NITROGEN AVAILABILITY AND ISOTOPIC SIGNATURE IN A *CEANOTHUS VELUTINUS*-*PSEUDOTSUGA MENZIESII* STAND IN THE WESTERN OREGON CASCADES

by

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By comparing several aspects of nitrogen cycling in a snowbrush (*Ceanothus velutinus*) and Douglas-fir (*Psuedotsuga menziesii*) forest stand, I addressed the issue of variations in nutrient availability and cycling. Soil extractable inorganic nitrogen, total soil nitrogen, litter percent nitrogen and enzyme activity values were measured between summer and fall seasons. Values of total soil N, litter percent N by weight, and soil extractable inorganic nitrogen were generally higher in *C. velutinus*, but the trends for extractable inorganic nitrogen depended on the N form (NO_3^- or NH_4^+), the season, and the soil depth analyzed. Enzyme activity was generally constant between seasons and species. I also determined the natural abundance isotope ratio of N in several of these ecosystem components and found a significant difference between ratios in the two species' litter N and the extractable inorganic N from soil, but not in total soil N. These different isotopic signatures may be used to trace nitrogen through the forest system in future studies.

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INTRODUCTION

As the trail ascends into the midst of a Pacific Northwest forest, a hiker is struck in awe by the enormous trees with dense mats of lichens draped over their branches and forest floor carpeted with moss and scattered decomposing logs. To observe and to understand the complexities of the interactions present in this scene would be a difficult task, even for the most astute observer. What we observe while hiking through the forest reveals only the above ground complexity, yet this is only part of the ecosystem with an equally important set of interactions is going on below ground as well. Included in these below ground interactions are nitrogen fixing bacteria which are just as vital for the functioning of the forest as the photosynthetic reactions in plants. My research attempts to further our understanding of the cycling of nitrogen from the atmosphere within the forest ecosystem.

A continual flux of energy and nutrients occurs between organisms in a forest and their surrounding physical environment, creating a complex three-dimensional ecological system, or ecosystem. Forest ecology studies attempt to characterize and to understand the dynamic interactions within and between ecosystems on both structural and functional levels. In order to enhance our knowledge of these intricate processes, my research has concentrated on nitrogen cycling in a secondary successional forest. Second growth stands following a severe disturbance such as logging do not look as complex as an old growth forest. Yet, the more sparse surroundings of second growth are misleading as proper function of these forests involves equally complex interactions. Therefore,

studying the storage and cycling of N in a second growth forest is crucial to our understanding of how ecosystems regenerate and function after disturbance.

Increasing our understanding of how N is stored and cycles within forests may improve our capacity to manage them as sustainable, diverse and productive systems (Perry, 1994). As the stress on forest ecosystems increase because of global population pressures, our ability to understand the intricacies of forest ecosystems may determine how effectively we can manage them.

BACKGROUND

Nitrogen cycle

Nitrogen (N) is a critical component of both proteins and nucleic acids, and organisms require N in relatively large amounts (Perry, 1994). Following carbon, oxygen and hydrogen, N has the next highest concentration in leaves, composing 2 to 2.5% by dry weight (Waring & Schlesinger, 1985). Unlike other biologically important nutrients such as phosphorus, sodium, potassium, calcium, magnesium, and manganese which originate from the weathering of rock material, only carbon (C) and N originate from the atmosphere. Unlike carbon dioxide gas (CO_2), however, which is readily available for uptake by biota via photosynthesis, inert N_2 gas must be transformed ("fixed") into an ionic form to be available for uptake by biota, despite composing over 78% of the atmosphere (Waring & Schlesinger, 1985). **Nitrogen fixation** is an energetically demanding process and is limited to specific organisms. As a result of the limitations of

the N fixing process, N deficiency is often observed in forest ecosystems (Johnson, 1992; Youngberg & Wollum, 1976). This makes it vitally important to understand and to study the processes that control the availability of nitrogen.

Nitrogen fixation converts gaseous nitrogen (N_2) to inorganic ammonium (NH_4^+), a form readily available to biota (Figure 1; Waring & Schlesinger, 1985). Several types of bacteria and cyanobacteria, the microbes that are capable of fixing N_2, possess the enzyme nitrogenase. Biological N_2 fixation therefore requires the presence of the nitrogenase enzyme in particular since other enzymes, such as those involved in other parts of the N cycle, are not capable of fixing the N_2 gas. Nitrogen may also be fixed to nitrate (NO_3^-) by volcanic action, lightning discharge, and industrial means. Despite these alternative forms of fixation, the majority of N fixation is biological (Waring & Schlesinger, 1985). Once fixed, NH_4^+ can also be oxidized to NO_2^- and NO_3^- by microorganisms in a process known as nitrification. Higher plants can utilize N only in the soluble ionic forms of NH_4^+ and NO_3^-.

Nitrogen fixation is an energy consuming process for plants with symbiotic bacteria. Plants capture and convert light energy from the sun into chemical energy in the form of **photosynthate** in a process known as photosynthesis. In a mutualistic relationship, the plant contributes the high-energy photosynthate to the bacteria, and the bacteria supplies fixed N to the plant. Since the amount of light a plant receives determines the amount of photosynthate the plant is able to give to the N fixers, the rate of N fixation is limited by light availability, assuming other factors such as nutrients and water are not limiting (Waring & Schlesinger, 1994). It is common, therefore, to find N fixing species in early successional forests following a recent disturbance (such as

clearcutting) where they can take advantage of the increased soil surface exposed to sunlight. Large amounts of N fixation often occur in these early successional stages when N fixing colonists are not overshadowed by the canopy of a mature forest (Waring & Schlesinger, 1994).

Fixation is not the only means by which N can enter a forest ecosystem. **Deposition** of NH_4^+ and NO_3^- also occurs, as either **wetfall** or **dryfall**. Yet, in a non-fertilized system (i.e. no addition of organic matter by means of a fertilizer such as compost), the amount of inorganic N that enters is comparatively low relative to that which enters by N-fixation (Waring & Schlesinger, 1985).

The bacteria in two genera, *Frankia* and *Rhizobium*, fix the majority of N in forest ecosystems (Kimmins, 1997). These bacteria form symbiotic associations with some higher plants and usually reside in nodules within the root of the associated plant (Solomon *et al.*, 1996). I will often refer to N fixing plants, yet this is not imply that the plant tissue is capable of N fixation, but instead refers to the mutualism between the plant and the N fixing bacteria residing in the plants' root nodules.

Nitrogen fixation also occurs in several species of lichens, organisms with a symbiotic relationship between a fungus and a photosynthetic component such as a cyanobacterium or a green alga. The cyanobacterial component of the lichen, as in the case of the lichen genus *Lobaria* that contains N fixing bacteria *Nostoc*, is responsible for N_2 fixation. Bacteria in other genera, such as *Bacillus*, are also capable of fixing N, yet do not form symbiotic relationships with higher plants. Instead these bacteria live freely in the soil, asymbiotically. The amount of N fixed asymbiotically is extremely low

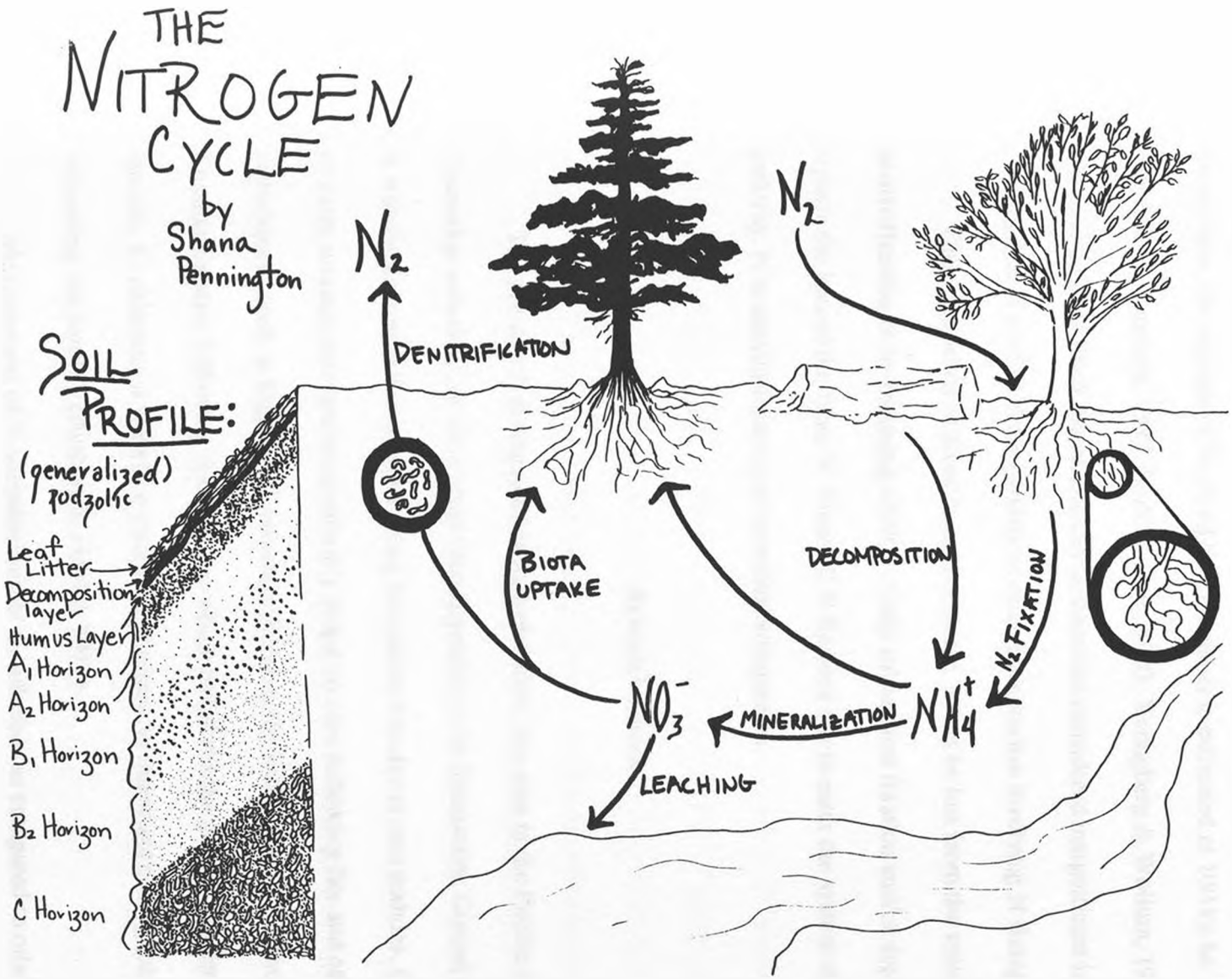


FIGURE 1. Schematic drawing of the Nitrogen Cycle in a forest ecosystem with a generalized soil profile.

compared to that fixed by symbiotic bacteria (Waring & Schlesinger, 1985), with values ranging from 0 to 3 **kg ha^{-1} yr^{-1}**in most temperate forest soils (Kimmins, 1997). In comparison, the amount of N_2 fixed by *Ceanothus* is estimated at 100 kg ha^{-1} yr^{-1} (McNabb & Cromack, 1983; Binkley *et al.*, 1982; Youngberg & Wollum, 1976). The amount of N fixed asymbiotically is therefore considered insignificant in its contribution of overall N availability in ecosystem studies involving N fixing plants.

Once deposited or fixed in an ecosystem, N can be lost from the system via either **denitrification** or by **leaching** of NO_3^-. Only subsequent fixation and/or deposition can replace the loss of the fixed N. Since all N fixation has to enter the system via a restricted pathway, N is usually limiting to terrestrial ecosystems.

Research Review

The primary N fixing plant in mid-elevation, dry sites in the Pacific Northwest is *Ceanothus velutinus*, or snowbrush (see appendix A for illustration; Conrad *et al.*, 1985). A woody plant with the nitrogen fixing bacterium *Frankia* in root nodules, *C. velutinus* is an early successional species commonly found on sites following fire and other severe disturbances such as logging. *C. velutinus* enriches the soil with the N_2 fixation activity of *Frankia* bacteria following disturbances which facilitates the growth of later successional species. *C. velutinus* is therefore considered to have an important ecological role in enhancing site fertility (Binkley & Husted, 1983).

Measurement of N **accretion** under *C. velutinus* as compared to other non-fixers has been the subject of research for the past 30 years. Zavitkovski and Newton (1968)

concluded that there were no significance differences in N accumulation in soils beneath *C. velutinus* compared to other shrubs. Later studies conducted by Youngberg and Wollum (1976) and Binkley *et al.* (1982) contradicted these earlier findings, and demonstrated higher N accretion in soils under *C. velutinus* compared to other shrubs. These studies estimated N accretion in biomass, litter, and soil to be 71.5 to 108 kg ha^{-1} yr^{-1}. These values closely resemble later estimates of N accretion in *C. velutinus* stands. McNabb and Cromack (1983) estimated an N fixation rate of 101 kg ha^{-1} yr^{-1}. Binkley *et al.* (1982) estimated a similar rate of 100 kg ha^{-1} yr^{-1}.

