

Seasonal patterns of climate controls over nitrogen fixation by *Alnus viridis* subsp. *fruticosa* in a secondary successional chronosequence in interior Alaska¹

Jennifer S. MITCHELL & Roger W. RUESS², Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska 99775, USA, e-mail: rwruess@alaska.edu

Abstract: Patterns of and controls over N₂ fixation by green alder were studied in post-fire, mid-succession, and white spruce upland forests in interior Alaska, focusing on the hypothesis that ecosystem-level nitrogen (N) inputs decrease with successional development. N₂-fixation rates tracked plant phenology during the 1997 (drought) and 1998 (normal precipitation) growing seasons. The best model for predicting acetylene reductase activity (ARA, μmol C₂H₄·g nodule_{DWT}⁻¹·h⁻¹) across all stands and sampling periods included a linear response to soil temperature and a quadratic response to Julian day ($r^2 = 0.23$, $P < 0.0001$). There were few significant relationships between seasonal maximum values for ARA and measured leaf traits; however, we did detect an inverse correlation between foliar N to P ratio and seasonal maximum ARA. During 1998, stands with higher maximum ARA had higher soil %N, and maximum ARA was positively correlated with subcanopy %P in O and A soil horizons. During 1997, leaf %P and N resorption were lower and leaves were thinner compared to 1998. Drought effects were most pronounced in mid-succession, where alder exhibited reduced ARA, leaf %P, leaf thickness, and lower leaf resorption of P and N. Although ARA and nodule biomass did not differ among stand types, increases in alder densities with successional time translated to increasing ecosystem-level N inputs across the chronosequence. These results contradict established theory predicting a decline in N₂-fixation rates and N₂-fixer abundance during successional stand development.

Keywords: alder, boreal, nitrogen cycling, nitrogen fixation, succession.

Résumé : Les patrons et les facteurs régissant la fixation du N₂ par l'aulne crispé ont été étudiés dans des peuplements après feu, de milieu de succession et de pessières blanches dans des forêts des hautes terres de l'intérieur l'Alaska, en se concentrant sur l'hypothèse que les niveaux d'apport d'azote (N) dans l'écosystème diminuent au cours de la succession. Les taux de fixation de N₂ suivaient la phénologie des plantes au cours des saisons de croissance de 1997 (sécheresse) et 1998 (précipitations normales). Le meilleur modèle pour prédire l'activité de la réductase de l'acétylène (ARA, μmol C₂H₄·g nodule_{poind sec}⁻¹·h⁻¹) parmi tous les peuplements et les périodes d'échantillonnage incluait une réponse linéaire à la température du sol et une réponse quadratique au jour julien ($r^2 = 0,23$, $P < 0,0001$). Il y avait peu de relations significatives entre les valeurs saisonnières maximales de l'ARA et les traits mesurés des feuilles; cependant, nous avons détecté une corrélation inverse entre le ratio foliaire N:P et le maximum saisonnier de l'ARA. En 1998, les peuplements ayant un ARA maximal plus élevé avaient un % N du sol plus grand; l'ARA maximal était corrélé positivement avec le % P des horizons O et A du sol en sous-canopée. En 1997, le % P des feuilles et la résorption foliaire de N étaient inférieurs et les feuilles plus minces qu'en 1998. Les effets de la sécheresse étaient les plus prononcés en milieu de succession là où les aulnes avaient des niveaux plus faibles d'ARA, de % P foliaire, d'épaisseur des feuilles et de résorption foliaire de P et N. Bien que l'ARA et la biomasse des nodules ne variaient pas entre les types de peuplements, l'augmentation de la densité des aulnes au cours de la succession s'est traduit par des apports accrus de N dans l'écosystème le long de la chronoséquence. Ces résultats contredisent la théorie reconnue prédisant un déclin des taux de fixation de N₂ et de l'abondance de fixateurs de N₂ au cours du développement successional des peuplements.

Mots-clés : aulne, boréal, cycle de l'azote, fixation de l'azote, succession.

Nomenclature: Viereck & Little, 2007.

Introduction

Although the significance of N₂-fixing plants to the nitrogen (N) economy of terrestrial ecosystems is broadly recognized, the ecological factors regulating inputs via N₂ fixation have never been fully characterized for any ecosystem (Vitousek & Field, 1999; Vitousek *et al.*, 2002). There are several reasons for this. First, many N₂-fixing plants

form additional symbioses with arbuscular (Shrihari *et al.*, 2000) or ectomycorrhizal (Yamanaka *et al.*, 2003) fungi, making the molecular, biochemical, and ecophysiological mechanisms affecting these tripartite relationships extremely complex. This complicates predictions of the role of ecological factors such as light availability, climate regime, herbivory, and soil nutrient status, all of which affect plant growth, demand for N, nitrogenase activity, and thus associated ecosystem N inputs (Vitousek & Field, 1999; Vitousek *et al.*, 2002; Ruess *et al.*, 2006). Secondly, several key parameters necessary for scaling physiological measures to

¹Rec. 2008-11-10; acc. 2009-05-11.

Associate Editor: Andrew Baird.

²Author for correspondence.

DOI 10.2980/16-3-3236

stand-level N inputs, particularly nodule biomass, are logistically challenging to sample. Finally, interactions among regulating factors are often ecosystem specific (Vitousek *et al.*, 2002) and can change substantially over short periods of successional time (Uliassi & Ruess, 2002). These reasons explain why temporal and spatial patterns of potentially limiting environmental factors typically account for less than half the variation in N₂-fixation rates at the nodule or stand scales.

Throughout interior Alaska, N₂ fixation by *Alnus* spp. is the primary pathway for ecosystem N accumulation during primary succession in floodplain environments and during secondary succession following fire in upland landscapes (Van Cleve *et al.*, 1983; Uliassi & Ruess, 2002). On river floodplains, where thin-leaf alder (*Alnus incana* subsp. *tenuifolia*, hereafter *A. tenuifolia*) is the dominant N₂-fixing species, fixation inputs decline from early to mid succession, due to reductions in both nitrogenase activity and alder stem density (Uliassi & Ruess, 2002; Anderson *et al.*, 2004). Uliassi and Ruess (2002) reported 30% reductions in acetylene reductase activities and 36% reductions in area-based N inputs in mid-succession stands, where *A. tenuifolia* dominates the sub-canopy of balsam poplar (*Populus balsamifera*), relative to early succession stands, where *A. tenuifolia* can form a near-continuous canopy. Reasons for this down-regulation of nitrogenase in mid-succession balsam poplar stands likely include reduced alder growth and alder plant N demand, driven by lower light availability and perhaps colder soils. As with many other N₂-fixing species, N₂-fixation rates in *A. tenuifolia* are governed by plant N to P stoichiometry (Huss-Danell, 1997; Uliassi & Ruess, 2002; Valverde & Wall, 2003) and are thus influenced strongly by soil P availability (Huss-Danell *et al.*, 2002; Valverde, Ferrari & Gabriel Wall, 2002; Vitousek *et al.*, 2002; Gentili & Huss-Danell, 2003). Uliassi and Ruess (2002) reported that stimulation of N₂ fixation by P fertilization was less during mid succession, also pointing to growth limitation due to other factors.

Green alder (*A. viridis* subsp. *fruticosa*, formerly *A. crispa*, hereafter *A. viridis*) dominates the shrub canopy in upland forests and is the most widely distributed alder species within interior, western, and northern Alaska. This species is a dominant shrub in both white spruce (*Picea glauca*) and black spruce (*Picea mariana*) forests, and it resprouts vigorously following fire, serving as a keystone species in early successional dynamics (Chapin *et al.*, 2006). *Alnus viridis* also dominates the shrub canopy at both latitudinal and elevational treelines and occupies broad expanses of arctic shrublands, where its range has expanded significantly over the past century (Sturm *et al.*, 2001; Tape, Sturm & Racine, 2006). The likelihood that this range expansion is a consequence of recent high-latitude warming (Sturm *et al.*, 2005b) is supported by correlations between regional climate gradients and modern pollen assemblages of *Alnus* throughout northern Alaska (Oswald *et al.*, 2003) and the expansion of *Alnus* concurrent with warmer and wetter conditions during the Holocene (Edwards *et al.*, 2001; Oswald *et al.*, 2003). Lloyd *et al.* (2005) reported both positive and negative growth responses of individual white spruce treeline stands against a backdrop of regional

warming and treeline advance. Whether there are similar thresholds of warming beyond which *Alnus* response to climate is no longer positive remains unknown.

Our foremost goal was to describe patterns of and controls over N₂ fixation by *A. viridis* along a gradient of stands representing 3 successional stages of post-fire forest development. The specific objectives were to (1) characterize seasonal patterns of N₂-fixation rates across a 200-y-old upland forest successional sequence and (2) evaluate climatic and stand-character controls over N₂-fixation rates and selected plant ecophysiological traits important to alder growth. We sought to test the hypothesis that soil nutrient availability (mainly N and P), in conjunction with microclimate (mainly soil temperature and moisture), regulates alder ecophysiology and associated N₂-fixation rates.

