Research paper

Conifer expansion reduces the competitive ability and herbivore defense of aspen by modifying light environment and soil chemistry

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Disturbance patterns strongly influence plant community structure. What remains less clear, particularly at a mechanistic level, is how changes in disturbance cycles alter successional outcomes in plant communities. There is evidence that fire suppression is resulting in longer fire return intervals in subalpine forests and that these lengthened intervals increase competitive interactions between aspen and conifer species. We conducted a field and greenhouse study to compare photosynthesis, growth and defense responses of quaking aspen and subalpine fir regeneration under light reductions and shifts in soil chemistry that occur as conifers increase in dominance. The studies demonstrated that aspen regeneration was substantially more sensitive to light and soil resource limitations than that of subalpine fir. For aspen, light reductions and/or shifts in soil chemistry limited height growth, biomass gain, photosynthesis and the production of defense compounds (phenolic glycosides and condensed tannins). Biomass gain and phenolic glycoside concentrations were co-limited by light reduction and changes in soil chemistry. In contrast, subalpine fir seedlings tended to be more tolerant of low light conditions and showed no sensitivity to changes in soil chemistry. Unlike aspen, subalpine fir increased its root to shoot ratio on conifer soils, which may partially explain its maintenance of growth and defense. The results suggest that increasing dominance of conifers in subalpine forests alters light conditions and soil chemistry in a way that places greater physiological and growth constraints on aspen than subalpine fir, with a likely outcome being more successful recruitment of conifers and losses in aspen cover.

Keywords: *Abies lasiocarpa*, aspen decline, disturbance, fire suppression, phenolic glycosides, *Populus tremuloides*, subalpine fir, succession.

Introduction

Disturbance is a key driver of secondary succession, which is critical to the assembly and development of plant communities (Attiwill 1994). Historically, succession cycles were driven by naturally timed disturbance events (Bengtsson et al. 2000). Increasingly, anthropogenic influences are changing the extent and timing of disturbance in plant communities (Brown and Smith 2000). Early succession species are particularly vulnerable to changes in disturbance cycles (Reyes et al. 2010).

Quaking aspen (*Populus tremuloides* Michx.), an early successional species, is a prominent and broadly distributed tree species that exerts a significant influence on the structure and function of subalpine and boreal forest systems (St. Clair et al. 2010). Recent patterns of aspen decline suggest that current management strategies and changing environmental conditions may be imposing constraints on aspen vigor and survival across portions of its range (Frey et al. 2004). Typically, aspen initiates secondary succession via root suckering following disturbance, usually fire (Fraser et al. 2004). In time, if seed

source is present, conifers begin to establish in aspen stands, which increases susceptibility to fire (St. Clair et al. 2010). This has historically resulted in fire cycles of 70–80 years (Strand et al. 2009). However, recent analysis suggests that both climate conditions (Buechling and Baker 2004, Beaty and Taylor 2008) and fire suppression by humans (Gallant et al. 2003, Van Wagner et al. 2006) have significantly lengthened fire return intervals in subalpine forests. There is evidence that fire suppression is favoring conifer expansion and displacement of aspen in the absence of disturbance (Gallant et al. 2003, Smith and Smith 2005), and drastically decreases aspen regeneration once fire does return (Smith et al. 2011).

What is lacking is a better understanding of how altered disturbance regimes impact competitive mechanisms underlying changes in forest composition and structure. As conifers establish and increase in height and basal area within aspen stands under longer fire return intervals, light penetration through the canopy decreases (Stadt and Lieffers 2000). Also conifer expansion in deciduous forests can change soil chemistry and reduce nitrogen mineralization via changes in leaf litter input (Nihlgård 1971). At the field sites included in this study we have observed that the bioavailability of macronutrients (N, P, K and Mg) decreases significantly as conifers, dominated by subalpine fir (Abies lasiocarpa Hooker Nuttall), expand into aspen stands (J. Buck and S.B. St. Clair, unpublished data). Do these changes differentially affect the success of early and late successional subalpine forest species?

The ability of aspen to limit herbivory and disease is critical to regeneration success (St. Clair et al. 2010). Quaking aspen produces two classes of phenolic-based allelochemicals from the shikimic acid pathway: condensed tannins and phenolic glycosides. Subalpine fir produces condensed tannins but not phenolic glycosides. In studies of aspen, foliar concentrations of phenolic glycosides are strongly correlated with reductions in insect (Donaldson and Lindroth 2007) and mammal (Wooley et al. 2008) herbivory, while condensed tannins may play a role in increasing resistance to microbial pathogens (Holeski et al. 2009). In controlled studies, both light conditions and nutrient availability have been shown to influence aspen defense chemistry production (Hemming and Lindroth 1999, Donaldson et al. 2006). Thus light and soil resource limitations associated with conifer expansion may compromise aspen defense against pathogens and herbivores.

The objective of this study was to determine how conifer expansion in subalpine forests differentially affects the photosynthetic capacity, growth and defense of aspen and subalpine fir (a mid-term to late successional species). The following predictions were tested: (i) subalpine fir photosynthesis, growth and defense responses are resilient to light and soil resource limitation associated with conifer expansion; (ii) light reduction and changes in soil chemistry associated with conifer expansion will significantly reduce rates of photosynthesis, growth

potential and defense of aspen; (iii) the secondary compounds in aspen that defend against pathogens and herbivores are reduced with increasing conifer dominance.

Materials and methods

Field study

Seven field locations that span the Fish Lake National Forest in central Utah were selected for a field study in July of 2008 to test our predictions under natural field conditions (field site geocoordinates: 38°74′30.38″N, 111°65′40.53″W; 38°48′21.16″N, 112°07′59.96″W; 38°58′85.64″N, 111°67′03.82″W; 38°76′80.71″N, 111°68′54.24″W; 38°69′67.14″N, 111°53′12.40″W; 38°53′95.73″N, 111°68′60.35″W; 38°1438.66″N, 112°20′51.67″W). Elevations ranged from 2700 to 3000 m. Sites were selected based on the presence of four adjacent stand conditions that varied in overstory composition: predominantly conifer, which was dominated by subalpine fir but also included spruce and other fir species (>75% conifer stems), predominantly aspen (>75% aspen stems), equal mix of aspen and conifer (~50% aspen and conifer stems) and a canopy gap with no overstory influence. The transitions in canopy composition at each field site were representative of stages in the typical pathway of secondary succession beginning with disturbance and ending with conifer dominance. In each stand there were multiple-aged cohorts of each species. The composition and density within each transition zone were determined using the point quarter method along a 50 m transect (Pollard 1971). The percentage of aspen to conifer in the aspen, mixed and conifer stands across the seven sites was 90:10, 51:49 and 24:76, respectively. Average stand density for the aspen, mixed and conifer stands was 2228 ± 472, 2806 ± 428 and 1978 ± 548. Aspen regeneration (<100 cm in height) nearest the 15, 30 and 45 m points along the transect was selected for measurement of gas exchange and leaf sample collection for foliar chemistry analysis.

