



Does inorganic nitrogen limit plant growth 3–5 years after fire in a Wyoming, USA, lodgepole pine forest?

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ABSTRACT

Nitrogen (N) is the major nutrient limiting plant growth and production in terrestrial ecosystems around the world. However, nutrient limitation is spatially variable, and different species within the same ecosystem may be limited by different nutrients. N constraints on plant growth have been investigated via fertilization experiments in a variety of ecosystems; however, recently burned coniferous forests are notably under-studied. Given the recent increase in fire activity across western North America, it is important to understand what limits plant growth and carbon sequestration in coniferous forests recovering from wildfire. We evaluated potential inorganic N limitation in four common native plant species, 3–5 years after stand-replacing wildfire in a lodgepole pine forest (*Pinus contorta* var. *latifolia*) in Wyoming, USA. Granular reagent grade ammonium nitrate was added around individual plants at a rate equal to the natural background rate of net N mineralization and at 10× this rate. The grass *Calamagrostis rubescens* exhibited clear evidence of inorganic N limitation: above-ground biomass and shoot:root ratio increased with the high-fertilizer treatment. Nitrogen:phosphorus (N:P) ratio in un-fertilized *C. rubescens* plants was <14, also consistent with N limitation, but N:P ratio shifted to >16 in the high-fertilizer treatment, suggesting the onset of P limitation. The upland sedge *Carex rossii* and seedlings of lodgepole pine were not limited by inorganic N: neither species showed any growth response to N fertilization; N:P ratios were only slightly <14; and foliar N concentrations were greater than critical values reported for mature lodgepole pine. The N-fixing forb *Lupinus argenteus* was not limited by N, for it showed no growth response to fertilization; rather its N:P ratio of 21 indicated P limitation. In this study, to our knowledge the first experimental evaluation of N limitation in subalpine coniferous forests following wildfire, N limitation was seen in only one of four species tested.

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1. Introduction

Nitrogen (N) is thought to be the major nutrient limiting plant growth and production in terrestrial ecosystems around the world (Vitousek and Howarth, 1991; Chapin et al., 2002; but see Elser et al., 2007). However, nutrient limitation is spatially variable, and can change with plant age and with primary succession (Chapin et al., 1986; Vitousek et al., 1993). Vitousek and Farrington (1997; p. 64–65) define nutrient limitation "... as occurring where the rate of a process is increased by additions of a nutrient in quantities sufficient to increase its biological availability ...". The most direct

and robust way to demonstrate nutrient limitation is by means of a fertilization experiment (Vitousek and Farrington, 1997; Eviner et al., 2000). N-fertilization experiments conducted at LTER (Long Term Ecological Research) sites around the U.S. have demonstrated consistent increases in above-ground net primary productivity at the stand level (Gough et al., 2000). However, responses of many individual species have been variable and context-specific, i.e., plant growth increased or decreased following N fertilization, depending on such variables as initial abundance of the species, geographic location, neighboring species, and total site productivity (Pennings et al., 2005).

Although experiments designed to test N limitation at the stand or species level have been conducted in a wide variety of ecosystem types (Cleland, 2008), many important ecosystems have not yet received adequate attention. Noteworthy among these under-studied ecosystems are recently burned coniferous forests. Of the experimental N limitation studies conducted at LTER sites, only two

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were in recently burned sites (both tall-grass prairie), and none were in coniferous forests (Cleland, 2008). Bennett et al. (2004) conducted N-fertilization experiments following clear-cutting and slash-burning in moist coniferous forests of coastal British Columbia, but we know of no comparable experiments in recently burned subalpine forests in the interior of North America. Soil pools and fluxes of available inorganic N are typically very low in mature subalpine coniferous forests (Fahey et al., 1985; Romme and Turner, 2004; Turner et al., 2007), and growth of lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.), a common dominant canopy species, is often N-limited in mature forests (e.g., Binkley et al., 1995; Yang, 1998; Brockley, 2000, 2003; Startsev et al., 2007). However, soil inorganic N typically increases after fire in these forests (Smithwick et al., 2005; Turner et al., 2007). This suggests that other nutrients (e.g., phosphorus) or other abiotic factors (e.g., temperature or moisture) could be more important in limiting plant growth during early post-fire succession, at least for some species. However, this hypothesis has not been tested. Given the recent increase in fire activity across the western U.S. and Canada (Westerling et al., 2006), it is important to understand what limits plant growth and carbon sequestration in coniferous forests recovering from wildfire.

The present study was designed to investigate inorganic N limitation on the growth of tree seedlings and several major herbaceous species in a recently burned lodgepole pine forest in northwestern Wyoming, USA. We studied only one site, but this apparently is the first experimental evaluation of inorganic N limitation in recently burned subalpine forests of western North America. We hope that this report will stimulate additional post-fire studies in similar ecosystems, because, as emphasized by Pennings et al. (2005), generalizations and predictions about limiting factors for species and ecosystems are not possible until a geographically widespread network of experimental studies is available.

