# Can nitrogen isotopes be used to detect the effects of burning on nitrogen cycling? A study on *Pinus resinosa* in Minnesota, USA

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## Abstract

*Pinus resinosa* stands in Minnesota, USA were surveyed and fine and coarse roots from stands were analysed for nitrogen isotopes in an attempt to determine if fire effects nitrogen cycling. Fire increased the % nitrogen of coarse roots and changed the ratio of C: N. It may also decrease the depth of the A-horizon. It did not however affect the nitrogen isotope ratio of stands. Isotope values of coarse roots were affected by the depth of the A-horizon, which is consistent with other studies. A correlation between the $^{15}N$ values of coarse and fine roots was found however fine roots were enriched proportionately to coarse roots. This suggests that they may be using a more enriched source of nitrogen such as ammonia although this is not conclusive. A PCA performed on site variables and isotope values determined the two major axes for sites were burn history and nitrogen cycle openness but that burning did not affect the second axis. A stepwise backwards regression showed the best predictors of a roots isotope value were, in decreasing order, the isotope value of coarse roots for fine roots and vice versa, the % nitrogen of roots and the age of stands. Coarse and fine roots showed opposite trends with % nitrogen. As the % nitrogen increased, fine roots were more $^{15}N$ enriched, again pointing to different nitrogen sources for coarse and fine roots. Older stands do appear to have a more open nitrogen cycle, as roots were $^{15}N$ enriched in these stands. The availability of different forms of nitrogen may influence the $^{15}N$ signal of roots especially if fine roots are preferentially using ammonia. Therefore, it is not possible to say if isotope values are due to stand openness, or due to different resources use by roots.

## Introduction

Itasca State Park in northern Minnesota, USA, was created in 1891 to protect the old-growth pine forests that surrounded Lake Itasca and its watershed. The site is also important as it contains the headwaters of the Mississippi. The park is on the terminal moraine of a glacier, thus the landscape is very hilly and has many water-filled depressions. The red pine (*Pinus resinosa*) is Minnesota's state tree. It is a tall conifer, up to 30m, with reddish-brown bark that is shallowly furrowed to form scaly plates. It can have a diameter of up to 100cm. It is hardy and found throughout north-central and northeastern Minnesota. It is shade intolerant, moderately fast growing and long lived. It is usually found growing on dry and highly acidic, sandy soils. Its recruitment appears to be fire dependant. It is an important timber tree and as a result was extensively logged, so much so that the Itasca Park was declared in order to preserve some of the last remaining old growth forest including the largest red pine in Minnesota about 36.5 m tall.

Since the park was declared, few *Pinus resinosa* seedlings have naturally recruited. In an effort to conserve the species, seedlings are planted into the forest and entire stands have been planted. However, the long-term survival of the species depends on an understanding of its recruitment requirements. Fire frequency and intensity are important aspects in determining successful recruitment and fire regimes in the park have drastically altered in the last 150 years. It was estimated that the pre-settlement fire regime was about one in every ten years (Frissell 1971). After the park was declared, fire was virtually eliminated. Currently a limited burning regime is applied. All of the current stands in the park are shown to have established after larger, stand clearing fires (Frissell 1971). *P.resinosa* seeds rapidly germinate on exposed soils (Rathke, 2001) and require open environments with little light competition for successful seedling establishment. This criterion is met when large fires remove all competing biomass and only red pines seed in. *P.resinosa* is also a poor competitor for other resources and is thus more successful in a nitrogen-limited system where it has a competitive advantage over other plants that require a more nutrient rich environment. Fire is also a disturbance that alters or resets the successional sequence within these forests thus allowing *P.resinosa* (a pioneer species) to exist amongst late-successional species if fire maintains a patchy landscape.

Nitrogen availability is usually the limiting factor for forest growth and productivity (Keeney, 1980). The majority of nitrogen found in soils is not immediately available for plant uptake as it is not in a useful form for plants (Binkley & Hart, 1989). In natural ecosystems, the nitrogen absorbed by plants comes from the decomposition of organic matter. This has been found in specifically in temperate forest by Whitaker *et al* (1979). Microbes break down dead organic matter and nitrogen is released as dissolved organic nitrogen. This source of nitrogen can be used by mycorrhizal fungi and decomposer microbes. Immobilization takes place when these microbes switch to using ammonia and nitrate and thus remove inorganic nitrogen from the system that would otherwise have been available for plants. When microbial growth is carbon limited, nitrogen mineralization takes place and microbes break down dissolved organic nitrogen and secrete ammonia. Ammonia can be converted into nitrates (which are more susceptible to volatilisation and loss due to leaching) through the process of nitrification. Thus, microbes can be either a consumer or a producer of ammonia depending on soil conditions. During the process of denitrification, denitrifying bacteria converts nitrates into nitrites, nitrous oxide, ammonia or elemental $N_2$ and often results in the loss of useful forms of nitrogen for plants from a system.

Nitrogen ($^{14}N$) has a naturally occurring isotope, $^{15}N$ that is 0.3663% as abundant as $^{14}N$. Natural processes that occur during nitrogen cycling can alter the proportion of the two forms. This is known as fractionation. The process of nitrogen mineralization does not discriminate between the two forms (Hogberg, 1997). Nitrification is associated with a change in the ratio of $^{14}N$ to $^{15}N$. The process of nitrification discriminates against ammonia, which is a heavier molecule. Thus, nitrate pools become depleted in $^{15}N$ and ammonium pools are relatively enriched (Koba *et al.* 2003). In a study by Feigin *et al* (1974), a difference of about 20 $^0/_{00}$ was observed between ammonia and nitrates in an agricultural field, although it is probably not usually as high as this. In nitrogen limited systems the demand for nitrogen is high and there is competition between plants and microbes for inorganic forms of nitrogen. If nitrogen were to become available, its uptake would be very efficient with all N taken up. If this were the case then fractionation between $^{14}N$ and $^{15}N$ would be very little. A further place where fractionation takes place is at the interface between microbes and plants. Soil microbes absorb nitrogen compounds from the soil and pass on nitrogen compounds that are depleted in$^{15}N$. The process is not fully understood and is dependant on the species of microbe and plant. Plants with ericoid and arbuscular mycorrhizae associations showed lower foliar $^{15}N$ values than non-mycorrhizal reference plants (Spriggs *et al*, 2003). This suggests that mycorrhizal fungi discriminate against $^{15}N$ during the transfer of N to plants and become relatively enriched compared to their associated plants. If there is long term cycling in a system, in this case a forest stand, and nitrogen products that are $^{15}N$ depleted are leaving the system in some form or other (e.g. leaching) then one would expect the system to have a higher ratio of $^{15}N$ to $^{14}N$. Forest systems are often closed with regard to nitrogen cycling as nitrogen is in such limited supply. However, with increasing anthropogenic nitrogen deposition, the introduction of earthworms, and increases in deciduous species, nitrogen supply has likely increased in Itasca State Park

Burning has been shown to influence the N cycle; increasing total N, potentially mineralizable N and $NH_4^+$ and $NO_3^-$ availability in surface soils (Choromanska & DeLuca, (2002). It can also cause severe N losses from temperate forests (Grogan *et al*, 2000) as well as carbon losses (Caldwell *et al* 2002). Burning would increase the openness of the nitrogen cycle through increased nitrogen inputs and nitrogen losses. However, in the long term, fire removes N from the ecosystem by volatising N from biomass. It also favours plants with low N concentrations, which promotes microbial immobilization (Reich et al. 2001). Yet, in the short-term, fire can increase N availability. Fire kills microbes in the top layer of soil due to the action of heat during a fire. This would increase certain pools of nitrogen compounds in relation to others depending on the nature of the microbial community. It also kills fire-sensitive plants, which would decrease the uptake potential of vegetation and increase pools of available N. Fire also alters the pH of soils through the addition of ash often making soils less acidic. This in turn affects the cycling of nutrients and the pools of different forms of nitrogen within the soil. If plants use less nitrate (which tends to be strongly depleted in $^{15}N$ due to fractionation during nitrification and as often leached from the system) after burning, due to the increased availability of ammonia, then plants will not be enriched. However, when nitrates are incorporated in plants in unburned sites then these would be $^{15}N$ depleted compared to burned sites as found by Grogan *et al* (2000).

