

Effects of defoliation on growth and N fixation in *Alnus tenuifolia*: Consequences for changing disturbance regimes at high latitudes¹

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Abstract: Alder plays an important role in the nitrogen (N) economy of boreal forests because of its high capacity for atmospheric N fixation. Range expansion and increased insect and/or pathogen attack are two potential consequences of climate change that may result in significant, albeit opposite, effects on these systems through influences on alder-mediated N inputs. This study contrasted the effects of weekly defoliation at different intensities on growth and N fixation in *Alnus tenuifolia* seedlings with recovery of these traits following a single but intensive defoliation event. Weekly removal of 15, 25, or 40% leaf area for 9 weeks reduced total plant weight by 7, 13, and 29%, respectively, and led to progressive increases in leaf weight ratio at the expense of shoot growth. Although maximum photosynthetic rates (P_{max}) were similar among treatments between defoliation events, increasing levels of defoliation led to progressive short-term declines in P_{max} immediately following treatments. Plants with 40% leaf removal had N fixation rates ($48.3 \pm 2.4 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) that were 67% less than undefoliated plants ($147.6 \pm 8.9 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$), and even the lowest level of leaf removal led to significant reductions in fixation rates relative to controls. In the recovery experiment, N fixation rates in defoliated plants ($158.4 \pm 12.1 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) were 40% less than control values ($264.1 \pm 18.3 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) 24 h following defoliation. After 28 d of regrowth, the total biomasses of defoliated and control plants were indistinguishable; however, N fixation rate in defoliated plants ($39.2 \pm 2.0 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) remained 73% less than that of control plants, suggesting a strong competition between symbiont and host sinks for photosynthate.

Keywords: Alaska, alder, climate change, defoliation, nitrogen fixation, ecosystem.

Résumé : L'aulne joue un rôle important dans la balance d'azote (N) des forêts boréales grâce à sa capacité élevée à fixer l'azote atmosphérique. L'expansion des aires de répartition et l'augmentation des attaques d'insectes et/ou de pathogènes sont des conséquences possibles du changement climatique qui pourraient avoir des effets significatifs quoique opposés sur ces systèmes via leurs influences sur les apports de N par l'aulne. Dans cette étude, les effets contrastés de défoliations hebdomadaires de différentes intensités sur la croissance et la fixation de N ont été examinés pour des semis de *Alnus tenuifolia* avec récupération de ces traits suite à un unique épisode de défoliation intense. Le retrait hebdomadaire de 15, 25 ou 40 % de la surface des feuilles pour neuf semaines a causé une réduction du poids total de la plante de 7, 13 et 29 %, respectivement et a conduit à une augmentation progressive du rapport de la masse foliaire au détriment de la croissance de la tige. Même si les taux maximum de photosynthèse (P_{max}) étaient similaires pour tous les traitements entre les épisodes de défoliation, l'augmentation du niveau de défoliation causait des déclin progressifs à court terme de P_{max} tout de suite après les traitements. Les plantes ayant perdu 40 % de leurs feuilles avaient des taux de fixation de N ($48.3 \pm 2.4 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) 67 % moins élevés que ceux des plantes n'ayant pas été défoliées ($147.6 \pm 8.9 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) et même la plus faible défoliation provoquait des réductions significatives du taux de fixation de N en comparaison avec les contrôles. Dans l'expérience de récupération, les taux de fixation de N des plantes défoliées ($158.4 \pm 12.1 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) étaient 40 % plus faibles que ceux des plantes contrôles ($264.1 \pm 18.3 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) 24 heures après la défoliation. Après 28 jours de croissance, les biomasses totales des plantes défoliées et contrôles étaient similaires, cependant, le taux de fixation de N des plantes défoliées demeurait 73 % moins élevé que celui des plantes contrôles, ce qui suggère une compétition importante entre les puits du symbionte et de l'hôte pour le photosynthétat.

Mots-clés : Alaska, aulne, changement climatique, défoliation, écosystème, fixation de l'azote.

Nomenclature: USDA, 2001; Flora of North America Editorial Committee, 2004; Index Fungorum Partnership, 2004.

Introduction

The expansion of shrubs in the Alaskan arctic over the past 50 y represents one of the most significant vegetation shifts linked with climate warming throughout northwestern North America (Silapaswan, Verbyla & McGuire, 2001; Sturm, Racine & Tape, 2001). Contributing to this large-scale vegetation change is the spread of *Alnus*, a nitrogen-fixing woody shrub found throughout Alaska and the

boreal forest. In addition to influencing land-atmosphere energy exchange and snow accumulation (Chapin *et al.*, 2000; 2005), this structural change in vegetation can potentially alter the nitrogen (N) balance of boreal and arctic ecosystems through atmospheric N inputs via N fixation (Vogel & Gower, 1998; Rhoades *et al.*, 2001; Myrold & Huss-Danell, 2003). For example, along the Tanana River floodplain in interior Alaska, nearly all the N accumulated during 150 y of forest succession can be accounted for by N fixation by *Alnus incana* ssp. *tenuifolia* (hereafter, *Alnus tenuifolia*) during the first 15–30 y of vegetation develop-

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ment (Uliassi & Ruess, 2002). Long-term N additions to arctic tundra are known to modify soil microbial processes, the production and cover of shrubs, and ecosystem carbon storage (Chapin *et al.*, 1995; Shaver *et al.*, 2001; Mack *et al.*, 2004). Increased N inputs following alder expansion can also modify watershed biogeochemistry and aquatic trophic dynamics (Goldman, 1961; Engstrom *et al.*, 2000), as occurred throughout southern and northwestern Alaska during the early- to mid-Holocene (Anderson & Brubaker, 1994; Oswald, Brubaker & Anderson, 1999; Hu, Finney & Brubaker, 2001).

