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Nitrogen acquisition strategies of mature Douglas-fir: a case study in the northern Rocky Mountains

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Abstract. Nitrogen (N) limits plant growth in temperate ecosystems, yet many evergreens exhibit low photosynthetic N use efficiency, which can be explained in part by their tendency to store more N than to use it in photosynthesis. However, it remains uncertain to what extent mature conifers translocate internal N reserves or take up N from soils to support new growth. In this study, we explored N dynamics within mature Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) trees by linking N uptake in field-grown trees with seasonal soil available N. We used a branch-level mass balance approach to infer seasonal changes in total N among multiple needle and stem cohorts and bole tissue, and used foliar $\delta^{15}\text{N}$ to evaluate N translocation/uptake from soils. Soil resin-exchangeable N and net N transformation rates were measured to assess whether soils had sufficient N to support new needle growth. We estimated that after bud break, new needle biomass in Douglas-fir trees accumulated an average of 0.20 ± 0.03 mg N/branch and 0.17 ± 0.03 mg N/branch in 2016 and 2017, respectively. While we did find some evidence of translocation of N from older stems to buds prior to bud break, we did not detect a significant drawdown of N from previous years' growth during needle expansion. This suggests that the majority of N used for new growth was not reallocated from aboveground storage, but originated from the soils. This finding was further supported by the $\delta^{15}\text{N}$ data, which showed divergent $\delta^{15}\text{N}$ patterns between older needles and buds prior to leaf flushing (indicative of translocation), but similar patterns of depletion and subsequent enrichment following leaf expansion (indicative of N originating from soils). Overall, in order to support new growth, our study trees obtained the majority of N from the soils, suggesting tight coupling between soil available N and N uptake in the ecosystem.

Key words: conifer; evergreen; nitrogen availability; nitrogen storage; nitrogen translocation; nitrogen uptake; *Pseudotsuga menziesii* var. *glauca*/Rocky Mountain Douglas-fir.

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INTRODUCTION

It is widely accepted that temperate ecosystems are nitrogen (N) limited (Reich et al. 2006, Elser et al. 2007, LeBauer and Treseder 2008), which implies that soil available N should govern plant N uptake and assimilation (Chapin et al. 1988). N availability in soils, in turn, is controlled by numerous environmental factors. In

higher elevation western U.S. forests, snow is a dominant abiotic factor that influences N cycling. Throughout the winter, snow insulates the soil and allows soil temperatures to remain above freezing so that N transformation can occur (Brooks et al. 2011, Maurer and Bowling 2014). As a result, N accumulates in the soils over winter and is then released in a pulse during snowmelt (Brooks et al. 1998). Given that N limits

photosynthesis and growth, plants are expected to take up this large pool of N as soon as it becomes readily available (Freschet et al. 2018, Reich 2018). However, evergreen plants may not always use this readily available pool in spring to support new growth; some studies have found evergreen conifers to rely on N taken up in the previous autumn to support new growth, suggesting an asynchrony between N availability in the soils and N uptake by trees (Nasholm and Ericsson 1990, Proe and Millard 1994). However, because many of these studies were conducted in controlled glasshouses or plantations, it remains unclear how prevalent this asynchrony is under field settings, or under conditions where snow is the dominant form of precipitation.

Growing season plant N uptake is strongly controlled by water availability and temperature (Chapin and Kedrowski 1983, Gessler et al. 2002, Rennenberg et al. 2009, Soggi and Templer 2011). Nutrients move through the soil to the root interface primarily via diffusion, and when soils dry down, nutrient transfer to roots slows (Lambers et al. 1998, Matson et al. 2002). Similarly, low water availability constrains transpiration, indirectly inhibiting N uptake (Gessler et al. 2002). N uptake in the spring can also be inhibited by low soil temperatures and root damage during the preceding winter (Millard and Grelet 2010, Sanders-DeMott et al. 2018). Thus, for forests that experience large seasonal shifts in temperature and soil moisture, internally stored N may be an important source supporting new growth. However, previous field studies that demonstrate the importance of internally stored N have focused on tundra and temperate deciduous forest ecosystems, where mid-summer (June–August) moisture limitation may not be the most important factor limiting tree growth (e.g., Chapin et al. 1980, Chapin and Kedrowski 1983, Soggi and Templer 2011). As a result, a different pattern of N storage and uptake may exist in ecosystems where mid-season moisture is limiting.

Plants store N throughout different plant parts, including stems, branches, old leaves, new leaves, buds, or roots (Nambiar and Fife 1991, Warren and Adams 2004). In evergreens, N is primarily stored in previous year leaves or stems (Chapin 1980, Millard and Grelet 2010). N storage in roots is a less important source of N in evergreen compared with deciduous trees, which

can store more than 60% of N in the roots (Nambiar 1987, Millard and Proe 1992, Gessler et al. 1998b, Proe et al. 2000, Stephens et al. 2001, Millard and Grelet 2010). N is mainly stored in the form of protein, which can represent up to 70% of total N (Millard 1988, Nasholm and Ericsson 1990). Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase), the main enzyme associated with carbon fixation during photosynthesis, makes up 50% of the total protein in C₃ plants, and it may function in N storage (Camm 1993, Warren and Adams 2004, Chapin et al. 2011, Tegeder and Masclaux-Daubresse 2018). Along with protein storage, amino acids, such as arginine, asparagine, and glutamine, as well as ribosomal proteins, play a role in transient N storage across the season (Nasholm et al. 1994, Masclaux-Daubresse et al. 2017). However, it is still uncertain if N stored in these pools is always reallocated to support new growth. In this study, our discussions are limited to evergreen and evergreen conifer species, unless otherwise noted, because N allocation patterns vary between deciduous and evergreen trees. Further, we refer specifically to evergreen conifers in order to differentiate from evergreens in general, which can include woody, evergreen shrubs.

While soil available N is relatively easy to measure across time and across a range of ecosystems, including tropical and temperate forests, grasslands, and arctic and alpine tundra systems (Vitousek and Matson 1988, Davidson et al. 1992, Turner et al. 1997, Jaeger et al. 1999, Schimel et al. 2004, Campbell et al. 2014), measuring N uptake by plants remains more difficult. The in situ depletion method, where intact roots are immersed in a solution of known N concentration, can offer a direct measure of the magnitude of N uptake across a season (Gessler et al. 1998a, Soggi and Templer 2011, Campbell et al. 2014), but the method can be time intensive and difficult to implement in mixed species forests. Alternatively, a mass balance approach helps track changes in different plant growth fractions across an entire year to assess whether nutrients accumulating in new tissues are translocated from parts of the plant or directly taken up by the roots, but this approach has traditionally been used on smaller woody plants, such as shrubs (Chapin and Kedrowski 1983, Silla and Escudero 2003, Furze et al. 2019). For example, by

accounting for changes in biomass as total N accumulated in the new growth of *Picea mariana* across a season, Chapin and Kedrowski (1983) found that by bud break, the current season's growth had already accumulated 70% of its maximum N content. According to this mass balance approach, if trees primarily use internally stored N to support new needle growth, then total N content in the past season needles, the branches, or the bole (i.e., growth fractions; Fig. 1a) would decline as new growth accumulates N throughout the season (Fig. 1b). In contrast, if trees use primarily newly acquired N from the soil, then N in the past season growth fractions is not expected to decline as new growth accumulates N throughout the season (Fig. 1c). Thus, applying the mass balance approach is one way to determine whether trees use newly acquired N or N from storage to support new growth.

Given that N uptake, storage, and remobilization are important processes that regulate tree growth, refining our knowledge of these patterns is essential for our basic understanding of how

trees acquire N under a natural setting. Thus, the main objective of this study was to explore tree N uptake dynamics of *Pseudotsuga menziesii* var. *glauca* (Rocky Mountain Douglas-fir) across two growing seasons, and to link N uptake with soil available N. More specifically, we asked the following questions: (1) Is translocated N from storage or newly acquired N from the soil more important in supporting new growth in Douglas-fir? (2) Do these patterns of N translocation versus uptake differ at contrasting elevations (and climate)? (3) If newly acquired N from the soil is more important in supporting new growth, do trees synchronize their N uptake with the timing of new growth? In order to address these questions, we applied an N mass balance approach in Douglas-fir trees growing in western Montana in order to estimate changes in N of different growth fractions. While this approach has been successfully demonstrated in shrubs and small trees (Chapin et al. 1980, Chapin and Kedrowski 1983, Gray and Schlesinger 1983) and in trees grown on plantations (Silla and Escudero 2003),

