lajtha 1987, Vitousek 1998). Control values of P resorption efficiencies were intermediate compared to other reported values (consistent with low P availability and a high P demand), but our fertilized values were among the lowest (Chapin and Kedrowski 1983). Nitrogen resorption efficiencies were low regardless of fertilization and similar to those of other actinorhizal species (Dawson and Funk 1981, Killingbeck 1993, but see Chapin and Kedrowski 1983). Both low N resorption, and low P resorption with fertilization, were likely artifacts of luxury consumption of these nutrients (Chapin et al. 1990, Chapin and Moilanen 1991). It is interesting to note that alders retain leaves for several weeks beyond other floodplain species. Extended leaf retention time may increase P resorption in alder because leaf P declined steadily until senescence.

Senescent leaf N concentrations were high and relatively invariant across plots ( $1.5\text{--}1.8 \text{ g N}/\text{m}^2$ ). However, green leaf N concentrations fell below these values during midsummer but then increased by the time leaves senesced. This midseason reduction of N may represent dilution of N in a growing canopy, or it may represent true resorption of N to support a large N sink in root growth, which is known to lag behind leafout for up to 6 wk (Ruess et al. 1998). Late-season increases in leaf N concentrations were likely driven by late-season  $\text{N}_2$  fixation in excess of plant N demands, possibly determined by the carbon demands of *Frankia*. Source-sink interactions are known to regulate resorption in Alaska paper birch (*Betula papyrifera*; Chapin and Moilanen 1991) and the absence of a strong N sink prior to leaf abscission may further reduce N resorption in alder. Fixation in excess of plant N demands may be an unavoidable trade-off incurred by N fixing plants that also promotes rapid ecosystem N accumulation and potentially low nutrient use efficiency (Vitousek 1982).

#### Conclusions

We have provided direct evidence that N and P cycles are coupled through  $\text{N}_2$  fixation in primary succession on the Tanana River floodplain. Phosphorus limited  $\text{N}_2$  fixation in alder stands but not in poplar stands. Nitrogen fixation is a dynamic process regulated by a suite of stage-dependent factors including P availability, soil

temperature, and soil moisture; the effects of soil moisture on N<sub>2</sub> fixation should be directly tested in this system. Nitrogen fixation adds substantial amounts of N into this system. However, generalizations about the magnitude of N inputs and N losses must be re-evaluated and we should more closely examine pathways of N loss in situ on the floodplain. Our data also challenge the traditional hypothesis that N<sub>2</sub> fixers do not need to resorb N because of their ability to fix N. Continued N<sub>2</sub> fixation late in the season, in excess of plant N demands, may lead to accretion of N into leaves, high senescent-leaf N concentrations, and rapid ecosystem incorporation of fixed N. The variability in N inputs from fixation seen in this system, and the potential for large N losses, may help explain the lack of correlation found between the presence of dominant stands of N fixers and soil N accumulation across a range of primary successional seres (Walker 1993).

## ACKNOWLEDGMENTS

We thank P. Groffman, P. Vitousek and an anonymous reviewer for their thoughtful reviews on a previous draft of this manuscript. We also thank J. Bryant, K. Kielland, and J. Schimel for critical reviews and discussions of earlier drafts of this manuscript and C. Mulder and K. Huss-Danell for continued support, encouragement, and discussions. Discussions with Keith Van Cleve were instrumental in the initial design of this project. We are particularly indebted to K. Barber and S. Schell for their tireless field assistance, and to L. Butler for his exacting laboratory assistance. We also thank A. Doyle, L. Oliver, and T. Quintel for a large amount of technical support and advice. This study was financially supported by the National Science Foundation's Long Term Ecological Research Program.

## LITERATURE CITED

- Aerts, R. 1996. Nutrient resorption from senescing leaves of perennials—are there general patterns. *Journal of Ecology* **84**(4):597–608.