Leaf area index (LAI) in each of the three stand understory types (conifer, aspen and mixed) was measured using the AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, WA, USA) during July of 2008 between 11 a.m. and 3 p.m. every 7 m along the transect above the understory vegetation. Two measurements were made at each point and then averaged for the stand

Greenhouse study

A controlled experiment was used as a validation of field-tested predictions and to examine the individual and interactive effects (which could not be controlled in the field) of soil chemistry and light environment on aspen and subalpine fir physiology and growth. The experiment was a completely randomized, factorial split plot design (two light levels × two soil types × two species × two plants per species) replicated four times. Light

level was the split plot treatment. The high light blocks had 75% full sunlight using 25% shade cloth and the low light blocks had 25% full sunlight using 75% shade cloth. This resulted in average LAI values of 2.1 m^2 m^{-2} for the high light blocks (simulating light conditions under aspen stands) and 4.7 m^2 m^{-2} for the low light blocks (simulating darker conditions under conifer-dominated stands). There were two aspen and subalpine fir plants on each of two soil core types (described below) within each light block. The data generated for each of the species replicates within each light block were averaged.

Soil cores in which aspen and subalpine fir were planted were collected from Telephone Hollow in the Uinta National Forest in May 2007 (40°18′29.67″N, 111°14′35.64″W, elevation 2491 m). Soil cores were collected underneath either a dominant conifer stand (>80% conifer) or a dominant aspen stand (>80% aspen) that were immediately adjacent to each other. The soil cores were collected by driving PVC pipe (10 cm in diameter and 20 cm in length) into the soil and carefully removing them with a shovel. Caps with drainage holes were placed on the bottom of each core.

Aspen was grown from root cuttings collected in May 2007 from an aspen stand in the vicinity of Telephone Hollow. Aspen root sections ~10 cm in length and ~0.5 cm in diameter were placed in vermiculite for 10 days, at which point emerging suckers developed. Suckers (1 cm in height) were excised from the root section using a razor blade and were dipped in a solution of 0.4% indolebutyric acid (to encourage root initiation) in ethanol for 5 s before being transferred to peat moss plugs. These transplants were then placed in a growth chamber under low light (100 μmol m^{-2} s^{-1}), 80% relative humidity at 20 °C. After 10 days, when root formation was visible, the roots were carefully washed and the young plants were carefully transferred into the soil cores. At the same time aspen suckers were being transferred to the soil cores, subalpine fir seedlings of uniform height (6 cm) were collected at Telephone Hollow and planted into the soil cores. The establishing aspen and subalpine fir trees in soil cores were maintained in the growth chamber for another week while root establishment occurred.

On 20 June 2007 the aspen suckers and subalpine fir seedlings in the soil cores were transferred into the greenhouse and the study was initiated. The aspen suckers and subalpine fir seedlings were grown for two seasons in a climate-controlled greenhouse at Brigham Young University in Provo, Utah (40°14′41.32″N, 111°38′56.94″W). The trees were watered using an automated watering system that delivered 300 ml of water twice a week. At the end of the first growing season (after the aspens had lost their leaves), the experimental units were moved to a climate-controlled cooler and kept at 2.7 °C to maintain dormancy through the winter. Light levels in the cooler were maintained at ~20 μmol m^{-2} s^{-1} for 8.5 h a day (8:30 a.m. to 5 p.m.). They were returned to the greenhouse

on 8 May 2008 under the same treatment conditions imposed during the summer of 2007. The experiment was terminated on 29 July 2008.

In the greenhouse, mean temperature and relative humidity in the high light plots (maximum light levels were 1200 μmol m^{-2} s^{-1}) during the day were 25 ± 0.08 °C and 42 ± 0.23%. In the low light plots (max photosynthetic photon flux density (PPFD) 350 μmol m^{-2} s^{-1}) mean temperature and relative humidity during the day were 24 ± 0.07 °C and 45 ± 0.2%. During the night, mean temperature and relative humidity were uniform in the two light treatments (19 ± 0.08 °C and 51 ± 0.3%). These climate parameters were selected to simulate average temperatures at our field sites.

Leaf analysis

Leaf tissue collected from both the greenhouse and field experiments were placed in freezer bags and transported back to the lab between blocks of dry ice and stored in the lab at −80 °C. Leaf area was determined using a leaf area meter (Li-Cor 3000; Li-Cor Environmental, Inc., Lincoln, NE, USA) and leaf mass on a dry weight basis was quantified using an analytical balance. Specific leaf area (SLA) was calculated based on the cm^2 of leaf area per gram dry weight of leaf tissue. Aspen leaves were freeze dried to preserve phenolic glycosides. Subalpine fir needles were oven dried at 60 °C for 72 h. Leaf and needle material was homogenized separately in a Wiley Mill using a #10 screen.

Condensed tannins were extracted from ~50 mg of leaf material suspended in 1 ml of 70% acetone–10 mM ascorbic acid solution in 2 ml screw-cap micro-centrifuge tubes. The samples were then vortexed on high at 4 °C for 20 min. The liquid supernatant was then removed and placed in a separate micro-centrifuge container, and the extraction was repeated. The concentration of tannins was then quantified spectrophotometrically (SpectraMax Plus 384, MDS, Toronto, Canada) using the modified butanol–HCl method described in Porter et al. (1986) using purified tannin isolated from aspen leaves as a standard.