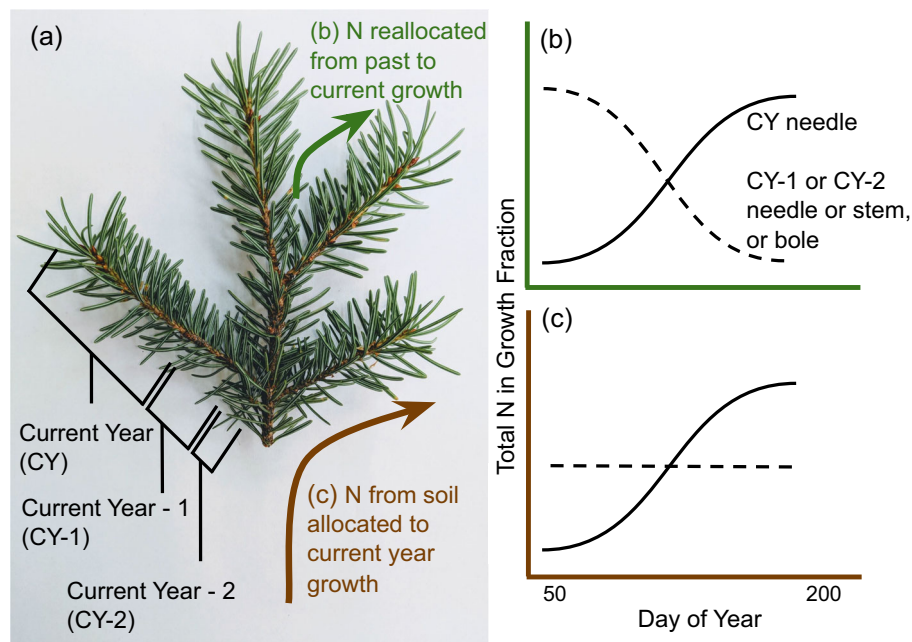


Fig. 1. Conceptual figure of potential expected outcomes, where (a) displays the needle cohorts and potential allocation patterns. Current year (CY) is the current year's growth, current year-1 (CY-1) is the one-year-old growth, and current year-2 (CY-2) is the two-year-old growth. (b) represents a drawdown of stored N from the past season growth, and (c) represents that the tree bypasses storage and instead uses newly acquired N from the soil.

to our knowledge, this is the first study to use this approach to measure N allocation in trees growing in a temperate conifer forest.

METHODS

Site description

The study was conducted during the 2016 and 2017 growing seasons in a mixed conifer forest of the North Fork of Elk Creek watershed at Lubrecht Experimental Forest in western Montana, USA. We sampled both trees and soils on southeast facing hillslopes at both a high (~1700 m a.s.l.) and a low (~1400 m a.s.l.) elevation site. Tree species at the lower elevation consisted of *Pseudotsuga menziesii* var. *glauca* and *Pinus ponderosa* var. *scopulorum*; at higher elevations, tree species consisted of *Pseudotsuga menziesii*, *Pinus contorta*, *Picea engelmannii*, *Abies lasiocarpa*, and *Larix occidentalis*. The mean annual temperatures from 1981 to 2010 were 4.2°C and 3.0°C at the low and high elevation sites, respectively. Snowfall represents 24% and 41% of annual average precipitation at the low and high elevations, respectively (NRCS SNOTEL, stations 604 and 657). The underlying lithology of the watershed is predominantly composed of quartz monzonite, with the periphery of the watershed composed of Mesoproterozoic metasedimentary mudstones and fine-grained sandstones (Belt supergroup). The soil texture ranged from extremely gravelly sandy loam and gravelly sandy loam to gravelly ashy loam, depending on sampling site and soil depth (NRCS 2017). To record air temperature, relative humidity, and soil volumetric water content (VWC) at both sites, we used VP3 and 5TE sensors connected to EM50 data loggers recording at 30-min intervals (METER group, Pullman, Washington, USA). To record precipitation, we used a tipping bucket rain gauge (METER, Pullman, Washington, USA). The data logger at the high elevation site malfunctioned between mid-August and the end of September 2017, so only low elevation data are reported for that period.

Western spruce budworm outbreak and drought

Between 2004 and 2017, western spruce budworm (WSBW, *Choristoneura occidentalis*) defoliated conifers, particularly Douglas-fir, at a high rate across Montana, including the forest

surrounding our field site (J. Egan, *personal communication*). The most severe defoliation (i.e., the percent of area defoliated) in the recent outbreak was during 2012, which affected 251 km² in Missoula County, Montana, USA, where our study site was located. During 2015, the year before our study began, the area of defoliation was larger, affecting 755 km² in Missoula County, but the severity of defoliation was lower. In 2016, the area affected dropped to 141 km² (DeNitto et al. 2016) and decreased even more in 2017 to 36 km² (DeNitto et al. 2017). However, although the county-wide severity of defoliation was lower in 2015, the defoliation severity at our site was very high (>75% new needles defoliated), while during 2016 and 2017, needle defoliation was moderate (25–50% of new needles were defoliated) to severe (50–75% of new needles were defoliated; Appendix S1: Fig. S1; DeNitto et al. 2016, C. Qubain, *personal observation*).

Drought and western spruce budworm severity have been shown to be directly linked (Anderegg et al. 2015, Xu et al. 2019). In 2015, the area surrounding the field sites experienced moderate to extreme drought conditions between May and November (U.S. Drought Monitor 2015). In 2016, the area experienced abnormally dry to severe drought conditions between the beginning of June through September, and in 2017, moderate-to-severe drought conditions lasted between the end of July through the middle of September.

Growth fraction collection and analysis

In order to examine seasonal changes in percent N (%N), carbon-to-N ratio (C:N), and the nitrogen isotope ratio ($\delta^{15}\text{N}$) among different plant parts, we collected different growth fractions, including needles, buds, stems, litter, and bole tissue. We sampled from ten healthy Douglas-fir trees, ranging from 40 to 80 yr old (diameter at breast height, DBH from 7.5 cm to 22.0 cm), hereafter referred to as N-collection trees, throughout the 2016 and 2017 growing seasons. Five trees were located at the high elevation site, and five were located at the low elevation site. In 2016, we sampled during 13 periods, starting in mid-April and ending in the beginning of November. In 2017, we sampled during 12 periods, starting at the end of February and ending in mid-September. During the February

sampling period in 2017, only trees at the low elevation site were sampled because snowpack at the high elevation site was not yet isothermal and trees there were assumed to be dormant. Beginning in September 2016, litter samples were collected using litter traps, constructed using 0.12-m² bins that were staked under the trees and covered with mesh screens.

During each sampling period, we collected samples from the tree bole, branches, and needles. For the bole sample, we collected one approximately 3.4 cm long sample using a 5-mm diameter tree borer. We also collected sun needles from three small branches from the middle canopy using a pole pruner (Fig. 2a1). Directly after clipping, we divided needles and stems by cohort (Fig. 2a2). In 2016, the samples were divided between buds or current year (CY, 2016) needles (depending on the timing of bud break relative to sampling period), previous year (CY-1, 2015) needles, CY-1 stems, and CY stems if present. In 2017, we divided samples into buds or CY needles, CY-1 (2016) needles, two-year-old (CY-2, 2015) needles, CY-1 stems, CY-2 stems, and CY stems if present. New stem growth did not suberize until August in both years, so new stem growth was considered needle material until the August sampling date for both years. We determined the beginning and end of each needle and stem cohort by the annual bud-scale scars on the stems. If both buds and needles were present during a collection period, both growth fractions were collected for that period. Bud break occurred during the week of May 31 in 2016 and June 15 in 2017 at the low elevation site, and it occurred during the week of June 15th in 2016 and June 25 in 2017 at the high elevation site.

After samples were collected and divided in the field, they were transported back to the laboratory and dried at 60°C for 48 h. All growth fractions except for the bole tissue were homogenized and handground using liquid nitrogen. Bole samples were ground in a cyclone sample mill (UDY corporation, Colorado, USA) and then pulverized using a tissue lyser (Qiagen TissueLyser II, Hilden, Germany) for 12 min. After all samples were ground and weighed, the needles and buds were analyzed for percent N (%N) and C (%C) contents, as well as $\delta^{15}\text{N}$, using continuous flow dual isotope analysis with a

CHNOS Elemental Analyzer interfaced to an IsoPrime100 mass spectrometer at the University of California Berkeley (Fig. 2a3). Because woody tissues were typically too low in N for accurate isotope analysis, and because we did not think these fractions would be as dynamic across the growing seasons, stem, bole, and needle litter samples were only analyzed for total N and total C using a CHNOS Elemental Analyzer at Montana State University.

Mass balance: tree branch harvest and calculations

In addition to commonly used parameters such as %N or C:N to infer seasonal patterns of N allocation, we used a tree branch N mass balance approach by multiplying %N by the total estimated biomass of the different growth fractions. In order to estimate biomass of the different growth fraction, at the end of July 2016, we harvested six trees, ranging in DBH from 7.5 to 15.8 cm (Fig. 2b1). We divided a subset of six branches from each tree (three each from the upper and lower canopy) into separate growth fractions (Buds, CY needles, CY-1 needles, CY stems, CY-1 stems, all other needles, and all other stems; Fig. 2b2). In the field, we weighed the entire tree trunk as well as all the cut branches from the tree and then brought the subsamples back to the laboratory to dry at 60°C. The needles and stems were dried for one week, and the boles were dried for two months.