In ecosystems where various N fixers are present in early successional stages, the range of N accretion is 10 to 160 kg ha^{-1} yr^{-1} (Boring *et al.*, 1988). *C. velutinus*, therefore, has a relatively high rate of N fixation compared to that of other N fixing species. This can also be demonstrated with comparison to the N fixation rate of red alder (*Alnus rubra*), another common N fixing tree in the Pacific Northwest, which is estimated to fix N at of a rate of 42 to 65 kg ha^{-1} yr^{-1} (Binkley, 1982). Accelerated nitrification, increased NO_3^- leaching, and soil acidification have been observed in the soils under red alder stands (Brozek, 1990; Binkley, 1982). Because *C. velutinus* has an even higher N fixation rate than red alder, we might expect a similar trend in *C. velutinus* soils. But a study investigating *C. velutinus* soils did not find this, but rather found a greater concentration of N compared to the plots containing no N fixers (Johnson, 1995). Because accelerated leaching was not observed by the Johnson study (1995), the fate of the large amount of accreted N within the ecosystem deserves further study.

Enzyme Activity

Enzymes secreted by microorganisms **catalyze** biochemical reactions involved in decomposition (Tabatabai, 1994). Plant tissues are decomposed by microorganisms into a form that is available to other plants. The rate of decomposition indicates how readily nutrients such as N, C, and phosphorus (P) become available to the ecosystem. In the process of decomposition, different enzymes must be present in order to break down the different compounds within the **substrate**. Therefore, measuring enzyme activities can indicate C, N or P nutrient cycling within the sample area. The faster nutrients become available, the more readily they can be taken up by other organisms. Thus, high levels of enzyme activity can be a cause of rapid nutrient turnover. Examining the enzymatic activity in the soils of a *C. velutinus-P. menziesii* (Douglas-fir, a non-fixing plant) stand might indicate the rate of litter decomposition, and ultimately nutrient cycling.

Due to the difficulty of studying the enzymes responsible for N fixation, nitrification, and denitrification, these enzymes are not typically analyzed directly. Instead, the level of activity of enzymes responsible for other processes in the soil is examined to indicate an overall level of activity. In order for nutrient cycling rates to be accelerated, several conditions must be fulfilled, including an adequate supply of water and nutrients, as well as moderate temperatures. Since N is often a limiting nutrient in forest ecosystems nutrient cycling rates may increase with additional availability of N. Therefore, it is common to examine other enzymes, such as phosphatase and β-

glucosidase, to indicate overall increase enzyme activity which may be due to several factors, including increased N availability.

Natural Abundance Isotopic Ratio

All elements of biological interest contain one or more stable **isotopes**: atoms with the same number of protons but a different number of neutrons (Rundel *et al.*, 1989). Fluctuations in the number of neutrons either increase or decrease an atom's total mass. The two stable nitrogen isotopes, ^{15}N and ^{14}N, can be distinguished as "heavy" and "light" referring to the difference in number of neutrons (indicated by the superscript).

Stable isotopes can be useful to ecologists because organisms may contain enzymes which preferentially utilize one isotope over another in their metabolism and other biological processes such carbon uptake (Rundel *et al.*, 1989). This selective discrimination towards a specific isotope leads to isotopic fractionation. Different processes may lead to differences in isotopic composition as a result of isotopic fractionation. For example, the ratio of ^{15}N to ^{14}N present in a plant may result from the preference for either the light or heavy isotope in the biochemical process by nitrogenase, the enzyme present in symbiotic bacteria which are capable of fixing N. Moreover, other proteins associated with nitrogenase could alter the isotopic ratio and the proteins may vary depending upon the species of N fixing bacteria. Since the enzyme activity may have resulted in discrimination of one of the isotopes, the isotope ratio may be quantitatively measured.

An organism with a different series of biochemical reactions, such as a non-N fixing species, may contain a different isotopic ratio compared to that of the N fixing plant. Therefore, an organism may contain a distinct isotopic ratio, often referred to as a "signal," compared to that of other species that can be useful in ecological studies. Determining an organism's isotopic composition, known as its natural abundance signature, can be used to trace the mechanisms and patterns beyond the individual to an ecosystem level (Lajtha & Michener, 1994).

It is possible to analyze whether a plant with N-fixing cyanobacteria has a distinct isotope compared to that of other species which may utilize "older" (soil) N sources that have undergone repeated internal cycles since having been fixed. If a difference in isotope ratios exists, it is possible to determine how long the signal lasts in the N of the N fixer by analyzing different compounds: litter percent N, total soil N, and extractable NH_4^+ and NO_3^-. This series of N compounds represents N in the leaf litter, N in the soil as it is decomposed into organic matter, and N in the extractable soil inorganic forms after the organic matter has been decomposed.

Research Questions

My research attempted to develop a tool that could enhance our ability to measure and quantify the intra- and inter-system cycling of N in a *Ceanothus velutinus-Pseudotsuga menzeisii* (Snowbrush-Douglas-fir) successional forest. Three major questions were addressed: 1. Does the high N fixation rate of *C. velutinus* result in greater N availability in soils beneath *C. velutinus*? 2. Does the presence of *C. velutinus* affect

microbial community activity? 3. Does the N fixed by *C. velutinus* have a distinct signal, a natural abundance isotopic signature? If a signal is present, it may also be possible for future studies to trace this signal through the ecosystem to determine the fate of the fixed N. These questions seek to further our understanding of N cycling within a *C. velutinus* - *P. menziesii* stand, as well as explore a research technique that may be applied to nutrient cycling within and between a plethora of ecosystems.

MATERIALS AND METHODS

Site Description

The study site is located in the H. J. Andrews Experimental forest in of the Blue River Ranger district (T15S R5E, Section 14) of the Willamette National Forest of the central western Cascade Mountains of Oregon. The climate is mild with an average precipitation of 250 cm yr^{-1}, with ten percent of that falling as snow. Over 70% of the annual precipitation occurs between November and March. The average elevation is approximately 1,000 m on southern slopes that range from 20 to 40 percent. The soil is a deep, well-drained sandy loam, classified as a Drystrandept soil (Binkley, 1982). A type of Inceptisol, Drystandept soils typically have a low base saturation.

All samples were collected from the area chosen for study, watersheds 6 and 7. Both watersheds were initially logged and burned under low intensity broadcast burning in 1974. Both watersheds were then planted with *P. menziesii* seedlings (see appendix A for illustration of *P. menziesii* cone). Watershed 6, encompassing 32 acres, was planted

with *P. menziesii* seedlings in 1976. Watershed 7, covering 37 acres, was planted in 1975. Each watershed was planted with approximately the same number of saplings (Anonymous, 1976).

C. velutinus produces many hard-coated, resistant seeds which remain viable within the soil for many years. In order for germination to occur, a heat treatment (such as fire) followed by a cold period is required (Youngberg et al., 1976). Thus, the *C. velutinus* plants in the study site grew from seeds which were dormant in the soil until the broadcast burning (Binkley, 1982). There were no lichens with N fixing cyanobacteria (such as certain species of *Lobaria*) observed at the study site, and *C. velutinus* is believed to be the only shrub with N fixing cyanobacteria within the site.

Soil Sampling

Soil and **litter** samples were collected on July 15, 1998, August 11, 1998, and November 7, 1998. All collection was directly beneath *C. velutinus* and *P. menziesii* plants in relative close proximity and the same individual plants were resampled. Ten samples of the upper soil horizon A (0-5 cm) from each species were collected for the two summer samples (for soil profile description, see figure 1). In addition, litter was collected from the duff layer on July 15, 1998. The fall sample consisted of collection from the upper A horizon (0-5 cm), lower A horizon (5-10 cm), and upper B soil horizon (10-15 cm, figure 1). Litter was collected at each plant in a 0.10 m^2 area, and the depth of the duff layer was recorded. All samples from each plant were collected in breathable

bags, and were refrigerated as quickly as possible after collection (within 12 hrs). All soils were sieved using a 2 mm mesh screen.

Summer samples remained refrigerated and were analyzed for extractable inorganic N (NH_4^+ and NO_3^-) and enzyme activity within a week of collection. Fall samples were refrigerated as well and analyzed within two weeks of collection for inorganic N. Samples were then air dried and analyzed for total N.

See appendix B for explanation of the importance of soil refrigeration.

Analytical techniques

Several techniques were performed in order to determine soil extractable inorganic N, total soil N, litter percent N (by weight) and N isotopic ratios. The amount of extractable inorganic N was determined for the July and November samples. Total soil N was determined for the August and November samples and the values were compiled for analysis. Litter percent N (by weight) was determined for the fall samples. Enzyme activity was determined in the August and November sample from 0-5 cm depth. The N isotopic ratio was determined in the July samples of litter and soil. The analysis of the samples is summarized below:

TABLE 1. Summary of Sample Collection and Analysis.

date	extractable inorganic N	total soil N	litter percent N	enzyme activity	isotopic ratio
July 15, 1998	✓	✓			✓
August 11, 199				✓	
November 7, 1998	✓	✓	✓	✓	

Extractable Soil Inorganic N Analysis

Sieved soil samples analyzed for extractable inorganic N were placed in 1N KCl, and shaken every 4 hours for a 24-hour period. The samples were then gravity filtered to remove any dissolved material from soil particles. Filtrates were then analyzed for extractable NH_4^+ and NO_3^- colorimetrically using a Scientific Instruments Autoanalyzer (Scientific Instruments, 1987).

See appendix C for further explanation of soil extractable inorganic N analysis.

Total Soil N and Total Percent N (by Weight) Analysis

Litter samples were dried at 65°C and then ground in a Wiley Mill to pass a 20-mesh screen. Litter and soil samples were digested on a block digestor using sulfuric acid-hydrogen peroxide flux and analyzed for total N (μg·N/g·litter) using a Scientific Instruments Autoanalyzer methods (Scientific Instruments, 1987).

See appendix D for details on total soil N and litter percent N analysis.

Enzyme Activity Analysis

Potential enzymatic activity in the soil was measured following a procedure adapted from Tabatabai (1994). Two assays were run to test the activity of β-glucosidase and phosphatase. For both assays, 5.0 g of soil was added to 50 mL deionized water and stirred. Aliquots (1mL) of the slurry were taken for analysis in replicate plus one control per sample.

For the β-glucosidase analysis, 1 mL of the β-glucosidase substrate (a buffered *p*-nitrophenyl-*β*-D-glucoside (PNG) and toluene solution) was added to all samples (except controls) and placed in a heat bath for 3 hrs. One ml aliquots of the β-glucosidase substrate were then added to all controls. One-half mL $CaCl_2$ and 2 mL THAM 12 (0.1M, pH 12 solution of Tris (Hydroxymethyl) Amino Methane) were added to stop the reaction of all samples and controls.

In the phosphatase analysis, a phosphate substrate was added (a buffered sodium *p*-nitrophenyl phosphate and toluene solution) to the 1 mL sample aliquot (except controls) and placed in a heat bath for 1 hour. The samples and controls were then removed from the heat bath. The phosphate substrate was then added in 1 mL aliquots to the controls. Immediately following, 0.5 mL $CaCl_2$ and 2.0 mL 0.5M NaOH were added to all of the samples and the controls to stop the reaction.

Once the reaction was stopped, all samples and controls were centrifuged for 10 min. at 2500 sp. They were then analyzed colorimetrically using a spectrometer for *para-*

nitrophenol (*p*NP) concentration. Conversion of absorbance reading to μmol *p*NP for samples was determined from a standard curve of a 1:10 dilution.

See appendix E for further explanation of the enzyme procedure.

Natural Abundance Isotopic Ratio Analysis

Summer litter samples used for isotope analysis were dried at 65°C and ground in a Wiley Mill to pass a 20-mesh screen. Soils for isotope analysis were prepared by extracting the soils for a period of 24 hrs. in 2N KCl solution. The samples were then sent to the Stable Isotopes Analysis Laboratory at Boston University where the $^{15}N/^{14}N$ values were determined, expressed as $\delta^{15}N$ (parts per thousand of excess ^{15}N relative to atmospheric nitrogen standards).

See appendix F for further explanation of the isotope procedure and the significance of units measuring isotopic ratio ($\delta^{15}N$).

Statistical Analysis

I tested for differences in soils under the two species for extractable inorganic N (NO_3^- and NH_4^+ concentrations) and enzyme activity within the same season samples as well as between seasonal samples (i.e. comparison between summer and fall). Isotopes were compared within and between tree species for the first summer sample for extractable soil NH_4^+, extractable NO_3^-, total soil N and litter percent N. Soil total N was

analyzed between species by compiling the soil total N results for both seasons. Data were analyzed using the General Linear Models procedure of SAS (SAS Institute Inc., 1982).

See appendix G for description of statistical methods.

RESULTS

Soil Extractable Inorganic Nitrogen

Extractable NO_3^- in *C. velutinus* soils was significantly greater than in *P. menziesii* soils in the summer (Table 2; figure 2). In the fall, *C. velutinus* soils were not significantly different in extractable soil NO_3^- at depth 0-5 cm (Table 2; figure 2), but extractable soil NO_3^- was significantly greater under *C. velutinus* at depth 10-15 cm (Table 2; figure 3).

For extractable NH_4^+, *C. velutinus* soils in the summer only have marginally higher values of NH_4^+ that *P. menziesii* due to the high variability in the *C. velutinus* sample (Table 2; figure 4). Fall sample results suggest significantly higher values of extractable NH_4^+ in the soils beneath *C. velutinus* than that of *P. menziesii* at depth 0-5 cm (Table 2; figure 3) as well as depth 10-15 cm (Table 2; figure 3).

There is an overall trend in both species of increased NO_3^- from summer and fall, accompanied by a subsequent decrease in extractable NH_4^+ between the seasons (Figure 5).

Total Soil N

Total soil N does not vary significantly between seasons under either species (Table 2; figure 6). There is significantly more total N in the soil at depth 0-5 cm under *C. velutinus* than under *P. menziesii* (Table 2; figure 6).