The phenolic glycosides salicortin and tremulacin were extracted from ~50 mg of aspen leaf tissue (subalpine fir does not contain any measurable levels of phenolic glycosides), which was placed in methanol in 2 ml screw-cap micro-centrifuge tubes. The samples were then vortexed on high for 5 min. The liquid supernatant was removed and placed in a separate micro-centrifuge tube, and the extraction was repeated. Final concentrations of salicortin and tremulacin were quantified using high-performance liquid chromatography (Agilent 1100 Series, Santa Clara, CA, USA) with a Luna 2, C18 column (150 × 4.6 mm, 5 μm) at a flow rate of 1 ml min^{-1}. Compound peaks were detected using a UV lamp at a wavelength of 280 nm using purified salicortin and tremulacin isolated from aspen leaves as a standard.

For phosphorus analysis, leaf samples were ashed in a muffle furnace at 495 °C for 12 h, dissolved in 2 ml of 100 mM HCl, and analyzed spectrophotometrically (Spectra-Max Plus 384; MDS) according to the methods of Murphy and Riley (1962). Leaf nitrogen concentrations were determined in a nitrogen analyzer (TruSpec CN Determinator; LECO Corporation, St Joseph, MI, USA) using the combustion method.

Gas exchange

Light response curves were conducted on the youngest fully expanded leaf or needles (that filled the entire chamber area) using a gas exchange system with a blue–red light source (Li-Cor 6400 and 6400-40; Li-Cor Biosciences, Lincoln, NE, USA) at ambient temperature and humidity. Leaf chamber CO_2 concentrations were controlled at 385 ppm using a CO_2 mixer. The light response curve was measured at each of the following light levels: 2000, 1500, 1000, 500, 200, 100, 50 and 0 μmol m^{-2} s^{-1}. Measurements were initiated by sealing the leaf tissue in the sample chamber. After CO_2 and water vapor concentrations in the leaf chamber reached a steady state (typically 60–90 s), rates of photosynthesis were logged and then the light was adjusted to the next PPFD. Light response curves in the greenhouse were taken from 9:45 to 14:30 on 3 and 4 July, 2008 and field measurements were taken from 10:00 to 14:30 on 9–17 July 2008.

Growth

The plants grown in the greenhouse study were harvested for biomass measurements on 29 July 2008. Aboveground plant biomass was clipped at the soil surface, measured for height using measuring tape and then placed in a paper bag. Roots were carefully removed from the soil by rinsing. Both shoot and root samples were placed in a drying oven at 60 °C for 72 h and then measured for mass using an analytical balance.

Soil analysis

Five soil cores (10 × 20 cm) collected under both the aspen and conifer stand at Telephone Hollow were analyzed for pH, total nitrogen, macro- and micro-nutrients, organic matter and texture. Soil analysis from each core was subdivided into O and A horizon samples for aspen cores and OA and B horizon samples for conifer cores based on the visual transition in the soil column, which occurred ~10 cm below the soil surface. A pH meter was used to determine pH in a saturated soil paste. To determine bioavailable phosphorus, soil samples were extracted in a sodium bicarbonate solution and analyzed with the methods of Olsen et al. (1954). Total nitrogen was determined using a nitrogen analyzer (C and N Determinator; LECO Corporation). Exchangeable Ca, Mg, K and Na were extracted with ammonium acetate according to the method of Normandin

et al. (1998) and Cu, Zn, Fe and Mn were extracted with DTPA. Soil cations were quantified using inductively coupled plasma spectroscopy (Iris Intrepid II XSP; Thermo Electron Corporation, Waltham, MA, USA). Organic matter was determined using the dichromate oxidation method from Walkley and Black (1934). Soil texture was quantified using a hydrometer.

Statistical analysis

Measurements of growth, photosynthesis (at the 2000 μmol m^{-2} s^{-1} light point), foliar chemistry and soil chemistry were tested for differences using an analysis of variance (ANOVA) model. Mean comparisons among treatment groups were determined using a Tukey adjusted t-test. Homogeneity of variance and normality were examined using Shapiro–Wilk W statistics and equal variance tests. Data that did not meet the assumptions for the parametric tests were transformed using a Box–Cox transformation. For the soil data we transformed P, organic matter, Zn, Fe, Mn, Mg, Na, N and K. From the field analysis, condensed tannins, nitrogen and phosphorus were transformed. In the controlled experiment, aspen biomass, condensed tannins, phosphorus and leaf nitrogen were transformed. Specific leaf area data from the greenhouse were unable to meet the parametric assumption, so a Kruskal–Wallis test was run with Tukey adjusted multiple comparisons. Statistical analysis was performed using JMP version 7 statistical software (SAS Institute, Cary, NC, USA) and NCSS version 7.1.4 (NCSS, LLC, Kaysville, UT, USA).

Results

Soils

Soils collected under the dominant aspen stand had nearly six times more total N and significantly higher levels of K, Mg, Ca, Zn, Mn, Cu and organic matter than conifer-dominated soils (Table 1). Most of these differences were most pronounced in the OA horizon. Soils collected under the conifer-dominated stand had greater P than aspen soil (Table 1). There was no difference in pH between the two soil types (Table 1). There were slightly higher fractions of silt in conifer soils but, in general, soil texture was similar (Table 1).

Light response curves

In the greenhouse study, aspen and subalpine fir grown in high light had significantly greater rates of photosynthesis in the light saturating portion of the light response curves than those grown in low light conditions (Figure 1). Aspen had significantly higher rates of photosynthesis when grown on aspen soil (Figure 1). In contrast, the rates of photosynthesis of subalpine fir seedlings were not significantly affected by soil type. In the field study, photosynthesis rates were significantly greater in aspen growing in gaps than aspen suckers growing under conifer, mixed or aspen canopy types (Figure 2).

Table 1. Soil chemistry of the OA and B soil horizons of the soil cores used in the greenhouse study.