Because we could not sample entire trees, we performed our N mass balance approach based on the six branches that were representative of the tree. Our rationale for using this approach included the following: (1) Given that Douglas-fir can retain needles up to 10 yr (Cole 1981), any N that was being translocated to current year needles would likely originate from older needles on the same branch (Millard and Grelet 2010), (2) conifers tend to store little N in the bole or roots, so the most active location of N translocation is likely in the stem and old leaves (Millard and Grelet 2010), and (3) scaling to the branch level and not to the whole tree level can minimize errors associated with scaling up. To scale N content to the branch level, we first established a linear relationship between the DBH of the harvest trees and the average growth fraction mass per branch (Fig. 2b4, Appendix S1: Eqs. S1,

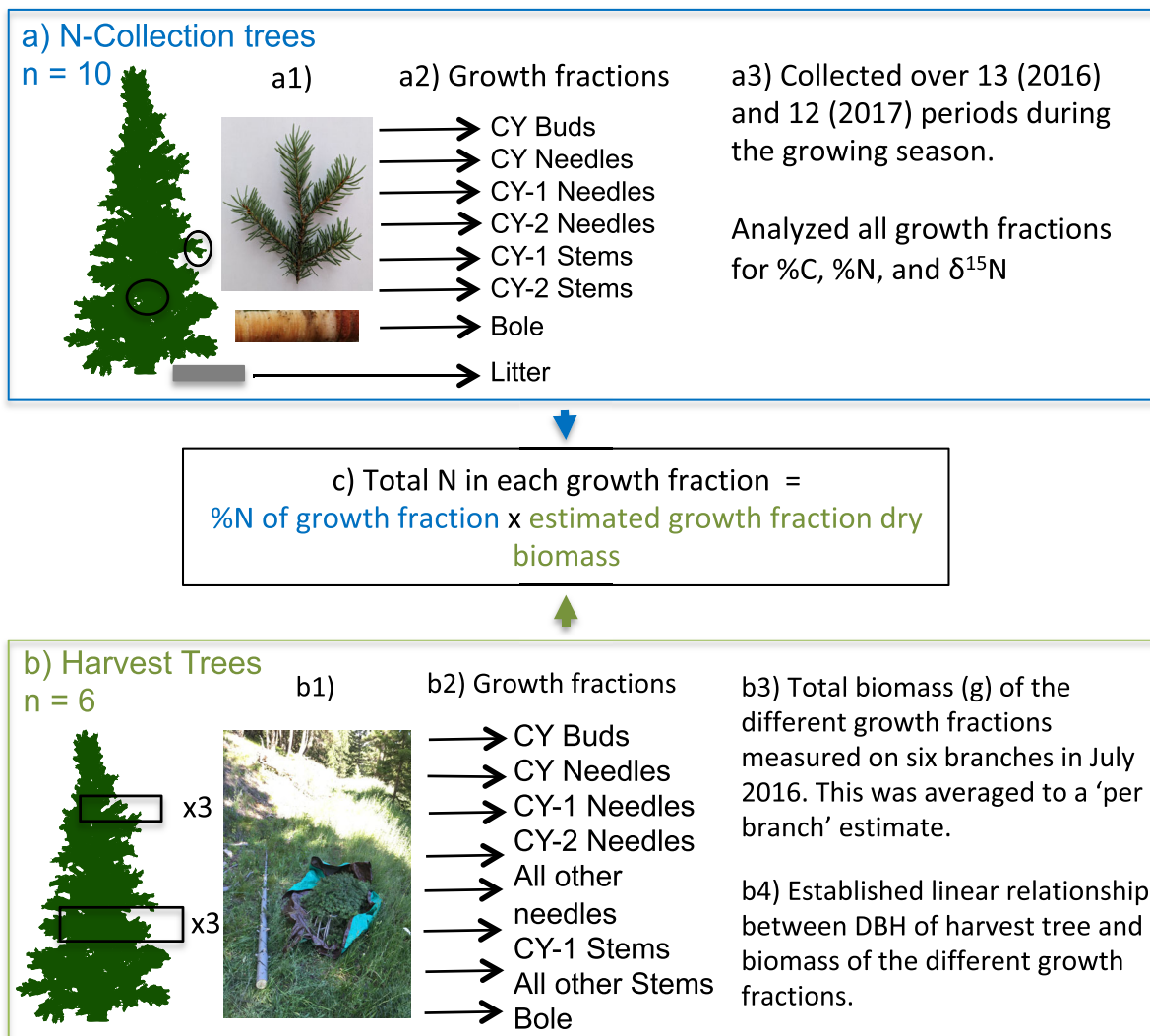


Fig. 2. Biomass harvesting methods used to calculate branch-level total N. Current year is the current year's growth, current year-1 is the one-year-old growth, and current year-2 is the two-year-old growth. (a1) Branches of the 10 N-collection trees were harvested approximately biweekly, (a2) divided into growth fractions, and (a3) dried, ground, and analyzed for %C, %N, and $\delta^{15}\text{N}$. Six off-plot trees were (b1) harvested and (b2) divided into growth fractions and b3) weighed. (b4) Then, allometric relationships between DBH and growth fraction dry mass were used to estimate the growth fraction dry mass in the N-collection trees. (c) The %N in each growth fraction (from a) was multiplied by the estimated growth fraction dry biomass (from b) in order to estimate total N in each growth fraction within whole tree branches.

S2). From this model, we predicted the estimated growth fraction branch masses in the N-collection trees, and we multiplied the predicted dry biomass (Appendix S1: Figure S2) by the N concentration of each growth fraction from each sampling period in order to calculate branch N content (mg/branch; Fig. 2c). We harvested trees

in late July to ensure that new needles were fully elongated (maximum biomass); however, we needed to account for the change in biomass as new needles flushed. To do so, we used published data from a mixed conifer forest in the North Cascade Mountains (Emmingham 1977) to estimate the percent of maximum elongation in

new growth as a function of the number of days after bud break, using our own observed dates of bud break to maximum needle elongation as the start and end dates.

Lastly, in order to account for any N lost through litterfall, we had to scale to the whole tree instead of branch level; this was due to the fact that we could not account for needles loss at the individual branch scale. To scale litter N to the whole tree level, we estimated how many litter traps fit under each tree canopy and multiplied that area times the total dry biomass of each litter sample after collection. We estimated the crown area of each tree using relationships developed between crown diameter and DBH following Curtis and Reukema (1970).

Soil N sampling

In order to estimate soil N supply rates, at both high and low elevation sites, we inserted eight soil ion exchange resin membrane (IER) PRS probes (4 cation and 4 anion membranes, Western Ag) every month from April until August 2016 at six locations across the entire site on the hillslopes, and less than 100 m from where sampled trees were growing (Binkley and Matson 1983, Jaeger et al. 1999). The resin-exchangeable N is often used to infer soil N supply and plant N uptake rates in agricultural settings because soil N supply and plant N uptake are highly correlated (Qian and Schoenau 1994). However, because N supply and uptake can be fairly heterogeneous within small areas, and the PRS probes were not placed directly beneath the N-collection trees during the first study season, we assumed that the PRS probes best represented the general seasonal trend of N supply rates for each study site. The PRS probes were inserted at a depth of approximately 10 cm in order to capture the most N dynamic portion of the soil profile. Additionally, in 2017, monthly from April through October, we inserted four PRS probes (2 cation and 2 anion membranes) under the canopy of each N-collection tree. After removal, the probes were kept cool and cleaned with distilled water within 48 h, refrigerated until the end of the season, and then analyzed for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ using flow injection analysis.

To measure net N mineralization and nitrification rates, we used the buried bag method (Eno 1960). We collected pairs of soil samples to a

15 cm depth under each N-collection tree from May until October 2017. We buried one of the samples in a polyethylene bag under the canopy for a one-month incubation period, and the other was cooled and transported back to the lab for processing. After the month long incubation period ended, we removed the buried samples. To process both sets of samples, we removed any live plant material and then extracted the samples using 1M KCl within 48 h of sampling. Extracts were gravity filtered through p8 coarse paper filters, and then syringe filtered through 0.7- μm glass filter tips. After filtration, the samples were analyzed for ammonium (NH_4^+) and nitrate (NO_3^-) using flow injection analysis (Lachat Quik-Chem Series 2400, Colorado).

Statistical analysis

For the growth fraction biomass scaling, we used R (R Core Team 2017) to fit a linear model to estimate the biomass for each growth fraction. We tested how dry biomass in CY needles, CY-1 needles, CY-1 stems, and boles varied as a function of DBH (Appendix S1: Section S1). In this model, normal-QQ and residual plots were used to visually assess how each model met the assumptions of normality and constant variance, and the model was log-transformed to better meet those assumptions.

For the %N, C:N ratio, total N, and $\delta^{15}\text{N}$, we fit linear mixed effects models using the lme function from the nlme package in R (R Core Team 2017, Pinheiro et al. 2018). We used a backwards stepwise model selection procedure to select each model. For %N, C:N, total N, and $\delta^{15}\text{N}$, we started with a full model containing the main fixed effects (timing, where days at or before bud break were considered before bud break; growth fraction; year; and elevation), as well as all combinations of interactions of each fixed effect. Fixed terms or interactions were removed from the model in a stepwise fashion until all remaining terms had a *P* value of <0.05. A random effect of individual tree was also included in each model in order to better account for the lack of independence in the study design, and we reported the intra-class correlation (ICC) for each model to explain the variability between observations in individual trees. Following this stepwise model selection process, we tested how %N and the C:N ratio changed as a function of

sample year, site, and an interaction between growth fraction and timing relative to bud break, after accounting for a random effect of individual tree. In the total N and $\delta^{15}\text{N}$ model selection process, elevation was not an important factor determining variation (P value >0.05), so the term was not included in the models. The total N of the bole and litter was modeled separately from the needle fractions due to the different scaling approaches used. Further, the bole and litter fractions were modeled separately because we only sampled litter for the entire season in 2017. All response variables except $\delta^{15}\text{N}$ and total N in the separate bole and litter models were log-transformed to better meet the assumptions of normality and constant variance. In 19 bole samples, %N was too low to measure using the elemental analyzer, so they were treated as not available (NAs) in each model.

