

NITROGEN UPTAKE AND TURNOVER IN RIPARIAN WOODY VEGETATION: A
¹⁵N TRACER EXPERIMENT.

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ABSTRACT

The nutrient balance of streams and adjacent riparian ecosystems may have been modified by the elimination of anadromous fish runs and perhaps by forest fertilization. To better understand nitrogen dynamics within stream and riparian ecosystems, and the nitrogen transfer between them, we fertilized two streams and their adjacent riparian corridors in central Idaho. On each stream two nitrogen doses were applied to a swathe approximately 35 m wide centered on the stream. The fertilizer N was enriched in ^{15}N to 18‰. This enrichment is light relative to many previous labeling studies, yet sufficient to yield a traceable signal in riparian and stream biota. This paper reports pre-treatment differences in $\delta^{15}\text{N}$ and the first-year N response to fertilizer within the riparian woody plant community. Future manuscripts will describe the transfer of allochthonous litter N to the stream and its subsequent processing by stream biota. Pre-treatment $\delta^{15}\text{N}$ differed between the two creeks ($p = 0.00016$), possibly due to residual salmon nitrogen on one of the creeks. Pre-treatment $\delta^{15}\text{N}$ of current-year needles was enriched compared to leaf litter, which was in turn enriched compared to needles aged 4 years and older. This leads us to conclude that fractionation due to retranslocation occurs in two phases. The first, which optimizes allocation of N in younger needle age classes, is distinctly different from the second, which conserves N prior to abscission. The $\delta^{15}\text{N}$ difference between creeks was eliminated by the fertilization ($p = 0.42$). In the two dominant conifer species, *Abies lasiocarpa* and *Picea engelmannii*, most fertilizer N was found in the current-year foliage; little was found in older needles and none was detected in litter ($p = 0.53$). In the only N-fixing shrub species, *Alnus incana*, little fertilizer N was detected (mean percent N derived from fertilizer (%Ndff) $5.0 \pm 1.6\%$ (se)). Far more fertilizer N was taken up by other deciduous shrubs (mean %Ndff = $33.9 \pm 4.5\%$).

Fertilizer N made up 25 (\pm 4.2%) of the N in deciduous shrub litter. These results demonstrate the importance of pre-treatment measurements, the feasibility of light labeling with ^{15}N , and the potential influence of riparian plant species composition on stream nutrient dynamics via allochthonous leaf litter inputs.

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INTRODUCTION

Nitrogen (N) availability limits growth in many terrestrial and aquatic ecosystems worldwide (Vitousek and Howarth 1991). In some streams, nitrogen deficiencies could be ameliorated by the N delivered in the decaying corpses of anadromous fish (Cederholm et al. 1989, Kline et al. 1990, Bilby et al. 1996). The effects of these N additions ramify throughout the ecosystem including the riparian vegetation. N derived from salmon can account for up to 24% of the N incorporated into riparian foliage of Pacific Northwest coastal rivers (Helfield and Naiman 2001). However, inputs of marine derived nutrients (MDN) from salmon runs have been reduced or eliminated by the construction of dams over much of the Pacific Northwest of the USA (Cederholm et al. 1989). At the same time, forest fertilization has become more common in this region (Mandzak and Moore 1994). Fertilization may provide additional nutrients to riparian zones. It is difficult to assess the net effect of salmon decline and fertilization gain without better knowledge of nutrient cycles in rivers and riparian zones.

Nutrient dynamics in riparian zones differ from those in non-riparian ecosystems (Hill 1996, Ettema et al. 1999, Pinay et al. 1995). Riparian vegetation is linked to the stream channel through allochthonous inputs, flooding, and hyporheic exchange (Dahm et al. 1998, Devito et al. 2000). Litter falling into the stream from adjacent riparian vegetation represents a net loss of nutrients from the riparian ecosystem, but a gain in carbon and nutrients by the stream ecosystem. This redistribution can have particular importance in heterotrophic streams where autochthonous productivity is low (Minshall et al. 1983). The stream/riparian linkage is not unidirectional however (Stanford and Ward 1993). Hyporheic zones can be nutrient rich areas of stream water and groundwater exchange; they have the potential to provide

riparian vegetation with supplemental nutrients (Cirimo and McDonnell 1997, Dahm et al. 1998, Devito et al. 2000). Flood pulse events can also redistribute biomass and nutrients between stream water and the riparian zone (Junk et al. 1989).

Species composition in riparian zones also differs from that in upland vegetation (Pabst and Spies 1998). Community composition can be determined by species differences in preferred forms of N (Turnbull et al. 1996), and can in turn affect the forms of N available (Miller and Bowman 2002). In addition, species differ in the efficiency with which they acquire N (Bothwell et al. 2001), the efficiency with which they produce biomass given some amount of N acquired (Bothwell et al. 2001, Nakamura et al. 2002, Yasumura et al. 2002), and the efficiency with which they re-cycle N (Epstein et al. 2001, Bothwell et al. 2001). Different plant communities can indirectly affect N dynamics by the quality of litter they produce and the composition of the soil biota they support (Blair et al. 1992, Stump and Binkley 1993, Garten and Van Miegrot 1994, Korsath et al. 2001). Analysis of composition effects is further complicated by symbiotic relationships with organisms such as atmospheric nitrogen-fixers and mycorrhizal fungi (Binkley et al. 1985, Turnbull et al. 1996, Gebauer and Taylor 1999, Hobbie et al. 2000).

Nitrogen stable isotopes have proven useful in N cycling studies. Isotopic signatures can differ among nitrogen pools, allowing inference of the N sources of plants (Nadelhoffer and Fry 1994). Such inferences are based on mixing models, an algebraic means of estimating the relative contributions of one or more sources, given knowledge of the isotopic composition of the source pools (Robinson 2001, Comstock 2001, Brooks et al. 2002).

However, differences in natural abundance of ^{15}N are often on the same order of magnitude as biological or physical processes that fractionate the ratio of ^{15}N to ^{14}N ($\delta^{15}\text{N}$), which can confound inference of source pools (Handley and Raven 1992, Evans 2001, Robinson 2001).

One source of background variation is historic N sources with distinct $\delta^{15}\text{N}$ signatures (Cederholm et al. 1989, Koerner et al. 1999, Chang and Handley 2000). Atmospheric deposition of N, marine-derived nutrients, and nitrogen derived from atmospheric fixation all have unique $\delta^{15}\text{N}$ signatures that result from the mechanism by which the N is brought into the system (Binkley et al. 1985, Cederholm et al. 1989, Kline et al. 1990). Once N deposition has occurred, a net change in the $\delta^{15}\text{N}$ can only occur if N exits the system, e.g., by leaching, runoff, or denitrification. Isotopic change in the residual N requires that the N be somehow fractionated (Evans 2001, Robinson 2001, Comstock 2001). When systems with high N availability lose large amounts of N through leaching, they are likely to display long-term enrichment in $\delta^{15}\text{N}$ (Högberg and Johannisson 1993). Closed systems have no net fractionation, although there may be internal shifts among pools. Such closed systems might be expected to retain historic $\delta^{15}\text{N}$ differences long after the N source is gone.

This project was part of a larger effort to track N derived from fertilizer (N_{dff}) as it was assimilated and cycled through the riparian and stream-channel biota. Long-term objectives emphasize the transition and exchange of N between riparian and aquatic ecosystem components. This paper details the pre-treatment N status of riparian vegetation and reports the initial incorporation of fertilizer N into riparian plants in the growing season following

fertilization. Fertilizer assimilation into aquatic N compartments will be addressed in future work.

We tested four sets of hypotheses: (1) **Creek Effects**: two creeks with different historic sources of nitrogen would display background differences in $\delta^{15}\text{N}$. Clear Creek supported a significant salmon run until 1924, when the Black Canyon Dam was constructed. Since MDN is generally enriched in ^{15}N relative to terrestrial sources (Kline et al 1990, Bilby et al. 1996, Helfield and Naiman 2001), we expect this source to have left a residual enrichment in vegetative $\delta^{15}\text{N}$ relative to Bogus Creek, which lacks historic MDN inputs due to a migration-blocking waterfall. (2) **Species effects**: different species would differ in pre-treatment $\delta^{15}\text{N}$ and foliar uptake of fertilizer N. In particular, we focused on *Abies lasiocarpa* and *Picea engelmannii*, the two most common overstory trees at the site. Although they commonly grow together, there is a dearth of literature detailing N dynamics in ecosystems dominated by these two trees. We expected no significant difference between *P. engelmannii* and *A. lasiocarpa* in uptake of fertilizer N as expressed by %Ndff. We also focused on *Alnus incana*, which utilizes atmospheric pools of N_2 as a nitrogen source in ecosystems where soil N is limiting (Huss-Danell 1986). We hypothesized that its $\delta^{15}\text{N}$ would be near zero prior to fertilization, but that it would take up fertilizer after the fertilization, perhaps to the exclusion of N-fixation. Finally, we expected deciduous shrubs to have a higher %Ndff than coniferous trees in the first year following fertilization (Stump and Binkley 1993). (3) **Allocation/retranslocation effects**: We expected no fractionation in $\delta^{15}\text{N}$ between green foliage and leaf litter. We hypothesized that most of the fertilizer taken up by the conifers would be allocated to current year needles. Since the conifers on our

sites commonly retain needles for several years, conifer litter was not expected to show fertilizer enrichment in this first year after treatment. Across all species, we expected the $\delta^{15}\text{N}$ of leaf litter to be indistinguishable from that of green foliage. (4) **Assumption assessment:** we hypothesized that in the first year after fertilization, the %N derived from fertilizer, calculated from a mixing model of $\delta^{15}\text{N}$, would be similar to the percentage of “new” N in the leaf among all species and sites. If supported, this result would argue for even mixing of the fertilizer and minimal fractionation upon uptake.