Soil horizon	pH	N (%)	P ($\mu g\ g^{-1}$)	K ($\mu g\ g^{-1}$)	Mg ($\mu g\ g^{-1}$)	Ca ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)
Aspen O	5.5 ± 0.1^a	0.44 ± 0.03^a	23 ± 2.7^c	840 ± 84^a	595 ± 90^a	5570 ± 421^a	0.75 ± 0.03^a
Aspen A	5.7 ± 0.2^a	0.23 ± 0.01^b	7.6 ± 0.9^d	452 ± 17^b	291 ± 24^b	3427 ± 214^b	0.56 ± 0.02^{bc}
Conifer OA	5.7 ± 0.1^a	0.07 ± 0.016^c	90 ± 6.0^a	561 ± 31^b	194 ± 21^c	2558 ± 156^{bc}	0.63 ± 0.05^{ab}
Conifer B	5.9 ± 0.1^a	0.06 ± 0.007^c	50 ± 8.2^b	287 ± 36^c	187 ± 17^c	1749 ± 137^c	0.47 ± 0.04^c
F-value	1.56	95.47***	81.08***	28.31***	21.54***	40.70***	10.23***

Soil horizon	Zn ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	%OM	% Sand	% Clay	% Silt
Aspen O	6.4 ± 0.6^a	67 ± 7.8^a	48 ± 14^a	9.2 ± 0.8^a	44 ± 1.3^{ab}	14.9 ± 0.84^a	40.9 ± 0.89^b
Aspen A	1.8 ± 0.5^b	85 ± 11^a	12 ± 1.6^b	5.3 ± 0.4^b	42 ± 0.9^{ab}	16.4 ± 0.54^a	40.8 ± 1.07^b
Conifer OA	$1.6^b \pm 0.7^c$	120 ± 21^a	16 ± 3.1^b	3.2 ± 0.4^c	38 ± 1.7^b	16.0 ± 0.63^a	45.1 ± 1.32^a
Conifer B	0.35 ± 0.13^c	104 ± 25^a	2.1 ± 0.4^c	1.8 ± 0.3^d	44.4 ± 1.1^a	14.9 ± 1.08^a	40.7 ± 0.54^b
F-value	20.52***	2.17	39.01***	36.23***	3.73*	0.92	4.74*

Means ± SE presented. Significant differences denoted as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Statistical differences among pairwise comparisons denoted by superscript letters.
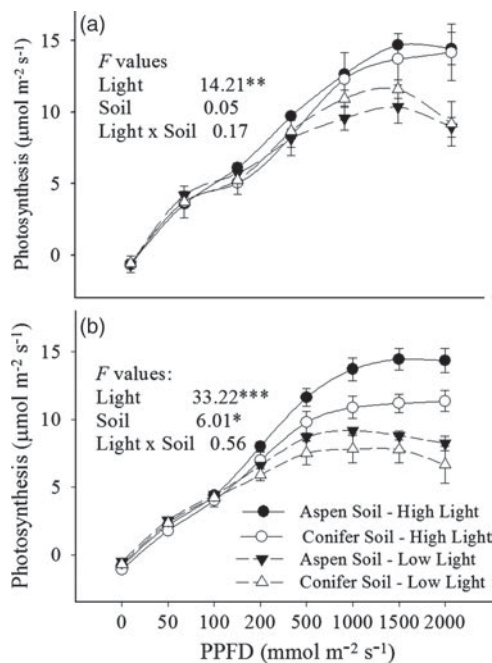

Figure 1. Photosynthetic light response curves for (a) subalpine fir and (b) aspen under all four treatment combinations in the greenhouse study. High light was 75% of full sunlight and low light was 25% of full sunlight, which is representative of light conditions underneath a pure aspen and pure conifer stand, respectively. Means ± SE presented. Main and interactive treatment effects in the ANOVA model were assessed on the highest light value of the light response curves. Significant differences are denoted as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Growth responses

In the high light treatment, aspen had significantly greater height and biomass growth on aspen soil when compared with growth on conifer soil. In contrast, there was no significant soil effect on aspen growth responses under the low light treatment, which resulted in a significant light by soil interaction in the ANOVA model (Figure 3). Neither light nor soil treatments significantly influenced the root to shoot ratio of aspen (Figure 4).
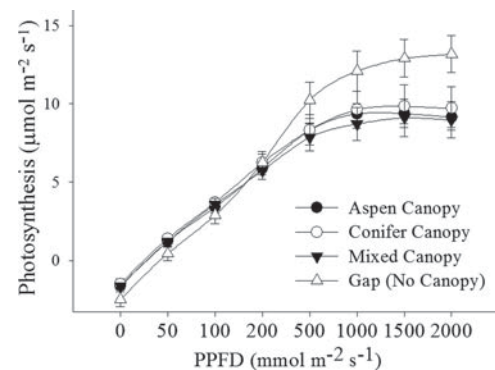

Figure 2. Photosynthetic light response curves of aspen suckers from the field study underneath aspen-dominant stands (>80% aspen), mixed aspen and conifer stands (50/50 of each), conifer-dominant stands (>80% conifer) and gaps with no overstory influence. Means ± SE presented. ANOVA analysis of stand effects at the highest light value in the light response curve was statistically significant ($P = 0.044$).

For subalpine fir, high light conditions resulted in significantly greater biomass accumulation but no difference in height growth (Figure 3). The only measure of growth significantly influenced by soil conditions was an increase in the root to shoot ratio in subalpine fir seedlings growing on conifer soil (Figure 4).