Methods

STUDY AREA

Stands representing seral stages of upland secondary successional forests were selected within the Bonanza Creek Experimental Forest (BCEF), located approximately 35 km southwest of Fairbanks, Alaska (64.8° N, 148.0° W). Replicate stands of the post-fire chronosequence of successional stages are maintained within the BCEF by the Bonanza Creek Long-Term Ecological Research program (BNZ LTER); more detailed information regarding the stages described below can be found on the BNZ LTER web page (<http://www.lter.uaf.edu>). Our study stands included a subset of these seral successional stages ($n = 3/\text{stage}$), corresponding to BNZ LTER-designated successional stages "UP1" (post-fire), "UP2" (mid succession), and "UP3" (white spruce) (Chapin *et al.*, 2006); these are described in detail on the above-mentioned web page. Briefly, early successional (14 y post-fire) stands had open, rapidly developing deciduous canopies with a dense herbaceous and graminoid ground cover surrounding numerous *A. viridis* and willow (*Salix* spp.) shrubs and isolated recruitment pockets of white spruce, paper birch (*Betula neoalaskana*), and trembling aspen (*Populus tremuloides*) saplings. Mid-succession stands (~60 y old) were dominated primarily by paper birch, with recruiting white spruce and an understory tall shrub community consisting of *A. viridis* and willow. On drier, south-facing slopes in early- and mid-succession communities, aspen dominated the overstory and *A. viridis* was restricted to more mesic soils at the base of hillslopes. We avoided these stands and selected more mesic stands dominated by birch, where alder was more abundant. Late succession conifer stands (~220 y old) were dominated by white spruce (basal area ~ 30 m²·ha⁻¹) (<http://www.lter.uaf.edu>), with an understory of *A. viridis* and a near-continuous ground cover of feathermoss (*Hylocomium splendens* and *Pleurozium schreberi*). The density of *A. viridis* averaged 90 ± 10, 179 ± 30, and 290 ± 62 shrubs·ha⁻¹ in early-, mid-, and late-successional stands, respectively, which translated to an alder canopy cover of 1725 ± 322, 3001 ± 723, and 5058 ± 1981 m²·ha⁻¹ in the 3 successional stages, respectively. Across the successional sequence, soils consist of deep loess deposits with shallow, poorly developed surface horizons and no permafrost. These upland soils have a pale brown B horizon with multiple bands of lamellae, are weakly leached with a base saturation of generally > 60%,

and are classified as Lamellic Haplocryepts according to the US soil classification system (Mulligan, 2006) and Lamellic Cambisols according to the World Reference Base for Soil Resources (ISSS Working Group WRB, 2006).

Climate in interior Alaska is strongly continental, with extremely cold winters and dry warm summers. Air temperature ranges from $-50\text{ }^{\circ}\text{C}$ to $+33\text{ }^{\circ}\text{C}$, with an annual average of $-2.9\text{ }^{\circ}\text{C}$ and a frost-free growing season averaging approximately 140 d. Summer daylight hours are long, and the region receives an average of 289 mm of precipitation annually, 60% of which falls as rain (Viereck *et al.*, 1993). Well-drained soils are associated with the topographically variable upland stands within the BCEF, where vegetation on south-facing slopes is particularly subject to drought stress during low precipitation years. In response to the regional warming and drying of recent decades, periodic drought stress has reduced aboveground growth of white spruce across interior Alaska (Barber, Juday & Finney, 2000).

We measured a number of climatically sensitive and seasonally variable ecophysiological traits of *A. viridis* over the 1997 and 1998 growing seasons. It is noteworthy that interior Alaska received an unusually low amount of both annual and summer precipitation during 1997 compared with 1998, when rainfall was close to the decadal average (<http://climate.gi.alaska.edu>). Our study area received approximately 3 times more summer precipitation in 1998 than in 1997 (<http://www.lter.uaf.edu>). Moreover, seasonal maximum soil temperatures at 10 cm soil depth were higher in the wetter year of 1998 ($11.5 \pm 0.3\text{ }^{\circ}\text{C}$) than in dry 1997 ($9.9 \pm 0.5\text{ }^{\circ}\text{C}$). Across both years, soil temperatures during the growing season were higher in mid-succession stands ($12.1 \pm 0.4\text{ }^{\circ}\text{C}$) compared to white spruce ($10.3 \pm 0.5\text{ }^{\circ}\text{C}$) and post-fire ($9.7 \pm 0.6\text{ }^{\circ}\text{C}$) stands (<http://www.lter.uaf.edu>).

EXPERIMENTAL DESIGN

In May of 1997 we selected 3 replicate stands within each of the 3 successional stages described above. All stands were located within 10 km of one another and had slopes ranging from 10° to 30° with a predominantly southerly aspect. *Alnus viridis* was absent in one replicate from the BNZ LTER post-fire (UP1C) and mid-succession (UP2C) replicate stands, likely due to competition for water with a dense aspen overstory. These stands most likely represent an alternate successional trajectory, whereby aspen matures, burns, and is self replaced without the development of a mature white spruce canopy (Johnstone, 2005; Kurkowski *et al.*, 2008). Therefore, we replaced these stands with 2 other stands where *A. viridis* was present that closely resembled and were near to the established BNZ LTER stands.

At each of the 9 stands, we selected a total of 70 *A. viridis* shrubs over an area of approximately 2.5 ha. During each of 7 sampling periods over 2 growing seasons (Table I), we randomly selected 10 of the 70 *A. viridis* shrubs from each replicate stand for measurement of N_2 fixation, foliar morphology, and chemistry, as well as concurrent soil temperature. Density of *A. viridis* (see above) was such that canopies of individual shrubs never overlapped, ensuring that measurements of N_2 fixation from nodules collected beneath a canopy could be reliably paired with

foliar characteristics of that individual shrub. An average of 1.5 d was required to complete the sampling of 10 shrubs for each replicate. To ensure sufficient resolution of seasonal trends for each stage, we staggered sampling of replicate stands within each stage; that is, we visited 1 post-fire replicate, then 1 mid-succession replicate, then 1 white spruce replicate stand, repeating this pattern until all 9 stands had been visited once within a sampling period. All sampling took place between 1000 and 1900 Alaska Standard Time.

NITROGENASE ACTIVITY

Nitrogen-fixation rate per unit nodule biomass was assessed using a modified short-term acetylene reduction assay (ARA) following the protocol of Uliassi and Ruess (2002). Nodule samples of 1–2 g dry weight were collected from the top 15 cm of soil within 2 m of the shrub base, dusted free of soil, and placed in a 60-mL incubation syringe and temporarily stored below the soil surface to maintain subsurface nodule temperatures. Nodule collection typically required less than 10 min per shrub. Assays were initiated by injecting acetylene (C_2H_2) (generated from CaC_2 in a Bliss generator) to create a 10% acetylene to air (v/v) gas mixture within the syringe. Gas samples (6 mL) were withdrawn at 30 and 90 s and stored in clean 10-mL syringes fitted with stopcocks. Gas samples were transported to the laboratory, stored at $4\text{ }^{\circ}\text{C}$ for a maximum of 3 d, and then analyzed for ethylene (C_2H_4) concentration. Nodule samples utilized in the assay were transported to the laboratory, thoroughly washed of soil, dried at $60\text{ }^{\circ}\text{C}$, and weighed to the nearest 0.1 mg.

Gas analyses were conducted using a Shimadzu 14A gas chromatograph (Shimadzu Scientific, Houston, Texas, USA) equipped with a flame ionization detector fitted with a 2-m Poropak N column and a back-flush valve to vent excess C_2H_2 . To calculate nitrogenase activity, we regressed the molar concentration of C_2H_4 in each 6 mL syringe against sampling time, forcing the regression through the origin. The slope of this relationship for each plant was then divided by the associated nodule dry weight (DWT) and reported as acetylene reductase activity ($\text{ARA} = \mu\text{mol C}_2\text{H}_4 \cdot \text{g nodule}_{\text{DWT}}^{-1} \cdot \text{h}^{-1}$). Concurrent to each field ARA measurement we recorded soil temperature at a depth of 10 cm at 2 undisturbed locations beneath the alder canopy using an analog soil thermometer.

FOLIAR CHEMISTRY AND MORPHOLOGY

Immediately following each field sampling of ARA, we collected leaf samples from associated alder individuals. All leaf samples were from the third fully expanded leaf

TABLE I. Sampling periods for determination of alder ecophysiological parameters within uplands of the Bonanza Creek Experimental Forest. Each replicate was visited once during the following dates.

	1997	1998
Sampling period 1	19 Jun – 3 Jul	11 Jun – 25 Jun
Sampling period 2	28 Jul – 9 Aug	4 Jul – 21 Jul
Sampling period 3	3 Sept – 30 Sept	3 Aug – 17 Aug
Sampling period 4		3 Sept – 24 Sept

on branches of arbitrarily chosen alder stems. One 2-cm-diameter punch was removed from the centre of each of 5 leaves for a pooled calculation of leaf specific weight (SLW = $\text{g}\cdot\text{m}^{-2}$), and an additional 5 intact leaves were collected and pooled from each plant for chemical analyses. Pooled leaf samples were oven-dried for 48 h at 60 °C and ground using a Wiley mill (850 μm mesh). Leaf N content (%N) was determined using a LECO CNS 2000 autoanalyzer (LECO, St. Joseph, Michigan, USA), and leaf P content (%P) was determined colorimetrically following perchloric acid digests using a modified Technicon autoanalyzer. Leaf nutrient content ($\text{g}\cdot\text{m}^{-2}$) was calculated by multiplying foliar nutrient concentrations by SLW.

We report foliar N and P resorption as resorptive pool size (mass of nutrient resorbed per unit leaf area) and resorption efficiency (percentage of pool size resorbed). Resorptive pool sizes were calculated by subtracting senescent foliar nutrient content from seasonal maximum foliar nutrient content. In the rare case where senescent leaf nutrient content was higher than seasonal maximum leaf nutrient content, we defined resorption as zero.