MATERIALS AND METHODS

Site

Two streams, Clear and Bogus Creek, were selected near Cascade, ID. These streams manifest a range of environmental variables including N source history, topography (Table 1), and plant community composition (Table 2). Clear Creek is unconfined, with a gentle gradient. A forested slope dominates the south bank, while the vegetation on the north bank is comprised of herbaceous and graminoid meadows mixed with open forest. The north bank also has an intermittent floodplain. The construction of the Black Canyon Dam in 1924 effectively eliminated salmon runs on Clear Creek. It has a history of cattle grazing, which was interrupted for the 2001-2002 sampling years. It is also used for camping and tourism.

Bogus Creek is narrow and confined, with a steeper channel gradient than Clear Creek. The dominant vegetative cover type is coniferous forest. It has a history of logging and a resident elk population. There is no record of anadromous fish runs on the area of interest on Bogus Creek, ostensibly due to a waterfall downstream of the study area.

Treatments

Two treatments were applied per stream: 224 kgN/ha, which is similar to operational forest management fertilizations in this area, and 448 kgN/ha. Each treatment reach measured one kilometer in length in order to have a continuous stretch of treated riparian vegetation as a source of allochthonous material for stream channel studies. One kilometer of untreated stream was left between treatments on Clear Creek. Due to geographic limitations on Bogus Creek, the light treatment was only 100 m long, and 800 m of stream were left untreated

between the shorter light treatment and the full 1km heavy treatment (Fig. 1). The total treated area measured 9.3 ha. Treatments were applied on November 1st and 2nd, 2001 via helicopter. Treatment application required 3-4 helicopter passes.

Fertilizer Mixture

The fertilizer mixture is the same as that commonly used in forest fertilization in the area (John Mandzak, Boise Corporation, pers. comm.). The primary nitrogen component was urea. Ammonium sulfate was added as a ¹⁵N label vector. Five atom-percent ¹⁵N enriched ammonium sulfate was added to reach a target $\delta^{15}\text{N}$ of 18 per mil for total nitrogen in the mixture. An industrial mixer (Ranco Fertiliservice, Inc. volumetric blender) was used to prepare two fertilizer batches, one for each treatment level. The mixture also contained potassium, copper, magnesium, boron, and other micronutrients. Phosphorus (P) was not included in the mixture because of the high inputs of P from weathering of parent material on Idaho batholith soils. The fully mixed batches were shipped to the research site approximately 4 hours away in a compartmented hopper truck with an auger arm. The fertilizer was applied from a helicopter-supported bucket to a swath 35 m wide centered on the stream.

Sampling

Vegetation was sampled both before and after treatment application. Pre-treatment field sampling began in mid-August of 2001 and was completed in mid-September 2001. Post-treatment sampling occurred in late July of 2002. Ten 10-by-10 meter plots were installed per treatment, beginning 300 m upstream from each treatment reach. Plots were 175 m apart

on alternating sides of the stream. This resulted in six plots falling within the treatment reach, two untreated plots upstream of the reach, and two untreated plots downstream beyond the end of the reach (Fig. 1). The plot design on the shorter light treatment on Bogus Creek was modified to have 4 plots 50m apart on alternating sides of the stream. Two plots fell within the reach with one plot upstream, and one plot beyond the downstream bound. Plots were constructed on a tangent to the stream edge by placing stakes 10 m apart on the stream bank. The boundary of the plot adjacent to the stream was allowed to vary with the stream edge.

Foliar samples were collected from the lowest branch of the contiguous live crown on every tree > 5 cm diameter at 1.3 m (dbh), usually with a pruning pole. Gebauer and Schulze (1991) reported no effect of canopy position on $\delta^{15}\text{N}$. A 0.22 caliber rifle was used to collect samples if the live crown began greater than 5 m above the ground. Species, dbh, height, distance from the stream edge, and height at which a foliar sample was collected were recorded on each plot. Heights and distances were recorded with a laser rangefinder (Impulse Laser Rangefinder, Laser Technology, Inc., Englewood, CO). Sun leaves were collected where possible and visibly unhealthy foliage was not sampled. Needles were separated into age classes. Tree foliar samples collected in 2002 were harvested at the same height as in 2001. Saplings (< 5 cm dbh) were sampled for current year needles and batched by species in 2001. 2002 sapling samples were batched by species, but current and 1-year old needles were separated. A batch sample comprised of foliage from several individuals was analyzed collectively. Every species of shrub was sampled on each plot, and foliage samples were batched by species so that there was one observation per species per plot. Leaf

litter was also sampled on each plot. One screen tray was randomly placed on each plot in the middle of September and litter was collected in late October of each year. Litter samples were then classed by species.

Stable Isotope Analysis

Isotope ratios were determined at the University of Idaho Stable Isotope Laboratory using a CN-2400 EA (Carlo Erba) coupled to a Finnigan-MAT delta+. Foliar samples were oven-dried (60°C for 48 hours) and ground in a ball mill prior to analysis. Average values of standard deviations of repeated standard (egg albumin) measurements were 0.10. Results are expressed in delta notation ($\delta^{15}\text{N}$) relative to atmospheric N_2 .

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (1)$$

where R is the ratio of ^{15}N to ^{14}N .

The known $\delta^{15}\text{N}$ of stable isotope labels incorporated into a simple mixing model yields the percent nitrogen derived from fertilizer ($\% \text{Ndff}_{\text{label}}$) in N sinks (Mugasha and Pluth 1994, Hood et al. 1999, Robinson 2001). Automated analysis of $\delta^{15}\text{N}$ allows for more intensive sampling regimes than past studies that we have used to our advantage to calculate $\% \text{Ndff}_{\text{label}}$ for each observational unit:

$$\% \text{Ndff}_{\text{label}} = \frac{\delta^{15}\text{N}_{\text{t2}} - \delta^{15}\text{N}_{\text{t1}}}{\delta^{15}\text{N}_{\text{fertilizer}} - \delta^{15}\text{N}_{\text{t1}}} * 100 \quad (2)$$

where $\delta^{15}\text{N}$ of fertilizer is 18‰, t1 is pre-treatment, and t2 is the first year after treatment. Calculated in this way, %Ndff_{label} provides a powerful test of our hypotheses because it accounts for background variation between individual trees, and removes spatial variability.

We devised another estimation of %Ndff based on the percentage of “new” N in the leaf following fertilization (%Ndff_{conc}):

$$\%Ndff_{\text{conc}} = \frac{N_{t2} - N_{t1}}{N_{t2}} * 100 \quad (3)$$

Where N is foliar nitrogen concentration by mass, and the subscripts t1 and t2 are as above.

Statistical Analysis

Statistical analyses were carried out in R (Ihaka and Gentleman 1996). A “full” model was constructed incorporating all data points to test creek and treatment hypotheses relating to $\delta^{15}\text{N}$ and %Ndff_{label} (Appendix I). Separate “partial” models for trees, shrubs, and litter were used to test hypotheses for which the full model became too unwieldy. All models were fit in a mixed effects framework, where measurements were nested within sampling year to account for repeated measures. %Ndff_{label} models, for which sampling year is implicit in the calculation, nested species within life form (tree, shrub) and within plot to build a covariance matrix that accounts for spatial variability of species composition. In this manner repeated measures and hierarchical data structure were appropriately modeled. Untreated plots were coded as the intercept in all models. Residuals of all partial models appeared independent and normal. A marginal heavy-tailedness was visible in the full %Ndff_{label} model, and the full $\delta^{15}\text{N}$ model was skewed slightly left. Although statistically insignificant (Table 3) to the

$\%N_{\text{dff}_{\text{label}}}$ model, creek and creek x treatment were retained in the model because they improved the distribution of the residuals (Appendix II). Bars on all graphs represent ± 1 standard error. Multiple comparisons were carried out using the multcomp library in R. A subset of *A. lasiocarpa* and *P. engelmannii* from the post-treatment sampling was randomly selected and balanced between creek, treatment, and species; to test hypotheses relating to older needle age classes.

To test the similarity between $\%N_{\text{dff}_{\text{label}}}$ and $\%N_{\text{dff}_{\text{conc}}}$, we used a test of equivalence rather than a traditional test of differences. In an equivalence test, the null hypothesis states that the means between samples are different rather than similar (Wellek 2003, Robinson and Froese, in review). This reverses the role of the null hypothesis and is better suited to our goal of determining whether the models agreed, not on whether they differed.