Leaf nutrients and morphology

In the greenhouse study, aspen suckers had higher leaf N and P concentrations when grown under low light conditions (Table 2). Foliar P concentrations were greater in aspen seedlings grown on conifer soil while foliar N was not affected by soil type (Table 2). For subalpine fir seedlings, light and soil treatments in the greenhouse study had no significant effects on foliar N levels (Table 2). Foliar P levels were significantly greater in subalpine fir seedlings grown on conifer soils. In the field, foliar N concentrations of aspen were greater under mixed stands than in aspen stands and gaps (Table 3). Foliar
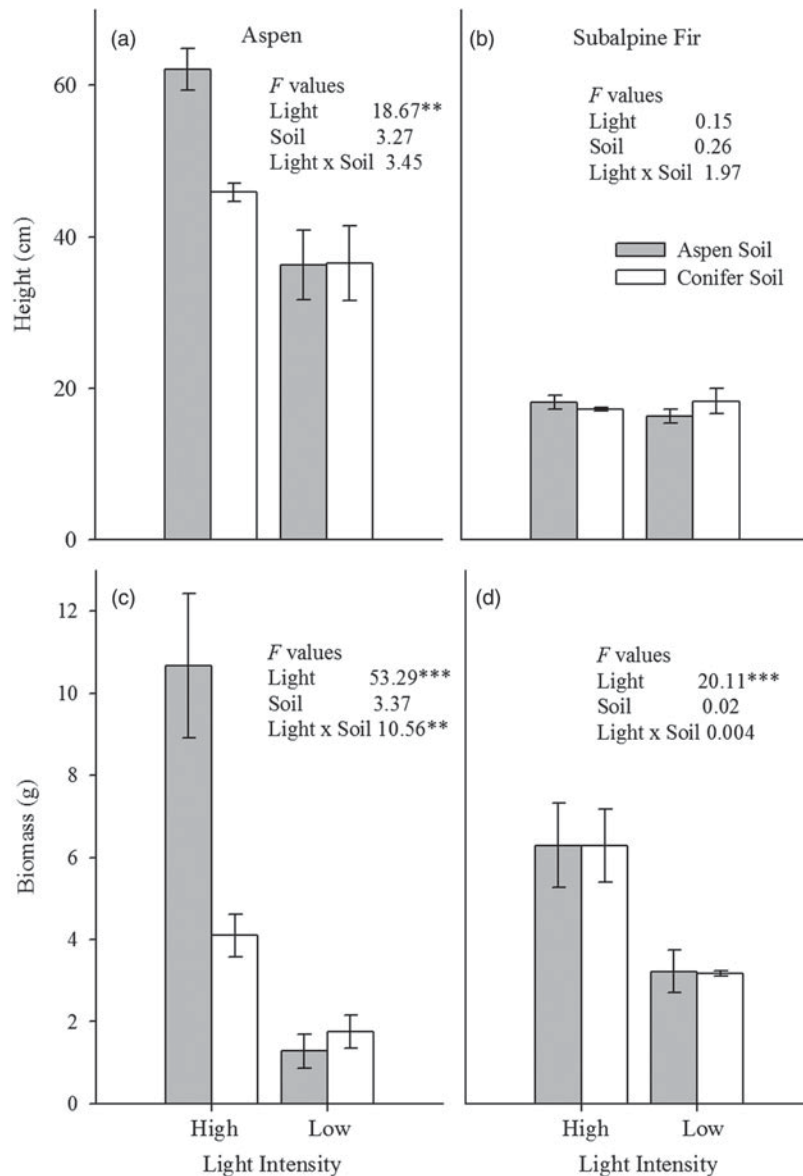
Figure 3. Growth responses (means ± SE) of aspen and subalpine fir grown in the greenhouse under contrasting light and soil conditions. Aspen height (a) was significantly reduced by light limitation while aspen biomass (c) was strongly constrained by light reduction and soil conditions under high light conditions (significant soil × light interaction). In contrast, subalpine fir height (b) and biomass (d) responses showed greater tolerance of light and soil limitations.

phosphorus concentrations were significantly lower in the gap compared with aspen, mixed and conifer stands (Table 3).

In the field, aspen leaves became thinner as conifer density increased (Table 3). Specific leaf area for aspen was found to be significantly higher (thinner leaves) under low light in the greenhouse (Table 2).

Foliar defense chemistry

In the field study, phenolic glycosides and condensed tannins decreased in aspen suckers with increasing conifer dominance (gap > aspen > mixed > conifer) (Table 3). In the greenhouse study, phenolic glycosides and condensed tannins in aspen decreased under low light conditions (Table 2). The main effect

of soil type was not significant for condensed tannins or phenolic glycosides. However, conifer soils did significantly reduce phenolic glycoside concentrations in aspen leaves, but only under high light conditions as indicated by the significant light by soil interaction term in the ANOVA model (Table 2). Condensed tannin levels in subalpine fir seedlings were greatest under low light conditions but were not significantly influenced by soil type (Table 2).

Discussion

Species responses to soil and light effects

Certain plant species have been shown to modulate environmental conditions in ways that decrease the growth potential of

competitor species (Van Breemen and Finzi 1998). This is consistent with Connell and Slatyer's (1977) model of inhibition and adds to Grime's (1977) model of competition where mid-term to late successional species have an active role in creating stress for other species. Conifer dominance in our study system created light and soil resource limitations to which aspen was substantially more sensitive than subalpine fir.
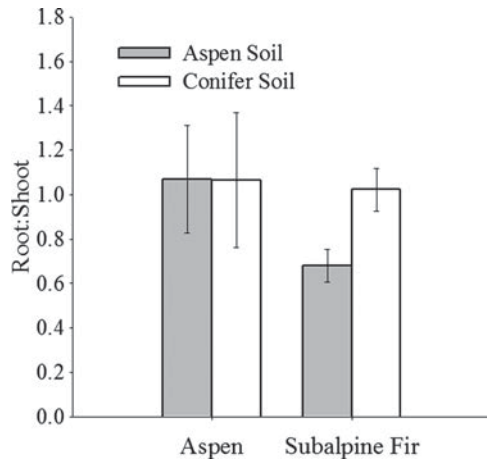

Figure 4. Root to shoot ratios (means ± SE) of aspen and subalpine fir grown on contrasting soil types in 75% full sunlight. There was no significant difference in aspen but subalpine fir showed a significant increase in the root to shoot ratio when growing on conifer soil cores ($t = 2.5$, $P = 0.030$).

While it has been established that shade tolerance plays a role in succession (Kobe and Coates 1997), less is known regarding the mechanisms of tolerance to observed shifts in soil chemistry exhibited by subalpine fir seedlings in this study. Subalpine fir exhibited phenotypic plasticity in root traits that may have accounted for observed tolerance to soil resource limitation. Unlike aspen, subalpine fir seedlings increased their root to shoot ratio when growing on conifer soil (Figure 4). We further observed that subalpine seedlings growing in conifer soils maintained their mycorrhizal associations in contrast to aspen roots which had drastic reductions in infection frequency (Clark and St. Clair 2011). Increases in the root to shoot ratio and mycorrhizal associations can dramatically increase the acquisition of limiting soil nutrients resulting in the maintenance of photosynthesis and growth on nutrient-limited soils (Aerts and Chapin 2000, St. Clair and Lynch 2005a).

