AN ABSTRACT OF THE THESIS OF

Kathleen A. Johnson for the degree of Doctor of Philosophy in Forest Science presented on April 30, 1998. Title: Effects of Vegetation and Soil Organisms on Soil Nutrient Dynamics in Subalpine *Abies lasiocarpa* Forests.

Abstract Approved: Signature redacted for privacy.

James M. Trappe

The broadest goal of the research covered in this thesis was to contribute to our limited knowledge of high elevation forest soil ecology. Based on the needs of funding agencies, specific objectives were to examine 1) how climate-induced *Abies lasiocarpa* ([Hook.] Nutt.) forest expansion affects soil nutrient pools, 2) the effects of commonly observed non-ectomycorrhizal fungal endophytes on *A. lasiosiocarpa* foliar nutrients and 3) how human activity impacts soil foodweb components and associated nutrient dynamics in backcountry recreation areas located in *A. lasiocarpa* forests. Experimental and observational emphasis was placed on major plant nutrients and the soil organisms that strongly control the storage and cycling of those nutrients.

Major plant nutrients were measured in soils collected in Washington and Montana where high elevation *A. lasiocarpa* forests are expanding into adjacent meadows. Analyses indicated a consistent pattern of meadow-to-forest decline in P and an increase in the ratio of C to N, despite differences in parent material and topography. The increasing C:N ratio likely reflects changes in litter chemistry associated with forest expansion. The decrease in extractable P at the three locations is consistent with estimates of annual P accrual in subalpine forest

biomass reported in other studies, supporting a hypothesis that the meadow-to-forest decline represents a shift in P from abiotic to biotic pools.

As is typical of the Pinaceae, *A. lasiocarpa* is known to be ectomycorrhizal, but recent observations by others of seedlings collected in subalpine meadows revealed abundant vesicular-arbuscular mycorrhizae (VAM) and unidentified, non-ectomycorrhizal, dark-septate endophytes (DSE). Root colonization by these two endophytes was induced in laboratory-grown seedlings of *A. lasiocarpa*. In the case of VAM, the presence of a graminoid meadow species grown together with the tree seedlings led to abundant root colonization and increased foliar P. Foliar P in a treatment where growing medium contained DSE fungal inoculum plus organic material was double that in treatments containing one or the other, but not both.

Soils from a degraded subalpine recreation site were analyzed to determine whether nutrient limitation or soil foodweb shifts might be a factor in poor site recovery. Samples collected from a severely compacted, devegetated campsite were compared to samples from adjacent undisturbed areas. Disturbance-related shifts were detected in soil nutrient pools and in the soil foodweb.

Effects of Vegetation and Soil Organisms on Soil Nutrient Dynamics in Subalpine *Abies lasiocarpa* Forests

by

Kathleen A. Johnson

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A remarkable group of women, some of whom are founding members of the Slime Mold Recovery Team (you know who you are), patiently helped me collect my wits whenever I felt them slipping away: Suzanne Simard, Marla Gilham, Jane Smith, Kristin Vanderbilt and Meg Ruby. May we be lifelong friends. Thom O'Dell and Ari Jumpponen not only offered ample technical help and kind criticism, they too buffered my angst. Carol Glassman, Efren Cásares and Doni Mckay, despite busy schedules, always provided excellent technical assistance.

Through it all, whether I was coming or going or couldn't tell which, my best friend and husband, Jim Reardon, was my port in the storm. This work is fondly dedicated to my Mom and Dad, who let me run wild in the woods, and to my brother, who was my companion there.

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Effects of Vegetation and Soil Organisms on Soil Nutrient Dynamics in Subalpine *Abies lasiocarpa* Forests

Introduction

Chapter 1

The research presented in this thesis addresses soil ecology in forests dominated by subalpine fir (*Abies lasiocarpa* [Hook] Nutt.). These forests have become a focus of attention for the U.S. Forest Service and National Park managers who funded much of this research because 1) montane treelines are predicted to shift in response to global warming and 2) recreationist's use of high elevation backcountry sites is increasing, leading to excessive damage in many areas. Though not addressed in these studies, it is also noteworthy that timber harvest is increasing in high elevation regions. The experimental and observational emphasis of this thesis is placed on major plant nutrients (C, N and P), and the soil organisms that strongly influence the cycling of those nutrients.

High elevation stands dominated by *A. lasiocarpa* extend from the Yukon Territory to Arizona, occurring in both the Rocky Mountains and the Northwest Pacific Coast Range (Alexander et al. 1990). The species has received recent attention as an upper elevation tree sensitive to climate change (Rochefort et al. 1994, Villalba et al. 1994, Peterson and Peterson 1994, Woodward et al. 1995, Hessel and Baker 1997). In western North America subalpine forest boundaries appear to be shifting in response to recent decades of warming, including extensive

subalpine meadow invasion (Franklin et al. 1971, Rochefort et al. 1994, Jakubos and Romme 1993). Because they were not widely exposed to commercial timber harvest until recent years, information on nutrient cycling and productivity in *A. lasiocarpa* forests is limited (Vogt et al. 1989, Aurthur and Fahey 1992).

Subalpine forests are characterized by cold temperatures, heavy snowpacks, slow growth rates and limited soil development (Vogt et. al.1989; Baron 1992). Because forest floor microbial activity that contributes to soil fertility through N mineralization is temperature limited (Vogt et al. 1989, Davis et al. 1991, Aurthur 1992), the organic horizons become important sinks of total ecosystem nitrogen, and the role of mycorrhizae may be particularly important, especially during periods of ecological stress (Read and Haselwandter 1981, Read 1991). Observations of ectomycorrhizae in *A. lasiocarpa* forests are reported in Trappe (1962) and Harvey et al. (1979,1987).

When high elevation forest preserves are degraded by excessive or inappropriate recreation activities, they are extremely difficult to restore, especially if heavy use is ongoing (see Cole and Hall 1992; Cole and Trull 1992; Rochefort and Gibbons 1992). In addition to severe climate, access is often poor and choices for revegetation species are limited.

Each chapter of this dissertation was written as a stand-alone document in manuscript format. Chapter 2 focuses on subalpine fir expansion into adjacent meadows in the mountains of Olympic National Park in Washington state and Glacier National Park in Montana. The study was designed to test the hypothesis that forest expansion leads to surface-soil shifts in the abundance and relationships

of major plant nutrients. Seven soil variables (C, N, P, Ca, Mg, K and pH) were measured in plots established in adjacent forest, sapling and meadow communities. All three sites were at forest/meadow boundaries where young subalpine fir are encroaching into meadow openings. Sapling ages in all three meadows range from 40-60 years (though rarely exceeding 3 meters in height), corresponding to recent decades of warmer drier climate following the Little Ice Age (Rochefort et. al. 1994).

Chapter 3 pertains to the mycorrhizal ecology and mineral nutrition of subalpine fir seedlings. Though members of the Pinaceae are generally regarded as ectomycorrhizal, recent observations of wild seedling roots have revealed abundant vesicular-arbuscular (VA) and non-ectomycorrhizal, dark-septate (DS) fungal structures. The physiological effects of these endophytes on *A. lasiocarpa* is unknown. In the first of two experiments covered in this chapter, a laboratory study was designed to determine if VA would form on subalpine fir seedlings and whether a VA companion plant was necessary to induce colonization. In the second experiment a DS fungus isolated from *Phyllodoce glanduliflora* (Ericaceae), and tentatively identified as *Phialocephala fortinnii* Wang and Wilcox, was introduced into soil-less growing medium with young germinants of *A. lasiocarpa* in the presence and absence of an organic substrate. Foliar N and P levels were measured in the seedlings after 14-week exposure to the fungal inoculants.

Chapter 4 addresses the degradation of soil biology and nutrition in a subalpine fir site by coupling laboratory analysis of field soils with a greenhouse bioassay. The broad objective was to examine the importance of soil biological activity to the maintenance of a dynamic plant-soil nutrient cycle (presented as a

hypothesis by Anderson et. al. 1981). Soil C, N, P and plant tissue N and P were analyzed in relation to the activity of two major foodweb functional groups (N-immobilizing decomposers and N-mineralizing consumers) along a gradient of site degradation. Because they are so fragile, degraded backcountry recreation areas in national parks and other preserves are often subject to the careful scrutiny of resource mangers. Understanding how degradation unravels critical plant-soil linkages will contribute to more effective amelioration of poor site conditions.

Chapter 5 summarizes results and concludes the overall study.

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Changes in Soil Carbon and Nutrients at Present-day Subalpine Forest Expansion sites in Montana and Washington

Chapter 2

Abstract

To investigate changes in the abundance and relationships of major soil nutrients associated with high elevation forest expansion, we measured C, N, P, Ca, Mg, K and pH in surface soils at two sites in Washington and one in Montana, where coniferous subalpine forests dominated by Abies lasiocarpa are expanding into adjacent meadows. Subsamples were collected from three 10m² plots in adjacent forest, sapling and meadow communities at all three locations. Analyses if variance indicated a significant meadow-to-forest decline in extractable P and an increase in the ratio of total C to N, despite differences in parent material, topography and vegetation among the three locations. Principle components analysis generated three clear composite factors: the first comprising the base cations, the second comprising C and N, and the third consisting of P alone. We suggest that the increasing C:N ratio reflects changes in litter chemistry associated with forest expansion. Changes in litter chemistry can be expected to influence decomposition rates and pathways. The average decline in extractable soil P at the three locations (0.15kg/ha/yr) is consistent with estimates of annual P accrual in subalpine forest biomass reported in other studies, supporting a hypothesis that the meadow-to-forest decline represents a shift in P from abiotic to biotic pools.

Introduction

Vegetation boundaries have attracted increased interest as a result of recent attention to climatic controls on broad classes of vegetation. Boundaries or ecotones may be sensitive indicators of the effects of changing climate because they often reflect climatic constraints on species (Emanuel et al. 1985, Neilson 1991, Gosz 1991). In a rapidly changing climate, transition zones such as forest edges at elevational and latitudinal treelines may be sites of the first perceptible changes in global vegetation patterns (Emanuel et al. 1985, Woodman 1989). Montane treelines could shift faster since latitudinal shifts may be blocked or slowed by physical barriers (Franklin et al. 1991). Field studies by several authors (cited in Pastor and Post 1986) suggest that species replacement along climatic or geologic gradients leads to changes in ecosystem properties.

Boundaries of subalpine forests in western North America are currently shifting in response to recent decades of climate warming (Franklin et al. 1971, Rochefort et al. 1994, Jakubos and Romme 1993) and altered disturbance regimes (Richardson and Bond 1991). At high elevations in the coastal Northwest and northern Rocky Mountains, subalpine meadows are undergoing extensive invasion by the dominant tree species, subalpine fir (*Abies lasiocarpa* [Hook] Nutt).

To test the hypothesis that forest expansion results in surface-soil shifts in the abundance and relationships of major plant nutrients, two subalpine meadows in the Olympic Mountains of western Washington (Olympic National Park) and one in the northern Rocky Mountains of Montana (Glacier National Park) were chosen

as study sites. The specific objective was to determine whether common patterns of change occur in surface soil (0-15cm) nutrients as high elevation forests expand into unforested areas, despite marked differences between sites in parent material and topography. Seven soil variables (C, N, P, Ca, Mg, K and pH) were measured in plots established in adjacent forest, sapling and meadow communities. All three sites were at forest/meadow boundaries where young subalpine fir are encroaching into meadow openings. Sapling ages in all three meadows range from 40-60 years (though rarely exceeding 3 meters in height), corresponding to recent decades of warmer drier climate following the Little Ice Age (Rochefort et al. 1994). Scattered younger seedlings are present, although not abundant. Mature trees in surrounding forests have been dated from 150 to several hundred years (author, unpublished).

Site Descriptions

Soils at the **Heather Park** site (1600m elev.) in Olympic Park are derived from Tertiary volcanic basalt pillows and breccias mixed with uplifted and eroded Tertiary sandstone and shale beds (Tabor 1975). The site is steep and rocky with areas of active surficial movement. The sapling and seedling encroachment pattern is both patchy and diffuse throughout the meadow opening. Meadow vegetation is strongly dominated by lupine and heather. Grasses, sedges and forbs are present but less prominent than the other two sites. A partial list of dominant plant species for each of the three locations appears in Appendix Table A.1

The **Obstruction Point** site (1700m) in Olympic Park sits at the crest of an uplifted and eroded Tertiary sandstone and shale ridge top (Tabor 1975). The topography is gently sloping and irregular, with conifer encroachment restricted to meadow edges and occasional dense bands of saplings extending out from forest edges. The meadow community is predominantly graminoid and herbaceous, with sparsely scattered, low-stature woody shrubs.

The **Preston Park** site (2100m) sits in the floor of a one kilometer long, sheltered Pleistocene cirque (Butler and Malanson 1989, Carrara 1990). Valley bottom soils have developed from mass wasting deposits and glacial till carved from uplifted and metamorphosed pre-Cambrian sediments. Limestone dominates the parent bedrock, with lesser amounts of argillite, quartzite and diorite (Nimlos and McConnel 1965, Bamberg and Major 1968, Butler and Malanson 1989). The sapling and seedling encroachment pattern at this site is similar to that at Obstruction Point, with dense bands of saplings spreading from forest edges. Occasional dense bands extend many meters into meadow openings. Saplings and seedlings are restricted to the dense edges or bands; isolated individuals occur infrequently in meadow openings. Life forms of vegetation at Preston Park are similar to those at Obstruction Point. Graminoids and forbs dominate, with occasional dwarf woody shrubs. Several genera of graminoids and forbs are common to both sites.

Methods and Materials

Experimental design and soil sampling

Soil samples were collected in a randomized complete block design with multiple observations (subsamples) per treatment per block (Steele and Torrie 1980). Each site (Preston Park, Obstruction Point and Heather Park) served as a block; each community (forest, sapling, meadow) served as an experimental unit and was replicated once within each block. In each block a 10 meter square gridded plot was established within each of the three experimental units. To avoid complications caused by edges or transition zones, the plots were selectively placed well inside (>5m) each community type. The plots fell along a contiguous invasion gradient from meadow to forest, but were separated in every case by at least 8 meters. Within each grid, at ten randomly selected intersections, surface litter was removed and 10cm diameter cores were extracted to a depth of 15cm. Samples were not composited.

Soil chemical analyses

Samples were removed from the site in plastic bags, air dried at room temperature and sieved through 2cm hardware cloth. Samples were stored dry at room temperature until laboratory analysis.

For total C and N analysis samples were finely ground, sieved to 250 mm and oven dried 24 hours at 105 C. The samples were analyzed by dry combustion

in a Carlo-Erba 1500 Series II Analyzer, following standard manual procedures. Ratios of C to N were derived from the data. Extractable P was analyzed using the dilute acid-fluoride procedures in Olsen and Sommers (1982), and exchangeable Ca, Mg and K by ammonium acetate extraction (Thomas 1982). Soil pH was determined following McClean (1982), with a glass electrode in a 1:10 soil/water solution.

Analyses of variance

Two-way (block by treatment) univariate analyses of variance (ANOVAS) were computed for each of the soil variables by taking the mean of the 10 subsamples for each plot, so that n=3 for each community type/experimental unit. Ca, Mg and K were log 10 transformed to stabilize variance. Pairwise comparisons were Fisher's LSD pairwise comparisons were used to test for significant differences among community types. All statistical analyses were conducted with SYSTAT Version 6.1 (1996).