RESULTS

Pre-treatment foliar $\delta^{15}\text{N}$ of Clear Creek was $1.75 (\pm 0.53\text{‰})$ (1 se) more enriched than that of Bogus Creek ($p = 0.00016$). The relative enrichment between creeks was generally uniform across life forms (trees, shrubs) and species--with one exception. *Alnus incana* was the only N_2 -fixing plant on this study site. Pre-treatment $\delta^{15}\text{N}$ of *Alnus incana* closely resembled that of air and did not reflect the creek differences displayed by the other species (mean *A. incana* $\delta^{15}\text{N}$: Bogus = $-1.12 \pm 0.55\text{‰}$, Clear = $-0.95 \pm 1.4\text{‰}$). $\delta^{15}\text{N}$ was similar between *Abies lasiocarpa* and *Picea engelmannii*, the 2 dominant trees on both streams (Fig. 2).

Following treatment, the enrichment of Clear Creek relative to Bogus Creek vanished ($p = 0.42$). $\% \text{N}_{\text{dff}_{\text{label}}}$ did not differ between *A. lasiocarpa* and *P. engelmannii* (Fig. 3). Most of the fertilizer N assimilated by trees was found in their current-year needles (Fig. 4). Post-treatment $\delta^{15}\text{N}$ of shrubs was generally higher than that of conifers (Fig. 5). *A. incana* showed only a minimal response to fertilization (Fig. 3). $\% \text{N}_{\text{dff}_{\text{label}}}$ in shrubs accounted for $20.3 (\pm 5.2\%)$ and $28.3 (\pm 4.1\%)$ of N in leaf material for the 224 kgN/ha and 448 kgN/ha treatments respectively, but $\% \text{N}_{\text{dff}_{\text{label}}}$ of *A. incana* leaves averaged $-4.4 (\pm 4.4\%)$ and $4.0 (\pm 3.7\%)$ respectively. In the full model with all observational points accounted for, a significant difference between the 224 kgN/ha and 448 kgN/ha treatments was not detectable ($p = 0.31$).

Pre-treatment $\delta^{15}\text{N}$ of leaf litter was depleted compared to current-year live foliage, except in the case of *A. incana*. The intercept of a regression using solely $\delta^{15}\text{N}$ of live foliage to

predict $\delta^{15}\text{N}$ of litter was $-1.1 (\pm 0.19\text{‰})$, with a slope of 0.91 ± 0.07 (Fig. 6). On average, leaf litter contained only 56% of the N in current year needles. Since conifers display ^{15}N -depletion in older age classes compared to current year live foliage on untreated plots, and leaf litter is also depleted compared to current year live foliage (Fig. 4), we tested the hypothesis that conifer needles older than 4 years would have $\delta^{15}\text{N}$ similar to that of leaf litter. Conifer litter was in fact enriched by 1.0‰ compared to foliage more than four years old (paired t-test, $n = 7$, $p = 0.002$). There was no difference in $\delta^{15}\text{N}$ of leaf litter on untreated plots between sampling years (paired t-test, $n = 25$, $p = 0.24$).

The proportion of $\text{Ndff}_{\text{label}}$ in leaf litter varied by species. Deciduous shrubs ranged from 20 to 40 % $\text{Ndff}_{\text{label}}$. None of the conifer species released measurable amounts of fertilizer N in their litter (Fig. 7).

An equivalence test between % $\text{Ndff}_{\text{label}}$ and % $\text{Ndff}_{\text{conc}}$ failed to detect a significant similarity between sample means (difference in means = 3.66, standard deviation = 20.03, $n = 380$, critical value = 3.22, t-value = 3.56) at a conservative region of indifference (25% of standard deviation). A less conservative test (30% of standard deviation), however, yielded a statistically significant similarity (critical value = 4.19, t-value = 3.56).

Estimates of $\delta^{15}\text{N}$ were not statistically different in control plots between years ($p = 0.96$). Covariance analysis of distance downstream from initial point of fertilization found no evidence that upstream treatments had contaminated plots downstream in the first year after treatment ($p = 0.92$).

DISCUSSION

Creek Effects

We interpret the observed difference in pre-treatment $\delta^{15}\text{N}$ between creeks as a legacy of historic differences in ecosystem N sources. A prominent N source on Bogus Creek was probably N derived from atmospheric fixation by *Alnus*, while anadromous fish probably provided a major source of N to Clear Creek. The practice of sheep and cattle grazing on Clear Creek might also have influenced $\delta^{15}\text{N}$ by exporting significant amounts of depleted N. Grazing effects seem unlikely to explain the differences, however, given that the $\delta^{15}\text{N}$ of graminoids on the study site closely resembled that of cow dung (data not shown). Further, we would expect the $\delta^{15}\text{N}$ of the N carried off the site in herbivore biomass to be enriched rather than depleted (Schoeninger and DeNiro 1984).

Different rates of denitrification might also explain the enrichment of Clear Creek relative to Bogus. Anaerobic conditions, such as in floodplain soils with high water contents, facilitate higher denitrification rates (Ettema et al. 1999). The process of denitrification fractionates against ^{15}N , leaving an enriched ^{15}N pool in the soil, while the lighter ^{14}N takes to the air in gaseous form (Chien et al. 1977, Blackmer and Bremer 1977, Mariotti et al. 1981, Kellman and Hillaire-Marcel 1998). Clear Creek has, on much of its length, a sizeable floodplain that may have higher rates of denitrification than Bogus Creek. In an attempt to discern whether floodplain soils significantly affected $\delta^{15}\text{N}$, we separated plots into two slope classes, flat (<5%) and steep (>5%), and analyzed the significance of these two classes to pre-treatment $\delta^{15}\text{N}$. Not only was slope class not significant ($p = 0.07$), but the inclusion of this variable had no influence on our ability to detect the creek effect. These arguments lead us back to

original N sources as a probable explanation for the creek difference. Now that the treated reaches do not differ in $\delta^{15}\text{N}$ due to the homogeneity of $\delta^{15}\text{N}$ of the applied fertilizer N source, future sampling may reveal whether differences between creeks re-emerge.

Species Effects

Foliar fertilizer N assimilation was similar among species within functional groups and differed between functional groups. The two dominant tree species showed no differences in either pre-treatment $\delta^{15}\text{N}$ or assimilation of fertilizer N in foliage. *Alnus* took up miniscule amounts of fertilizer N, and shrub species such as *Salix* and *Amelanchier* took up a slightly greater proportion of fertilizer N than trees. Hooper and Vitousek (1999) similarly concluded that composition of functional groups in plant communities plays an important role in ecosystem N dynamics.

Alnus incana was the only species that took up negligible amounts of fertilizer N. *Alnus* has its own N source via atmospheric N fixation, yet reliance on atmospheric N usually declines after fertilization of N_2 -fixers, implying some uptake of fertilizer (Pastor and Binkley 1998, Vázquez et al. 2001). This shift of N source, and concomitant reduction in metabolic cost, is presumed to cause the increase in productivity typical of N_2 -fixing plants following fertilization (Huss-Danell 1986, Ledgard et al. 2001). In 1992, Mead and Preston reported that *Alnus sinuata* took up fertilizer in the first year, then rapidly turned it over. After eight years, little fertilizer N remained in the leaves. The amount of fertilizer we added should have provided plenty of available N for *Alnus*, as evidenced by N uptake by other shrubs, yet *Alnus* did not utilize this source in the first year. Another year's sampling of foliar $\delta^{15}\text{N}$

would indicate whether this near complete reliance on atmospheric N-fixation is a long-term phenomenon.

Allocation/Retranslocation Effects

In the conifers, fertilizer N was allocated almost exclusively to current-year and one year-old needles. Preferential allocation of fertilizer to current-year needles in conifers has been reported in previous studies (Mugasha and Pluth 1994, Nadelhoffer et al. 1999, George et al. 1999). *P. engelmannii* retained needles on our sites for up to 9 years. The fertilizer N may be redistributed among needle cohorts over the next several years. However, if it remains in the 2002 cohort, it will take several years for fertilizer N to cycle back to the soil, or the stream, as leaf litter. In contrast, shrubs returned a significant portion of fertilizer N to the soil or stream as they abscised last year's foliage. This difference between conifers and shrubs highlights the importance of plant community composition to ecosystem N dynamics.