RESULTS

Growth fraction %N and C:N

The timing of snowmelt between the high and the low elevation sites can differ by almost one month (NRCS SNOTEL, stations 604 and 657), and the high elevation site stayed cooler and received more rainfall than the low elevation site (Fig. 3). These differences in climate between the two sites led to an offset in the timing of bud break. Therefore, in order to compare the %N and C:N ratios of the different growth fractions, we normalized time to reflect before and after bud break, where negative values represent before bud break. Our model examined how %N varied as a function of elevation, year, an interaction between time relative to bud break and growth fraction, and a random effect of individual tree. We found that median %N was both greater at the high elevation and during 2016 compared to in 2017 (Appendix S1: Table S1; Figs. 4a,b, 5a,b, 6a,b). The median %N in each growth fraction was also dependent on the timing relative to bud break after accounting for the random effect of individual tree (ICC = 0.03; Appendix S1: Table S1). The %N in CY needles, and in CY-1 and CY-2 stems decreased after bud break (Figs. 4a,b, 5a,b, 6a,b). We did not detect any seasonal changes in %N in CY-1 or CY-2 needles or in the bole or litter. Among the different growth fractions, %N was highest in buds and

CY needles during the bud break period, followed by CY-1 needles, CY-2 needles, and CY-1 stems (Figs. 5a,b, 6a,b). Bole tissue, litter, and CY-2 stems had the lowest %N (Figs. 4a,b, 5a,b, 6a,b).

The C:N ratio is often used to evaluate nutrient allocation within plants. Woody tissues have higher C:N ratios than photosynthetic tissue, and we confirmed these patterns by examining C:N among the different growth fractions. The median C:N ratio in all growth fractions was both greater at the low elevation and during 2017 compared to 2016 (Appendix S1: Table S2, Fig. S3). Further, the median C:N ratio of all growth fractions varied depending on the timing relative to bud break after accounting for the random effect of individual tree (ICC = 0.02; Appendix S1: Table S2). In 2016, the bole had the highest C:N ratio, followed by litter, stems, and then needles (Appendix S1: Fig. S3). In 2017, the bole had the highest C:N ratio, followed by stems, litter, and then needles. Mean C:N in CY-1 stems, and to a lesser degree in CY-2 stems, increased across the seasons. On the other hand, the C:N of bud/CY needle declined prior to bud break, reaching the lowest point just prior to bud break, but then increased following bud break. As expected, this suggests that the addition of new biomass following bud break diluted needle N (Appendix S1: Fig. S3).

Branch mass balance calculations

In order to apply the mass balance approach at the branch scale, we followed pools of N among different growth fractions within branches throughout two growing seasons (Figs. 4c,d, 5c, d, 6c, d). In the needles and stems, we found that median total N was greater in 2017 compared to in 2016 (Appendix S1: Table S3). Further, we found that differences in total N between growth fractions depended on the day of year relative to bud break after accounting for the random effect of individual tree (ICC = 0.4; Appendix S1: Table S3). Our model indicated that CY needles accumulated a median of 0.04 ± 0.01 mg N/branch after bud break, while median total N in CY-1 stems decreased by 0.013 ± 0.018 mg N/branch after bud break (Fig. 7). In all other remaining needle and stem growth fractions, total N did not show important changes across the season. While we did not detect differences

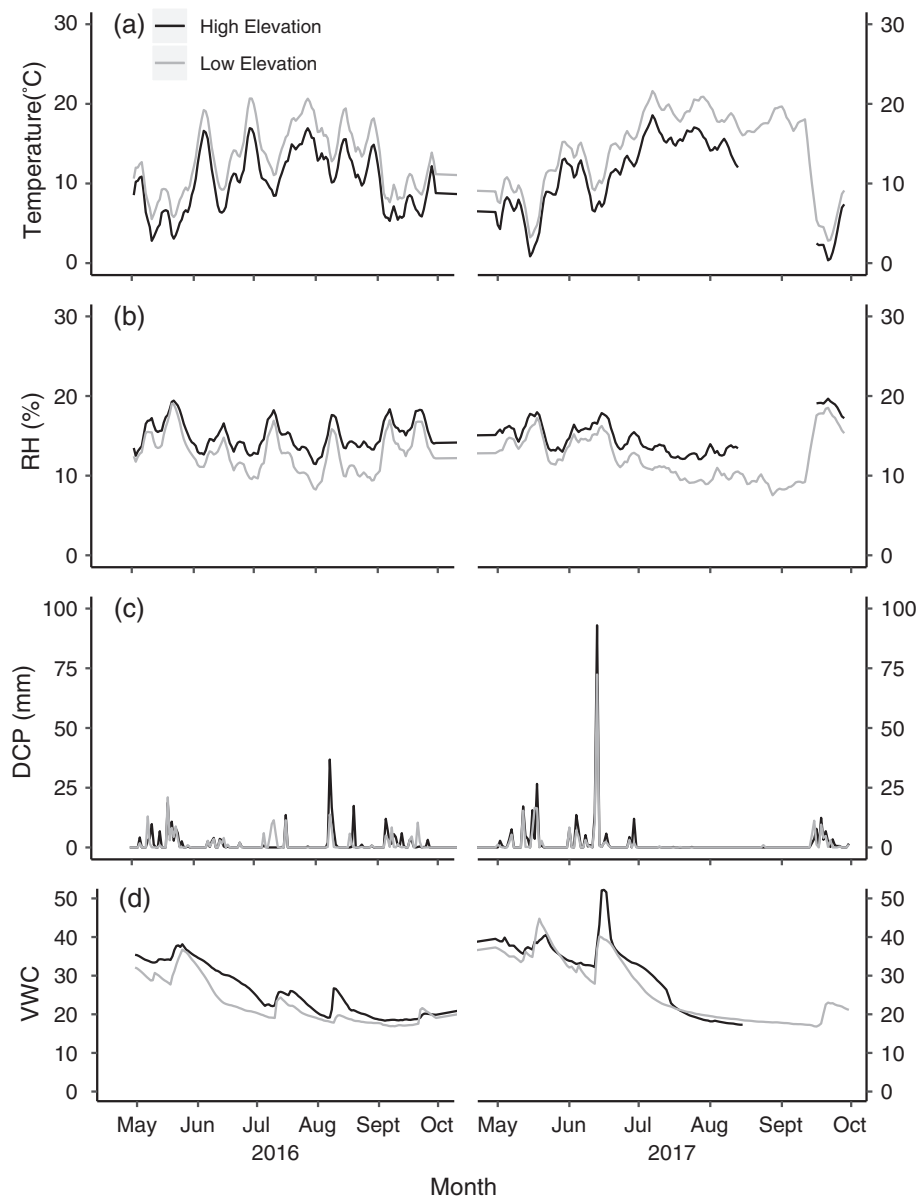


Fig. 3. 5-d moving averages of (a) temperature (°C), (b) relative humidity (%), (c) daily cumulative precipitation (mm), and (d) daily volumetric water content (VWC) averaged by 10 cm, 30 cm, and 50 cm soil depths. The broken *x*-axis between years represents the overwinter period.

in total N depending on elevation overall, average total N in mature CY needles was consistently higher at the high elevation (0.001–0.665 mg N/branch) than the low elevation site (0.001–0.50 mg N/branch; Figs. 4c, d, 5c, d). In 2016, the highest total N occurred in CY needles, followed by CY-1 needles, CY-1 stems, and then buds (Figs. 4c, 5c). In contrast, during 2017, we

found the greatest total N in CY-1 needles (which were CY needles in 2016), CY needles, CY-2 needles, CY-1 and CY-2 stems, and then buds (Figs. 4d, 5d). We also calculated the differences in average total N of each growth fraction before (day after flush < 0) and after (day after flush ≥ 0) bud break. We found the largest difference occurred in the bud/CY needles at