We did not measure total ecosystem production. Instead, our focus was on individualistic responses of five common species to augmented inorganic N, three to five years after a lightning-ignited wildfire. The species included naturally germinated seedlings of lodgepole pine, the dominant tree species in this area; and sprouts of three native herbs: the rhizomatous grass *Calamagrostis rubescens* Buckl., the upland sedge *Carex rossii* Boott, and the N-fixing leguminous forb *Lupinus argenteus* Pursh. We also included the forb *Chamaenerion (Epilobium) angustifolium* (L.) Scop. at the beginning of the experiment, but this species declined severely over the course of the study, as initially healthy-appearing plants died or persisted only as low stunted forms. This decline affected both treated and untreated plants within our treatment area, as well as untreated plants outside the treatment area. The reasons for the decline are unknown, but apparently unrelated to N, so this species was dropped from the analyses and interpretations.

We added a low and a high dose of inorganic N to individual plants of each species in two successive years, measured plant leaf area in each year, and harvested the plants in the third year to measure above-ground and below-ground dry weight. In this study we fertilized only with inorganic N. Additional experiments would be needed to evaluate other potentially limiting nutrients by this method. However, foliar nutrient concentrations and ratios of different nutrients also have been used to infer nutrient limitation in the absence of experimental fertilization treatments. Brockley (2001; reported in Table 6 in Blevins et al., 2005) has summarized “foliar critical values” of various nutrients for lodgepole pine; for N this value is 1.35% of dry weight. Koerselman and Meuleman (1996) reported that nitrogen:phosphorus (N:P) ratios >16 were typically associated with P limitation whereas N:P ratios <14 indicated N limitation in wetland ecosystems. Timmer and Stone (1978) used a combination of changes in biomass and foliar nutrient concentrations following fertilization to evaluate

nutrient limitation. Therefore, in this study we not only measured growth responses to N fertilization, but also examined foliar N and P concentrations and N:P ratios, and used this combined information to evaluate nutrient limitation in a recently burned lodgepole pine forest ecosystem.

2. Materials and methods

2.1. Study area

The fertilization experiment was conducted adjacent to one of the stands where we had carried out an earlier study of post-fire foliar N (the Glade site – Metzger et al., 2006). This area is located in the Rockefeller Parkway between Yellowstone and Grand Teton National Parks, northwestern Wyoming, USA. The Glade fire in 2000 burned 1280 ha of lodgepole pine forests that had developed following earlier stand-replacing fires in the late 1800 s. The substrate consists of Quaternary rhyolite bedrock and rhyolite-dominated glacial deposits, and soils are mostly Typic Cryumbrepts and Dystric Chryocrepts (Richard Easterbrook, GIS specialist, Grand Teton National Park, personal communication, 2005). Elevation is ca. 2150 m on gently rolling topography. The nearest weather station is ca. 25 km away, at Moran, WY, where records show average air temperatures of -9.6°C in December and 16°C in July, and average annual precipitation of 575 mm. Although one flank of the Glade fire was actively suppressed to protect park developments, most of the area (including our study site) burned without interference. Because fire retardant typically contains inorganic N, we were careful to select a study area where no fire retardant was applied. We conducted the fertilization experiment within a plot of ca. 0.25 ha where the land sloped very gently towards the southeast. The 2000 fire had burned as a crown fire in this area, consuming all needles and small twigs on the trees, as well as most of the ground-layer vegetation and litter; there was essentially no O horizon remaining after the fire. When we initiated the fertilization experiment in 2003, total plant cover was ca. 20% and litter accumulation was minimal. See Metzger et al. (2006) for more details about soils and vegetation at the study site.

2.2. Field procedures

We established six parallel 50-m transects, 10 m apart, running west to east, and permanently marked at both ends. Along each transect we selected three individuals of each of the five species to be used in the experiment, for a total of 15 experimental plants per transect. Selected plants were discrete individuals, surrounded by bare soil, and separated from other selected individuals by at least 2 m. All of the lodgepole pine were naturally germinated seedlings that had established in 2001, the first year after the fire. *Calamagrostis*, *Carex*, *Chamaenerion (Epilobium)*, and *Lupinus* plants were all individuals that had sprouted from surviving roots in 2001. It is possible that some of the individuals of these four herbaceous species were genetically identical ramets of the same root system; however, it is likely that most represented unique genets, since selected individuals of a given species were separated from each other by at least 2 m and usually by 4+ m. Each plant was marked by driving a steel nail into the ground nearby, and the position, distance, and direction from the transect were recorded to facilitate re-location. The three treatments (described below) were randomly assigned to the three individuals of each species along each transect. Thus, each of the six transects contained one individual plant representing each combination of species and treatment, such that $n = 6$ for each species-treatment combination. (One exception: only four *Carex* plants were used in the control and high-fertilizer treatments because of lack of suitable individuals along two of the transects.)

In early August, 2003, we laid a large black cloth around the base of each plant of interest to exclude other plants or the forest floor from view, then placed a 0.5 m × 0.5 m frame around each plant, and laid a ruler within the frame for calibration of photo-analysis (see below). Plants were small enough at this time to easily fit entirely within the frame. For lodgepole pine seedlings, we measured basal diameter and height, and for the herbaceous species we recorded presence/absence of flowers or fruits. We then photographed the frame and its contents from above using an Olympus 5.5 Mp digital camera, and used this photo to compute the plant's leaf area (see below). Finally, we applied the appropriate fertilizer treatment (see below).

