

SCALING NITROGEN RETENTION FROM TREES TO FORESTS
THROUGH SUCCESSION

by

Aaron James Scott-Klingborg

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Land Resources and Environmental Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2016

©COPYRIGHT

by

Aaron James Scott-Klingborg

2016

All Rights Reserved

DEDICATION

For the trees.

ACKNOWLEDGEMENTS

I am very grateful for the hard work and enthusiasm of my advisor Dr. Jack Brookshire in guiding this project and investing in my career as a scientist. Much appreciation goes to advisory committee members Dr. Cathy Zabinski and Dr. Travis Belote for their insightful questions, expertise, and dedication towards making this project great. I would not have accomplished much without the help of a few terrific individuals: my partner Meg Housman for her delightful and expert assistance in the field, Erik Norderud for his unparalleled root sorting ability, and Dr. Jane Klassen for her invaluable help running many hundreds of my samples, sometimes repeatedly, in the laboratory.

TABLE OF CONTENTS

1. INTRODUCTION	1
Scientific Background	1
Study Goals	6
References	7
2. LARGE TREES DOMINATE NITROGEN RETENTION ACROSS FOREST SUCCESSION	8
Contribution of Authors and Co-authors	9
Manuscript Information Page	10
Abstract	11
Introduction	11
Methods	14
Study Sites	14
Field Measures	16
Calculations and Model Simulations	19
Root Uptake Simulation	21
Carbon Growth Simulations	22
Scaling Uptake by Individual Trees to ecosystem-level N Cycling	23
Results	23
Tree and Stand Results	23
Simulation Results	25
Carbon Growth Rate Simulation	26
Discussion	26
References	39
3. EXPRESSION OF SINK-DRIVEN AND TRANSACTIONAL NITROGEN LIMITATION FOLLOWING STAND-REPLACING DISTURBANCE IN AN INLAND PACIFIC NORTHWEST CONIFEROUS FOREST	40
Contribution of Authors and Co-authors	40
Manuscript Information Page	41
Abstract	42
Introduction	43
Methods	46
Results	51

TABLE OF CONTENTS – CONTINUED

Discussion	55
References	69
4. CONCLUSIONS	73
References	81
REFERENCES	82

LIST OF FIGURES

Figure	Page
2.1 Forest stand inventory (a) comparison of core and allometric fine root biomass estimates (b) and stand aboveground and fine root biomass to 15 cm (c).....	31
2.2 Ammonium and nitrate concentrations from water and potassium chloride extracts.....	32
2.3 Scaling of N uptake by trees v. aboveground tree biomass (a) and foliar N resorption v. aboveground tree biomass (b)	33
2.4 Annual N uptake by forest stands and large trees.....	34
2.5 Annual C growth rates for individual trees (a) and annual C growth rates for stands compared to Pacific Northwest forests (b).....	35
3.1 Total ecosystem C pools (a) and N pools (b).....	62
3.2 Soil C pools (a) and N pools (b)	63
3.3 Changes in C concentration with depth (a), and N concentration with depth (b), and C:N changes with depth (c)	64
3.4 Changes in C concentration with depth relative to conifer litter (a), and changes in N concentration with depth relative to conifer litter (b)	65
3.5 Turnover times of C and N	66
3.6 DIN concentrations (a) and DON concentrations (b)	67

LIST OF TABLES

Table	Page
3.1 Rates of change in biomass, soil, and turnover N pools	68

ABSTRACT

We seek to understand how the ability of trees to acquire and retain nitrogen (N) changes throughout their lifetimes. This capacity enables trees to act as carbon (C) sinks individually and collectively in forest ecosystems over successional time scales. We evaluate how properties that govern nutrient retention change with tree size and forest age, and how allometric relationships scale up to influence ecosystem-level patterns of N cycling and retention. Most generally, we hypothesized that changes in N uptake and recycling efficiency with increasing tree size would vary with forest age and N availability. Additionally, we evaluated changes in ecosystem-level C and N accumulation throughout secondary forest succession following clear-cut logging disturbances in an effort to understand how N limitation may become expressed over time and interact with forest successional dynamics.

Our findings highlight the importance of large trees in ecosystem N cycling and growth. We find that increasing mass growth rates are matched by an increasing capacity to acquire and retain N without necessitating increases in growth efficiency. Research findings indicate that mortality of single trees may hold profound consequences for stand-level N retention in addition to C storage. At the ecosystem scale, we find N accumulation and limitation are dynamic processes that fluctuate in strength and source over forest succession, and that ecosystem accumulation of N was driven predominately by increasing N in plant biomass rather than in soil pools.

CHAPTER ONE

INTRODUCTION

Scientific Background

The terrestrial biosphere removes about one-third of the anthropogenic CO₂ produced from fossil fuel combustion annually, but great uncertainties about the future magnitude and trajectory of this sink exist (Canadell *et al.* 2007). Saturation of the terrestrial C sink may in part be caused by nutrient limitation, particularly nitrogen (N) limitation, wherein positive growth responses associated with elevated CO₂ are limited by N availability (Finzi *et al.* 2007; Norby *et al.* 2010). It is therefore critical to understand the ability of plants to acquire and retain nutrients when they are limiting if we are to accurately predict the magnitude of the terrestrial C sink. Forests dominate the terrestrial C sink, but maintaining the current rate of C storage may be contingent upon continued forest productivity despite N limitation. Under limiting conditions, trees can retain N via root uptake and internal recycling, but the allometric scaling properties underlying any potential size-dependency of N uptake or recycling are unclear. These relationships are vital to understanding how tree-level processes translate into ecosystem-level patterns of N-availability and forest growth over time, and enable us to integrate small-scale physiological processes such as uptake by roots and foliar resorption with large-scale patterns of N cycling and C productivity.

A broadly used framework for the integration of wide-ranging biological scales can be found in the metabolic theory of ecology (MTE). The MTE describes how

biological properties of organisms (biomass, leaf area, stem diameter) and communities (population density, size structure) scale to inform physiological and biogeochemical patterns observed at the ecosystem level (West *et al.* 1997). Scaling relationships derived from MTE take the form $Y = Y_0 M^\theta$, where M is organism mass, Y may be metabolic rate, or any other parameter of interest (such as leaf mass, photosynthetic rate, etc.). Y_0 (a constant) differs according to the relationship (as a means of translating units of M into units of Y) of interest, and θ is the scaling exponent that describes the relationship between Y and M . For many biological relationships, metabolic scaling theory is useful for understanding the functioning and organizing principles of organisms and ecosystems across widely varying life histories and environments (Brown *et al.* 2004). But perhaps the most coveted asset of this framework is its potential to elucidate important mechanisms across a spectrum of biological scale. From relating the photosynthetic activity of a single leaf to the growth rates of entire trees, metabolic scaling theory uses fundamentals of the geometry of energy flow through transport networks in organisms to relate the variation in physical properties of organisms to their metabolic rates (West *et al.* 1997). Ultimately, MTE provides numerous theoretical predictions that can assist in explaining empirically observed relationships.

For plants, metabolic rate is proportional to resource supply, and thus the active (photosynthesizing or absorbing) surface areas of leaves or roots, respectively (West *et al.* 1999). Nutrient uptake supplies available soil nutrients, and theoretically should align with the biomass allocation and resource supply scaling predictions described by metabolic scaling theory. In spite of the potential utility of this metabolic scaling

approach, the scaling properties of nutrient uptake or recycling via foliar resorption are unclear.

Current understanding of nutrient uptake processes by trees derives from experiments conducted at the root-scale, which are then extrapolated to fine root biomass allocation sampling conducted at the forest or stand scale to estimate forest-level uptake fluxes. Relating the root to the ecosystem scale in this manner has proven difficult. Measuring root uptake in the field is notoriously difficult and fraught with potential artifacts. For example, depletion techniques can significantly alter the diffusion environment and concentration-dependent kinetics and isotope tracers are subject to competition for N by microbes, all of which can make scaling uptake fluxes over time and space difficult (Lucash *et al.* 2007). Numerous models have been used to estimate uptake by roots to avoid such complications posed by in situ methods. However, many of these models omit or overlook the implications of nutrient uptake at the tree scale, or how uptake influences forest N cycling aside from stand productivity. Understanding such a relationship may be key to elucidating the mechanisms by which trees and forests exhibit various patterns of N-cycling dynamics throughout their growth and development.

Root morphological properties such as specific root length (SRL) and diameter fundamentally dictate absorptive surface area of a root system. Empirical data support the notion that root morphology is a strong indicator of nutrient-acquisition capability, as high SRL species are able to proliferate root growth to a greater degree within nutrient-rich areas of soils compared to lower SRL species (Hodge 2004), and can possess a greater absorptive area per investment in root mass given low SRL and root tissue

density. Knowledge of root morphology can therefore inform our estimation of the ability of a root segment to absorb nutrients in a given area of soil, and its inclusion in the calculation of nutrient uptake places mechanistic and physical constraints on uptake.

Uptake by roots is not the sole flux of nitrogen within the tree-soil system. Foliar N resorption represents a vital mechanism by which trees can retain N (Aerts 1996). In N-limited systems with minimal inputs from deposition or fixation and low hydrologic losses, litterfall N is one of the few inputs of N to the plant-soil system and ultimately contributes to the mineralization rate of N (Scott & Binkley 1997). As such, understanding foliar N resorption at the individual tree scale is essential to completing the picture of the tree-soil N cycling system.

Understanding the mechanisms dictating the prevalence of N limitation in forests is paramount to predicting the response of terrestrial vegetation to rising CO₂ concentrations. Forests are the largest terrestrial C sinks, and N limitation is pervasive in temperate forests. Fundamentally, the balance of inputs and losses of N govern N availability and the degree to which it may limit plant productivity. Temperate forests are characterized by low N availability primarily due to low inputs from N deposition and largely absent N-fixing species over long periods of time. These forests are also highly retentive for N, typically displaying low N export from leaching losses or denitrification fluxes. Throughout forest succession, N is thought to accumulate to an extent due to the high retentiveness and steady, though low, inputs from deposition and/or N fixation. However, N limitation can persist indefinitely throughout succession when, despite strong retention by plants, N is lost from pools that plants cannot control, such as through

loss of dissolved organic nitrogen, fire, or physical loss of soil (Menge *et al.* 2009). Such disturbances thus can alter the trajectory of N accumulation in subsequently recovering forests. A particularly extreme N-budget-altering disturbance is the anthropogenic intervention of clear-cut logging. Many natural disturbances such as windthrow, landslides, or even wildfire leave behind a substantial proportion of biomass, though it may be killed. Clear-cut logging removes virtually all standing biomass, and a substantial proportion of the ecosystem N pool stored in biomass is removed simultaneously. As the world's forested landscape is increasingly falling under human management (Magnani *et al.* 2007)—in the U.S. alone, clear-cut logging accounts for nearly 40% of annual harvested forest area (Forest Inventory and Analysis National Program)—the recovery of these forests and the associated changes in N availability and limitation following management activities are therefore vital considerations in assessing the trajectory of terrestrial carbon (C) storage.

Here, we combine original field measures across a >300 year-old chronosequence of conifer forest development with a theoretical model of root uptake (Gerber & Brookshire 2014) and analysis of N allocation patterns in trees to investigate the scaling properties between individual tree uptake and recycling and forest-level patterns of N retention and C accumulation. These scaling relationships will provide insight as to how the ability of trees to acquire and retain N change throughout their lifetimes enable trees to act as C sinks individually, and collectively in forest ecosystems. We next evaluate changes in N availability and limitation in a coniferous forest following clear-cut disturbance, to assess the potential recovery trajectory awaiting many similar forests.

We conducted our work across a chrono-sequence of >300 years of time since clear-cutting in a temperate, coniferous forest. The chrono-sequence permits us the substitution of space for time, with each study site representing a distinct stage in forest succession. The chrono-sequence also provides an extensive range of tree sizes with which we are able to construct scaling relationships between our parameters of interest. Furthermore, our study area in Northwestern Montana represents the eastern geographical extent of the Pacific Northwest forest belt, a forest biome which possess some of the most productive and valuable timber lands and commercial conifer species on earth. Our sites also lie within the boundaries of the Montana Legacy Project, a landmark, multi-stakeholder land conservation initiative designed to protect critical forest habitat.

Study Goals

1. Construct scaling relationships between tree size and nutrient uptake and foliar N resorption, and evaluate how these findings align with the conceptual framework of metabolic scaling theory.
2. Use these scaling relationships to infer how individual tree-level processes may emerge as ecosystem level patterns of N cycling and retention, and C growth in trees.
3. Evaluate how ecosystem C and N pools change after clear-cutting disturbances in a Northwestern Montana coniferous forests.
4. Assess the ability of N-limited forests to retain N when it is limiting.

5. Understand the relationships between forest succession and changes in the strength and mechanisms causing N-limitation over time.