Characterization of the acquisition and retranslocation of N over the course of a plant's life cycle has been the focus of several studies (Fife and Nambiar 1982/1984, Nambiar and Fife 1991, Millard and Proe 1992/1993, Aerts 1996, Kolb and Evans 2002). We found evidence of a significant depletion of leaf litter $\delta^{15}\text{N}$ compared to current-year live foliage (Fig. 6). Needles of age four and older also displayed a depletion compared to current year needles (Fig. 4) consistent with results from Gebauer and Schulze (1991) for healthy stands of *Picea abies*. Compared to four years and older needles, leaf litter is enriched in $\delta^{15}\text{N}$. N is commonly retranslocated from the leaf to storage pools in another organ prior to leaf abscission (Chapin and Kedrowski 1983). One might expect isotope ratio discrimination in

this process (Kolb and Evans 2002). These data suggest that the mechanisms for retranslocation prior to abscission (fractionation in older needles) and for optimization of N to support new growth (fractionation in young needles) are two very different processes, as argued by Nambiar and Fife in 1991. In contrast to our results, Kolb and Evans (2002) did not observe an isotopic change between green leaves and litter. Perhaps this discrepancy can be explained as a consequence of species differences in translocation; their work focused on deciduous *Encelia* and *Quercus* species. Fractionations upon retranslocation might also result from differences in the ratio of soluble to insoluble leaf proteins, which can depend on N status of the growing site (Pugnaire and Chapin 1983). While pre-treatment nutrient status on these sites is arguably N limited, *Alnus*, which may well be free from N-limitation due to its N₂-fixing capacity, showed no difference between live foliage and leaf litter $\delta^{15}\text{N}$ (Fig. 6).

Assessment of Assumptions

Our interpretation of fertilizer effects rests on two key assumptions. The first and most critical is that the ¹⁵N label was evenly mixed into the fertilizer prior to application. We diluted 5 atom % ¹⁵(NH₄)₂SO₄; ~ 13,300‰, to 18‰. This required the mixing of 22kg of ammonium sulfate into ~13 Mg of the total fertilizer mixture. Mixing small amounts of one substance into large amounts of another is risky. Multiple passes by the helicopter during application should have helped reduce this risk. Uneven mixing would increase the variability in our treatments, but should be free from bias. The near 1:1 ratio of %N_{dff_{label}} and %N_{dff_{conc}} argues that the mixing and dispersion of label were adequate.

The second key assumption is that there was no fractionation against ^{15}N as nitrogen was assimilated. We labeled the fertilizer with a signature slightly enriched over natural abundance. Our estimates of $\% \text{Ndff}_{\text{label}}$ would therefore be sensitive to fractionation. For example, suppose a plant takes up enough fertilizer to represent 37% of the N in its leaves ($\% \text{Ndff}_{\text{label}}$). If this hypothetical plant fractionated against ^{15}N on uptake by 1‰, then the observed $\% \text{Ndff}_{\text{label}}$ for this plant would be 32% (equation 2), just outside our experimentally determined standard error (4-5% for most species). Högberg et al. (1999) report that ecto-mycorrhizal and non-mycorrhizal *Pinus sylvestris* discriminate against ^{15}N on uptake of ammonium by 0.9 to 6.5‰. $\delta^{15}\text{N}$ of leaf tissue was positively correlated with fractionation on uptake. However, these authors argued that fractionation on the upper end of the range would not occur unless a substantial amount of available nitrogen was not taken up (Högberg et al. 1999). We do not believe that much N escaped capture by ecosystem biota, again because of the near 1:1 relationship between $\% \text{Ndff}_{\text{label}}$ and $\% \text{Ndff}_{\text{conc}}$. If fractionation had occurred, it would be expected to influence the $\% \text{Ndff}_{\text{label}}$ estimate, but not the $\% \text{Ndff}_{\text{conc}}$, and these two variables would be dissimilar. The equivalence test between $\% \text{Ndff}_{\text{label}}$ and $\% \text{Ndff}_{\text{conc}}$ supports the conclusion that fractionation on uptake is borderline insignificant. $\% \text{Ndff}_{\text{label}}$ and $\% \text{Ndff}_{\text{conc}}$ are not statistically similar, but they are borderline since a slightly less conservative test picks up a significant similarity. The pre-treatment N limitation on our study sites appears to have worked to our advantage in this case, because N-limited plants and microbes are unlikely to release much N to the stream, even under fertilization.

Conclusion

This study departs from previous ^{15}N labeling studies on many points. To ensure a readily detectable signal in ecosystem compartments, nearly all previous studies labeled their N inputs with a ^{15}N signature several orders of magnitude larger than that of natural abundance. The Harvard Forest chronic N addition experiments labeled their sites with treatments targeted at 1000‰ (Nadelhoffer et al. 1999). Indeed, most ^{15}N labeling experiments add tracers at concentrations greater than 2 atom%, or 4,551‰ (Mugasha and Pluth 1994, George et al. 1999, Epstein et al. 2000). Studies labeled at this concentration of ^{15}N pay little heed to fractionations in $\delta^{15}\text{N}$, because the error introduced by such is several orders of magnitude smaller than that caused by the experimental error. In all of the above studies N was added in liquid form to ensure even mixing, where our study applied the fertilizer in solid pellet form and relied on ambient precipitation to disperse the label. The advantage to this project is that our treatments are measured in hectares where past studies are measured in square meters. This is all the more powerful considering the ecosystem to which these treatments were applied: the interface between land and water.

The inclusion of a stable isotope tracer to the fertilizer allows us to quantify the fraction of fertilizer N within each ecosystem component, and eventually, trace its transition to the aquatic system and impact on the stream biota. This is important because of the contention over N dynamics in these systems; specifically, the relative importance of allochthonous N sources to stream and riparian ecosystems (Cederholm 1989, Bilby 1996, Helfield and Naiman 2001), the impact of fertilizer N pollution to these systems (Hill 1996, Devito et al. 2000), and the theory of how nutrients behave at the interface between riparian and stream

ecosystems (Minshall et al 1983, Cirimo and McDonnel 1997, Dahm et al 1998). The ultimate goal of this project is to characterize nutrient dynamics across the stream-riparian interface, so that we may eventually learn how to treat these distinct ecosystems as an integrated unit.

TABLES

	Bogus	Clear
Channel Gradient (%)	3.4	1.5
Base Flow Discharge (m ³ /s)	.007	.334
Elevation Range of Reaches (m)	1450 - 1550	1560 - 1592

Table 1. Some stream channel characteristics.

(a) Trees			
Species	Bogus	Clear	Total
<i>Abies grandis</i>	1		1
<i>Abies lasiocarpa</i>	27	13	40
<i>Pinus contorta</i>	5	9	14
<i>Picea engelmannii</i>	35	46	81
<i>Pinus ponderosa</i>		4	4
<i>Pseudotsuga menziesii</i>		4	4
Total	68	76	144

(b) Shrubs			
Species	Bogus	Clear	Total
<i>Alnus incana</i>	13	16	29
<i>Amelanchier alnifolia</i>	6	3	9
<i>Cornus stolonifera</i>	5	5	10
low shrubs*	8	2	10
<i>Ribes spp.</i>	4	1	5
<i>Rosaceae spp.</i>	8		8
<i>Salix spp.</i>		13	13
<i>Spiraea betulifolia</i>	8	3	11
<i>Vaccinium spp.</i>	8	1	9
Total	60	44	104

Table 2. Table of counts and distributions of tree (a) and shrub (b) species.

* Low shrubs are a composite of *Linnaea borealis* and *Chimaphila umbelata*.

	numDF	denDF	F	p-value
Species	15	80	10.45	<0.0001
Creek	1	27	0.49	0.4917
Treatment	2	27	15.86	<0.0001
%Ndff _{conc}	1	290	115.07	<0.0001
Leaf Age	2	290	76.12	<0.0001
Creek x Treatment	2	27	1.27	0.2973
Treatment x %Ndff _{conc}	2	290	36.07	<0.0001

Table 3. Results of Analysis of variance for %N derived from fertilizer_{label} as the response variable. Species are nested within lifeform class (trees/saplings, shrubs) within plot as random effects.

	numDF	denDF	F	p-value
Species	20	385	19.60	<0.0001
Life-form class	3	171	14.00	<0.0001
Treatment	2	57	13.22	<0.0001
Year	1	1	41.18	0.0984
Creek	1	57	27.01	<0.0001
%N	1	454	430.10	<0.0001
Leaf Age	2	454	72.51	<0.0001
Treatment X Year	2	57	13.23	<0.0001
Treatment X Creek	2	57	2.72	0.0742
Treatment X %N	2	454	26.77	<0.0001
Year X Creek	1	57	2.41	0.1264

Table 4. Results from analysis of variance of all observations of $\delta^{15}\text{N}$.