Accompanying the recent warming of high-latitude ecosystems is the potential for increased frequency of insect outbreaks, ostensibly through a shortening of time necessary for life-cycle completion (Malmstrom & Raffa, 2000). Aboveground tissues of N-fixing woody plants are typically well defended chemically against vertebrate and invertebrate herbivores. Presumably, the costs associated with regrowth coupled with the carbon and nutrient demands of N-fixing and mycorrhizal symbionts have selected for chemical defence in these plants. In interior Alaska, both *Alnus viridis* ssp. *fruticosa* and *Alnus tenuifolia* are generally avoided by vertebrate and invertebrate herbivores (Buttler, 2003), a behaviour that promotes the dominance of alders during early succession and leads to high rates of ecosystem N input (Kielland, Bryant & Ruess, 1997; Uliassi & Ruess, 2002). However, periodic outbreaks of a number of herbivorous insects, notably the introduced alder woolly sawfly (*Eriocampa ovata*), can partially or completely defoliate *A. tenuifolia* over large landscapes (R. W. Ruess, pers. observ.; USDA, 2005). The population dynamics of these outbreaks are largely unknown, as are the impacts of defoliation on growth and N-fixation potential of *Alnus*. Recently, *A. tenuifolia* has also suffered outbreaks of a fungal stem canker (putatively *Valsa melanodiscus* [anamorph *Cytospora umbriana*]), resulting in widespread growth suppression, branch dieback, and ramet mortality throughout south-central and interior Alaska (see below) (USDA, 2005; Lori Trummer, pers. comm.). If defoliation reduces growth and N fixation in *Alnus* to the degree it can in leguminous trees (Nygren *et al.*, 2000), then ecosystem-level N inputs throughout the Alaskan boreal forest could be reduced substantially.

The purpose of this study was to examine the impacts of simulated insect herbivory on growth, photosynthesis, and N fixation in *Alnus tenuifolia* seedlings. We were particularly interested in comparing responses to long-term chronic defoliation at different intensities with recovery following a single intensive defoliation event, as both patterns of insect defoliation occur periodically in interior Alaska.

Methods

PLANT GROWTH CONDITIONS

During late fall, seeds of *Alnus tenuifolia* were collected from 10–20 individuals at three early successional, alder-dominated sites (10–20 y age) scattered along the Tanana River floodplain and stored in bulk at 4 °C. Collection sites are included within a larger network of permanent sites under study by the Bonanza Creek Long Term Ecological Research Program (BNZ LTER), approximately 30 km

southwest of Fairbanks, Alaska (64° 48' N, 147° 52' W). Further information regarding these sites can be found on the BNZ LTER webpage (<http://www.lter.uaf.edu/>). Plants were grown in a greenhouse in 1-L Styrofoam cups filled with soil collected from the surface layers (top 10 cm) at the same sites from which seeds were collected. Our previous experiments have shown that native organic soil results in high levels of nodulation and mycorrhizal infection without the need for supplemental *Frankia* inoculation. Five to 10 seeds were germinated on the moistened soil surface with pots covered with clear plastic wrap. After germination and seedling establishment, plants were thinned to one individual per pot and grown in a greenhouse under an 18-h photoperiod under high-pressure sodium lights that provided approximately 450 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR at canopy height. Plants were watered every other day and given a N-free nutrient solution weekly containing standard concentrations of micro- and macronutrients. Volumes of water and nutrients were increased throughout the experiment to meet plant demands.

After four weeks of growth, plants were randomly assigned to one of two experiments or a set of allometric controls: continuous defoliation (“Defoliation”), recovery from chronic defoliation (“Recovery”), or “Allometric controls”. All plants were seeded and received a final harvest on the same dates.

For the defoliation experiment, plants were grown for 9 weeks with 0, 15, 25 or 40% of leaf area removed ($n = 12$ pots per treatment). Each week, leaf area was removed from new fully emerged cohorts of leaves using a standard paper punch (5 mm diameter punch). We first established a relationship between leaf length (cm) and leaf area (cm^2) (Leaf Area = $0.456 \cdot \text{Length}$, $r^2 = 0.94$) and then calculated the number of punches required to achieve a given treatment level for each leaf based on length. Our original plan was to have much higher levels of defoliation (*e.g.*, 75%); however, we found that 40% was the maximum area that could be removed with punches and still retain an intact leaf. Leaves punched one week were not punched again.

For the recovery experiment, 60 plants received a one-time defoliation (40% leaf removal) on week 5 of the experiment. Thereafter, cohorts ($n = 12$) were sampled and harvested after 6 h, 24 h, and then in weeks 6, 7, and 9.