STATISTICAL ANALYSES

SAS 9.1 (SAS, 2002) was used for all statistical analyses. Main treatment effects on plant parameters (successional stage, sampling period, and year) were tested using ANOVA (PROC GLM), with individual alder shrubs nested within stands, with stands being the level of replication. Any significant effects from general linear models were subsequently examined with a Tukey's HSD test. We used Spearman's correlation (PROC CORR) to examine possible relationships between plant parameters (*i.e.*, seasonal maximum values of plant parameters and alder stem density) and soil chemical characteristics for these stands obtained from Mitchell and Ruess (in press). These soil data are archived on the BNZ LTER website. To explore relationships between plant parameters and stand-level soil properties, we used soil chemical parameters averaged across canopy (samples collected beneath alder canopies) and interspace (samples collected several meters away from alder canopies) soils. Annual seasonal maximum values of plant parameters (*i.e.*, ARA, leaf %N, leaf %P, SLW, and soil temperature) were obtained from the highest average value for a single sampling period at a given stand ($n = 10$ alders).

To define primary controls over N_2 fixation, stepwise regression was used to evaluate the combined effects of linear and quadratic terms for soil temperature and Julian day across all plants. Data were tested for normality using PROC UNIVARIATE and transformed where necessary to meet statistical assumptions (Zar, 1998). Significance for all tests was set at $P < 0.05$; however, we included "marginally significant" values ($P < 0.10$) that we believe were of particular ecological importance. Unless otherwise stated, values throughout the text are untransformed data and represent means ± 1 standard error.

Results

PATTERNS AND CONTROLS OVER NITROGEN FIXATION

Seasonal patterns of ARA closely tracked plant phenology throughout the growing season, with highest rates

measured during the third week of July ($7.93 \pm 0.89 \mu\text{mol C}_2\text{H}_4\cdot\text{g nodule}_{\text{DWT}}^{-1}\cdot\text{h}^{-1}$). Across all samples, ARA varied more over the course of growing seasons ($P < 0.0001$) (Figure 1a–c) than between years ($P = 0.92$), stages ($P < 0.005$), or replicates within stages ($P < 0.005$).

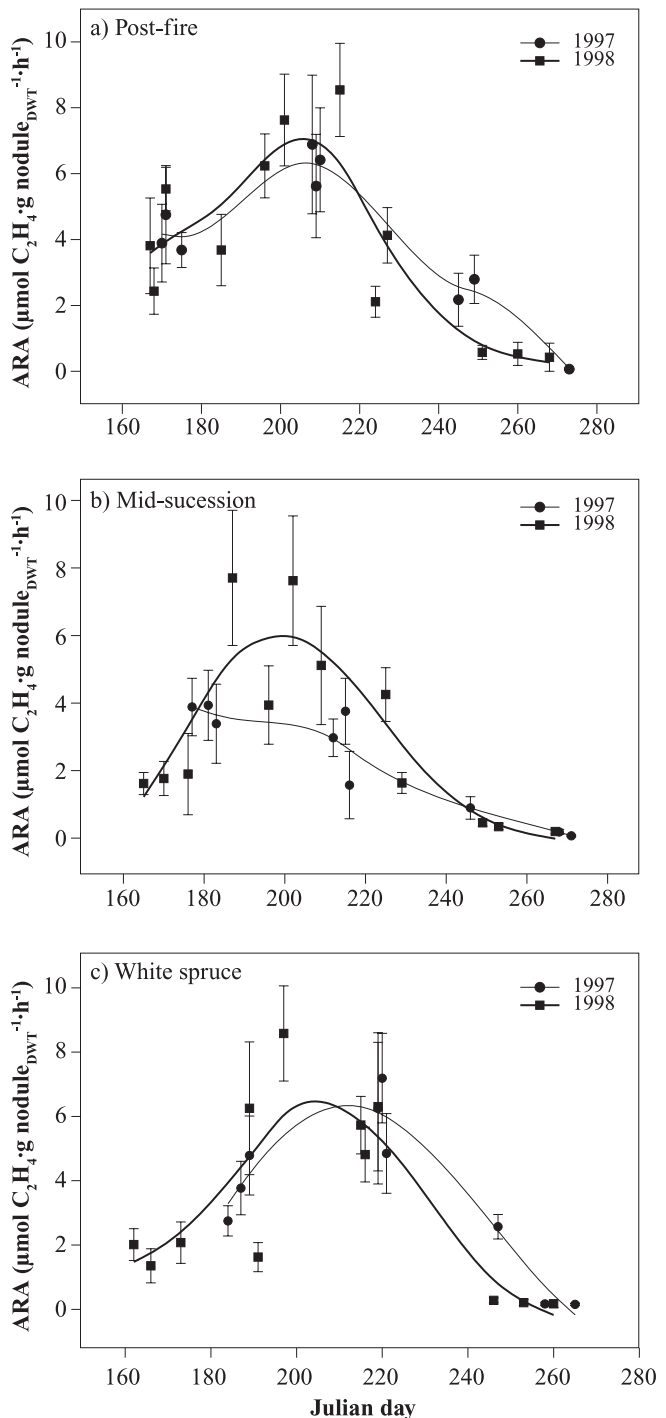


FIGURE 1. Seasonal patterns of acetylene reduction activity (ARA) by *A. viridis* during study years for (a) post-fire, (b) mid-succession, and (c) white spruce upland forest stages within the Bonanza Creek Experimental Forest. Curves represent spline interpolation functions through all data points for each year. Values are means ± 1 SE ($n = 10$).

The best model for predicting ARA ($\mu\text{mol C}_2\text{H}_4 \cdot \text{g nodule}_{\text{DWT}}^{-1} \cdot \text{h}^{-1}$) across all stands and sampling periods included a linear response to soil temperature (SOILT, $^{\circ}\text{C}$) and a quadratic response to Julian day (DAY) ($\text{ARA} = -0.113 \cdot \text{SOILT} + 0.757 \cdot \text{DAY} - 0.0018 \cdot [\text{DAY}]^2 - 71.897$; $r^2 = 0.23$, $P < 0.0001$). Seasonal maximum rates of ARA correlated positively with soil temperature across years and stages (Figure 2) and across years among mid-succession stands ($r^2 = 0.79$, $P < 0.05$). We believe these positive correlations were partially the response of alder to water availability since rainfall and soil temperature were both elevated in 1998 compared to 1997.

We detected no successional trend in seasonal maximum values of ARA, which averaged 6.54 ± 0.63 , 5.13 ± 0.81 , and $6.32 \pm 0.59 \mu\text{mol C}_2\text{H}_4 \cdot \text{g nodule}_{\text{DWT}}^{-1} \cdot \text{h}^{-1}$ for post-fire, mid-succession, and white spruce stages, respectively (Figure 3). Nor was there a significant overall difference in maximum ARA between study years or among replicates within stages when all data were combined. Interannual difference in seasonal maximum ARA within

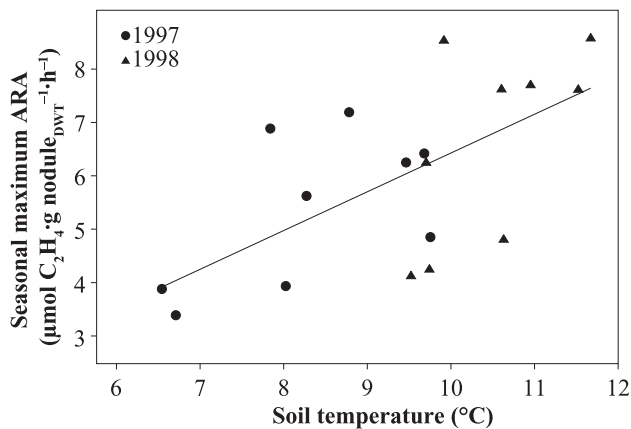


FIGURE 2. Relationship between seasonal maximum rates of ARA by *A. viridis* and concurrent soil temperature across upland forest stages within the Bonanza Creek Experimental Forest ($y = 0.72x - 0.80$, $r^2 = 0.40$, $P < 0.005$). Values are stand-level means for ARA ($n = 10$).

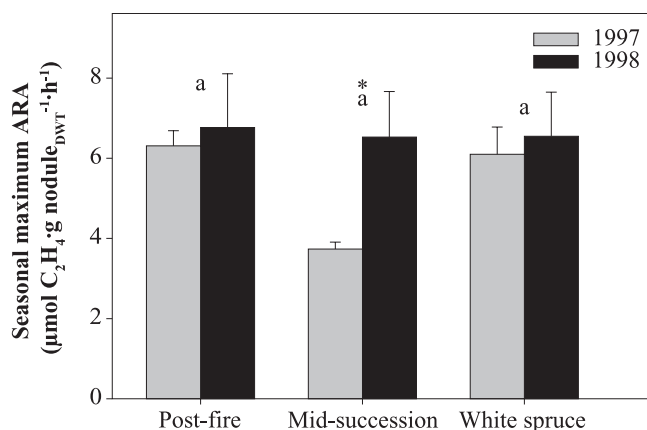


FIGURE 3. Seasonal maximum rates of acetylene reduction activity (ARA) by *A. viridis* in upland forest stages within the Bonanza Creek Experimental Forest. Letters indicate differences among stages and asterisk indicates difference between years within a stage at $P < 0.05$. Values are means ± 1 SE ($n = 30$).

a successional stage was only evident in mid-succession, where alder exhibited significantly lower ARA during the dry year, 1997, compared to 1998, the year of normal precipitation (Figure 3).