FIGURES

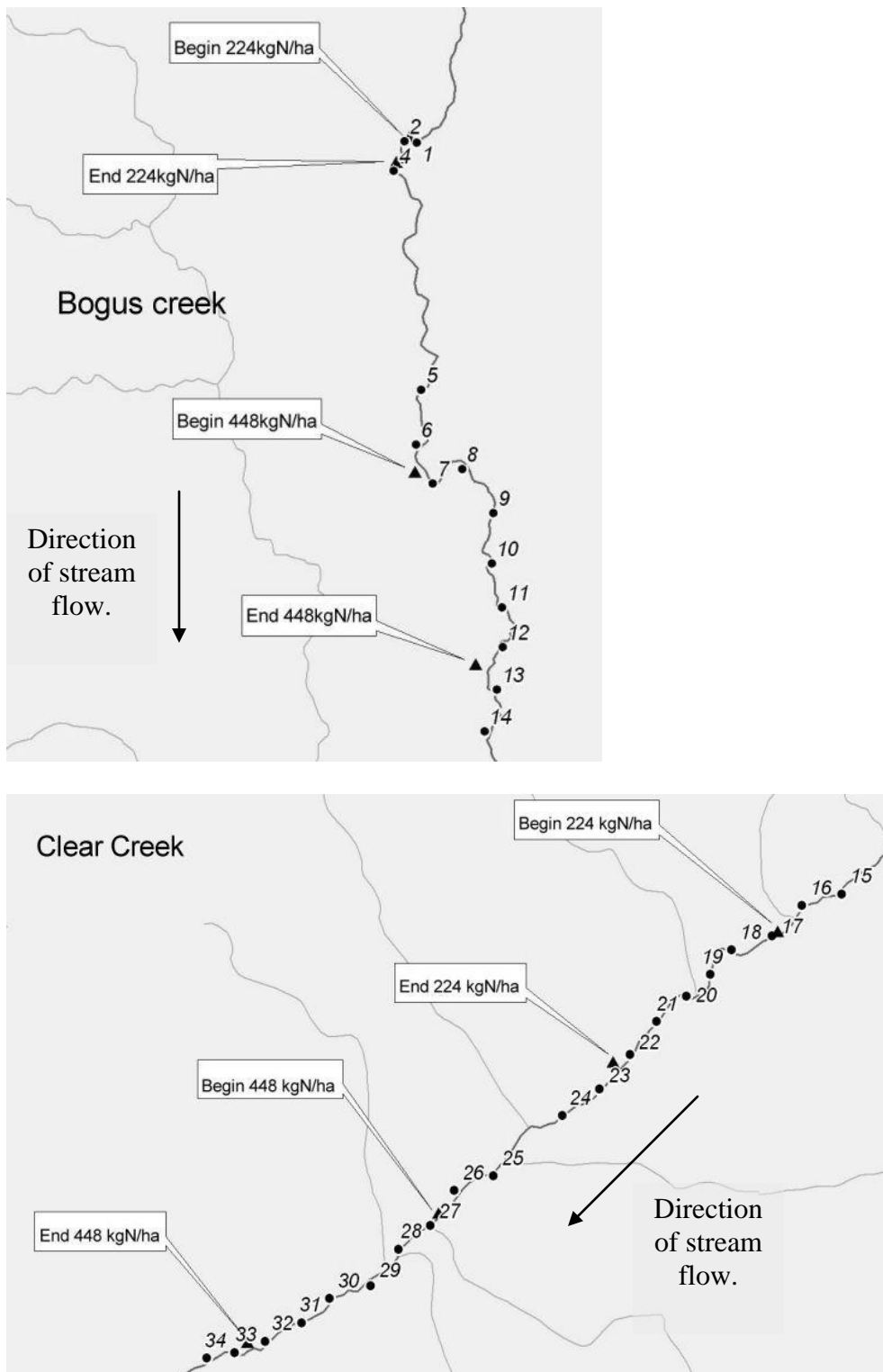


Fig. 1. Maps of study sites: treatments and plots (numbered). Maps are on different scales.

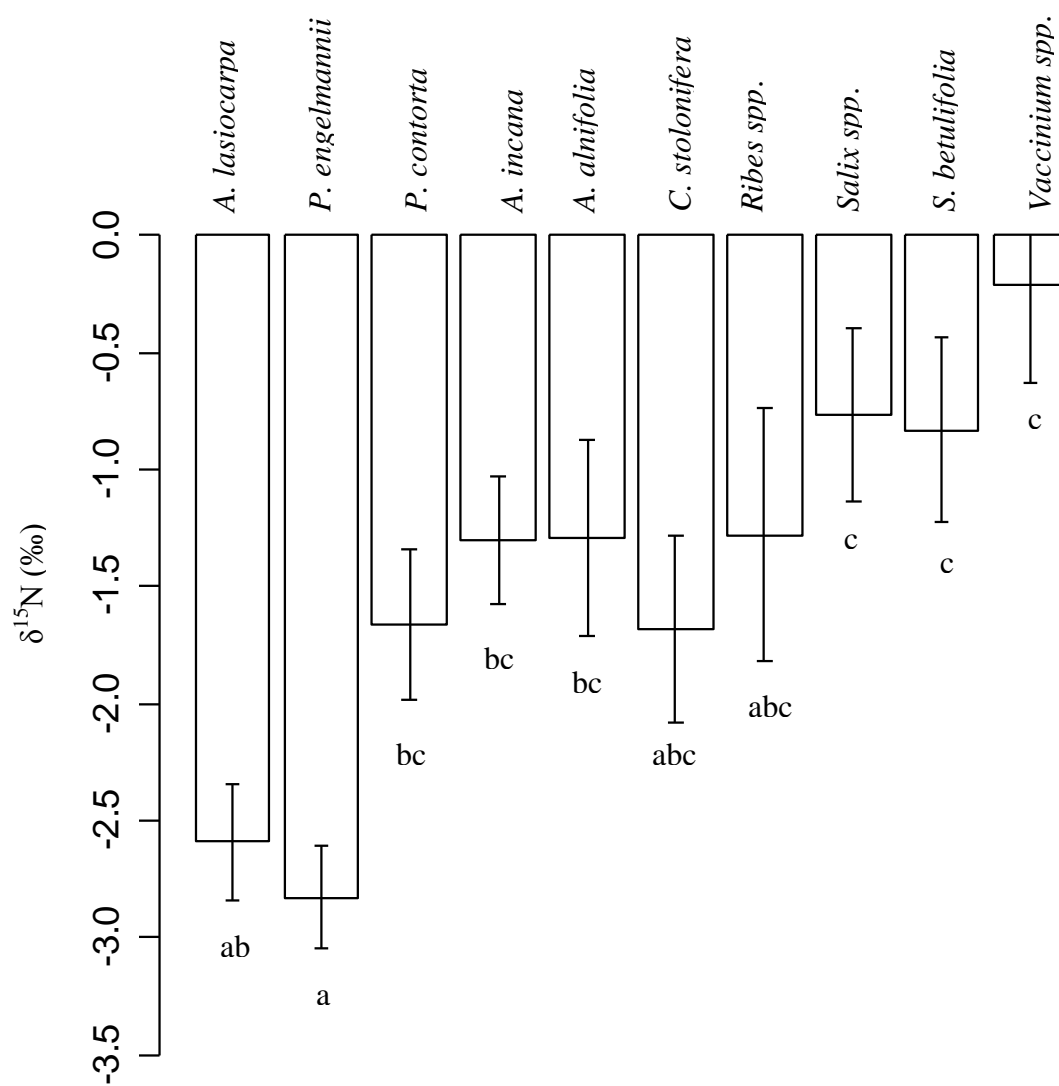


Fig. 2. Average values of pre-treatment $\delta^{15}\text{N}$ by species (bars represent ± 1 standard error). Results of multiple pairwise comparisons are given in lowercase letters. Species with the same letter have no statistical difference.