The same measurements and fertilizer applications were repeated one year later, in late July of 2004. This is the approximate time of peak biomass in this ecosystem. In late July of 2005 we again made the same measurements, but then harvested each marked individual (including roots ≥ 0.5 mm diameter) by carefully excavating with a shovel, dried the plants to measure dry weight of above-ground and below-ground parts, and collected leaf samples for determination of foliar N and P concentrations. Winter precipitation (October–April) was 92%, 73%, and 52% of average in 2002–2003, in 2003–2004, and in 2004–2005, respectively; summer precipitation (May–September) was 75%, 122%, and 119% of average in 2003, 2004, and 2005, respectively; and summer temperatures were 95–101% of average in 2003–2005 (Moran climate station; raw data provided by Phil Farnes, Snowcap Hydrology, Bozeman, MT). All of the experimental plants persisted throughout the two years of the experiment, except for one *L. argenteus* individual in the control treatment.

2.3. Fertilizer treatments

We tested three levels of inorganic N fertilization: (i) *control* = no N addition; (ii) *low fertilizer treatment* = adding an amount equal to the natural background rate of net N mineralization at this site; and (iii) *high-fertilizer treatment* = adding 10 times the background rate. We computed the amount of N fertilizer needed to augment the natural background rate of net N mineralization as follows. We assumed that our fertilizer would affect the area within the 0.5 m × 0.5 m sampling frame to a depth of 12 cm, which represents approximately 27 kg of soil given the bulk density of soils in this area. We had measured net N mineralization in the adjacent Glade site using open-top resin cores (Binkley and Hart, 1989; Binkley et al., 1992) as part of a larger study of N dynamics in this ecosystem (Turner et al., 2007), and found it to be 11.4 mg N per kg soil per year in 2001–2003. Thus, multiplying the mineralization rate by the soil weight, we determined that natural background N mineralization would equal approximately 0.3078 g N per year within the upper 12 cm of soil beneath a 0.5 m × 0.5 m sampling frame. Ten times the background rate would be 3.078 g N per year. These rates of N augmentation were achieved by sprinkling either 0.775 g or 7.75 g of dry reagent grade ammonium nitrate around individual plants, taking care to stay within the 0.5 m × 0.5 m sampling frame. Soil surfaces were dry at the time of fertilization; we expect that the granular fertilizer was dissolved and carried into the soil during the first substantial precipitation event following application.

2.4. Laboratory procedures

We knew from earlier studies that herbaceous leaf area is strongly correlated with total above-ground biomass in the

herbaceous species examined in this study (Turner et al., 2004). Therefore, we computed leaf area of each target plant from the photos taken in the field, and used leaf area as a surrogate for total above-ground biomass.

2.4.1. Image analysis

All digital images were analyzed for total leaf area using WinCAM color based area software (Regent Industries, 2005). WinCAM estimates leaf area based on user-defined supervised classification of image colors that represent the desired plant components for area measurement. For each individual image, all above-ground photosynthetic plant components were carefully identified for measurement, while all non-photosynthetic plant components, along with all other unrelated aspects of the image (e.g., quadrat frame, ruler) were excluded. Leaf area (mm²) for each image was then calculated from the newly classified image.

2.4.2. Nitrogen and phosphorus concentrations

Plant foliar samples were powder ground prior to analysis. Total nitrogen concentrations were determined with dry combustion on a Costech 4010 Elemental Analyzer (Valencia, CA). Phosphorous was determined on samples dry ashed at 500 °C. Ashed plant samples were mixed with 10 mL of 1 N HCl and then diluted to 50 mL with deionized water. The ascorbic acid method was then used according to Murphy and Riley (1962) for colorimetric determination of P with a Spectronic Genesys 20 Visible Spectrophotometer (Waltham, MA).

2.5. Statistical analyses

Because sample sizes were small ($n=6$ for each species-treatment combination) and data were not normally distributed, we tested the measured variables of each species for differences among treatments using the non-parametric Kruskal–Wallis one-way analysis of variance. Where Kruskal–Wallis indicated significant differences among all three treatments, pairs of treatments were evaluated again with the same test to identify specific differences. All statistical analyses were conducted using the program *Statistix 8.0*.

3. Results

Growth responses of *C. rubescens* to N fertilization were consistent with inorganic N limitation (Table 1). All of the plants grew during the two years of the experiment, and there were no significant differences between the control and low-fertilization treatments in any of the growth parameters measured. However, the magnitude of increase in leaf area over two years was greater in the high-fertilization treatment than in the control and low-fertilizer treatments. Above-ground biomass and shoot:root ratios at the end of the experiment also were greater in the high-fertilizer treatment, although total plant biomass was not significantly different (Table 1).

For *C. rossii*, *L. argenteus*, and lodgepole pine, there were no significant differences in any of the measured growth responses among the control, low-fertilizer, and high-fertilizer treatments (Table 1), consistent with the idea that inorganic N was *not* limiting growth of these species. For lodgepole pine seedlings we also measured height and basal diameter growth from 2003 to 2005, neither of which differed among treatments ($P \leq 0.10$). Over this two-year period, median height increment among lodgepole pine seedlings of all treatments combined was 219% ($n = 18$); median diameter increment was 168% ($n = 18$). Median height was 20 cm in 2003 and 66 cm in 2005; median basal diameter was 0.6 cm in 2003 and 1.7 cm in 2005.