In this study, we investigated if the nitrogen isotope ratio of roots could be used as an indicator of the openness of the nitrogen cycle within stands. Stands that had been burned were compared to stands that had not been burnt in order to see if they differed in the isotope composition of their roots. This study also investigated if coarse and fine roots differ in terms of their isotope signal or could be used interchangeably in future studies. Other factors were investigated as well such as the nitrogen concentration of roots, the carbon isotopic signal of roots and the depth and organic content of the A-horizon to see if these had possible influences on the $^{15}N$ value of roots. Stand densities were also recorded. Low-density stands (in terms of stem numbers) often have an open canopy and are thus a more open system with regard to nutrients and materials. Low-density stands are often older. This may mean that nutrients have been cycling within that stand for longer and thus may have a more enriched signal than younger stands, as a greater proportion of lighter elements have been lost from the system over time. The openness of the nitrogen cycle and the effects of burning have important consequences for *P.resinosa*. These trees are mostly found on sandy, nutrient poor sites. If stands are becoming nitrogen enriched due to nitrogen deposition from surrounding farming areas or due to the accumulation of organic matter in stands due to a infrequent and unnatural fire regime, then this may inhibit the recruitment of this tree.

## Methods

### Study site and sampling methods

Red pine (*Pinus resinosa*) stands within the Itasca State Park, Minnesota, USA were selected. Stands were selected by walking along official park trails. A site was considered appropriate when 90% of stems over 10cm diameter were *P.resinosa*. All stand sampled were at least 20m from the trail in order to avoid any possible effects of the trail on the site. Each site was over 100m from any other site but was usually separated by much larger distances (over a kilometre). 30 different sites were sampled (See Appendix 1 for GPS Co-ordinates and a brief site descriptions).

At each site a 10x10m plot was marked out. The diameter at breast height (dbh) for every tree over 10cm in diameter was determined using a dbh tape. Species other than red pine were also recorded (Appendix 1 contains the top three dominant species of the understory and midstory). The understory and midstory cover was quantified (low, medium or high). Evidence of recent burning was also noted. A soil corer was used in order to determine the depth of the A-horizon. This soil was then retained in order to determine the % organic content. A metal tube (diameter 53mm) was hammered into the ground up to a depth of 20cm at three places within each 10x10m site and the soil removed and combined. This bulk soil was retained for further analysis at the field station. At each hole created during this process I dug using a hand trowel to look for earthworms. If earthworms were found the number of worms was recorded.

Laboratory work

At the field station, each bulk soil sample was sieved and rinsed through a 2mm sieve. Any earthworms found at this stage were also recorded. Roots were separated into fine (<2mm) and coarse (>2mm) root material. This was done by floating the sieved material in a small bucket of water. Soil and stones were also removed from roots at this stage. The roots were then oven dried at 60°C for three days.

The A-horizon samples were sieved through a 2mm sieve and dried at 60°C for three days. They were then placed in crucibles and placed in a Muffle furnace that had been heated to over 400°C (The thermostat was not very accurate but the oven was at an acceptable temperature between 400°C and 600°C). Samples were heated in batches of eight as the oven space was limited and to avoid cross contamination of samples. Each batch was heated for an hour and a half. Samples were taken out of the furnace to cool and were re-weighed as soon as they were cool enough to work with. All samples were processed within a five-hour period. The loss on ignition (LOI) is determined by subtracting the post furnace weight from the pre-furnace weight and is an indicator of the organic content of the soil as the organic component ignites in the furnace and is lost as $CO_2$. This technique is accurate to with 1% for soils that have an organic content of over 10%.

The dried coarse and fine roots were analysed for nitrogen and carbon isotopes at the University of Cape Town's Mass Spectroscopy unit in the Archaeology department using a Finnegan MAT252 isotope ratio mass spectrometer attached to an elemental analyser (model NA 1500, Carlo Erba, Milan Italy). Samples were first ground using a grinder with a sieve size of 5microns. A subsample of 2mg was weighed using a scale with an accuracy of five decimal points. 16 Nasturtium standards (expected $^{15}N$ of 7.1, $^{13}C$ of –27.72) and 16 Acacia Saligna standards (expected $^{15}N$ of 0.43, $^{13}C$ of –27.56) were included in the analysis in order to correct for drift in the reference gas. $\delta^{13}C$ and $\delta^{15}N$ values were referred to the international standard VPDB (Vienna Pee Dee Belemnite). $\delta^{13}C$ ($^{0}/_{00}$) = ($R_S$ /$R_{VPDB}$ –1) x 1000 where $R_S$ and $R_{VPDB}$ are the molecular abundance ratios of carbon isotopes ($^{13}C$/ $^{12}C$) of the sample and the standard VPDB respectively. The same equation is used for nitrogen but the isotopes are $^{15}N$ and $^{14}N$. (See appendix 2 for the $\delta^{13}C$ and $\delta^{15}N$ for coarse and fine roots for each plot).

## Statistical Methods

Variables were tested for normality using the STATISTICA programme and Lilliefors test for normality. The only non-normal variable was the C: N ratio for coarse roots (Lilliefors $p<.01$) therefore the log of this data was used. The JMP statistical package was used for all other statistical analyses. These included regressions, stepwise backwards elimination regression and a principal components analysis. ANOVAs were also run on site factors between burned and unburned sites. General Linear Models were also run to determine which site factors contributed to the variation observed in $^{15}$N and %N.

# Results

## Isotope values for coarse and fine roots

The regression between $^{15}$N values for coarse and fine roots was significant (F=6.3, p=0.01) however there was much scatter in the data ($R^2$= 0.15). The regression between $^{13}$C values for coarse and fine roots was also significant (F= 6.9 p=0.01) but again there was a lot of scatter in the data ($R^2$ = 0.17). Neither %N nor %C correlated significantly with their respective isotopes for both coarse and fine roots (p>0.05, data not shown).

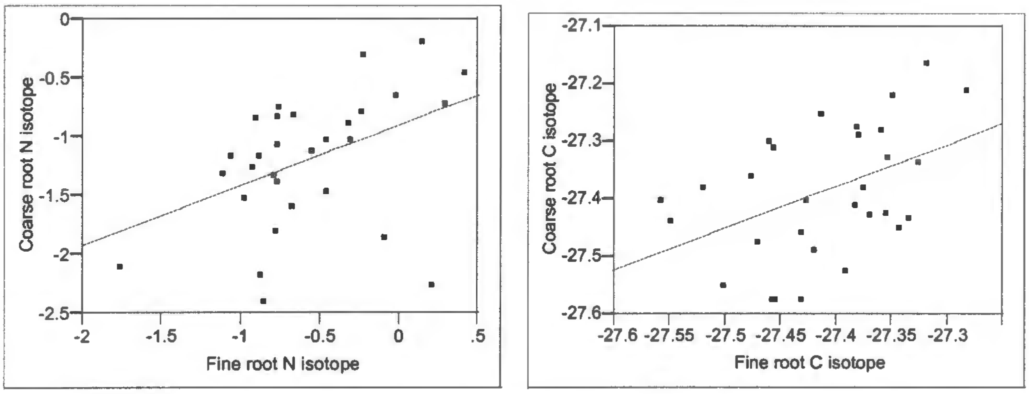

Figure (1). The correlation between fine and coarse roots for both nitrogen and carbon isotopes. Both correlations were significant at the p=0.01 level.

## The effects of burning

A one-way analysis of variance (ANOVA) was performed on each of the variables and for whether the site had been burned or not.

The %N in coarse roots differed between burnt and unburned sites but not significantly ($F = 3.72$ $P = 0.06$). %N in fine roots did not significantly differ between burned and unburned sites although unburned site do appear to have a larger range in % nitrogen than burnt sites.