Allometric controls were established to track plant growth and N fixation throughout the 9-week experimental period in order to distinguish defoliation responses from phenological changes in growth and N fixation occurring throughout the experiment. These controls were initiated at the same time as the defoliation and recovery experiments, with cohorts ($n = 10$) sampled and harvested on weeks 2, 5, 6, and 7. Samples on weeks 5, 6, and 7 served as control plants for the recovery experiment, and the “0%” treatment for the defoliation experiment served as the week 9 cohort for the allometric controls.

PHOTOSYNTHETIC RATES

During the ninth week of the experiment, photosynthetic rates were measured on plants from each of the four treatment levels of the defoliation experiment using a LI-6400 Photosynthesis System (LiCOR, Lincoln, Nebraska, USA). Maximum photosynthetic rates (P_{max}) and the photosynthetic

time response to defoliation were measured on a separate cohort of five randomly selected plants on different days. For P_{\max} , measurements were made on one fully expanded, untreated leaf from each plant the day prior to the scheduled weekly defoliation treatment. Photosynthesis was logged after rates had stabilized under the following conditions: PPFD = 500 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, CO₂ concentration = 400 ppm, leaf temperature = 20 °C. To measure short-term photosynthetic response to defoliation, one fully expanded leaf was placed in the cuvette, and photosynthetic rates were allowed to stabilize. All other leaves were then defoliated according to the treatment level (0, 15, 25, or 40% leaf removal) during which time photosynthesis of the intact leaf was monitored continuously for an additional 30 min.

PLANT HARVEST AND NITROGEN FIXATION

At harvest, plants were separated into leaves, shoots, roots, and nodules, which were dried for 48 h at 60 °C and weighed to the nearest mg. Specific leaf area ($\text{cm}^2\cdot\text{g}^{-1}$) was measured on five fully expanded, untreated leaves on each plant during harvest using a 1-cm-diameter cork borer.

Nitrogen fixation rate of each plant was measured at harvest using a ¹⁵N₂ uptake method (Anderson *et al.*, 2004). First, the stem was cut at the soil surface, and then the entire belowground mass was immersed in water so that soil could be teased free of the root system. Approximately 2.5 g of fresh nodule with attached fine roots was harvested, placed in a 60-mL polyethylene syringe fitted with a septum, and buried in wet sand maintained at 15 °C. Ten mL of 99 atom% ¹⁵N₂ (Isotec Inc., Miamisburg, Ohio, USA) was then added to the syringe to produce an incubation atmosphere of approximately 15% ¹⁵N₂. Immediately after the addition of the ¹⁵N₂, a 15-mL sample of the incubation atmosphere was removed to provide a quantitative measure of atom percent enrichment (APE) of ¹⁵N₂ at time zero (T₀). These samples were stored in 10-mL exetainers (Labco, High Wycombe, Buckinghamshire, UK) before analysis using a dual-inlet isotope ratio mass spectrometer (PDZ Europa Scientific Instruments, Crewe, Cheshire, UK). After the removal of the incubation atmosphere sample, the syringe containing the root nodules was immediately returned to wet sand for 10 minutes. Nodules were then removed from the syringe and immediately frozen in liquid N₂. The nodules were thoroughly rinsed through a fine sieve to remove all adhering soil and organic material, dried for 48 h at 60 °C, and ground using a Wig-L-Bug ball mill (Reflex Analytical, Ridgewood, New Jersey, USA) in preparation for mass spectrometry analysis. An additional dried nodule sample from each plant not incubated with ¹⁵N₂ was used as a control for the determination of atom percent enrichment (APE) for each nodule sample according to the following equation:

$$\text{APE}_{\text{nodule}} = \frac{^{15}\text{N}_{\text{enriched nodules}} - ^{15}\text{N}_{\text{control nodules}}}{^{15}\text{N}_{\text{atmosphere}}} \quad [1]$$

where both ¹⁵N content measures are in atom%. By combining APE with total nodule N content, dividing by incubation time, and correcting for the composition of the initial incubation atmosphere as determined by mass spectrometry, we calculated the specific N fixation activity of the nodule samples ($\text{SNF} = \mu\text{mol N assimilated}\cdot\text{g}_{\text{DWT}}^{-1}\cdot\text{h}^{-1}$) as follows:

$$\text{SNF} = \frac{(\text{APE}_{\text{nodule}} \times \%N_{\text{nodule}})}{(\text{incubation time (h)} \times \%^{15}N_{\text{atmosphere}})} \quad [2]$$

where $\%N_{\text{nodule}}$ is the mass percent N content of the enriched nodule sample and $\%^{15}N_{\text{atmosphere}}$ is the atom percent ¹⁵N content of the incubation atmosphere at the beginning of the assay.

STATISTICAL ANALYSES

Variations among treatments within the defoliation experiment and among time periods within the allometric controls and the recovery experiment were analyzed separately by ANOVA (PROC GLM) (SAS, 2002). Data were square-root or log₁₀(X + 1) transformed where necessary to meet ANOVA assumptions. To contrast the allometric controls and recovery experiment, each population designation was entered as a class variable, and least-squares means (LSM) of dependent variables were compared by time period. A number of relationships among growth-response variables were explored using linear regression. Statistical significance was determined at $\alpha = 0.05$ for all analyses. Unless otherwise stated, data presented are means (± 1 SE) of untransformed data.