Across years and successional stages there were few significant relationships between seasonal maximum values for ARA and measured plant parameters (*i.e.*, leaf %N, leaf %P, and leaf N and P resorptive pools and efficiencies). However, we did detect an inverse correlation between foliar N to P ratios and seasonal maximum ARA (Figure 4), which was most pronounced for mid-succession stands ($r = -0.84$, $P < 0.05$).

Significant relationships between ARA and subcanopy soil chemical characteristics, which we did not find for interspace soils, were either interpreted as a direct effect of N_2 fixation on soil properties or a direct effect of soil properties on ARA. We report statistically significant relationships between subcanopy soil parameters and seasonal maximum ARA which are ecologically meaningful. Several of these relationships were specific to the 1998 growing season, when water was likely not limiting alder growth and nitrogenase activity.

Although we detected no relationships between soil N parameters and ARA during 1997, %N in O ($r^2 = 0.64$, $P < 0.05$), A ($r^2 = 0.43$, $P = 0.05$), and C ($r^2 = 0.49$, $P < 0.05$) horizon soils correlated positively with ARA across all stands during 1998. Notably, seasonal maximum ARA also correlated positively with interspace soil %N in the O ($r^2 = 0.64$, $P < 0.05$) and C ($r^2 = 0.62$, $P < 0.05$) soil horizons in 1998. ARA was greater in stands where subcanopy soil %P was higher in the O ($r^2 = 0.42$, $P < 0.10$) and A ($r^2 = 0.40$, $P < 0.10$) soil horizons, but these effects were only evident in 1998. We also found positive overall correlations between ARA and soil % C in the O ($r^2 = 0.55$, $P < 0.10$) and C ($r^2 = 0.71$, $P < 0.05$) horizons; these correlations were particularly apparent in 1998 ($r^2 = 0.47$, $P < 0.05$ and $r^2 = 0.40$, $P < 0.10$, respectively). These relationships with soil C likely resulted from inter-correlations between ARA, rates of plant growth, and litterfall carbon contributions.

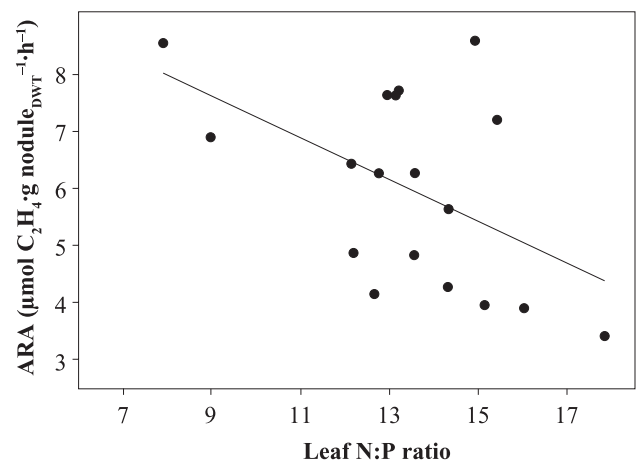


FIGURE 4. Relationship between seasonal maximum rates of ARA by *A. viridis* and foliar N to P ratio across upland forest stages within the Bonanza Creek Experimental Forest ($y = 10.91 - 0.37x$, $r = -0.50$, $P < 0.05$). Values are means for ARA ($n = 10$).

FOLIAR DYNAMICS

Green leaf N concentration varied little from early June through the first week of August. However, as a consequence of lower values after the first week of August, seasonal variation was significant in all successional stages during both

study years (all $P < 0.0001$). Seasonal maximum leaf N concentration was greater in white spruce stands than in mid-succession and post-fire stands, which did not differ (Table II). Seasonal maximum leaf %N did not vary between years for any successional stage (Table III).

TABLE II. *Alnus viridis* foliar parameters and nutrient resorption from senescent leaves for both study years combined in upland forest stages within the Bonanza Creek Experimental Forest. SLW = specific leaf weight. Numbers within rows followed by different letters are significantly different at $P < 0.05$. Values for maximum and senescent foliar parameters are means ± 1 SE ($n = 60$). Values for resorption pools and resorption efficiency are means ± 1 SE ($n = 6$).

Parameter	Post-fire	Mid-succession	White spruce	ANOVA
Maximum SLW ($\text{g}\cdot\text{m}^{-2}$)	67.35 \pm 3.78 ^a	39.31 \pm 4.40 ^b	37.08 \pm 3.77 ^b	$F_{2,17} = 270.28, P < 0.001$
Nitrogen				
Maximum (%)	2.71 \pm 0.09 ^{ab}	2.69 \pm 0.05 ^b	2.86 \pm 0.03 ^a	$F_{2,17} = 5.55, P < 0.05$
Maximum ($\text{g}\cdot\text{m}^{-2}$)	1.82 \pm 0.10 ^a	1.05 \pm 0.11 ^b	1.03 \pm 0.10 ^b	$F_{2,17} = 239.31, P < 0.0001$
Senescent ($\text{g}\cdot\text{m}^{-2}$)	1.14 \pm 0.11 ^a	0.82 \pm 0.05 ^b	0.75 \pm 0.08 ^b	$F_{2,17} = 8.10, P < 0.005$
Resorption pool ($\text{g}\cdot\text{m}^{-2}$)	0.68 \pm 0.11 ^a	0.23 \pm 0.07 ^b	0.28 \pm 0.10 ^b	$F_{2,17} = 9.80, P < 0.005$
Resorption efficiency (%)	36.92 \pm 5.55 ^a	19.43 \pm 4.45 ^a	25.99 \pm 7.42 ^a	$F_{2,17} = 2.41, P = 0.13$
Phosphorus				
Maximum (%)	0.27 \pm 0.04 ^a	0.21 \pm 0.01 ^b	0.23 \pm 0.01 ^b	$F_{2,17} = 13.76, P < 0.005$
Maximum ($\text{g}\cdot\text{m}^{-2}$)	0.17 \pm 0.02 ^a	0.08 \pm 0.01 ^b	0.08 \pm 0.01 ^b	$F_{2,17} = 148.07, P < 0.0001$
Senescent ($\text{g}\cdot\text{m}^{-2}$)	0.16 \pm 0.03 ^a	0.06 \pm 0.01 ^b	0.05 \pm 0.01 ^b	$F_{2,17} = 20.32, P < 0.005$
Resorption pool ($\text{g}\cdot\text{m}^{-2}$)	0.02 \pm 0.01 ^a	0.02 \pm 0.01 ^a	0.03 \pm 0.01 ^a	$F_{2,17} = 0.91, P = 0.10$
Resorption efficiency (%)	15.58 \pm 8.8a ^a	25.53 \pm 7.43 ^a	32.89 \pm 8.01 ^a	$F_{2,17} = 1.18, P = 0.36$

TABLE III. *Alnus viridis* foliar parameters and nutrient resorption from senescent leaves by study year in upland forest stages within the Bonanza Creek Experimental Forest. SLW = specific leaf weight. Numbers within columns followed by different letters indicate significant year effects for a given parameter at $P < 0.05$. Significant ANOVAs are in bold font. Values for maximum and senescent foliar parameters are means ± 1 SE ($n = 30$). Values for resorption pools and resorption efficiency are means ± 1 SE ($n = 3$).