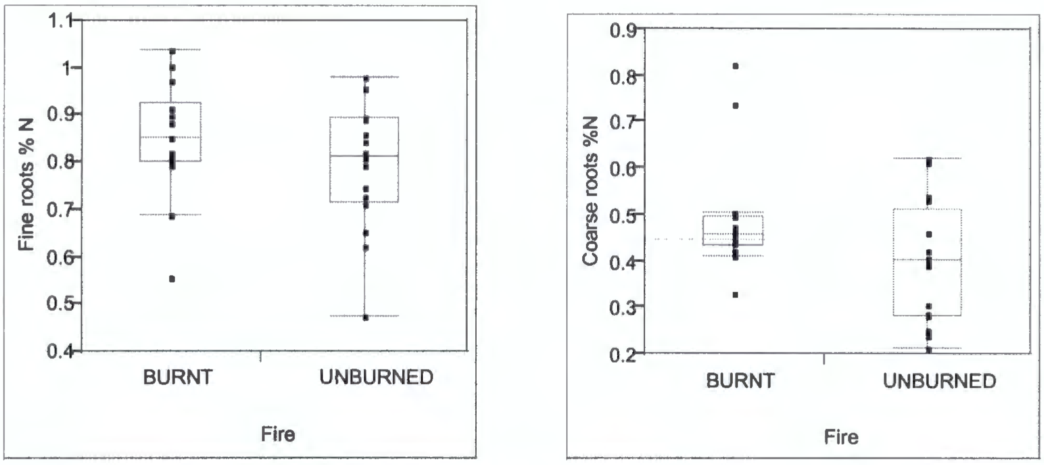

Figure (2) %nitrogen and the effects of fire on coarse and fine roots. The box and whiskers for all figures represent the data in quantiles.

Burnt and unburned sites differed significantly in their C: N for fine roots (F=6.6, p=0.01). This relationship was significant for the log of coarse roots (F=6.2 p<0.02). Unburned sites have more variation in the C: N ratio than burned sites. Burned sites have a significantly lower amount of carbon relative to nitrogen for coarse and fine roots.

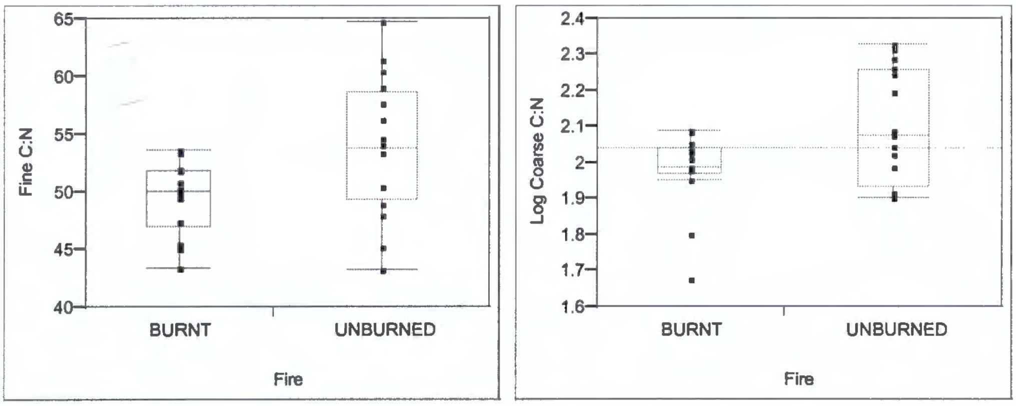

Figure (3). The relationship between the amount of carbon to the amount of nitrogen found in coarse and fine roots for burned and unburned sites. The log of C: N was used for coarse roots, as the data was not normally distributed.

The nitrogen and carbon isotope value for coarse and fine roots for burnt and unburned sites was also compared. The following figure shows the relationship (See Fig 4). There are no significant differences between burnt and unburned sites and their N isotope values for coarse or fine roots

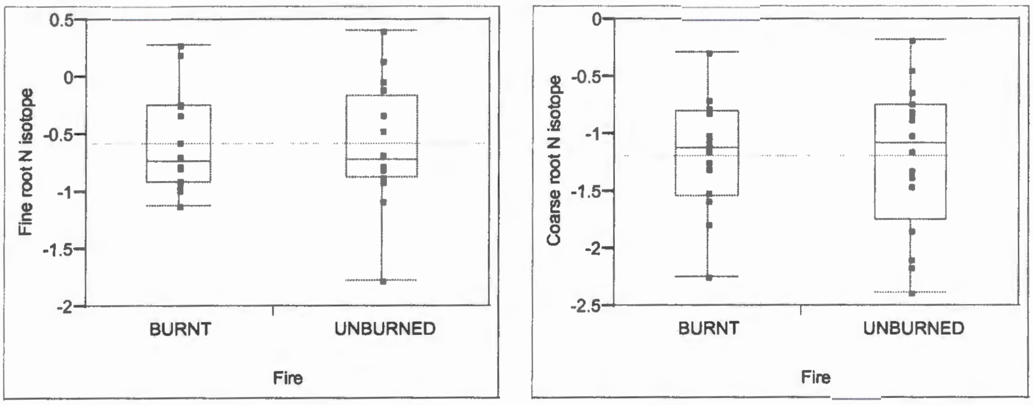

Figure (4). The N isotope range for coarse and fine roots for burnt and unburned sites.

The data was investigated to see if there was a relationship with the depth of the A-horizon and the N isotope signal for coarse and fine roots. Roots were extracted from a depth of up to 20cm while the depth of the A-horizon varied for each site. Figure (5) presents the results.

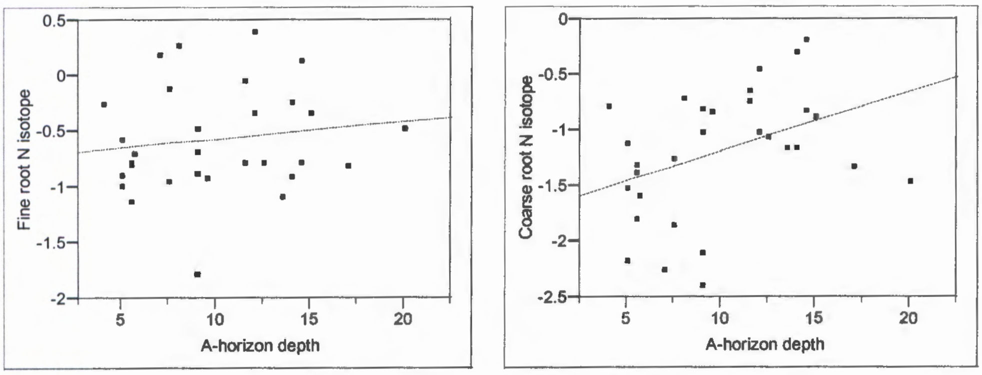

Figure (5). The relationship between the nitrogen isotopic signature of coarse and fine roots and increasing depth of the A-horizon (in cm). The relationship is not significant for fine roots ($F=0.50$ $p<0.5$) but is significant for coarse roots ($F= 4.96$, $p<0.05$).

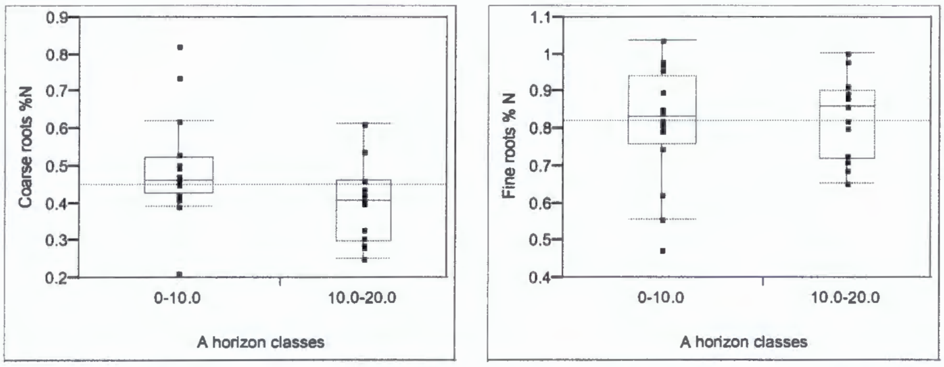

Figure (6). The effects of the depth of the A-horizon on coarse and fine roots % nitrogen. The A-horizon was grouped into two classes 0-10cm depth and 10-20 cm depth.

Fine roots have a higher % nitrogen than coarse roots and the depth of the A-horizon has no significant impact on the % nitrogen of fine roots. Coarse roots have a lower % nitrogen and this decreases as the depth of the A-horizon increases. This is almost significant (F= 3.94 p=0.057).