Table 1Growth responses to inorganic N fertilization by four species in a recently burned lodgepole pine forest in Wyoming, USA^a.

Species	%Change in leaf area 2003–2005 = [(2005–2003)/2003]*100	Above-ground biomass in 2005 (g/plant)	Total biomass in 2005 (g/plant)	Ratio of above- to below-ground biomass in 2005	Interpretation of nutrient limitation based on growth response
<i>Calamagrostis rubescens</i>	Control = 76 (a) Lo fert = 54 (a) Hi fert = 148 (b) (<i>P</i> = 0.024)	Control = 38 (a) Lo fert = 21 (a) Hi fert = 69 (b) (<i>P</i> = 0.060)	NS Median = 149 <i>n</i> = 18	Control = 0.47 (a) Lo fert = 0.34 (a) Hi fert = 0.85 (b) (<i>P</i> = 0.026)	N limitation
<i>Carex rossii</i>	NS Median = 118 <i>n</i> = 14	NS Median = 51 <i>n</i> = 14	NS Median = 120 <i>n</i> = 14	NS Median = 0.80 <i>n</i> = 14	No N limitation
<i>Pinus contorta</i> var. <i>latifolia</i>	NS Median = 808 <i>n</i> = 18	NS Median = 97 <i>n</i> = 18	NS Median = 110 <i>n</i> = 18	NS Median = 6.08 <i>n</i> = 18	No N limitation
<i>Lupinus argenteus</i>	NS Median = 56 <i>n</i> = 17	NS Median = 14 <i>n</i> = 17	NS Median = 25 <i>n</i> = 17	NS Median = 1.17 <i>n</i> = 17	No N limitation

^a Control treatments received no fertilizer; "lo fert" received additional N equal to the natural background net mineralization rate; "hi fert" received 10× the background mineralization rate. Values reported are medians, plus *P*-values derived from Kruskal–Wallis one-way analysis of variance and sample sizes (*n*). NS= not significant at *P* ≤ 0.10. Where Kruskal–Wallis indicated significant differences among all three treatments, pairs of treatments were evaluated again with the same test to identify specific differences (designated with lower case letters in parentheses: treatments with the same letter are not different at *P* ≤ 0.05). Where overall differences are not significant, the median value from all three treatments combined is reported.

Table 2Foliar nutrient concentrations and ratios after two years of experimental inorganic N fertilization for four species in a recently burned lodgepole pine forest in Wyoming, USA^a.

Species	Foliar N (%) in 2005	Foliar P (%) in 2005	C:N ratio in 2005	N:P ratio in 2005	Interpretation of nutrient limitation based on foliar concentrations
<i>Calamagrostis rubescens</i>	NS Median = 1.22 <i>n</i> = 18	Control = 0.11 (a) Lo fert = 0.12 (a) Hi fert = 0.07 (b) (<i>P</i> = .006)	NS Median = 35.2 <i>n</i> = 18	Control = 12.3 (a) Lo fert = 11.6 (a) Hi fert = 16.7 (b) (<i>P</i> = 0.008)	N limitation in Control and Lo fert plants P limitation (marginal) in Hi fert plants
<i>Carex rossii</i>	NS Median = 1.54 <i>n</i> = 14	NS Median = 0.12 <i>n</i> = 14	NS Median = 28.7 <i>n</i> = 14	NS Median = 13.1 <i>n</i> = 14	Possible N limitation (marginal)
<i>Pinus contorta</i> var. <i>latifolia</i>	NS Median = 1.87 <i>n</i> = 18 (FCV = 1.35)	NS Median = 0.15 <i>n</i> = 18 (FCV = 0.10)	NS Median = 27.0 <i>n</i> = 18	NS Median = 12.6 <i>n</i> = 18	Possible N limitation by N:P ratio No N or P limitation by FCV
<i>Lupinus argenteus</i>	NS Median = 3.87 <i>n</i> = 17	NS Median = 0.18 <i>n</i> = 17	NS Median = 11.4 <i>n</i> = 17	NS Median = 21.6 <i>n</i> = 17	P limitation

^a Control treatments received no fertilizer; "lo fert" received additional N equal to the natural background net mineralization rate; "hi fert" received 10× the background mineralization rate. Values reported are medians, plus *P*-values derived from Kruskal–Wallis one-way analysis of variance and sample sizes (*n*). NS= not significant at *P* ≤ 0.10. Where Kruskal–Wallis indicated significant differences among all three treatments, pairs of treatments were evaluated again with the same test to identify specific differences (designated with lower case letters in parentheses: treatments with the same letter are not different at *P* ≤ 0.05). Where overall differences are not significant, the median value from all three treatments combined is reported. Interpretations of N vs P limitation for all species are based on the N:P ratio, viz., N:P < 14 implies N limitation, N:P > 16 implies P limitation (Koerselman and Meuleman, 1996). Additionally, the "foliar critical value" (FCV) from Brockley (2001; reported in Table 6 in Blevins et al., 2005) is used to interpret N and P limitation in lodgepole pine.