Results

ALLOMETRIC CONTROLS

Harvests revealed significant changes in both growth and rates of N fixation of the allometric controls throughout the 9-week experimental period. The most notable changes were an increase in biomass allocation to shoots at the expense of leaf tissue as plants grew taller and a peak in N-fixation rates measured during week 5 (Figures 1 and 2).

DEFOLIATION EXPERIMENT

Relative to undefoliated plants, weekly defoliation by 15, 25, and 40% leaf removal reduced total plant weight by 7 (ns), 13 ($P < 0.01$), and 29% ($P < 0.0001$), respectively (Table I). Increasing levels of defoliation reduced

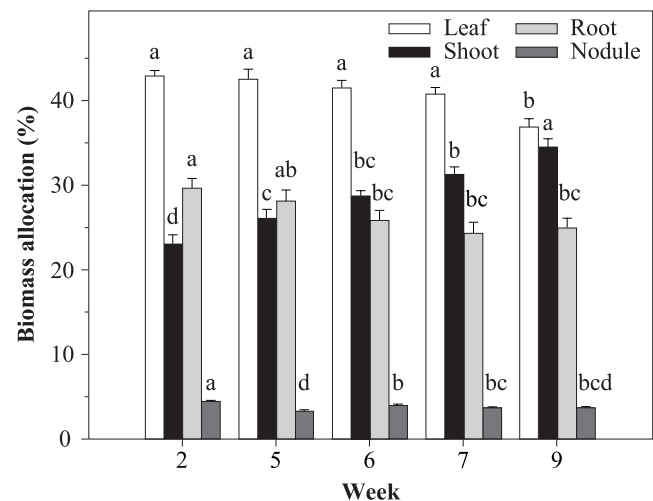


FIGURE 1. Biomass allocation (mean + 1 SE) to leaf, shoot, root, and nodule in untreated plants (= allometric controls) throughout the experimental period. Different letters above bars within a tissue type represent significant differences among time periods at $P < 0.05$.

biomass allocation to shoots but increased leaf weight ratio (Figure 3). Given that harvested leaf biomass values did not include tissue removed throughout the experiment, actual biomass allocation to leaves in defoliated plants was greater than reported. Plants with 25 and 40% leaf removal had lower allocation to root mass than other treatments, but no differences in allocation to nodules were found among treatments, which averaged $3.4 \pm 0.1\%$ across all plants. There were some differences, but no distinct pattern in specific leaf area among treatments (Table I).

Defoliation did not affect maximum photosynthetic rates, regardless of whether data were expressed per unit area (Table I) or per unit mass (not shown). However, immediately following a defoliation event of 25 or 40%, photosynthetic rates declined briefly relative to controls (Figure 4). The decline in photosynthetic rates was correlated with declines in stomatal conductance in these plants (data not shown).

Increasing levels of defoliation led to increasing reductions in N-fixation rates (Figure 5). Plants with 40% leaf removal had N-fixation rates ($48.3 \pm 2.4 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$)

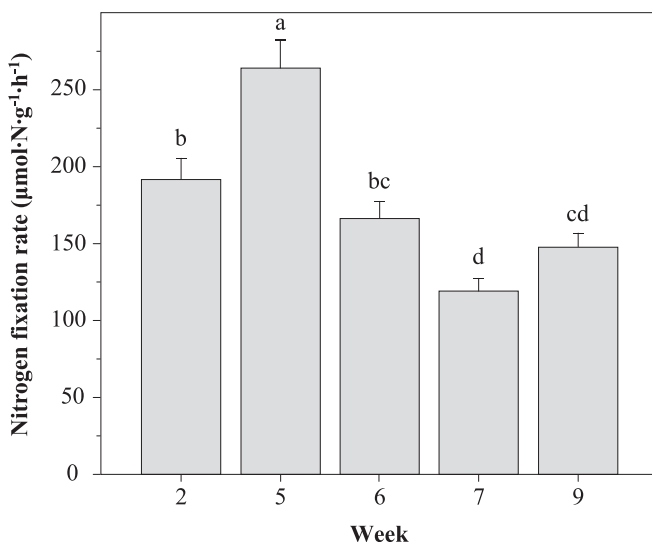


FIGURE 2. Nitrogen fixation rates (mean + 1 SE) of untreated plants measured throughout the experimental period. Statistical format follows Figure 1.

that were 67% less than undefoliated plants ($147.6 \pm 8.9 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$), and even plants with the lowest level of leaf removal (15%) had significantly lower fixation rates than controls. Across all plants, leaf N concentration increased with N-fixation rate (Leaf N [%] = $2.55 + 0.0023 \cdot \text{N-fixation rate} [\mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}]$; $r^2 = 0.12$, $P < 0.01$).

RECOVERY EXPERIMENT

One-time removal of 40% of the leaf area during week 5 reduced total plant biomass by 18% after 1 week of regrowth ($P < 0.0001$), and by 25% after 2 weeks of regrowth ($P < 0.0001$), relative to control plants (Figure 6). However, after a month of regrowth, the total biomasses of defoliated and control plants were indistinguishable, due principally to an increased biomass allocation to leaf area at the expense of height growth in defoliated plants (Figure 7).