References

- Aerts, R. (1996). Nutrient Resorption from Senescing Leaves of Perennials: Are there General Patterns? *J. Ecol.*, 84, 597–608.
- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85, 1771–1789.
- Canadell, J.G., Pataki, D.E. & Pitelka, L. (Eds.). (2007). *Terrestrial ecosystems in a changing world*. Global change--the IGBP series. Springer, Berlin ; New York.
- Forest Inventory and Analysis National Program (2016). *U.S. Forest facts and historical trends*. Available at: <http://www.fia.fs.fed.us/program-features/rpa/>. Last accessed: 01 April 2016.
- Gerber, S. & Brookshire, E.N.J. (2014). Scaling of Physical Constraints at the Root-Soil Interface to Macroscopic Patterns of Nutrient Retention in Ecosystems. *Am. Nat.*, 183, 418–430.
- Hodge, A. (2004). The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol.*, 162, 9–24.
- Lucash, M.S., Eissenstat, D.M., Joslin, J.D., McFarlane, K.J. & Yanai, R.D. (2007). Estimating nutrient uptake by mature tree roots under field conditions: challenges and opportunities. *Trees*, 21, 593–603.
- Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., *et al.* (2007). The human footprint in the carbon cycle of temperate and boreal forests. *Nature*, 447, 848–850.
- Nabuurs, G.-J., Lindner, M., Verkerk, P.J., Gunia, K., Deda, P., Michalak, R., *et al.* (2013). First signs of carbon sink saturation in European forest biomass. *Nat. Clim. Change*, 3, 792–796.
- Scott, N.A. & Binkley, D. (1997). Foliage litter quality and annual net N mineralization: comparison across North American forest sites. *Oecologia*, 111, 151–159.
- West, G.B., Brown, J.H. & Enquist, B.J. (1997). A General Model for the Origin of Allometric Scaling Laws in Biology. *Science*, 276, 122–126.
- West, G.B., Brown, J.H. & Enquist, B.J. (1999). The Fourth Dimension of Life: Fractal Geometry and Allometric Scaling of Organisms. *Science*, 284, 1677–1679.

CHAPTER TWO

LARGE TREES DOMINATE NITROGEN RETENTION ACROSS FOREST SUCCESSION

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Aaron Scott-Klingborg

Contributions: Collected and analyzed data. Wrote first draft of manuscript and contributed to further revisions.

Author: Dr. Jack Brookshire

Contributions: Conceived the study design, provided primary feedback on statistical analyses, and contributed to writing the manuscript.

Manuscript Information Page

Aaron Scott-Klingborg, Jack Brookshire

Ecology Letters

Status of Manuscript:

☐ Prepared for submission to a peer-reviewed journal

☒ Officially submitted to a peer-review journal

☐ Accepted by a peer-reviewed journal

☐ Published in a peer-reviewed journal

Wiley

Submitted April 28, 2016.

LARGE TREES DOMINATE NITROGEN RETENTION ACROSS FOREST SUCCESSION

Abstract

Trees have the capacity to retain N in ecosystems via root uptake and internal recycling when N limited, but how these processes change as trees get larger and ecosystems develop over time is unclear. By combining theoretical modeling with empirical measures of plant-soil N cycling across a forest chrono-sequence, we find that increasing mass growth rates in trees are matched by an increasing ability to acquire and recycle N without necessitating increases in growth efficiency. Our findings reveal a keystone role for large trees in N cycling, with the largest individuals contributing disproportionately to ecosystem-level N retention. Further, we document that while the proportion of forest N-flux delivered by N resorption does increase through time, root uptake remains the dominant means of N supply through hundreds of years of forest succession.

Introduction

Trillions of trees (Crowther *et al.* 2015) cover over 30-percent of Earth's land surface (Dixon *et al.* 1994) and remove about one-third of anthropogenic atmospheric CO₂ emissions annually (Le Quéré *et al.* 2015). To accomplish this, trees require light, water, space, and nutrients. A deficiency in any one of these resources can reduce the growth rate of individual trees and in turn, influence productivity at the scale of forest stands. Nitrogen (N) is generally limiting in extra-tropical forests (Vitousek & Howarth

1991), but the strength of this limitation depends on the net balance of inputs and losses over time (Vitousek & Reiners 1975; Gorham *et al.* 1979; Hedin *et al.* 1995). Over the time scale of ecological succession (decades to centuries), the size and density distributions of trees can change dramatically; individual tree size in mid- to late-successional forests often varies over several orders of magnitude (Ryan *et al.* 1997). Less clear is how such changes in stand structure interact with N availability over time. Under limiting conditions, trees collectively retain N in ecosystems via root uptake and internal recycling, but the allometric scaling properties underlying any potential size-dependency of N uptake or recycling are unclear. These relationships are vital to understanding how tree-level processes translate into ecosystem-level patterns of N-availability and forest growth over time.

Recent empirical analyses across the global range of tree size reveals that individual tree growth continues to increase as trees get larger (Stephenson *et al.* 2014) a pattern predicted by metabolic scaling theory (Enquist *et al.* 1999) but seemingly in conflict with observations of declining stand-level productivity over time. The apparent paradox of increasing individual mass growth with observed age-related declines in stand-level productivity (Ryan *et al.* 1997; Tang *et al.* 2014) is reconciled as increases in individual-tree leaf area are sufficient to overcome declines in leaf-level photosynthetic rates, and reductions in stem density via mortality are sufficient to reduce stand level productivity (Stephenson *et al.* 2014). These findings also support the notion that old forests composed of large trees can continue to act as sinks for C (Luyssaert *et al.* 2008).

Whether increasing mass growth in trees and C storage in forests are mechanistically linked to increases in N uptake or recycling efficiency is uncertain.

First, from a mass balance perspective, it seems reasonable that N uptake by roots must increase as trees increase in size in order to maintain internal stoichiometry and growth efficiency. Yet, for individual trees, empirical allometric (Chojnacky *et al.* 2014) and theoretical models (Enquist & Niklas 2002) predict decreasing proportional biomass allocation to fine roots with increasing tree size, while at the stand-level, fine root biomass allocation is predicted to decline after forests reach peak productivity (Ryan *et al.* 1997, Tang *et al.* 2014). These observations suggest that N uptake efficiency increases with tree size, but support for such a mechanism is lacking.

Second, how internal recycling of N within trees varies with tree size and forest age is similarly uncertain. Foliar resorption is a key mechanism by which N is retained by both trees and forests (Aerts 1996). At the stand level, foliar N resorption efficiency may increase with increasing forest age (Sun *et al.* 2016), and resorption may supply an increasing proportion of N demand throughout forest succession (Bond-Lamberty *et al.* 2006). However, such generalizations derive largely from even-aged stand level measures; less is known about how resorption efficiency relates to individual tree development or size. Thus, critical to resolving the role of individual trees in N retention and C accumulation in forest ecosystems is determining how fine root allocation and the efficiency of N uptake and foliar resorption change as trees grow larger and ecosystems mature.

Here, we combine original field measures across a >300 year-old chronosequence of conifer forest development with a theoretical model of root uptake (Gerber & Brookshire 2014) and analysis of N allocation patterns in trees to investigate the scaling properties between individual tree uptake and recycling and forest-level patterns of N retention and C accumulation. We ask the following questions: First, how do root morphology and mass (properties that govern uptake efficiency) change with tree size and forest age? Second, how do the scaling properties between tree size and root uptake and resorption align with the conceptual framework of metabolic scaling, which predicts $3/4$ power convergence with size? Third, how do these allometric relationships scale up to influence ecosystem-level patterns of N cycling and retention? Fourth, how does N allometry influence predictions of increasing growth with increasing tree size? Most generally, we hypothesized that changes in N uptake and recycling efficiency with increasing tree size would vary with forest age and N availability. We further investigated how flexibility in N use strategies influence predictions of forest productivity and ecosystem-level N retention as trees grow larger.

Methods

Study Sites

Field sites are in northwestern Montana in the Swan Valley located throughout a 100 km² area centered near the southern edge of Swan Lake (47°55'8"N 113°50' 35"W). Mean annual precipitation is ~750 mm yr⁻¹ and mean annual temperature is ~7 °C (Western Regional Climate Center, 2016). The Swan Valley was glaciated during the

Bull Lake and Pinedale glaciations and is characterized by skeletal, gravelly soils overlaying glacial till and Precambrian sedimentary mudstone. Soils are inceptisols (predominately dystric eutrochrepts) with high gravel content and ash common in the subsurface (Soil Survey Staff 2014). The Swan Valley harbors a diverse array of conifers and represents the eastern most extent of the maritime-influenced Pacific Northwest (PNW) forest belt, one of the most productive extra-tropical forest biomes on Earth (Waring & Franklin 1979). Dominant tree species in our study area are *Abies grandis*, *A. lasiocarpa*, *Larix occidentalis*, *Pinus monticola*, *Psuedotsuga mensezei* and *Thuja plicata*.

Sites known to have been clear-cut only once in the previous 100 years were identified from the US Forest Service and Wilderness Society Forest Activity Tracking System database (Natural Resource Manager, USFS) accessed via a Google Earth database (Natural Resource Manager, USFS) provided by The Wilderness Society. The four sites selected were clear-cut in 1918, 1955, 1969, and 2000, respectively, and the total area of each treatment was 15-50 ha. One undisturbed stand dated by prior work to be 350 years old (Lesica *et al.* 1991) was included as an old-growth site. US Forest Service records indicate the old-growth area has been free of fire and harvest activity since at least 1899 (Ayres 1900), and absence of fire scars, charcoal deposits, and stumps support our assumption that this stand has been largely undisturbed in recent history. Maximum distance between sites is 15 km. The elevations of field sites vary from 980-1080 MASL.

Field Measures

We established three inventory plots within each of the clear-cut treatments (hereafter, sites) and the old-growth site. For the 45-, 59-, 96-year-old and old-growth sites, inventory plots were 157 m². For the 14-year old site, plots were 40 m² to accommodate high stem densities. We measured diameter at breast height (1.37 m from ground, DBH) and tree height (using a clinometer) for all trees in each of the 15 inventory plots. All inventory plots were greater than 100 m from one another, and greater than 50 m from clear-cut treatment edges, roads, and riparian areas. From inventory data, tree aboveground biomass (AGB) and foliage biomass were estimated using species-specific allometric regression functions presented in Chojnacky et al. (2014) for all trees over 2.5 cm DBH for all 11 species present. Trees shorter than breast height were measured for diameter a proportional distance from ground level as larger trees. Across all sites this yielded a population of $n=405$ trees for analyses hereafter. We measured the projected crown area for 58 trees (across all study sites) that varied in DBH from 2.5 to 90.5 cm and fit a logarithmic function to these data to construct an allometric estimate of crown area for the remaining trees in the inventory.

We focused our analysis on *A. grandis* and *T. plicata*, two species common to the PNW. These species are prevalent throughout the study area and further represent two contrasting life-histories for conifers: *A. grandis* is a fast-growing, semi-shade tolerant, short-lived, ectomycorrhizal (ECM) host, and *T. plicata* is a slow growing, highly shade-tolerant, long-lived, arbuscular mycorrhizal (AM) host.

Fine root biomass (roots < 2mm) was first measured at the stand level from core samples taken to a depth of 15 cm. Three pairs of 8x15 cm auger cores were taken haphazardly within each inventory plot. Pairs of cores were composited into a single sample and fine roots were sorted from soils, rinsed, and oven dried to constant mass at 65 °C before weighing. Next, we estimated fine root biomass for individual trees using published allometric equations (Chojnacky *et al.* 2014). We summed allometric estimates of fine root biomass for individual trees within each inventory plot, and compared these allometric stand-level sums to our empirical stand-level estimates from cores. Importantly, we note that the allometric estimates of fine root biomass scale as the 0.7 power of AGB. We applied an extinction function derived for temperate coniferous forests (Jackson *et al.* 1997) to this allometric fine root biomass estimate, which yields 28.35% of total fine root biomass present to a depth of 15 cm.

To estimate how root morphology changes across a range of tree sizes, fine roots were excavated by hand for a subset of 44 trees with DBH varying from 0.7 cm to 127 cm across the inventory plots from all stand ages. These roots were traced from an identifiable lateral tree root or stem to intact terminal order root tips from the three most abundant species in the study sites (*A. grandis* $n=21$, *T. plicata* $n=18$, and *L. occidentalis* $n=6$). Roots were washed and dried at 65 °C to constant mass and then weighed. Roots were then rehydrated briefly and scanned using WinRHIZO (Regent Instruments, Inc. Quebec City, Canada) image analysis software to obtain total root length and average root diameter. Specific root length was calculated using the scanned fine root length / dry mass of scanned fine roots.

Foliar N was quantified by collecting live and senesced foliage from 40 trees across a range of sizes selected from the oldest and youngest sites (9 *A. grandis*, 12 *T. plicata* from the 14 year-old site; 8 *A. grandis*, 11 *T. plicata* from the old-growth) to span the range of tree size represented across the chrono-sequence. Foliage was collected by hand or with a shotgun for tall trees from sun-exposed areas of the canopy. Senesced foliage was collected in litter baskets suspended within smaller focal tree canopies or underneath the crown for large trees; for some trees, senesced ‘ready to fall’ foliage was removed by hand when litter baskets yielded insufficient litter mass for analysis. After oven drying at 65 °C to constant mass and grinding, green and senesced foliage was analyzed for N via elemental combustion (Costech Analytical Technologies, Inc., Valencia, CA, USA). Resorption efficiency was calculated as:

$$\frac{[N]_{live} - [N]_{dead} * MLCF}{[N]_{live}} * 100 = \% \text{ resorption efficiency} \quad (1)$$

Where $[N]_{live}$ is the N concentration in live foliage in mg N g^{-1} , $[N]_{dead}$ is the N concentration in dead foliage in mg N g^{-1} , and MLCF is a mass loss correction factor of 0.745 for conifers as described in (Vergutz *et al.* 2012). Average foliar turnover time specific to each species (*T. plicata* – 9 years, *A. grandis* – 5 years) was used to calculate foliar nutrient resorption rates (Balster & Marshall 2000; Harlow *et al.* 2005).