Foliar chemistry was consistent with N limitation in the control and low-fertilizer treatments for *C. rubescens*, with the N:P ratio < 14 in each (Table 2). In the high-fertilizer treatment for this species, however, foliar P concentration was significantly lower than in the other two treatments, and the N:P ratio was > 16 (Table 2)—consistent with P limitation. The N:P ratio for *L. argenteus* was high (median = 21, Table 2) in all treatments, consistent with P limitation in this species. There were no significant differences among treatments in N:P ratio for *C. rossii* or lodgepole pine. The median N:P ratio in both species was < 14, suggesting N limitation, though the ratios were only slightly < 14 (Table 2). And in the lodgepole pine seedlings, foliar N and foliar P were both higher than Brockley's (2001; reported in Table 6 in Blevins et al., 2005) "foliar critical value" for this species, indicating that neither N nor P was limiting.

Presence/absence of flowers or fruits on the herbaceous species proved to be uninformative regarding potential nutrient limitation, as no patterns related to treatment were apparent. For *C. rubescens*, every plant in every treatment was flowering in every year. For *C. rossii*, every plant was flowering in every year, except for one in each of the two fertilization treatments in the first year (before treatments were applied). For *L. argenteus*, every plant in the control and low fertilizer treatments were flowering in every year; in the high-fertilizer treatments one did not flower in 2003 and in 2004 (a different individual in each year), but all six were flowering in 2005.

4. Discussion

As in similar experiments in other kinds of ecosystems (e.g., Bennett et al., 2004; Pennings et al., 2005), we saw individualistic

responses to inorganic N fertilization among the four species that were evaluated. The rhizomatous grass *C. rubescens* showed the clearest evidence of inorganic N limitation, as indicated by both growth responses and foliar nutrient concentrations. The high level of N-fertilization stimulated increased growth, and the N:P ratio in un-fertilized plants was <14. Interestingly, the increase in growth came entirely in the above-ground parts of the plants: there was no significant difference among treatments in below-ground biomass (data not shown) or in total biomass (Table 1). The result of this uneven growth response was a higher shoot:root ratio in the high-fertilizer treatment. Median foliar N concentration was 1.22% with no difference between control and fertilized plants, suggesting that the plants were effectively taking up the additional N and incorporating it into biomass. Plants receiving the high-fertilizer treatment had lower foliar P and an N:P ratio >16 (Table 2), suggesting that their N requirements had been met and that P was now becoming limiting (Elser et al., 2007).

C. rubescens is widespread and often abundant at low to moderate elevations throughout the northern Rocky Mountain region, where it rapidly increases in cover following fire or clearcutting, and is regarded as a serious competitor for lodgepole pine and Douglas-fir (*Pseudotsuga menziesii*) seedlings (Doyle et al., 1998; Matthews, 2000; Simard et al., 2003). The closely related species, *Calamagrostis canadensis*, which usually grows in somewhat wetter and cooler sites, also responds positively to opening of the forest canopy and to inorganic N augmentation via fire or fertilization (Hangs et al., 2002, 2003a, 2003b; Goodman and Hungate, 2006).

Two species, *C. rossii* and lodgepole pine, showed no growth response to N fertilization in this study (Table 1), indicating no N limitation. Both species had N:P ratios <14 (Table 2), which does suggest N limitation, but the ratios were only slightly <14, and foliar N in the pine seedlings was well above Brockley's (2001; reported in Table 6 in Blevins et al., 2005) "critical foliar value" of 1.35% for this species. Because (i) growth response to nutrient addition is considered to be the most definitive indicator of nutrient limitation (Vitousek and Farrington, 1997); (ii) the N:P indicators of N limitation were only marginal; and (iii) it is not certain that Koerselman and Meuleman's (1996) N:P criteria, developed for wetland plants, can be applied uncritically to upland plants, we conclude that *C. rossii* and lodgepole pine were not limited by inorganic N in our study area three to five years after fire. It is possible that we simply did not apply enough fertilizer to see a growth response; perhaps all of the added inorganic N was immobilized by soil microorganisms before it could reach the plant roots, as has been reported in other fertilization experiments (e.g., Chang et al., 1997; Staples et al., 1999; Eviner et al., 2000). It is also possible that other kinds of species-specific interactions between plant roots and soil microbes (e.g., Bever, 1994) contributed to differential responses by the four plant species investigated in this study. Nevertheless, the strong response we saw in *C. rubescens* would seem to indicate that the added N was in fact available to the plant roots, yet it did not stimulate additional growth in *C. rossii* or lodgepole pine.

We found no other literature about possible nutrient limitation in *C. rossii*; this species is widespread and commonly increases after fire (Leege and Godbolt, 1985; USDA, 2007), but has received relatively little research attention. A considerable amount of research has focused on nutrient limitation in young and mature lodgepole pine trees, often within a silvicultural context, with different results reported in different settings (e.g., Yang, 1998 and references cited therein; Brockley, 2000, 2003; Startsev et al., 2007). Although N has often been found to be limiting, growth also may be limited primarily by other nutrients such as sulfur or boron (e.g., Brockley, 2000,

2003; Kishchuk et al., 2002; Blevins et al., 2005; Sanborn et al., 2005).