To explore patterns of change within each site (block), one-way ANOVAS and pairwise comparisons were computed by using the 10 subsamples as replicates (pseudoreplication *sensu* Hurlbert 1984). Results are presented and discussed, but inferences are restricted.

Correlations

Pearson product-moment correlation matrices were computed for all variables, both within and among locations. The coefficient of variation (standard deviation divided by the mean, expressed as a percent) is a relative measure of variability and allows direct comparisons between variables. It was calculated for each variable in each community at each location, to determine whether vegetation type affected the variability of the nutrients measured. Coefficients were also calculated for each variable using the means of combined samples, to examine which variables exhibited the greatest variability.

Principle components analysis

Briefly, in principle components analysis (PCA) the original set of variables is reduced to a lesser number of composite variables, termed principle components, or factors, in order to summarize the data with minimum information loss. All variables are considered simultaneously in order to examine interrelationships and underlying patterns or structure in the data. In a sense, each of the variables is considered as a dependent variable that is a function of some underlying, latent, and hypothetical set of factors (Hair et al. 1992). Naming and interpretation of the new composite factors depends on the analyst's ability to understand relationships represented in the new factors.

In this study PCA was employed as an exploratory, rather than confirmatory, technique to condense the data and expose any underlying structure. Latent root

(eigenvalue) criterion and scree tests were used to select the final number of composite factors (Hair et al. 1992). The factor extraction technique was an orthogonal Varimax rotation. Prior to the computations all nutrient units were standardized to parts per million (Jongman et al. 1987). pH was not included in the factor analysis, but was used subsequently to evaluate correlations with composite factors. First the data from all three locations was combined and analyzed in groups of forest, sapling and meadow. A second analysis was conducted for each location.

Results

Analyses of variance

The most consistent and significant differences resulted for P and the ratio of C to N when compared across communities ($p \leq 0.05$). Mean C:N increased consistently from meadow to forest and mean P increased from sapling to forest (Figures 2.1 and 2.2, Appendix Tables A.2 and A.3). Similar patterns were observed within each location (Appendix Table A.4).

Neither C nor N alone showed a significant trend, although means for C are consistently higher in each forest when compared to the adjacent meadow. Conversely, N means were consistently lower in forests than in meadows. pH decreased consistently and significantly from meadow to forest at Obstruction Point and Heather Park. The Preston Park decrease was not statistically significant.

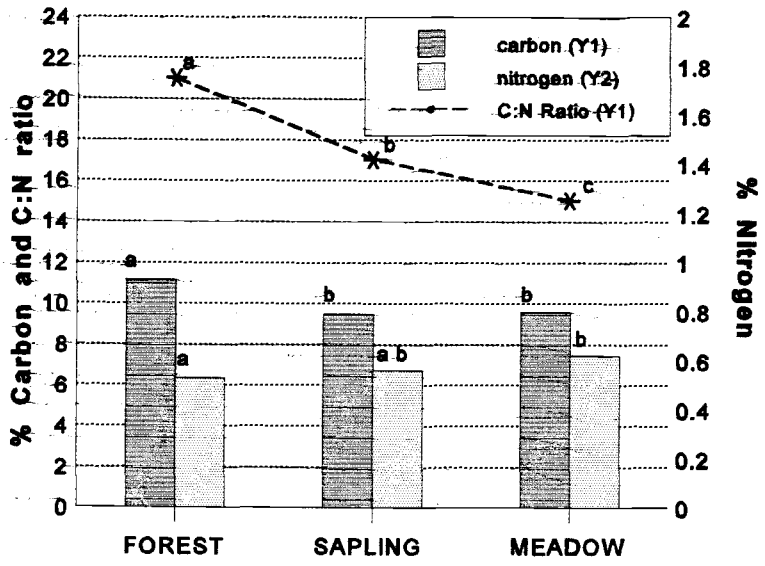


Figure 2.1. Mean percent C and C:N ratio (left axis) and percent N (right axis). Letters indicate results of Tukey pairwise comparisons among vegetation types for each variable separately. Bars topped by different letters are significantly different at p=0.05.

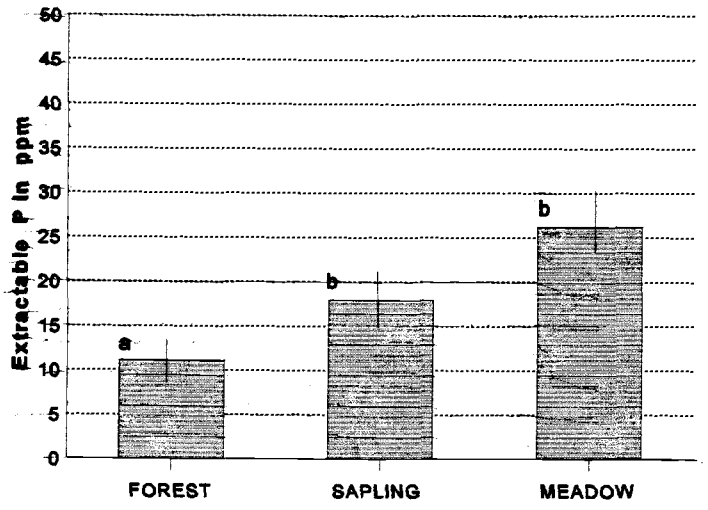


Figure 2.2. Mean values for extractable P in each vegetation type. Bars topped by different letters are significantly different at p=0.05. Error bars are standard error.

There was no pattern of increase or decline in Ca, Mg, or K common to all locations (Figure 2.3). However, within each location, the three cations behaved similarly (Appendix Table A.4). At Preston Park all were higher in the forest than in the meadow. At Obstruction Point and Heather Park all means were highest in the meadow and lower in the saplings, but diverged in the forests.

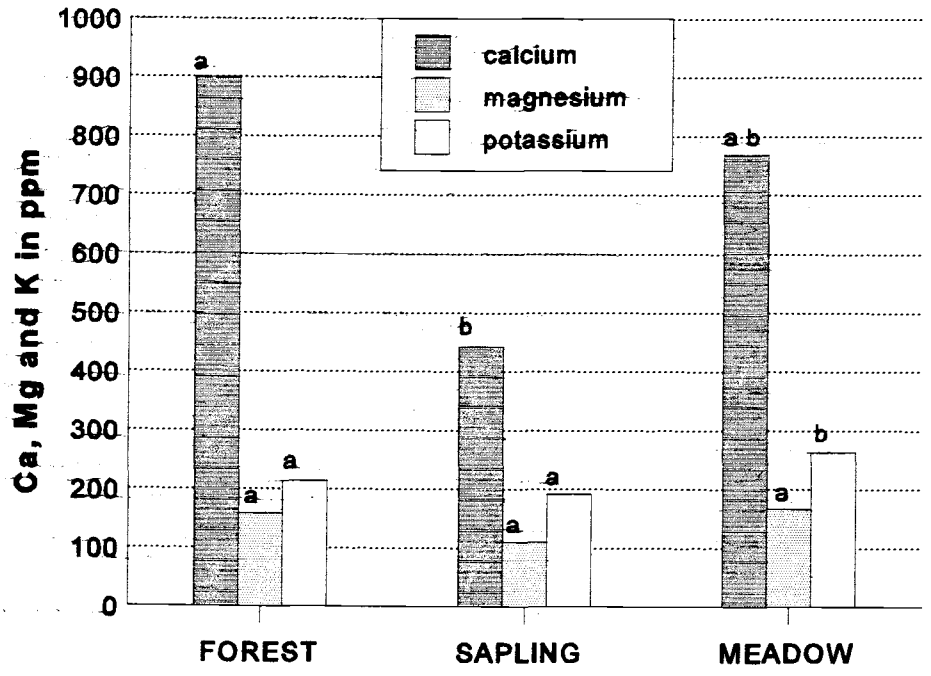


Figure 2.3. Mean Ca, Mg and K in each vegetation type. Letters indicate results of Tukey pairwise comparisons among vegetation types for each variable separately. Bars topped by different letters are significantly different at p=0.05.

Correlations

Within-location correlations (Table 2.1) between C and N were positive, consistently strong, and highly significant *r* values range from 0.62 to 0.86

($p \leq 0.001$). Within-location correlations among base cations were also positive and significant with *r* values ranging from 0.48 to 0.91. Other significant correlations emerged, though not as strongly and consistently. For example, the correlations between pH and cations were positive and significant for the entire data set, but that pattern did not hold within each location. Correlations for all variables (n=90) appear in Appendix Table A.5.

Principle components analysis

The selection of three composite factors extracted in this analysis was based primarily on two criteria: the latent root (eigenvalue) criterion and the scree test (Hair et al. 1992). An exact quantitative basis for deciding the number of factors to extract has not been developed (Hair et al. 1992). Using the latent root criterion, only factors having eigenvalues greater than 1 are considered significant. Figure 2.4 shows that 3 factors fall above the 1.0 cutoff. It also shows the factors in a format used to apply the scree test. In the scree test the curve of the plotted factors is used to evaluate the cutoff. The number of points above which the curve begins to straighten is considered to indicate the maximum number of factors to extract.

Preston Park	C	N	C:N	Mg	Ca	K	P	pH
C	1.00							
N	**0.86****	1.00						
C:N	0.32	-.021	1.00					
Mg	0.35*	-.003	**0.69***	1.00				
Ca	0.18	-.021	**0.72****	**0.89****	1.00			
K	0.44*	0.21	0.39*	**0.67****	**0.59****	1.00		
P	0.14	0.43*	**-0.53***	-0.47	**-0.57****	-0.16	1.00	
pH	-0.15	0.04	-0.41*	0.22	0.16	0.23	0.03	1.00

Heather Park	C	N	C:N	Mg	Ca	K	P	pH
C	1.00							
N	**0.63****	1.00						
C:N	**0.55***	-0.29	1.00					
Mg	0.42*	**0.67****	-0.13	1.00				
Ca	0.26	**0.77****	-0.42*	**0.84****	1.00			
K	**0.51***	**0.76****	-0.14	**0.63****	**0.66****	1.00		
P	0.16	0.30	-0.13	0.18	0.30	0.35	1.00	
pH	-0.13	**0.56**	**-0.69****	**0.58****	**0.75****	0.44*	0.19	1.00

Obstr. Point	C	N	C:N	Mg	Ca	K	P	pH
C	1.00							
N	**0.82****	1.00						
C:N	**0.59***	0.05	1.00					
Mg	0.21	0.28	-0.09	1.00				
Ca	0.27	0.38*	-0.09	**0.91****	1.00			
K	-0.26	0.31	**-0.55***	**0.50***	**0.53***	1.00		
P	-0.42*	-0.15	**-0.54***	0.27	0.21	0.35	1.00	
pH	-0.21	0.03	-0.41*	0.23	0.26	0.44*	0.24	1.00

Table 2.1. Within-location correlation matrices (n=30).
* $p \leq 0.05$; ** $p \leq 0.001$. Simple linear regression. Values ≥ 0.50 appear in boldface.

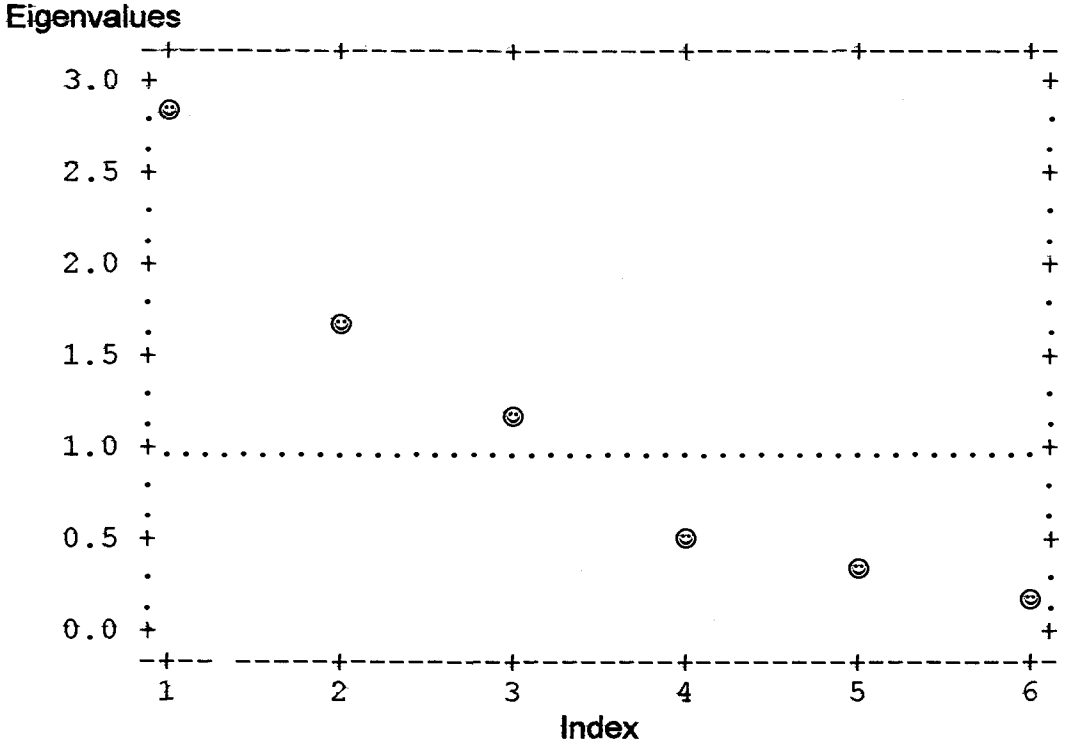


Figure 2.4. Factor scree plot.

Using a third approach to factor selection, several different factor structures were derived from trial rotations. In one trial the ratio of C to N was entered as a variable rather than C and N as separate variables. In another trial pH was included as a variable, and in a third both C:N and pH were included. The factor structure selected as the best representation of the data is shown in Table 2.2. Alternative factor matrices appear in Appendix Tables A.6-A.8

	Factor 1 "Cations"	Factor 2 "CN"	Factor 3 "P"
Mg	**0.931**	0.133	-.084
Ca	**0.917**	0.052	-.210
K	**0.710**	0.286	0.403
C	0.163	**0.919**	-0.198
N	0.118	**0.877**	0.338
P	-0.102	0.024	**0.942**
Eigenvalue	2.262	1.715	1.254
% Variance	37.71	28.59	20.90

Table 2.2. Varimax rotated factor loadings. Those appearing in boldface (>0.50) are considered significant. Percent variance is amount by each factor. Combined total variance explained is 87.2%.

The values associated with each nutrient in the factor columns (termed factor loadings or coefficients) represent the correlation between an original variable and its factor. The eigenvalue is the sum of squared factor loadings. Factor loadings appearing in boldface (>0.50) are considered very significant and identify which nutrient or group of nutrients are prominent in a factor. The prominent variables serve as the basis for naming the factor. Based on the configuration of factor loadings, the names "Cations" "CN" and "P" were assigned to the three composite factors.