Soil extracts were used to quantify dissolved inorganic N (DIN; $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) and phosphate ($\text{PO}_4\text{-P}$) availability in the study sites. We performed water and 2M potassium chloride (KCl) extracts in 2014 and water extracts again in 2015. For water and KCl extracts in 2014, soil was separated into organic horizons, and mineral soil from 0-15 cm and 15-30 cm. We sampled a 10 x 10 cm area of organic horizon material

excluding surface litter to the depth of contact with mineral soil at four locations around a larger sampling plot centered on, but not restricted to the inventory plots. For each of these sampling plots, the four organic horizon samples were composited into one sample. The mineral soil was sampled below this section of forest floor from 0-15 cm and 15-30 cm using a 5 x 15 cm corer. Samples were composited as above. In total, this yielded $n=45$ extracts each for water and KCl. For water extracts in 2015, we took four 2x15 cm cores from the surface of the organic horizon at the drip line in a NESW pattern around 72 individual trees growing within and directly adjacent to one inventory plot for each site (22 trees in 14-year old, 9 trees in the 45-, 59-, and 96-year old sites, and 23 trees in the old-growth).

Forest floor samples for extractions were mixed, sieved to 2 mm, and a 15 g sub-sample was shaken in 75 ml of ultra-pure water (or 2M KCl) for 3.5 hours, filtered with a Whatman 41 20- μ m filter, and kept on ice until frozen within 48 hours. This process was repeated for mineral soil samples. Samples were then thawed and filtered to 0.7 μ m prior to analysis for water extracts via a segmented flow auto-analyzer (SEAL Analytical, Mequon, WI, USA), KCl extracts were analyzed via flow injection auto-analyzer (Lachat Instruments, Loveland, CO, USA).

Calculations and Model simulations

Nutrient uptake by roots was quantified using a nutrient uptake model (Gerber & Brookshire 2014). The model allows for the calculation of uptake affinity (the uptake rate constant, k_p , Eq. 2, (Gerber & Brookshire 2014) for N based on root properties of specific

root length (σ), radius (r), and root mass per area (M_r). Soil parameters included in the model are impedance to nutrient diffusion (κ'), and an active soil depth of 15 cm (h):

$$k_p = \frac{2\pi\kappa'M_r}{h[\log\left(\frac{h}{\pi M_r \sigma r^2}\right) - \left(\frac{3}{2}\right)]} \quad (2)$$

The diffusion transport mechanisms used in the model require N-limiting conditions, which ensure a sufficiently strong depletion zone is created by uptake to allow for diffusion of N across the concentration gradient to the root surface. Uptake is calculated as k_p * mass N in the volume of soil exploited by roots in the rooting area (15 cm depth * rooting area) to yield mass N year⁻¹. Sequential coring in the study sites indicated that the majority of fine roots were concentrated in the upper 15 cm of soil.

Our theoretical framework of N uptake circumvents many of the complications posed by in situ uptake methods, and relies on a small set of morphological parameters that are relatively easy to measure and has proven capable of predicting inorganic N leaching losses from soils (Gerber & Brookshire 2014). Ideally, our estimates would be compared to empirical measures of uptake. However, measuring root uptake in the field is notoriously difficult and fraught with potential artifacts. For example, depletion techniques can significantly alter the diffusion environment and concentration-dependent kinetics and isotope tracers are subject to competition for N by microbes, all of which can make scaling uptake fluxes over time and space difficult (Lucash *et al.* 2007). In contrast, our modeled estimates based on uptake affinity are limited in their ability to capture transient responses to pulse increases in N availability, particularly early in the growing season.

Root Uptake Simulation

Root uptake was estimated at the individual tree level. To allow for plasticity in morphological traits and rooting characteristics, N uptake simulations were run by conducting 10,000 Monte Carlo realizations while allowing SRL, root diameter, and rooting area to vary simultaneously within ranges observed in the field and/or reported in the literature. For each of the 405 individual trees with a DBH > 2.5 cm, SRL was allowed to vary between 700 and 13,000 m kg⁻¹, root diameter varied as a function of SRL assuming a constant root tissue density (the mean value of 389.6 kg m⁻³, which prevents random combinations of SRL and diameter generated in the Monte Carlo from yielding unrealistically high or low tissue densities), and based on an estimate from the literature (Eis 1987), rooting area varied between 2 and 5 times the projected crown area. Active rooting depth was considered fixed at 15 cm.

Within the Monte Carlo, a log-log Reduced Major Axis (RMA) linear regression (after Niklas & Enquist 2001) was performed using AGB as the explanatory variable and uptake in kg N year⁻¹ as the response variable for each of 10,000 Monte Carlo iterations across the entire range of tree biomass (n=405). The median slope of all iterations of the log-log linear regression was used as the estimate of the scaling exponent. For each individual tree, the median of the 10,000 estimates of total N uptake rate was used as that individual's uptake rate, and these were summed by site to calculate stand level N uptake rates. We used site level means from the 2015 water extracts to define the DIN concentration in the rooting area for each tree across all Monte Carlo iterations.

We performed the log-log RMA regression for scaling resorption N flux v. AGB only for the 40 individuals from which foliage samples were taken and N availability in the rooting area was measured from 2015 soil water extracts. We estimated stand-level resorption fluxes by applying our observed live foliar N concentrations and resorption efficiencies respective to *A. grandis* and *T. plicata*, and the global average resorption efficiency for conifers (62.7%, Vergutz *et al.* 2012) and average of *A. grandis* and *T. plicata* live foliar N concentration to all other species.

Carbon Growth Simulations

To address how allometric scaling properties of N uptake and resorption may inform estimates of aboveground C growth, we employed a simple allocation scheme and compared the relationship between our observed aboveground stand biomass C and aboveground estimated stand C growth derived from N uptake to published forest stand data. Aboveground C growth rates for trees were calculated as the sum of foliar and stem C allocation using the allocation scheme presented in Cleveland *et al.* (2013). This scheme assumes that ~73% of N acquired via root uptake is allocated to foliage production and ~9.8% of N taken up is allocated to stem production with a C:N mass ratio of 42:1 for foliage and 250:1 for stems. To examine the sensitivity of our growth estimates to a given allocation scheme, we applied a 33% reduction in NUE from the allocation scheme above for all individuals. We additionally forced NUE to decline linearly by 33% from the smallest to largest individual by prescribing the NUE from the above scheme to the smallest individual and the same NUE reduced by 33% to the largest

individual, and with a linear model to assign NUE to intermediate trees with AGB as the predictor variable. We reversed this simulation to project C growth for linearly increasing NUE by 33% over the range of tree size in study sites. A growing season length was imposed limiting uptake to 172 days per year for all simulations (MODIS phenology data). We compared our N-uptake based growth estimates to reported growth rates for similar forest stands ('PNW forests', Law and Berner 2015) containing the same dominant or co-dominant tree species as our study sites. We then compared estimated scaling exponents for aboveground C growth rate v. aboveground C mass plots of each NUE simulation above with the estimated slope of the PNW forests using maximum likelihood estimation (MLE).

Scaling uptake by individual trees to ecosystem-level N cycling

We estimated the contribution of individual large trees to the total N-uptake flux in a stand by comparing the median annual N-uptake value from the Monte Carlo N-uptake simulation for the largest AGB tree in each inventory plot to the sum of the annual uptake of all trees present in the inventory plot. All analyses were performed in R 3.2.0 (R Core Team 2015), and RMA analyses used package lmodel2 (Legendre 2014).

Results

Tree and Stand Results

Across sites, individual trees spanned a large range (Fig. 1a) in height (0.75 to 42.6 m), diameter (0.3 to 90.5 cm) indicating a transition toward larger individuals in

older stands. From core sampling, stand-level root biomass showed a positive linear relationship with stand age ($P < 0.001$). Comparison of allometric estimates of stand-level fine root biomass to core-sampled stand-level fine root biomass indicated that there was no difference between allometric and core samples within sites, based on a non-significant interaction between sample type (core and allometric) and stand age (two-way ANOVA, $P = 0.89$). This analysis indicated that allometric estimates may under-predict fine root biomass compared to core estimates at the stand-level (regression slope of core v. allometric estimates = 0.77, $P = 0.001$; Fig. 1b) in the smaller stands. Aboveground biomass (Fig. 1c) had a significant positive correlation with stand age (Pearson's $r = 0.93$, $P < 0.001$).

SRL varied from 1,560 to 12,490 m kg^{-1} across the 44 morphology samples. Species did not differ in SRL (one-way ANOVA: $F(2,42) = 1.55$, $p = 0.22$), and there was no correlation between tree size (DBH) and SRL (Pearson's $r = 0.087$, $P = 0.57$) across species. For a subset of root morphology samples for which local nutrient concentrations were taken ($n = 14$), there was no correlation between DIN and SRL (Pearson's $r = 0.07$, $P = 0.80$) or N:P (Pearson's $r = -0.22$, $P = 0.45$).

Soil DIN concentrations were low and similar across stands (Fig. 2) and did not change significantly with increasing stand age for ammonium ($P = 0.27$) or nitrate ($P = 0.97$). Additionally, ammonium was in excess of nitrate for $> 94\%$ of samples across all field campaigns. Nitrogen: P ratios (molar DIN:PO₄-P) in water extracts were low (~ 2 -3.5) in all sites and did not change with increasing age across the chrono-sequence ($P = 0.93$).

Foliar N resorption efficiency was higher (one-way ANOVA: $F(1,38):108.9$, $p<0.001$) for *T. plicata* ($n=23$, mean 62.2%) than for *A. grandis* ($n=17$, mean 28.6%). There was no correlation between AGB and foliar N resorption efficiency (Pearson's $r=-0.06$, $P=0.80$ for *A. grandis*; Pearson's $r=-0.09$, $P=0.68$ for *T. plicata*) or foliar N concentration (Pearson's $r=-0.038$, $P=0.88$ for *A. grandis*; Pearson's $r=-0.39$, $P=0.06$ for *T. plicata*) in either species. Additionally, no correlation was found between resorption efficiency and DIN (Pearson's $r=-0.12$, $P=0.43$) or N:P (Pearson's $r=0.037$, $P=0.82$) or foliar N concentration and DIN (Pearson's $r=0.01$, $P=0.94$) or N:P (Pearson's $r=-0.12$, $P=0.46$) for either species. In effect, changes in resorption fluxes were proportional to changes in foliar biomass. Further, we found no correlation between soil N:P and resorption efficiency in our study sites.

Simulation Results

Results of the uptake simulation for all 405 trees indicated that annual uptake flux increased with increasing AGB, and scaled as the 0.73 power of AGB, with associated 2.5 and 97.5-percent quantiles of 0.72 and 0.74, respectively from the Monte Carlo simulation (Fig. 3a). Data for 40 trees with paired local DIN, resorption efficiency, and AGB data indicated that annual foliar N resorption flux increased with AGB and also scaled as the 0.80 power of AGB (Fig. 3b). Uptake estimates indicated that for each age class, the largest individual tree was responsible for between 20-77% of the entire annual uptake N flux for the stand (Fig. 4).

Carbon growth rate simulation

Estimates of aboveground C growth rates from our simulations indicated that C growth increased continuously with AGB given a conserved allocation scheme (Fig. 5a). When summed to the stand-level, our estimates compared well with other PNW forests, but tended to over-predict C growth for the highest biomass stands, indicating our uptake estimates or allocation scheme may be generous (Fig. 5b). The MLE estimated exponent for the reduced and normal NUE scheme was $\theta = 0.59$, and the increasing NUE scheme had an estimated exponent of $\theta = 0.65$. The MLE estimated exponent for the declining NUE scenario ($\theta = 0.53$) compared most favorably to observed patterns of C growth in other PNW forests ($\theta = 0.46$).

Discussion

Our results indicate that as individual trees get larger over time, increases in C productivity are matched by an increasing ability to acquire N. This relationship was driven by theoretically predicted (Enquist & Niklas 2002) and empirically supported (Chojnacky *et al.* 2014) increases in fine root biomass with increasing AGB, as root morphology (the primary driver of uptake affinity in our model) did not change directionally with tree size across our sites. Congruent with the idea (Stephenson *et al.* 2014) that total increases in leaf area are sufficient to outpace declines in leaf level photosynthesis and enable increasing mass growth rates, we find that although the proportional allocation to fine roots declined from 8% to 1% of AGB from the smallest to

largest trees, overall increases in fine root biomass were sufficient to yield continuous increases in N uptake.

Our findings suggest that if foliar N resorption were to play an increasing role in N retention through succession, it is because N resorption fluxes increase in proportion to foliar biomass, and not due to strategic shifts in resorption efficiency at the leaf level. We found that foliar N concentrations and resorption efficiency did not change directionally with tree size for our two focal species, and N resorption efficiency did not appear to be influenced by local nutrient availability. Our results also identify that while resorption contributes an increasing proportion to forest scale N flux through succession, it contributes at maximum less than one-fifth (~5-17%) of total stand N flux. We conclude that N uptake by roots dominates N supply to trees throughout succession, at least for our sites.