Litter N

C. velutinus litter has significantly higher percentage of N by weight than litter found under *P. menziesii* (Table 2; figure 7). The amount of N was also determined as concentration of N per m^2 area (Table 2). The average mass of the litter beneath *C. velutinus* was 103.12 mg litter/m^2, and for *P. menziesii* was 289.8 mg litter/m^2. When expressed as mg N/m^2, there was no significant difference between *C. velutinus* and *P. menziesii* (Table 2).

TABLE 2. Extractable NO_3^- and NH_4^+, Total Soil N, and Litter N Trends between Species based on Season and Area.

N compound*	*C. velutinus*	*P. menziesii*	df	p-value
NO_3^- (μg N/ g soil)				
summer	1.59 ± 0.13	1.22 ± 0.01	19	0.04
fall (0-5 cm)	5.71 ± 0.54	5.24 ± 0.57	19	0.55
fall (10-15 cm)	5.60 ± 0.39	3.92 ± 0.56	19	0.02
NH_4^+ (μg N/ g soil)				
summer	22.7 ± 3.44	15.53 ± 1.8	19	0.08
fall (0-5 cm)	8.04 ± 1.13	4.18 ± 0.88	19	0.01
fall (10-15 cm)	2.99 ± 0.38	1.74 ± 0.30	19	0.02
total soil N (mg N/ g soil)	2.60 ± 0.47	1.99 ± 0.038	38	0.03
litter N (mg N/ g litter)				
fall	17.94 ± 1.16	5.34 ± 0.37	19	0.0001
litter mass per m^2 (g litter m^{-2})				
fall	103.12	289.81	19	----
soil total N per m^2 (g N m^{-2})	1847.50	1635.08	38	>0.05

*all samples from 0-5cm.

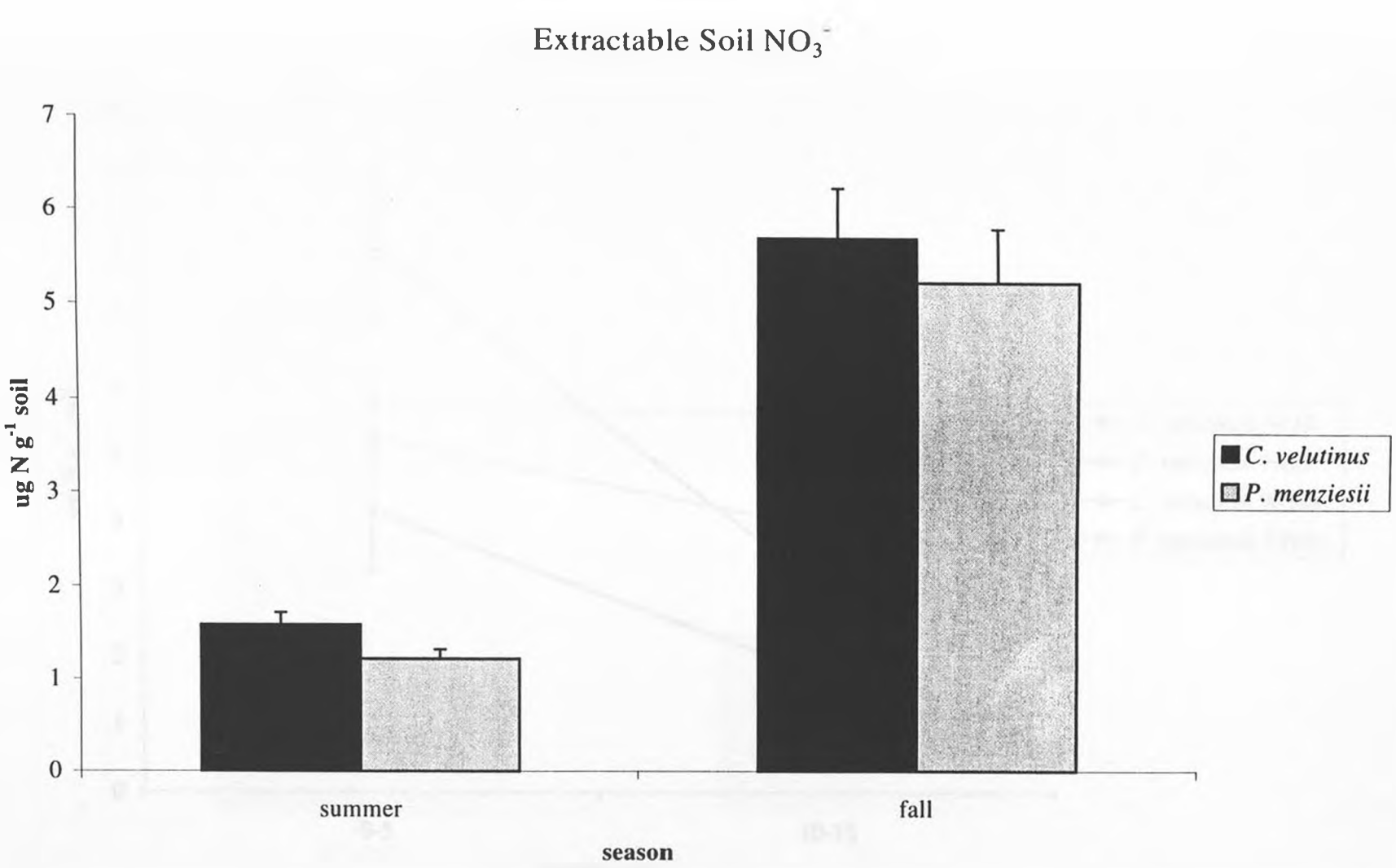


FIGURE 2. Comparison of extractable NO_3^- (μg N g^{-1} soil) from soil between species and between seasons. Bars indicate ± one standard error.

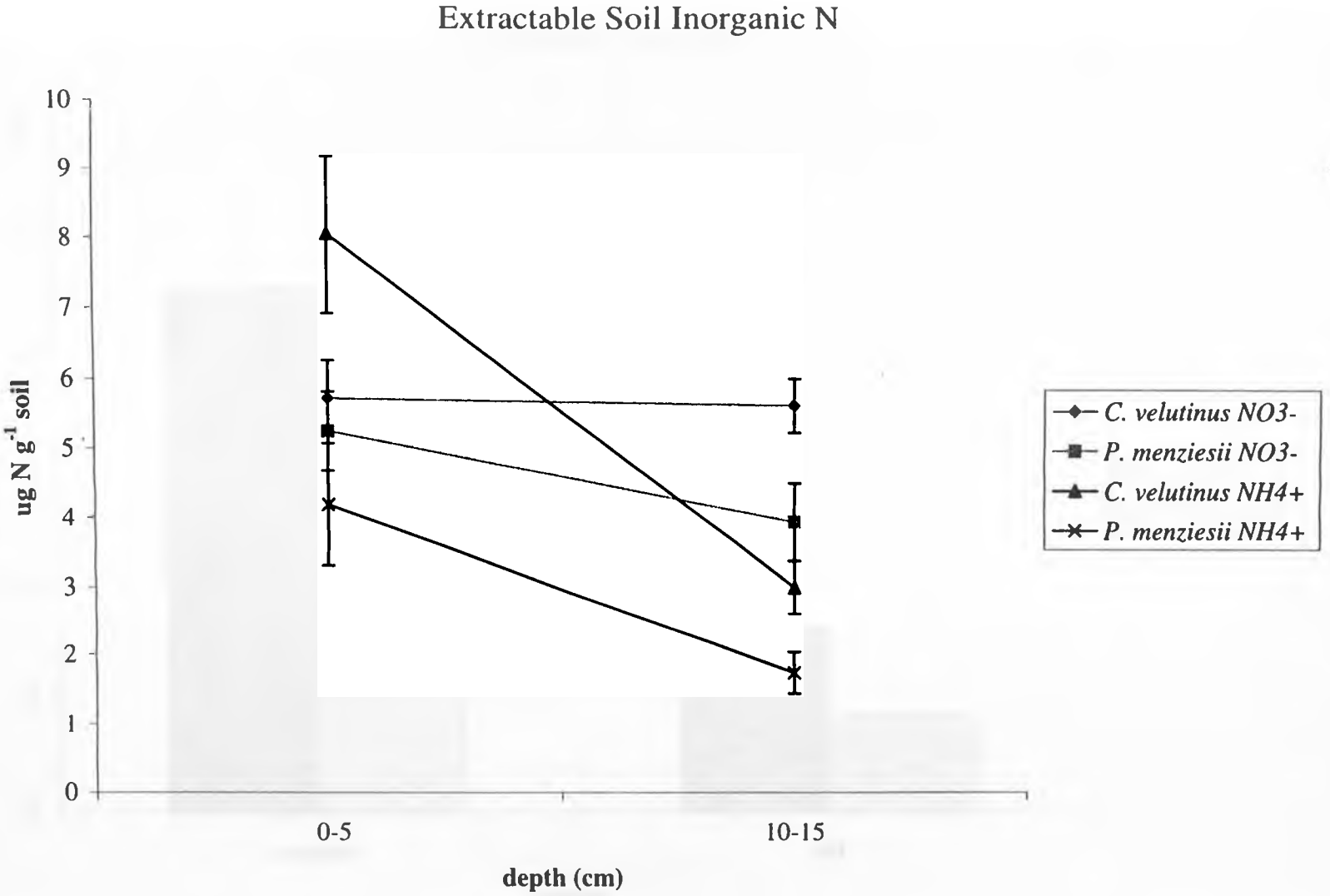


FIGURE 3. Amount of extractable inorganic N (μg N g^{-1} soil) between two soil depths (cm) beneath *C. velutinus* and *P. menziesii*. Bars indicate ± one standard error.

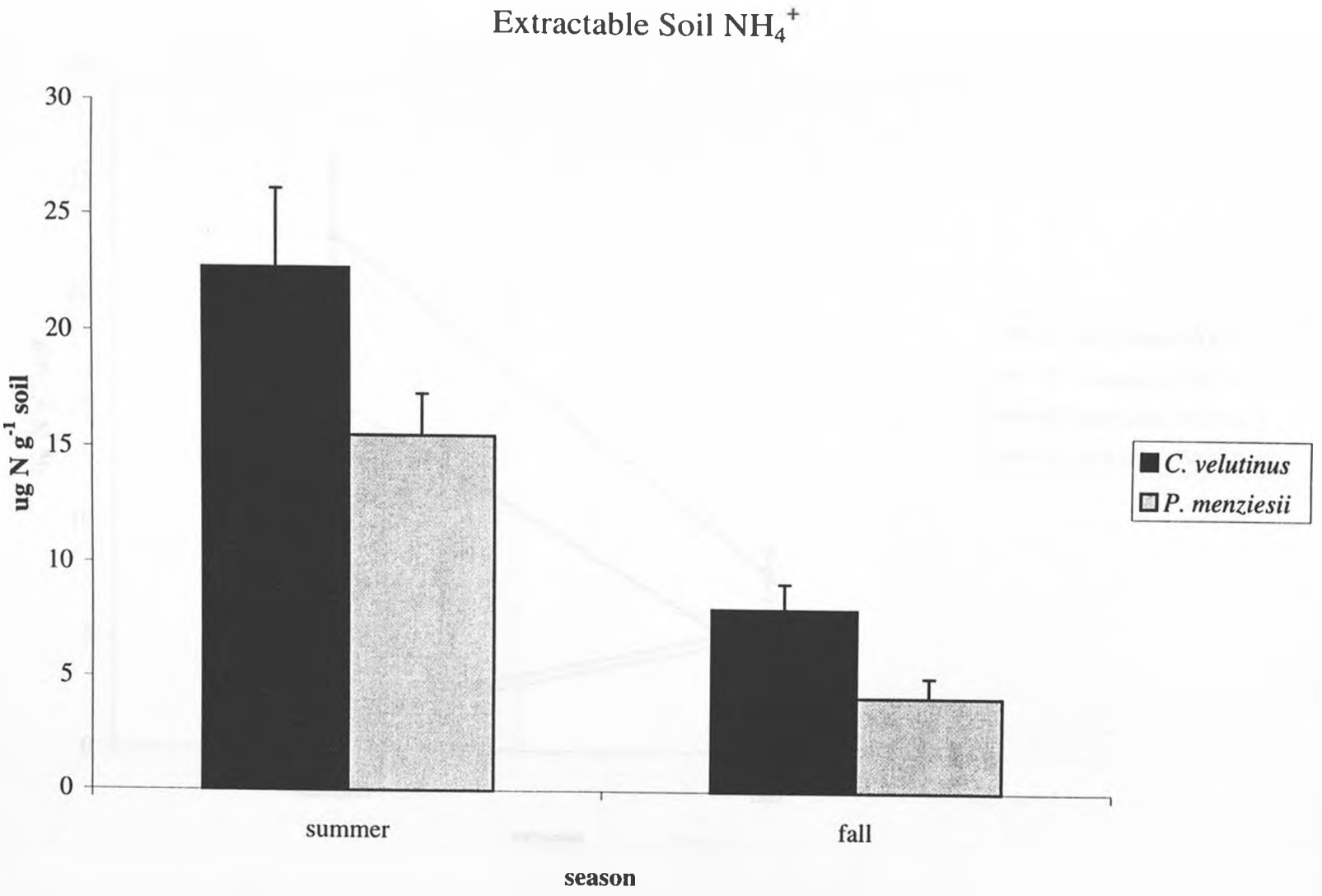


FIGURE 4. Comparison of extractable NH_4^+ values (μg N g^{-1} soil) from soil between species and seasons. Bars indicate ± one standard error.