The effects of burning on the depth of the A-horizon are not significant but there does seem to be a strong trend with burned sites having a shallower A-horizon (see Fig 7).

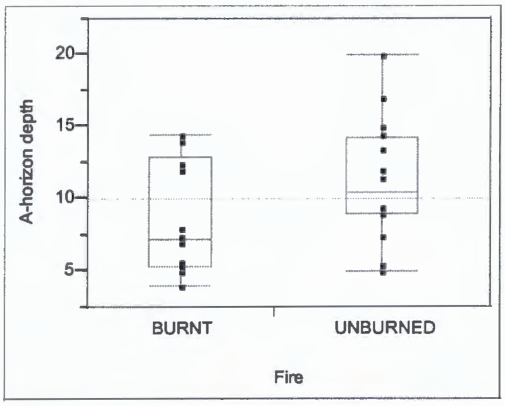

Figure (7). The depth of the A-horizon (in cm) for burned and unburned sites.

Unburned sites have a larger variance around the mean than burned sites and there is a trend that they have a deeper A-horizon as well although this is not significant (F=3.08, $p<0.1$).

The correlation between % Organic content and depth of the A-horizon is significant ($F= 5.92$ $p< 0.05$) but there is much variation ($R^2= 0.14$). It is unexpected that as the A-horizon gets deeper the % organic content decreases.

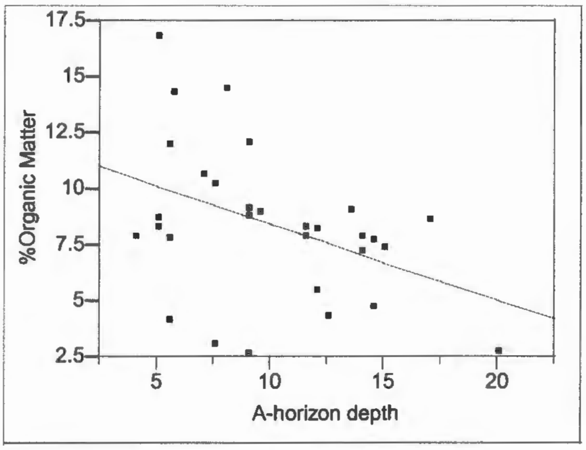

Figure (8). The relationship between the A-horizon depth (in cm) and the % organic content of the A-horizon.

Environmental factors, $^{15}N$ and %N

Models were run to see the effects of the different variables on both $^{15}N$ and %N for both coarse and fine roots. The depth of the A-horizon significantly affected the $^{15}N$ of coarse roots ($R^2= 0.5344$, $F=4.96$ $p< 0.05$). % Organic matter explained a significant amount of the variance in %N in fine roots ($R^2 = 0.12$, $F=4.97$, $p<0.05$). % Organic matter also explained a significant amount of the variance in %N for coarse roots ($R^2=0.17$, $F=7.02$ $p=0.01$). The ratio of the % Carbon to % Nitrogen in sites was also affected by burning ($R^2=0.16$, $F=6.6$ $p=0.01$) for fine roots.

A stepwise backwards elimination regression was performed using the JMP model function for isotope values for fine and coarse roots. It was found that for fine roots the best predictors of their isotope signature were (in decreasing importance) the isotopic signature of the coarse roots, the % nitrogen of the fine roots and the average dbh for all tree species on the site. The model was significant ($F= 3.58$, $p<0.05$) but explained only 21% of the variation observed in the nitrogen isotope value for fine roots. As the isotope value for fine roots increased, so did the value for coarse roots.

As the % nitrogen of fine roots increased, fine roots were more enriched in ${}^{15}$N. As the average dbh for all tree species on the site increased so fine roots were more ${}^{15}$N enriched.

For coarse roots, similar factors explained the variation in their isotopic signature. The factor that best predicted the isotope value of coarse roots was the value of fine roots at the same site. As the value for ${}^{15}$N increased for fine roots coarse roots ${}^{15}$N increased as well. The % of nitrogen in coarse roots was the next best predictor. However, the trend was opposite to that of fine roots. As the % nitrogen of coarse roots increased, they were relatively depleted in ${}^{15}$N. The average dbh of *P.resinosa* was the third factor. As the dbh of pines increased the coarse roots were relatively enriched in ${}^{15}$N. The model was significant ($F= 4.04$ $p<0.02$) and $r^2$ (adjusted) = 0.239 thus the model explains 24% of the variation in the isotope signal of coarse roots. For neither coarse nor fine roots was burning a significant factor that influenced the root isotope value.

The relationship between the number of tree stems per plot (stems over 10cm in diameter) and the average diameter of all trees on the plot and for *P.resinosa* alone was investigated.

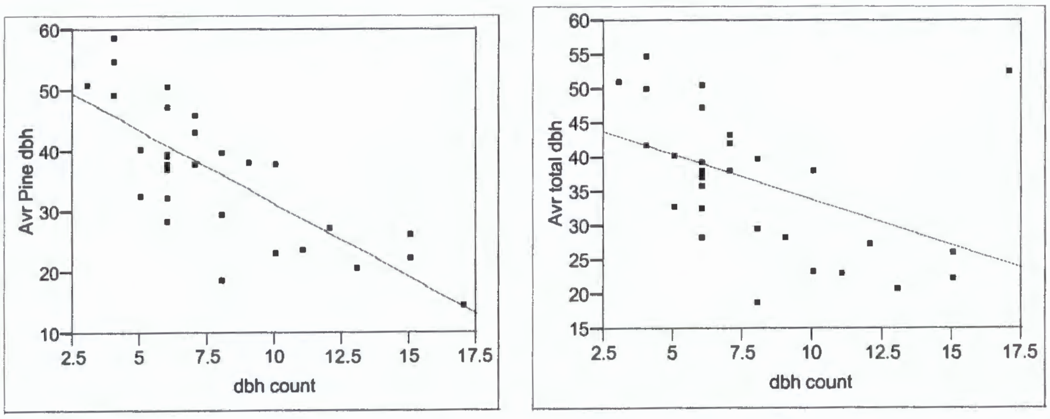

Figure (9). The relationship between the number of trees in a plot and the average dbh of trees in the plot. On the left, is the relationship for *P.resinosa* alone. On the right, we have the relationship for all trees over 10cm in diameter regardless of species.

The relationship for *P.resinosa* alone, and for all trees combined, is significant (F= 41.87, $p<0.0001$ and F= 7.80 $p<0.01$ respectively). Thus, the fewer trees in a plot the larger the average size of the trees.

## Principal Components Analysis

A principal components analysis (PCA) was run on the environmental data for each site and the nitrogen and carbon information (see Table 1). The two main axes were rotated to strengthen contrasts (see Table 2). The first axis explains 24% of the variation in sites; the second axis explains 20%. Based on the eigenvectors, the first axis separates sites based in part on burn history. Sites with few numbers of large trees have a deep A-horizon, large fine root biomass, high organic matter content, and both fine and coarse roots have high N concentrations. The PCA rotated factor scores were saved (Table 2) and used in analyses with burning and other variables. Burned sites score more positive on axis 1 (F= 3.45 p=0.07).