- Akkermans, A. D. L. 1971. Nitrogen fixation and nodulation of *Alnus* and *Hippophae* under natural conditions. Dissertation. University of Leiden, The Netherlands.
- Binkley, D., and P. Vitousek. 1991. Soil nutrient availability. Pages 75–96 in R. W. Percy, J. Ehleringer, H. A. Mooney, and P. W. Rundel, editors. *Plant physiological ecology*. Chapman and Hall, New York, New York, USA.
- Birk, E. M., and P. M. Vitousek. 1986. Nitrogen availability and nitrogen use efficiency in loblolly pine stands. *Ecology* **67**:69–79.
- Bormann, B. T., and J. C. Gordon. 1984. Stand density effects in young red alder plantations: productivity, photosynthate partitioning, and nitrogen fixation. *Ecology* **65**:394–402.
- Bormann, B. T., and R. C. Sidle. 1990. Changes in productivity and distribution of nutrients in a chronosequence at Glacier Bay National Park, Alaska. *Journal of Ecology* **78**:561–578.
- Bryant, J. P. 1987. Feltleaf willow–snowshoe hare interactions: plant carbon/nutrient balance and floodplain succession. *Ecology* **68**:1319–1327.
- Chapin, F. S., III, and R. A. Kedrowski. 1983. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous Taiga trees. *Ecology* **64**:376–391.
- Chapin, F. S., III, and L. Moilanen. 1991. Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. *Ecology* **72**:709–715.
- Chapin, F. S., III, E.-D. Schultze, and H. A. Mooney. 1990. The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* **21**:423–427.
- Chapin, F. S., III, and G. R. Shaver. 1985. Arctic. Pages 16–40 in B. F. Chabot and H. A. Mooney, editors. *Physiological ecology of North American plant communities*. Chapman and Hall, New York, New York, USA.
- Chapin, F. S., III, L. R. Walker, C. L. Fastie, and L. C. Sharmán. 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs* **64**:149–175.
- Clein, J. S., and J. P. Schimel. 1995. Nitrogen turnover and availability during succession from alder to poplar in Alaskan Taiga forests. *Soil Biology and Biochemistry* **27**:743–752.
- Cole, C. V., and R. D. Heil. 1981. Phosphorus effects on terrestrial nitrogen cycling. In F. E. Clark and T. Rosswall, editors. *Terrestrial nitrogen cycles*. *Ecological Bulletin* (Stockholm) **33**:363–374.
- Côté, B., C. S. Vogel, and J. O. Dawson. 1989. Autumnal changes in tissue nitrogen of autumn olive, black alder, and eastern cottonwood. *Plant and Soil* **118**:23–32.
- Crews, T. J. 1993. Phosphorus regulation of nitrogen fixation in traditional Mexican agriculture. *Biogeochemistry* **21**:141–166.
- Crews, T. J., K. Kitayama, J. H. Fownes, R. H. Riley, D. A. Herbert, D. Mueller-Dombois, and P. M. Vitousek. 1995. Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology* **76**:1407–1424.
- Crocker, R. L., and J. Major. 1955. Soil development in relation to vegetation and surface age at Glacier Bay, Alaska. *Journal of Ecology* **43**:425–448.
- Dawson, J. O., and D. T. Funk. 1981. Seasonal change in foliar nitrogen concentration of *Alnus glutinosa*. *Forest Science* **27**:239–243.
- Dixon, R. O. D., and C. T. Wheeler. 1983. Biochemical, physiological and environmental aspects of symbiotic nitrogen fixation. Pages 108–172 in J. C. Gordon and C. T. Wheeler, editors. *Biological nitrogen fixation in forest ecosystems: foundations and applications*. Martinus Nijhoff, The Hague, The Netherlands.
- Donald, C. M., and C. H. Williams. 1954. Fertility and productivity of a podzolic soil as influenced by subterranean clover (*Trifolium subterraneum* L.) and superphosphate. *Australian Journal of Agricultural Research* **5**:665–687.