Implicit in assumptions about the function of foliar N resorption is that plants able to resorb a substantial amount of N may be less reliant on uptake of N by roots, and vice versa (Van Heerwaarden *et al.* 2003). In this context we note that *A. grandis* (mean resorption efficiency 28.6%) associates with ECM fungi, a relationship that effectively increases the available N pool by enabling access to organic N (Lambers *et al.* 2008), and potentially diminishes the necessity of *A. grandis* using N resorption as a means to prevent N loss. In contrast, the AM fungi associated *T. plicata*, does not have access to this additional organic N pool, and accordingly shows much higher mean resorption efficiency (62.2%).

In light of the potential for mycorrhizal associations to influence resource acquisition strategies as discussed above, we recognize that omitting N uptake mediated by mycorrhizae may bias our estimates of N uptake and C growth. Specifically, we may be underestimating uptake by ECM trees in considering only DIN, given the capacity for some ECM species to access organic forms of N. However, further investigation into the uptake affinity per unit C cost benefits conferred by mycorrhizae is required to better understand whether our estimates may over- or underestimate uptake.

Our finding that N retention increases with increasing tree size identifies a keystone role for large trees in ecosystem level C and N cycling. We estimate that N uptake by the single largest individuals in most stands can account for nearly a quarter or more of total stand uptake despite these trees representing only 2-8% of the stems present in stands. Though the effects of watershed-level forest clearing on DIN losses have been known for some time (Bormann *et al.* 1968) our results emphasize the disproportionate role of individual large trees in natural gap-phase forest dynamics. At the high end of our estimates, we find that mortality of a single large tree could result in a pulse loss of 77% of a stand's uptake sink. The degree to which this would imprint on ecosystem-level losses would ultimately depend on the ability of neighboring trees to access any increases in soil N.

At the ecosystem-level, our N-uptake based forest C growth estimates tend to slightly over-predict those measured for other PNW forests in the TERRA data set. This may imply that other factors may limit growth efficiency for the PNW forests rather than N alone (which we assume here), or that the allocation scheme used employs a generous

NUE (which is the only parameter estimated by the allocation scheme). Because we assume that growth is strictly N-limited, growth in our simulations results directly from prescribed allocation schemes. While our measurements of dissolved N and P in soils do lend strong support to our assumption that N is limiting, these measures cannot exclude limitation by multiple resources. Including factors such as water or light limitation, or the effects of competition may allow our simulation to better approximate natural forests that may be experiencing limitation by multiple factors. Our findings nevertheless support theoretical predictions that even under strongly N limiting conditions forests can continue to increase in productivity (Dybzinski *et al.* 2014).

Our findings also illuminate how ecosystem level patterns of N-cycling emerge from tree-level N-use strategies. Given our uptake findings, invariant mineral N pools in soils across our chrono-sequence imply that mineralization rates increase and N residence times decrease with stand age. This agrees well with observations of faster N-cycling via higher gross mineralization rates in older vs. younger forests (Davidson *et al.* 1992) and more rapid turnover of DIN in undisturbed forests (Stark *et al.* 1997) which is required to supply high N demand by large trees.

Understanding the relationships between tree and forest size and nutrient retention may be especially important in predicting the response of the future forest C sink. Both N uptake and recycling efficiency are implicated in sustaining growth responses to elevated CO₂ (Finzi *et al.* 2007; Norby *et al.* 2010). Whether this implies that large statured, older forests will remain the most responsive to rising atmospheric CO₂ is uncertain. Our findings may hold particular importance as large trees appear particularly susceptible to

mortality from drought events (Bennett *et al.* 2015) and may be disappearing from forests globally (Lindenmayer *et al.* 2012). Forest management activities that include the removal of even small numbers of large trees may need to consider the long-term implications for ecosystem C and N retention.

Acknowledgments: We thank the Swan Lake Ranger District for sampling access, T. Belote for identifying chrono-sequence sites, M. Housman for invaluable field assistance, and N. Wurzbarger for helpful comments on an earlier version of this manuscript. This research was supported by funding from the Montana Agricultural Experiment Station and the Montana State University Graduate School and Office of the Vice President for Research and Economic Development.

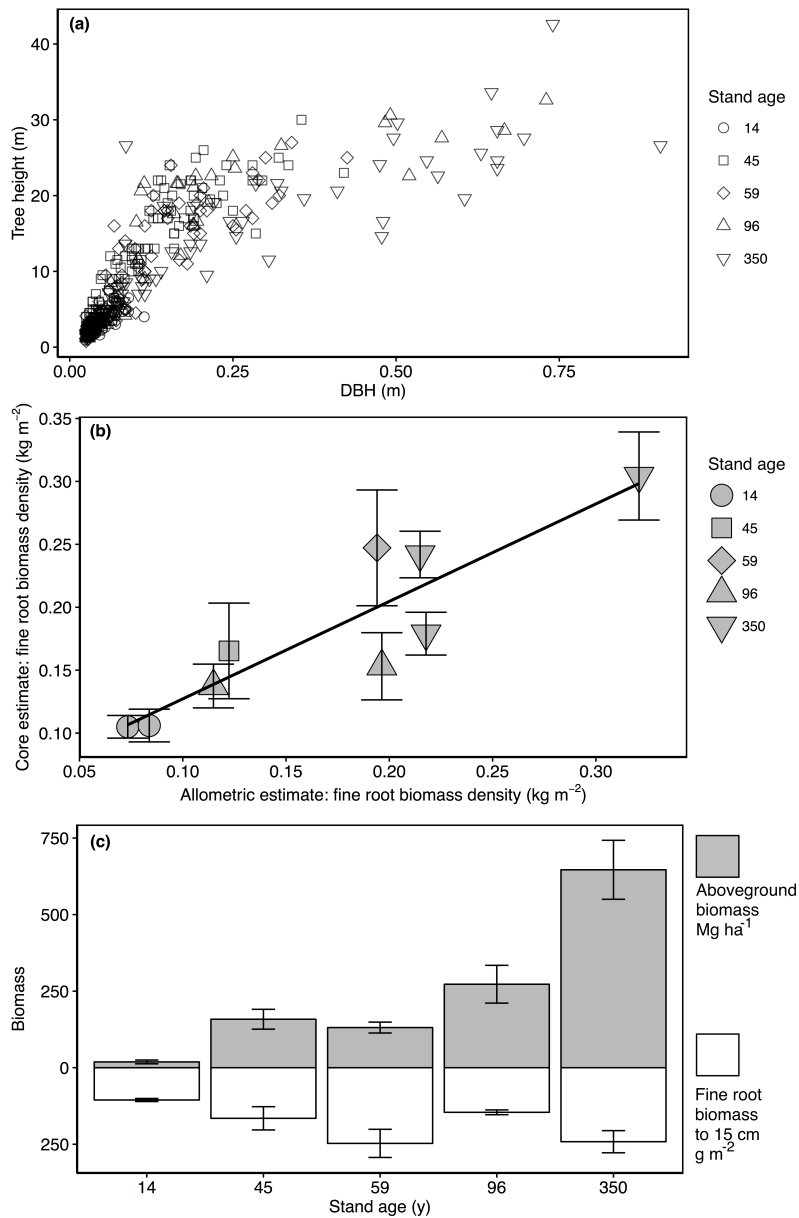


Figure 1. (a) Stand inventory of 405 individuals with DBH > 2.5 cm.

(b) Core sampling of fine root biomass compared well with allometric estimates summed to the stand level. The error bars represent standard error among cores from the same inventory plot. 3 pairs of cores per inventory plot were taken from all three inventory plots in each of the 350-, 96-, and 14-year old plots, and from 1 inventory plot in each of the 45 and 59 year old plots. The regression slope estimate of 0.77 indicates that allometric estimates when summed to the stand level may under-estimate fine root biomass compared to core samples.

(c) Aboveground biomass from allometric equations and fine root biomass to 15 cm from core sampling indicated general increases with increasing stand age.

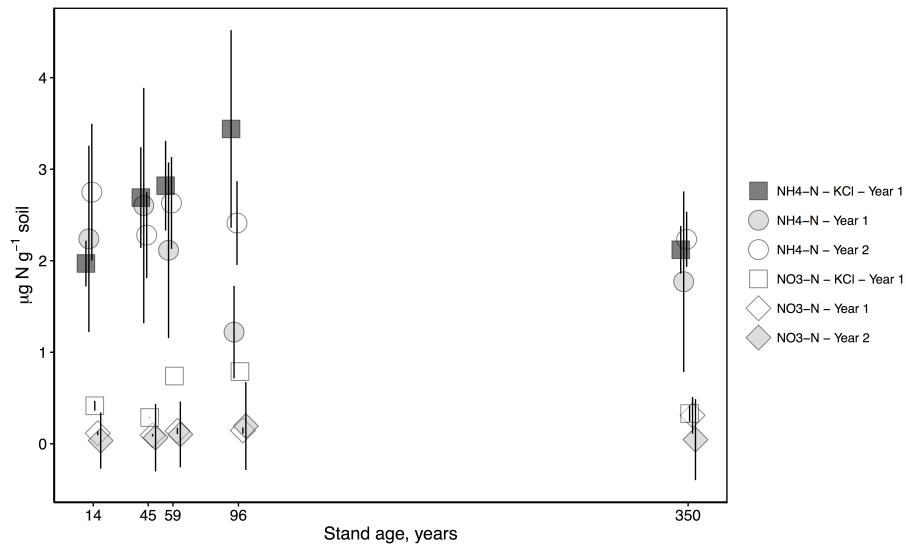


Figure 2. Ammonium and nitrate concentrations from water and KCL extracts in study sites for two sampling years. Inorganic nitrogen concentrations across the chrono-sequence did not vary in relation to stand age. Levels were low, and in 94% of samples ammonium exceeded nitrate. Year 1 (2014) samples are an average of the O horizon and 0-15 cm depth, while Year 2 (2015) samples were 15 cm depth from the top of soil surface, including the O horizon.

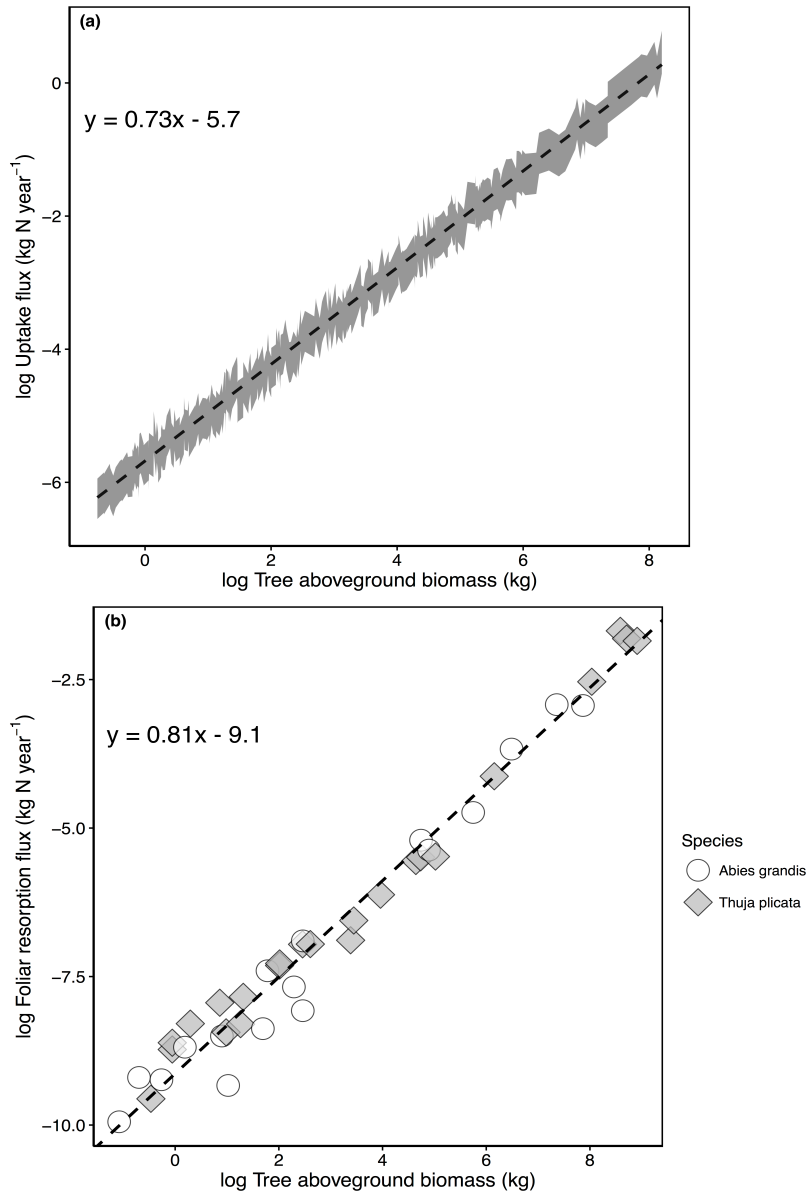


Figure 3. (a) Log-log RMA scaling of N uptake rates v. aboveground biomass from 10,000 Monte Carlo realizations allowing specific root length, root diameter, and rooting area to vary for 405 trees from stand inventory. Shaded area represents the 2.5- and 97.5-percent quantiles from the Monte Carlo simulation.