Table 1. Multivariate Principal Components Analysis for site and nitrogen variables. Isotope values are also included. Only axis 1 and 2 are shown.

| | Axis 1 | Axis 2 |
|---|---|---|
| Eigenvalue | 3.1206 | 2.5105 |
| Percent | 24.0045 | 19.3113 |
| Cum Percent | 24.0045 | 43.3159 |
| Eigenvectors | | |
| Dbh count | -0.44013 | -0.01878 |
| Average total dbh | 0.34849 | -0.00853 |
| Sum cross sectional surface area (m2) | 0.31269 | 0.14934 |
| % Organic Matter | 0.42639 | 0.18402 |
| A-horizon depth | -0.28348 | 0.29416 |
| Fine Root biomass | -0.19434 | 0.02327 |
| Coarse Root biomass | 0.07760 | 0.20200 |
| Fine root C isotope | 0.13319 | -0.44624 |
| Coarse root C isotope | 0.14813 | -0.37668 |
| Fine root N isotope | -0.01860 | 0.40631 |
| Coarse root N isotope | -0.17133 | 0.38717 |
| Fine roots % N | 0.24327 | 0.38837 |
| Coarse roots %N | 0.39056 | 0.09326 |

Table 2. The eigenvectors for site and isotope variables for the first two axes that have been rotated to strengthen contrasts.

| | | |
|---|---|---|
| Rotated Factor Pattern | -0.762279 | 0.155967 |
| Dbh count | | |
| Average total dbh | 0.594749 | -0.159514 |
| Sum cross sectional surface area (m2) | 0.592801 | 0.098497 |
| % Organic Matter | 0.800956 | 0.104102 |
| A-horizon depth | -0.375585 | 0.571791 |
| Fine Root biomass | -0.324698 | 0.117437 |
| Coarse Root biomass | 0.209258 | 0.278281 |
| Fine root C isotope | 0.060409 | -0.742709 |
| Coarse root C isotope | 0.112260 | -0.641930 |
| Fine root N isotope | 0.121166 | 0.633120 |
| Coarse root N isotope | -0.148121 | 0.667824 |
| Fine roots % N | 0.563732 | 0.495522 |
| Coarse roots %N | 0.705273 | -0.020515 |

The second axis separates stands based on the openness of the N cycle. Stands that are enriched in $^{15}N$ in coarse and fine roots are depleted in $^{13}C$, have a deep A-horizon, and high fine root N concentrations. There was no significant difference between burned and unburned sites in axis 2 scores (F= 0.14 p<0.8).

## Discussion

### The correlation between coarse and fine roots

Studies often focus on the N isotope values of soils, whole plants or leaves (Evans 2001) in an attempt to understand nitrogen acquisition and allocation from source to plant. Few studies have looked at the isotopic signature of roots. If roots are examined they are usually not separated into different sizes or fine roots alone are examined reported and the literature (e.g. Currie & Nadelhoffer 1999). In order to investigate if fine roots are representative of coarse root nitrogen isotope signals (and vice versa) the isotope signal of fine versus coarse roots was determined for nitrogen and carbon. Figure (1) presents the results. For both carbon and nitrogen isotopes there is a significant correlation between coarse and fine roots (F= 6.9 p=0.01 and F= 6.3 P= 0.01 respectively) however for nitrogen only 15% of the variation in coarse roots isotope values could be determined from the value of the fine roots. For carbon isotopes, the value was 17%. Thus, when evaluating the isotope signal of roots in this study both fine and coarse roots are considered separately.

There could be numerous reasons for the scatter observed in this data. A study by Nadelhoffer & Fry (1988) showed that the nitrogen isotope signal of the soil varied with depth as shallower soils were depleted in $^{15}N$ due to leaf litter inputs that were also depleted. Deeper soils were relatively enriched in $^{15}N$ due to increased decomposition of $^{15}N$-enriched products. Both fine and coarse roots within a single plot could thus be assimilating and incorporating nitrogen isotopes that have slightly different signal according to depth of the root. Högberg (1997) shows that there is also little transfer of N from roots in one soil horizon to another. Thus roots in the A-horizon could well have a different isotope value from roots below this as the A-horizon was never as deep as the depth sampled by the root corer (20cm).

The roots used in this analysis were also not all from *Pinus resinosa*. Other tree and forb species were present on most plots. Although *P.resinosa* made up at least 90% of all stems over 10cm in diameter many smaller trees and shrubs were present on some plots especially in the understory and their roots could well be included in root cores. Root cores were taken in three places within each plot up to a depth of 20cm. It has been shown that different species using the same nitrogen source can have different isotope values for leaf tissue that vary between 0-10$^{o}/_{oo}$ (Handley & Raven 1992). This may also be the case for roots although I have not found a study that reports this. Microbes associated with the roots may have been included with the roots and ground up as well. Studies have shown that plants and their associated mycorrhizae may differ as much as 8 $^{o}/_{oo}$ (Högberg 1997). Thus there are several possible reason for why there is a lot of scatter on the correlation between coarse and fine roots but the there is an overall significant trend with coarse and fine roots having similar values.

### The difference in % nitrogen of fine and coarse roots

Roots from sites that were burnt were compared with roots from sites that were unburned for differences in their % nitrogen. There was no significant difference for fine roots and an almost significant difference for coarse roots (F=3.72 p=0.06) see Fig (2). Roots from sites that had been burned had higher % nitrogen than unburned sites for fine roots and possibly for coarse roots as well. This is expected as burning creates a post burn flush in nitrogen. When a site is burnt, nitrogen that is in an organic from in either plant litter or humus is volatised or oxidised. Ash is also deposited on the forest floor from combusted plants and litter. The effects of wildfire ash residues on soils were investigated by Grogan *et al* (2000). It was found that ash stimulated post fire production and ecosystem nitrogen retention as well as increasing $NH^+_4$pools. All of these factors affect the nitrogen content of the soil. Most studies report an increase in $NH^+_4$-N and $NO_3^-$ -N in burned soils (Christensen 1973, Christensen & Muller 1975, Grogan *et al* 2000) especially in nitrogen limited systems (Stock & Lewis 1986). Fire can also alter the chemical and physical properties of soil and thus indirectly alter the cycling of nitrogen within the soil (Raison 1979). Grogan *et al* (2000) showed the ash increased the mean pH of soil from 4.6 to 5.2, a result also found to Raison (1979). This would increase the below ground cycling of nitrogen enhancing the $NH^+_4$ pools in burned soils.

*P.resinosa* are found on sandy nutrient poor soils. One would assume that inorganic nitrogen is in limited supply. If inorganic nitrogen was added to the system in small amounts, soil bacteria could rapidly assimilate it. If inorganic nitrogen was added in large amounts, as what happens during the post burn nitrogen flush, then the roots may be able to access some of it directly, otherwise they would later be able to access it from the bodies of decomposing soil bacteria. Thus, there is generally a fluctuating availability of inorganic nitrogen after the post burn flush due to the absorption and depletion of nitrogen followed by a later release of nitrogen by microbes when they die. $NH^+_4$ pools are originally higher after a fire but decrease as nitrifying bacteria converts them into $NO_3^-$.

Coarse roots % nitrogen is almost significantly different between burned and unburned sites (see Fig 2, F= 3.72 p=0.06) but this relationship is not found for fine roots. This could be due to the amount of time that has passed since the stands were burnt. The nutrient flush would have been assimilated into the roots shortly after the fire (Stock & Lewis, 1986). Coarse roots are normally longer-lived than fine roots (although little work has been done on the longevity of roots). Both coarse and fine roots may have absorbed the available post-burn inorganic N but the fine roots would have died sooner and their nitrogen either recycled into the soil or reabsorbed into the plant. The fine roots that were analysed are possibly from a later, more limited nitrogen environment. The coarse roots may still carry the signature of an earlier, more N rich soil environment, created by the post burn N flush. There is a large range in the % nitrogen of coarse roots in unburned sites while the range is much lower for burned sites. This trend exists for fine roots as well. This indicates that they may be other factors influencing nitrogen availability to roots. These could be topographic differences between sites (which would affect the openness of the nitrogen cycle) or site-specific nitrogen cycling differences

In tandem with the correlation between % nitrogen and fire status, there is also a relationship between fire and the % carbon to % nitrogen ratio found in coarse and fine roots (see Fig 3. For fine roots, burned sites had a significantly smaller C: N ratio than unburned sites (F=6.59, p=0.015) as did coarse roots (F=6.20, p=0.019). This could be explained for coarse roots due to the post-burn nitrogen flush increasing the amount of N relative to C. However, this explanation does not account for the result for fine roots as %N did not significantly differ between burned and unburned sites. This suggests that the other variable in the ratio could also be responsible for the significant relationship. With %N being higher in post-burn sites, %C could also have been lower as fire could have lowered the amount of carbon within the soil due to combustion (Caldwell *et el* 2001). This effect has been noted even after 50 years have passed since burning (Parker *et al* 2001). So not only does fire appear to cause a post burn nitrogen flush, it also lowers the amount of carbon available in soils.