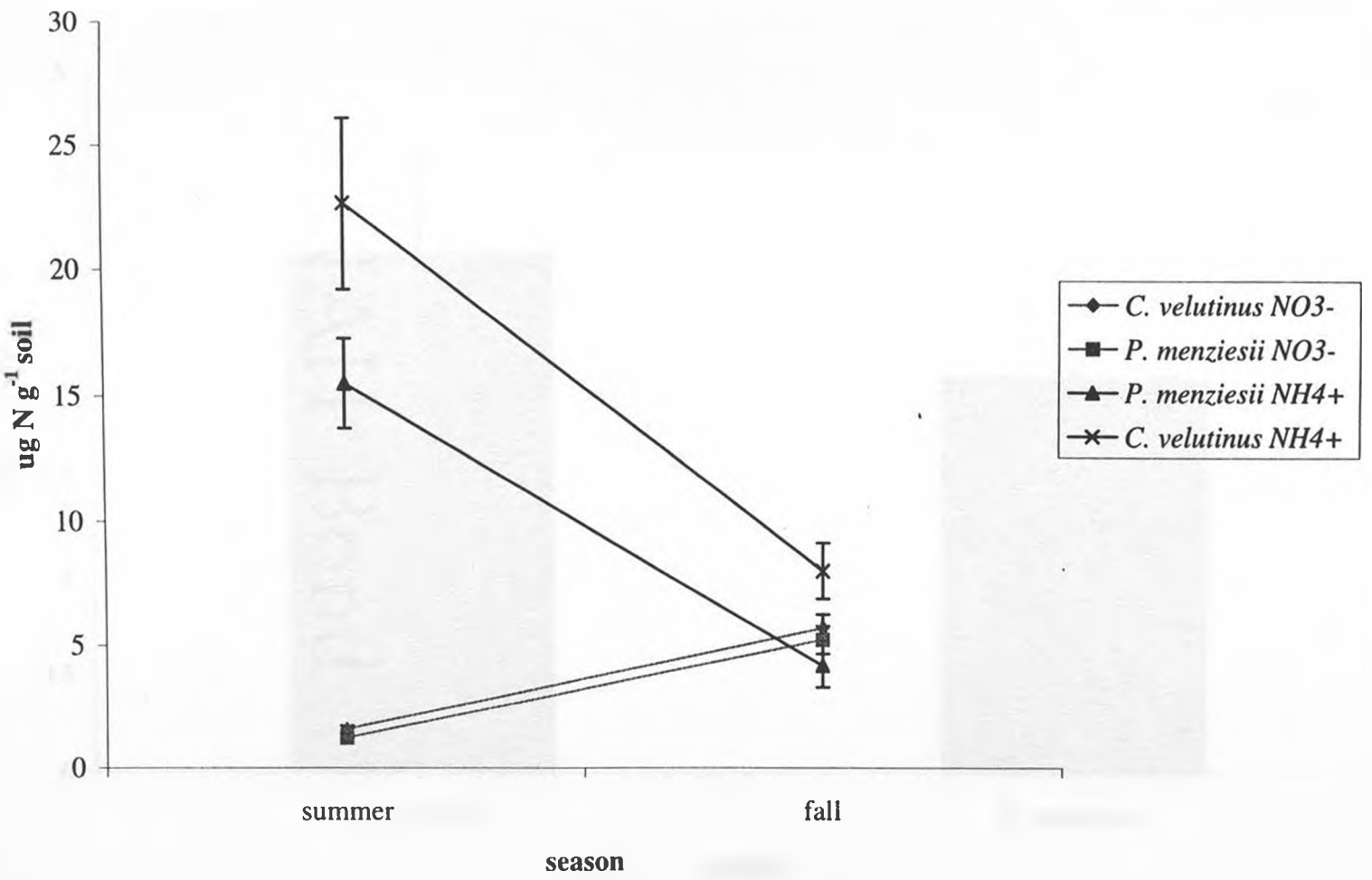


FIGURE 5. Comparison of seasonal nitrification trend in extractable inorganic N (μg N g^{-1} soil) in soils beneath *C. velutinus* and *P. menziesii*. Bars indicate ± one standard error.

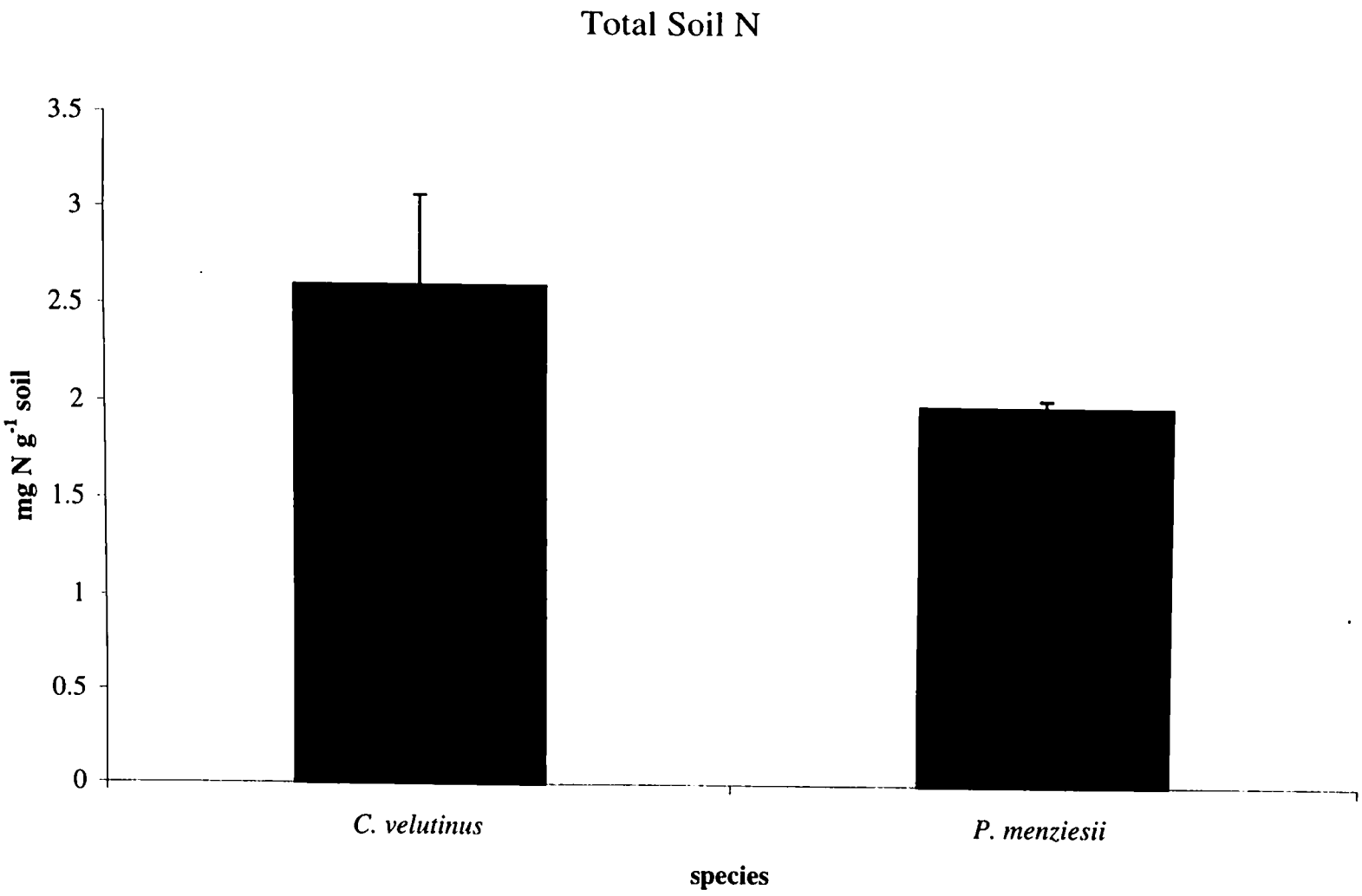


FIGURE 6. Amount of total N (mg N g-1 soil) in soils beneath *C. velutinus* and *P. menziesii*. Compilation of summer and fall samples. Bars indicate ± one standard error.

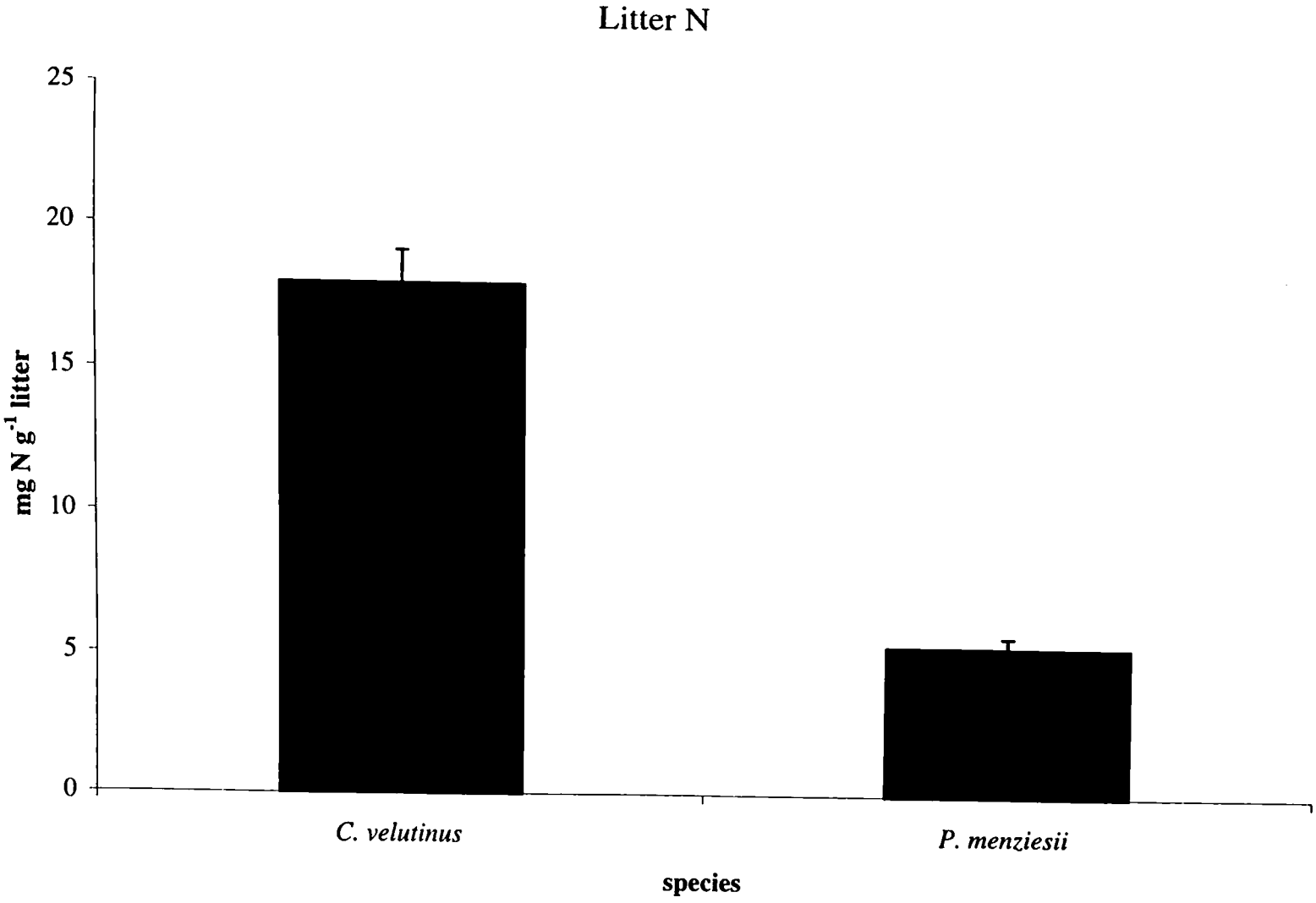


FIGURE 7. Comparison of *C. velutinus* and *P. menziesii* litter N (μg N g-1 litter) in fall samples. Bars indicate ± one standard error.

Enzyme activity

There were no significant differences in potential enzyme activity for each season for either β-glucosidase or phosphatase between species (Table 3). When comparing between seasons within species, in most cases there were no significant differences in potential enzyme activity for β-glucosidase or phosphatase (Table 4). The exception to this is that phosphatase activity was greater in the fall than the summer in the soil beneath *P. menziesii* (Table 4; figure 8). Comparison in overall activity (combining species) of each enzyme between seasons was not significant for phosphatase ($p=0.11$, $df=39$) or β-glucosidase ($p=0.16$, $df=39$).

TABLE 3. Comparison of the Potential Enzyme Activity between Species within Seasons.

Enzyme	Season	Species		df	p-value
		C. velutinus (μmol/g/h)	*P. menziesii* (μmol/g/h)		
β-glucosidase					
	summer	5.28 ± 0.69	3.93 ± 0.64	19	0.17
	fall	4.03 ± 0.45	3.59 ± 0.34	19	0.44
phosphatase					
	summer	14.47 ± 2.59	8.89 ± 1.75	19	0.09
	fall	15.37 ± 3.32	15.90 ± 1.61	19	0.89

TABLE 4. Comparison within Species of β-glucosidase and phosphatase Potential Activities between Seasons.