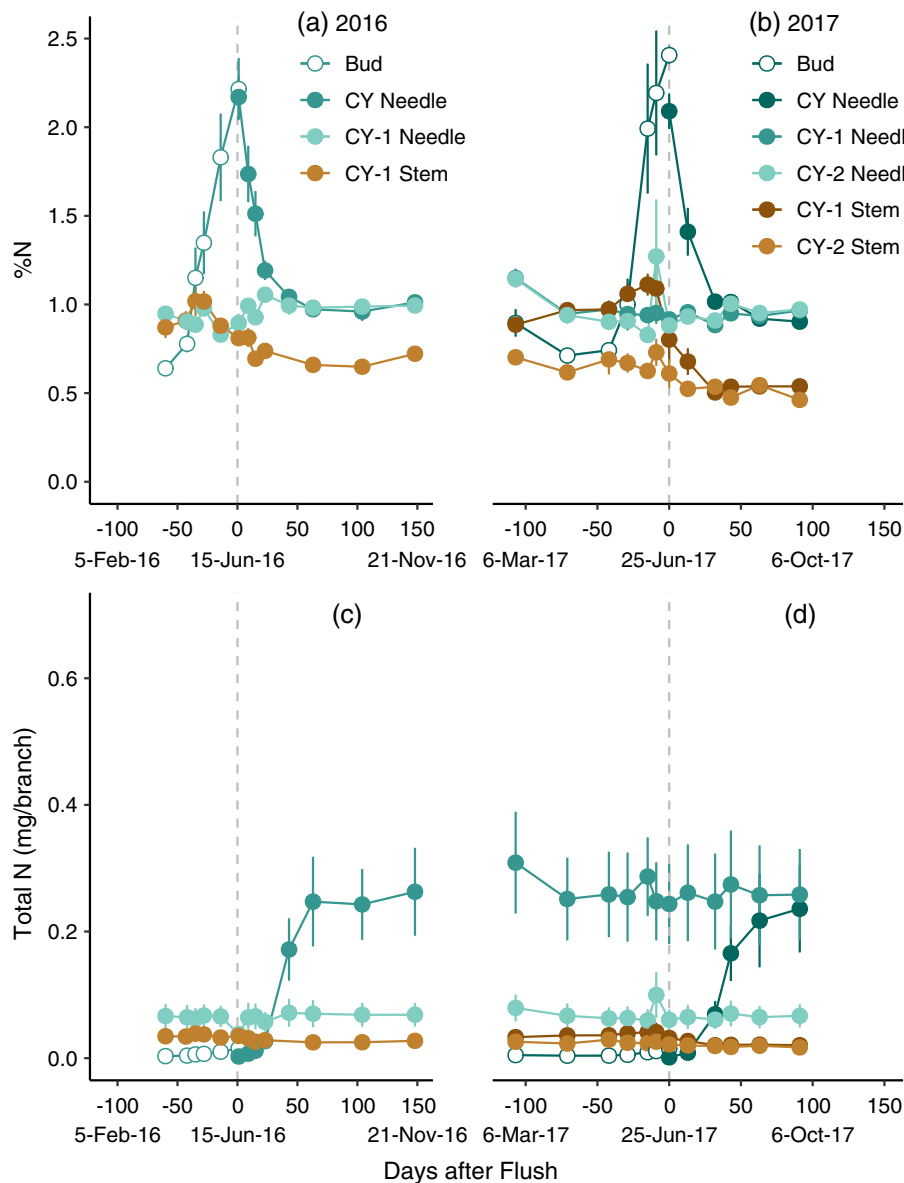


Fig. 4. Pattern of percent N (%N; a and b) and total N (mg/ branch; c and d) for all growth fractions in *Pseudotsuga menziesii* (mean \pm SE, $n = 5$) during 2016 (a and c) and 2017 (b and d) at the low elevation site relative to needle flush at day 0 (dotted gray line). Note: CY needles in 2016 become CY-1 needles in 2017, and CY-1 needles/stem in 2016 become CY-2 needles/stem in 2017.

both high and low elevations in both years (Fig. 7). In 2017, we also found an increase in CY-1 needles at the high elevation site after bud break. We note that the average difference in total N between the growth fractions from Fig. 7 has different values from Figs. 4 and 5. This is because Figs. 4 and 5 represented each

individual sampling date, while Fig. 7 is an average of all N sampling dates by each growth fraction before and after bud break.

We also traced N allocation within the bole and litter at a whole tree level (Fig. 6). In the bole, the average total N was greater during 2016 (Appendix S1: Table S4; Fig. 6) and was 8.0 g/

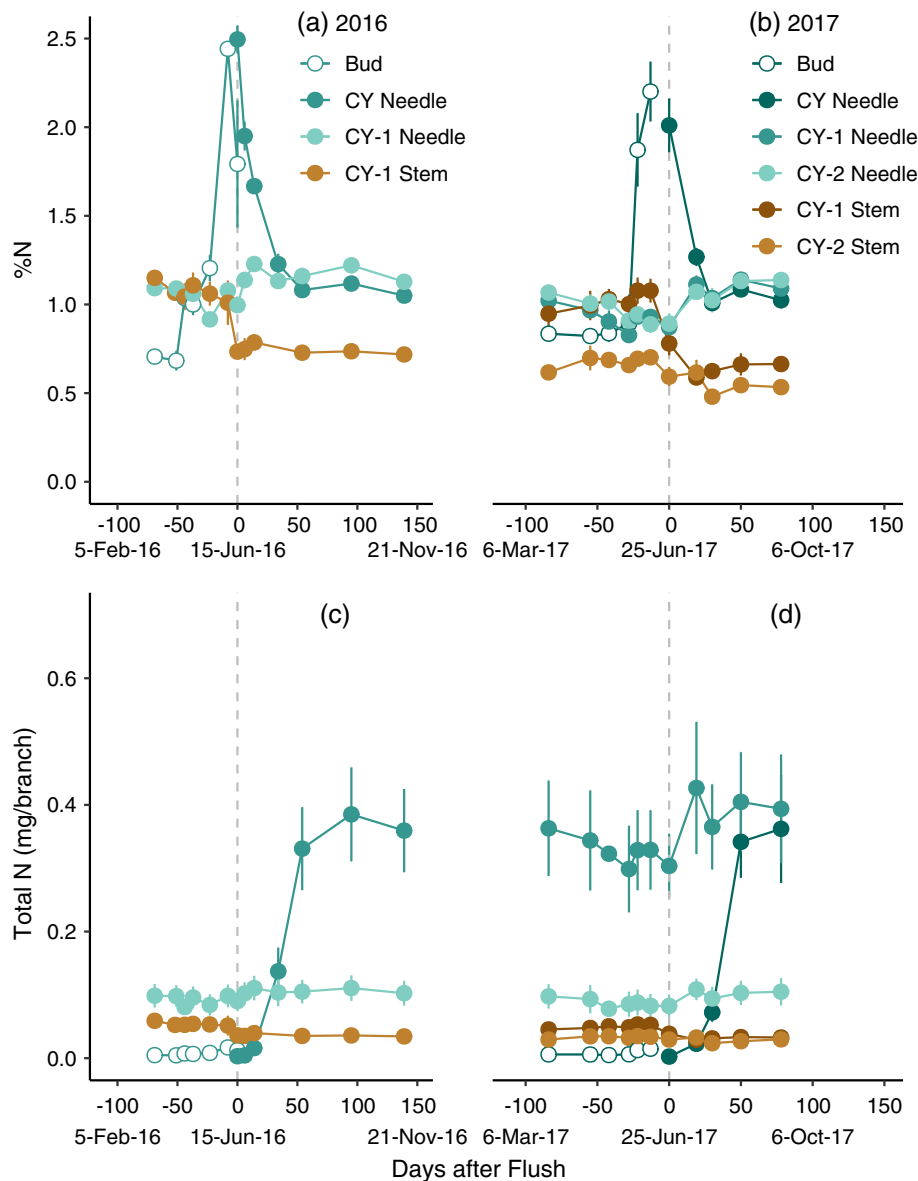


Fig. 5. Pattern of percent N (%N; a and b) and total N (mg/ branch; c and d) for all growth fractions in *Pseudotsuga menziesii* (mean \pm SE, $n = 5$) during 2016 (a and c) and 2017 (b and d) at the high elevation site relative to needle flush at day 0 (dotted gray line). Note: CY Needles in 2016 become CY-1 needles in 2017, and CY-1 needles/stem in 2016 become CY-2 needles/stem in 2017.

tree (95% CI of 7.0 to 10.0 g/tree) greater after bud break, but it did not vary between elevations accounting for the random effects of individual tree (ICC = 0.58; Appendix S1: Table S4; Fig. 6e, f). During 2017, the average total N returned to the soil as litterfall was greater at the high elevation (Appendix S1: Table S5; Fig. 4) and was

2.1 g/tree (95% CI 2.0–2.6) lower after bud break after accounting for the random effect of individual tree (ICC = 0.32; Fig. 6e, f). We did not collect litter before bud break in 2016, so we could only compare the average difference of total N in litter for 2017. The bole had higher total N compared to litter, but this was likely because that