### The effects of fire on nitrogen isotopes

Fire affects nitrogen cycling. When a fire moves through a forest it burns up organic matter and through chemical and physical process cause a nitrogen flush in the soil. This flush adds inorganic nitrogen to the soil that would be lacking in a low nutrient system. Soil microbes and plant roots would rapidly assimilate this resource causing a relative depletion in inorganic nitrogen. When these microrganisms die, they release this nitrogen back into the soil resulting in a second smaller flush in inorganic nitrogen. This process continues, resulting in the fluctuating availability of inorganic nitrogen. It will also result in the fluctuating availability of different forms of nitrogen as microbes and plants use and release different forms and at different times. The form of nitrogen used by plants also affects their $^{15}N$ signal. The process of nitrification discriminates against ammonia, which is heavier. Thus, nitrate pools become depleted in $^{15}N$ and ammonium pools are relatively enriched (Koba *et al.* 2003). If a plant was preferentially using ammonia pools then it would become $^{15}N$ enriched. If roots were being formed at this time, I would assume this signal would be incorporated in the root tissue. When the data for the *P.resinosa* stand is examined (see Fig 4) the fine and coarse root values for $^{15}N$ are not significantly affected by burning ($F=0.017$ $p>0.8$ and $F=0.008$ $p>0.9$). As the sites were burnt at different times they could be in different stages of post-burn inorganic nitrogen fluctuation and using different forms of nitrogen as described above and thus do not have a recognisable isotopic signal in common. If the fine roots of each site were sampled soon after a burn (within a month), it is more likely that they will show a $^{15}N$-enriched signal due to the use of post burn available nitrogen. Using the $^{15}N$ signal of coarse and fine roots to determine the openness of the nitrogen cycle is made complicated due to the changing nature of the resource used by roots within soil.

In another study on the effects of fire on ecosystem cycling of nitrogen in a bishop pine forest (*Pinus muricata*), $^{15}N$ abundance in plant foliage did not differ from the $^{15}N$ abundance of the bulk soil organic matter at sites that had been recently burned (Grogan *et al* 2000). However, unburned sites plant foliage was $^{15}N$ depleted relative to the total soil N pool at the site. This was thought to be due to the increased availability of $NH_4$ after burns. Plants preferentially used this and nitrites were lost to the system. However, in unburned sites, nitrites were being incorporated in plants and hence they had a depleted signal. In the current study it is possible it that if the $^{15}N$ abundance of the soil and leaves was determined a similar result may have been found. However, within roots alone no pattern of $^{15}N$ values and burning was found. It is possible that *P.resinosa* in this study differ in their nitrogen assimilation and discrimination to *P. muricata*. If a future study were made on *P.resinosa,* it would be useful to determine the $^{15}N$ abundance of the soil N pool and of the leaves in order to determine if the same patterns exist as those found for *P. muricata*. Nitrogen compounds may be so limiting in Itasca that any nitrogen added to the system, whether it be nitrite or ammonium would be assimilated by plants and thus post burn differences between sites would be more complex and thus no real pattern emerges with burning and $^{15}N$ values.

## The effects of depth on nitrogen isotopes

Microbes that decompose organic matter can also affect the $^{15}N$ content of a soil. If they preferentially pass on $^{14}N$ to $^{15}N$ to plants and these compounds were transported elsewhere e.g. incorporated within organic tissue, and thus temporarily removed from the soil system, then the soil would become $^{15}N$ enriched as a result. A study has shown that deeper soils are more $^{15}N$ enriched due to the microbial decomposition while shallower soils reflect the N signature of leaf litter inputs that are usually $^{15}N$ depleted (Nadelhoffer & Fry, 1988). Thus, one would expect a changing $^{15}N$ signature with depth. In this study, sites differed in their A-horizon depth. It was found that sites with a deeper A-horizon had a relatively enriched $^{15}N$ signal for coarse roots ($F=4.02$ $p<0.05$ see Fig 5). This relationship was not significant for fine roots.

Fine roots are already relatively enriched compared to coarse roots (see Fig 5). They do also not appear to differ in their % nitrogen with depth (see Fig 6). It is possible that fine roots are relatively enriched in $^{15}N$, and have a higher % of nitrogen that does not differ with depth of the A-horizon, as they are utilising a different resource to that of coarse roots that does not differ in availability with depth. Due to the $^{15}N$ enriched nature of fine roots one could speculate that this might be ammonia as nitrate pools become depleted in $^{15}N$ and ammonium pools are relatively enriched in $^{15}N$ when nitrification takes place (Koba *et al.* 2003). Coarse roots may become enriched with depth due to the effects of microbial decomposition in deeper soils creating a more enriched nitrogen product or it is possible that deeper A-horizons are formed in older soils where nitrogen has been cycling for longer and thus is more $^{15}N$ enriched. It is not possible to say which process accounts for the difference observed.

Fine roots may also be more enriched due to possible microbial associations that are uncommon in coarse roots. Mycorrhizae are formed on the roots of red pine seedlings by *Boletinus pictus, Tylopilus felleus, Cenococcum graniforme, Gomphidius superiorensis, G. vinicolor*, several species of *Suillus*, and *Scleroderma aurantium* (Hepting 1971, Peterson and Smith 1975, Palm and Stewart 1984, Richter and Bruhn 1986). These may improve the uptake of soil moisture and mineral nutrients. As these mycorrhizae pass on nitrogen to the roots of plants, they become $^{15}N$ enriched. If these mycorrhizae were ground up with the roots and included in the analysis, fine roots would appear more enriched. If they are using the decomposing microbes as a source of nitrogen, they will also be enriched in $^{15}N$. One would predict that these mycorrhizae are present regardless of the depth of the A-horizon. Thus, there are two possible reasons for the enrichment of fine roots relative to coarse roots. This is either due to the difference in nitrogen pools being used or due to microbial association with fine roots and not coarse roots. Further research could help clarify this issue to determine if fine roots are using different nitrogen source to coarse roots. It could also clarify if microbial association with fine roots is affected by depth. If so, then it is more likely that fine roots are enriched due to using a different source rather than microbial association. It is also possible that both effects are taking place and that fine roots are preferentially using ammonia as the associated microbes preferentially absorb this resource.

### The effects of burning on the A horizon

There is a trend that sites that were burnt had a smaller A-horizon than unburned sites (see Fig 7) but this is not significant ($F= 3.08$ $p<0.1$). Burnt sites also appear to have a smaller range in A-horizon depth. In these *P.resinosa* stands, earthworms are a new introduction and there is reason to believe that they have not invaded all stands (personal observation, transects dug during class work). This means that organic matter accumulates on the surface of the soil. Other agents may break down this litter but the action of earthworms is probably faster. If a stand burns then fire consumes this organic matter. Although there is a post-burn pulse in nitrogen, the organic matter is lost and thus the humus layer would probably decrease. This could explain why stands that have burnt have shallower A-horizons.

### The relationship between A-horizon depth and % organic matter

When the % organic matter was examined for soils with different A-horizon depths an interesting pattern was found (see Fig 8). Soils with a deeper A-horizon had a lower organic content ($F=5.92$, $p<0.05$). One might expect soils with a higher organic content to have a deeper A-horizon. It may be that the older soils are deeper and over time, more of the organic content has been decomposed and used by plants. Alternatively, it is possible that the absolute amount of organic matter for deeper soils is indeed greater but that the relative amount is less as deeper soils have a much larger soil volume. It is also possible that there is no cause and effect relationship between % organic content and A-horizon depth and that the depth of the A-horizon is determined by other factors such as the age of the stand. The % organic content might be a short-term effect of leaf litter inputs and thus soils that have a deeper A-horizon have a lower %organic content as the surface input has to be redistributed over a much larger volume of soil. As earthworms are not a natural agent in this ecosystem, few other organisms would be able to distribute organic mater from the surface deep into the soil. This relationship might change if earthworms become more abundant resulting in the organic content of soils with deep A-horizons increasing. This would have impacts on nutrient availability and might impact on the relative abundance of nitrogen in different soil pools when this matter decays.