The nitrogen-fixation rate in defoliated plants ($158.4 \pm 12.1 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) was 40% less than control values ($264.1 \pm 18.3 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) 24 h following defoliation (Figure 8). This rate remained relatively stable over the following 2 weeks in defoliated plants, at which time there was no difference in rates between control and treated plants. However, there was a significant decline in N-fixation rate in defoliated plants between weeks 7 and 9, and by the final harvest, N-fixation rate in defoliated plants ($39.2 \pm 2.0 \mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) was 73% less than those of control plants ($P < 0.0001$). Leaf N concentration in defoliated plants (2.59 ± 0.07) was also significantly less than that of control plants (2.98 ± 0.11) by the end of the experiment ($P < 0.001$).

Discussion

EFFECTS OF DEFOLIATION ON N-FIXATION

Our finding that increasing levels of defoliation leads to progressive declines in N-fixation rate in *A. tenuifolia* is consistent with findings from other clipping/defoliation studies with both legumes (Cralle & Heichel, 1981; Davidson, Culvenor & Simpson, 1990; Gordon & Kessler, 1990; Ta, MacDowall & Faris, 1990; Kim *et al.*, 1993; Nygren *et al.*, 2000) and actinorhizal (Huss-Danell & Sellstedt, 1985) plants. Weekly removal of 40% of the area of new leaf cohorts of *A. tenuifolia* reduced N-fixation rate

TABLE I. Growth characteristics and N-fixation rates of *Alnus tenuifolia* defoliated weekly at four levels of leaf removal. Numbers within rows followed by different letters are significantly different at $P < 0.05$.

	0%	15%	25%	40%
Biomass ($\text{g}\cdot\text{plant}^{-1}$)				
Leaf	$10.8 \pm 0.5\text{ab}$	$10.7 \pm 0.4\text{ab}$	$11.4 \pm 0.4\text{a}$	$9.7 \pm 0.2\text{b}$
Shoot	$10.1 \pm 0.5\text{a}$	$9.0 \pm 0.6\text{ab}$	$7.9 \pm 0.4\text{b}$	$5.9 \pm 0.2\text{c}$
Root	$7.3 \pm 0.5\text{a}$	$6.6 \pm 0.4\text{a}$	$5.4 \pm 0.3\text{b}$	$4.3 \pm 0.2\text{c}$
Nodule	$1.1 \pm 0.0\text{a}$	$0.9 \pm 0.1\text{b}$	$0.9 \pm 0.0\text{b}$	$0.7 \pm 0.0\text{c}$
Total	$29.3 \pm 1.2\text{a}$	$27.3 \pm 1.1\text{ab}$	$25.6 \pm 0.8\text{b}$	$20.7 \pm 0.6\text{c}$
Leaf N (%)	$2.98 \pm 0.11\text{a}$	$2.84 \pm 0.07\text{ab}$	$2.66 \pm 0.05\text{b}$	$2.67 \pm 0.04\text{b}$
Specific leaf area ($\text{cm}^2\cdot\text{g}^{-1}$)	$185.9 \pm 6.9\text{ab}$	$190.3 \pm 5.0\text{ab}$	$174.6 \pm 3.1\text{b}$	$195.2 \pm 7.8\text{a}$
Photosynthetic rate ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	$6.0 \pm 0.7\text{a}$	$5.8 \pm 0.5\text{a}$	$6.1 \pm 0.4\text{a}$	$6.1 \pm 0.4\text{a}$
N fixation rate ($\mu\text{mol N}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$)	$147.6 \pm 8.9\text{a}$	$114.3 \pm 9.1\text{b}$	$98.3 \pm 8.8\text{b}$	$48.3 \pm 2.4\text{c}$

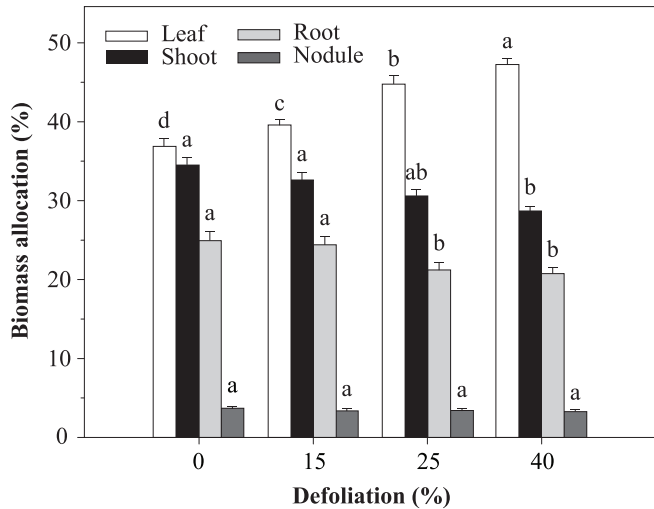


FIGURE 3. Biomass allocation (mean ± 1 SE) to leaf, shoot, root, and nodule in plants exposed to weekly removal of 0, 15, 25, or 40% of new leaves for 9 weeks. Statistical format follows Figure 1.