Parameter		Post-fire	Mid-succession	White spruce
Maximum SLW ($\text{g}\cdot\text{m}^{-2}$)	1997	59.23 \pm 1.52^b	29.63 \pm 1.24^b	28.93 \pm 0.55^b
	1998	75.48 \pm 1.75^a	48.98 \pm 1.34^a	45.23 \pm 2.05^a
	ANOVA	$F_{1,5} = 49.36, P < 0.005$	$F_{1,5} = 112.68, P < 0.0005$	$F_{1,5} = 88.28, P < 0.05$
Maximum leaf %N	1997	2.77 \pm 0.12 ^a	2.74 \pm 0.01 ^a	2.83 \pm 0.06 ^a
	1998	2.64 \pm 0.15 ^a	2.64 \pm 0.09 ^a	2.90 \pm 0.03 ^a
	ANOVA	$F_{1,5} = 6.32, P = 0.13$	$F_{1,5} = 1.20, P = 0.39$	$F_{1,5} = 1.07, P = 0.41$
Maximum leaf N ($\text{g}\cdot\text{m}^{-2}$)	1997	1.63 \pm 0.03^b	0.81 \pm 0.04^b	0.82 \pm 0.01^b
	1998	2.01 \pm 0.10^a	1.29 \pm 0.05^a	1.23 \pm 0.06^a
	ANOVA	$F_{1,5} = 13.16, P < 0.05$	$F_{1,5} = 61.46, P < 0.005$	$F_{1,5} = 38.13, P < 0.005$
Senescent leaf N ($\text{g}\cdot\text{m}^{-2}$)	1997	1.05 \pm 0.16 ^a	0.73 \pm 0.02^b	0.64 \pm 0.10 ^a
	1998	1.23 \pm 0.16 ^a	0.92 \pm 0.06^a	0.85 \pm 0.11 ^a
	ANOVA	$F_{1,5} = 0.31, P = 0.63$	$F_{1,5} = 7.71, P = 0.05$	$F_{1,5} = 1.17, P = 0.40$
N resorption ($\text{g}\cdot\text{m}^{-2}$)	1997	0.57 \pm 0.19 ^a	0.08 \pm 0.02^b	0.18 \pm 0.09 ^a
	1998	0.78 \pm 0.11 ^a	0.37 \pm 0.02^a	0.38 \pm 0.17 ^a
	ANOVA	$F_{1,5} = 0.90, P = 0.40$	$F_{1,5} = 140.25, P < 0.005$	$F_{1,5} = 1.00, P = 0.37$
N efficiency (%)	1997	34.85 \pm 10.84 ^a	9.94 \pm 1.81^b	22.49 \pm 11.22 ^a
	1998	39.00 \pm 5.70 ^a	28.92 \pm 2.43^a	29.49 \pm 11.72 ^a
	ANOVA	$F_{1,5} = 0.06, P = 0.91$	$F_{1,5} = 39.15, P < 0.005$	$F_{1,5} = 0.10, P = 0.78$
Maximum leaf %P	1997	0.24 \pm 0.04 ^a	0.19 \pm 0.01 ^a	0.21 \pm 0.01^b
	1998	0.30 \pm 0.07 ^a	0.22 \pm 0.01 ^a	0.25 \pm 0.02^a
	ANOVA	$F_{1,5} = 3.95, P = 0.19$	$F_{1,5} = 6.06, P = 0.10$	$F_{1,5} = 103.29, P < 0.05$
Maximum leaf P ($\text{g}\cdot\text{m}^{-2}$)	1997	0.14 \pm 0.02^a	0.05 \pm 0.01^b	0.06 \pm 0.00^a
	1998	0.20 \pm 0.03^b	0.10 \pm 0.01^a	0.10 \pm 0.00^b
	ANOVA	$F_{1,5} = 26.38, P < 0.05$	$F_{1,5} = 27.84, P < 0.005$	$F_{1,5} = 38.08, P < 0.005$
Senescent leaf P ($\text{g}\cdot\text{m}^{-2}$)	1997	0.12 \pm 0.04 ^a	0.05 \pm 0.00 ^a	0.04 \pm 0.01 ^b
	1998	0.19 \pm 0.05 ^a	0.07 \pm 0.01 ^a	0.07 \pm 0.00 ^a
	ANOVA	$F_{1,5} = 2.44, P = 0.26$	$F_{1,5} = 2.61, P = 0.25$	$F_{1,5} = 13.01, P < 0.05$
P resorption ($\text{g}\cdot\text{m}^{-2}$)	1997	0.02 \pm 0.02 ^a	0.01 \pm 0.01 ^b	0.02 \pm 0.01 ^a
	1998	0.02 \pm 0.02 ^a	0.03 \pm 0.01 ^a	0.03 \pm 0.00 ^b
	ANOVA	$F_{1,5} = 0.00, P = 0.96$	$F_{1,5} = 6.67, P < 0.10$	$F_{1,5} = 1.32, P = 0.37$
P efficiency (%)	1997	17.06 \pm 13.66 ^a	15.47 \pm 8.20 ^a	34.48 \pm 17.65 ^a
	1998	14.09 \pm 14.09 ^a	35.60 \pm 10.37 ^a	31.31 \pm 2.69 ^a
	ANOVA	$F_{1,5} = 0.02, P = 0.91$	$F_{1,5} = 4.24, P = 0.20$	$F_{1,5} = 0.04, P = 0.85$

Alder in post-fire stands resorbed a larger pool of foliar N compared to plants in mid-successional and white spruce stages, which did not differ (Table II). However, nitrogen resorption efficiency did not vary across successional stages, averaging $27.5 \pm 15.6\%$ across all sites. *Alnus viridis* resorbed a significantly smaller pool of N in 1997 compared to 1998 when averaged across stages ($P < 0.05$), particularly in mid-succession stands (Table III). N resorption efficiency in mid-succession was lower in 1997 than 1998; however, we detected no year effect on N resorption efficiency across successional stages. Averaged across years, alder resorbed a larger pool of foliar N in stands where subcanopy soil P content in the O ($r^2 = 0.22$, $P = 0.05$), A ($r^2 = 0.39$, $P = 0.005$), and C (Figure 5) horizons, and soil %P in the C horizon ($r^2 = 0.25$, $P < 0.05$) was greater.

Leaf %P varied seasonally among post-fire, mid-succession, and white spruce stages (all $P < 0.001$). Overall, seasonal maximum leaf %P was higher in post-fire than in mid-successional or white spruce stands (Table II). During

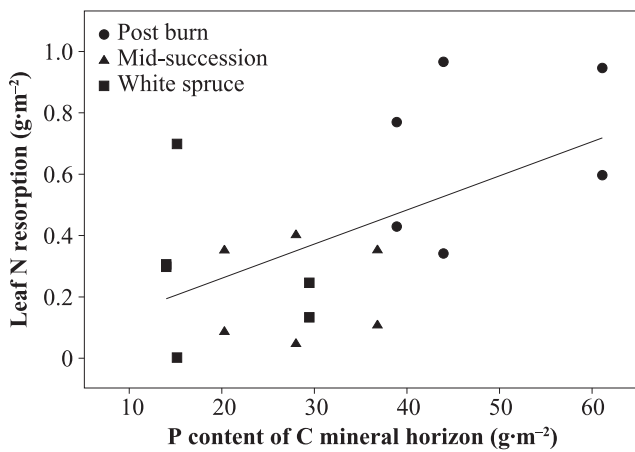


FIGURE 5. Relationship between foliar N resorption and total phosphorus content in C horizon soils over 2 y within upland forests stands of the Bonanza Creek Experimental Forest ($y = 0.01x + 0.04$, $r^2 = 0.55$, $P < 0.05$).

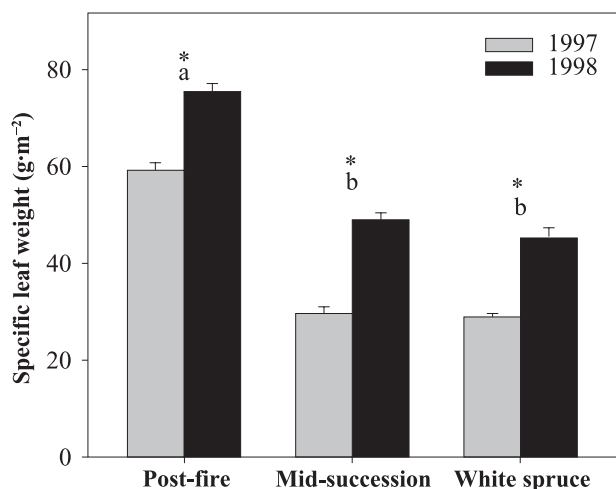


FIGURE 6. Specific leaf weight (SLW) of *A. viridis* in upland forest stages within Bonanza Creek Experimental Forest. Letters indicate differences among stages and asterisks indicate differences between years within stages at $P < 0.05$. Values are means + 1 SE ($n = 30$).

1998, the year of normal precipitation, *A. viridis* in all successional stages produced leaves with a higher seasonal maximum %P compared to 1997, particularly in mid- and late-successional stands (Table III).

Across all stands, leaf %P correlated positively with O horizon P content ($r^2 = 0.17$, $P < 0.10$), A horizon soil %P ($r^2 = 0.18$, $P < 0.10$), total soil N content ($r^2 = 0.22$, $P < 0.05$), and O horizon N content ($r^2 = 0.37$, $P < 0.05$). The positive correlation between leaf %P and O horizon N content was evident in both 1997 ($r^2 = 0.47$, $P < 0.05$) and 1998 ($r^2 = 0.44$, $P < 0.05$).

The pools of P resorbed during senescence were nearly identical among stages, and interannual difference was only evident in mid-succession stands, where P resorption pools in 1998 were more than double those in 1997 (Table III). However, senescent leaf P contents in mid- and late-succession stands were approximately a third of those in post-fire stands (Table II).

Alder produced thicker leaves (higher SLW) in 1998 than in 1997 when averaged across stands (Figure 6, Table III). Seasonal maximum SLW was greater in post-fire stands than in either mid-succession or white spruce stands (Table II, Figure 6) and varied little among replicates within stages. Seasonal variation in SLW was not evident in post-fire stands ($P = 0.35$), was somewhat greater in white spruce stands ($P < 0.05$), and was pronounced in mid-succession stands ($P < 0.0001$). Near the end of the 1997 growing season we measured a notable increase in SLW in mid-succession stands immediately following the first significant rainfall event (15 mm on August 11) of the dry 1997 growing season.

Discussion

N₂ FIXATION

Plant phenology (Julian day) explained the largest percent of variation in N₂-fixation rates among sampling periods across successional stages. Similar results have been reported for *A. tenuifolia* growing in interior Alaskan floodplain forests (Uliassi & Ruess, 2002; Anderson *et al.*, 2009) and *A. incana* in northern Sweden (Huss-Danell, 1991); these findings most simply reflect that N₂ fixation closely tracks plant growth and N demand within the growing season. Our most parsimonious model included a non-linear response to plant phenology (indexed by Julian day) and an independent, linear response to soil temperature. However, this combined model explained only 23% of the variation in N₂-fixation rates across successional stages, and it was not improved by inclusion of leaf chemical parameters, such as N, P, or N to P ratios. This low explanatory power over field N₂-fixation rates is not uncommon (Uliassi & Ruess, 2002; Anderson *et al.*, 2004; Anderson *et al.*, 2009) and probably results from a combination of ecophysiological factors and sampling limitations. In particular, we suspect that soil moisture data for each field ARA measurement would have improved our models substantially. The inclusion of nodules of different ages, or perhaps even dead nodules (Zitzer & Dawson, 1989; Ruess *et al.*, 2009), and inherent differences in the condition, age, and growth rates of roots to which nodule

clusters were attached may have also contributed to the high variability among our ARA measurements. In addition, the reduction ratio of C_2H_2 to N_2 is known to vary by over an order of magnitude in both *A. viridis* and *A. tenuifolia* (Anderson *et al.*, 2004), limiting the utility of ARA as a reliable index of N_2 -fixation rates. Another factor influencing nodule activity may be the differences in genetic structure of *Frankia* haplotypes within nodule clusters at the plant, stand, and landscape scales (Anderson *et al.*, 2009).