### Factors that effect the % nitrogen of roots

When a model was run to look at the effects of % organic matter on % nitrogen, it was found that there was a significant relationship for both coarse and fine roots (F= 7.02 $p=0.01$ and F= 4.97 $p<0.05$). It is not certain if the roots have a higher % nitrogen because there is more organic matter in the A-horizon to supply them with nitrogen or if it is that the roots are of a high quality and thus when they decompose produce organic matter with a higher % nitrogen. Either way, by removing organic matter from the system by burning, in the short-term increases the amount of available nitrogen, but in the long term, may decrease the amount of total nitrogen in the soil (Grogan 2000). This may favour *P.resinosa* as this tree is found on nutrient poor sites. It is a poor competitor for light and space and thus is successful in areas where it encounters little competition as one would find in a nutrient poor environment. It may be that the post fire environment initially favours other plants over *P.resinosa* but that once the post burn flush in nitrogen has been assimilated and plants can not longer survive in the nitrogen limited system, *P.resinosa* can take off. Fires could be used in stands where *P.resinosa* may recruit in order to limit the accumulation of organic matter and to minimise competition from other plants that are less fire tolerant.

### Factors that affect the $^{15}N$ of roots in this study

The factor that best explains the $^{15}N$ values of fine roots in this study was the $^{15}N$ of coarse roots (stepwise backwards regression, F= 3.58 $p<0.05$) and vice versa for coarse roots (F= 4.04 $p<0.02$). As the isotope value for fine roots increases, so does the coarse root value. This was shown in Fig (1) and is significant for both nitrogen and carbon isotopes for roots (F= 6.3 and F= 6.9 respectively, $p=0.01$ for both). This would indicate that they are using a similar source of nitrogen or using similar proportions of different sources of nitrogen. However, if one investigates the slope of the equation for the graph although the isotope value is increasing concomitantly, the value for fine roots is less negative than coarse roots (Coarse root N isotope = -0.89865 + 0.5106147 Fine root N isotope). For example for a fine root $^{15}N$ value of –0.5, coarse roots would have a value of –1.15. Thus, fine roots are relatively enriched in $^{15}N$ compared to coarse roots although the isotope signal for fine and coarse roots increases in step.

For both classes of roots % nitrogen was the next best predictor of the $^{15}N$ of the roots. As the % nitrogen increased for fine roots $^{15}N$ was more prevalent whereas for coarse roots as % nitrogen increases roots were $^{15}N$ depleted. Why would coarse and fine roots show the opposite trend? It is possible that again there is a difference in the source of nitrogen being used. Fine roots were possibly using a source of nitrogen that was $^{15}N$ enriched while coarse roots may be using a $^{15}N$-depleted source. Nitrite pools are depleted in $^{15}N$ while ammonium pools are relatively enriched (Koba *et al.* 2003). Thus, it is possible that fine roots are preferentially using ammonia and coarse roots are using nitrites. This could explain why there is such poor explanatory power ($r^2 = 0.15$) when predicting the N isotope value of coarse roots from fine roots (Fig 1). However there is still a significant correlation between fine and coarse roots isotope values thus sources of nitrites and ammonia must decreases and increase together. This would suggest that if fine and coarse roots are using different pools of nitrogen, the size of the nitrite and the ammonium pool should be similar, as otherwise one would expect there to be no relationship between coarse and fine root isotope values.

The third variable that explained some of the variation in the isotope values of roots was average dbh for all tree species for fine roots and the average dbh for *P.resinosa* for coarse roots. Fig (9) shows the relationship between the average dbh of pines and the average dbh of all species on a plot as related to the number of trees per plot. There is a significant relationship for both measures ($F= 41.87$, $p<0.0001$ and $F= 7.80$ $p<0.01$ respectively). Plots that have trees with larger average dbh have fewer trees per plot. Thus, the number of tree per plot could be affecting the $^{15}N$ of roots. This could be due to stand openness, or due to the age of plots, since trees with larger diameters are older. As the dbh of pines increased the coarse roots were relatively enriched in $^{15}N$. As the average dbh for all tree species on the site increased so fine roots were more $^{15}N$ enriched. Thus with increasing dbh, or stand openness, or stand age, roots were $^{15}N$ enriched. These three different factors are all related so they don't need to be treated separately. As the stand ages and opens up, and nitrogen is lost from the system, the soil becomes $^{15}N$ enriched (due to microbial discrimination transferring relatively $^{15}N$ depleted products to plants which are later lost). Thus, the stand becomes relatively enriched with $^{15}N$. This enrichment increases over time. In addition, as the stand opens up even greater losses of depleted nitrogen occur, thus adding to the enrichment effect.

### PCA data for site and isotope data

The two main axes of the PCA run on the environmental data for each site explained a little less than 45% of the variation in sites. The first axis separated out sites based on burn history. Sites with few large trees have a deep A-horizon, large fine root biomass, high organic content and both fine and coarse roots with high N concentrations. There is an almost significant difference between burned and unburned sites along axis 1 ($F= 3.45$ $p=0.07$). Stands may have fewer trees as some may have been burned during the fire. Burned sites may have a larger fine root biomass in order to better capture the post-burn nitrogen flush. The high nitrogen concentrations of roots could also be due to this effect. The high organic content of the soil could be due to the large fine root biomass. As roots die and break off, they become incorporated into the soil and part of the organic content of the soil.

The second axis of the PCA is based on the openness of the N cycle. Stands are enriched in $^{15}N$ in coarse and fine roots and are depleted in $^{13}C$, have a deep A-horizon, and high fine root N concentrations. There was no significant difference between burned and unburned sites in axis 2 scores ($F= 0.14$ $p<0.8$) thus burning does not affect this axis. These sites may have an open nitrogen cycle resulting in nitrogen being lost from the system and thus the stand become relatively $^{15}N$ depleted. These stands may also differ in the relative availability of nitrites to ammonia, which would also result in a higher isotope value for roots if ammonia were more abundant. It is not possible to disentangle the two effects. The stands are depleted in $^{13}C$, which implies a low water use efficiency (Pate, 2001). These stands could have a deep A-horizon due to the sites topography e.g. in a depression or the base of hills where organic matter and soil (and water) could accumulate. A higher water table would explain the low water use efficiency. Plants may have high root nitrogen concentrations as plants may be investing in roots in order to obtain more of the nitrogen resource or because there is a higher nitrogen availability in these stands. Elevated nitrogen availability also leads to $^{15}N$ enrichment of soil pools, as $^{14}N$ is lost through leaching and denitrification, leaving the soil N pool relatively enriched over time (Dawson *et al* 2002).

## Conclusion

Burning does not appear to affect the proportion of nitrogen isotopes found in roots in the Itasca State Park. Thus, nitrogen isotopes are not a good indicator of nitrogen cycling openness as caused by burning. Burning does decrease the C: N ratio for fine roots, and the log of coarse roots. It may also decrease the depth of the A-horizon (although this was not significant). It causes an increase in nitrogen availability after a fire but may ultimately decrease the total availability of nitrogen in a system as fire volatises organic matter (Caldwell *et al* 2001) and windblown ash is transported elsewhere (Grogan *et al* 2000). Thus, fire could be a useful management tool to combat increasing soil fertility due to anthropogenic inputs. More research would need to take place to establish if this is possible and what the effects of increased fire will be on other stand biota and nitrogen pools.

Factors other than burning appear to be more important in determining the isotopic signature of roots. The isotope values of fine and coarse roots are related but fine roots are relatively enriched. The reasons for this are unclear, as one would expect the openness of the nitrogen cycle to affect coarse and fine roots equally. Fine and coarse roots also differed in their response to increased nitrogen. As the % nitrogen increased for fine roots, $^{15}N$ was more prevalent, whereas for coarse roots as % nitrogen increases, roots were $^{15}N$ depleted. This suggests that fine roots may be preferentially using ammonium, which is relatively $^{15}N$ enriched. However, for the relationship between the fine and coarse roots isotope values to still hold, the proportion of ammonia to nitrite must be related. With the data that has been collected, it is not possible to determine which hypothesis is correct. A possible way to discriminate between the two in future studies would be to try and quantify the ammonia and nitrite pools in the different sites, and determine the relative importance of nitrification in stands. One would have to test if coarse and fine roots are using different sources of nitrogen or differ in isotope signal due to temporal variability in roots lifespan and fluctuating nitrogen availabilities of different nitrogen forms.