- Eisele, K. A., D. S. Schimel, L. A. Kapustka, and W. J. Parton. 1989. Effects of available P and N:P ratios on non-symbiotic dinitrogen fixation in tallgrass prairie soils. *Oecologia* **79**:471–474.
- Ekblad, A., and K. Huss-Danell. 1995. Nitrogen fixation by *Alnus incana* and nitrogen transfer from *A. incana* to *Pinus sylvestris* influenced by macronutrients and ectomycorrhiza. *New Phytologist* **131**:453–459.
- Ekblad, A., H. Wallander, R. Carlsson, and K. Huss-Danell. 1995. Fungal biomass in roots and extramatrical mycelium in relation to macronutrients and plant biomass of ectomycorrhizal *Pinus sylvestris* and *Alnus incana*. *New Phytologist* **131**:443–451.
- Fastie, C. L. 1995. Causes and ecosystem consequences of multiple pathways of primary succession at Glacier Bay Alaska. *Ecology* **76**:1899–1916.
- Fraga-Beddiar, A., and F. Le Tacon. 1990. Interactions between a VA mycorrhizal fungus and *Frankia* associated with alder (*Alnus glutinosa* (L.) Gaetn.). *Symbiosis* **9**:247–258.
- Frenzen, P. M., M. E. Krasny, and L. P. Rigney. 1988. Thirty-three years of plant succession on the Kautz Creek mudflow, Mount Rainier National Park, Washington. *Canadian Journal of Botany* **66**:130–137.
- Gates, C. T. 1974. Nodule and plant development in *Stylo-*

- santhes humilis* H. B. K.: symbiotic response to phosphorus and sulphur. *Australian Journal of Botany* **22**:45–55.
- Giller, K. E. 1987. Use and abuse of the acetylene reduction assay for measurement of “associative” nitrogen fixation. *Soil Biology and Biochemistry* **19**:783–784.
- Groffman, P. M., and J. T. Tiedje. 1989. Denitrification in north temperate forest soils: spatial and temporal patterns at the landscape and seasonal scales. *Soil Biology and Biochemistry* **21**:612–620.
- Groffman, P. M., and C. L. Turner. 1995. Plant productivity and nitrogen gas fluxes in a tallgrass prairie landscape. *Landscape Ecology* **10**:255–266.
- Gutschick, V. P. 1981. Evolved strategies in nitrogen acquisition by plants. *American Naturalist* **118**:607–637.
- Harrington, J. T., and J. R. Seiler. 1988. Acetylene reduction in black alder seedlings as affected by direct and indirect moisture deficits using a split-pot growing system. *Environmental and Experimental Botany* **28**:225–230.
- Högberg, P. 1989. Root symbioses of trees in savannas. Pages 121–135 in J. Proctor, editor. *Mineral nutrients in tropical forest and savanna ecosystems*. Blackwell Scientific, Oxford, UK.
- Huss-Danell, K. 1990. The physiology of actinorhizal nodules. Pages 129–156 in C. R. Schwintzer and J. D. Tjepkema, editors. *The biology of Frankia and Actinorhizal plants*. Academic Press, San Diego, California, USA.
- Huss-Danell, K., P.-O. Lundquist, and A. Ekblad. 1989. Growth and acetylene reduction activity by intact plants of *Alnus incana* under field conditions. *Plant and Soil* **118**:61–73.
- Huss-Danell, K., P.-O. Lundquist, and H. Ohlsson. 1992. N<sub>2</sub> fixation in a young *Alnus incana* stand on seasonal and diurnal variation in whole plant nitrogenase activity. *Canadian Journal of Botany* **70**:1537–1544.
- Huss-Danell, K., and A. Sellstedt. 1985. Nitrogenase activity in response to darkening and defoliation of *Alnus incana*. *Journal of Experimental Botany* **36**:1352–1358.
- Ingestad, T. 1981. Nutrition and growth of birch and grey alder seedlings in low conductivity solutions and at varied relative rates of nutrient addition. *Physiologia Plantarum* **52**:454–466.