While the Pearson correlation coefficients calculated earlier are measures of linear predictability, the composite factors represent the best (eigenspatial) association between variables or the "line of closest fit to systems of points in

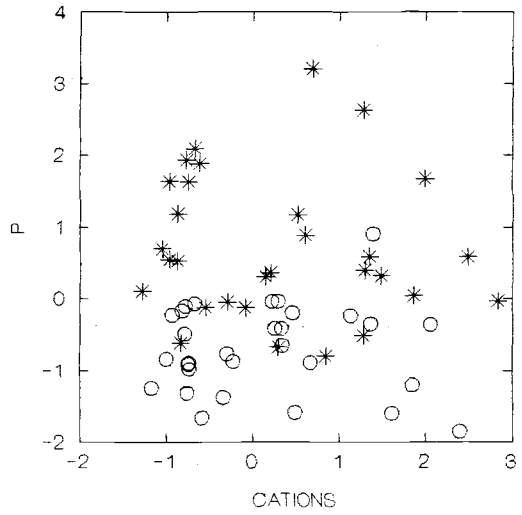
space" (in Wilkinson et al. 1992). The eigenvalues (also termed latent root) at the bottom of each column indicate the relative importance of each factor in accounting for the variance associated with the set of variables. The percentage values at the bottom of the columns indicate how much of the total variance is accounted for by each factor, followed by the cumulative total. In this analysis the total variance accounted for by the three factors was 87.2%.

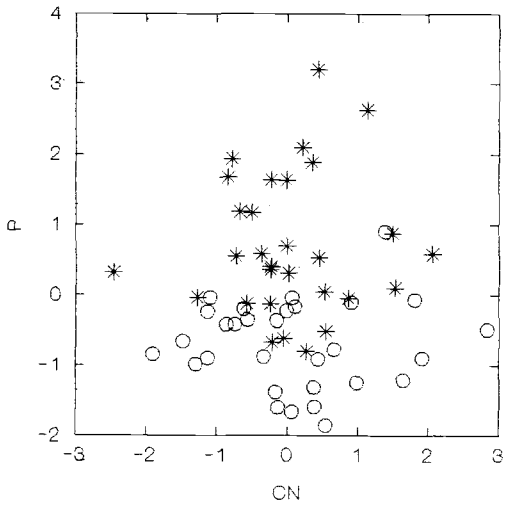
Figure 2.5 shows plots of individual factor scores, differentiating forest and meadow, plotted on all three possible combinations of factor axes. Sapling data were omitted to clarify the image (in most cases sapling points were distributed throughout the point cloud). Data from all three locations are combined.

The plots reveal tendencies for the scores to separate by forest and meadow: in the first two plots, meadow scores tend to load negatively on the P axis, and forests positively. The vegetation types do not show any pattern of separation along the cation axis. In the third plot, the meadow scores tend to cluster between 1 and -1 on the CN axis, forest scores are more evenly distributed. Again there is no clustering pattern along the cation axis.

Figure 2.5. Plots of individual factor scores, differentiating forest and meadow, plotted on all three combinations of factor axes. Sapling data was omitted to clarify the image (in most cases sapling points were distributed throughout the point cloud). Data from all three locations are combined.

Forest=O
Meadow = ✳





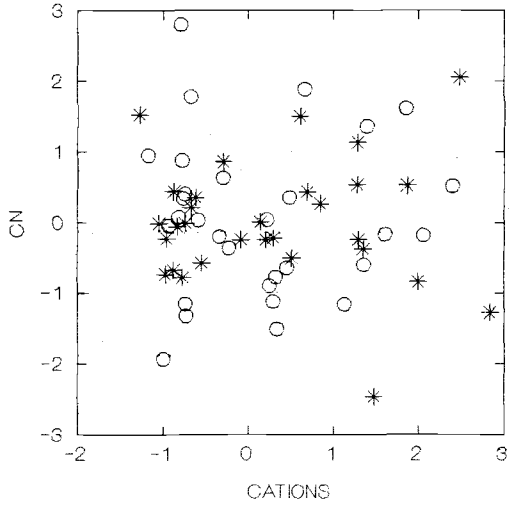
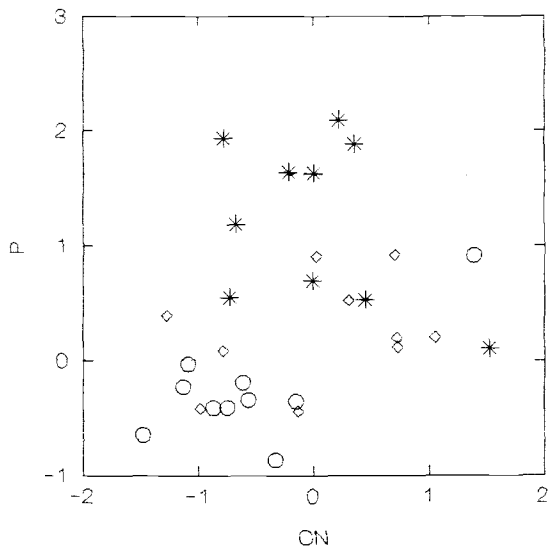


Figure 2.5

Figure 2.6. Is a plot of Preston Park scores only, plotted on the P and CN axes. It includes sapling scores and is presented here to show how the forest and meadow scores tend to cluster independently, while sapling scores intergrade into both regions. Figure 2.7 shows scores separated by locations and plotted on all three axis combinations, with sapling points removed. Notice that in all three locations, where scores are plotted on P and Cation axes forest and meadow tend to separate, with forest scores loading lower (usually negatively) on the P axis, and meadow scores loading high. Score distribution along the cation axis shows some grouping but the pattern is not consistent among locations.

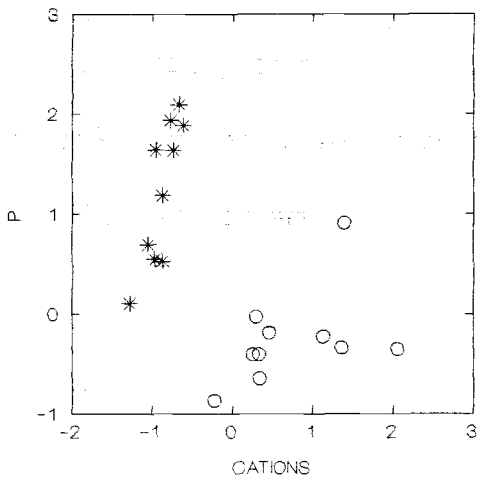


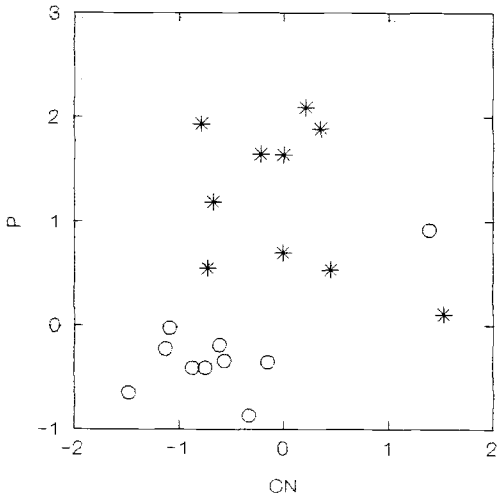
Forest = o Sapling = ◇ Meadow = ✳

Figure 2.6 Preston Park factor scores plotted on the "P" and "CN" axes. Sapling scores are plotted to show how forest and meadow scores tend to cluster independently, while sapling scores intergrade into both regions.

Figure 2.7 (Next 3 pages). Factor scores plotted on all three axis combinations, with sapling points removed.

Preston Park
Forest = o
Meadow = ✳





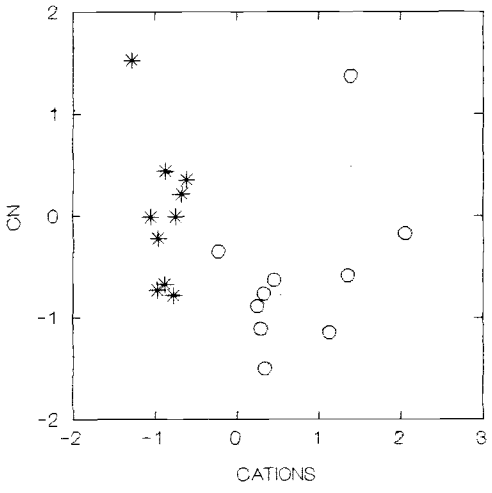
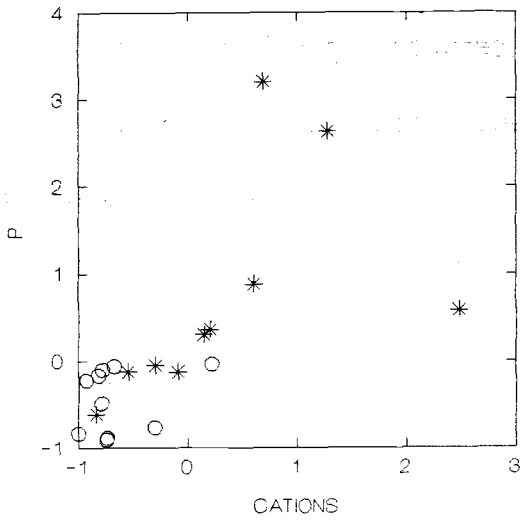
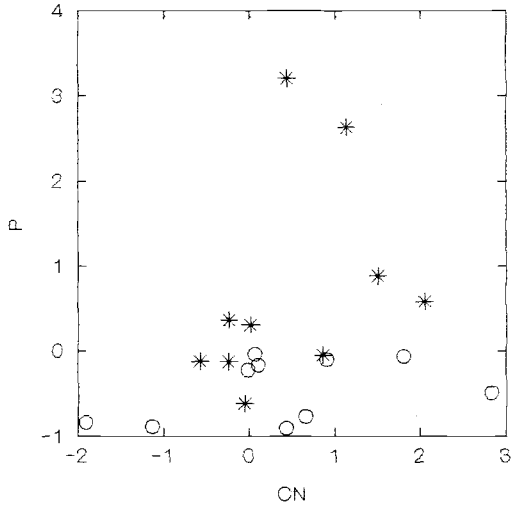


Figure 2.7

Obstruction Point
Forest = o
Meadow = ✳





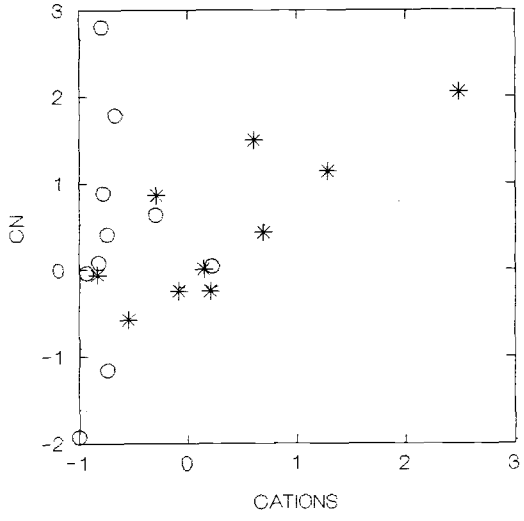
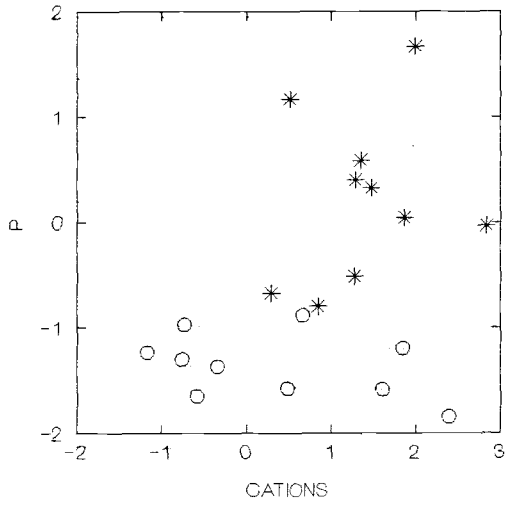
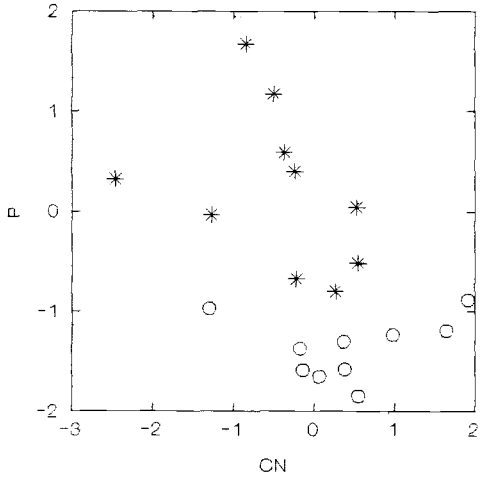


Figure 2.7 Continued

Heather Park
Forest = o
Meadow = ✳





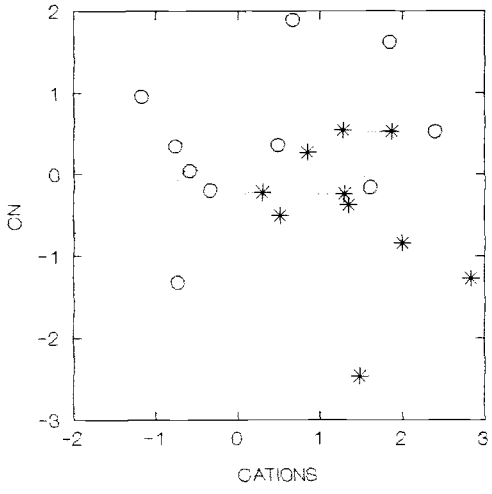


Figure 2.7 Continued

Where scores are plotted on P and CN axes meadows tend to load higher than forests on the P axis in all three locations. There is no consistent pattern along the cation axis. Scores plotted on the CN and cation axes show a slight tendency to cluster into meadow and forest groups, but the pattern is not consistent among locations.

To further examine relationships among the variables using PCA, a separate analysis was conducted for each of the three vegetation communities (forest, sapling and meadow). A guideline proposed by Grossman et al. (1991) suggests that for PCA to perform well, the number of samples should be at least 3 times the number of variables. In this case of separate community analyses, the total number of samples was 30 and number of variables was 6. The resulting factor matrices were quite similar to those for the entire data set. For the forest data, however, K paired with P, rather than Ca and Mg, to form the third factor. The highest percentage of variance (35%) was accounted for by Ca and Mg in the first factor. The P/K factor explained only 27%.

Correlation analyses with composite factors

One of the possible functions of PCA is to generate composite factors for use in subsequent statistical analyses. Because pH is not a measure of a plant nutrients *per se*, it was excluded from the factor analyses and subsequently included in a Pearson correlation analysis against each of the composite factors. None of the correlations is strong, but the *r* value of +0.52 for the Cation factor

is highly significant:

	CATIONS	CN	P
pH	0.523	-0.188	-0.011

Discussion

The meadow-to forest increase in C:N and sapling-to-forest decrease in P is evidence that these changes in nutrient status at the soil surface are associated with subalpine fir expansion at an interregional scale. The increasing C:N ratios may reflect changes in litter nutrient concentrations along the meadow-to-forest continuum because carbon:nutrient ratios widen in plant tissues as they become more woody (Perry 1994). Since carbon:nutrient proportions determine the immobilization-mineralization reactions of nutrients during decomposition (Waring and Schlesinger 1985, Paul and Clark 1989), the meadow-to-forest increase in C:N in this study should be an indicator of changing detrital resource quality that influences soil decomposition rates. C and N cycles are strongly and reciprocally linked because species-characteristic carbon compounds in litter control N release (through decomposition rate), which in turn controls the rate of net primary production (Pastor and Post 1986).