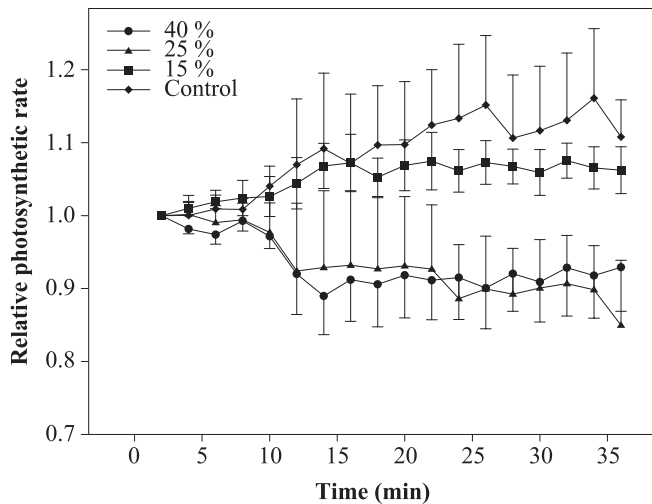


FIGURE 4. Response of photosynthesis (mean ± 1 SE) of an undefoliated leaf to defoliation (imposed at time = 10 min) of all other leaves at the level of 0, 15, 25, or 40% leaf removal. Statistical format follows Figure 1.

by 67% when defoliation was maintained over 9 weeks, although significant reductions in N fixation resulted from much lower levels of defoliation. We also found that reductions in N-fixation rate from a single defoliation event (recovery experiment) were immediate, resulting in a 40% reduction within 24 h. Moreover, even after 5 weeks of recovery from the one-time defoliation event, N-fixation rates in untreated plants were approximately 4 times that of defoliated plants of the same age.

A number of physiological mechanisms may reduce N fixation in defoliated alders. First, there is growing consensus that defoliation, like other disturbances that modify whole-plant carbon balance, such as changes in light regime and CO₂ concentration (Arnone & Gordon, 1990; Lundquist & Huss-Danell, 1991; Benamar, Thiery & Pizelle, 1995; Huss-Danell, 1997; Temperton *et al.*, 2003), reduces flux of reductant (notably sucrose) to nodule vesicles (Parsons & Sunley, 2001; Lundquist, Nasholm & Huss-Danell, 2003).

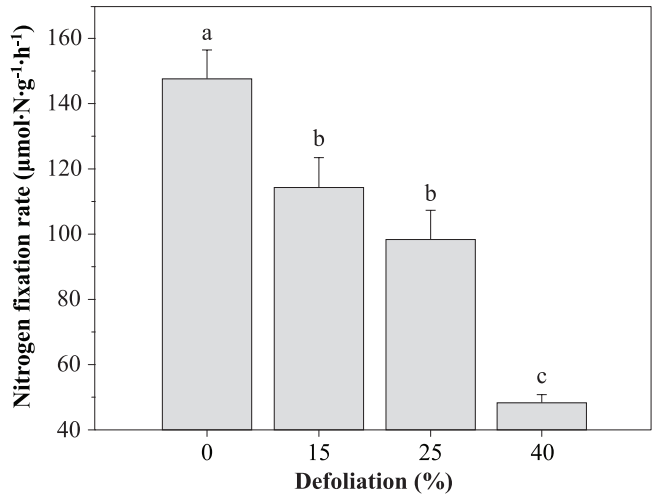


FIGURE 5. Nitrogen fixation rates (mean ± 1 SE) of plants exposed to weekly removal of 0, 15, 25, or 40% of new leaves for 9 weeks. Statistical format follows Figure 1.

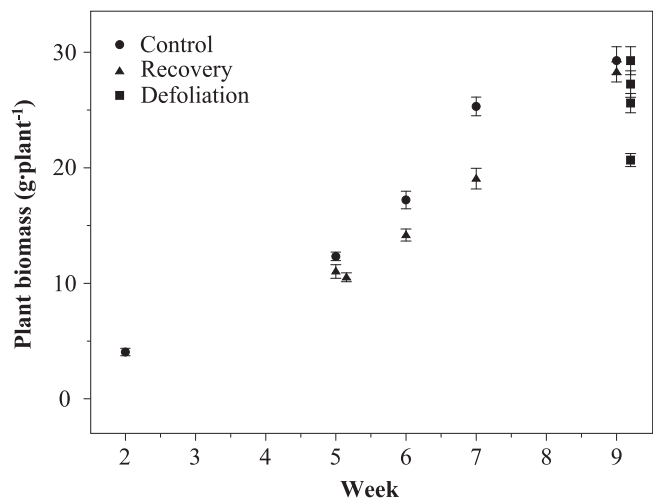


FIGURE 6. Total biomass of plants (mean ± 1 SE) from allometric controls and recovery and defoliation experiments measured periodically throughout the experimental period. Statistical format follows Figure 1.

Interrupted photoassimilate supply to nodules following severe defoliation may reduce respiratory consumption of oxygen within root nodules. For example, Lundquist, Nasholm and Huss-Danell (2003) found that exposing *Alnus incana* to dark stress for 22 h led to an oxygen-mediated degradation of the nitrogenase FeMo protein sub-unit and an associated inhibition of enzyme activity.