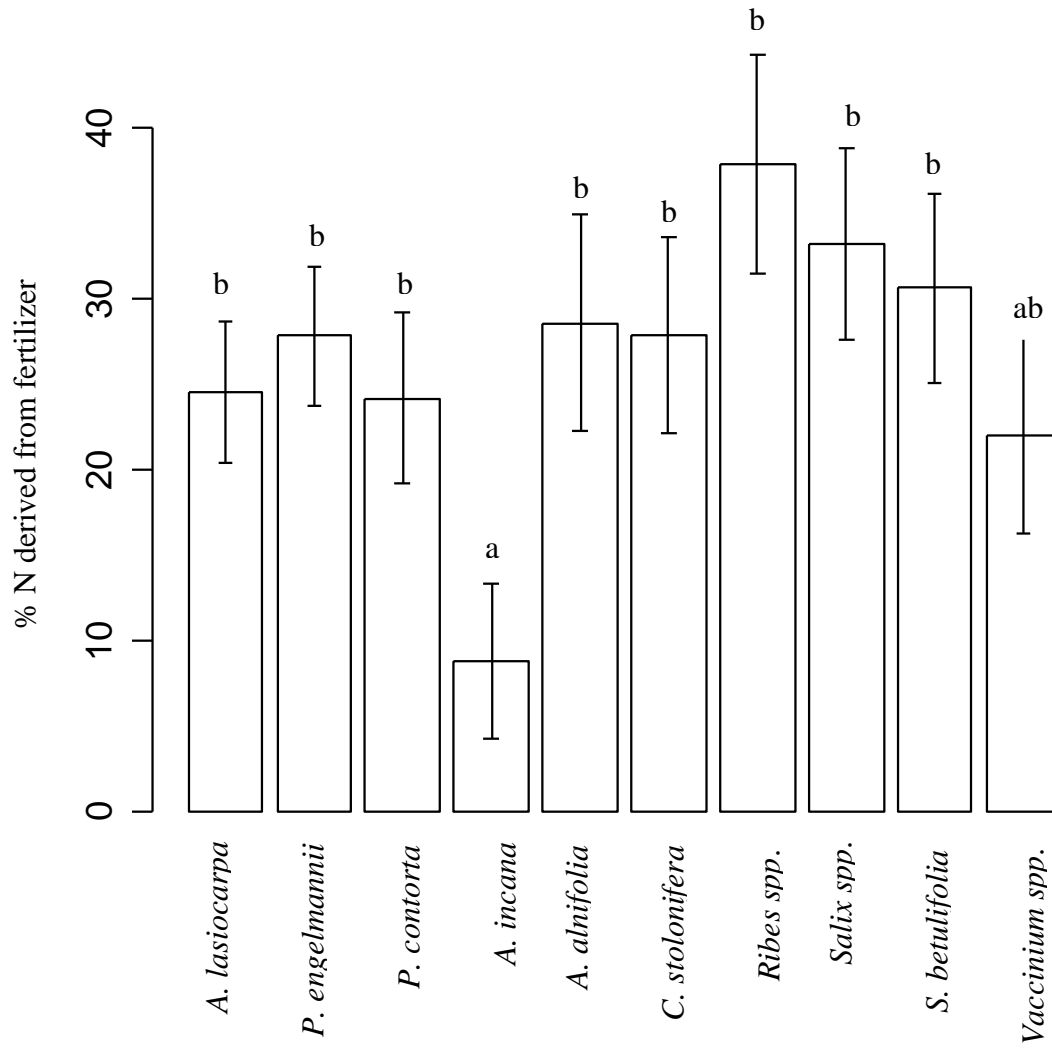


Fig. 3. Average values of %N derived from fertilizer by species.

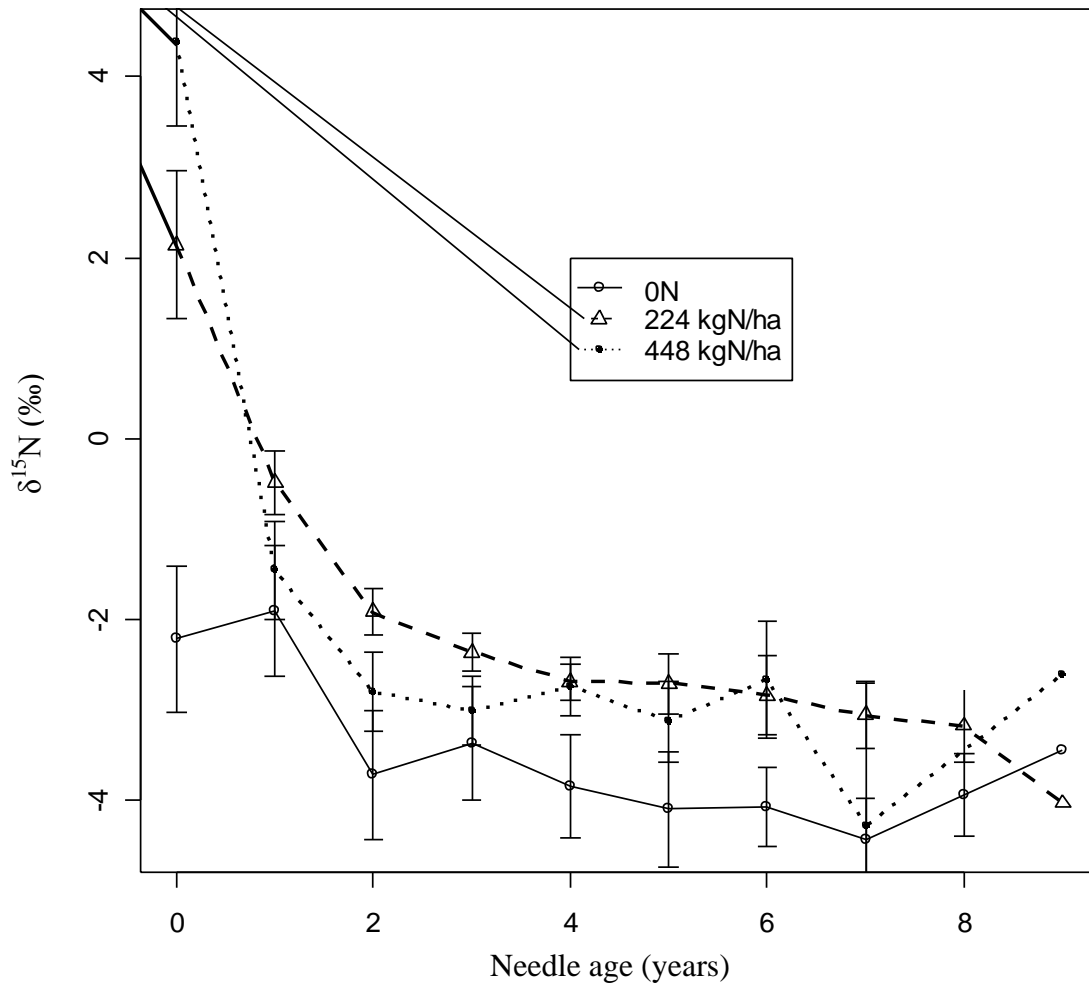


Fig. 4. Subset of $\delta^{15}\text{N}$ of *A. lasiocarpa* and *P. engelmannii* plotted against needle age by treatment.

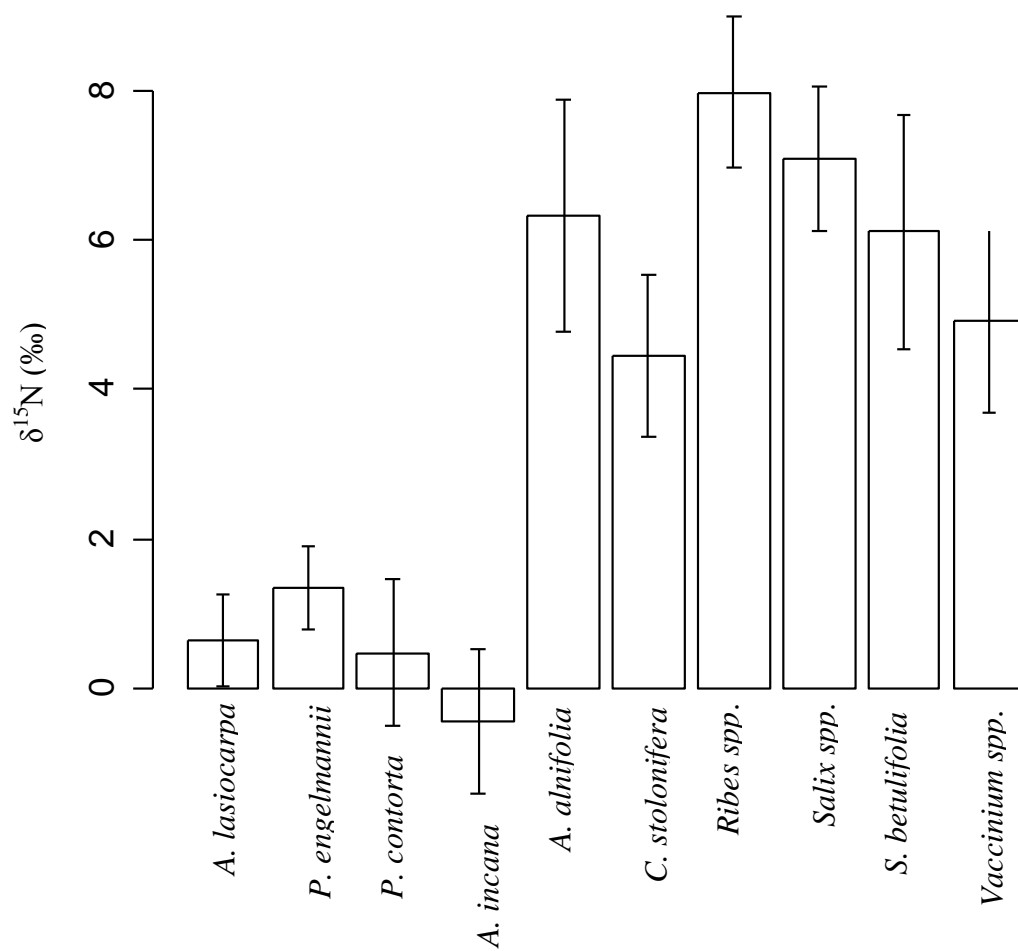


Fig. 5. Average values of post-treatment $\delta^{15}\text{N}$ of treated stream reaches by species.