The results are consistent with computer simulations of forest response to climate change in eastern North America (Pastor and Post 1988). Using a linked forest productivity/soil process model, simulated changes in vegetation composition led to altered soil N availability, via litter quality, which in turn amplified

vegetation changes. The simulated responses resulted from positive feedback between decomposition rate, hence N availability, and C:N ratios in foliage. Under conditions where both N and moisture became limiting, forest productivity and carbon storage declined. The potential for moisture limitation at high elevations in Montana is projected in climate change model developed by Running and Nemani (1991). Their simulations of upper montane regions, where summer soil moisture is almost entirely dependent on melting snowpack, led to a 69 day spring retreat of final snowmelt, tied to a growing season increase of 63 days, strongly suggesting that moisture could become limiting in some soils. The future productivity of subalpine vegetation will also be influenced by the efficiency of species in using nutrients and the extent to which warmer soils increase microbial decomposition.

At the p-value cutoff level used in this study, the 6.8ppm sapling-to-forest P decrease was significant ($p=0.01$), but the meadow-to-sapling decrease of 8.2 ppm was not ($p = 0.08$). In each of the 3 sapling stands the mean value for P fell between meadow and forest values. We suggest that the decline in extractable P represents a shift from a plant-available abiotic pool (easily soluble Ca, Fe and Al phosphates; Olson and Sommers 1982), to a biotic pool, largely conifer biomass, as forest expansion occurs.

By converting the ppm values in our study to kg/ha (calculations appear in Appendix Table A.9), we estimated that the average decline in available soil P in these forests was 22.5 kg/ha over approximately 150 years.

To determine whether the differences between meadow and forest P might be attributable to accumulation in forest biomass, we estimated potential annual

accrual in biomass, multiplied by the approximate age of the stand, and compared the resulting number to the measured differences in available P. For annual P accrual we used a conservative value of 0.1 kg/ha/yr derived from published reports of P uptake in subalpine fir/Engelmann forests (Prescott et al. 1988; Arthur and Fahey 1991; calculations in Appendix Table A.9). Multiplying 0.1 kg P/ha/yr by the stand age (150 yr) gives an overall accrual of 15 kg P/ha, somewhat less than the 22.5 kg/ha mean decline in soil P from meadow to forest. Substituting a mean, rather than conservative value, derived from the two publications (0.15 kg/P/ha/yr annual accrual times 150 y), gives an overall accrual of 22.5 kg/ha. That the two numbers agree exactly is certainly fortuitous, but the close agreement supports our hypothesis that available P shifted from soil to biomass as a result of forest expansion.

Prescott et al. (1988) and Arthur and Fahey (1991) report annual subalpine forest P uptake (requirement minus resorption) at 1.8 and 1.7 kg/ha/yr, respectively. By extrapolating a conservative 1.0 kg/ha/yr uptake estimate to our sites, and multiplying by 150 years, the total uptake would have been 150 kg/ha. If 22.5 kg/ha has actually accrued in biomass, 127.5 kg/ha (150 minus 22.5) has recycled over the years, at an average of 0.85 kg/ha/yr. A yearly accrual of 0.15 kg/ha/yr, coupled with the recycle rate of 0.85 kg/ha indicates the magnitude of the P sink in forest biomass. Because these estimates are based on differences between existing meadow and forest, they do not account for any initial P uptake during early post-glaciation community development.

The phosphorus data are consistent with those of other authors, and suggest

that forests in these high subalpine ecosystems constitute a net P sink: the removal of soil P (ie. its transfer into biomass) gradually outpaces its resupply through recycling and mineral weathering.

Our main objective was to look for forest-to-meadow nutrient shifts consistent at an interregional scale, but between-site differences in cation patterns are noteworthy. Preston Park in was the only location where Ca and Mg showed a relation to vegetation cover by increasing significantly from meadow to forest (though pH remained unchanged). Correlations between cations and C:N ratios are also much higher at Preston Park, supporting the idea of a connection between cations forest expansion.

Changes in the soil exchange complex (and therefore exchangeable Ca, Mg and K) can result from the sequestration of nutrient cations in forest biomass or the addition of cations taken up from deeper horizons or released by mineral weathering (Binkley 1995, Homann 1992). Information presented in the Homann et al. (1992) paper allows a comparison between our data and cation transfer from mineral to organic soil in a Douglas fir forest in western Washington. By dividing total O horizon cations by stand age, they report an annual net cation shift of 4.5,0.60 and 0.7 kg/ha/yr of Ca, Mg and K, respectively, from mineral to organic horizons. By determining the difference between forest and meadow mean values, and converting the Preston Park data from ppm to kg/ha (see appendix) the same calculation (using means from top 15cm of soil and 150yr) gives estimates of similar magnitude: 5.9, 1.1 and 0.4 kg/ha/yr for Ca, Mg and K, respectively. This comparison assumes that the meadow-to-forest transition is a chronosequence and

that forest vegetation is more effective than meadow vegetation in moving cations upward in the soil profile. We are unable to determine whether the forest stand was once meadow, but it is clear that the sapling stand has established in the meadow community. Popenoe et al. (1992), reported exchangeable decreasing Ca and K with depth (0-150cm) under conifer forest, but not under open prairie. Exchangeable Ca also decreased with depth under conifer-invaded prairie. It is unclear why the Preston Park pattern of cation increase with forest expansion would be unique among the three sites, and the unreplicated results must be regarded as a single case study.

Principle components analysis

The 3 composite factors extracted with PCA appear to represent meaningful chemical and ecological relationships among the variables in our study sites. The composite Cations factor explained the largest amount of variance (38%) in the data set as a whole. That they combine to form a single factor, correlate well within locations, yet do not consistently track forest expansion reflects their similar chemistries and the lesser role of vegetation influence. The positive and significant correlation between the cation factor and pH may be a reflection of the relatively young age of the communities, since as soils weather, acidity typically correlates negatively with base cations (Perry 1994).

The pairing of C and N in a single composite factor, explaining 29%of the variance, reflects their close links as they cycle through litter and belowground

pools. (Paul and Clark 1989; Sprent 1987; Waring and Schlesinger 1985).

The separation of P into a singular factor, explaining 21% of the variance indicates the lack of any strong linear or eigenspatial association with the other variables or factors. This occurred despite the clear tendency of available P to decline from meadow to forest at all three locations. However, the weak but significant Pearson correlation between P and C/N (r= -0.47; $p \leq 0.001$) is noteworthy since links between C,N and P cycles in biotic -(including decomposition) pathways have been established (Schlesinger 1991), though P links to C and N are not as tightly coupled as C to N (Perry 1994). Whereas P is usually bound to oxygen in organic material, most N atoms in litter are bound directly to carbon so microbial access to either C or N requires breaking C-N bonds.

Graphical PCA displays of the three factors revealed that the two most distinct vegetation types (forest and meadow) tend to cluster separately within each location, when plotted on all three axis combinations, but the spatial arrangement of clusters is not consistent among locations (refer back to Figure 2.7). In Preston Park, for example, meadows score negatively and forests tend to score positively on the Cation factor, but the distribution does not hold for the other two locations. PCA showed that forest and meadow soils could be distinguished when the analysis was conducted within-location, but not for the combined data set. Within each location the arrangements of the clusters appears to reflect the data as presented in the bar charts. For example, unlike the other two locations, the Preston Park soils tended to have highest Ca, Mg and K means in the forest, and lowest means in the meadow. This pattern is echoed in Figure 2.7, where the

positions of the meadow points load lower negatively and forest points load positively on the Cation axis.

When used to detect latent structure in the data, results of the principle components analysis are consistent with our current understanding of cation and carbon/nitrogen biogeochemistry. The structure of the component factors reinforces the results of the other statistical analyses in this study.

The restricted sampling and analyses in this study preclude detailed inferences about causal mechanisms, but the results suggest that sites where subalpine forests are currently expanding can be important focal points for understanding indirect effects of climate change on forest productivity.

We agree with recommendations by Anderson (1991), Agren et al. (1991) and others that integrated studies on plant and soil processes, especially in areas where vegetation shifts are already occurring, are necessary to understand ecosystem carbon and nutrient dynamics in relation to climate change.

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Appendix

APPENDIX TABLE A.1

Dominant meadow species at each location

Preston Park, Glacier National Park, Montana

Luzula hitchcockii (Cyperaceae)
Erithronium grandiflorum (Liliaceae)
Deschampsia atropurpurea (Poaceae)
Senecio triangularis (Asteraceae)
Valeriana sitchensis (Valarianaceae)
Hypericum formosum (Hypericaceae)
Clatonia lanceolata (Portulacaceae)
Carex Nigricans (Cyperaceae)
Castilleja rhexifolia (Scrophulariaceae)
Aster foliaceous (Asteraceae)
Trollius laxus (Ranunculaceae)
Anemone occidentalis (Ranunculaceae)
Sibbaldia procumbens (Rosaceae)

Heather Park, Olympic National Park, Washington

Cassiope mertensiana (Ericaceae)
Rhododendron albiflorum (Ericaceae)
Phyllodoce empetriformis (Ericaceae)
Deschampsia atropurpurea (Poaceae)
Hieracium gracile (Asteraceae)
Erigeron perigrinus (Asteraceae)
Lupinus latifolius (Fabaceae)
Carex spectabilis (Cyperaceae)
Carex nigricans (Cyperaceae)
Antennaria lanata (Asteraceae)
Veronica cusickii (Scrophulariaceae)
Luetkea pectinata (Rosaceae)

Obstruction Point, Olympic National Park, Washington

Erithronium grandiflorum (Liliaceae)
Carex phaeocephala (Cyperaceae)
Festuca idahoensis (Poaceae)
Vaccinium deliciosum (Ericaceae)
Cassiope mertensiana (Ericaceae)

APPENDIX TABLE A.2

C:N Anova and LSD pairwise comparison p-values

	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	339.95	2	169.98	8.96	.03
Block	290.87	2	145.43	7.66	.04
Error	75.90	4	18.07		

	Forest	Sapling	Meadow
Forest	1.00		
Sapling	0.01	1.00	
Meadow	0.13	0.08	1.00

Phosphorus Anova and LSD pairwise comparison p-values

	Sum-of-Squares	df	Mean-Square	F-ratio	P
Treatment	50.85	2	25.42	15.55	.01
Block	30.70	2	15.35	9.39	.03
Error	6.54	4	1.63		

	Forest	Sapling	Meadow
Forest	1.00		
Sapling	0.01	1.00	
Meadow	0.02	0.20	1.00

Appendix Table A.2. Analyses of Variance and LSD pairwise comparison matrices for C:N and phosphorus.

APPENDIX TABLE A.3

	%C	%N	C:N	ppm P	ppm Ca	ppm Mg	ppm K	pH
Forest	11.15	0.52	21.18	11.10	890.20	157.80	213.53	4.83
	1.11	*0.01*	*1.87*	*2.56*	*266.45*	*46.68*	*21.91*	*0.28*
Sapling	9.48	0.56	17.02	17.93	468.13	108.80	191.17	5.00
	0.09	*0.04*	*1.50*	*5.19*	*140.06*	*19.56*	*6.98*	*0.21*
Meadow	9.57	0.62	15.75	26.14	806.20	165.80	263.20	5.28
	0.41	*0.04*	*0.73*	*5.25*	*395.71*	*56.39*	*35.15*	*0.30*

Appendix Table A.3. Means (s.e. in italics) for each variable in each vegetation type, where the 10 samples from each plot were averaged, such that n=3.

APPENDIX TABLE A.4

PRESTON PARK	Forest	Sapling	Meadow	p-values
%C	9.01 ±.67	9.33 ±0.63	8.98 ±0.45	≤0.895
%N	0.51 ±0.03	0.61 ±0.03	0.62 ±0.03	≤0.034
C:N	17.61 ±0.440	15.35 ±0.26	14.42 ±0.13	≤0.001
P (ppm)	12.92 ±1.67	22.59 ±2.36	35.98 ±3.33	≤0.001
Ca (ppm)	1019.60 ±171.50	422.80 ±71.29	132.60 ±12.90	≤0.001
Mg (ppm)	215.60 ±18.012	95.00 ±13.21	55.40 ±5.09	≤0.001
K (ppm)	253.50 ±23.74	177.20 ±14.48	195.60 ±11.76	≤0.012
pH	5.07 ±0.09	5.16 ±0.07	5.11 ±0.04	≤0.646

OBSTR. POINT	Forest	Sapling	Meadow	p-values
%C	11.69 ±1.22	9.47 ±0.60	10.36 ±0.64	≤0.211
%N	0.53 ±0.04	0.60 ±0.03	0.69 ±0.04	≤0.033
C:N	22.03 ±0.97	15.69 ±0.27	15.92 ±0.96	≤0.001
P (ppm)	14.35 ±2.02	23.63 ±3.65	24.41 ±5.83	≤0.064
Ca (ppm)	377.80 ±71.64	251.40 ±90.30	783.20 ±188.62	≤0.001
Mg (ppm)	65.40 ±11.17	84.00 ±12.27	201.00 ±42.12	≤0.001
K (ppm)	209.10 ±1	198.40 ±24.81	280.30 ±32.80	≤0.098
pH	4.27 ±0.08	4.58 ±0.08	4.665 ±0.09	0.001

continued next page

Appendix Table A.4 (continued)

HEATHER PARK	Forest	Sapling	Meadow	p-values
%C	12.74 ±0.81	9.63 ±0.95	9.36 ±0.70	≤0.013
%N	0.53 ±0.04	0.48 ±0.04	0.55 ±0.03	≤0.388
C:N	23.91 ±0.41	20.02 ±0.53	16.92 ±0.61	≤0.001
P (ppm)	6.04 ±0.17	7.57 ±2.87	18.02 ±4.03	≤0.001
Ca (ppm)	1273.20 ±325.95	730.20 ±110.73	1502.80 ±163.684	≤0.036
Mg (ppm)	192.40 ±36.03	147.40 ±17.87	241.00 ±19.40	≤0.060
K (ppm)	178.00 ±21.34	197.90 ±27.00	313.70 ±25.21	≤0.001
pH	5.16 ±0.03	5.26 ±0.04	5.87 ±0.22	≤0.001

Appendix Table A.4. Means, standard errors (±) and univariate p values for each variable within each location. Means were calculated from the 10 samples. Ca, Mg, and K were log 10 transformed before analysis to stabilize variance.

APPENDIX TABLE A.5

Preston Park	C	N	C:N	Mg	Ca	K	P	pH
C	1.00							
N	**0.66**	1.00						
C:N	**0.54**	-0.23	1.00					
Mg	0.28	0.07	0.26	1.00				
Ca	0.26	0.24	0.09	**0.85**	1.00			
K	0.28	0.43	-0.15	0.48	**0.56**	1.00		
P	-0.11	0.29	-0.47	-0.21	0.15	0.19	1.00	
pH	-0.13	-0.04	-0.13	0.49	0.47	0.28	-0.08	1.00

Appendix Table A.5. Pearson correlation matrix of all variables (n=90). Values ≥0.50 appear in boldface.