Species	Enzyme	Season summer (μmol/g/h)	Season fall (μmol/g/h)	df	p-value
C. velutinus					
	β-glucosidase	5.28 ± 0.69	4.03 ± 0.45	19	0.15
	phosphatase	14.47 ± 2.59	15.37 ± 3.32	19	0.83
P. menziesii					
	β-glucosidase	3.93 ± 0.64	3.59 ± 0.34	19	0.65
	phosphatase	8.89 ± 1.75	15.90 ± 1.61	19	0.01

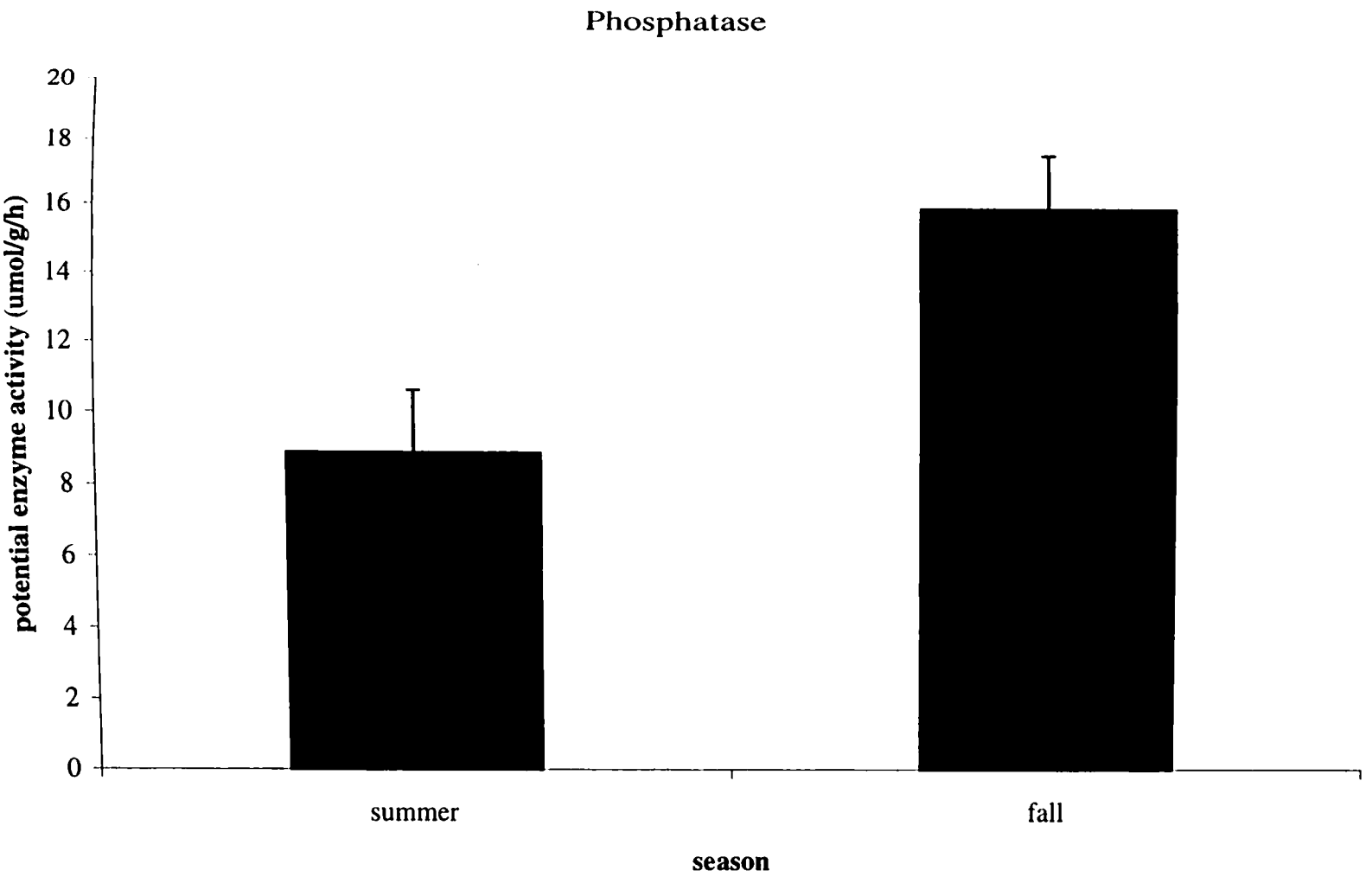


FIGURE 8. Potential phosphatase enzyme activity (μmol/g/h) of *P. menziesii* between seasons. Bars indicate ± one standard error.

Natural Abundance Isotopic ratio

There were significant differences in the isotopic ratio ($\delta^{15}N$) of soil extractable NO_3^-, soil extractable NH_4^+, and N of the litter between *C. velutinus* and *P. menziesii* (Table 5; figures 9 & 10). An outlying data point was removed which changed the initial result of a non-significant difference to a significant difference between *C. velutinus* and *P. menziesii* extractable NO_3^- $\delta^{15}N$ values ($p=0.03$, $df=18$; table 5). There was no significant difference in the isotopic ratios in the total soil N between the two species (Table 5).

The $\delta^{15}N$ values were not correlated with the concentration of extractable soil NO_3^- (Figure 11). The $\delta^{15}N$ values of extractable soil NH_4^+ were correlated with the concentration of extractable NH_4^+, yet with a low r^2 value (Figure 12).

TABLE 5. $\delta^{15}N$ values of Extractable NO_3^- and NH_4^+, Soil total N, and Litter N.

N compound	*C. velutinus* (‰)	*P. menziesii* (‰)	df	p-value
NO_3^-	5.77 ±1.14	6.31 ± 0.56	19	0.03
NH_4^+	-2.21 ± 0.76	-0.17 ± 0.40	19	0.04
Soil Total N	3.78 ± 0.68	3.92 ± 0.36	19	0.86
Litter N	-0.86 ± 0.17	-1.57 ± 0.24	19	0.03

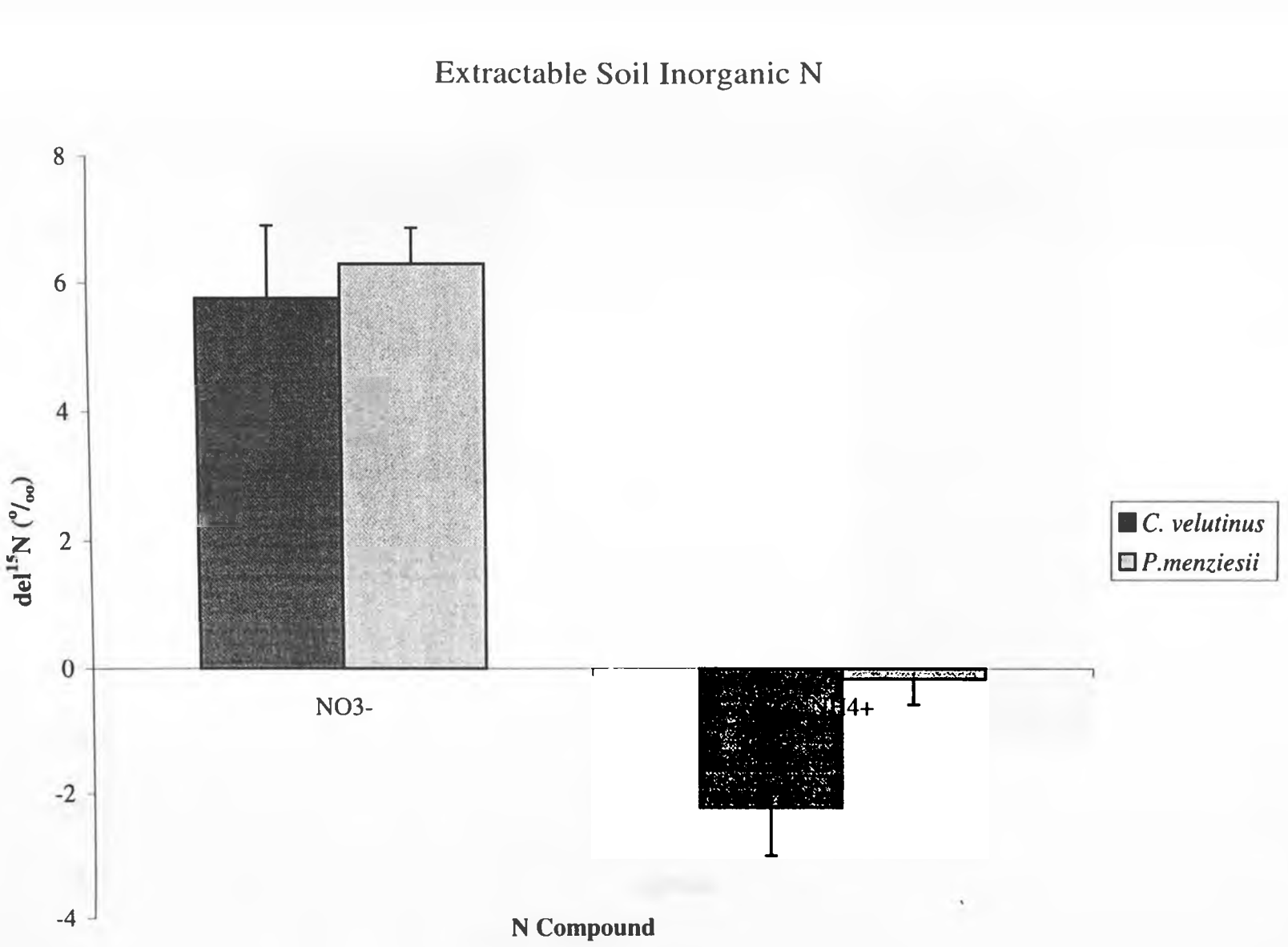


FIGURE 9. Comparison of *C. velutinus* and *P. menziesii* extractable soil NO_3^- and NH_4^+ del^{15}N values ($^o/_{oo}$). Bars indicate ± one standard error.

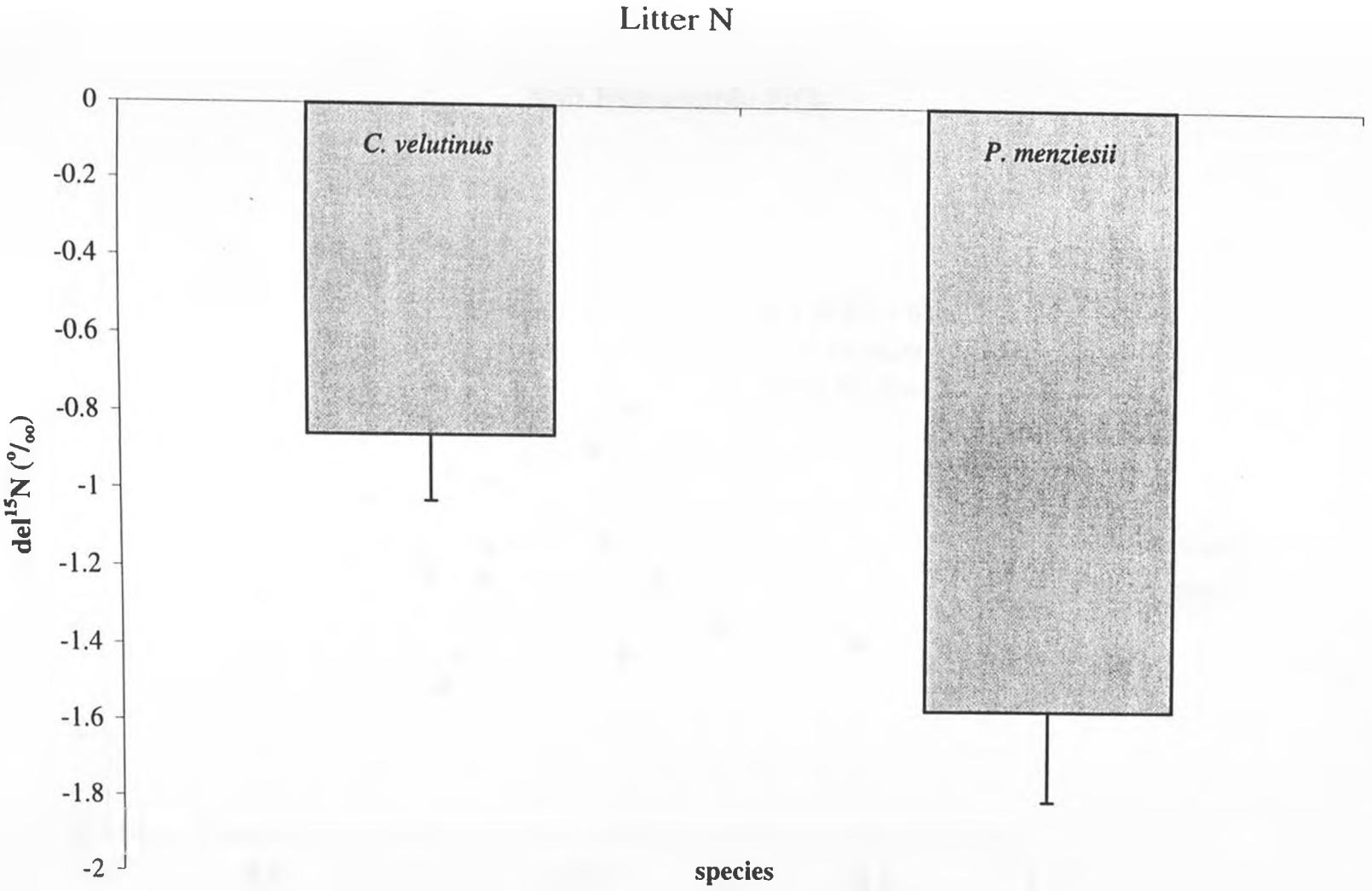


FIGURE 10. Comparison of *C. velutinus* and *P. menziesii* fall litter N del^{15}N values ($^{o}/_{oo}$). Bars indicate ± one standard error.