We are unaware of any studies that have mechanistically examined how the interplay between shifts in light environment and soil chemistry that occur under conifer expansion in subalpine forests affects successional outcomes. For aspen, photosynthetic and growth sensitivity to nutrient limitations on conifer soils was strongest under high light conditions (light × soil interaction) (Figure 3). This suggests that light reduction was the primary constraint to aspen growth and that soil stress was an important secondary limitation, which supports our second prediction. An alternative explanation that is also consistent with these results is that nutrient deficiency can

Table 2. Greenhouse results showing the main and interactive effects of soil type and light conditions on foliar chemistry for aspen and subalpine fir

Treatments	Nitrogen (% dry weight)	Phosphorus (mg g^{-1})	SLA (cm^2 g^{-1})	Phenolic glyc. (% dry weight)	Tannins (% dry weight)
Aspen					
High light					
Aspen soil	0.94 ± 0.13[b]	1.16 ± 0.03[c]	137 ± 5.9[b]	24.1 ± 0.73[a]	2.42 ± 0.81[a]
Conifer soil	0.92 ± 0.11[b]	1.41 ± 0.17[bc]	149 ± 3.4[b]	21.8 ± 1.65[ab]	1.91 ± 0.70[a]
Low light					
Aspen soil	1.99 ± 0.11[a]	1.64 ± 0.14[ab]	303 ± 8.0[a]	16.3 ± 1.39[c]	0.34 ± 0.05[b]
Conifer soil	1.96 ± 0.04[a]	2.02 ± 0.46[a]	372 ± 32[a]	17.9 ± 1.85[bc]	0.29 ± 0.04[b]
F-values					
Light	105***	19.8**	78***	50***	41***
Soil	0.1051	6.3*	41***	0.04	1.03
Light × soil	0.0031	0.38	NA	4.59*	0.09
Subalpine fir					
High light					
Aspen soil	1.22 ± 0.07[a]	1.48 ± 0.11[b]	NA	NA	8.1 ± 0.28[ab]
Conifer soil	1.25 ± 0.07[a]	1.85 ± 0.11[ab]	NA	NA	7.6 ± 0.23[b]
Low light					
Aspen soil	1.07 ± 0.07[a]	1.43 ± 0.12[b]	NA	NA	9.5 ± 0.54[a]
Conifer soil	1.24 ± 0.11[a]	2.12 ± 0.22[a]	NA	NA	9.2 ± 1.02[ab]
F-values					
Light	1.03	0.62	NA	NA	6.2*
Soil	1.31	12.9**	NA	NA	0.52
Light × soil	0.78	1.18	NA	NA	0.03

Means ± SE presented. F-values from the ANOVA models are presented with significance for the main effects and interactions denoted as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Differences in pairwise comparisons within species are denoted by superscript letters.

Table 3. Field results showing the effects of stand type on foliar chemistry

Stand type	Nitrogen (% dry weight)	Phosphorus (mg g^{-1})	Specific leaf area (cm^2 g^{-1})	Phenolic glyc. (% dry weight)	Tannins (% dry weight)
Gap	1.53 ± 0.14[c]	2.37 ± 0.31[b]	125.46 ± 4.67[c]	24.05 ± 2.52[a]	3.42 ± 1.44[a]
Aspen	1.62 ± 0.14[bc]	3.16 ± 0.18[a]	159.47 ± 6.09[b]	21.52 ± 4.50[ab]	1.41 ± 0.44[a]
Mixed	2.31 ± 0.22[a]	3.78 ± 0.34[a]	195.51 ± 10.68[a]	18.16 ± 1.68[bc]	0.46 ± 0.22[b]
Conifer	2.3 ± 0.42[ab]	3.79 ± 0.57[a]	204.08 ± 7.60[a]	14.50 ± 1.40[c]	0.41 ± 0.25[b]
F-value	3.21*	4.99**	20.71***	4.64*	8.61***

Means ± SE presented. Significant differences denoted as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Statistically significant differences between pairwise comparisons at the 0.05 level are denoted by superscript letters.

promote oxidative stress under high light conditions as has been observed in maples (St. Clair and Lynch 2005b, St. Clair et al. 2005).

Light and soil conditions also impacted foliar nutrient status. Interestingly, light tended to have a stronger impact on foliar nutrient status than soil type in aspen (Table 2). Lower foliar N and P concentrations in aspen leaves under high light conditions were likely the result of nutrient dilution in tissues experiencing increased growth rates (Roumet and Roy 1999). This provides additional evidence that nutrient constraints were secondary to light limitation for aspen growth. In contrast to aspen, foliar nutrition of subalpine fir showed no sensitivity to light conditions.

Based on these data patterns, the only conditions under which aspen growth outpaced subalpine fir was on aspen soil in high light, a condition found only in relatively pure aspen stands. Clearly changes in light and soil chemistry associated with conifer expansion, which is widespread and increasing under longer fire return intervals (Strand et al. 2009), is likely to favor further subalpine fir growth and recruitment.

Impacts on herbivory escape

Aspen defense chemistry decreased with increasing conifer dominance as outlined in our third prediction (Tables 2 and 3). This is the first study that we are aware of that demonstrates that chemical defenses can be compromised through the competitive effects of a late successional species on an early successional species (Table 3). However, since light and soil nutrient reductions covary with increasing conifer dominance in the field, the controlled greenhouse study was necessary to identify the independent and interactive effects of light and soil limitations on foliar defense chemistry. The greenhouse results demonstrated that defense chemistry in aspen was strongly reduced under low light conditions (Table 2), which is consistent with studies by Osier and Lindroth (2006). However, as we observed in the growth response data, soil stress was also an important constraint to the production of phenolic glycosides in aspen under high light conditions (Table 2, significant light × soil interaction). In contrast, condensed tannins were greater under low light conditions in subalpine fir, which may be an adaptive response under conifer expansion.