APPENDIX TABLE A.6 Factor loadings for within-community data

FOREST	Factor 1	Factor 2	Factor 3
P	**0.886**	-0.287	0.144
K	**0.840**	0.389	0.263
Mg	0.043	**0.981**	-0.006
Ca	-0.038	**0.924**	0.277
C	0.093	0.035	**0.976**
N	0.403	0.269	**0.834**
Eigenvalue	1.665	2.122	1.815

SAPLING	Factor 1	Factor 2	Factor 3
Mg	0.932	0.132	-0.148
Ca	0.921	-0.156	-0.173
K	0.719	0.458	0.224
N	-0.078	0.968	0.135
C	0.227	0.924	-0.222
P	-0.108	-0.029	0.975
Eigenvalue	2.310	2.044	1.021
% of total variance explained	38.36	34.06	18.68 Total variance explained : 91.10%

MEADOW	Factor 1	Factor 2	Factor 3
Ca	**0.924**	-0.078	-0.214
K	**0.858**	0.129	0.120
Mg	**0.850**	0.365	-0.191
N	0.019	**0.972**	0.127
C	0.224	**0.945**	-0.029
P	-0.107	0.067	**0.978**
Eigenvalue	2.374	1.997	1.070
% of total variance explained	39.57	33.28	17.82 Total variance explained: 90.67%

Appendix Table A.7 Alternate factor loadings, including Ph

	Factor 1	Factor 2	Factor 3
Mg	**0.866**	0.241	-0.103
Ca	**0.877**	0.161	-0.231
pH	**0.755**	-.305	0.053
K	**0.654**	0.385	0.380
C	0.083	**0.922**	-0.197
N	0.089	**0.844**	0.355
P	-0.107	0.026	**0.938**
Eigenvalue	2.567	1.888	1.255
% of total variance explained	36.68	26.97	17.93 Total variance explained: 81.58%

Appendix Table A.8 Alternate factor loadings, including C:N

	Factor 1	Factor 2	Factor 3
Mg	**0.932**	0.152	0.014
Ca	**0.891**	0.172	0.225
K	**0.779**	-0.340	-0.146
P	**-0.058**	**-0.938**	-0.240
C:N	0.044	0.235	**0.966**
Eigenvalue	2.276	1.103	1.064
% of total variance explained	45.52	22.07	21.28 Total variance explained: 88.87%

APPENDIX TABLE A.9 Phosphorus cycling calulations

PPM to kg/ha conversion:

1) one 10cm deep ha^2 = 1.0 X $10^9 cm^3$

2) (1.0 X 10^9) / (1X10^6) = 1,000cm^3

3) assuming soil bulk density of 1.0 g/cm^3, 1000cm^3 = 1kg

4) so (where BD = 1.0), ppm = kg/ha

Because our measurements were to 15cm depth, rather than 10, we multiplied the mean meadow-to-forest P decline of 15ppm by 1.5, giving 22.5ppm or kg/15cm deep hectare.

Uptake and cycling calculations:

Using Prescott (1988) data:

(1.8 kg/ha/yr reported P uptake)(350 yr-old forest) = 630 kg/ha total P uptake over 350 years

630 kg/ha total uptake - 29.28 kg/ha reported in biomass = 600.72 kg/ha recycled

(600.72 kg/ha) / 350 years = 1.17 kg/ha/yr recycled

1.8 kg/ha/yr reported uptake - 1.7 kg/ha/yr recycled = 0.1 kg/ha/yr annual accrual

Using Aurthur and Fahey (1991) data:

(1.7 kg/ha/yr reported P uptake)(500 yr-old forest) = 850 kg/ha total P uptake over 500 years

850 kg/ha total uptake - 80 kg/ha reported in biomass = 770 kg/ha recycled

(770 kg/ha) / 500 years = 1.5 kg/ha/yr recycled

1.7 kg/ha/yr reported uptake - 1.5 kg/ha/yr recycled = 0.2 kg/ha/yr annual accrual

Non-ectomycorrhizal Fungal Endophytes Improve Foliar Nutrient Status in *Abies lasiocarpa*: the Role of Companion Plants and Organic Matter

Chapter 3

Abstract

Roots of wild *Abies lasiocarpa* seedlings have recently been reported to contain abundant vesicular-arbuscular mycorrhizae and unidentified, non-ectomycorrhizal, dark-septate endophytes. The extent and ecological importance of the symbioses is unknown. We induced root colonization by these endophytes and measured effects on foliar nutrients. In the first of 2 experiments, spores of *Glomus intraradices* were introduced into soil-less planting media with fir growing alone and in combination with *Calamagrostis rubescens*, acting as an alternate mycorrhizal host. The same two treatments without the spores served as controls. All inoculated fir seedlings grown in combination with *Calamagrostis* were colonized. One inoculated fir seedling grown without grass was sparsely colonized. Phosphorus concentration in the colonized fir seedlings grown with the grass was 10 times higher than in the noninoculated fir grown with grass. Tissue nitrogen did not differ among treatments. In the second experiment fir germinants were transplanted into soil-less media with and without organic material and a dark-septate fungal isolate. Seedlings treated with inoculum were heavily colonized. Fungus and organic material appeared to interact to effect both N and P in seedling foliage. Foliar P in the treatment containing both fungus and organic material was

double that in the other two treatments. Inoculated seedlings had lower foliar N than the noninoculated seedlings, but of the two inoculated treatments, seedlings with organic matter had significantly higher N. The evidence suggests that at least some species of these two non-ectomycorrhizal symbionts may play a role in the ecology of subalpine fir seedlings. It supports other research findings on mycorrhizal mediation of plant interactions and the role of organic matter in mycorrhizal functioning.

Introduction

Subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) has received recent attention as an upper elevation species sensitive to climate change (Rochefort et al. 1994, Villalba et al. 1994, Peterson and Peterson 1994, Woodward et al. 1995, Hessel and Baker 1997), but information on nutrient cycling and productivity of *A. lasiocarpa* forests is still limited (Vogt et al. 1989, Aurthur and Fahey 1992). High elevation stands dominated by the species extend from the Yukon Territory to Arizona, occurring the Rocky Mountains and the Northwest Pacific Coast Range (Alexander et al. 1990). Findings reported here address its mycorrhizal ecology and seedling nutrient status.

Because nutrient mineralization and availability to plants is often temperature-limited in high elevation soils (Vogt et al. 1989, Davis et al. 1991, Aurthur 1992), the role of mycorrhizae may be particularly important, especially during periods of ecological stress (Read and Haselwandter 1981, Read 1991).

Observations of ectomycorrhizae in *A. lasiocarpa* forests are reported in Trappe (1962) and Harvey et al. (1979,1987).

Though Pinaceae in general is regarded as an ectomycorrhizal taxon (Gerdemann 1968, Read 1993), we note recent reports of vesicular-arbuscular mycorrhizae (VAM) in wild and laboratory-grown Pinaceae (Cázares 1992, Cázares and Trappe 1993, Smith et al. 1998). Smith et al. (1998) observed experimentally increased foliar P in *Pseudotsuga menziesii* (Mirb.) Franco, following inoculation with spores of VAM fungus. This is the second report of physiological effects of VAM colonization in any species of Pinaceae.

In addition to VAM and ectomycorrhizae in *A. lasiocarpa*, Cázares (1992) also observed root colonization by unidentified dark-walled, septate endophytes (DSE) that did not form usual mycorrhizal structures. In that study the endophyte, or class of endophytes (reviewed in and Trappe, 1998) was observed in 37 other subalpine and alpine species in 14 plant families in the North Cascades of Washington. In a survey of Austrian alpine plant communities, Read and Haselwandter (1981) characterized DSE forms as "major root-inhabiting fungi in alpine herbaceous vegetation." Although this class of endophytes appears common in cold regions (see also Currah and Van Dyk 1986, Bledsoe et al. 1990, Gardes and Dahlberg 1996), it is not restricted to those habitats (Jumpponen and Trappe 1998). Taxonomic and morphologic characterizations of DSE types appear in Wang and Wilcox (1985), Stoyke and Currah (1991), Stoyke et al. (1992), Currah and Stoyke (1993) and O'Dell et al. (1993). Studies of its physiological effects are few and inconclusive. Both pathogenic and beneficial effects have been reported

(Wilcox and Wang 1987, Haselwandter and Read 1982). Its ecological functions are unknown, but Caldwell et al. (1996) experimentally determined that 10 of 10 DSE isolates tested for the production of decomposition enzymes were able to process major detrital carbon nitrogen and phosphorus polymers, suggesting a potential mechanism for indirect DSE host plant access to detrital pools.

In the first of two experiments, we designed a greenhouse study to determine if VAM would form on subalpine fir seedlings and whether a companion VAM host was requisite. We tested the capacity of *Glomus intraradices* to colonize subalpine fir from a spore inoculum, the effect of an alternate VAM host on colonization, and foliar N and P in colonized and uncolonized seedlings. The alternate host selected was pinegrass, (*Calamagrostis rubescens* Buckl.), which occurs commonly in forests and forest openings from valleys to subalpine zones in Pacific and Rocky Mountain forests (Lackschewitz 1991). In the second experiment a DSE fungus isolated from *Phyllodoce glanduliflora* (Ericaceae), and tentatively identified as *Phialocephala fortinnii* Wang and Wilcox, was introduced into soil-less growing medium with young germinants of *A. lasiocarpa* in the presence and absence of an organic substrate. Both experiments were fertilized with abundant N but limited P to test for colonization and treatment interaction effects on P uptake. Foliar assays provide a temporally integrated index of nutrient uptake and availability, and are useful for identifying limiting nutrients (Binkley 1986, Binkley and Hart 1989, van den Driessche 1974).

Materials and Methods

Experiment 1: VAM fungal spores and pinegrass

Treatments

Treatments consisted of fir grown alone with no spore inoculum (treatment F), fir grown with pinegrass (treatment F + G), fir grown alone with spores (treatment F + S), and fir grown with both pinegrass and spores (treatment F + G + S). Each was replicated 49 times in a completely randomized fashion, but mortality reduced replications to 41, 41, 37, and 34, respectively.

Seed germination and growing medium

Subalpine fir seeds were surface sterilized in 30% hydrogen peroxide for 30 min, then cold/moist stratified in vermiculite for 10 days at 4 °C. Seeds were germinated at room temperature, and one 10-day-old seedling was transplanted into each 150 cc Supercell™ leach tube (Stuewe & Sons, Corvallis, OR). Seeds of pinegrass that had been treated briefly with a Captan™ fungicide solution (Zeneca, Wilmington, DE) were planted in the leach tubes at the same time the fir seedlings were transplanted. The growing medium consisted of four parts horticultural grade vermiculite and one part horticultural grade heat-expanded pumice (perlite).

Inoculation

The Nutrilink™ inoculum (Native Plants Inc., Salt Lake City, UT) in a dried-clay carrier contained approximately 500 spores of *Glomus intraradices* per gram. Uninoculated controls were treated with an equal amount of dried-clay carrier containing no spores.

The growing medium was moistened before planting and a narrow glass rod was set in the center of the mix to create an open column for inserting 0.4 ml of inoculum followed by the fir germinant. Pinegrass seeds were scattered on the surface and covered lightly with growing mix.

Growing conditions

The plants were grown in a laboratory at 20-22 °C under 16-h days of artificial Gro Lux™ light (Sylvania, Hillsboro NH). Seedlings were watered as needed to maintain media moisture and were fertilized four times with Long-Ashton nutrient solution (Hewitt 1966), containing full strength N but modified to reduce P concentration to one-quarter strength (11ppm). Each seedling received a total of 100 mls of nutrient solution over the course of the experiment.

Clearing and staining

Seedlings were harvested after 14 weeks. The clearing and staining procedures are modified from Cázares and Trappe (1993) and Phillips and Hayman

(1970). Entire root systems were washed in running tap water, placed in Tissue-Tek™ plastic capsules (Fisher Scientific Co., Pittsburgh, Pa.), and soaked in 10% KOH solution for 14 hours. After the KOH solution was decanted, samples were rinsed with distilled water and soaked again in 10% KOH for 7 hours. Roots were then rinsed again with distilled water, placed in a fresh 10% KOH solution, steamed for 40 minutes, rinsed, steamed for 30 minutes in 1% HCL, and rinsed again. Cleared samples were steamed for 30 minutes in a solution of 0.05% trypan blue in lactoglycerol, then rinsed and stored in lactoglycerol until microscopic examination. A small subsample of grass roots was also cleared and stained by the same methods for later comparison of inoculated and uninoculated treatments.

Foliar analysis

All needles were removed from ten randomly selected seedlings in each of the four treatment groups and dried overnight at 70 °C. Tissue was analyzed for the percentage of N and P with the micro-Kjeldahl digest method of Bremner and Mulvaney (1982). Included in the analysis as a basis for nutrient-level comparisons were needles from three wild subalpine fir seedlings.

Assessing colonization

Each 5 mm section of the root system of all fir seedlings was systematically examined from root collar to tip with a Zeiss binocular microscope for the presence of vesicles, arbuscules, or hyphae. Root systems were not heavily lignified, so that

evidence of fungal activity was easily discerned. If any portion of a 5 mm segment contained evidence of VAM, it was tallied as colonized. Each root system was counted as one sample. The percentage of colonization given here represents the percentage of segments in a root that were occupied by at least one form of the fungus. This estimate of colonization of course does not provide a complete estimate of the percentage of root length colonized because not all segments were entirely colonized, but it provides a measure of the distribution of colonization throughout the root system (Read et al. 1976).

Experiment 2: DSE and organic matter

Inoculum and growing medium

The DSE isolate we used (strain EC11, isolated by Cázares (1992) maintained at the UDSA-PNW Research Station, Corvallis, OR), has been tentatively identified as *P. fortinii* on the basis of strong morphological similarities to the type culture (Jumpponen, personal communication). To produce a large quantity of inoculated growing medium, four 1cm diameter agar plugs of DSE were propagated for 30 days in 100 mls sterile liquid Modified Melin Nordkrans (MMN) solution (Marx and Zak 1965). The resulting culture was macerated 10-15 seconds in sterile blender then added to autoclaved 2L flasks containing 400mls liquid MMN and 1L horticultural grade vermiculite. The mixture was moist but not saturated. Flasks were capped with sterile gauze and inverted sterile beakers, then stored at

room temperature. Fungal mycelium spread visibly through the substrate, and after 20 days contents were emptied into fine mesh strainers and rinsed thoroughly for several minutes with distilled water to remove remaining nutrient solution. Microscopic examination of the inoculated media revealed abundant hyphae wefting around and through particle layers.

For two of the three treatments the inoculated vermiculite was mixed with equal parts of the other media component. For treatment 1 the other component consisted of equal parts perlite, vermiculite and horticultural grade sphagnum peat moss (treatment +PM+DSE). For treatment 2 the second component consisted of equal parts perlite and vermiculite (treatment +DSE). Treatment 3 incorporated an equal quantity of uninoculated vermiculite into the second component mixture of perlite, vermiculite and peat (treatment +PM). Each treatment was replicated with 28 seedlings but mortality reduced replications to 27, 27 and 25, respectively.