Although lodgepole pine seedling growth does not appear to be N-limited in our study area at this very early stage of post-fire succession, it is possible that it will become so at a later stage. Binkley et al. (1995), working in southeastern Wyoming, reported no N limitation in young lodgepole pine trees (23–43 years old) but evident limitation in older trees (57–117). At the time of our experiment, total plant cover was low and the increase in available N that typically is produced by fire (Ahlgren and Ahlgren, 1960; Smithwick et al., 2005) still may have been adequate to support the growth of lodgepole pine seedlings. In addition, it is known that ectomycorrhizal fungi play a role in nutrient acquisition in lodgepole pine, such that inorganic nutrients may be less important than organic sources for plant nutrition in some settings (Lindahl et al., 2002); and an intriguing recent study has suggested that associative nitrogen fixation might occur within some ectomycorrhizae associated with lodgepole pine (Paul et al., 2007). However, additional study is needed to test these various hypotheses regarding the mechanism(s) underlying the lack of N limitation in the lodgepole pine seedlings that we studied. Whatever the mechanism, it is notable that the median foliar N concentration (1.87%) found in these young seedlings is rather high for lodgepole pine in general (Moore et al., 2004) and well above the 1.35% level considered critical (Brockley, 2001; reported in Table 6 in Blevins et al., 2005). The values are also considerably higher than what we have measured in lodgepole pine saplings growing in similar conditions 16 years after stand-replacing fire (0.88–1.39%; M.G. Turner et al., unpublished data).

Growth of the N-fixer *L. argenteus* clearly was not limited by inorganic N in our study: none of the growth responses differed among treatments, and the N:P ratio was well above 16 (Tables 1 and 2). The high N:P ratio indicated that *L. argenteus* probably was limited by availability of P. This finding is consistent with nutrient limitation studies conducted with other species of *Lupinus*, all of which indicate that atmospheric fixation provides adequate N (Lee et al., 2003; Myrold and Huss-Danell, 2003; Canals et al., 2005) and some of which further suggest that P limits the rate of N-fixation (Crews, 1993; Vitousek, 1999).

Three limitations of our study should be noted. First, we experimentally augmented the supply of only one nutrient, inorganic N. Additional experiments are needed to test the hypothesis of P limitation in *L. argenteus* and in *C. rubescens* when this latter species' N needs are met. Moreover, lodgepole pine may be limited by P or by trace elements, notably sulfur or boron (Brockley, 2000, 2003; Kishchuk et al., 2002; Blevins et al., 2005; Sanborn et al., 2005), none of which was tested in this study. Second, our sample sizes were small ($n=6$ for each species-treatment combination) and our statistical analyses relatively simple. We note, however, that where the Kruskal–Wallis test indicated no significant differences ($P \leq 0.10$) in this study, median values were very close and it was apparent from inspection of the data that significant differences were not likely to be seen even with data transformations followed by more robust parametric tests. Finally, we examined only one stand in one stage of post-fire succession. Given the spatial and temporal variability that characterizes nutrient relations in plants (Chapin et al., 1986) and nutrient resources in soils (Robertson et al., 1993, 1997; Turner and Chapin, 2005; Turner et al., 2007), additional studies are needed to better understand the heterogeneity of nutrient limitations in recently burned coniferous forests, and to make broader generalizations about this type of ecosystem (Pennings et al., 2005). Despite these limitations, the study reported here apparently is the first experimental test of nutrient constraints on

plant growth following recent wildfires in subalpine coniferous forests of western North America. Hopefully these results will stimulate additional investigations in a wide range of recently burned environments.