When data were pooled across all stands, seasonal maximum rates of N_2 fixation were slightly lower during the dry year of 1997 ($5.4 \pm 0.5 \mu\text{mol } C_2H_4 \cdot \text{g nodule}_{DWT}^{-1} \cdot \text{h}^{-1}$) compared to 1998 ($6.6 \pm 0.6 \mu\text{mol } C_2H_4 \cdot \text{g nodule}_{DWT}^{-1} \cdot \text{h}^{-1}$), a year of relatively normal precipitation. This non-significant result was largely driven by higher N_2 -fixation rates in mid-succession stands (+76%), and there were non-significant increases in early (+7%) and late (+7%) succession during the wetter year (Figure 3). Our measured peak rates were less than those measured by Anderson *et al.* (2004), who reported averages across these same stands ranging from 7.0 to 16.3 $\mu\text{mol } C_2H_4 \cdot \text{g nodule}_{DWT}^{-1} \cdot \text{h}^{-1}$ during a later, normal-precipitation year. Given that their study was not designed to measure seasonal maximum rates, the differences between the two studies were likely conservative. Thus, it appears that we may not have captured the N_2 -fixation potential of *A. viridis* in either 1997, as a direct consequence of drought, or in 1998, perhaps as a result of resource allocation to growth recovery following disturbance the previous year (Ruess *et al.*, 2006).

One of our most striking results was the lack of variation in maximum N_2 -fixation rates across successional stages, which averaged 6.8 ± 1.3 , 6.5 ± 1.1 , and $6.5 \pm 1.1 \mu\text{mol } C_2H_4 \cdot \text{g nodule}_{DWT}^{-1} \cdot \text{h}^{-1}$ in early-, mid-, and late-succession stages, respectively, during 1998 (Figure 3). This contrasts with studies of *A. tenuifolia*, which showed a pronounced decline in fixation rates with the progression from early ($25.0 \pm 8.0 \mu\text{mol } C_2H_4 \cdot \text{g nodule}_{DWT}^{-1} \cdot \text{h}^{-1}$), to mid ($17.5 \pm 3.1 \mu\text{mol } C_2H_4 \cdot \text{g nodule}_{DWT}^{-1} \cdot \text{h}^{-1}$) (Uliassi & Ruess, 2002), to late ($10.8 \pm 1.3 \mu\text{mol } C_2H_4 \cdot \text{g nodule}_{DWT}^{-1} \cdot \text{h}^{-1}$) succession stands (Anderson *et al.*, 2004). We have already mentioned 2 factors that may contribute to the absence of a successional pattern in ARA. The first is drought, which may have masked otherwise greater differences among stages. The second factor is the reduction ratio of C_2H_2 to N_2 , which Anderson *et al.* (2004) found to be inversely correlated with N_2 -fixation rates as determined by $^{15}N_2$ uptake. This means that if there were higher rates of N_2 fixation in early succession stands, as we initially hypothesized because of high levels of light and perhaps higher soil P, these might go undetected as the inhibition of N_2 reduction by C_2H_2 declines at high rates of electron flux through the nitrogenase complex (Anderson *et al.*, 2004). Finally, lower potential relative growth rates, plant N demand, and ARA in *A. viridis* relative to *A. tenuifolia* (R. W. Ruess, unpubl. data) may also contribute to the differences between these 2 species and landscapes. If water is less limiting in floodplain relative to upland forests, *A. tenuifolia* in early succession floodplain stands may be growing closer to its maximum potential and therefore be much more sensitive to other growth limitations later in succession compared with the more slow-growing *A. viridis*.

CLIMATE SENSITIVITY

Across all stands *A. viridis* produced thinner leaves (Figure 6) during the dry year, which likely translated to reduced litterfall inputs of C, N, and P in the low precipitation year. Given the importance of *A. viridis* for soil properties as mediated through litterfall inputs, we predict that periodic reductions of alder litterfall inputs to subcanopy soils in response to water limitation could have significant implications for ecosystem-level N cycling dynamics (Knops, Bradley & Wedin, 2002).

It seems likely that our result of lower foliar P concentration in the dry year was related to reduced photosynthesis and associated soil nutrient uptake in response to drought (Minoletti & Boerner, 1994; Wright, Reich & Westoby, 2001). Drought-stress has also been shown to negatively affect the ability of alder to allocate photosynthate to other physiological processes necessary for maintaining plant nutrient balance, including C supply to *Frankia* (Lundquist, 2005), ectomycorrhizae (Rygielwicz & Andersen, 1994), and the formation and maintenance of both fine roots (Ruess *et al.*, 2003) and cluster roots (Shane & Lambers, 2005). Support of ectomycorrhizal symbionts may be particularly important for maintaining optimal N to P balance in N_2 -fixing woody plants growing under both P-limiting and water-limiting conditions (Querejeta, Egerton-Warburton & Allen, 2003).

The drought of 1997 had the most severe effects on alder in mid-succession stands, where, as previously described, N_2 -fixation rates and leaf thickness were significantly reduced relative to values in 1998 (Table III). Notably, our finding of thinner leaves in this and other successional stages (Figure 6) during the drought year (Table III) has not been previously documented. Furthermore, we noted that alder in mid-succession stands responded dramatically to a late growing season precipitation event that ended the drought of 1997. Following 15 mm of rainfall on 11 August, alders in mid-succession stands produced a cohort of leaves that were 26% thicker ($39 \text{ g} \cdot \text{m}^{-2}$) than the previous cohort sample in July ($31 \text{ g} \cdot \text{m}^{-2}$). Interestingly, resorption of foliar N and P from this younger leaf cohort during fall was significantly lower than values recorded in mid-succession stands in 1998 (or in the other 2 stages in 1997) (Table III). We believe this resulted from prolonged leaf retention in order to extend the photosynthetic life of leaves. Tateno (2003) argued that the benefits for non- N_2 -fixing plants of N resorption in fall outweigh the potential C gains of retaining leaves longer in the growing season but speculated that in N_2 -fixing plants, such benefits may not be necessary. But this argument overlooks the inherently high P demands in N_2 -fixing species and their capacity for efficient P resorption. For example, working in interior Alaskan upland forests, Chapin and Kedrowski (1983) reported less N resorption for *A. viridis* relative to *Betula neoalaskana* (61% versus 75%, respectively) but greater P resorption in *A. viridis* (81% versus 44%, respectively). Uliassi and Ruess (2002) showed that P resorption efficiency in *A. tenuifolia* growing in early succession floodplain stands was reduced from 51% to 11% in response to P fertilization. For reasons we can't explain, our values for both N (27%) and P (24%) resorption

efficiency (averaged across all stands for both 1997 and 1998) are considerably less than those measured by Chapin and Kedrowski (1983). Given that alders typically retain leaves longer than other deciduous woody plants in interior Alaska, a flexible strategy of prolonging C gain to support *Frankia*, but also ectomycorrhizae for the acquisition of P (Read, Leake & Perez-Moreno, 2004), may be an important adaptation for maintaining C and nutrient balance under variable climatic conditions.

Although we report some evidence that alder was both light- and P-limited, our results strongly suggest that water availability was the primary factor limiting alder growth on both annual and successional timescales. For example, we found that while response to low water availability was most pronounced in the mid-succession stage, *A. viridis* was less productive in a low precipitation year throughout the chronosequence. We attribute the near absence of alder from aspen-dominated mid-succession stands to competitive exclusion related to water limitation and suggest that the density of green alder could be reduced within other mid-succession stands if the current trend of warming and drying throughout interior Alaska (Barber, Juday & Finney, 2000) continues. Given the substantial N inputs by *A. viridis* to late succession stands, failure to persist through mid-succession stands could substantially influence the N economy of this vegetation chronosequence.

IMPLICATIONS OF CLIMATE CHANGE

Studies of modern shrub expansion in the arctic tundra have focused on potential alterations to hydrological and energy balances (Chapin *et al.*, 2000; Sturm, Racine & Tape, 2001; Chapin *et al.*, 2005; Tape, Sturm & Racine, 2006). For example, greater snow accumulation promoted by large shrubs has been found to alter surface albedo and stimulate a positive feedback loop associating the insulating effect of greater snow accumulation around shrubs with warmer temperatures, increased soil microbial activity (summer and winter), and thus enhanced nutrient availability (Sturm, Racine & Tape, 2001). There is also strong evidence that *A. viridis* stimulates the N economy (Vogel & Gower, 1998; Rhoades *et al.*, 2001; Densmore, 2005) and hence growth of associated species. For instance, alder stimulation of plant-available soil N may partially explain the observed “halos” of dwarf birch and willow surrounding alder clumps throughout the north slope of the Brooks Range (Tape, Sturm & Racine, 2006). Despite evidence from this and other studies that *A. viridis* (the dominant component of shrub expansion) stimulates proximal and stand level soil N in boreal and arctic ecosystems (Vogel & Gower, 1998; Rhoades *et al.*, 2001; Mitchell, 2006; Mitchell & Ruess, in press), the N₂-fixing capacity of alder has been overlooked by most studies discussing arctic shrub expansion. Given the pronounced effects of alder on ecosystem structure and function throughout western and northern Alaska during the last significant shrub expansion, which took place during the mid-Holocene (Anderson & Brubaker, 1994; Oswald, Brubaker & Anderson, 1999; Hu, Finney & Brubaker, 2001), it is reasonable to assume that significant N inputs to tundra may substantially alter similar properties of these systems.