Seeds of *A. lasiocarpa* were stratified and germinated as above and grown in trays of vermiculite. For each treatment, twenty-eight 1mo old seedlings were transplanted into leach tubes containing the growing mixture. Seedlings were grown under the same moisture and fertilization regime described for experiment number 1.

Seedlings were harvested after 14 weeks. Roots were observed microscopically for surface colonization then cleared and stained as in experiment 1. Colonization was not quantified. Foliar analysis was conducted as above on 10 randomly selected seedlings from each treatment.

Statistical analysis

Univariate ANOVAS were computed in SYSTAT™ 6.1 for percent concentration of foliar N and P, followed by Bonferroni pairwise comparisons to determine significance of differences between treatment. To stabilize variance, phosphorus values were square-root transformed (Zar 1984) using the equation

$$X' = \sqrt{X} + \sqrt{X} + 1$$

Results

Experiment 1: VAM spores and pinegrass

No fungal colonization was detected in fir roots from F or F + G treatments, nor was colonization detected in a subsample of pinegrass roots from the uninoculated treatment. One fir root sample from the F + S treatment contained two vesicles and a 1mm length of hypha.

All root samples from the F + G + S treatment were occupied by vesicles or hyphae, usually both. Colonization was abundant. Ninety-seven percent of the root samples were at least 25% colonized, half the samples were more than 50% colonized and 15% of samples were more than 75% colonized. Colonization was denser in main roots than lateral roots. Seventy-nine percent of main roots were more than 50% colonized, while only 21% of lateral roots had greater than 50% colonization. Vesicles and hyphae were infrequent in root tips. No arbuscules

were observed. Examination of a subsample of pinegrass roots from this treatment showed expected VAM colonization. Visual assessment at harvest time revealed no obvious differences in seedling size or vigor among treatments.

Nitrogen concentrations did not differ significantly among treatments (Table 3.1) Phosphorus concentrations were significantly higher in seedlings from the F + G + S treatment. Differences among the other three treatments were not significant at $p \leq .05$. N and P concentrations in the three wild specimens were consistently lower than in the experimental seedlings, but the N:P ratio was similar to that for the F + G + S treatment.

	Experiment 1				Experiment 2			
	F	F+G	F+S	F+S+G	+DSE	+PM	+DSE +PM	Field Samples
% Foliar N	**2.88a**	**2.49a**	**2.61a**	**2.48a**	**2.79a**	**4.06b**	**3.23a**	**0.11**
	0.14	*0.09*	*0.17*	*0.18*	*0.14*	*0.16*	*0.12*	*0.02*
% Foliar P	**0.08a**	**0.04a**	**0.14a**	**0.40b**	**0.19a**	**0.22a**	**0.48b**	**0.01**
	0.02	*0.02*	*0.05*	*0.06*	*0.05*	*0.04*	*0.03*	*.003*
N:P ratio	**90.48**	**167.35**	**55.08**	**7.51**	**18.55**	**33.70**	**7.07**	**8.80**
	30.61	*35.84*	*26.75*	*1.03*	*3.26*	*11.94*	*0.52*	*0.66*

Table 3.1. Mean percent concentration (untransformed) of foliar N and P. Standard errors are in parentheses. Within each experiment. Values followed by different letters are significantly different at $p < .05$ using Least Significant Difference pairwise comparisons.

Experiment 2: DSE and organic matter

After 14 weeks seedlings were harvested and observed for root colonization before and after clearing. No fungal colonization was observed in any roots from the unioculated treatment. All root systems from the two inoculated treatments were abundantly colonized with numerous loose wefts of superficial DSE runner hyphae following depressions between epidermal cells, identified in (Jumpponen and Trappe 1998) as early stage colonization. Hyphae were always most abundant in epidermal cells in older areas of the root. Colonization of root tips was usually sparse or absent. Structures characteristic of late stage colonization, such as hyphal penetration into root hairs and cortical cells, or intracellular sclerotia of multiple thick-walled cells, were not detected.

Mean foliar P in the +PM+DSE treatment was double that in the other two treatments (Table 1). Foliar N was significantly higher in the +PM treatment than in the two inoculated treatments, but in the 2 inoculated treatments, N was significantly higher in the one with peat moss.

Discussion

Seedlings in both experiments were small at the time of harvest (±3cm), and there was no visible difference in seedling size among treatments, but increased tissue P in the absence of growth increases are not unusual in VAM studies (Haselwandter and Read 1982, Stribley et al. 1980). In both experiments the application of full-strength N fertilizer led to foliar N concentrations considerably

higher than those in the three wild seedlings sampled for this study, those reported for *A. lasiocarpa* by Barrick and Schoettle (1996), and those reported for other conifers (Ingestad 1979, Binkley 1986, Munson and Bernier 1993, Barrick and Schoettle 1996). Nutrient concentrations higher than optimal are characterized as luxury consumption that supports growth during later periods of nutrient stress (Chapin 1980). Improved nutrient status derived from mycorrhizal symbioses could be more important for survival of establishing seedlings than increases in growth, especially during periods of ecological stress (Francis et al. 1986, Read 1991).

In the first experiment the general absence of colonization in the F + S treatment suggests that colonization of fir roots in the F + G + S treatment occurred via root-to-root contact or hyphae growing from the roots of colonized pinegrass. This conclusion is supported by evidence for interspecific hyphal colonization by VAM (Francis et al. 1986) and evidence that not spores but fungal hyphae associated with established plants are the major source of VAM inoculation in natural communities (Read et al.. 1976; Read 1993). Spore abundance in natural communities can be low despite abundant colonization of most plants (Read et al. 1976). The presence of hyphae and vesicles in one fir from the F + S treatment indicates that at least one spore germinated in the absence of grass.

Smith et al. (1998) reported abundant VAM colonization in 36wk-old *Pseudotsuga menziesii* grown with grass, but the effect on seedling P nutrition was notably different than in this experiment. In this experiment foliar P in the colonized seedlings ranged from 3 to 10 times higher than in the uninoculated treatments. In contrast, foliar P in colonized *P. menziesii* seedlings in the Smith et al. study was

significantly lower when compared to two treatments where the tree grew alone. However, mean P in the colonized seedlings (grown with grass) was significantly higher (3X) than in the uninoculated seedlings grown with the grass. To summarize, in both experiments colonization appeared to mediate competition with the grass for limited P, but in the case of 14wk-old *A. lasiocarpa*, colonization also markedly improved P nutrition over seedlings grown without competition.

Fir and grass competition for foliar N appeared not to be affected by colonization. In this experiment the N levels in the trees grown alone were slightly higher but differences were not significant. Smith et al. (1998) reported significantly higher foliar N when uncolonized tree seedlings grew alone. Colonization did not improve foliar N over any treatment in either experiment.

Optimum foliar ratios of N to P have been determined for many conifers (e.g. Ingestad 1979), but have not been experimentally determined for *A. lasiocarpa*. Our comparisons with ratios in the wild seedlings and those reported in other conifer studies (Ingestad 1979, Comerford and Fisher 1984, Barrick and Schoettle 1996) suggest that the ratio in fir under the F + G + S treatment was probably within the normal, and perhaps optimal, range for growth. For *Pinus elliottii* Comerford and Fisher (1984) established that foliar N:P greater than 14 or 15 indicated a sufficiency of N with respect to P, while a lower ratio indicated that N was limiting. Barrick and Schoettle suggested optimum N:P ratios of 8 to 10 for the range of species they studied, which included *A lasiocarpa*. The very high N:P ratios in the three uncolonized treatments indicates the extent of P limitation in those seedlings.

Because the experiments were not conducted in sterile microcosms, we have not proven a direct causal link between *Glomus* colonization and increased tissue P. However, it is widely accepted that VAM improve host P nutrition. Other VA mycorrhizosphere organisms also influence plant nutrient uptake (Barea et al. 1975, Hayman 1982), and their role, if any, should be determined.

The evidence that plants from different families share compatibility with a common mycorrhizal fungus demonstrates the potential for linkage between those plants in the wild. The findings support the hypothesis by Read et al. (1976) that VAM plants establish and survive by linking into existing hyphal networks. Observations by Cásares and Trappe (1993) that VAM colonization was lowest in *A. lasiocarpa* seedlings collected under a closed forest canopy led them to hypothesize that the higher colonization in seedlings collected from openings was related to greater abundance of VAM hosts and propagules, and that the VAM hyphal network is sparser under the forest canopy.

If VAM colonization in the wild improves P nutrition of *A. lasiocarpa* when competition is absent, and mediates competition for P between *A. lasiocarpa* and other VAM hosts, as supported by these results, the symbiosis should facilitate seedling establishment in subalpine and alpine meadows where ectomycorrhizal hosts are scarce or absent. Results of a soil-transfer experiment near treeline show that ectomycorrhizae-bearing forest soils improved survival and growth of transplanted *Pinus contorta* (var. *latifolia* Engelm.) germinants in both subalpine and alpine meadows, but not of *A. lasiocarpa* (Author, unpublished). Though overall *A. lasiocarpa* mortalilty was high in that study, the implication is that *A. lasiocarpa* may

be less dependent on ectomycorrhizae for seedling establishment. Smith et al. (1998) were unable to induce colonization of *P. contorta* with *Glomus* spores (though no companion plant was used), and unlike *A. lasiocarpa*, VAM has not been observed in wild *P. contorta* seedlings (Author, unpublished).

The results of the second experiment indicate either a direct or indirect interaction between the organic substrate and the fungus that increased plant seedling P uptake over the other two treatments. Jumpponen et al. (1998) induced colonization of *P. contorta* with *Phialocephala fortinnii*, significantly increasing foliar P in a series of treatments with organic matter and N fertilizer. Haselwandter and Read (1982) significantly increased foliar P in two *Carex* species following inoculation with a dark septate endophyte.

The most direct explanation for the results seen in this experiment is that fungal enzyme production released additional P from the organic substrate, rendering it available to the seedlings. The demonstrated capacity of DSE to produce detritus-degrading enzymes (Caldwell et al. 1996) supports this hypothesis, but it has not been shown that the enzymes act to increase plant or fungal nutrient acquisition in nature.

An alternative explanation is that some property of the peat moss, possibly moisture retention or loosely bound nutrient ions, facilitated hyphal proliferation from roots, thereby effectively increasing the ion-absorbing surface. This would be especially true with respect to P, which is much less mobile than N in soil solution. The *Sphagnum* spp. peat mosses are not nutrient rich and are typically added to propagation mixtures to improve moisture holding capacity. Three samples of the

peat moss used in this experiment were analyzed with the foliar samples and found to contain very small amounts of N and P (.013% and .007%, respectively).

The decrease in foliar N in the two inoculated treatments suggests that N otherwise available for plant uptake was sequestered in fungal biomass and perhaps an associated mycorrhizosphere community. Of the two inoculated treatments, those seedlings in the +DSE+PM treatment had higher N, again suggesting an interaction between fungus and peat moss that improved plant nutrition. Jumpponen et al. (1998) also demonstrated increased N uptake in inoculated *P. contorta* when abundant fertilizer N and organic matter were present.

The ratio of N to P in all treatments was at or above normal (as discussed above), indicating that if fungal N sequestration occurred, it did not lead to seedling nutrient deficiency. It is noteworthy that the very high foliar N:P ratios in observed in experiment 2 did not occur in experiment 1, and that the interaction between DSE and organic matter that improved P nutrition also resulted N and P proportions resembling those of wild specimens.

Because results of both experiments in this paper are supported by numerous observations of these endophytes in wild specimens, and laboratory study of other plants, they suggest a more complex ecology of symbioses for *A. lasiocarpa* and other ectomycorrhizal Pinaceae. Further study should test whether VAM- or DS -induced alleviation of P, and perhaps N deficiency improves *A. lasiocarpa* seedling survival in its harsh montane habitat.

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Soil Foodweb Shifts and Reduced Plant Nutrient Supply in a Degraded Subalpine Recreation Site

Chapter 4

Abstract

Soils from a degraded wilderness recreation site at Cobalt Lake in Glacier National Park were analyzed to determine whether nutrient loss or limitation might be a factor in poor site recovery, and whether limitation could be related to changes in the activity of soil organisms. Surface soils were sampled in a severely compacted, devegetated campsite, then compared to samples from adjacent undisturbed and moderately damaged areas. Whole soil samples were extracted for available P and combusted for total C and N. Ion exchange resin bags embedded at 30 sampling points for 2 time intervals were assayed for NO_3, NH_4 and P. A greenhouse bioassay for foliar N and P was conducted using a native grass grown in soils collected at the site. Organisms representing two foodweb trophic levels were assessed microscopically. Active bacterial and fungal biomass were quantified, together representing first-level decomposers. Nematodes and protozoa were counted, together representing second-level consumers. Spores of vesicular-arbuscular mycorrhizal fungi were enumerated in the same samples, and roots of the bioassay grasses were examined for mycorrhizal colonization.

Measures of total N, extractable P and exchange resin NO_3 and NH_4 did not support the hypothesis that N and P availability was limited in the degraded soil.

Resin NO_3, NH_4 and whole-soil extractable P were, in fact, significantly higher in those soils. However, P extracted from resin bags buried in the degraded site for the 5-week, late summer interval was significantly lower than from the undisturbed site. There were no differences in resin P over the 10-month interval. The resins may be the more realistic indicator of plant-available P in degraded soils, at least for comparative purposes. Results of the greenhouse bioassay support this suggestion because foliar P levels were significantly higher in plants grown in soil form the undisturbed area. Exchange resins were not an accurate indicator of N availability, however, because bioassay plants also took up more N from the undisturbed soils. No fungal colonization of roots was detected in the greenhouse plants, so increased N and P uptake cannot be attributed to mycorrhizae. There was no difference in vesicular-arbuscular spore counts among the three soil types. Disturbance-related shifts were detected, however, in decomposer and consumer trophic levels of the soil foodweb. Active bacterial and fungal biomass was lowest in the area of intermediate disturbance. Total fungal biomass was lowest in the degraded soil. Nematode individuals were 10 times higher in undisturbed soil than degraded soil. Protozoa were highest in the area of intermediate disturbance. Since mycorrhizae were not a factor in nutrient uptake in the greenhouse bioassay, yet plants in undisturbed soils had higher foliar nutrients, it appears the diminished role of nematodes functioning as mineralizers may have reduced nutrient supply to bioassay plant roots. Determining whether human impact leads to disruption in the structure and function of the second trophic level, via soil compaction, could prove pivotal in understanding and ameliorating poor site recovery.

Introduction

Remote montane preserves degraded by excessive or inappropriate recreation activities are extremely difficult to restore, especially if heavy use continues (see Cole and Hall 1992; Cole and Trull 1992; Rochefort and Gibbons 1992). In addition to severe climate, access is poor and choices for revegetation species are limited. Little is known about the ecology of soil fertility and degradation in these native ecosystems because they are so remote and, until recently, have been infrequently utilized for commercial timber harvest.

Subalpine forests are characterized by cold temperatures, heavy snowpacks, slow growth rates and limited soil development (Vogt et al.1989; Baron 1992). Because the forest floor microbial activity that contributes to soil fertility through N mineralization is temperature limited, the organic horizons become important sinks of total ecosystem nitrogen, increasing the importance of functionally distinct mycorrhizae in nutrient decomposition and uptake (Read and Haselwandter 1981; Vogt et al. 1989; Read 1991).