The observed reductions in defense compounds of aspen in response to altered light and soil conditions by conifers can drastically increase susceptibility to herbivory. While condensed tannins have shown limited effects on aspen-adapted herbivores (Ayres et al. 1997), there is some evidence that they may confer resistance to microbial pathogens (Holeski et al. 2009). Phenolic glycosides, however, have shown significant biological activity against aspen-adapted herbivores (Hwang and Lindroth 1997, Donaldson and Lindroth 2007) and elk preferentially consume aspen genotypes that have lower concentrations of phenolic glycosides (Wooley et al. 2008). Studies indicate that the reductions in phenolic glycosides from 24% down to 16–21% in response to light and soil limitations observed in this study (Tables 2 and 3) may increase aspen susceptibility to insect defoliation and mammal herbivory by ≥50% (Donaldson and Lindroth 2007, Wooley et al. 2008).

Aspen height growth was drastically reduced under light reduction and soil stress while subalpine fir height growth was not (Figure 3). Subalpine fir's height growth insensitivity to lower light conditions is consistent with shade-tolerant species, as they generally alter lateral growth over height under decreasing light (Parent and Messier 1995). The ability of aspen ramets to quickly grow above the mammal browse line is an important herbivory escape strategy in areas where browsing pressure is high. Studies examining aspen dieback have found that lack of aspen recruitment and poor regeneration commonly occur in areas of intense browsing pressure (Kaye et al. 2005), which further promotes succession to conifers (Strand et al. 2009). Our data suggest that lower light levels and changes in soil chemistry with conifer expansion increase the amount of time needed to grow above the mammal browse line by 20–30%. With reductions in defense chemistry and slower growth, aspen becomes more susceptible to herbivory, making it less likely that there will be recruitment into the overstory and persistence of aspen with conifer dominance in late successional stands (Kashian et al. 2007).

Synthesis and conclusions

This study shows a consistent pattern in which reductions in light levels and shifts in soil chemistry with conifer expansion
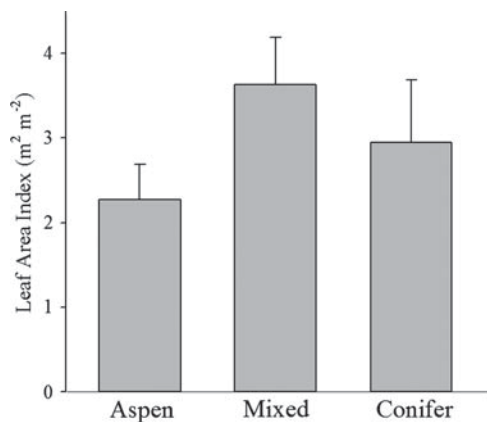
Figure 5. Leaf area index (means ± SE) values (a measure of canopy light interception) in dominant aspen (>80%), dominant conifer (>80%) or mixed aspen–conifer stands (50% of each). Note the substantial variation in means as denoted by the large error bars. This was largely a function of large numbers of canopy gaps particularly underneath conifer-dominant stands.

negatively impact aspen regeneration success through mechanisms of decreased competitive ability and herbivory escape. The field study documents the general effects of increasing conifer dominance on regeneration success, while the greenhouse study characterizes the unique influences of light and soil chemistry and their important interactions. The greenhouse study was conducted on independent suckers in contrast to aspen suckers in the field, which often maintain connections to a parental root system in the first few years of growth (Shepperd 1993). While defense responses were consistent between independent aspen plants in the greenhouse and suckers in the field, there were differences in photosynthetic responses (Figures 1 and 2). Additional studies are needed to examine whether clonal root connections of young aspen suckers can mitigate the light and soil resource limitations that develop with increasing conifer dominance.

The role of gap dynamics in aspen–conifer succession is intriguing. Canopy gaps form and expand as fire intervals lengthen (Hill et al. 2005). Gap dynamics in mixed conifer–deciduous forests can create light heterogeneity, which allows for the recruitment of shade-intolerant tree species (de Römer et al. 2007). Measurement of LAI at our field sites shows mean differences in light penetration, but there were no significant differences between canopy types. This appeared to be the result of canopy gaps, which created substantial variability within stands (Figure 5). Gap dynamics in mixed and pure conifer stands at later successional stages may allow aspen regeneration to persist in the understory until disturbance returns, although the regeneration response will be reduced (Smith et al. 2011). However, if gap conditions indeed alleviate light limitations, our data suggest that aspen's sensitivity to soil conditions under conifer stands will still place significant

constraints on growth potential, and defense against herbivores through reduction in phenolic glycosides.

While more research needs to be done, a conceptual model is emerging in which longer fire return intervals increase competitive interactions between an early (aspen) and late (subalpine fir) successional species in subalpine forests that favors subalpine fir and reduce the competitive ability of aspen.

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References

Aerts, R. and F. Chapin. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30:2–67.

Attiwill, P.M. 1994. The disturbance of forest ecosystems-the ecological basis for conservative management. For. Ecol. Manag. 63:247–300.

Ayres, M.P., T.P. Clausen, J.S.F. MacLean, A.M. Redman and P.B. Reichardt. 1997. Diversity of structure and antiherbivore activity in condensed tannins. Ecology 78:1696–1712.

Beaty, R. and A. Taylor. 2008. Fire history and the structure and dynamics of a mixed conifer forest landscape in the northern Sierra Nevada, Lake Tahoe Basin, CA, USA. For. Ecol. Manag. 255:707–719.

Bengtsson, J., S.G. Nilsson, A. Franc and P. Menozzi. 2000. Biodiversity, disturbances, ecosystem function and management of European forests. For. Ecol. Manag. 132:39–50.

Brown, J.K., J.K. Smith eds. 2000. Wildland fire in ecosystems: effects of fire on flora.General Technical Report RMRS-GTR-42-vol. 2. Ogden, UT: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. 257 p.

Buechling, A. and W. Baker. 2004. A fire history from tree rings in a high-elevation forest of Rocky Mountain National Park. Can. J. For. Res. 34:1259–1273.

Clark, A. and S.B. St. Clair. 2011. Mycorrhizas and secondary succession in aspen–conifer forests: light limitation differentially affects a dominant early and late successional species. For. Ecol. Manage. (in press) doi:10.1016/j.foreco.2011.03.024.

Connell, J. and R. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. Am. Nat. 111:1119–1144.

de Römer, A., D. Kneeshaw and Y. Bergeron. 2007. Small gap dynamics in the southern boreal forest of eastern Canada: do canopy gaps influence stand development? J. Veg. Sci. 18:815–826.