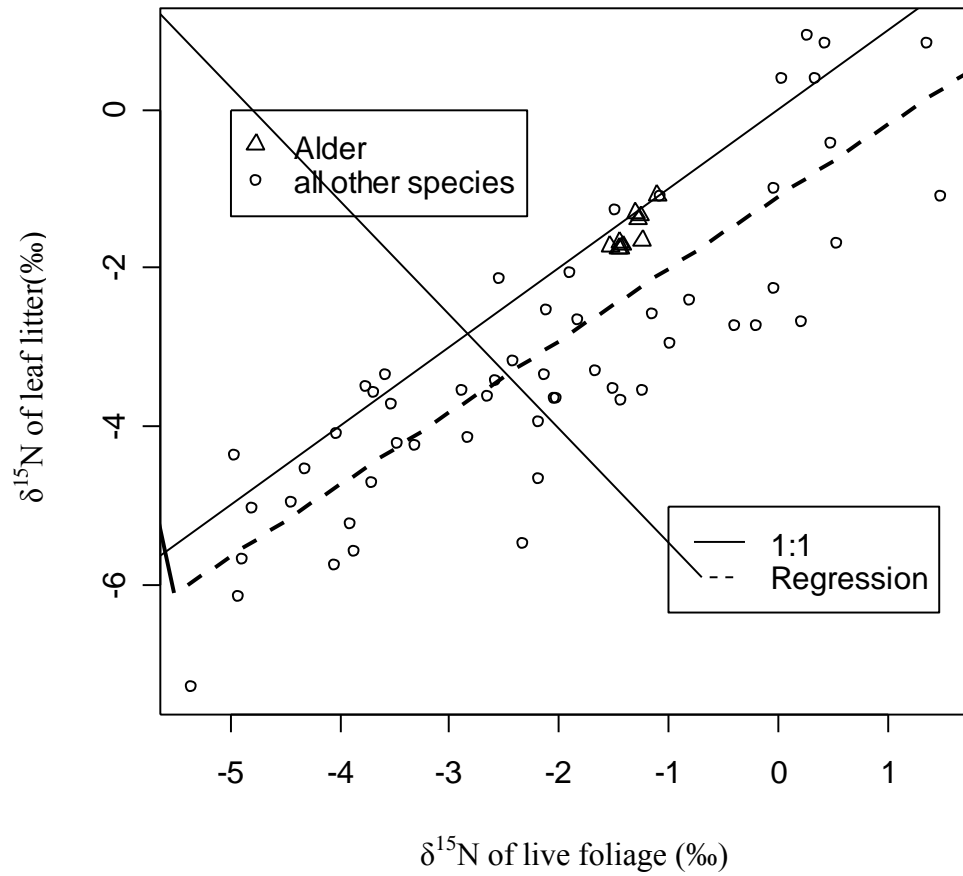


Fig. 6. Pre-treatment $\delta^{15}\text{N}$ of leaf litter plotted against $\delta^{15}\text{N}$ of current-year live foliage for all species. $R^2 = 0.71$.

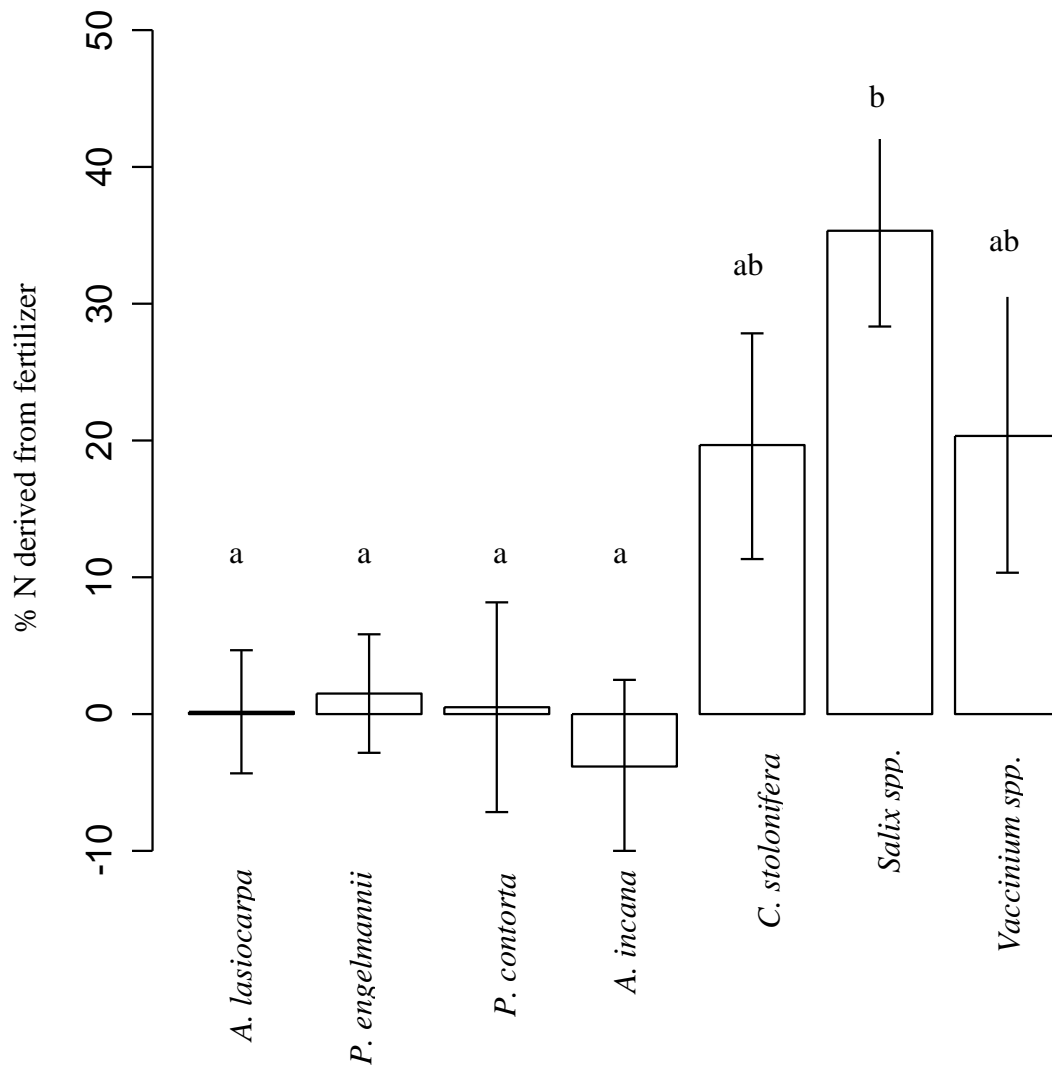


Fig. 7. Average values of %N derived from fertilizer_{label} of leaf litter by species.

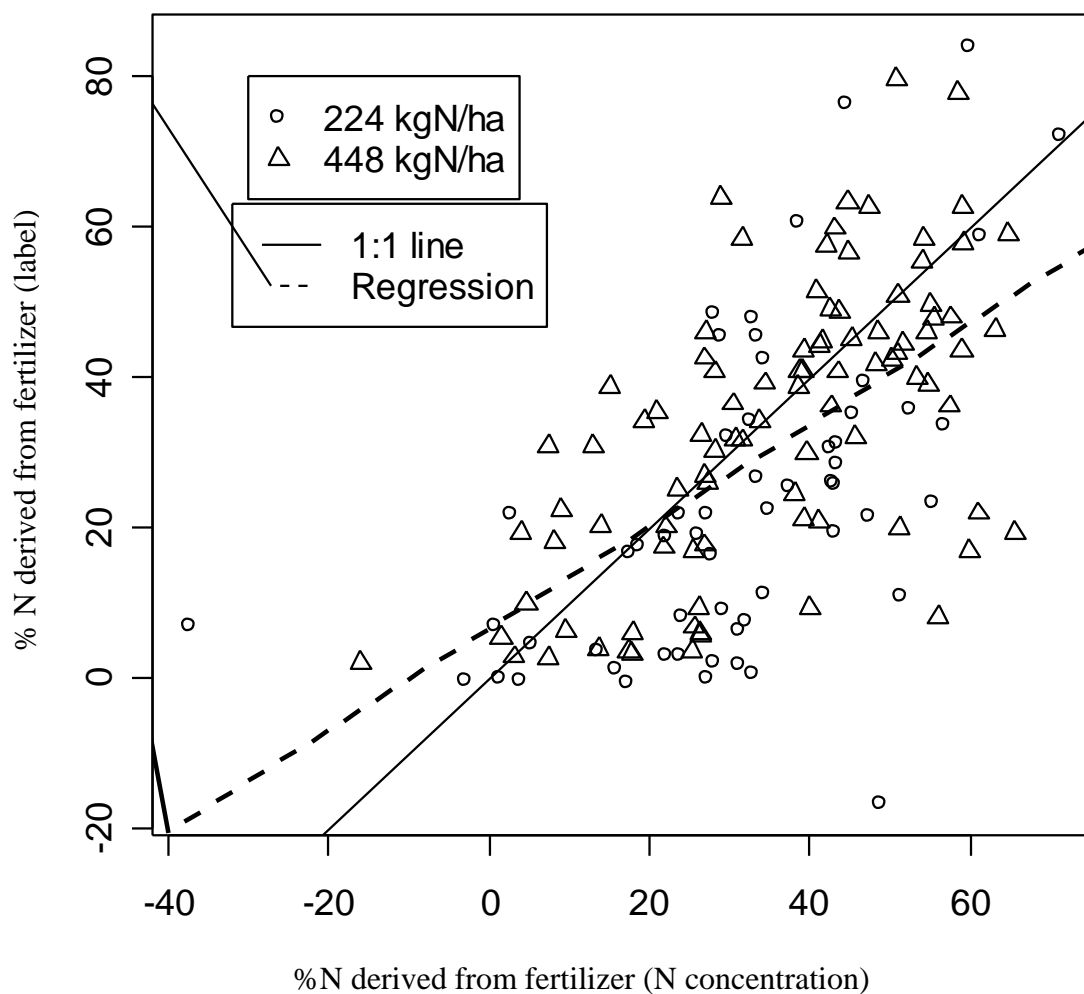


Fig. 8. %N derived from fertilizer_{label} plotted against %N derived from fertilizer_{conc} for current-year foliage. Regression line represents %N derived from fertilizer predicted solely by %N increase. Intercept = $6.5 \pm 2.9\%$, slope = 0.68 ± 0.07 , root mean square error (root MSE) = 4.04. Three outliers of high influence (Cook's distance > 0.05) were deleted (model prior to deletion: intercept = $12.6 \pm 2.52\%$, slope = 0.52 ± 0.07 , root MSE = 4.12).