(b) Log-log RMA scaling of aboveground biomass vs. foliar N resorption rate for 40 trees with measured N resorption efficiencies and foliar N concentration paired to each tree. We assumed annual turnover of 1/5 of all foliage for *Abies grandis* and 1/9 of all foliage for *Thuja plicata*. The scaling slope of the RMA regression indicates that the relationship scales close the theoretically predicted value of $3/4$ from metabolic scaling theory.

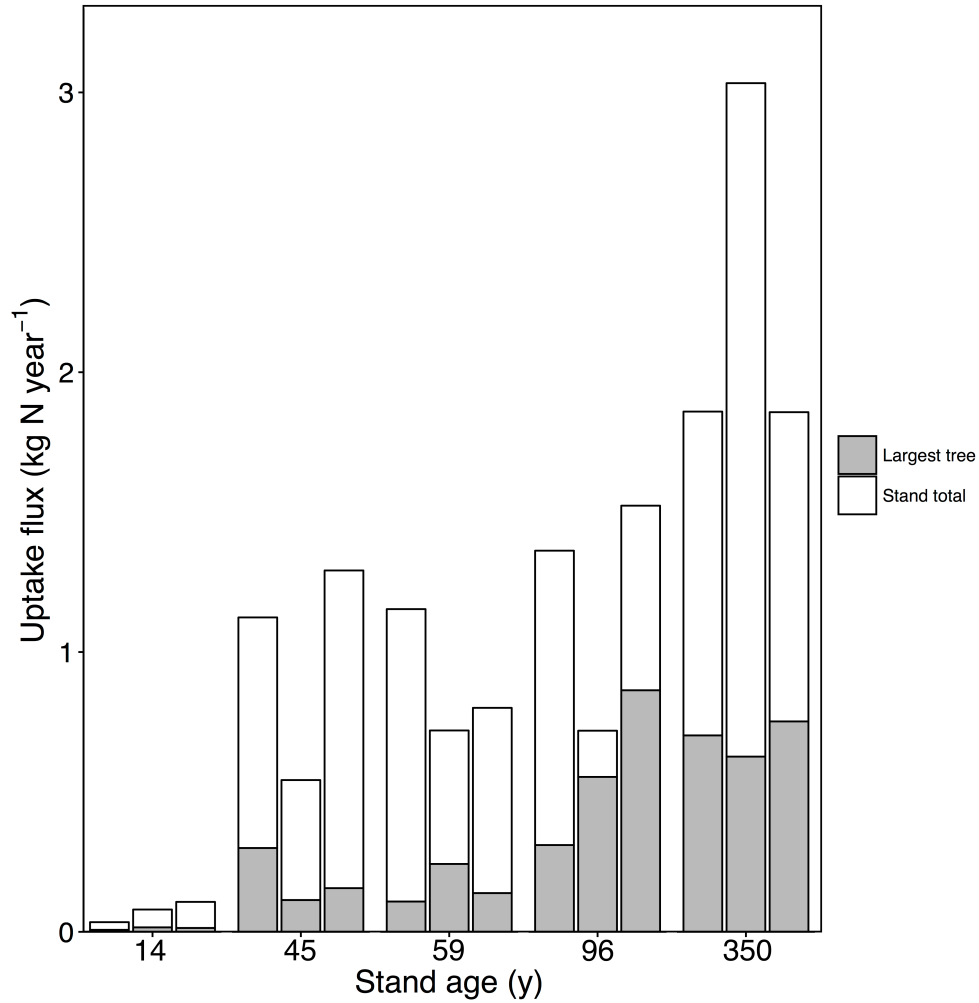


Figure 4. Annual uptake by forest stands and large trees. Tops of white bars represent total annual N uptake flux by a forest inventory plot on a per plot basis. Tops of grey bars indicate annual N flux by the largest aboveground biomass tree within that inventory plot. Uptake fluxes are the median values from the Monte Carlo simulation for individuals, and the median uptake values per individual are summed within inventory plot to obtain inventory plot level uptake fluxes.

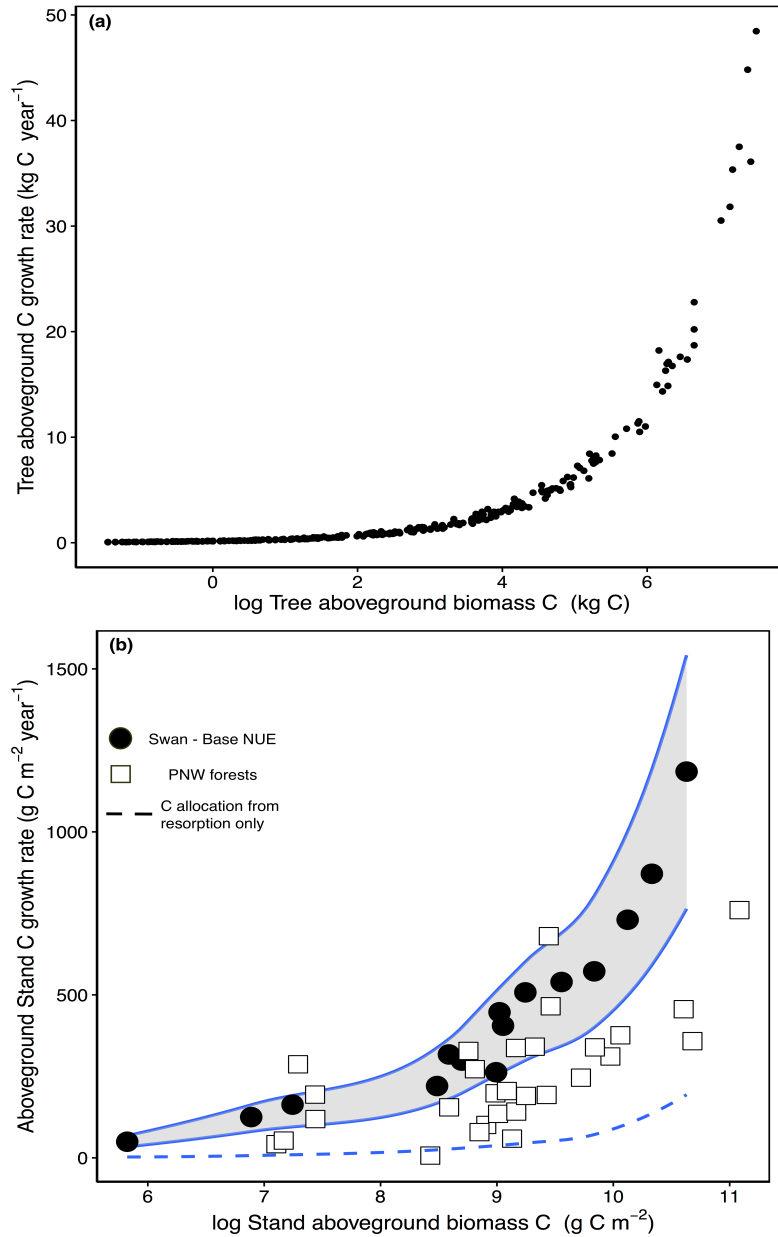


Figure 5. (a) Annual C growth rates for individual trees using the median value of uptake from the Monte Carlo simulation and estimated foliar N resorption as described in the text. (b) Annual aboveground (stem + foliage) C growth rates for stands calculated from N uptake + foliar N resorption fluxes. Shaded region represents smoothed maximum and minimum values from 10,000 Monte Carlo realizations allowing the nitrogen use efficiency scheme to vary $\pm 33.3\%$ from the Normal NUE scheme, shown in black circles. White triangles are stand data from Pacific Northwest forest plots with similar species compositions. The dashed line indicates growth derived solely from N resorption fluxes based on the Normal NUE scheme.

References

- Aerts, R. (1996). Nutrient Resorption from Senescing Leaves of Perennials: Are there General Patterns? *J. Ecol.*, 84, 597–608.
- Ayres, H.B. (1900). The Lewis and Clark Forest Reserve, Montana. In: *21st Annual Report of the USGS, 1899-1900*. US Government Printing Office, Washington, D.C., pp. 47–78.
- Balster, N.J. & Marshall, J.D. (2000). Decreased needle longevity of fertilized Douglas-fir and grand fir in the northern Rockies. *Tree Physiol.*, 20, 1191–1197.
- Bennett, A.C., McDowell, N.G., Allen, C.D. & Anderson-Teixeira, K.J. (2015). Larger trees suffer most during drought in forests worldwide. *Nat. Plants*, 1, 15139.
- Blasius, D. & Oberwinkler, F. (1989). Succession of mycorrhizae: a matter of tree age or stand age? In: *Annales Des Sciences Forestieres*. EDP Sciences, p. 758s–761s.
- Bond-Lamberty, B., Gower, S.T., Wang, C., Cyr, P. & Veldhuis, H. (2006). Nitrogen dynamics of a boreal black spruce wildfire chronosequence. *Biogeochemistry*, 81, 1–16.
- Bormann, F.H., Likens, G.E., Fisher, D.W. & Pierce, R.S. (1968). Nutrient Loss Accelerated by Clear-Cutting of a Forest Ecosystem. *Science*, 159, 882–884.
- Chojnacky, D.C., Heath, L.S. & Jenkins, J.C. (2014). Updated generalized biomass equations for North American tree species. *Forestry*, 87, 129–151.
- Cleveland, C.C., Houlton, B.Z., Smith, W.K., Marklein, A.R., Reed, S.C., Parton, W., *et al.* (2013). Patterns of new versus recycled primary production in the terrestrial biosphere. *Proc. Natl. Acad. Sci.*, 110, 12733–12737.
- Crowther, T.W., Glick, H.B., Covey, K.R., Bettigole, C., Maynard, D.S., Thomas, S.M., *et al.* (2015). Mapping tree density at a global scale. *Nature*, 525, 201–205.
- Davidson, E.A., Hart, S.C. & Firestone, M.K. (1992). Internal Cycling of Nitrate in Soils of a Mature Coniferous Forest. *Ecology*, 73, 1148–1156.
- Dighton, J. & Mason, P.A. (1985). Mycorrhizal dynamics during forest tree development. In: *Developmental Biology of Higher Fungi*, British Mycological Society Symposium. Cambridge University Press, Cambridge, pp. 117–139.
- Dixon, R.K., Solomon, A.M., Brown, S., Houghton, R.A., Trexler, M.C. & Wisniewski, J. (1994). Carbon Pools and Flux of Global Forest Ecosystems. *Science*, 263, 185–190.

- Dybzinski, R., Farrior, C.E. & Pacala, S.W. (2014). Increased forest carbon storage with increased atmospheric CO₂ despite nitrogen limitation: A game-theoretic allocation model for trees in competition for nitrogen and light. *Glob. Change Biol.*, n/a–n/a.
- Eis, S. (1987). Root systems of older immature hemlock, cedar, and Douglas-fir. *Can. J. For. Res.*, 17, 1348–1354.
- Enquist, B.J. & Niklas, K.J. (2002). Global Allocation Rules for Patterns of Biomass Partitioning in Seed Plants. *Science*, 295, 1517–1520.
- Enquist, B.J., West, G.B., Charnov, E.L. & Brown, J.H. (1999). Allometric scaling of production and life-history variation in vascular plants. *Nature*, 401, 907–911.
- Finzi, A.C., Norby, R.J., Calfapietra, C., Gallet-Budynek, A., Gielen, B., Holmes, W.E., *et al.* (2007). Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO₂. *Proc. Natl. Acad. Sci.*, 104, 14014–14019.
- Gerber, S. & Brookshire, E.N.J. (2014). Scaling of Physical Constraints at the Root-Soil Interface to Macroscopic Patterns of Nutrient Retention in Ecosystems. *Am. Nat.*, 183, 418–430.
- Gorham, E., Vitousek, P.M. & Reiners, W.A. (1979). The regulation of chemical budgets over the course of terrestrial ecosystem succession. *Annu. Rev. Ecol. Syst.*, 10, 53–84.
- Harlow, B.A., Duursma, R.A. & Marshall, J.D. (2005). Leaf longevity of western red cedar (*Thuja plicata*) increases with depth in the canopy. *Tree Physiol.*, 25, 557–562.
- Hedin, L.O., Armesto, J.J. & Johnson, A.H. (1995). Patterns of Nutrient Loss from Unpolluted, Old-Growth Temperate Forests: Evaluation of Biogeochemical Theory. *Ecology*, 76, 493–509.
- Jackson, R.B., Mooney, H.A. & Schulze, E.-D. (1997). A global budget for fine root biomass, surface area, and nutrient contents. *Proc. Natl. Acad. Sci.*, 94, 7362–7366.
- Lambers, H., Iii, F.S.C. & Pons, T.L. (2008). Mineral Nutrition. In: *Plant Physiological Ecology*. Springer New York, pp. 255–320.
- Le Quéré, C., Moriarty, R., Andrew, R.M., Peters, G.P., Ciais, P., Friedlingstein, P., *et al.* (2015). Global carbon budget 2014.
- Lesica, P., McCune, B., Cooper, S.V. & Hong, W.S. (1991). Differences in lichen and

bryophyte communities between old-growth and managed second-growth forests in the Swan Valley, Montana. *Can. J. Bot.*, 69, 1745–1755.