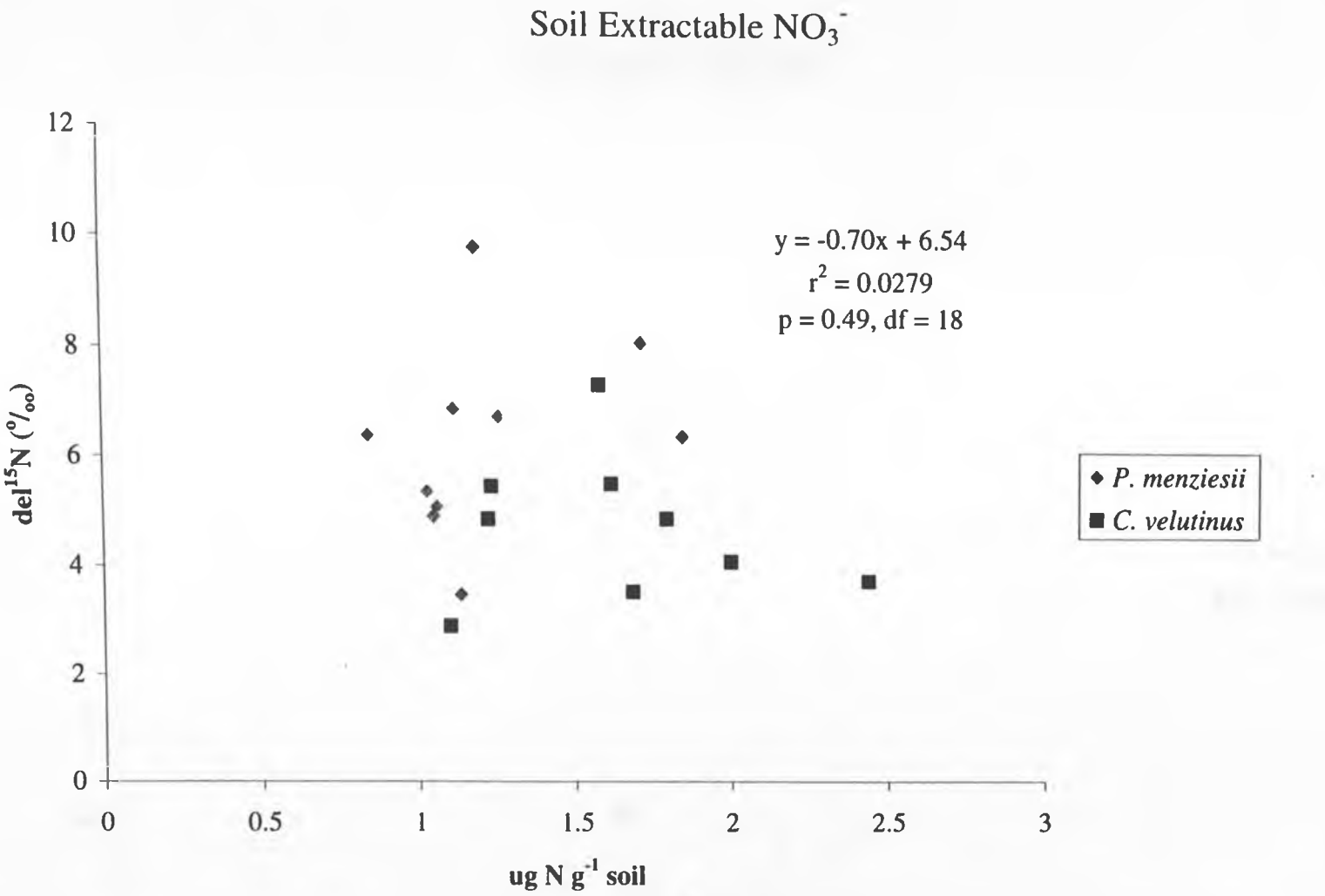


FIGURE 11. Linear regression of the del^{15}N extractable NO_3^- ($^o/_{oo}$) versus the amount of extractable NO_3^- (ug N g^{-1} soil) present in the soil.

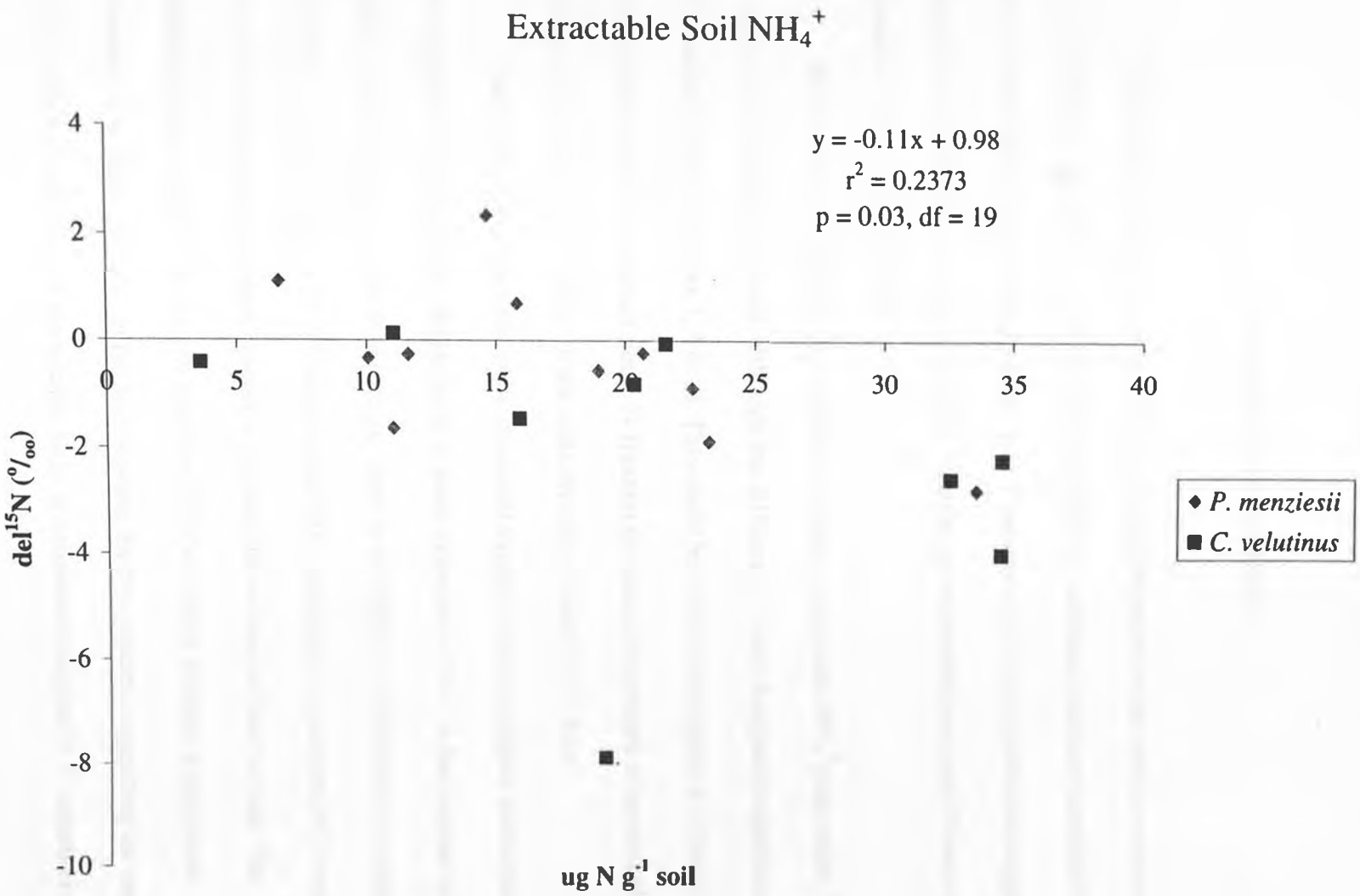


FIGURE 12. Demonstration of the regression of $del^{15}N$ extractable NH_4^+ (‰) versus the amount of extractable NH_4^+ (ug N g^{-1} soil)

CONCLUSION

Extractable Soil Inorganic N

The greater amounts of extractable inorganic N present in the soils is consistent with the suggestion that a N-fixing species, such as *C. velutinus*, has more available N in the soils than does a non-fixing plant such as *P. menziesii*. Yet, the results also suggest that the relative difference between the two species in extractable inorganic N varies with N form as well as with season.

Soils under *C. velutinus* consistently had more extractable NH_4^+ than under *P. menziesii* at all depths analyzed, although the difference is only marginally significant in the summer (Table 2; figures 3, 4 & 5). This could be a direct consequence of the N-fixing ability of the *C. velutinus* plant: N fixation increases the amount of extractable inorganic N in the form of NH_4^+ in the soils directly beneath the N fixer.

The results for extractable NO_3^- from soil suggest a more complex interaction with regard to N availability. While there is more extractable NO_3^- in the summer in soils under *C. velutinus* than under *P. menziesii*, there is no significant difference in extractable soil NO_3^- in the fall (Figure 2). For extractable NO_3^-, therefore, the amount of N available is contingent not only on which species is present, but on time of year as well. The additional depth analyzed in the fall samples (10-15cm) does indicate a significant difference in extractable NO_3^- in the fall between the two species, suggesting the amount of N available in the form of extractable NO_3^- is consistently higher in *C. velutinus* soils

(Figure 3). Yet, different seasons may dictate at which depth the relative difference occurs.

Even though there is a difference in extractable NO_3^- availability, it cannot be concluded that this difference, which is not drastic, has any biological significance with respect to regards to nutrient requirements. Nevertheless, the species difference does exist in the summer time in extractable NO_3^- concentration from the soil.

The trend for NO_3^- availability may be due to several factors. *P. menziesii* may have a decreased ability or necessity for NO_3^- in the fall and winter season and therefore uptake less. The difference in the relative amount available between depths has yet to be investigated, and no reasonable conclusions can be made without further study. Several studies could include examination of how nutrient concentration changes with depth over when sampled more frequently for a longer time period and if nutrients are taken up equally at all depths. Another possibility is that the seasonal difference in the litter production between the two species may influence the availability of extractable soil N between seasons as well.

The amount of extractable soil NH_4^+ decreases and the amount of extractable soil NO_3^- increases from summer to fall in both species (Figure 5). This trend has been observed in different studies, and may be a result of an increase in activity of the microbial community responsible for nitrification with the increase in precipitation with the onset of the winter season. Although this is a reasonable argument, the enzyme results do not support this trend as there was no significant differences in any of the enzyme activity between seasons, with the exception of *P. menziesii* soil microbial phosphatase activity (Figure 8). This discrepancy could be a result of the analysis of only two types of

bacteria, neither of which are directly involved in the nitrification process, and were used instead to indicate overall nutrient cycling rates. Other studies could therefore perhaps look at enzymes more directly responsible for the nitrification process, such as enzyme activity in *Nitrosomonas* and *Nitrobacter*, which may more clearly indicate an increase in nitrification as a result of microbial activity. Yet, these enzymes are difficult to isolate and to measure, and it may not be possible until different enzyme activity sampling techniques are discovered.

Total Soil N

Results for total soil N also support the hypothesis that a N-fixing species will have more N in its soil (Table 2, figure 7). The total soil N accounts for all N, including that found in organic matter in the soil, and was greater overall in the *C. velutinus* soils than those of *P. menziesii*. Presumably the decomposers would be able to convert this N into inorganic N which would then accrete in the *C. velutinus* soils. It cannot be concluded, though, that the aforementioned inorganic N trends are a direct result of an increase in total soil N in *C. velutinus* soils. In order to draw this conclusion further studies would have to trace the movement of N through the cycle, perhaps with the use of isotopes.

Litter N

The litter N results support the hypothesis that a N-fixing species will have more N content in its leaves as well (Table 2, figure 7). Although not surprising, it is important to establish that a N-fixer such as *C. velutinus* does have significantly more N on a per gram basis than *P. menziesii*. There is more N available on a per gram litter basis to be decomposed with *C. velutinus* litter than *P. menziesii*. When analyzed on an area basis, the higher amount of litter biomass under *P. menziesii* compensates for the lower concentration of N in the litter so there is not a significant difference between the two species in the amount of N in the litter (Table 2). Thus, *P. menziesii* has much of its N sequestered in needles on the forest floor, an accumulation that could either be due to more abscission of needles or else due to needles which are more difficult to decompose than *C. velutinus* litter. Examination of the different litter inputs as well as the varying decomposition rates of the two species' litter should be an area of future study.

Enzyme Activity

The results of the potential enzyme activity assays were surprising in that there was only one significant increase in activity between season (*P. menziesii* soil phosphatase activity), and there were no other differences between species or seasons (Tables 3 & 4). Even though enzyme activity has been found to increase exponentially with increasing temperatures (Waring & Schlesinger, 1985), soil moisture can often be a limiting factor in the dry season (Waring & Franklin, 1979). Since the majority of the

rainfall in the Western Cascades occurs between November and March, an increase in enzyme activity was predicted in the fall sample as compared to the summer. One possible explanation of the overall lack of change in enzyme activity is that at the time of the fall sample collection (November 15, 1998) enough precipitation had not yet fallen to significantly increase the enzyme activity.

Future studies could conduct more frequent seasonal studies at this site to determine if, after a prolonged period of precipitation, there is a significant difference in enzyme activity compared to the summer, as well as within species. Sampling at this site would be difficult because of restricted accessibility after snow falls. Another possible way to measure microbial activity would be to install filters of a known weight in a permeable mesh bag at the beginning of the winter season. After an extended time, in spring for example, the bags could be recollected and the filter papers reweighed. The percent difference in weight would indicate the amount of microbial activity during the particular time span. This experiment was installed last fall and results may be available within a year.