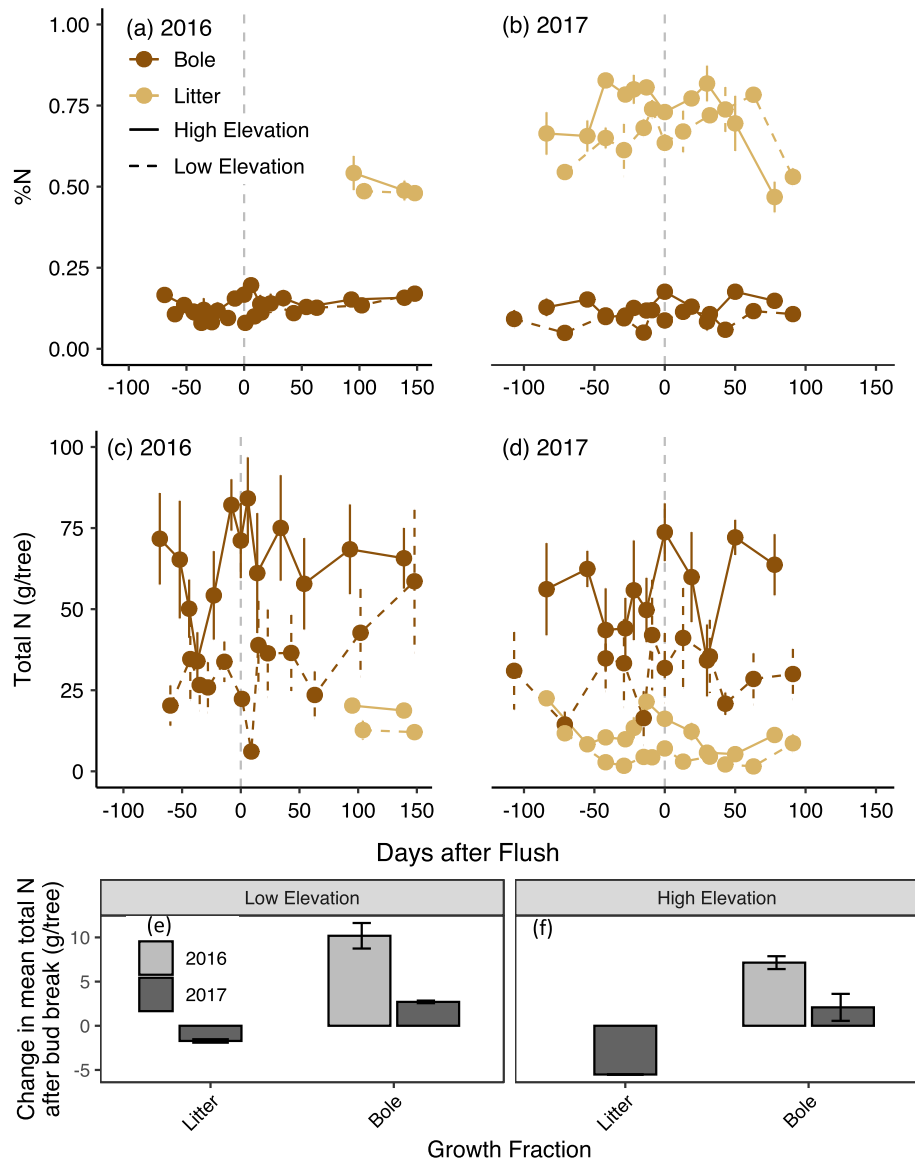


Fig. 6. Pattern of percent N (%N; a, b; mean \pm SE) and total N (g/tree; c, d; mean \pm SE) for bole (dark brown) and litter (light brown) growth fractions in *Pseudotsuga menziesii* ($n = 5$ per sample date) across 2016 (a, c) and 2017 (b, d) relative to needle flush at day 0 (dotted gray line). For the bole, differences in average total N before and after bud break are shown for both 2016 (light gray) and 2017 (dark gray) at the low elevation (e) and the high elevation (f). For the litter, differences in average total N before and after bud break are only from 2017 (dark gray; e, f).

fraction made up a larger portion of biomass in the tree (Fig. 6).

Growth fraction $\delta^{15}N$

We also analyzed the $\delta^{15}N$ of buds and needles to evaluate qualitative changes in N pools that

may occur due to uptake versus reallocation of N. Mean $\delta^{15}N$ was more negative during 2017 compared to 2016, but did not differ by elevation (Appendix S1:Table S6; Fig. 8). Further, variability in $\delta^{15}N$ between growth fractions was dependent on timing relative to bud break

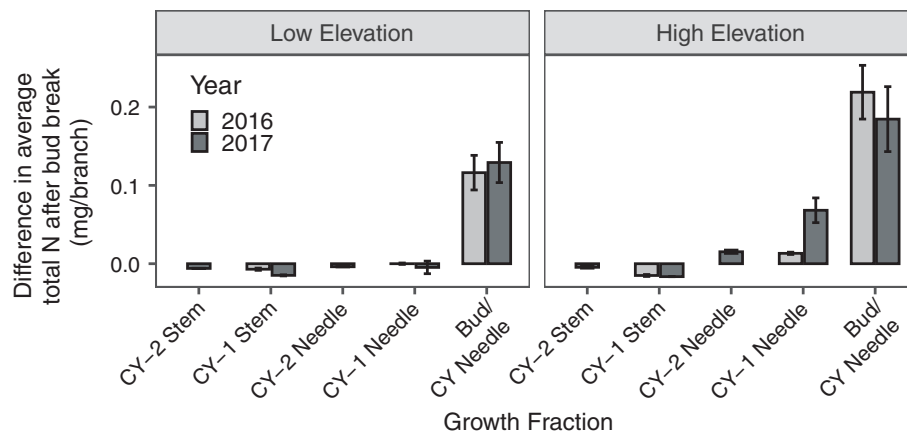


Fig. 7. Differences in average total N after bud break for the different needle growth fractions for low and high elevation sites.

(Appendix S1:Table S6) after accounting for the random effect of individual tree ($ICC = 0.55$). In both years, $\delta^{15}N$ in buds, CY needles, and CY-1 needles were similar during the first sampling date (~1.5 months prior to bud break); however, bud and needle $\delta^{15}N$ began to diverge leading up to bud break, with bud $\delta^{15}N$ becoming more isotopically enriched, and CY and CY-1 needle $\delta^{15}N$ becoming more depleted (from $\sim -2.5\text{‰}$ to -3.2‰ ; Fig. 8). Directly before and directly after bud break, all needles, including CY, CY-1, and CY-2, had similar $\delta^{15}N$ values, where the $\delta^{15}N$ pattern typically became more depleted before becoming increasingly enriched. We did not detect differences in mean $\delta^{15}N$ between needle cohorts, except at the low elevation in 2017, where buds were more depleted than CY-1 and CY-2 needles (Fig. 8).

Soil N transformation and supply

We observed net mineralization and nitrification rates using the buried bag approach in 2017. Using this approach, we did not detect differences in transformation rates across the season (Fig. 9). We also used the PRS probes to examine how soil NH_4-N and NO_3-N supply responded at different elevations and across the seasons. During 2016, both NH_4-N and NO_3-N supply increased toward July at both elevations, though seasonal patterns after July 2016 are unknown (Fig. 10). During 2017 at the low elevation, NH_4-N and NO_3-N supply rates were highest during the mid-June and late-September burial periods,

while they peaked both at the beginning and end of the growing season at high elevation during 2017 (Fig. 10) when soils were wet (Fig. 3).

DISCUSSION

Nitrogen is a limiting nutrient for many temperate plants, but storage can play a more important role in evergreen plants compared with deciduous plants, because of their differences in leaf longevity and less efficient use of N (Warren and Adams 2004). Conifers can use this stored N to provide a buffer from inter-annual variation in soil N availability (Warren and Adams 2004) and to support new growth by remobilizing the stored N (Millard and Grellet 2010). However, in our study, we found that over two years, the majority of N used to support new needles in Douglas-fir was acquired from soils during the growing seasons, and not from aboveground storage in older growth fractions. While some translocation from older stems to the buds appeared to have occurred prior to bud break, our study found that most of the N accumulating in the current year needles was newly acquired from the soils. Furthermore, we did not find this seasonal pattern of N supplying new growth to change between the drier, warmer low elevation site, and the wetter, cooler high elevation site. Our findings suggest that while Douglas-fir do have the ability to store N internally, soil available N is still an important source to supply N needed for

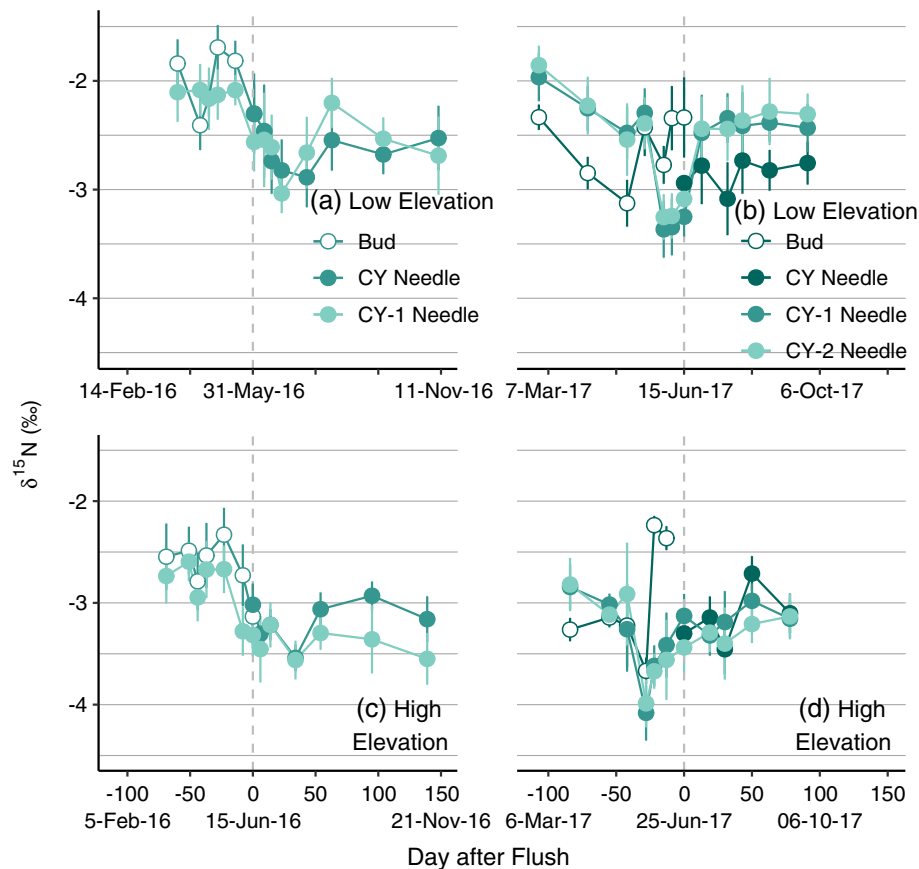


Fig. 8. Pattern of $\delta^{15}\text{N}$ (mean \pm SE) for all growth fractions at the low (a, b) and high (c, d) elevation sites across the 2016 (a, c) and 2017 (b, d) seasons relative to needle flush at bud break (dotted gray line).

new biomass production, especially under disturbance and drought conditions.