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References

- Ahlgren, I.F., Ahlgren, C.E., 1960. Ecological effects of forest fires. *Botanical Review* 26, 483–533.
- Bennett, J.N., Laphorne, B.M., Blevins, L.L., Prescott, C.E., 2004. Response of *Gaultheria shallon* and *Epilobium angustifolium* to large additions of nitrogen and phosphorus fertilizer. *Canadian Journal of Forest Research* 34, 502–506.
- Bever, J.D., 1994. Feedback between plants and their soil communities in an old field community. *Ecology* 7, 1965–1977.
- Binkley, D., Hart, S.C., 1989. The components of nitrogen availability assessments in forest soils. *Advances in Soil Science* 10, 57–112.
- Binkley, D., Bell, R., Sollins, P., 1992. Comparison of methods for estimating soil nitrogen transformations in adjacent conifer and alder-conifer forests. *Canadian Journal of Forest Research* 22, 858–863.
- Binkley, D., Smith, F.W., Son, Y., 1995. Nutrient supply and declines in leaf area and production in lodgepole pine. *Canadian Journal of Forest Research* 25, 621–628.
- Blevins, D.P., Prescott, C.E., Allen, H.L., Newsome, T.A., 2005. The effects of nutrition and density on growth, foliage biomass, and growth efficiency of high-density fire-origin lodgepole pine in central British Columbia. *Canadian Journal of Forest Research* 35, 2851–2859.
- Brockley, R.P., 2000. Using foliar variables to predict the response of lodgepole pine to nitrogen and sulphur fertilization. *Canadian Journal of Forest Research* 30, 1389–1399.
- Brockley, R.P., 2001. Fertilization of lodgepole pine in western Canada. In: Bamsey, C. (Ed.), *Proceedings, Enhanced Forest Management Fertilization and Economics Conference*, Edmonton, Alberta, 1–2 March 2001.
- Brockley, R.P., 2003. Effects of nitrogen and boron fertilization on foliar boron nutrition and growth in two different lodgepole pine ecosystems. *Canadian Journal of Forest Research* 33, 988–996.
- Canals, R.M., Eviner, V.T., Herman, D.H., Chapin III, F.S., 2005. Plant colonizers shape early N-dynamics in gopher-mounds. *Plant and Soil* 276, 327–334.
- Chang, S.X., Preston, C.M., McCullough, K., 1997. Transformation of residual ^{15}N in a coniferous forest soil humus layer in northern Vancouver Island, British Columbia. *Plant and Soil* 192, 295–305.
- Chapin III, F.S., Vitousek, P.M., Van Cleve, K., 1986. The nature of nutrient limitation in plant communities. *American Naturalist* 127, 48–58.
- Chapin III, F.S., Matson, P.A., Mooney, H.A., 2002. *Principles of terrestrial ecosystem ecology*. Springer, New York.
- Cleland, E.E., 2008. Species responses to nitrogen fertilization in herbaceous plant communities, and associated species traits. *Ecology* 89, 1175.
- Crews, T.E., 1993. Phosphorus regulation of nitrogen fixation in a traditional Mexican agroecosystem. *Biogeochemistry* 21, 141–166.
- Doyle, K.M., Knight, D.H., Taylor, D.L., Barmore Jr., W.J., Benedict, J.M., 1998. Seventeen years of forest succession following the waterfalls Canyon fire in Grand Teton National Park, Wyoming. *International Journal of Wildland Fire* 8, 45–55.
- Elsler, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* 10, 1135–1142.
- Eviner, V.T., Chapin III, F.S., Vaughn, C.E., 2000. Nutrient manipulations in terrestrial ecosystems. In: Sala, O.E., Jackson, R.B., Mooney, H.A., Howarth, R.W. (Eds.), *Methods in Ecosystem Science*. Springer, New York, pp. 291–307.
- Fahay, T.J., Yavitt, J.B., Pearson, J.A., Knight, D.H., 1985. The nitrogen cycle in lodgepole pine forests, southeastern Wyoming. *Biogeochemistry* 1, 257–275.
- Goodman, L.F., Hungate, B.A., 2006. Managing forests infested by spruce beetles in south-central Alaska: effects on nitrogen availability, understory biomass, and spruce regeneration. *Forest Ecology and Management* 227, 267–274.
- Gough, L., Osenberg, C.W., Gross, K.L., Collins, S.L., 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos* 89, 428–439.
- Hangs, R.D., Knight, J.D., Van Rees, K.C.J., 2002. Interspecific competition for nitrogen between early successional species and planted white spruce and jack pine seedlings. *Canadian Journal of Forest Research* 32, 1813–1821.
- Hangs, R.D., Knight, J.D., Van Rees, K.C.J., 2003a. Nitrogen accumulation by conifer seedlings and competitor species from ^{15}N -labeled controlled-release fertilizer. *Soil Science Society of America Journal* 67, 300–308.
- Hangs, R.D., Knight, J.D., Van Rees, K.C.J., 2003b. Nitrogen uptake characteristics for roots of conifer seedlings and common boreal forest competitor species. *Canadian Journal of Forest Research* 33, 156–163.
- Kishchuk, B.E., Weetman, G.F., Brockley, R.P., Prescott, C.E., 2002. Fourteen-year growth response of young lodgepole pine to repeated fertilization. *Canadian Journal of Forest Research* 32, 153–160.
- Koerselman, W., Meuleman, A.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33, 1441–1450.
- Lee, T.D., Tjoelker, M.G., Reich, P.B., Russelle, M.P., 2003. Contrasting growth response of an N_2 -fixing and non-fixing forb elevated CO_2 : dependence on soil N supply. *Plant and Soil* 255, 75–486.
- Lege, T.A., Godbolt, G., 1985. Herbaceous response following prescribed burning and seeding of elk range in Idaho. *Northwest Science* 59, 134–143.