- Israel, D. W. 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant Physiology* **84**:835–840.
- Jobidon, R., and J.-R. Thibault. 1982. Allelopathic growth inhibition of nodulated and unnodulated *Alnus crispa* seedlings by *Populus balsamifera*. *American Journal of Botany* **69**:1213–1223.
- Killingbeck, K. T. 1993. Inefficient nitrogen resorption in genets of the actinorhizal nitrogen fixing shrub *Comptonia peregrina*: physiological ineptitude or evolutionary trade-off? *Oecologia* **94**:542–549.
- Klingensmith, K. M., and K. Van Cleve. 1993. Patterns of nitrogen mineralization and nitrification in floodplain successional soils along the Tanana River, Interior Alaska. *Canadian Journal of Forest Research* **23**:964–965.
- Lajtha, K. 1987. Nutrient reabsorption efficiency and the response to phosphorus fertilization in the desert shrub *Larrea tridentata* (DC.). *Biogeochemistry* **4**:265–276.
- Lawrence, J. W., and J. D. Tjepkema. 1990. Techniques for measuring nitrogenase activity in *Frankia* and Actinorhizal plants. Pages 264–280 in C. R. Schwintzer and J. D. Tjepkema, editors. *The biology of Frankia and Actinorhizal plants*. Academic Press, San Diego, California, USA.
- Luken, J. O., and R. W. Fonda. 1983. Nitrogen accumulation in a chronosequence of red alder communities along the Hoh River, Olympic National Park, Washington. *Canadian Journal of Forest Research* **13**:1228–1237.
- Mann, D. H., C. L. Fastie, E. L. Rowland, and N. H. Bigelow. 1995. Spruce succession, disturbance, and geomorphology on the Tanana River floodplain, Alaska. *Ecoscience* **2**(2):184–199.
- Marion, G. M., K. Van Cleve, C. T. Dyrness, and C. H. Black. 1993. The soil chemical environment along a forest primary successional sequence on the Tanana River floodplain, interior Alaska. *Canadian Journal of Forest Research* **23**:914–922.
- Olsen, S. R., and L. E. Sommers. 1982. Phosphorus. Pages 403–430 in A. L. Page, R. H. Miller, and D. R. Keeney, editors. *Methods of soil analysis, part two: chemical and microbiological properties*. American Society of Agronomy, and Soil Science Society of America, Madison, Wisconsin, USA.
- Paul, E. A., and R. M. N. Kucey. 1981. Carbon flow in plant microbial associations. *Science* **213**:473–474.
- Peoples, M. B., D. M. Lilley, V. F. Burnett, A. M. Ridley, and D. L. Garden. 1995. Effects of surface application of lime and superphosphate to acid soils on growth and N<sub>2</sub> fixation by subterranean clover in mixed pasture swards. *Soil Biology and Biochemistry* **27**:663–671.
- Reddell, P., P. A. Rosbrook, G. D. Bowen, and D. Gwaze. 1988. Growth responses in *Casuarina cunninghamiana* plantings to inoculation with *Frankia*. *Plant and Soil* **108**:79–86.
- Reichardt, P. B., F. S. Chapin, III, J. P. Bryant, B. R. Mattes, and T. P. Clausen. 1991. Carbon/nutrient balance as a predictor of plant defense in Alaskan balsam poplar: potential importance of metabolite turnover. *Oecologia* **88**:401–406.
- Reiners, W. A. 1981. Nitrogen cycling in relation to ecosystem succession. Pages 507–528 in F. E. Clark and T. Rosswall, editors. *Terrestrial nitrogen cycles*. Ecological Bulletin (Stockholm), Sweden.
- Rice, E. L. 1974. *Allelopathy*. Academic Press, San Diego, California, USA.
- Ruess, R. W., R. L. Hendrick, and J. P. Bryant. 1998. Regulation of fine root dynamics by mammalian browsers in early successional taiga forests of interior Alaska. *Ecology* **79**:2706–2720.