High elevation restoration research typically focuses on the decline or recovery of plant species and communities (e.g. Chambers et al. 1984; Densmore and Holmes 1987; Chambers et al. 1990; but see Allen et al. 1987). Where failure to establish plant cover in native ecosystems is a persistent problem, closer examination of plant-soil interactions are necessary.

Perry et al. (1989) characterized the relationship between vegetation and soil as a dynamic, self-maintaining system with many linkages and feedbacks

operating within environmental constraints, and compared it to the analogous boy who pulls himself up by his own bootstraps (see also Perry and Amaranthus 1990). Within a bootstrapping framework, internal linkages and interactions mediate system resilience and productivity. Seemingly redundant pools and pathways confer stability. Biological components of soil, including plant roots, mycorrhizae and rhizosphere organisms are major contributors to internal nutrient linkages and pathways. For example, plant litter production and the active release of carbon compounds from roots (Paul and Clark 1989) provides energy to microbial decomposers (bacteria and fungi) that in turn are a food resource for larger grazers and consumers (nematodes, protozoa and microarthropods). Because the concentration of N in decomposer tissue is high, their consumers excrete excess N, typically in forms available for plant uptake and growth (Anderson et al. 1981), allowing for continued photosynthesis and litter production. For the techniques of restoration ecology to evolve beyond revegetation, an understanding of how plant-soil linkages and feedbacks unravel during degradation is critical.

Because of the strong influence of soil biota on major plant nutrients (Paul and Clark 1989; Baere et al. 1995), functional characterizations or models of soil organisms have been developed and tested. (Coleman 1985; Ingham et al. 1985; Moore et al. 1988; Coleman et al. 1990; Walter et al. 1991). As a basis for a foodweb conceptual model, Ingham et al. (1985) summarized system responses to the consumption of decomposers by consumers: increased N and P mineralization, increased CO_2 evolution, increased plant N uptake and growth, and increased or decreased bacterial populations. In a grassland study of seasonal relationships

between decomposers and consumers, Ingham et al. (1986) provided evidence that consumer populations tracked their resource base through time, and further, that increased N mineralization occurred concomitant with higher consumer numbers. During a late-summer period when bacterial numbers increased fourfold, N was immobilized. Figure 4.1 shows simplified component groups of the first two soil foodweb trophic levels.

A broad objective of the study described here was to examine the importance of soil biological activity to the maintenance of a dynamic plant-soil nutrient cycle (presented as a hypothesis by Anderson et al. 1981), within the conceptual framework of an internally consistent bootstrapping system. Soil C, N, P and plant tissue N and P were analyzed in relation to the activity of two major foodweb functional groups (N-immobilizing decomposers and N-mineralizing consumers) along a gradient of site degradation.

A final objective was to determine whether the measurement of major component groups in one or two soil foodweb trophic positions offered, a functional characterization of site degradation useful to restorationists, based on the findings of Curry and Good (1992), Wardle et al. (1993), and Ingham and Thies (1995), who suggest that the condition of the detrital foodweb can be a useful indicator of disturbance regimes.

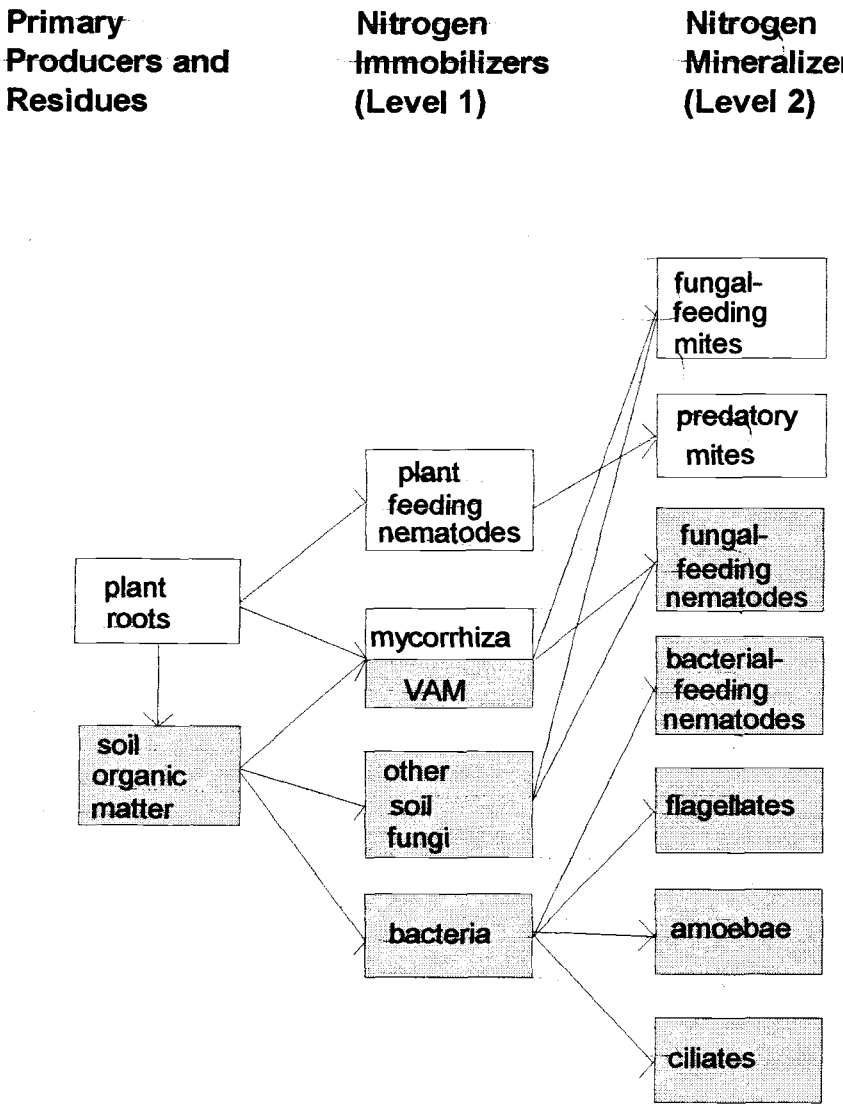

Figure 4.1. Simplified component groups of the first two soil foodweb trophic levels. Arrows indicate directions of energy and/or nutrient flows between components. Shaded blocks are the points of focus in this study. Modified from Ingham et al. 1986.

Methods and Materials

Study Site

Cobalt Lake backcountry campground in Glacier National Park is near alpine treeline at 2012m. Elevation. *Abies lasiocarpa* is the only tree species in the area, with patchy groves occurring around the campground. Stunted trees were present in and around the sampling area. Understory vegetation is dominated by *Luzula hitchcockii, Xerophyllum tenax, Vaccinium scoparium,* and various grasses and sedges.

An area of heavy camper use was selected for intensive study and soil samples were collected along a gradient of impact ranging from slight to severe, as indicated by visual observations of plant cover and soil compaction. At the least disturbed end, vegetative cover was intact and consisted mostly of *Abies lasiocarpa, Vaccinium scoparium* and *Xerophyllum tenax.* The adjacent intermediate area had sparse vegetation and a high proportion of bare, compacted soil. The degraded area was a tent campsite completely denuded of vegetation with compacted soil.

Sampling Design

Gridded plots (3.5m^2, 0.5m grid) were established in adjacent "undisturbed", intermediate and severely degraded areas. Surface soils were sampled to 15cm

at ten randomly selected intersections within each of the three areas, for a total of 30 samples. Samples were not composited. Nylon mesh bags containing 5g wet weight mixed-bed ion exchange resins (Rexyn 300 H-OH, Fisher Scientific) were placed 5cm deep near each sampling hole. The first set was in place for 5 weeks in late August and September, then removed and replaced by a second set which was in place for the subsequent 10 months

Nutrient Analyses

Total C and N were determined by high temperature dry combustion in a Carlo-Erba 1500 Series II Analyzer, following standard manual procedures. Extractable P was assayed using the dilute acid-flouride technique in Olson and Sommers (1982). Soil pH was measured following McClean (1982) in a 1:10 soil/water solution. Resin analysis for P and N was conducted using 1.5g dry weight resin from each bag extracted in 2N KCL (Hart and Firestone 1989).

Organisms

Active bacteria and fungi were enumerated in diluted soils stained with fluorescein diacetate using epiflorescent microscopy (Ingham 1992; Ingham and Klein 1984; Babiuk and Paul 1970). Most Probable Number enumeration of protozoa followed Ingham (1992) and Darbyshire et al. (1974). Nematodes were extracted on Baermann funnels (Anderson and Coleman 1977) and counted and identified using DIC microscopy. VAM spores were extracted following Ianson

and Allen (1986). Organisms, biomass and spores are reported on a per gram dry soil basis, except nematodes, which are reported on a per 10g dry soil basis.

Greenhouse Bioassay

For the greenhouse bioassay soils were collected from the site in late September, sieved to 1cm and mixed with a standard greenhouse mix of peat, perlite and vermiculite in a 2 to 1 soil/medium ratio. Six-week old *Deschampsia caespitosa* seedlings were germinated in vermiculite and transplanted into the media mixture in 150 cc leach tubes. Thirty five leach tubes were planted for each of the three soil types. The plants were watered daily and harvested after 12 weeks. Foliage was oven-dried overnight at 70C and roots were cleared and stained with trypan blue modified from Càsares and Trappe (1993) and Phillips and Hayman (1970). Foliar tissue was analyzed for percent N and P using the micro-Kjeldahl digest described in Bremner and Mulvaney (1982), then processed on a Technicon Autoanalyzer.

Statistical Analyses

Variables were analyzed with one-way analyses of variance followed by Tukey pairwise comparisons using SYSTAT™ Version 5.0 software. Resin data were log-10 transformed to stabilize variance. Due to a laboratory error that reduced nematode analysis to small and uneven samples sizes of 3,4 and 5 for undisturbed, intermediate and degraded soils respectively, non-parametric analysis

of ranks was employed on all of the organism variables, for consistency. Non-parametric analyses of variance are appropriate where sample sizes are small and equal population variance assumptions are not met (Zar 1984).

Results

Nitrogen

Total soil N at the site ranged from 0.38 to 0.46% and did not differ significantly ($p \geq .05$) among the three site conditions (Table 4.1). Mean resin NO_3 in the degraded site for the 5-week and 10-month intervals was 7.6 and 67 times higher, respectively, than resin NO_3 in the undisturbed soils (Table 4.2). Both differences were highly significant ($p \leq .001$). Mean resin NH_4 did not differ significantly among the three sites during the short interval. Over the 10-month interval, however, resin NH_4 was 14 times higher in the degraded site than in the undisturbed site ($p \leq .001$).

Carbon and C:N Ratio

Total soil C ranged from 6.3 to 9.6% (Table 4.1). Declining C with level of disturbance, coupled with constant N, resulted in a constant decline in the ratio of C to N from undisturbed to degraded. Differences between the undisturbed and degraded areas were highly significant ($p \leq .001$).

	Degraded	Deg *vs* Int p value	**Intermed.**	Int *vs* Und p value	**Undisturb.**	Und *vs* Deg p value
%C	**6.93** 1.45	0.849	**6.27** 1.85	0.028	**9.63** 1.97	0.089
%N	**0.457** 0.089	0.403	**0.380** 0.092	0.384	**0.459** 0.188	0.999
P ppm	**41.78** 9.85	0.002	**26.87** 10.7	≤ 0.001	**6.95** 1.53	≤ 0.001
C:N	**15** 0.82	0.036	**16.3** 1.25	≤ 0.001	**20.9** 1.2	≤ 0.001

Table 4.1. Nutrient means (standard errors listed beneath) and p values for Tukey pairwise comparisons.

	Degraded	Deg *vs* Int p value	**Intermed.**	Int *vs* Und p value	**Undist.**	Und *vs* Deg p value
PO4 5wks	**1.59** 0.41	0.264	**1.87** 0.71	0.525	**2.18** 0.39	0.032
PO4 10mos	**1.63** 0.31	0.754	**1.56** 0.26	0.893	**1.47** 0.24	0.459
N03 5wks	**23.7** 13.7	≤0.001	**80.39** 117.1	0.883	**3.03** 3.65	≤0.001
N03 10mos	**543.7** 317.8	≤0.001	**144.5** 148.3	0.011	**8.06** 7.51	≤0.001
NH4 5wks	**36.75** 32.77	0.663	**101.9** 135.5	0.293	**50.14** 49.32	0.786
NH4 5mos	**516.1** 479.9	0.413	**64.86** 48.24	0.001	**36.37** 6.44	≤0.001

Table 4.2. Ion exchange resin results (in μg/g resin). Untransformed means (standard deviations beneath). P values are for Tukey pairwise comparisons made on log-10 transformed data.

Phosphorus

Whole-soil extractable P was 6 times higher in the degraded soils ($p \leq .001$) than in the undisturbed soils during September (Table 4.1). Conversely, resin P was lower in the degraded soils than in the undisturbed ($p \leq .05$), and not significantly different over the October-July interval (Table 4.2).

Organisms

Bacteria, fungi and nematodes all showed significant disturbance-related declines (Table 4.3) while protozoa increased with disturbance. Active bacterial and fungal biomass did not differ statistically between the degraded and undisturbed soils, though active fungal biomass in the degraded soil averaged less than half that in the undisturbed soil ($p = 0.116$). Active biomass of both bacteria and fungi were significantly lower in the area of intermediate disturbance, however. The decline in the ratio of active fungi to bacteria from undisturbed to degraded soil was not significant. Total fungal biomass, including both live and dead hyphae, was 3 times higher in the undisturbed soil than in the degraded soil.

Protozoa (mostly flagellates and amoebae) were highest in the intermediate soils, where they were 7 times higher than in the undisturbed soils. Few ciliates were detected in the samples.

Nematodes, dominated by bacterial and fungal feeders, were nearly 10 times more abundant in the undisturbed soils than in the degraded soils, and more than twice as abundant in the intermediate soils.

VAM spores were present in all three soils, but counts were highly variable and there were no significant differences in quantity.

	Degraded	Deg *vs* Int p value	**Intermed.**	Int *vs* Und p value	**Undist.**	Und *vs* Deg p value
***ug* Active Bacteria per g soil**	**3.61** 1.6 n = 10	0.037	**2.02** 0.86 n = 10	0.015	**3.67** 1.22 n = 10	0.641
***ug* Active Fungi per g soil**	**12.71** 10.52 n = 10	0.492	**8.98** 7.57 n = 10	0.029	**28.42** 25.01 n = 10	0.116
Active Fun:Bac ratio	**3.78** 3.17 n = 10	0.508	**4.73** 3.53 n = 10	0.262	**7.4** 5.08 n = 10	0.083
***ug* Total Fungi per g soil**	**667.2** 517.8 n = 7	0.225	**933.9** 441.1 n = 7	0.063	**2155** 913 n = 3	0.010
Protozoa per g soil	**743.2** 465.1 n = 5	0.042	**2748** 2581 n = 5	0.001	**388.1** 229.2 n = 5	0.072
Nematodes per 10 g soil	**5.78** 2.9 n = 5	0.416	**13.62** 25.39 n = 4	0.013	**47.37** 23.41 n = 3	0.036
VAM Spores per g soil	**16.2** 13.04 n = 10	0.323	**22.4** 19.61 n = 10	0.776	**21.67** 18.99 n = 10	0.495

Table 4.3. Mean values for each organism class. Standard deviations and sample sizes are listed beneath. P values are for Fisher's LSD pairwise comparisons of ranks.