An equally plausible mechanism for the decline in N fixation following defoliation is the activation of an autoregulatory inhibition of nitrogenase, driven by elevated whole plant N:P ratios (Huss-Danell, 1997; Valverde & Wall, 2003). This down regulation of nitrogenase activity and nodule production and growth is thought to be mediated by a phloem-transported signal inhibitor responsive to plant nitrogen balance. Because of the high phosphate demand in leguminous and actinorhizal plants, autoregulation of nodule growth and function are more closely linked to plant N:P ratio than to N balance alone (Huss-Danell *et al.*, 2002; Valverde, Ferrari & Wall, 2002; Vitousek *et al.*,

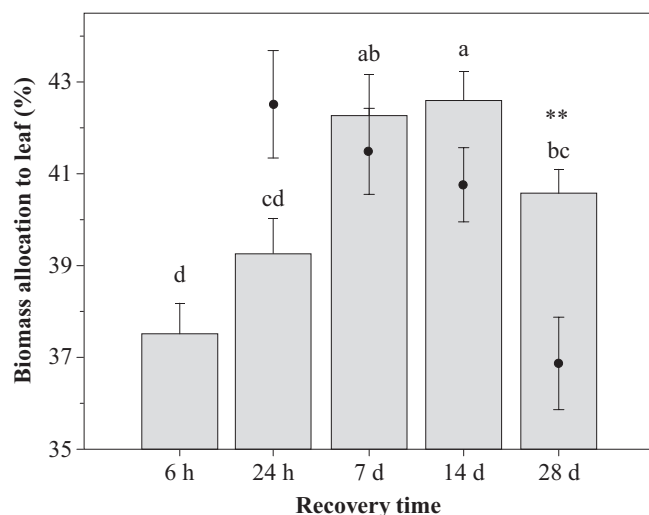


FIGURE 7. Biomass allocation to leaf (mean \pm 1 SE) measured at 6 h and 24 h (week 5), and then after 7 d (week 6), 14 d (week 7), and 28 d (week 9) following a one-time defoliation treatment of 40% leaf removal (bars). Different letters above bars represent significant differences among time periods at $P < 0.05$. Asterisks above bars represent differences between allometric controls (points) and defoliated plants (bars) for a given time period at $P < 0.05$.

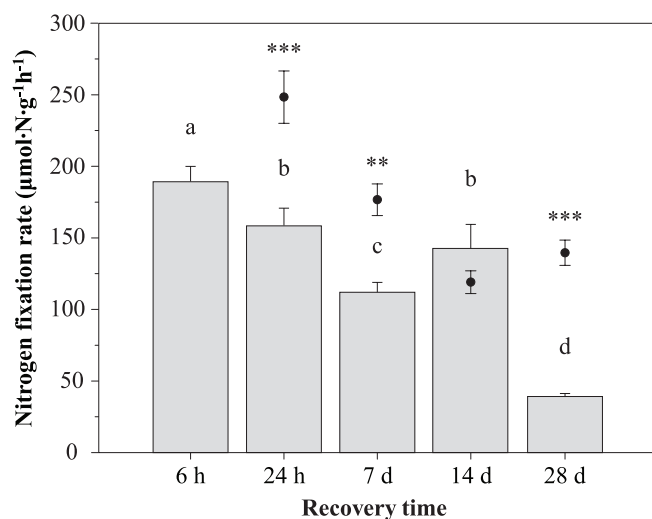


FIGURE 8. Nitrogen fixation rates (mean \pm 1 SE) measured at 6 h and 24 h (week 5), and then after 7 d (week 6), 14 d (week 7), and 28 d (week 9) following a one-time defoliation treatment of 40% leaf removal (bars). Different letters above bars represent significant differences among time periods at $P < 0.05$. Asterisks above bars represent differences between allometric controls (points) and defoliated plants (bars) for a given time period at $P < 0.05$.

2002; Gentili & Huss-Danell, 2003). Wall, Valverde, and Huss-Danell (2003) discussed the importance of the direct positive effect of P on nodule activity distinct from a more general response to whole-plant P status. Two systemic signalling processes are thought to occur at different stages of root growth and development (Wall, 2000). The first occurs during the early stages of infection prior to the initiation of N fixation, while the second, which varies among actinorhizal genera, is a whole-plant response regulating stimulation or inhibition of nodule function that occurs once the symbiosis has been established. In *Alnus*, a change in plant N balance can release this inhibitory down regulation to increase

new nodule production and stimulate nitrogenase activity, as Wall, Valverde, and Huss-Danell (2003) showed for *Alnus incana* when plants were switched to a N₂-free atmosphere. Plant N demand can also be elevated through alleviation of plant P stress. For example, Gentili and Huss-Danell (2003) showed increased nodulation and nitrogenase activity in greenhouse-grown *Alnus incana* following P fertilization, as did Binkley, Senock, and Cromack (2003) working with *Facaltaria moluccana* seedlings. We previously found a similar response in mature *Alnus tenuifolia* growing in the field, where P fertilization of alder stands increased the production of new (single-lobed) nodules by > 200% and stand-level N-fixation rates by 138% (Uliassi & Ruess, 2002).