- Lindahl, B.O., Taylor, A.F.S., Finlay, R.D., 2002. Defining nutritional constraints on carbon cycling in boreal forests—towards a less 'phyto-centric' perspective. *Plant and Soil* 242, 123–135.
- Matthews, R.F., 2000. *Calamagrostis rubescens*. Fire Effects Information System (online), USDA Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (<http://www.fed.us/database/feis/>). Accessed October 16, 2007.
- Metzger, K.L., Romme, W.H., Turner, M.G., 2006. Foliar nitrogen patterns following stand-replacing fire in lodgepole pine (*Pinus contorta* var. *latifolia*) forests of the Rocky Mountains, USA. *Forest Ecology and Management* 227, 22–30.
- Moore, J.A., Mika, P.G., Shaw, T.M., Garrison-Johnston, M.I., 2004. Foliar nutrient characteristics of four conifer species in the interior northwest United States. *Western Journal of Applied Forestry* 19, 13–24.
- Murphy, J., Riley, J.P., 1962. A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* 27, 31–36.
- Myrold, D.D., Huss-Danell, K., 2003. Alder and lupine enhance nitrogen cycling in a degraded forest soil in Northern Sweden. *Plant and Soil* 254, 47–56.
- Paul, L.R., Chapman, B.K., Chanway, C.P., 2007. Nitrogen fixation associated with *Suillus tomentosus* Tuberculate ectomycorrhizae on *Pinus contorta* var. *latifolia*. *Annals of Botany* 99, 1101–1109.
- Pennings, S.C., Clark, C.M., Cleland, E.E., Collins, S.L., Gough, L., Gross, K.L., Milchunas, D.G., Suding, K.N., 2005. Do individual plant species show predictable responses to nitrogen addition across multiple experiments? *Oikos* 11, 547–555.
- Robertson, G.P., Crum, J.R., Ellis, B.G., 1993. The spatial variability of soil resources following long-term disturbance. *Oecologia* 96, 451–456.
- Robertson, G.P., Klingensmith, K.M., Klug, M.J., Paul, E.A., Crum, J.R., Ellis, B.G., 1997. Soil resources, microbial activity, and primary production across an agricultural ecosystem. *Ecological Applications* 7, 158–170.
- Romme, W.H., Turner, M.G., 2004. Ten years after the 1988 Yellowstone fires: Is restoration needed? In: Wallace, L.L. (Ed.), *After the Fires: The Ecology of Change in Yellowstone National Park*. Yale University Press, New Haven & London, pp. 318–361.
- Sanborn, P.T., Prietzel, J., Brockley, R.P., 2005. Soil and lodgepole pine foliar responses to two fertilizer sulphur forms in the Sub-Boreal Spruce zone, central interior British Columbia. *Canadian Journal of Forest Research* 35, 2316–2322.
- Simard, S.W., Jones, M.D., Durall, D.M., Hope, G.D., Stathers, R.J., Sorensen, N.S., Zimonick, B.J., 2003. Chemical and mechanical site preparation: effects on *Pinus contorta* growth, physiology, and microsite quality on grassy, steep forest sites in British Columbia. *Canadian Journal of Forest Research* 33, 1495–1515.
- Smithwick, E.A.H., Turner, M.G., Mack, M.C., Chapin III, F.S., 2005. Post-fire soil N cycling in northern conifer forests affected by severe, stand-replacing wildfires. *Ecosystems* 8, 163–181.
- Staples, T.E., Van Rees, K.C.J., van Kessel, C., 1999. Nitrogen competition using ^{15}N between early successional plants and planted white spruce seedlings. *Canadian Journal of Forest Research* 29, 1282–1289.
- Startsev, N.A., Lieffers, V.J., McNabb, D.H., 2007. Effects of feathermoss removal, thinning and fertilization on lodgepole pine growth, soil microclimate and stand nitrogen dynamics. *Forest Ecology and Management* 240, 79–86.
- Timmer, V.R., Stone, E.L., 1978. Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. *Soil Science Society of America Journal* 42, 125–130.
- Turner, M.G., Chapin III, F.S., 2005. Causes and consequences of spatial heterogeneity in ecosystem function. In: Lovett, G.M., Jones, C.G., Turner, M.G., Weathers, K.C. (Eds.), *Ecosystem Function in Heterogeneous Landscapes*. Springer-Verlag, New York, pp. 9–30.
- Turner, M.G., Tinker, D.B., Romme, W.H., Kashian, D.M., Litton, C.M., 2004. Landscape patterns of sapling density, leaf area, and aboveground net primary production in postfire lodgepole pine forests, Yellowstone National Park (USA). *Ecosystems* 7, 751–775.
- Turner, M.G., Smithwick, E.A.H., Metzger, K.L., Tinker, D.B., Romme, W.H., 2007. Inorganic nitrogen availability following severe stand-replacing fire in the Greater Yellowstone Ecosystem. *Proceedings of the National Academy of Sciences* 104, 4782–4789.

- USDA, 2007. Fire Effects Information System (online), USDA Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (<http://www.fed.us/database/feis/>). Accessed October 16, 2007.
- Vitousek, P.M., 1999. Nutrient limitation to nitrogen fixation in young volcanic sites. *Ecosystems* 2, 505–510.
- Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13, 87–115.
- Vitousek, P.M., Farrington, H., 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. *Biogeochemistry* 37, 63–75.
- Vitousek, P.M., Walker, L.R., Whiteaker, L.D., Matson, P.A., 1993. Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry* 23, 197–215.
- Westerling, A.L., Hidalgo, H.G., Cayan, D.R., Swetnam, T.W., 2006. Warming and earlier spring increase western U.S. forest wildfire activity. *Science* 313, 940–943 (18 August 2006).
- Yang, R.C., 1998. Foliage and stand growth responses of semimature lodgepole pine to thinning and fertilization. *Canadian Journal of Forest Research* 28, 1794–1804.