Donaldson, J.R. and R.L. Lindroth. 2007. Genetics, environment, and their interaction determine efficacy of chemical defense in trembling aspen. Ecology 88:729–739.

Donaldson, J.R., E.L. Kruger and R.L. Lindroth. 2006. Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (Populus tremuloides). New Phytol. 169:561–570.

Fraser, E., S. Ländhausser and V. Lieffers. 2004. The effect of fire severity and salvage logging traffic on regeneration and early growth of aspen suckers in north-central Alberta. For. Chron. 80:251–256.

Frey, B.R., V.J. Lieffers, E.H. Hogg and S.M. Ländhausser. 2004. Predicting landscape patters of aspen dieback: mechanisms and knowledge gaps. Can. J. For. Res. 34:1379–1390.

Gallant, A.L., A.J. Hansen, J.S. Councilman, D.K. Monte and D.W. Betz. 2003. Vegetation dynamics under fire exclusion and logging in a Rocky Mountain watershed. Ecol. Appl. 13:385–403.

Grime, J. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111:1169–1194.

Hemming, J.D. and R.L. Lindroth. 1999. Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. J. Chem. Ecol. 25:1687–1714.

Hill, S.B., A.U. Mallik and H.Y.H. Chen. 2005. Canopy gap disturbance and succession in trembling aspen dominated boreal forests in northeastern Ontario. Can. J. For. Res. 35:1942–1951.

Holeski, L.M., A. Vogelzang, G. Stanosz and R.L. Lindroth. 2009. Incidence of Venturia shoot blight in aspen (*Populus tremuloides* Michx.) varies with tree chemistry and genotype. Biochem. Syst. Ecol. 37:139–145.

Hwang, S.Y. and R.L. Lindroth 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. Oecologia 11:99–108.

Kashian, D.M., W.H. Romme and C.M. Regan. 2007. Reconciling divergent interpretations of quaking aspen decline on the northern Colorado Front Range. Ecol. Appl. 17:1296–1311.

Kaye, M.W., D. Binkley and T.J. Stohlgren. 2005. Effects of conifers and elk browsing on quaking aspen forests in the central Rocky Mountains, USA. Ecol. Appl. 15:1284–1295.

Kobe, R. and K. Coates. 1997. Models of sapling mortality as a function of growth to characterize interspecific variation in shade tolerance of eight tree species of northwestern British Columbia. Can. J. For. Res. 27:227–236.

Murphy, J. and J. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31–36.

Nihlgård, B. 1971. Pedological influence of spruce planted on former beech forest soils in Scania, South Sweden. Oikos 22:302–314.

Normandin, V., J. Kotuby-Amacher and R.O. Miller. 1998. Modification of the ammonium acetate extractant for the determination of exchangeable cations in calcareous soils. Comm. Soil. Sci. Plant Anal. 11:1785–1791.

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ. 939:1–19.

Osier, T.L. and R.L. Lindroth. 2006. Genotype and environment determine allocation to and costs of resistance in quaking aspen. Oecologia 148:293–303.

Parent, S. and C. Messier. 1995. Effects of overstory and understory on the understory light environment in mixed boreal forests. Can. J. For. Res. 25:878–885.

Pollard, J.H. 1971. On distance estimators of density in randomly distributed forests. Biometrics 27:991–1002.

Porter, L.J., L.N. Hrstich and B.G. Chan. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25:223–230.

Reyes, G.P., D. Kneeshaw, L. De Grandpre and A. Leduc. 2010. Changes in woody vegetation abundance and diversity after natural disturbances causing different levels of mortality. J. Veg. Sci. 21:406–417.

Roumet, C. and J. Roy. 1999. Leaf structure and chemical composition as affected by elevated CO_2: genotypic responses of two perennial grasses. New Phytol. 143:73–81.

Shepperd, W.D. 1993. Initial growth, development, and clonal dynamics of regenerated aspen in the Rocky Mountains. RM-RP-312. US Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, 8 p.

Smith, A.E. and F.W. Smith. 2005. Twenty-year change in aspen dominance in pure aspen and mixed aspen/conifer stands on the Uncompahgre Plateau, CO, USA. For. Ecol. Manag. 213:338–348.

Smith, E.A., D. O'loughlin, J. Buck and S.B. St. Clair. 2011. The influences of conifer succession, physiographic conditions and herbivory on quaking aspen regeneration after fire. For. Ecol. Manage. (in press) doi:10.1016/j.foreco.2011.03.038.

St. Clair, S.B. and J.P. Lynch. 2005*a*. Base cation stimulation of mycorrhization and photosynthesis of sugar maple on acid soils are coupled by foliar nutrient dynamics. New Phytol. 165:581–590.

St. Clair, S.B. and J.P. Lynch. 2005*b*. Differences in the success of sugar maple and red maple seedlings on acid soils are influenced by nutrient dynamics and light environment. Plant, Cell Environ. 28:874–885.

St. Clair, S.B., J. Carlson and J.P. Lynch. 2005. Evidence for oxidative stress in sugar maple on acidic, nutrient imbalanced forest soils. Oecologia 145:258–269.

St. Clair, S.B., J. Guyon and J. Donaldson. 2010. Quaking aspen's current and future status in western North America: the role of succession, climate, biotic agents and its clonal nature. Prog. Bot. 71:371–400.

Stadt, K.J. and V.J. Lieffers. 2000. MIXLIGHT: a flexible light transmission model for mixed-species forest stands. Agric. For. Meteorol. 102:235–252.

Strand, E., L. Vierling, S. Bunting and P. Gessler. 2009. Quantifying successional rates in western aspen woodlands: current conditions, future predictions. For. Ecol. Manag. 257:1705–1715.

Van Breemen, N. and A.C. Finzi. 1998. Plant-soil interactions: ecological aspects and evolutionary implications. Biogeochemistry 42:1–19.

Van Wagner, C., M. Finney and M. Heathcott. 2006. Historical fire cycles in the Canadian Rocky Mountain parks. For. Sci. 52:704–717.

Walkley, A. and I.A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29–38.

Wooley, S., S. Walker, J. Vernon and R. Lindroth. 2008. Aspen decline, aspen chemistry, and elk herbivory: are they linked? Rangelands 30:17–21.