At least two factors would contribute to increased plant N:P ratios following severe defoliation. First, an immediate reduction in whole plant assimilation rate would depress growth rates and plant demand for N, leading to a short-term accumulation of N through atmospheric fixation. Second, defoliation would likely impose competition among leaf meristems for plant phosphorus (P), a large portion of which is contained in leaves, leading to plant P stress. Collectively, both factors would increase whole-plant N:P ratio and trigger the down regulation of nitrogenase activity. Similar mechanisms can be invoked to explain reductions in N fixation as plants age. For example, Voisin *et al.* (2003) found progressively lower N-fixation rates per unit carbon allocated to nodulated roots in *Pisum sativum* among vegetative, flowering, and seed-filling stages, suggesting a prioritized shift in carbon allocation relative to nitrogen demand.

RECOVERY OF PLANT GROWTH AND N FIXATION FOLLOWING DEFOLIATION

During recovery from defoliation, the carbon and nutrient costs of N fixation impose constraints on regrowth in actinorhizal plants. Similar to non N-fixing plants (Louahlia *et al.*, 2004), N and carbon required for tissue regrowth following defoliation in N-fixing plants are derived from the remobilization of reserves (Ta, MacDowall & Faris, 1990; Kim *et al.*, 1993; Ourry, Kim & Boucaud, 1994; Belanger & Richards, 2000; Simon *et al.*, 2004). And while the tripartite associations involving both ectomycorrhizae and *Frankia* have been shown to enhance growth and N-fixing capacity in *Alnus tenuifolia* when compared to *Frankia* infection alone (Yamanaka *et al.*, 2003), it is likely that supporting both symbionts imposes substantial carbon and nutrient costs following defoliation. We found that biomass recovery following a single severe defoliation event was aided by an increased allocation to leaf area (Figures 6 and 7), but this appears to have occurred at the expense of carbon flux to nodules. By the end of the recovery experiment, total biomasses of defoliated and control plants were indistinguishable, but N-fixation rates in once-defoliated plants were over 70% less than controls. Since leaf N concentration of defoliated plants was also less than controls, it appears that rather than being down regulated, N-fixation rates in defoliated plants were relying on soil N to meet plant N demands. Plants were fed N-free fertilizer but grown in soil excavated from the surface organic horizons beneath dense alder stands along the Tanana River floodplain. Since most roots on experimental plants were infected with ectomycorrhizae

(R. W. Ruess, pers. observ.), this symbiosis may have aided in N uptake (Read, Leake & Perez-Moreno, 2004). We are not aware of any studies documenting the energy costs of supporting both ectomycorrhizae and *Frankia*, but ectomycorrhizae are known to consume a significant portion of photosynthates (Wu, Nara & Hogetsu, 2002), and Tjepkema (1985) estimated that N fixation consumed approximately 16% of gross photosynthesis in *Alnus rubra* seedlings.

ECOSYSTEM LEVEL CONSEQUENCES OF PEST OUTBREAKS

Our results suggest that recent increases in insect and pathogen outbreaks on *Alnus tenuifolia* throughout south-central and interior Alaska (USDA, 2005) may have substantial effects on ecosystem-level N inputs to the Alaskan boreal forest. We are not aware of any studies examining the impacts of defoliators on N-fixation rates by alder under field conditions, and we are hesitant in translating results from our seedling experiment to natural stands of mature shrubs. However, we predict that current rates of pathogen and defoliator attack have substantially reduced N-fixation inputs below our earlier estimates of 59 ± 11 (early successional) and $38 \pm 11 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$ (mid-successional) made over a decade ago along the Tanana River (Uliassi & Ruess, 2002). The current stem canker outbreak is greatest in south-central Alaska, where the co-occurrence of canker infection and sawfly defoliation has resulted in nearly complete stand mortality along major drainages, such as the Eagle River northwest of Anchorage (R. W. Ruess, pers. observ.). At the BNZ LTER research sites along the Tanana River floodplain in interior Alaska, stem canker is most prominent in early successional stands, where *Alnus tenuifolia* dominates the canopy understory and 82% of recruitment-size stems (< 4 cm diameter) and 72% of larger stems (> 4 cm diameter) are either infected with or dead from canker (Table II). We are currently examining whether this level of stem mortality has resulted in an associated loss of stand-level N-fixation inputs through fixation.

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TABLE II. Stem density and levels of stem canker pathogen infection in *Alnus tenuifolia* (mean \pm SE) growing in early successional shrub-dominated stands, balsam poplar stands, and white spruce stands along the Tanana River (BNZ LTER sites). At each site ($n = 3/\text{stand type}$), canker infection was assessed on all stems within a 30- \times 30-m plot during July 2005.

	Early shrub	Balsam poplar	White spruce
Stem density (stems \cdot ha ⁻¹) and basal area (m ² \cdot ha ⁻¹) in parentheses)	17,942 \pm 2212 (95.9 \pm 3.0)	7119 \pm 495 (21.4 \pm 1.7)	6055 \pm 585 (13.7 \pm 2.0)
% of ramets < 4 cm diameter either infected with (upper value) or dead from (in parentheses) canker	38 \pm 16 (43 \pm 20)	6 \pm 1 (22 \pm 2)	9 \pm 6 (28 \pm 2)
% of ramets > 4 cm diameter either infected with (upper value) or dead from (in parentheses) canker	66 \pm 8 (6 \pm 2)	25 \pm 6 (37 \pm 2)	24 \pm 16 (29 \pm 6)

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