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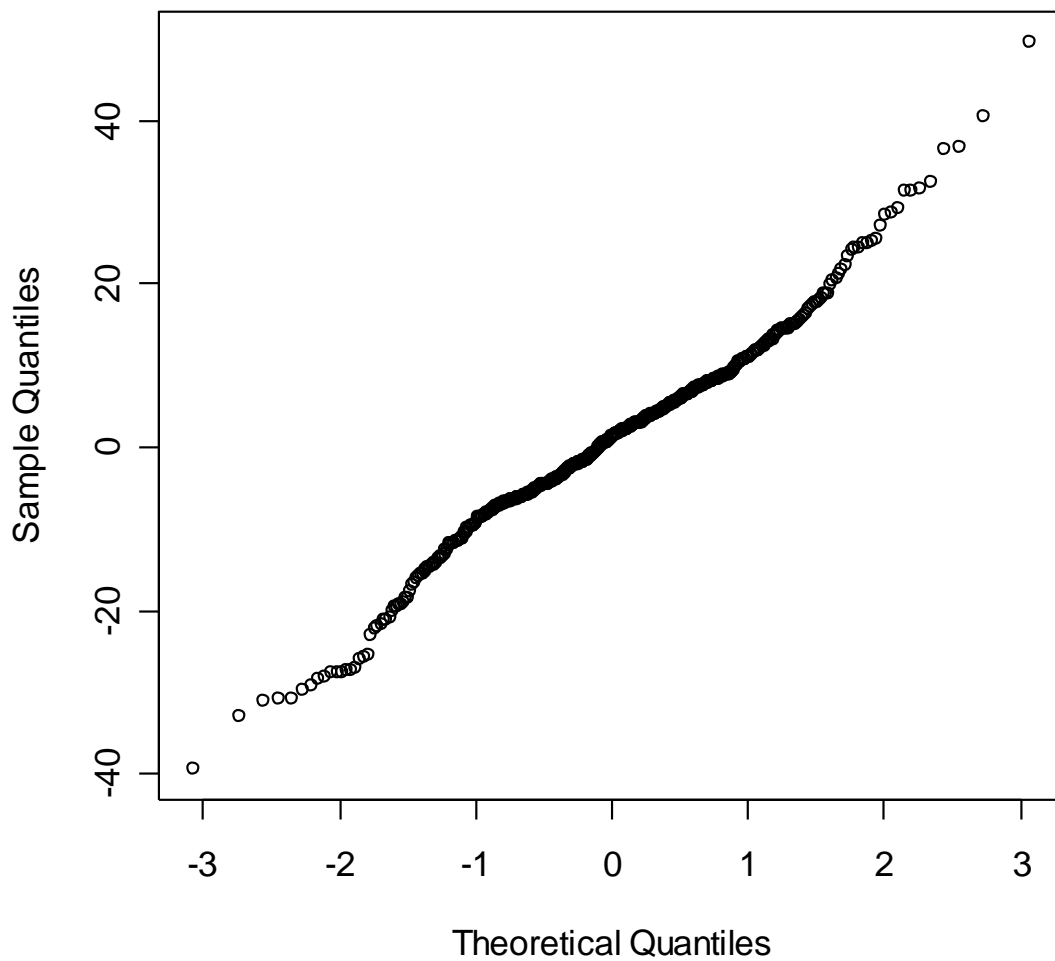
APPENDIX 1

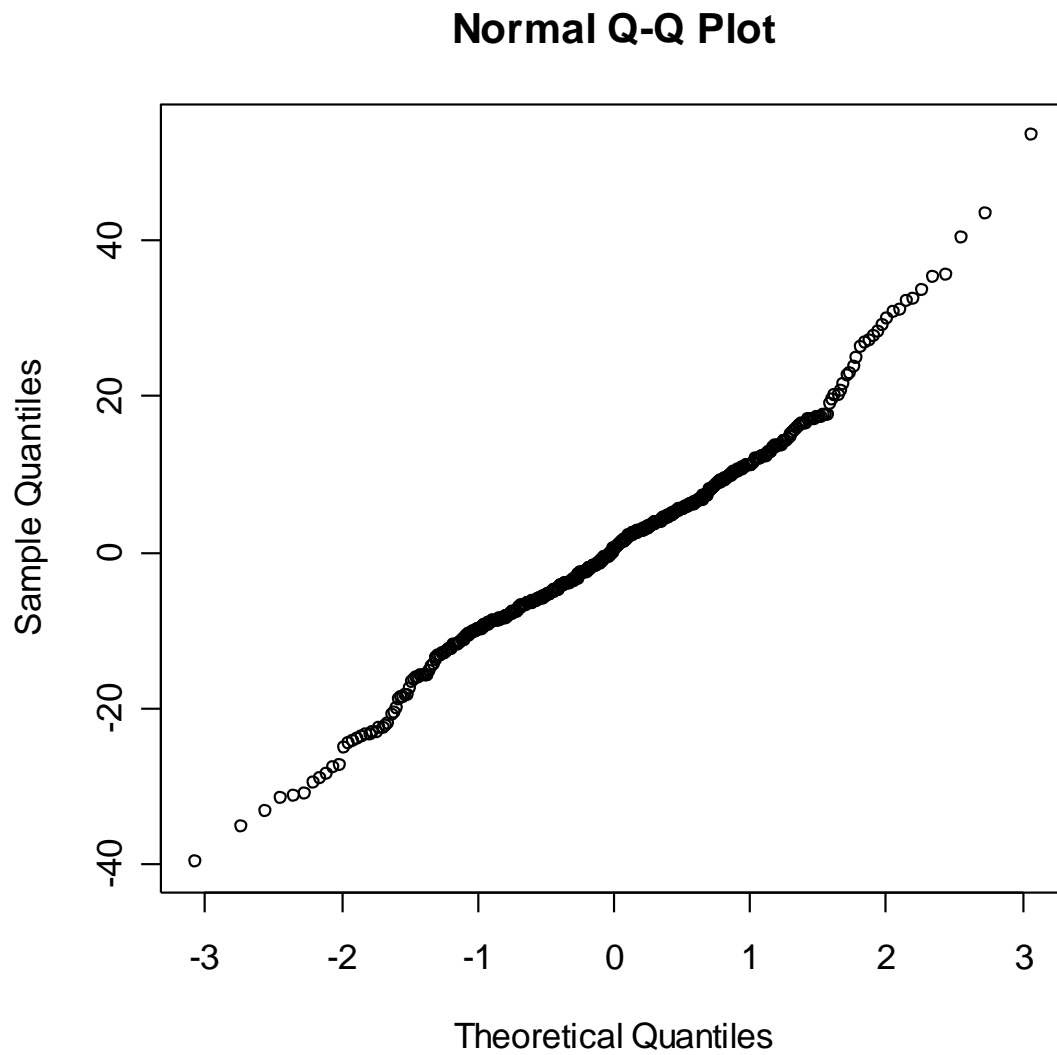
Model Structures

$$\%Ndf_{\text{label}} \sim \text{species} + \text{creek} * \text{treatment} + \text{treatment} * \%Ndf_{\text{conc}} + \text{age}$$
$$\text{random effects} = \text{plot/lifeform-class/species}$$
$$\delta^{15}\text{N} \sim \text{species} + \text{life-form class} + \text{treatment} * \text{year} + \text{creek} * \text{treatment} + \text{treatment} * \text{N}$$
$$+ \text{creek} * \text{year} + \text{age: random effects} = \text{year/plot/life-form class/species}$$

APPENDIX II

Normal Q-Q Plot

Appendix.II.1. Q-Q normal plot of full %N derived from fertilizer_{label} model.



Appendix.II.2. Q-Q normal plot of %N derived from fertilizer_{label} model removing creek and creek x treatment.