Lindenmayer, D.B., Laurance, W.F. & Franklin, J.F. (2012). Global Decline in Large Old Trees. *Science*, 338, 1305–1306.

Lucash, M.S., Eissenstat, D.M., Joslin, J.D., McFarlane, K.J. & Yanai, R.D. (2007). Estimating nutrient uptake by mature tree roots under field conditions: challenges and opportunities. *Trees*, 21, 593–603.

Luyssaert, S., Schulze, E.-D., Börner, A., Knohl, A., Hessenmöller, D., Law, B.E., *et al.* (2008). Old-growth forests as global carbon sinks. *Nature*, 455, 213–215.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., *et al.* (2007). The human footprint in the carbon cycle of temperate and boreal forests. *Nature*, 447, 848–850.

Niklas, K.J. & Enquist, B.J. (2001). Invariant scaling relationships for interspecific plant biomass production rates and body size. *Proc. Natl. Acad. Sci.*, 98, 2922–2927.

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E. & McMurtrie, R.E. (2010). CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proc. Natl. Acad. Sci.*, 107, 19368–19373.

Ryan, M.G., Binkley, D. & Fownes, J.H. (1997). Age-Related Decline in Forest Productivity: Pattern and Process. In: *Advances in Ecological Research* (ed. Fitter, M.B. and A.H.). Academic Press, pp. 213–262.

Soil Survey Staff. (2014). *Web Soil Survey*. Available at: <http://websoilsurvey.nrcs.usda.gov/>. Last accessed 01 April 2016.

Stark, J.M., Hart, S.C. & others. (1997). High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature*, 385, 61–64.

Stephenson, N.L., Das, A.J., Condit, R., Russo, S.E., Baker, P.J., Beckman, N.G., *et al.* (2014). Rate of tree carbon accumulation increases continuously with tree size. *NATURE*, 507, 90–92.

Sun, Z., Liu, L., Peng, S., Peñuelas, J., Zeng, H. & Piao, S. (2016). Age-Related Modulation of the Nitrogen Resorption Efficiency Response to Growth Requirements and Soil Nitrogen Availability in a Temperate Pine Plantation. *Ecosystems*.

Tang, J., Luyssaert, S., Richardson, A.D., Kutsch, W. & Janssens, I.A. (2014). Steeper declines in forest photosynthesis than respiration explain age-driven decreases in forest growth. *Proc. Natl. Acad. Sci.*, 111, 8856–8860.

Twieg, B.D., Durall, D.M. & Simard, S.W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytol.*, 176, 437–447.

Van Heerwaarden, L.M., Toet, S. & Aerts, R. (2003). Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. *Oikos*, 101, 664–669.

Vergutz, L., Manzoni, S., Porporato, A., Novais, R.F. & Jackson, R.B. (2012). Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecol. Monogr.*, 82, 205–220.

Vitousek, P.M. & Howarth, R.W. (1991). Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, 13, 87–115.

Vitousek, P.M. & Reiners, W.A. (1975). Ecosystem Succession and Nutrient Retention: A Hypothesis. *BioScience*, 25, 376–381.

Vogt, K.A., Moore, E.E., Vogt, D.J., Redlin, M.J. & Edmonds, R.L. (1983). Conifer fine root and mycorrhizal root biomass within the forest floors of Douglas-fir stands of different ages and site productivities. *Can. J. For. Res.*, 13, 429–437.

Waring, R.H. & Franklin, J.F. (1979). Evergreen coniferous forests of the Pacific Northwest. *Science*, 204, 1380–1386.

Western Regional Climate Center. (2016). *Swan Lake, Montana*. Available at: <http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?mt8087>. Last accessed: 01 April 2016.

CHAPTER THREE

EXPRESSION OF SINK-DRIVEN AND TRANSACTIONAL NITROGEN
LIMITATION FOLLOWING STAND-REPLACING
DISTURBANCE IN AN INLAND PACIFIC NORTHWEST CONIFEROUS FOREST

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Aaron Scott-Klingborg

Contributions: Collected and analyzed data. Wrote first draft of manuscript and contributed to further revisions.

Author: Dr. Jack Brookshire

Contributions: Conceived the study design, provided primary feedback on statistical analyses, and contributed to writing the manuscript.

Manuscript Information Page

Aaron Scott-Klingborg, Jack Brookshire

Ecosystems

Status of Manuscript:

☒ Prepared for submission to a peer-reviewed journal

☐ Officially submitted to a peer-review journal

☐ Accepted by a peer-reviewed journal

☐ Published in a peer-reviewed journal

Springer

EXPRESSION OF SINK-DRIVEN AND TRANSACTIONAL
NITROGEN LIMITATION FOLLOWING STAND-REPLACING DISTURBANCE IN
AN INLAND PACIFIC NORTHWEST CONIFEROUS FOREST

Abstract

Stand-replacing logging disturbances re-initiate forest succession and in doing so, influence the re-accumulation of carbon (C) and nitrogen (N) in the ecosystem. Dynamics of N accumulation and demand dictate the expression of sink-driven and transactional N limitation following disturbances, but the relationships between succession, disturbance, and these two types of N limitation are unclear. Here, we assess recovery of ecosystem C and N pools following clear-cut logging disturbance using a coniferous forest chronosequence. We find that for a particularly N-deficient forest ecosystem, net N and C accumulation are only found in biomass sinks, with variable patterns of loss and accumulation throughout other ecosystem components in litter, organic horizons, and mineral soils. We find that N is strongly retained relative to C along the pathway from plant litter inputs with depth through the soil profile, inducing transactional N limitation primarily via accumulation in plant-unavailable forms and slow N turnover in the litter and organic horizons.

Introduction

Forests collectively constitute the largest carbon (C) sink on land (Le Quéré *et al.* 2015), and productivity in these ecosystems is widely limited by nitrogen (N) availability (Vitousek & Howarth 1991). Fundamentally, the balance of inputs and losses of N govern its availability and the degree to which N may limit plant productivity, but disturbances can severely disrupt this balance. Disturbances can alter the trajectory of N accumulation and strength of N limitation through succession when they initiate losses of N uncontrollable by plants (Menge *et al.* 2009) or by triggering oscillations in the degree of limitation by multiple elements that require extended periods of time to re-equilibrate (Rastetter *et al.* 2013). In general, the recovery dynamics of ecosystem N cycles depend on initial conditions, the disturbance type, intensity and frequency, and the species pool available for re-colonization. For example, early colonization by N-fixing species following fire can rapidly replenish disturbance-induced N losses (Binkley 2003; Perakis *et al.* 2011). Other disturbances such as windthrow retain residual biomass on site, which effectively re-distributes nutrients from living to detrital pools without altering total ecosystem C and N pools (Pickett & White 1985).

In contrast, harvest-related stand-replacing disturbances impose a unique long-term imprint on forest C and N cycles as they instantaneously remove the plant sink for both C and N via rapid wholesale removal of biomass from an ecosystem (Bormann *et al.* 1968). These disturbances also re-initiate plant community succession at a higher frequency than natural stand-replacing disturbances considering that harvest rotations occur on far shorter than return intervals for disturbances such as fire (Seymour *et al.*

2002; McLauchlan *et al.* 2014). A long history of work describes the recovery dynamics of N pools throughout succession and the variable effects on the strength of N limitation during recovery (Vitousek & Reiners 1975; Menge *et al.* 2009; Rastetter *et al.* 2013). However, the absolute strength of N limitation is difficult to assess directly for entire ecosystems (Vitousek & Howarth 1991), as N limitation may be expressed differently between microbes and plants across ecosystem N pools and change dynamically over the course of succession.

In particular, the dynamics of coupled recovery of C and N cycles is strongly shaped by the relative strengths of sink-driven versus transactional N limitation. Sink-driven N limitation occurs when N accumulates in forms unavailable for plants. Sinks include N assimilated in plant biomass, organic N from litterfall or stem turnover, and N associated with soil organic matter (Vitousek *et al.* 2010; Thomas *et al.* 2015). Transactional limitation describes the turnover of these sinks into plant-available forms via mineralization (Vitousek *et al.* 2010; Thomas *et al.* 2015), which constitute time lags between N release and immediate demand by plants.

Successional dynamics further influence C and N pools and the potential for different types of N limitation over time. Drawing from a large body of forest chronosequence research, aboveground biomass generally increases through time following stand initiation (Kira & Shidei 1967; Pregitzer & Euskirchen 2004) which, depending on external N supply, may indicate the strengthening of sink-driven limitation as N accumulates in biomass (Hooker & Compton 2003). Conversely, the strength of sink-driven N limitation may ultimately wane as competition precludes boundless biomass

accumulation, as evidenced by declines in forest biomass accumulation late in succession (Ryan *et al.* 1997). Increases in litterfall and stem turnover associated with biomass accumulation may indicate the potential for transactional limitation to emerge as N in litterfall must be mineralized to plant-available forms (Berg & McClaugherty 2014). In soils, forest floor and mineral soil changes throughout succession may drive both sink-driven and transactional limitation as N associated with soil organic matter accumulates as the forest floor builds through time (Pregitzer & Euskirchen 2004; Sun *et al.* 2004), and a strongly N-limited microbial community prevents sink-driven uptake by plants via immobilization and re-uptake of N (Vitousek & Matson 1984; Knops *et al.* 2002). However, definitive mechanistic relationships between time since disturbance and soil N pools have proven elusive to demonstrate in chrono-sequence work.

Coniferous forests in the Pacific Northwest (PNW) are well-suited to investigate these dynamics, as they are characterized by high productivity, gradients in N availability, and perhaps most importantly, various widespread disturbance regimes including intensive harvest activity. Chrono-sequence work here has investigated interactions between forest growth and N and C cycling (Sollins *et al.* 1980; Law *et al.* 2003; Sun *et al.* 2004; Law & Waring 2015), disturbances (Prescott 1997; Giesen *et al.* 2008; Perakis *et al.* 2015) and successional dynamics (Waring & Franklin 1979; Binkley 2003). However, how distinct types and periods of N-limitation emerge from these dynamics through succession has been difficult to assess. Here, we use a post clear-cut logging chrono-sequence for an inland PNW coniferous forest to investigate how the relative strengths of sink-driven vs. transactional N-limitation change through the course

of ecosystem recovery. To evaluate the emergence of sink-driven vs. transactional N-limitation, we track C and N pool dynamics over the timescale of succession in biomass and soils, and identify potential sinks and loss vectors of N. To assess the relative strengths and identify predominant locations of each type of N-limitation, we examine C and N dynamics through the soil profile within sites, from the continuum of plant litter inputs through forest floor material, to mineral soil C and N. We ask 1) on what time scales and in what pools are sink-driven and transactional N limitation expressed throughout forest succession, 2) what are the relative strengths of each type of limitation at different stand ages, and 3) how do the combined effects of forest aggradation and disturbance influence the expression of N-limitation? We consider pools of N and C in tree aboveground biomass, litter, organic horizons, mineral soils, and dissolved N across >300 years of forest succession following a clear cutting disturbance.

Methods

We established a chrono-sequence of time since clear-cutting in the Swan Valley of Northwestern Montana. The Swan Valley harbors a diverse array of conifers and represents the eastern most geographical extent of the maritime-influenced PNW forest belt. Dominant tree species in our study area are *Abies grandis*, *A. lasiocarpa*, *Larix occidentalis*, *Pinus monticola*, *Psuedotsuga mensezei* and *Thuja plicata*. Regional estimates of atmospheric N deposition are low, at around $1.6 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (National Atmospheric Deposition Program 2016). Similar forest types commonly harbor symbiotic N-fixing shrub *Shepherdia canadensis*, which can input N at a rate of $0.8 \text{ kg N ha}^{-1} \text{ year}^{-1}$

(Hendrickson & Burgess 1989), and non-symbiotic N-fixation in the range of 0.7 kg N ha⁻¹ year⁻¹, mostly in woody debris (Jurgensen *et al.* 1992), yielding potential total external N inputs to the system in the range of 2.4 kg N ha⁻¹ year⁻¹.

Field sites are located throughout a 100 km² area centered near the southern edge of Swan Lake (47°55'8"N 113°50' 35"W). Mean annual precipitation is ~750 mm yr⁻¹ and mean annual temperature is ~7 °C (Western Regional Climate Center, 2016). The Swan Valley was glaciated during the Bull Lake and Pinedale glaciations and is characterized by skeletal, gravelly soils overlaying glacial till and Precambrian sedimentary mudstone. Soils are inceptisols (predominately dystic eutrochrepts) with high gravel content and ash common in the subsurface (Soil Survey Staff 2014).

Sites known to have been clear-cut only once in the previous 100 years were identified from the US Forest Service and Wilderness Society Forest Activity Tracking System database (Natural Resource Manager, USFS) accessed via a Google Earth database (Natural Resource Manager, USFS) provided by The Wilderness Society. The four sites selected were clear-cut in 1918, 1955, 1969, and 2000, respectively, and the total area of each treatment was 15-50 ha. One undisturbed stand dated by prior work to be 350 years old (Lesica *et al.* 1991) was included as an old-growth site. US Forest Service records indicate the old-growth area has been free of fire and harvest activity since at least 1899 (Ayres 1900), and absence of fire scars, charcoal deposits, and stumps support our assumption that this stand has been largely undisturbed in recent history. Maximum distance between sites is 15 km. The elevations of field sites vary from 980-1080 MASL.