Remobilization versus uptake

The storage of resources, including nutrients, is an important plant function. Deciduous and evergreen species display large differences in where and how they store nutrients, and when they store and remobilize nutrients. Further, within evergreen species, there is still no clear consensus on how much N is remobilized versus newly acquired from the soils in order to support new growth (Millard and Grelet 2010). Some studies have found that almost all N in new tissue is translocated from other plant growth fractions (Chapin and Kedrowski 1983), while others suggest that evergreens instead rely more on N that was taken up directly from the soil (Chapin 1980, Aerts and Chapin 1999). Additionally,

other studies find a temporal shift where N remobilization is important at the start of the growing season, but uptake becomes more important later in the growing season (Millard and Proe 1992).

In our observational study, we found very little evidence that the N accumulating in the newly expanding needles could be sufficiently supplied from remobilized N alone because we did not observe a large decline in N in the older needles, stems, litter, or bole tissue (Figs. 4–7). However, at the start of the growing season when soil temperatures were cold and root N uptake was minimal, some remobilization from older stems to buds likely occurred, as demonstrated in our data where %N in buds increased while %N and total N in CY-1 stems decreased (Figs. 4, 5). This is consistent with other studies that observed the peak concentrations of remobilized amino acids

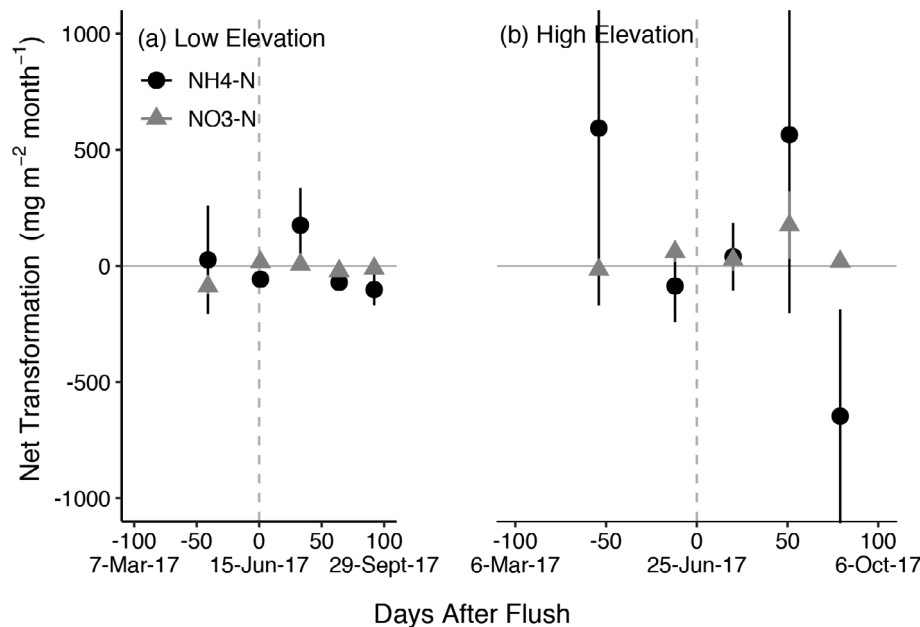


Fig. 9. Net N transformation rates (mean \pm SE) from the buried bags ($n = 5$) at the low (a) and high elevation (b) sites during 2017, where the dotted gray line marks bud break and the solid horizontal line marks 0, where values <0 represent periods of immobilization while values >0 represent periods of mineralization. Each point represents the first day of a month-long burial period.

in xylem sap to occur around bud break in deciduous trees (Guak et al. 2003, Millard et al. 2006). However, N taken up from soil following bud break dwarfed the magnitude of N remobilization; by the time bud break occurred (late May to mid-June in 2016, mid-June to late June in 2017), root uptake of N from the soils was likely the dominant source. During this period, soil moisture levels were high (Yano et al. 2019), soil N supply and availability rates were high (Figs. 9, 10), and transpiration rates were high (Looker et al. 2018), enhancing rates of N mass flow to the roots (Lambers et al. 1998, Matson et al. 2002).

The $\delta^{15}\text{N}$ of needle tissue also demonstrates that the bud and needle N pools undergo changes around bud break. The consistent temporal pattern between different needle cohorts and between low and high elevation tree needle $\delta^{15}\text{N}$ suggests similar patterns of N translocation and uptake (Fig. 8). We suggest that the temporal variation in needle $\delta^{15}\text{N}$ reflects changes in N sink-source dynamics in two discrete phases. The first phase was immediately before bud break when stored N was internally translocated from

older needles to buds (as seen in increasing bud %N; Figs. 4, 5). During this period, there was little N uptake by roots due to low soil temperature (Alvarez-Uria and Körner 2007), and sink strength was limited by bud break. We used the divergence in the $\delta^{15}\text{N}$ between buds and CY/CY-1 needles prior to bud break to infer translocation of N. Consistent with the principles of isotopic mass balance, the data suggest that N accumulating in the buds (which was becoming more isotopically enriched) was translocated from the CY/CY-1 needles (which were becoming more isotopically depleted). The second phase occurred following bud break, when the divergence in buds, CY, and CY-1 $\delta^{15}\text{N}$ stopped and instead began to change in the same direction. This change in $\delta^{15}\text{N}$ pattern likely represents uptake of N directly from the soils. Studies have found ^{15}N discrimination in the plants to occur when soil N availability exceeds plant N uptake (Hogberg 1997, Evans 2001, Dawson et al. 2002). Glutamine synthetase and nitrate reductase operate to perform NH_4^+ and NO_3^- assimilation, and in the process, they fractionate against ^{15}N . When soil N availability is high (e.g., early

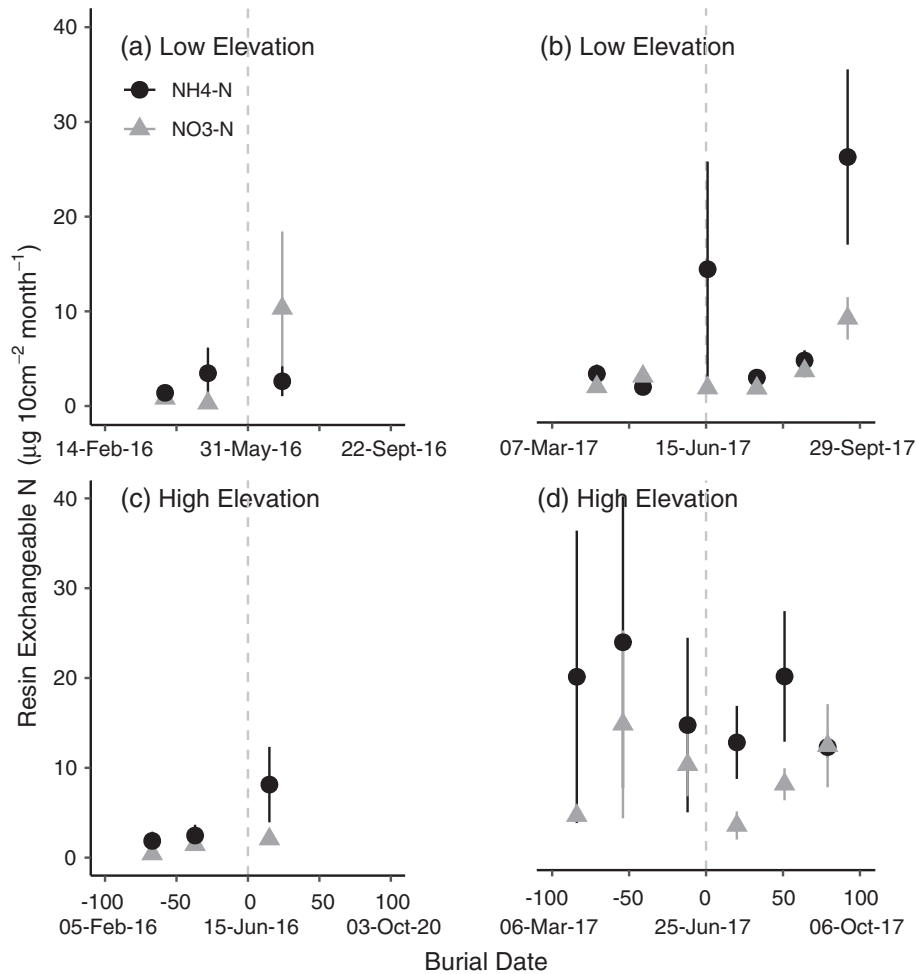


Fig. 10. Resin-exchangeable N (mean ± SE) from the IER probes at the low (a) and high (b) elevation sites across the 2016 (a, c) and 2017 (b, d) seasons, where the dotted gray line marks bud break. Each point represents the first day of a month-long burial period.

growing season, March–May, prior to bud break), some efflux of inorganic N from the root occurs, and the N assimilated into the plant is more depleted in ¹⁵N relative to the N efflux (more negative δ¹⁵N; Evans 2001). Under lower soil N availability (e.g., late growing season, August–September, postbud break), most N taken up is assimilated into the needles with little lost via root efflux, and thus, the N assimilated into the plant N pool more closely reflects the δ¹⁵N of the soil (less negative δ¹⁵N). Our observations of seasonal δ¹⁵N in plant tissues reflect this source-sink dynamic. In the early growing season, the more negative δ¹⁵N needle tissue (CY, CY-1) suggests that soil N was high and plants

were actively taking up this depleted δ¹⁵N pool; however, by mid-summer (June–July), needle tissue δ¹⁵N became more positive, suggesting that soil available N decreased (Fig. 8).