Large-scale shifts in primary production and N cycling associated with alder invasion into areas occupied by tussock tundra could also modify ecosystem C budgets (McGuire *et al.*, 2002). For example, long-term experimental N fertilization of tundra plots near Toolik Lake in Arctic Alaska led to loss of C from deep soil horizons, mediated through microbially stimulated decomposition that exceeded increases in net primary production (Mack *et al.*, 2004). Alternatively, N-fixation inputs might be expected to increase rates of C storage by reducing soil decomposition rates through inhibition of lignolytic enzyme activities (Resh, Binkley & Parrotta, 2002). Although we found no evidence for changes in mineral soil C beneath alder canopies in boreal upland forests, it would be interesting to examine whether shifts in sub-surface soil C stocks have occurred in arctic landscapes where alder has expanded dramatically over the past several decades (Tape, Sturm & Racine, 2006).

Recent shrub expansion throughout western (Silapaswan, Verbyla & McGuire, 2001) and northern Alaska has correlated with climatic warming; however, it has been suggested that shrub range expansion may have been initiated over a century ago (Tape, Sturm & Racine, 2006), prior to the warming trend of the last 30 y (Keyser *et al.*, 2000; Serreze *et al.*, 2000; Hinzman *et al.*, 2005). The current explanation involves an increase in soil nutrient availability brought on by elevated winter soil temperatures resulting from increased snowpack (Sturm *et al.*, 2005a). Support for this hypothesis comes from increases in shrub density within the long-term N fertilization plots at Toolik Lake (Shaver *et al.*, 2001). However, our observations regarding the sensitivity of *A. viridis* to water stress, and evidence that alder expansion during the Holocene was related to warmer temperatures and increased effective moisture (Hu, Finney & Brubaker, 2001; Mann *et al.*, 2002), suggest that modern alder expansion may also be influenced by soil moisture regime.

Acknowledgements

We would like to thank the Institute of Arctic Biology at the University of Alaska Fairbanks for providing logistical support to this project and J. J. Frost for his hard-working enthusiasm and mild-mannered presence over two field seasons. We also sincerely thank the staff at the UAF Forest Soils Lab and the UAF Experimental Greenhouse for their instruction and assistance. We greatly appreciate the comments and suggestions by reviewers (S. Payette and F. Gentili) and the Associate Editor (A. Baird), which substantially improved the manuscript. Funding for the research was provided by the Bonanza Creek Long-Term Ecological Research program (funded jointly by NSF grant DEB-0423442 and USDA Forest Service, Pacific Northwest Research Station grant PNW01-JV11261952-231).

Literature cited

- Anderson, M. D., R. W. Ruess, D. D. Uliassi & J. S. Mitchell, 2004. Estimating N₂ fixation in two species of *Alnus* in interior Alaska using acetylene reduction and ¹⁵N₂ uptake. *Écoscience*, 11: 102–112.
- Anderson, M. D., R. W. Ruess, D. D. Myrold & D. L. Taylor, 2009. Host species and habitat affect nodulation by specific *Frankia* genotypes in two species of *Alnus* in interior Alaska. *Oecologia*, 160: 619–630.

- Anderson, P. & L. Brubaker, 1994. Vegetation history of northcentral Alaska: Mapped summary of late-Quaternary pollen data. *Quaternary Science Review*, 13: 71–92.
- Barber, V. A., G. P. Juday & B. P. Finney, 2000. Reduced growth of Alaskan white spruce in the twentieth century from temperature-induced drought stress. *Nature*, 405: 668–673.
- Chapin, F. S., III & 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, A. D. McGuire, J. Randerson, R. Pielke Sr., D. Baldocchi, S. E. Hobbie, N. Roulet, W. Eugster, E. Kasischke, E. B. Rastetter, S. A. Zimov & S. W. Running, 2000. Arctic and boreal ecosystems of western North America as components of the climate system. *Global Change Biology*, 6 (Suppl. 1): 1–13.
- Chapin, F. S., III, M. Sturm, M. C. Serreze, J. P. McFadden, J. R. Key, A. H. Lloyd, A. D. McGuire, T. S. Rupp, A. H. Lynch, J. P. Schimel, J. Beringer, W. L. Chapman, H. E. Epstein, E. S. Euskirchen, L. D. Hinzman, G. Jia, C.-L. Ping, K. D. Tape, C. D. C. Thompson, D. A. Walker & J. M. Welker, 2005. Role of land-surface changes in arctic summer warming. *Science*, 310: 657–660.
- Chapin, F. S., III, T. N. Hollingsworth, D. F. Murray, L. A. Viereck & M. D. Walker, 2006. Floristic diversity and vegetation distribution in the Alaskan boreal forest. Pages 81–99 in F. S. Chapin III, M. W. Oswood, K. Van Cleve, L. A. Viereck & D. L. Verbyla (eds.). *Alaska's Changing Boreal Forest*. Oxford University Press, New York, New York.
- Densmore, R. V., 2005. Succession on subalpine placer mine spoil: Effects of revegetation with *Alnus viridis*, Alaska, USA. *Arctic, Antarctic, and Alpine Research*, 37: 297–303.
- Edwards, M. E., C. J. Mock, B. P. Finney, V. A. Barber & P. J. Bartlein, 2001. Potential analogues for paleoclimatic variations in eastern interior Alaska during the past 14,000 yr: Atmospheric-circulation controls of regional temperature and moisture responses. *Quaternary Science Reviews*, 20: 189–202.
- Gentili, F. & K. Huss-Danell, 2003. Local and systemic effects of phosphorus and nitrogen on nodulation and nodule function in *Alnus incana*. *Journal of Experimental Botany*, 54: 2757–2767.
- Hinzman, L. D., N. D. Bettez, W. R. Bolton, F. S. Chapin III, M. B. Dyrgerov, C. L. Fastie, B. Griffith, R. D. Hollister, A. Hope, H. P. Huntington, A. M. Jensen, G. J. Jia, T. Jorgenson, D. L. Kane, D. R. Klein, G. Kofinas, A. H. Lynch, A. H. Lloyd, A. D. McGuire, F. E. Nelson, M. Nolan, W. C. Oechel, T. E. Osterkamp, C. H. Racine, V. E. Romanovsky, R. S. Stone, D. A. Stow, M. Sturm, C. E. Tweedie, G. L. Vourlitis, M. D. Walker, D. A. Walker, P. J. Webber, J. M. Welker, K. S. Winker & K. Yoshikawa, 2005. Evidence and implications of recent climate change in northern Alaska and other arctic regions. *Climatic Change*, 72: 251–298.
- Hu, F.-S., B. P. Finney & L. B. Brubaker, 2001. Effects of Holocene *Alnus* expansion on aquatic productivity, nitrogen cycling and soil development in southwestern Alaska. *Ecosystems*, 4: 358–368.
- Huss-Danell, K., 1991. Influence of host (*Alnus* and *Myrica*) genotype on infectivity, N₂ fixation, spore formation and hydrogenase activity in *Frankia*. *New Phytologist*, 119: 121–127.
- Huss-Danell, K., 1997. Tansley Review No. 93: Actinorhizal symbioses and their N₂ fixation. *New Phytologist*, 136: 375–405.
- Huss-Danell, K., F. Gentili, C. Valverde, L. G. Wall & A. Wiklund, 2002. Phosphorus is important in nodulation of actinorhizal plants and legumes. Pages 163–166 in T. Finan, N. O'Brian, D. Layzell, K. Vessey & W. Newton (eds.). *Nitrogen Fixation: Global Perspectives*. CAB International, Wallingford.
- ISSS Working Group WRG, 2006. *World Reference Base for Soil Resources 2006*. 2nd Edition. World Soil Reports No. 103. FAO, Rome.
- Johnstone, J. F., 2005. Effects of aspen (*Populus tremuloides*) sucker removal on postfire conifer regeneration in central Alaska. *Canadian Journal of Forest Research*, 35: 483–486.
- Keyser, A. R., J. S. Kimball, R. R. Nemani & S. W. Running, 2000. Simulating the effects of climatic change on the carbon balance of North American high-latitude forests. *Global Change Biology*, 6 (Suppl. 1): 185–195.
- Knops, J. M. H., K. L. Bradley & D. A. Wedin, 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecology Letters*, 5: 454–466.
- Kurkowski, T. A., D. H. Mann, T. S. Rupp & D. L. Verbyla, 2008. Relative importance of different secondary successional pathways in an Alaskan boreal forest. *Canadian Journal of Forest Research*, 38: 1911–1923.
- Mack, M. C., E. A. G. Schuur, M. S. Bret-Harte, G. R. Shaver & F. S. Chapin III, 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature*, 431: 440–443.
- Lloyd, A. H., A. E. Wilson, C. L. Fastie & R. M. Landis, 2005. Population dynamics of black spruce and white spruce near the arctic treeline in the southern Brooks Range, Alaska. *Canadian Journal of Forest Research*, 35: 2073–2081.
- Lundqvist, P. O., 2005. Carbon cost of nitrogenase activity in *Frankia-Alnus incana* root nodules. *Plant and soil*, 273: 235–244.
- Mann, D. H., D. M. Peteet, R. E. Reanier & M. L. Kunz, 2002. Responses of an arctic landscape to Lateglacial and early Holocene climatic changes: The importance of moisture. *Quaternary Science Reviews*, 21: 997–1021.
- McGuire, A. D., C. Wirth, M. Apps, J. Beringer, J. Klein, H. Epstein, D. W. Kicklighter, J. Bhatti, F. S. Chapin III, B. de Groot, D. Efremov, W. Eugster, M. Fukuda, T. Gower, L. Hinzman, B. Huntley, G. J. Jia, E. S. Kasischke, J. M. Melillo, V. Romanovsky, A. Shvedenko, E. Vaganov & D. Walker, 2002. Environmental variation, vegetation distribution, carbon dynamics, and water/energy exchange in high latitudes. *Journal of Vegetation Science*, 13: 301–314.
- Minoletti, M. L. & R. E. J. Boerner, 1994. Drought and site fertility effects on foliar nitrogen and phosphorus dynamics and nutrient resorption by the forest understorey shrub *Viburnum acerifolium* L. *American Midland Naturalist*, 131: 109–119.
- Mitchell J. S., 2006. Patterns of and controls over N inputs by green alder (*Alnus viridis* ssp. *fruticosa*) to a secondary successional chronosequence in interior Alaska. M.S. thesis. University of Alaska Fairbanks, Fairbanks, Alaska.
- Mitchell, J. S. & R. W. Ruess, in press. N₂-fixing alder (*Alnus viridis* spp. *fruticosa*) effects on soil properties across a secondary successional chronosequence in interior Alaska. *Biogeochemistry*.
- Mulligan, D., 2006. Soil survey of the Fairbanks and North Star Borough areas. US Department of Agriculture Natural Resources Conservation Service, Palmer, Alaska.
- Oswald, W., L. Brubaker & P. Anderson, 1999. Late Quaternary vegetational history of the Howard Pass Area, northwestern Alaska. *Canadian Journal of Botany*, 77: 570–581.
- Oswald, W. W., L. B. Brubaker, F. S. Hu & D. G. Gavin, 2003. Pollen–vegetation calibration for tundra communities in the Arctic foothills, northern Alaska. *Journal of Ecology*, 91: 1022–1033.
- Querejeta, J., L. M. Egerton-Warburton & M. F. Allen, 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia*, 134: 55–64.