Since the enzyme results were not very indicative of cycling rate, the amount of C in the soils was also determined by Julie Spears, a Ph.D. student in an Oregon State University Botany lab. It was our hope that with the C information, we could calculate the C/N ratio of the soils from the enzyme analysis. Since enzyme activity is targeted to organic matter instead of mineral soil, analyzing the enzyme activities based on a per gram organic matter, rather than per gram soil would hopefully demonstrate significant differences in enzyme activity. Yet the results from this analysis did not demonstrate any other significant differences in enzyme activity (Spears, personal communication).

The selection of β-glucosidase and phosphatase enzymes was based on the feasibility of conducting in vitro experiments to obtain potential enzyme activities. These two enzymes, although not directly catalyzing N compound reactions, were chosen as a possible indicator of overall nutrient cycling. It is possible, therefore, that these two enzymes in particular are not indicative of much of the nutrient cycling in the area of study.

The significant difference in the phosphatase enzyme in *P. menziesii* soils between seasons should not be overlooked (Figure 8). Although a biological explanation of this is not clear, it may be worth further investigation as differences in nutrient availability other than N between the two species soils.

Lastly, it should be mentioned that another possibility is that species, for the most part, do not significantly affect the rate of nutrient cycling, as shown by my results. In order to determine if these results are truly reflective of the nutrient cycling rates, further studies should investigate a wider range of enzymes as well as *in vivo* studies, if possible.

Natural Abundance Isotopic Ratio

The significant difference in $^{15}N/^{14}N$ ratio between *C. velutinus* and *P. menziesii* suggest that isotopic signatures could be used to trace the flow of N through the system (Table 5; figures 9 & 10). The presence of the signal, or difference in isotopic ratios, in the litter and the extractable inorganic N suggest the signal does persist into the soil.

It is not clear why the soil total $\delta^{15}N$ did not differ between the two species, whereas the inorganic $\delta^{15}N$ did. One possibility is that there is a greater amount of

residual (old) N that no longer contains a detectable signal that, in a sense, may have swamped our ability to detect any distinct signal.

Nevertheless, my results suggest that the use of N isotopes may be a feasible way to trace N recently fixed by *C. velutinus* since the signal is present in the inorganic forms. Future studies should investigate how long the signal persists in this system, and if it is present in other plants in the area or in the streams. The signal is lost as the N is continually taken up by other organisms whose biochemical processes alter the $^{15}N/^{14}N$ ratio. It is not known, then, how long the signal will persist for in the system and should be investigated further. Being able to track the N fixed by *C. velutinus* would greatly enhance our understanding of nutrient cycling in this ecosystem.

Summary

This discussion of the various elements involved in nutrient cycling addresses the larger question, how do ecosystems function? My research contributes only a small glimpse as to what may be used to heighten our knowledge of ecosystem processes. In addition, I discuss a few questions regarding species interactions, and whether the presence of a particular species affects different elements in that system. Yet the questions are many, and answers, if obtainable, are complex. So why continue to study ecosystem level processes? A key component to an ecosystem is the interaction of species, as well as biotic and abiotic factors. Therefore, to not address these issues would inhibit our understanding of the individual organism in relation to the connected system it

thrives in. Furthering our understanding of these processes is intricately linked with our ability to manage and protect sustainable and diverse ecosystems.

APPENDIX A

ILLUSTRATIONS

ILLUSTRATION 1. Typical *C. velutinus* branch with simple leaves and three distinct veins from the base of the leaf.

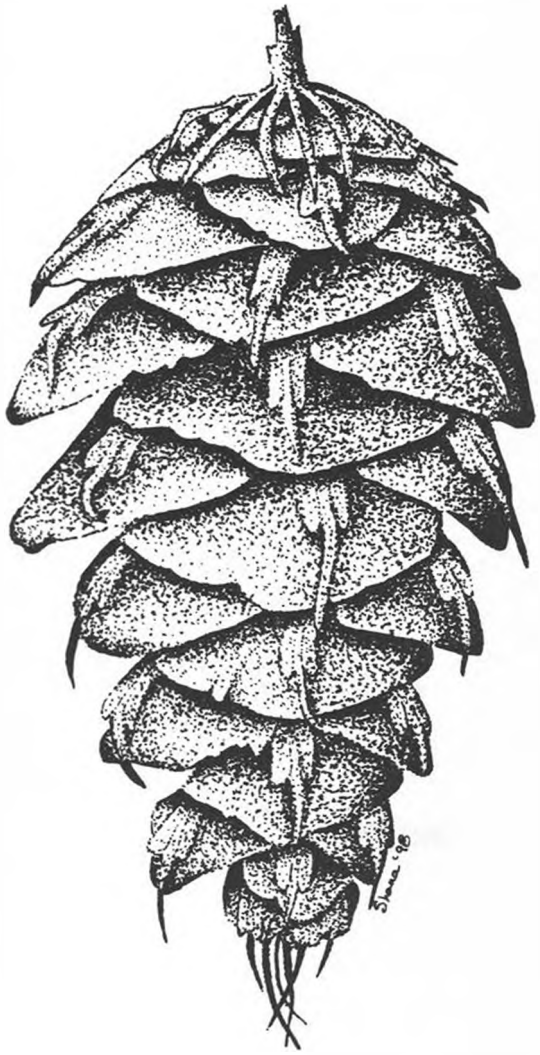


ILLUSTRATION 2. *P. menziesii* cone; distinct with its mouse tail-like bracts.

APPENDIX B

SOIL SAMPLING

Why Refrigerate?

Soil samples can change chemically when they are kept moist and warm in a closed bag. It is important to specify whether, or how soon after being collected samples were refrigerated. This is especially important for enzymatic activity, which is temperature dependent. When chilled at the cooler temperature, the enzymes are not able to carry out their metabolic processes. Thus, if the samples are chilled, the amount of enzymatic activity upon time of analysis will reflect amount present at the time of sampling. Refrigerated samples do not need to be analyzed immediately because of this.

Similarly, if the microbes in the soil are allowed to continue their metabolic processes the relative amounts of N in certain forms can change. In other words, the chemical properties of the soil can be drastically altered if microbe activity is allowed to persist, which is important as the soils are kept in bags where water tends to condense. Even though the soil bags used in this experiment are breathable, the levels of moisture can increase within the enclosed space. Since microbial activity is limited by water, activity could potentially increase at room temperature with elevated moisture levels. That is why it is important to refrigerate all soils as soon as possible after collection, and keep them refrigerated unless they are dried.

APPENDIX C

EXTRACTABLE SOIL INORGANIC N ANALYSIS

What does Extractable Inorganic N Indicate in our Soils?

The levels of extractable inorganic N (NH_4^+ and NO_3^-) indicate the amount of N available for organismal uptake. Soil samples were analyzed for extractable NO_3^- and NH_4^+ content in order to find out whether more available N was present beneath *C. velutinus*. Since N accretion has been shown in previous studies to be high in *Ceanothus* stands (McNabb & Cromack, 1983; Binkley et al., 1982; Youngberg & Wollum, 1976), I expect that the soils beneath *Ceanothus* will be higher in extractable NH_4^+ and NO_3^- than those beneath the non-fixing Douglas-fir.

KCL Extraction: How does it Work?

Extractable inorganic forms of N, NH_4^+ and NO_3^-, are made available for analysis by placing them in solution. By definition, inorganic N ions are in solution within the soil as compared to organic forms of N which, as the name implies, are predominately found within the organic matter in the soil (there is dissolved organic N in the soil as well, but most plants cannot use N in this form). Because of its positive charge, NH_4^+ is retained due to the soil's high cation exchange capacity (CEC). This refers to the attraction of positively charged ions to negative electrical charges found on secondary mineral soil surfaces (Perry, 1994). By placing the soil sample in KCl, NH_4^+ will be displaced by the K^+ ions on the CEC sites. The binding of K^+ ions displaces the extractable NH_4^+ into the solution that can then be measured.

Once the extraction, or displacement of NH_4^+ molecules, is complete, the samples (filtrates) are gravity filtered to prepare them for analysis. In the filtrates there are both the extractable NH_4^+ molecules as well as the extractable NO_3^- molecules (which, due to their negative charge, tend to remain in soil solution and do not bind to CEC sites). Filtrates were analyzed on the Autoanalyzer separately in order to determine relative amounts of extractable soil NO_3^- and NH_4^+ in solution.

Autoanalyzer: How does it Work?

The Autoanalyzer is a convenient method to determine the quantity of a specific molecule through a series of chemical reactions. By adding a specific reagent to the filtrate, controlled chemical reactions will occur with the molecule of interest. The product of these reactions will produce a color. The Autoanalyzer measures the intensity of the color (known as colorimetry), which is proportional to the concentration of the molecule of interest. This is recorded and transformed into peaks, the height of which corresponds to relative concentrations.

A series of standards, samples of known concentrations, are then run and measured colorimetrically. Since both samples and standards are run under the same conditions, the height of the standard peaks can be used to determine the concentrations of the molecule of interest. Measuring standard peak heights and graphing these values (peak height versus standard concentration) yields a linear relationship. By determining the equation of this line, the other sample values can be plugged into the x value, relative peak height. Thus, the concentration of product (in $\mu g \cdot L$) can be determined for any given sample.

Since samples are not air dried before determining the concentration of N, the dry weight must be determined for each sample. The dry weight was determined by multiplying the fraction of dry weight by the wet weight of the sample. Fraction dry weight is determined by the following calculation:

Fraction dry weight = (dry weight (g) - tin weight (g))/(wet weight (g) - tin weight (g))

The tin weight refers to the tin that the soils were measured and dried in.

How is Extractable NO_3^- Analyzed?

Total extractable NO_3^- was determined using a standard Scientific Instruments procedure (Scientific Instruments, 1987). Extractable NO_3^- is first reduced to nitrite (NO_2^-) in a copper-cadium reductor column in the presence of ammonium chloride. Once this reduction takes place, a color-producing reagent, that contains sulfanilamide, is added in acidic conditions to the sample stream, forming a diazo compound when the NO_2^- ions react with the sulfanilamide reagent. The diazo compound then couples with N-1-naphthylethylenediamine to form a reddish-purple azo dye. It is the intensity of the azo dye color that the Autoanalyzer measures colorimetrically. The original amount of extractable NO_3^- is directly proportional to the amount of dye present at the end of the chemical reactions because substrate is the limiting factor. In other words, excess reagent is added to all of the solutions so all of the sample nitrate will be reduced and react completely.

How is Extractable NH_4^+ Analyzed?

Using the same machinery, samples can be run on the Autoanalyzer with a different set of reagents to measure extractable NH_4^+. Total extractable NH_4^+ was determined for each sample using the Berthelot Reaction following a Scientific Instruments procedure (Scientific Instruments, 1987). In this reaction, extractable NH_4^+ reacts with dichloroisocyanurate and sodium phenoxide to form a blue colored compound that is closely related to indophenol (another compound known for its distinct blue coloration). To prevent formation of precipitates from the hydroxides of calcium and magnesium, a solution of potassium sodium tartrate and sodium phenoxide is added to the sample stream. The blue colored compound is then measured colorimetrically by the Autoanalyzer.

For both the extractable NO_3^- and NH_4^+ runs, the Autoanalyzer produces a print-out consisting of a series of peaks, described in the Autoanalyzer section. These peaks are then converted to $\mu g \cdot L$ N soil dry weight as described in Autoanalyzer section.

APPENDIX D

TOTAL SOIL N AND LITTER N ANALYSIS

Block Digestor: One Heck of a Stomach

By digesting organic materials it is possible to determine the total N. As described in the introduction, there are two forms in which N exists in the soil: inorganic N (NH_4^+, NO_3^-) and organic N. The analysis of the amount of inorganic N has already been described. To determine how much total N is in the soil, it is also necessary to analyze how much N is contained within the organic matter.

In organic matter, N is primarily incorporated as amide groups that are N compounds attached to a carbon chain. It is not possible to extract the N off of the carbon compounds by the procedure used for the inorganic N compounds. Nitrogen in organic matter is bound much more tightly than inorganic N, which is soluble. Thus, a much stronger reaction (oxidation) is necessary to remove the N from amide form and place it in a measurable, ionic form in solution.

By placing the soil and litter into a tube with 5 mls H_2SO_4 and 2 mls H_20_2, an oxidation reaction takes place due to the interaction of the peroxide and acid with reduced C. This reaction begins to 'digest' the carbon, meaning the carbon compounds in organic matter are oxidized to CO_2. This displaces the N that was in the amide form into solution in the form of NH_4^+. The presence of the acid is important for this reaction, as H+ ions must be available to bind to N atoms. Otherwise, the N would escape from the solution as ammonia gas, NH_3. The reaction is accelerated by heating the samples to 320°C. Once cooled, an additional 2 mls of H_20_2 are added to the solution, and then heated again to 340°C to volatilize any additional H_20_2, bringing the reaction to completion. It is important to remove any excess H_20_2 because it is a powerful oxidant and may interfere

with the colorimetry measured with the Autoanalyzer. In summary, a digestion removes the N from the organic matter in a soluble form (NH_4^+).

Measurement of N

In this procedure, the N in solution will remain in the NH_4^+ form, and will not be oxidized to NO_3^-. Therefore, it is possible to use the Autoanalyzer in a similar manner as was previously done for the measurement of NH_4^+ to determine the amount of total NH_4^+ in the solution. These samples not only contain the NH_4^+ from the organic matter digestions, but also the NH_4^+ from the original soil solution. Therefore, the amount of NH_4^+ determined previously in the inorganic N analysis must be subtracted from the total NH_4^+ measured in this procedure. The difference will yield the amount of N contained in organic matter.