Greenhouse Bioassay

Foliar N and P levels in the greenhouse-grown grass were significantly higher (1.7x and 1.3x, respectively) in plants grown in soils from the undisturbed

site when compared to those in the degraded soils (Figure 4.2, Table 4.4). Values for foliage from the intermediate soils were similar to those in the degraded soils. No mycorrhizal colonization was detected on grass roots grown in any of the soils.

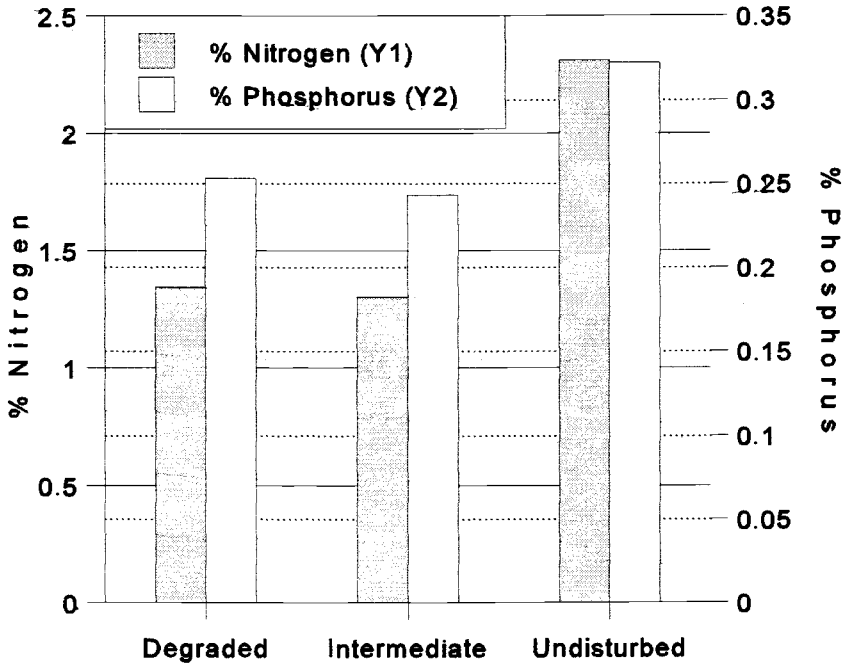

Figure 4.2. *Deschampsia* foliage N and P concentration.

	Degraded	Deg *vs* Int p value	**Intermed.**	Int *vs* Und p value	**Undisturb.**	Und *vs* Deg p value
%N	1.344 0.145	0.762	1.302 0.111	≤ 0.001	2.311 0.273	≤ 0.001
%P	0.253 0.025	0.603	0.243 0.026	≤ 0.001	0.322 0.042	≤ 0.001

Table 4.4. Mean values for *Deschampsia caespitosa* foliage N and P. Standard errors are listed beneath. P values are for Tukey pairwise comparisons.

Discussion

Foodweb

The analyses revealed disturbance-related shifts in both decomposer and consumer trophic levels of the soil foodweb. The activity of both bacteria and fungi was lowest in the area of intermediate disturbance. Belowground heterogeneity may have been higher there because relatively sparse vegetation was present and soil exposed between remaining plants was bare and compacted. it is unclear why the activity of the first trophic level would be even lower than in the degraded soil. Higher protozoan numbers in the intermediate site, attributable mostly to amoebae, suggest that all or a portion of that taxon may have been especially adapted to disturbance. High numbers of bactivorous protozoa could explain the decrease in their food base.

Taken together, the 10-fold decline in nematodes and the reduced supply of bioassay plant N and P in the degraded soils suggest discontinuity in the normal nutrient pathway from second trophic level output to plant uptake. That is, if bacteria and fungi are functioning as nutrients sinks and their consumers are absent, the nutrients tied up in decomposers will be unavailable to plants or the rate of supply will be diminished.

Soil disturbance associated with recreationists' trampling (exacerbated by vegetation loss) at Cobalt Lake may be such that soil pores have collapsed to a point where the mobility of meso- and macrofauna is restricted. Organisms

belonging to the second trophic level typically require larger soil pore spaces than bacteria and fungi. Small pores (<2.5μm) associated with soil microaggregates are inhabited largely by bacteria, while large macroaggregates are richer in nutrients, with pores (25-100μm) more intensively colonized by larger organisms (Paul and Clark 1989). Hassink et al. (1993) found close correlations between bacteria and pores <1.2μm and nematodes and pore sizes 30-90μm. They also found more N mineralized in soils where the activity of bactivorous nematodes was highest. Smeltzer et al. (1986) reported significant declines in bacteria, fungi, nematodes and microarthropods following artificially induced forest soil compaction. Microarthropods (largely springtails and Collembola) that consume bacteria and fungi are the third major component of the nutrient-N-releasing second level (Coleman et al. 1990). Recent research in a subalpine forest in British Columbia revealed a sharp decline in fungivorous microarthropods and nematodes following logging disturbance (Author, unpublished).

Physical problems associated with forest soil compaction are well known (Greacon and Sands 1980; Childs et al. 1989). Cole (1978) and Cole and Trull (1982) studied the experimental effects of controlled trampling on aboveground vegetation. Smelzer et al. (1986) studied the effects of compaction on four classes of soil organisms, and Hassink et al. (1993) studied the distribution of soil biota in soils of differing texture and pore size. We found no studies examining the effects of compaction on biologically mediated components of the belowground nutrient cycle.

Total Carbon and Nitrogen

Despite a significant decrease in total C in the degraded soil, 6.9% is still a relatively high level of soil C. However, the remaining C stores may be in recalcitrant forms unavailable to the microflora causing them to be carbon (rather than nitrogen) limited. The decline in C:N ratio supports this. The absence of carbon compounds normally exuded by plant roots (Paul and Clark 1989) is likely to limit the large microbial populations living within rhizospheres. The lack of significant difference in total N indicates that whatever changes occurred in nutrient cycle pathways as a result of degradation, the overall soil N content has not declined.

Nitrogen and Phosphorus

In general, the resin results indicated higher mineral N in the degraded site, but bioassay plant uptake was significantly lower in the degraded soil. Higher mineral N is frequently observed in disturbed soils (e.g. Khanna 1981; Vitousek 1981). It is unclear whether the resin assay of the undisturbed site accurately reflected the same or less N availability to roots during the assay periods, or failed to recover N as it cycled tightly from pool to pool. N supply and limitation are often closely linked and differences between measurement methods stem from differences in the sensitivity of each method to factors influencing soil N transformations (Binkley and Hart 1989). Despite generally high levels of solution N present in the degraded soils, the rate of N turnover and supply to roots appears

be higher in the undisturbed soils, as indicated by the bioassay results. Because temperature and moisture were standardized across all treatments in the greenhouse, the factors contributing to increased plant uptake were likely to be largely biological. The undisturbed soils in the greenhouse bioassay probably had higher levels of both immobilizers (fungi) and mineralizers (nematodes) when they were collected. Whether the organisms persisted throughout the growing period is unknown.

VAM colonization was absent from the bioassay plant roots, indicating that the relatively high uptake in soils from the undisturbed site was not attributable to mycorrhizae.

Phosphorus extracted from whole soil using dilute acid was also highest in the degraded site, again contradicting the foliar bioassay. Foliar P in the greenhouse bioassay was highest in the undisturbed soil. In the case of P however, resin measures did appear to reflect plant availability: it was significantly higher in the undisturbed soil during the 5-week interval. There was no difference among the sites over the 10-month interval. It is unclear why resin sorption of P would be a more accurate index of plant availability than resin sorption of N. The difference may be related to differences in ion mobility: nitrogen ions are much more mobile in soil solution than phosphorus.

Because plant bioassays are an index of nutrient availability integrated over time (Binkley and Hart 1989), thus incorporating supply rate, it appears that for this comparison between degraded and undegraded soils, the bioassay was the indicator of nutrient availability most relevant to restoration questions.

This study lends supports the value of foodweb analyses in understanding nutrient cycling. Identifying critical disruptions in key nutrient pathways is a step toward ameliorating those disruptions with ecologically appropriate restoration techniques. In future research the questions raised should be addressed with replicated studies using additional native plant species in paired field and greenhouse bioassays, combined with observations of the structure and function of the second trophic level. It will be necessary to determine whether the reduced nutrient availability or supply rate demonstrated in the greenhouse is a significant limitation on seedling establishment in field sites. Successful cultivation of native bioassay plants in degraded sites is quite labor intensive, and may require close attention to watering, shading, protection from damage etc, but in addition to testing the hypotheses presented here, the effort itself will undoubtedly produce further hypotheses about factors limiting site recovery.

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Summary and Conclusions

Chapter 5

The research presented in this thesis addresses soil ecology in forests dominated by *Abies lasiocarpa*. Experimental and observational emphasis was placed on major plant nutrients and the soil organisms that strongly control the storage and cycling of those nutrients. Issues addressed included changes in nutrient pools as subalpine fir forests expand into adjacent meadows, the mycorrhizal ecology of subalpine fir, and changes in soil nutrient pools and foodweb structure in damaged subalpine habitat.

In Chapter 2, the meadow-to-forest increase in C:N and sapling-to-forest decrease in P is evidence that these changes are associated with subalpine fir expansion at an interregional scale. The increasing C:N ratios probably reflect changes in litter nutrient concentrations along the meadow-to-forest continuum because carbon:nutrient ratios widen in plant tissues as they become more woody. The results are consistent with computer simulations of forest response to climate change, where simulated changes in vegetation composition led to altered soil N availability, via litter quality, which in turn amplified vegetation changes. The observed meadow-to-forest decline in extractable P leads to the hypothesis that P is moving from a plant-available abiotic pool into a biotic pool, largely conifer biomass, as forest expansion occurs. The estimates of P uptake by subalpine fir are consistent with those of other authors, and suggest that high subalpine forests

constitute a net P sink. Though the objective was to look for broad nutrient-shift patterns by replicating the measurements at an interregional scale, within-site patterns not consistent across regions (especially those of Ca, Mg and K), should provide bases for hypotheses concerning forest expansion at local scales, The application of principal components analysis revealed structure in the data consistent with our understanding of carbon and nitrogen relationships: C and N emerged together as a single factor. C and N cycles are closely linked in nature because their relative proportions determine mineralization/immobilization reactions during decomposition. The 3 cations also emerged combined as a single factor. Though their behavior was not interregionally consistent, Ca, Mg and K tended to behave in concert within each location. The overall results suggest that changes in C, N and P cycles caused by climate-induced forest expansion should be measurable on an immediate time scale and can perhaps be extrapolated to regional expanses of high-elevation forest.

Some authors have suggested that mycorrhizal ecology in cold, high elevation forests may be particularly important because the microbial activity that renders nutrients available to plants is temperature limited, thereby increasing plants' reliance on mycorrhizal partners. Subalpine fir, like all members of the Pinaceae, hosts numerous ectomycorrhizal fungi, but the results of Chapter 3, together with others' observations, indicate that vesicular-arbuscular and dark-septate, *Phialocephala*-like fungi may be major players in its nutritional ecology. The versatility conferred by an ability to form symbioses in these major mycorrhizal classes (which is not common among many plant families), may contribute to its

success as a dominant high-elevation species. The findings from the first of the two experiments suggests that not only does subalpine fir co-host VAM fungi with graminoid meadow species, but that it does so by linking into existing hyphal networks. The results of the second experiment indicate either a direct or indirect interaction between the fungus and soil organic matter that led to improved seedling nutrition. Because numerous DS isolates have been shown to produce detritus-degrading enzymes in the laboratory, it is reasonable to hypothesize that the improved seedling nutrient uptake in this experiment was due in part, at least to fungal enzyme access to recalcitrant organic material in the growing medium, and that the same phenomenon occurs in the wild. Again, this supports the findings and hypotheses of other researchers in this area. The extent to which the VAM/DSE/Ectomycorrhizal habit of subalpine fir is unique to the species or high elevation habitats is unclear. There are limited reports of VAM appearing in other Pinaceae, and data showing that the DSE types are unusually abundant in subalpine and alpine habitats, but both phenomena are worthy of much more study.

As described in Chapter 4, measures of total N, extractable P and exchange resin NO_3 and NH_4 did not support the hypothesis that N and P availability to plants was limited in the degraded soil. Resin N and whole-soil extractable P was, in fact, higher in the degraded soil, but resin P was lower. Despite this, bioassay plant uptake of both nutrients was higher in the undisturbed soil.

Because ion exchange resins are thought to be a reasonable indicator of plant availability in some cases, we expected parallel patterns between plant uptake and resin adsorption. The pattern was parallel in the case of P, but

opposite for N. That is, lower resin P in the degraded soil was mirrored by lower bioassay plant P uptake, but highest resin N in the degraded soil was counterpoint to lowest plant N uptake from degraded soil. So in situations that compare disturbed and undisturbed soil, resins, rather than whole-soil extracts, may be the more realistic indicator of plant-available P. Because N adsorption by resins was not mirrored by plant uptake, the benefit of resins for comparing N availability in disturbed and undisturbed soil may be limited. No fungal colonization of bioassay plant roots was detected, so the increased nutrient uptake cannot be attributed to mycorrhizae.

Soils were also analysed for foodweb structure. The effect of disturbance was to reduce total fungal biomass and nematode numbers. The loss of fungal biomass means the reduction of an N-immobilizing pool. Were fungal biomass to increase, the pool of mineral N indicated by the resins might shrink. Taken together, the 10-fold decline in nematodes and the reduced supply of bioassay plant N and P in the degraded soils suggest discontinuity in the normal nutrient pathway from second trophic level output to plant uptake. That is, if bacteria and fungi are functioning as nutrients sinks and their consumers are absent, the nutrients tied up in decomposers will be unavailable to plants or the rate of supply will be diminished. Soil disturbance associated with recreationists' trampling (exacerbated by vegetation loss) at Cobalt Lake may be such that soil pores have collapsed to a point where the mobility of meso- and macrofauna is restricted. This study lends supports the value of foodweb analyses in understanding nutrient cycling. Identifying critical disruptions in key nutrient pathways is a step toward

ameliorating those disruptions with ecologically appropriate restoration techniques.

Stresses unique to subalpine forest soils include short growing seasons, cold temperatures, long snowpack, and in the case of Glacier National Park, a pronounced period of soil moisture stress during late summer, because soil moisture is derived almost entirely from snowmelt. The relatively high soil C and N levels observed in these studies (even in the degraded soil) do not necessarily indicate that the soils are particulary fertile. Low temperature and moisture, coupled with the recalcitrant chemical composition of coniferous detritus, can lead to severe nutrient limitation. Nutrient turnover by the soil biological community is slow. Damage to the organic horizons exacerbates limitation by disrupting the normal structure and function of the soil foodweb. It is important to note that almost all of the research described here was conducted during the summer or under greenhouse conditions. In many cases soils under winter snowpack are insulated and do not freeze, so biological activity, including plant uptake, proceeds at unknown levels during winter months. Inferences from these studies must be restricted, therefore, because they do not take into account winter soil conditions.

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