Within each of the clear-cut treatments and the old-growth area (hereafter, sites) we established 3 inventory plots. For the 45-, 59-, 96-year-old and old-growth sites, inventory plots were 157 m². For the 14-year old site, plots were 40 m² to accommodate high stem densities. We measured diameter at breast height (1.37 m from ground, DBH) and tree height (using a clinometer) for all trees in each of the 15 inventory plots. All inventory plots were greater than 100 m from one another, and greater than 50 m from clear-cut treatment edges, roads, and riparian areas. From inventory data, tree aboveground biomass (AGB) and foliage biomass were estimated using species-specific allometric regression functions presented in Chojnacky et al. (2014) for all trees over 2.5 cm DBH for all 11 species present. Trees shorter than breast height were measured for diameter a proportional distance from ground level as larger trees. Across all sites this yielded a population of $n=405$ trees for analyses hereafter. Fine root biomass (roots < 2mm) was first measured at the stand level from core samples taken to a depth of 15 cm. Three pairs of 8x15 cm auger cores were taken haphazardly within each inventory plot. Pairs of cores were composited into a single sample and fine roots were sorted from cores, rinsed, and oven dried to constant mass at 65 °C before weighing.

To measure total C and N and dissolved N and P of various forest pools, we took a 30 x 30 cm sample of the litter layer to the depth of contact with the forest organic horizon at four locations around a larger sampling plot, centered at the inventory plot. We then took a 10 x 10 cm sample of organic horizon below the surface litter to the depth of contact with mineral soil at the same four locations. For each of these sampling plots, the four litter samples were composited into one sample, and this was repeated with the

organic horizon samples. The mineral soil was sampled below this section of forest O horizon from 0-15 cm and 15-30 cm using a 5 x 15 cm corer. Samples were composited as above, and subsampled in duplicate. Approximately 10 mL of material (litter, organic horizon, or mineral soil) was ground using a mechanical mill, and 5-50 mg of the resulting sample was analyzed for C and N via elemental combustion (Costech Analytical technologies, Inc. Valencia, CA, USA). Dissolved N and P was measured on the organic horizon and mineral soil samples. We performed soil water extracts to quantify dissolved inorganic N (DIN; $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) and phosphate ($\text{PO}_4\text{-P}$) availability. We also performed 2M potassium chloride (KCl) extracts in 2014 to measure dissolved inorganic N concentrations in these pools. For water and KCl extracts, soil organic horizons, mineral soil from 0-15 cm, and mineral soil from 15-30 cm were extracted separately. Organic horizon samples for extractions were mixed, sieved to 2 mm, and a 15 g sub-sample was shaken in 75 ml of ultra-pure water (or 2M KCl) for 3.5 hours, filtered with a Whatman 41 20- μm filter, and kept on ice until frozen within 48 hours. This process was repeated for mineral soil samples. Samples were then thawed and filtered to 0.7 μm prior to analysis for water extracts via a segmented flow auto-analyzer (SEAL Analytical, Mequon, WI, USA), KCl extracts were analyzed via flow injection auto-analyzer (Lachat Instruments, Loveland, CO, USA). Water extracts were also analyzed for total dissolved nitrogen (TDN) via chemiluminescence detection (Shimadzu Corp., Tokyo, Japan). Total dissolved inorganic nitrogen concentrations were subtracted from TDN to obtain dissolved organic nitrogen concentrations.

Foliar N was quantified by collecting live and senesced foliage from 40 trees across a range of sizes selected from the oldest and youngest sites (9 *A. grandis*, 12 *T. plicata* from the 14 year-old site; 8 *A. grandis*, 11 *T. plicata* from the old-growth) to span the range of tree size represented across the chrono-sequence. Foliage was collected by hand or with a shotgun for tall trees from sun-exposed areas of the canopy. Senesced foliage was collected in litter baskets suspended within smaller focal tree canopies or underneath the crown for large trees; for some trees, senesced ‘ready to fall’ foliage was removed by hand when litter baskets yielded insufficient litter mass for analysis. After drying at 65 °C to constant mass and grinding, green and senesced foliage was analyzed for N via elemental combustion (Costech Analytical Technologies, Inc., Valencia, CA, USA). Resorption efficiency was calculated as:

$$\frac{[N]_{live} - [N]_{dead} * MLCF}{[N]_{live}} * 100 = \% \text{ resorption efficiency} \quad (1)$$

Where $[N]_{live}$ is the N concentration in live foliage in mg N g^{-1} , $[N]_{dead}$ is the N concentration in dead foliage in mg N g^{-1} , and MLCF is a mass loss correction factor of 0.745 for conifers as described in (Vergutz *et al.* 2012). Average foliar turnover time specific to each species (*T. plicata* – 9 years, *A. grandis* – 5 years) was used to calculate foliar nutrient resorption rates (Balster & Marshall 2000; Harlow *et al.* 2005).

To estimate turnover rates of N pools, we estimated the average input from foliar litterfall using allometric estimates of foliage biomass and a foliar turnover time of 9 years for *T. plicata* foliage, 5 years for *A. grandis* foliage, and 7 years for foliage of all other species. We applied our observed foliar N concentration in live foliage and resorption efficiency for *T. plicata* (62.2%) and *A. grandis* (28.6) and used the mean N

concentration (8.6 mg N / g) and global average for conifer resorption efficiency (62.7%) for all other species (Vergutz *et al.* 2012). These estimates of foliar N inputs were used to calculate the turnover time as: $(N_{\text{Litter}} + N_{\text{Oi}})/N_{\text{litterfall}}$ where $N_{\text{Litter}} + N_{\text{Oi}}$ is the N pool in the combined litter and organic horizons and $N_{\text{litterfall}}$ is the litterfall N input rate described above. We used the average of the N input rate between two adjacent stand ages to calculate the rate of N supplied from turnover between age intervals.

Results

Total ecosystem C (Fig. 1a; aboveground biomass, fine roots to 15 cm, litter, organic horizon, and mineral soils to 30 cm) was highest in the 350-year stand with $368.2 \pm 54 \text{ Mg C ha}^{-1}$ (mean $\pm$ SE). Total ecosystem N (Fig. 1b) in our sites was also highest in the 350-year old stand with $3,279.9 \pm 461 \text{ kg N ha}^{-1}$ (mean $\pm$ SE). In aboveground tree biomass, C increased significantly with age ($p=0.002$) as did N ($p=0.003$), though increases in C were faster than those for N (Fig. 1c). Aboveground biomass C in the 350-year old site was $323.5 \pm 48 \text{ Mg C ha}^{-1}$. Aboveground biomass N in the 350-year-old site was $1.5 \pm 0.2 \text{ Mg N ha}^{-1}$. Biomass C:N in tree aboveground biomass increased with stand age from 206.5 to 245.3. Total ecosystem C increased significantly with stand age ($p=0.002$) as did total ecosystem N ($p=0.002$), driven largely by the increases in aboveground biomass. Total ecosystem C:N (aboveground + all soil components) also increased significantly with increasing stand age (Fig. 1c) from 38.6 to 131.7 ($p=0.03$).

The total soil C pool (litter layer, organic horizon, and mineral soil to 30 cm) showed a distinct period of loss between the 14- and 59 year-old sites, followed by a

period of significant accumulation between the 59- and 96-year old sites (Fig. 2a). N accumulation and loss rates were low compared to C (Fig. 2b), and followed the same pattern of initial loss followed by re-accumulation. This pattern was in large part driven by the loss of the organic horizon, which was lowest in the 59-year-old site. Considering all soil pools combined (litter, organic horizon, and mineral soil from 0-30 cm), C:N did not vary significantly across the chrono-sequence.

Within a single soil pool, there were variable periods of gain and loss for both C and N. In the litter layer, the total C pool had a significant positive linear relationship with total litter mass, but for N, a decline in concentration with increasing stand age resulted in a significant ($P=0.07$), but very minimal decline in total litter N pool with increasing stand age. In the mineral soil, the fluctuations in C and N pools reflected soil bulk density, which was not constant across sites, nor did it change directionally with stand age. In these horizons, there was a significant positive linear relationship between soil bulk density and soil C pool ($p = 0.02$) and soil N pool ($p < 0.001$). There were no significant correlations between soil bulk density and soil C concentration ($p=0.42$) or N concentration ($p=0.90$) in the mineral soil. Bulk density of the organic horizon also fluctuated, but there were no correlations between bulk density and C pools ($p=0.67$) or N pools ($p=0.31$) or C concentrations ($p=0.44$) or N concentrations ($p=0.48$), as the depth and total mass of the organic horizon also changed with age, which drove the declines and accumulation periods of C and N in the organic horizon.

Within ages, average C concentration (Fig. 3a) changed significantly through the ecosystem profile (litter layer, organic horizon, and mineral soils to 30 cm) for all site

ages (one-way ANOVA; $F(4,69)$: 1848, $p < 0.001$), but across sites age did not have a significant effect on these concentrations (two-way ANOVA; $F_{\text{age}}(4,65)$: 1.28, $p = 0.3$, $F_{\text{layer}}(4,65)$: 1878, $p < 0.001$). Mean C concentration in conifer litter was 391.92 mg C / g ($SE \pm 1.39$), litter 490.69 mg C / g ($SE \pm 4.08$), organic horizon 320.28 ($SE \pm 17.35$), and mineral soil 19.20 ($SE \pm 2.49$). Differences in C concentration among all layers were significant at the $P = 0.05$ level from least-squares means adjusted pairwise comparisons, except between the 0-15 cm and 15-30 cm mineral soil layers ($P = 0.6$).

Across all site ages, average N concentration (Fig. 3b) changed significantly through the ecosystem profile (Fig. 5b; one-way ANOVA; $F(4,69)$: 53.9, $p < 0.001$). Average N concentration in conifer litter was 5.09 mg N/ g ($SE \pm 0.28$), in the litter layer was 13.15 mg N/g ($SE \pm 1.35$), in the organic horizon was 9.89 mg N /g ($SE \pm 0.36$), and in the mineral soil 0.88 mg N / g ($SE \pm 0.09$). Differences of N concentration among all layers were significant at the $p = 0.05$ level from least-squares means adjusted pairwise comparisons, except for between the 0-15cm and 15-30cm mineral soil layers ($P = 0.99$), and the litter layer and organic horizon ($P = 0.07$). Age did not have a significant effect on these concentrations (two-way ANOVA; $F_{\text{age}}(4,65)$: 0.5, $p = 0.7$, $F_{\text{layer}}(4,65)$: 38.8, $p < 0.001$). Across and within all sites, C:N declines with depth were significant between the conifer litter inputs and all other layers (Fig. 3c) from least squares means adjusted pairwise comparisons, and indicated a decreasing trend with depth.

To compare whether the differences in N and C concentrations between soil horizons are the result of preferential N retention or accumulation, we examined the fractional change in average C and N concentration for each component relative to the

average of the concentration in conifer litter concentrations. This showed that average C concentration (Fig. 3d) in the litter layer had a fractional change significantly greater than 1, indicating that the litter layer was the only soil C pool with a higher C concentration than conifer litter inputs. All other C pools showed a significant decrease compared to the conifer litter. N concentration (Fig. 3e) for the litter layer and organic horizon was significantly higher than the conifer litter, indicative of strong net N accumulation in organic matter relative to the source, while mineral soil N concentrations were significantly lower than conifer litter.

Analysis of N turnover indicated that turnover was fastest in the old-growth site and slowest in the 14-year-old site (Fig. 4). Estimated turnover of N was sufficient to balance the aboveground uptake sink in all sites except between the 14- and 45- and between the 59-96 year old sites, where 26 and 63% of the N required by biomass respectively during this time was balanced by N turnover (Table 1). In sites where uptake was balanced by N turnover, excess N did not appear to be balanced as an input flux to the 15-30 cm soil layer, and may constitute losses from the system. Losses between the 0-14-, 45-59-, and 96-350-year age intervals are estimated at 1.3, 9.6, and 1.4 kg N ha⁻¹ year⁻¹, respectively.

Soil DIN concentrations (Fig. 5a) were low and similar across stands and did not change significantly with increasing stand age for ammonium ($P=0.27$) or nitrate ($P=0.97$). From 2014 water extract samples, there was no relationship between C:N and ammonium in the organic horizon ($P=0.95$), or mineral soil ($P=0.93$), or nitrate in these components ($P=0.07$ and $P=0.59$) respectively. There was also no relationship between

total N concentration and ammonium concentration in organic horizon ($P=0.5$) or mineral soils ($P=0.5$), or for nitrate concentration ($P=0.63$ and $P=0.9$) in these components respectively. Average ammonium pools were similar across the chrono-sequence, as were nitrate pools. Nitrogen: P ratios (molar DIN:PO₄-P) in water extracts were low (~ 2 -3.5) in all sites and did not change with increasing age across the chrono-sequence ($P=0.93$). Average dissolved organic nitrogen concentrations exceeded DIN concentrations in all sites and across most soil horizons (Fig. 5b).