- Russo, O. R. 1989. Evaluating alder–endophyte (*Alnus acuminata*–*Frankia*–*Mycorrhizae*) interactions. *Plant and Soil* **118**:151–155.
- Sanginga, N., S. K. A. Danso, and G. D. Bowen. 1989. Nodulation and growth responses of *Alloccasuarina* and *Casuarina* species to phosphorus fertilization. *Plant and Soil* **118**:125–132.
- SAS Institute. 1995. *SAS/STAT user’s guide*. Release 6.1 edition. SAS Institute, Cary, North Carolina, USA.
- Scheiner, S. A. 1993. MANOVA: multiple response variables and multispecies interactions. Pages 94–112 in S. M. Scheiner and J. Gurevitch, editors. *Design and analysis of ecological experiments*. Chapman and Hall, New York, New York, USA.
- Schimel, J. P., R. G. Cates, and R. W. Ruess. 1998. The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. *Biogeochemistry* **42**:221–234.
- Schimel, J. P., K. Van Cleve, R. G. Cates, T. P. Clausen, and P. B. Reichardt. 1995. Effects of balsam poplar (*Populus balsamifera*) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. *Canadian Journal of Botany* **74**:84–90.
- Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. *Science* **195**:260–262.
- Schwintzer, C. R., A. M. Berry, and L. D. Disney. 1982. Seasonal patterns of root nodule growth, endophyte morphology, nitrogenase activity, and shoot development in *Myrica Gale*. *Canadian Journal of Botany* **60**:746–757.
- Shaver, G. R., and F. S. Chapin, III. 1991. Production: bio-

- mass relationships and element cycling in contrasting arctic vegetation types. *Ecological Monographs* **61**:1–31.
- Shaver, G. R., and J. M. Melillo. 1984. Nutrient budgets of marsh plants: efficiency concepts and relation to availability. *Ecology* **65**:1491–1510.
- Stewart, W. D. P., G. P. Fitzgerald, and R. H. Burris. 1967. In situ studies on N<sub>2</sub> fixation using the acetylene reduction technique. *Biochemistry* **58**:2071–2078.
- Uliassi, D. D. 1998. The regulation of symbiotic nitrogen fixation by thinleaf alder in primary successional forests of the Tanana River floodplain, interior Alaska. Thesis. University of Alaska, Fairbanks, Alaska, USA.
- Uliassi, D. D., K. Huss-Danell, R. W. Ruess, and K. Doran. 2000. Biomass allocation and nitrogenase activity in *Alnus tenuifolia*: responses to successional soil type and phosphorus availability. *Ecoscience* **7**:73–79.
- Van Cleve, K., F. S. Chapin, III, C. T. Dyrness, and L. A. Viereck. 1991. Element cycling in Taiga forests: state-factor control. *BioScience* **41**:78–88.
- Van Cleve, K., C. T. Dyrness, G. M. Marion, and R. Erickson. 1993a. Control of soil development on the Tanana River floodplain, interior Alaska. *Canadian Journal of Forest Research* **23**:941–955.
- Van Cleve, K., L. Oliver, R. Schlentner, L. A. Viereck, and C. T. Dyrness. 1983. Productivity and nutrient cycling in taiga forest ecosystems. *Canadian Journal of Forest Research* **13**:747–766.
- Van Cleve, K., L. A. Viereck, and R. L. Schlentner. 1971. Accumulation of nitrogen in alder (*Alnus*) ecosystems near Fairbanks, Alaska. *Arctic and Alpine Research* **3**:101–114.
- Van Cleve, K., J. Yarie, and R. Ericson. 1993b. Nitrogen mineralization and nitrification in successional ecosystems of the Tanana River floodplain, interior Alaska. *Canadian Journal of Forest Research* **23**:970–978.
- Viereck, L. A. 1966. Plant succession and soil development on gravel outwash of the Muldrow Glacier, Alaska. *Ecological Monographs* **36**:189–199.