Stand age also has a small effect on the $^{15}N$ value of roots. This suggests that older stands may indeed be more open in their nitrogen cycling. This would result in older stands' soils being more $^{15}N$ enriched as $^{15}N$ depleted products have been preferentially lost from the system over time due to the discrimination of mycorrhizae associated with roots. Other factors may also be affecting the $^{15}N$ signal of roots though it is not clear what these factors are. They could be the rate of nitrogen cycling or the topography of sites. Isotopically enriched sites are associated with high water availability indicating that topography may be the preliminary factor resulting in secondary elevated nitrogen cycling, although this will require further investigation. Determining the rate of nitrogen cycling in stands of different ages and different site characteristics would aid the interpretation of the results.

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## Appendix 1. Site descriptions

| Site | Trail | Latitude (`N) | Longitude (`W) | % Red pine | Understory density | Understory species | Midstory density | Midstory species | Fire | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | La Salle | 47.13.65 | 95.11.15 | 100 | low | None | none | None | UNBURNED | Sparse |
| 2 | La Salle | 47.13.74 | 95.11.10 | 100 | low | Tillia | none | None | UNBURNED | Sparse |
| 3 | La Salle | 47.13.78 | 95.10.68 | 90 | low | None | none | 1 aspen | UNBURNED | Marginal, v sandy, till |
| 4 | La Salle | 47.14.02 | 95.11.31 | 90 | low | Grass, oak, ash | med | Fir, ash | UNBURNED | Soil v compact |
| 5 | Beach | 47.14.26 | 95.11.90 | 100 | med | Forbs, oak, tillia | low | Birch | BURNT | |
| 6 | Beach | 47.14.26 | 95.11.90 | 100 | med | Forbs, tillia, oak | none | None | UNBURNED | |
| 7 | Indian mounds | 47.14.43 | 95.12.44 | 95 | high | Forbs, tillia, grass | none | None | UNBURNED | |
| 8 | Headwaters | 47.14.43 | 95.12.44 | 95 | med | Forbs, Tillia, fir | low | Fir, ash, | UNBURNED | |
| 9 | Bert's cabins | 47.14.30 | 95.14.00 | 100 | med | Ferns, hazel | none | None | BURNT | |
| 10 | Bert's cabins | 47.14.33 | 95.14.12 | 100 | low | Sedge, hazel | none | None | BURNT | |
| 11 | Landmark | 47.13.51 | 95.15.27 | 100 | med | Ferns, | none | None | BURNT | |
| 12 | Bohall | 47.12.80 | 95.15.27 | 100 | low | Hazel | low | Hazel | UNBURNED | Contrast to site 13. |
| 13 | Bohall | 47.12.81 | 95.15.22 | 100 | med | Herbs | none | None | BURNT | Burnt contrast to site 12 |
| 14 | CCC | 47.12.53 | 95.15.44 | 100 | low | Herbs | low | Maple | UNBURNED | 1937 plantation, CCC |
| 15 | Largest Red pine | 47.13.91 | 95.13.91 | 100 | low | Grass | none | None | BURNT | Open, burnt often |
| 16 | Largest Red pine | 47.11.16 | 95.13.92 | 90 | low | Herbs | high | Fir | UNBURNED | Unburned contrast to site 15 |
| 17 | Deer Exclosure | 47.11.09 | 95.09.76 | 100 | low | Forbs, ash | low | Oak, maple, white pine | UNBURNED | Inside exclosure, 25 deg slope |
| 18 | Deer exclosure | 47.11.05 | 95.09.78 | 100 | low | Forbs, hazel | med | Fir, white pine, maple | UNBURNED | Inside exclosure |
| 19 | Deer exclosure | 47.11.06 | 95.09.72 | 100 | med | Hazel, forbs | none | None | BURNT | Open canopy. Next to exclosure |
| 20 | South entrance | 47.09.50 | 95.09.19 | 100 | low | Hazel, aspen, forbs | none | None | BURNT | Above small lake |
| 21 | Red pine trail | 47.09.85 | 95.10.40 | 90 | high | Hazel, maple | med | Maple, hazel | BURNT | 6cm organic layer. Rocky. In mixed forest |
| 22 | Red pine trail | 47.09.92 | 95.09.98 | 95 | low | Grass, aspen, forbs | none | None | BURNT | Difficult to define A horizon. Sandy and rocky |
| 23 | Mary Lake | 47.11.08 | 95.09.87 | 95 | med | Hazel, oak, maple | low | Oak, aspen | BURNT | Next to Mary Lake. Steep |
| 24 | Preacher's grove | 47.12.03 | 95.10.42 | 95 | none | None | low | 1wpine | BURNT | Open. 200yr trees. |
| 25 | Headwaters | 47.14.44 | 95.12.81 | 100 | none | None | none | None | UNBURNED | A horizon based on texture. Sandy |
| 26 | Landmark | 47.13.44 | 95.15.16 | 100 | low | Ferns, grass/sedge, ash | none | None | BURNT | Overlooking bog. Sparse |
| 27 | Nicollet | 47.11.58 | 95.13.76 | 90 | low | Forbs, ferns | med | Dead and dying spruce | UNBURNED | Slope of 25deg, dead and dying spruce |
| 28 | Nicollet | 47.11.41 | 95.13.79 | 95 | low | Grass, dandelions, ash | low | Fir | UNBURNED | Quite open site with dead spruce |
| 29 | East entrance | 47.11.66 | 95.09.26 | 95 | med | Hazel, ferns, forbs | med | Maple, white pine, oak | UNBURNED | Slope of 15deg. Unburned contrast to site 30 |
| 30 | East entrance | 47.11.62 | 95.09.33 | 100 | med | Bramble, hazel, sedge | none | None | BURNT | Tall trees, burnt contrast to 29 |

Appendix 2. The isotope values for $\delta^{13}C$ and $\delta^{15}N$ for coarse and fine roots for each plot.

| **Plot number** | Fine root $\delta^{13}C$ | Coarse root $\delta^{13}C$ | Fine root $\delta^{15}N$ | Coarse root $\delta^{15}N$ |
|---|---|---|---|---|
| **1** | -27.50 | -27.55 | -0.79 | -1.31 |
| **2** | -27.42 | -27.49 | -0.33 | -0.87 |
| **3** | -27.33 | -27.33 | -1.77 | -2.09 |
| **4** | -27.48 | -27.36 | -0.77 | -0.73 |
| **5** | -27.47 | -27.47 | -0.23 | -0.28 |
| **6** | -27.55 | -27.44 | 0.14 | -0.17 |
| **7** | -27.32 | -27.16 | -0.03 | -0.64 |
| **8** | -27.56 | -27.40 | 0.41 | -0.43 |
| **9** | -27.46 | -27.57 | -0.77 | -0.81 |
| **10** | -27.43 | -27.40 | -0.90 | -1.15 |
| **11** | -27.45 | -27.57 | 0.28 | -0.70 |
| **12** | -27.36 | -27.42 | -0.91 | -0.82 |
| **13** | -27.43 | -27.57 | -0.32 | -1.01 |
| **14** | -27.39 | -27.52 | -0.46 | -1.45 |
| **15** | -27.41 | -27.25 | -0.79 | -1.79 |
| **16** | -27.35 | -27.22 | -0.88 | -2.15 |
| **17** | -27.34 | -27.45 | -0.77 | -1.37 |
| **18** | -27.52 | -27.38 | -0.86 | -2.38 |
| **19** | -27.38 | -27.41 | -0.68 | -1.58 |
| **20** | -27.37 | -27.42 | -0.78 | -1.05 |
| **21** | -27.46 | -27.29 | 0.20 | -2.24 |
| **22** | -27.43 | -27.45 | -1.12 | -1.30 |
| **23** | -27.36 | -27.28 | -0.98 | -1.51 |
| **24** | -27.38 | -27.27 | -0.94 | -1.24 |
| **25** | -27.38 | -27.38 | -0.10 | -1.83 |
| **26** | -27.46 | -27.31 | -0.56 | -1.10 |
| **27** | -27.35 | -27.32 | -0.67 | -0.79 |
| **28** | -27.28 | -27.21 | -0.46 | -1.01 |
| **29** | -27.38 | -27.29 | -1.07 | -1.15 |
| **30** | -27.34 | -27.43 | -0.25 | -0.78 |