- Read, D., J. Leake & J. Perez-Moreno, 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, 82: 1243–1263.
- Resh, S. C., D. Binkley & J. A. Parrotta, 2002. Greater soil carbon sequestration under nitrogen-fixing trees compared with Eucalyptus species. *Ecosystems*, 5: 217–231.
- Rhoades, C., H. Oskarsson, D. Binkley & B. Stottlemeyer, 2001. Alder (*Alnus crispa*) effects on soils in ecosystems of the Agashashok River valley, northwest Alaska. *Ecoscience*, 8: 89–95.
- Ruess, R. W., R. L. Hendrick, A. J. Burton, K. S. Pregitzer, B. Sveinbjornsson, M. F. Allen & G. Maurer, 2003. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. *Ecological Monographs*, 74: 643–652.
- Ruess, R. W., M. D. Anderson, J. S. Mitchell & J. W. McFarland, 2006. Effects of defoliation on growth and N fixation in *Alnus tenuifolia*: Consequences for changing disturbance regimes at high latitudes. *Ecoscience*, 13: 402–412.
- Ruess, R., J. McFarland, L. M. Trummer & J. K. Rohrs-Richey, 2009. Disease-mediated declines in N-fixation inputs by *Alnus tenuifolia* to early-successional floodplains in interior and south-central Alaska. *Ecosystems*, 12: 489–501.
- Rygielwicz, P. T. & C. P. Andersen, 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. *Nature*, 369: 58–60.
- SAS, 2002. SAS 9.1.3 for Windows, 4th Edition. SAS Institute Inc., Cary, North Carolina.
- Serreze, M. C., J. E. Walsh, F. S. Chapin III, T. Osterkamp, M. Dyrgerov, V. Romanovsky, W. C. Oechel, J. Morison, T. Zhang & R. G. Barry, 2000. Observational evidence of recent change in the northern high-latitude environment. *Climatic Change*, 46: 159–207.
- Shane, M. & H. Lambers, 2005. Cluster roots: A curiosity in context. *Plant and Soil*, 274: 101–125.
- Shaver, G. R., M. S. Bret-Harte, M. H. Jones, J. Johnstone, L. Gough, J. Laundre & F. S. Chapin III, 2001. Species composition interacts with fertilizer to control long-term change in tundra productivity. *Ecology*, 82: 3163–3181.
- Shrihari, P. C., K. Sakamoto, K. Inubushi & S. Akao, 2000. Interaction between supernodulating or non-nodulating mutants of soybean and two arbuscular mycorrhizal fungi. *Mycorrhiza*, 10: 101–106.
- Silapaswan, C. S., D. L. Verbyla & A. D. McGuire, 2001. Land cover change on the Seward Peninsula: The use of remote sensing to evaluate potential influences of climate warming on historical vegetation dynamics. *Canadian Journal of Remote Sensing*, 27: 542–554.
- Sturm, M., C. Racine & K. Tape, 2001. Increasing shrub abundance in the Arctic. *Nature*, 411: 546–547.
- Sturm, M., J. P. McFadden, G. E. Liston, F. S. Chapin III, J. Holmgren & M. Walker, 2001. Snow–shrub interactions in arctic tundra: A feedback loop with climatic implications. *Journal of Climate*, 14: 336–344.
- Sturm, M., T. Douglas, C. Racine & G. E. Liston, 2005a. Changing snow and shrub conditions affect albedo with global implications. *Journal of Geophysical Research - Biogeosciences*, 110: G01004, doi:10.1029/2005JG000013.
- Sturm, M., J. Schimel, G. Michaelson, J. M. Welker, S. F. Oberbauer, G. E. Liston, J. Fahnestock & V. E. Romanosky, 2005b. Winter biological processes could help convert arctic tundra to shrubland. *BioScience*, 55: 17–26.
- Tape, K., M. Sturm & C. Racine, 2006. The evidence for shrub expansion in northern Alaska and the Pan-Arctic. *Global Change Biology*, 12: 1–17.
- Tateno, N., 2003. Benefit to N₂-fixing alder of extending growth period at the cost of leaf nitrogen loss without resorption. *Oecologia*, 137: 338–343.
- Uliassi, D. D. & R. W. Ruess, 2002. Limitations to symbiotic nitrogen fixation in primary succession on the Tanana River floodplain, Alaska. *Ecology*, 83: 88–103.
- Valverde, C., A. Ferrari & L. Gabriel Wall, 2002. Phosphorus and the regulation of nodulation in the actinorhizal symbiosis between *Discaria trinervis* (Rhamnaceae) and *Frankia* BCU110501. *New Phytologist*, 153: 43–51.
- Valverde, C. & L. G. Wall, 2003. The regulation of nodulation, nitrogen fixation and ammonium assimilation under a carbohydrate shortage stress in the *Discaria trinervis*–*Frankia* symbiosis. *Plant and Soil*, 254: 155–165.
- Van Cleve, K., L. Oliver, R. Schlentner, L. A. Viereck & C. T. Dyrness, 1983. Productivity and nutrient cycling in taiga forest ecosystems. *Canadian Journal of Forest Research*, 13: 747–766.
- Viereck, L. A. & E. L. Little, 2007. *Alaska Trees and Shrubs*. 2nd Edition. University of Alaska Press, Fairbanks, Alaska.
- Viereck, L. A., K. Vanclve, P. C. Adams & R. E. Schlentner, 1993. Climate of the Tanana River floodplain near Fairbanks, Alaska. *Canadian Journal of Forest Research*, 23: 899–913.
- Vitousek, P. M. & C. B. Field, 1999. Ecosystem constraints to symbiotic nitrogen fixers: A simple model and its implications. *Biogeochemistry*, 46: 179–202.
- Vitousek, P. M., K. Cassman, C. Cleveland, T. Crews, C. B. Field, N. B. Grimm, R. W. Howarth, R. Marino, L. Martinelli, E. B. Rastetter & J. I. Sprent, 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry*, 57: 1–45.
- Vogel, J. G. & S. T. Gower, 1998. Carbon and nitrogen dynamics of boreal jack pine stands with and without a green alder understory. *Ecosystems*, 1: 386–400.
- Wright, I. J., P. B. Reich & M. Westoby, 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology*, 15: 423–434.
- Yamanaka, T., C. Y. Li, B. T. Bormann & H. Okabe, 2003. Tripartite associations in an alder: Effects of *Frankia* and *Alpova diplophloeus* on the growth, nitrogen fixation and mineral acquisition of *Alnus tenuifolia*. *Plant and Soil*, 254: 179–186.
- Zar, J. H., 1998. *Biostatistical Analysis*. 4th Edition. Prentice-Hall Inc., Englewood Cliffs, New Jersey.
- Zitzer, S. F. & J. O. Dawson, 1989. Seasonal changes in nodular nitrogenase activity of *Alnus glutinosa* and *Elaeagnus angustifolia*. *Tree Physiology*, 5: 185–194.