- Viereck, L. A., C. T. Dyrness, and M. J. Foote. 1993a. An overview of the vegetation and soils of the floodplain ecosystems of the Tanana River, interior Alaska. *Canadian Journal of Forest Research* **23**:889–898.
- Viereck, L. A., K. Van Cleve, P. C. Adams, and R. E. Schlentner. 1993b. Climate of the Tanana River floodplain near Fairbanks Alaska. *Canadian Journal of Forest Research* **23**:899–913.
- Vitousek, P. M. 1982. Nutrient cycling and nutrient use efficiency. *American Naturalist* **119**:553–572.
- Vitousek, P. M. 1998. Foliar and litter nutrients, nutrient resorption, and decomposition in Hawaiian *Metrosideros polymorpha*. *Ecosystems* **1**:401–407.
- Vitousek, P. M. 2000. Nutrient limitations to nitrogen fixation in young volcanic sites. *Ecosystems* **2**:505–510.
- Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* **13**:87–115.
- Vitousek, P. M., and L. R. Walker. 1989. Biological invasion by *Myrica Faya* in Hawaii: plant demography, nitrogen fixation, ecosystem effects. *Ecological Monographs* **59**:274–265.
- Vitousek, P. M., L. R. Walker, L. D. Whiteaker, and P. A. Matson. 1993. Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry* **23**:197–215.
- Walbridge, M. R. 1991. Phosphorus availability in acid organic soils of the lower North Carolina Coastal Plain. *Ecology* **72**:2083–2100.
- Walker, L. R. 1993. Nitrogen fixers and species replacements in primary succession. Pages 249–272 in J. Miles and D. W. H. Walton, editors. *Primary succession on land*. Blackwell Scientific, Oxford, UK.
- Walker, L. R., and F. S. Chapin, III. 1986. Physiological controls over seedling growth in primary succession on an Alaskan floodplain. *Ecology* **67**:1508–1523.
- Walker, L. W. 1989. Soil nitrogen changes during primary succession on a floodplain in Alaska, U.S.A. *Arctic and Alpine Research* **21**:341–349.
- Walker, L. W., J. C. Zasada, and F. S. Chapin, III. 1986. The role of life history processes in primary succession on an Alaskan floodplain. *Ecology* **67**:1243–1253.
- Walker, T. W., and J. K. Syers. 1976. The fate of phosphorus during pedogenesis. *Geoderma* **15**:1–9.
- Warembourg, F. R., and C. Roumet. 1989. Why and how to estimate the cost of symbiotic N<sub>2</sub> fixation? a progressive approach based on the use of <sup>14</sup>C and <sup>15</sup>N isotopes. *Plant and Soil* **115**:167–177.
- Weisz, P. R., and T. R. Sinclair. 1988. Soybean nodule gas permeability, nitrogen fixation and diurnal cycles in soil temperature. *Plant and Soil* **109**:227–234.
- Whitledge, T. E., S. C. Mallow, C. J. Patton, and C. D. Wirrick. 1981. Automated nutrient analysis in seawater. Technical Report, Ocean Science Division, Brookhaven National Laboratory, Upton, New York, USA.
- Winship, L. J., and J. D. Tjepkema. 1990. Techniques for measuring nitrogenase activity in *Frankia* and actinorhizal plants. Pages 263–280 in C. R. Schwintzer and J. D. Tjepkema, editors. *The biology of Frankia and actinorhizal plants*. Academic Press, San Diego, California, USA.
- Younger, P. D., and L. A. Kapustka. 1983. N<sub>2</sub>(C<sub>2</sub>H<sub>2</sub>)ase activity by *Alnus incana* ssp. *rugosa* (Betulaceae) in the northern hardwood forest. *American Journal of Botany* **70**:30–39.
- Zitzer, S. F., and J. O. Dawson. 1989. Seasonal changes in nodular nitrogenase activity in *Alnus glutinosa* and *Elaeagnus angustifolia*. *Tree Physiology* **5**:185–194.