Our observation that newly acquired N is important for supporting new biomass production has been observed in a range of ecosystems and different evergreen plants, though rarely in conifers. In a tundra ecosystem, *Ledum palustre*, an evergreen shrub, did not use any stored N from past season leaves, stems, or roots for new growth (Chapin et al. 1980). In another study, even after old leaves were manually defoliated from evergreen species in both tundra and Mediterranean environments, N accumulation in

new leaves still continued (Jonasson 1989), further demonstrating that N used to support new growth in evergreens is not necessarily derived from N stored in older growth fractions. On the other hand, other studies have shown that evergreens do allocate N from storage to support the current season's growth, indicating an ability to decouple foliar N demand from soil nutrient availability across seasons (Schlesinger et al. 1982, Chapin and Kedrowski 1983, Gray 1983, Nambiar and Fife 1987, Rapp et al. 1992, 1999, Wendler et al. 1995, Cherbuy et al. 2001). In some cases, translocation of N can be important when soils are too dry for nutrient acquisition, as was the case for *Ceanothus megacarpus*, an evergreen shrub (Gray 1983, Gray and Schlesinger 1983). *Ceanothus* even stored more N than deciduous species (Chapin and Shaver 1989), though this could be due to the species' N-fixation strategy (Gray 1983). N translocation may also be more likely to occur in seedlings, when root development is not sufficient to acquire all the nitrogen needed for new growth (Proe and Millard 1994).

Differences in storage versus uptake patterns between this study and others are likely due to differences in life stage, growth habit, climate, or leaf longevity (Millard and Grelet 2010). First, studies examining seedlings are typically performed in glasshouses, where growing conditions are ideal compared to in the field. N allocation strategies between seedlings and adult trees could differ because they allocate biomass differently (Mooney 1972, Dickson 1989, Espeleta and Eissenstat 1998). Secondly, while the literature focuses on leaf longevity (i.e., deciduous versus evergreen habits) as the most important trait controlling N residence time in leaves (Aerts and Van Der Peijl 1993, Aerts and Chapin 1999, Silla and Escudero 2003, Takashima et al. 2004), life form (specifically broadleaf evergreens versus evergreen conifers) is an informative trait distinguishing N allocation strategies. Some studies evaluated N-fixing evergreen shrubs in Mediterranean environments with leaf longevity ranging between 2 and 3 yr (Schlesinger et al. 1982, Gray 1983), while another examined an evergreen conifer growing on the tundra with an average leaf life span of 30–40 yr (Chapin and Kedrowski 1983). These differences between functional traits and variability in climate conditions could affect plants' N allocation strategies by, for example,

influencing plants' nutrient uptake capacity (Lambers et al. 1998), changing the amount of N allocated for cell structure versus photosynthetic function (Takashima et al. 2004), or affecting evergreen's ability to repair and regenerate roots (Campbell et al. 2014). However, further investigation is needed to elucidate the relative importance of each.

In order to assess the importance of stored versus newly acquired soil N for new needle growth, we relied on using the mass balance approach at the branch scale because the nutrient depletion method was not feasible at our site. However, the mass balance approach had limitations because it is difficult to scale up to an entire mature tree, or properly track below ground processes (Nambiar and Fife 1991, Proe and Millard 1994). Because previous studies have found that total N in conifer roots and older needles does not fluctuate seasonally, we assumed that all roots and needles older than three years provided minimal N to support new growth (Nambiar 1987, Gessler et al. 1998b). Further, nutrient pools in older needles are relatively inert (Aerts and Chapin 1999). It is possible, however, that the importance of roots as an N reservoir in larger trees may have been overlooked and that some N could have been reallocated from roots and older needles and stems. Thus, without measuring these growth fractions directly, we cannot completely rule out N translocation from those growth fractions.

Synchrony between soil N availability and tree N accumulation

Given that several studies had found the highest levels of plant-available N in soil following spring snowmelt (Brooks et al. 1998, 2011, Maurer and Bowling 2014, Yano et al. 2015), we expected that plant-available N would be highest in the spring directly after snowmelt. However, we did not consistently observe this pattern. In fact, at the low elevation in 2017, the mean $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ supply rates were highest at the end of the growing season, and during 2016 the rates increased toward July. However, at the high elevation site during 2017, mean $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ supply rates were relatively high from the spring to the fall, with peaks at the beginning and at the end of the growing season. Our sampling periods may not have been early enough to

capture the soil N peak after snowmelt, especially during 2016, or, in the case of the low elevation site, the winter snowpack may not have been deep enough to insulate the soil and promote N mineralization throughout the winter (Campbell et al. 2014). Nevertheless, the net N transformation (Fig. 8), and to a greater degree, the resin exchange results (Fig. 9), suggests that the highest rates of soil N supply (particularly $\text{NH}_4\text{-N}$) coincided with needle expansion (approximately 0–50 d after bud break). The pattern of peak soil available N and peak tree N uptake is not perfectly synchronized, and previous studies have observed these asynchronies. Asynchronies can develop either because of sink N dynamics related to plant growth (Nambiar and Fife 1987) or because internal source N pools are more important controls on needle N remobilization than the size of the available N pool in soils (Millard and Grelet 2010). In cases where soil N availability is low, remobilization rates in plants tend to be higher. In contrast, in cases when soil available N is relatively high (such as in 2017 compared to 2016), soil N can be an important driver in increasing N uptake rates (Cole 1981, Nambiar and Fife 1987).

Spruce budworm outbreak

Older needle and stem cohorts can serve as N reservoirs that can be remobilized within conifers during needle expansion (Millard and Grelet 2010). However, the results from 2016 show that there was minimal remobilization of N from 2015 needles or stems, therefore suggesting that the N accumulating in the CY needles primarily came from the soil. Given that our results contrasted previous studies showing strong translocation within conifer needles, we hypothesized that this lack of translocation could have resulted from a spruce budworm (*Choristoneura occidentalis*) outbreak that lasted between 2004 and 2017, and was widespread during 2015. In 2016, we observed that a large percentage of the 2015 needles (CY-1 needles in 2016), which would have been the largest reservoir of stored N, were severely defoliated (Appendix S1: Fig. S1). Spruce budworm can defoliate between 8% and 17% of a tree's gross volume in a given outbreak (Alfaro et al. 1985), and outbreaks are most severe in drought-stressed trees with relatively low foliar

N concentrations (Cates et al. 1983, Redak and Cates 1984). If the 2015 (CY-1) mature needles served as the primary N reservoir, and that reservoir was lost through budworm defoliation, the trees would depend more on N acquired from the soils to support new biomass production (Millard et al. 2001, Millard and Grelet 2010).

In 2017, we observed that fewer 2016 (CY-1) needles were defoliated by spruce budworm than in 2016. Because there was less spruce budworm defoliation on the CY-1 needle cohort, we expected to see higher rates of translocation of N from the CY-1 to 2017 (CY) needles. However, in 2017, we did not observe evidence that a large portion of N in CY needles was translocated from CY-1 needles. While some N was likely translocated from CY-1 stems, most N in the CY needles was acquired from the soils. Thus, defoliation did not appear to be the main factor influencing translocation strategies. Nevertheless, it is possible that it may take more than two years after insect defoliation for trees to sufficiently recover N storage in needles. Longer-term sampling would be needed to address this more directly.

CONCLUSION

Our findings suggest that conifer trees growing in snow-dominated semi-arid ecosystems rely on soil available N during the growing season to support new needle growth. This was true for both the cool, wet high elevation site and the warm, dry low elevation site. Tree N accumulation in new growth synchronized with seasonal patterns of soil N supply. While we observed that Douglas-fir can meet their N demand by drawing from the soil, the trees may not be as resilient under multiple prolonged stressors. Changes in soil N availability due to declining snowpack, more severe and long-lasting droughts, combined with more frequent disturbance from insect outbreaks, such as spruce budworm, could compound to hinder trees' ability to take up enough N to support new growth (Brooks et al. 1995, Brooks and Williams 1999, Pederson et al. 2011, Campbell et al. 2014, Flower et al. 2014). Future research should address how changes in snowpack and insect disturbance will directly influence N accumulation in evergreens.

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