In order to determine the concentration of N in solution, all samples are then measured colorimetrically on the Autoanalyzer following a Scientific Instruments procedure (1987). In this procedure, a similar reaction (Berthelot reaction) was set up as for NH_4^+ analysis, but the reagents must be modified because the digestion of the samples is in acid. Despite these differences, the general principles guiding the basis of the reaction and colorimetric measurement are the same for both procedures. As described in previous sections, NH_4^+ measurements were converted to $\mu g \cdot L$ N soil dry weight.

Why Grind the Litter?

When the litter is first collected, there are many different sized particles. Grinding the litter samples provides a homogenous sample (containing standard, similar sized particles) for analysis. By grinding the litter prior to the analysis, the surface area is increased making these bonds more accessible to the chemicals. Additionally, the relatively small sub-sample analyzed will contain representatives of many different types of leaves thereby resulting in a homogenous mix. Thus, by grinding to prepare the litter, a homogenous sample is obtained that is more easily and quickly digested.

APPENDIX E

ENZYME ANALYSIS

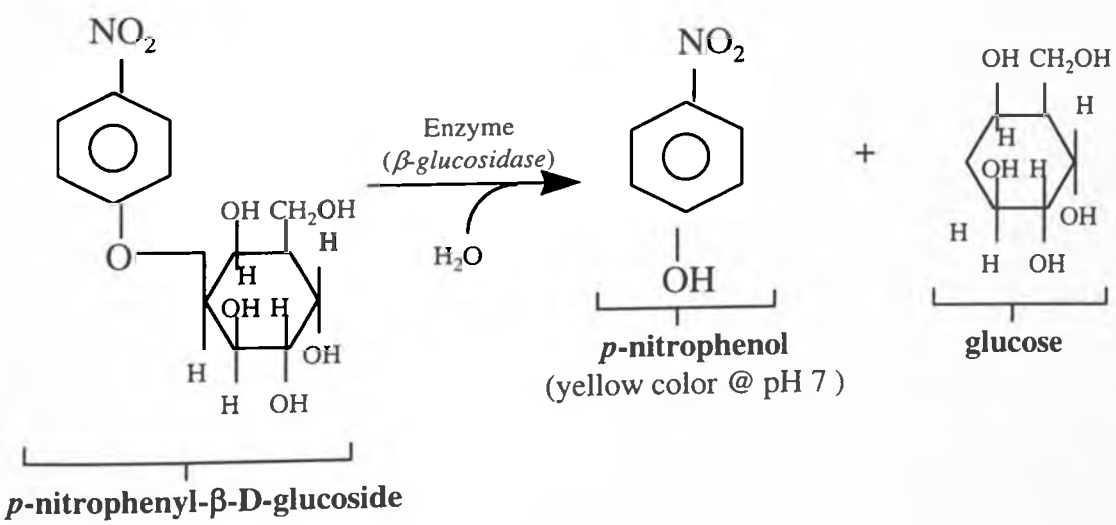
Description of the Enzymes Analyzed:

β-glucosidase is responsible for one of the steps required in the breakdown of cellulose, a major component of organic material (e.g. in leaves). The enzymatic activity of β-glucosidase makes an energetically useful form of carbon (glucose) available to organisms. Another type of enzyme, phosphatase is a general name for a broad group of enzymes that catalyze the hydrolysis of both **esters** and **anhydrides** of phosphoric acid, H_3PO_4. It is through this process that organic phosphorus is converted to inorganic phosphorus, thereby becoming available for organismal uptake.

What Causes the Color?

Both assays, or experimental runs, involved the colorimetric analysis of the amount of *p*-nitrophenol (*p*NP) when released from soil incubated in sodium *p*-nitrophenol phosphate or glucose buffer solution.

For β-glucosidase activity analysis, the reaction is as follows:

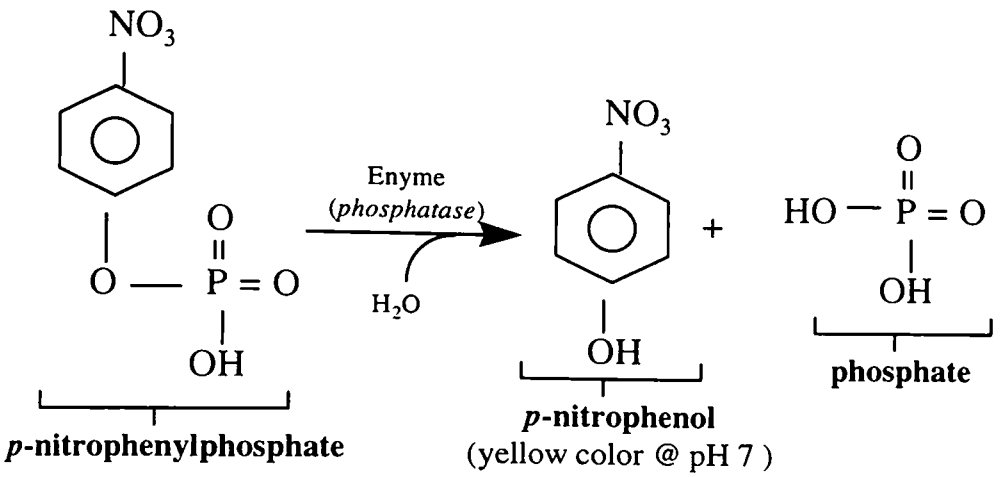


***p*-nitrophenyl-β-D-glucoside**

***p*-nitrophenol**
(yellow color @ pH 7)

glucose

The reaction for phosphatase activity is as follows:



In both reactions, the group attached to *p*NP is hydrolyzed (or cleaved by adding water across the bond) to produce two separate molecules, *p*NP and either a phosphate or glucose molecule. This cleavage can only be realized in the presence of the respective enzyme. The microorganisms take up the phosphate or glucose molecule, and the *p*NP molecules remain in solution. Therefore, the concentration of *p*NP, measured by the color intensity of the sample, is indicative of enzyme activity.

APPENDIX F

NATURAL ABUNDANCE ISOTOPIC RATIO ANALYSIS

What is an Isotopic Ratio and How is it Determined?

Isotopic ratios are determined by comparing the ratio of heavy to light isotopes in a sample to the ratio in a standard (in the case of N, the isotopic ratio of N_2 in the atmosphere is the standard) using a **mass spectrometer**. The use of a reporting ratio as compared to a primary standard is so that confusion or misleading results can be avoided. The absolute abundance of isotopes within a sample can in fact be measured, but it is such a small amount that reflections of day-to-day fluctuations or sample preparation could dramatically influence the results. Thus, the differences in the ratios are represented by 'del' (δ) notation and have the units of per mil, $^{o}/_{oo}$ (Lajtha & Michener, 1994), and is described by the following equation:

$$\delta\ (^{o}/_{oo}) = \frac{R_{sa} - R_{std}}{R_{std}}$$

Where R_{sa} is the isotopic ratio of the sample, R_{std} is that of the standard, which in the case of all N isotope studies is the atmosphere, which is assumed to be relatively constant throughout the year ($^{15}N/^{14}N$ is estimated to be 1:100).

Use of Isotopes as a Signal

By determining the isotopic ratio of the soil and litter, it is possible to determine if *C. velutinus* has a distinct "signal", or isotopic abundance signature, compared to that of non-fixing plants. It can be determined by comparing δ values between different organisms, in this study between *C. velutinus* and *P. menziesii*. If statistical analyses

determine that the δ values are significantly different between the organisms studied, then perhaps this can be used to trace the fate of these materials in the ecosystem.

If a signal is present in the N that *C. velutinus* has fixed compared to the N profile in *P. menziesii* litter and soil, it may be possible in future studies to trace it to other parts of the ecosystem. The isotopic analysis of the soil and leaf litter beneath *P. menziesii* may contain a signal distinct from that of the signal from *C. velutinus*. It is hypothesized that since *P. menziesii*, a non-N fixer, is subsisting off of different N sources it would have a different signal present in its isotopic ratio. Different sources refers to how "old" the N in the system is. If the N has undergone a high degree of intrasystem cycling it may have an isotopic signature different than that of N recently fixed. This is based on the observation that soil N is usually more abundant in ^{15}N than is atmospheric N. A plant such as *P. menziesii* that cannot fix its own N is completely dependent on N in the soil, and would be expected to have a higher amount of ^{15}N. Determining whether or not *C. velutinus* has a distinct signal from *P. menziesii*, and if that signal can be traced through the ecosystem into the watershed, will indicate whether or not this procedure can be use to trace nutrient cycling through various processes in a forest ecosystem.

APPENDIX G

STATISTICAL ANALYSIS

Why do Statistics? What do the Numbers Mean?

Statistical analysis of results does not indicate whether a relationship is true, instead it expresses the probability of it not being true. The probability of being wrong expressed by these percentages is known as a **p-value**. The 5% level of significance is convention commonly used as a cutoff. There are two other main "tiers" of significance, 1% (or 0.01) and 0.1% (or 0.001). The 1% level of significance is referred to "highly" significant; the 0.1% is "very highly significant." When a study claims a p value of 0.001, then there is only one-in-one-thousand chance they are incorrect. Therefore, the trend suggested by their data is highly supported and that the values obtained could have come by chance. In summary,

$P \geq 0.05$ non-significant or not significant
$P < 0.05$ significant
$P < 0.01$ highly significant
$P < 0.001$ very highly significant

In order to determine whether or not my data set has significant relationships between certain variables, I analyzed the data with parametric statistics. Parametric statistics are based on the mean of observations and the variance about that mean (Bradshaw, 1998).

One of the most powerful tools in statistics is to look at the analysis of variance, otherwise known as ANOVA (the ANalysis Of VAriance). In short, ANOVA is a parametric statistical test which determines the difference between two mean samples. In

other words, it addresses the question, "Is the variation among samples greater than variation within samples?" By applying this statistical method to a data set, I determined relationships in the data to certain degrees of significance.

Another important term to address for aid in a basic understanding of statistics is degrees of freedom (df). Degrees of freedom are the number of independent observations within the data set. For example, if the sum weight of five dogs were known, how many of the individual weights would have to be known in order to state the weight of each individual dog? Four. (The fifth weight determined by subtracting the sum of the four from the total). Thus in this example there are four degrees of freedom.

APPENDIX H

GLOSSARY

Accretion-term referring to when a compound or substance is still aggrading, i.e. continuing to gain biomass, or in terms of inorganic N, still increasing.

Biogeochemistry-the study of the movement of chemical elements between organisms and non-living compartments of the atmosphere, lithosphere and hydrosphere.

Catalyze-to noticeably increase the rate of a reaction. Catalysts are the substances (such as enzymes) which accomplish the increase in the reaction rate without themselves being permanently alter or consumed in the reaction.

Denitrification-the conversion of nitrate (NO_3^-) to nitrogen gas (N_2) by denitrifying bacteria in the soil; part of the nitrogen cycle.

Deposition-the laying down of material on the earth's surface. Includes materials that have been derived elsewhere.

Dryfall-the result of gravitational sedimentation of particles during periods without rain.

Ecology-the study of ecological systems and their interacting abiotic and biotic components.

Intersystem cycle-the nutrient and energy cycle *between* two or more ecosystems.

Intrasystem cycle-the nutrient and energy cycle *within* ecosystems.

Isotopes-atoms with the same number of protons but with a different number of neutrons; Isotopes have the same chemical properties but differ in mass and in the physical properties that depend on their mass.

Kg/ha/yr-"Kilograms per hectare per year;" a useful unit of measurement for ecosystem ecologists. Approximately equivalent to pounds per acre per year.

Litter-the accumulation of fallen leaves on the forest floor; typically defined as the upper part of the organic horizon (for different horizons, see the soil profile, figure 1).

Leaching-the process by which dissolved materials are washed away or carried with water down through the various levels of the soil.

Mass Spectrometer-an instrument that separates charged atoms and molecules on the basis of their mass differences.

Nitrogen cycle-the worldwide circulation of nitrogen from the abiotic (non-living, physical) environment into living things and back to the abiotic environment.

Nitrogen Inorganic Forms:

N_2- Nitrogen gas
NO_3^-- Nitrate
NO_2^-- Nitrite
NH_3- Ammonia gas
NH_4^+- Ammonium
* Nitrogen can also be incorporated into various organic forms, such as in amine groups and amino acids.

Photosynthate-the product of photosynthesis in all land plants and most photosynthetic species; high-energy carbon compound, typically a sugar (such as glucose).

Reagent-a substance used in a reaction for purpose of testing, analyzing, or detecting other substances.

Secondary succession-succession following a disturbance (such as clearcutting) that removes some but not all of the biological imprints on a site (Perry, 1994).

Substrate-a compound that reacts with a reagent in any enzyme catalyzed reaction.

Symbiotic relationship (symbioses)-a close interaction between individuals of different species. In the case of nitrogen fixing bacteria in *C. velutinus*, the symbiosis is mutualistic as well since both species benefit.

Watershed-total land area that drains directly or indirectly into a particular river, stream, or lake.

Wetfall-the deposition of nutrients by precipitation.

Total N- refers to the total amount of N samples after all of the carbon of the compounds has been removed (referred to as 'digestion'). All organic N is converted to NH^{4+} in the digests. The initial soil NH^{4+} is still in the solution. By measuring the total amount of NH^{4+} in a digested sample, the total amount of N in the soil (both organic and inorganic) can be determined. By this method, NO^{3-} is not taken into account, but the relative values are low and would not affect the relative significance of the results.

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