Discussion

Our findings make for an interesting comparison to other coniferous PNW forests and place our sites at the low end of N availability regionally. The largest soil N pool observed across our sites ($1,727 \text{ kg N ha}^{-1}$) is strikingly low compared to that reported for similar forests regionally, which for the same depths can harbor $\sim 3,000 \text{ kg N ha}^{-1}$ (Jurgensen *et al.* 1997), and are dwarfed by the high-productivity PNW stands of coastal Oregon (up to $10,200 \text{ kg N ha}^{-1}$; Perakis *et al.* 2011). In part, this may be due to the conspicuous lack of N-fixing plant species across our chrono-sequence. Gains from N-fixing species early in succession can be considerable (Hendrickson & Burgess 1989; Binkley 2003), and the absence of these inputs in our sites may have prevented any considerable accumulation of N in soils following disturbance. If these species have been absent from the area for even longer time scales than our chrono-sequence represents, this may partially explain the exceptionally low soil N totals, and undoubtedly the relatively young age of soils in our sites contributes to the low soil N pools. Overall, we find that

soil N pools in our sites appear resistant to change following stand-replacing disturbance. While both C and N are lost initially with the decline in organic horizon mass, each nearly recoups this initial loss by 350 years. Relative to the prior pool size during this loss-accumulation period, C is both lost faster, and recouped faster than N during these periods. This is indicative of preferential N retention throughout the chrono-sequence, but also the far slower turnover of N that may explain the apparent resistance to disturbance shown by N pools in our sites. The low soil water and KCl extracts for DIN further support our assumption that the forests in our sites are N-limited, as do the low dissolved DIN:PO₄-P ratios from water extracts.

Despite the slow turnover and stability of the soil N pools and low N availability, we estimate an increasing sink for N in aboveground biomass. Aboveground biomass N accumulation may effectively operate as a positive feedback on N-limitation for our sites where external N inputs are low. As the aboveground biomass N sinks increase, N must be removed from the soil N pool, which in turn reduces the amount available for subsequent turnover. We do not detect the removal of N from the soil pool associated with an increasing biomass N sink in our sites, potentially because the absolute rate of aboveground biomass N accumulation ($4.4 \text{ kg N ha}^{-1} \text{ year}^{-1}$) over the entire chrono-sequence nearly matches the combined input rates from N deposition ($1.6 \text{ kg N ha}^{-1} \text{ year}^{-1}$, NADP) and heterotrophic and symbiotic biological N fixation ($2.4 \text{ kg N ha}^{-1} \text{ year}^{-1}$; Hendrickson & Burgess 1989; Jurgensen *et al.* 1992). Inputs from deposition and fixation may offset the removal of N via plant uptake, effectively adding sufficient N capital to the system to enable N accumulation in aboveground biomass.

We expect that the 350-year old site possesses the greatest degree of sink-driven limitation given the absolute amount of N sequestered in biomass, and likewise the 14-year-old site the least. However, the most rapid increases in biomass N accumulation occur between the 14- and 45-year-old sites and 59- and 96-year-old sites, which indicates that this source of N-limitation becomes expressed more rapidly for these periods than any other. The sink lessens due to the decline in aboveground biomass between the 45- and 59-year-old sites, though we do not observe a reciprocal increase in soil N pools during this period.

The fact that we do not observe accumulations of N despite periods of N surplus from turnover (see below) indicates the existence of an additional sink for N in the ecosystem. DON dominates the dissolved N pool in all of our sites, a feature common among N-limited forests. Such losses of DON are common in unpolluted forests where DON dominates the dissolved N pool, and function as a key mechanism by which N is lost and N-limitation is maintained (Hedin *et al.* 1995; Perakis & Hedin 2002). Losses of N on the order of $3\text{--}8 \text{ kg N ha}^{-1} \text{ year}^{-1}$ from soil N pools in our sites are necessary to balance surplus N from turnover and prevent the accumulation of N given our current budget, and though we did not measure DON losses directly, these values fall well within the reported range of losses for unpolluted temperate forests (Perakis & Hedin 2002).

Turnover estimates of N indicate variable periods of transactional limitation across the chrono-sequence. As N appears to be accumulating in both the litter and organic horizons given the elevated N concentrations, transactional N limitation periods would thus approximate the turnover time of these pools. Transactional N limitation is

greatest where N turnover fails to balance the biomass N increment between the 14- and 45-year-old sites and the 59- and 96-year-old sites. Adding inputs from deposition and fixation alleviates the deficit between the 59-96-year old sites, but between the 14- and 45-year-old sites an N deficit remains. In the remaining age intervals, turnover of N appears to balance the necessary uptake N sink. We estimated that the old-growth N pool turnover time is the shortest among all the sites, resulting in the smallest degree of transactional N limitation among sites. The soil pool in the old-growth may therefore supply N more efficiently than the other sites. Other work supports faster N turnover in older, undisturbed forests (Davidson *et al.* 1992; Stark *et al.* 1997), and may thus offset the large biomass N sink in the old-growth sites.

Mechanisms responsible for transactional N limitation can be deduced via our analysis of the soil profiles within sites. Our analysis comparing the N and C concentrations of these reservoirs to the N and C concentrations of the litter source material revealed that N is indeed retained within the sites, with preferential retention of N over C. As the litter and organic material showed increased N concentrations compared to litter inputs, we find that N is immobilized and/or accumulating due to the slow turnover of the soil N pools. Assuming the N concentrations of inputs remain relatively stable, the concentration increases indicate that N is accumulating faster than it is being released via decomposition, thus initiating a period of transactional N limitation. We see elevated N concentrations in the litter and organic horizons in all sites, indicating that to some degree transactional N-limitation does exist. However, as our turnover estimates indicate, net turnover of N may be sufficient to meet plant demand.

For C, concentrations remain elevated only in the litter layer, which is reasonable considering the likelihood that inputs such as fine and small woody debris have contributed significantly greater amounts of C compared to conifer litter alone. Despite the loading of C in this reservoir, the signature of C accumulation is absent by the time the litter pool effectively transfers to the organic horizon pool. As such, we find that N is retained preferentially to C during this transfer, evidenced by the still-elevated N concentration in the organic horizon pool.

The expression of sink-driven and transactional N limitation through the chronosequence appeared to have been directly influenced by forest stand dynamics and time-since disturbance. The two periods of rapid biomass N accumulation, indicative of strong sink-driven limitation, also appeared to be the most transactionally N-limited. The period from the 14-45-year old sites may represent forest release from a reorganizational aggrading phase where rapid biomass increases induced a strong period of transactional N limitation and rapid expression of sink-driven limitation as biomass N increases outpaced N turnover. The period from the 59 to 96-year-old sites where biomass N increases did not appear to be balanced by N turnover followed a decline in aboveground biomass (from 45-59 years), potentially indicating a period of self-thinning had occurred, thereby reducing N inputs and inducing a lagged effect of reduced litter inputs to the soil N pool available for turnover.

The removal of N (and C) from the organic horizon from 14-45 years followed by a steep recovery was driven by the loss in depth and mass of the organic horizon, which approximates a pattern termed the “Covington curve” observed by many others

(Covington 1981; Yanai *et al.* 2003; Sun *et al.* 2004) in forest chrono-sequence work. Prior work indicates that the initial loss of organic horizon material is the result of enhanced decomposition promoted by the removal of canopy cover, resulting in an extended and warmer growing season that promotes decomposition (Covington 1981). However, others have since speculated that numerous other factors may cause this decline, such as mechanical mixing of the litter or organic matter into mineral soil, exposure of litter material to novel decomposer communities that promote decomposition, or physical removal of the organic horizon to some degree during harvest (Yanai *et al.* 2003; Noormets *et al.* 2015). Whether the mechanisms behind this pattern are tied to biotic successional processes or mechanical disturbances in our sites and others remain unclear, but the ubiquity of the pattern, and its appearance in our sites, is nonetheless an important factor controlling periods of C and N loss and accumulation across our chrono-sequence. The rapid biomass removal and large-scale “reset” of N inputs to the system caused by clear-cutting directly influences our turnover time estimates for N. Our high turnover time estimate in the 14-year-old site stems from a low early-successional litterfall input N flux compared to what amounts to a late-successional litter and organic horizon N pool, as it differs little from the pools across the chrono-sequence.

Whether the variation we observed in N and C accumulation rates among our sites was related to clear-cutting disturbance is difficult to disentangle. The effects of forest harvest on ecosystem N pools are highly variable across studies, but one meta-analysis has shown that there is little effect of logging on total soil N pools (Johnson & Curtis

2001), as our results suggest. N pools in our sites change predominately with changes in bulk density (for the mineral soil N pools) or overall mass changes in the litter and organic horizon rather than time since disturbance, as others have noted (Giesen *et al.* 2008). Some studies report that immediately following harvesting, mechanical disturbance can affect bulk density (Foote *et al.* 2015), but how this should change over time is unclear.

Our findings support the idea that N sequestration into plant biomass represents a long-term sink that may sustain ecosystem-level N limitation, but also reveal how stability in overall soil N pools emerges from the dynamic exchanges of N among active-cycling pools. Despite C inputs that appeared to outpace those of N given the increases in ecosystem C:N through time, we found that processing of these inputs resulted in preferential retention of N. Across all sites there was a decreasing trend in C:N with increasing depth relative to the conifer litter inputs from the litter layer, to the organic horizon, and to mineral soil layers indicating the preferential retention of N with this progression. Strong preferential retention of N over long time scales as indicated across our chrono-sequence ultimately resulted in slow turnover as N is tightly held, preventing rapid perturbation and N loss following disturbance.

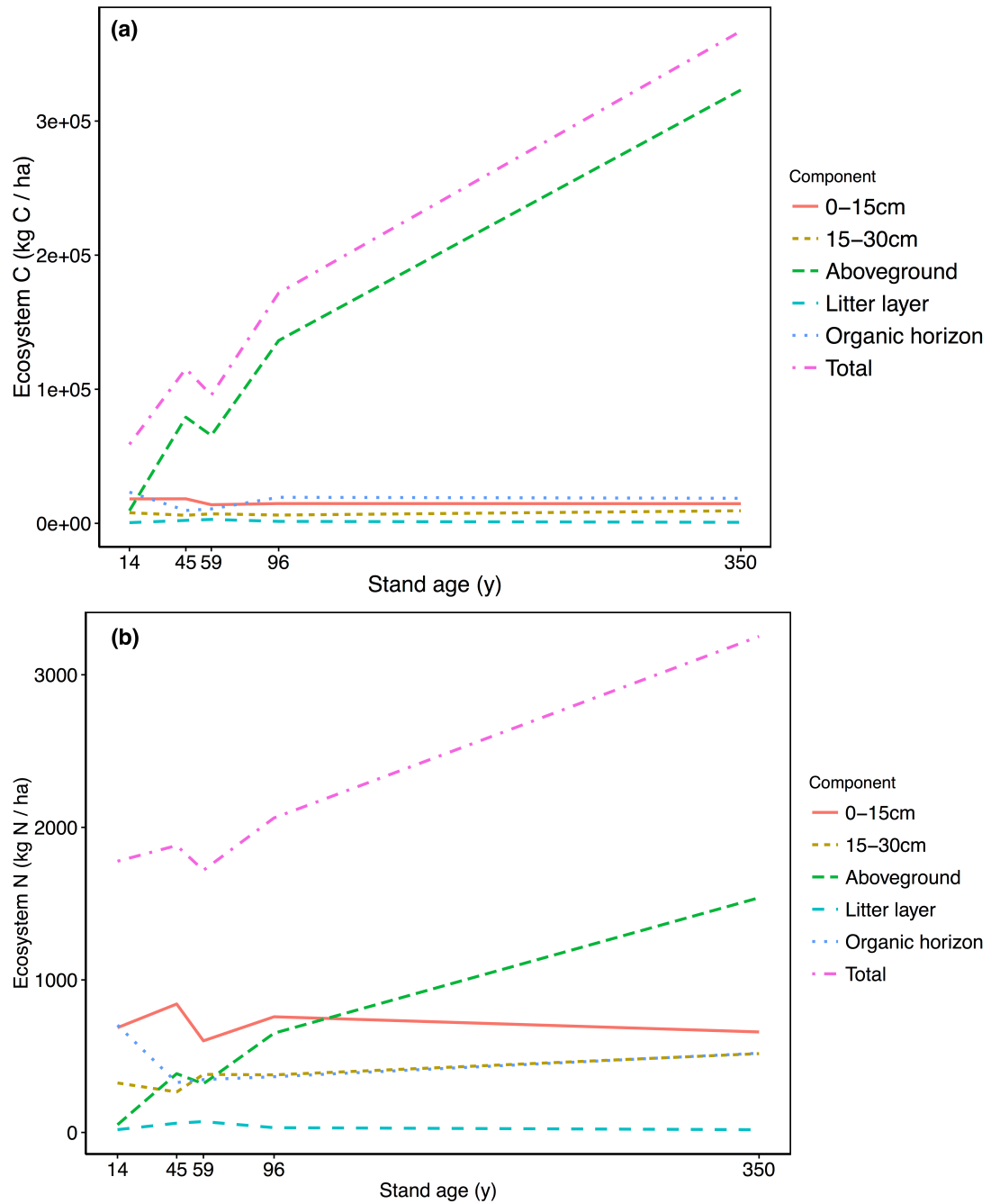


Figure 1. Total ecosystem C (a) and N (b) pools and trajectories for biomass including fine roots to 15 cm depth, litter layer, organic horizons, and mineral soils.

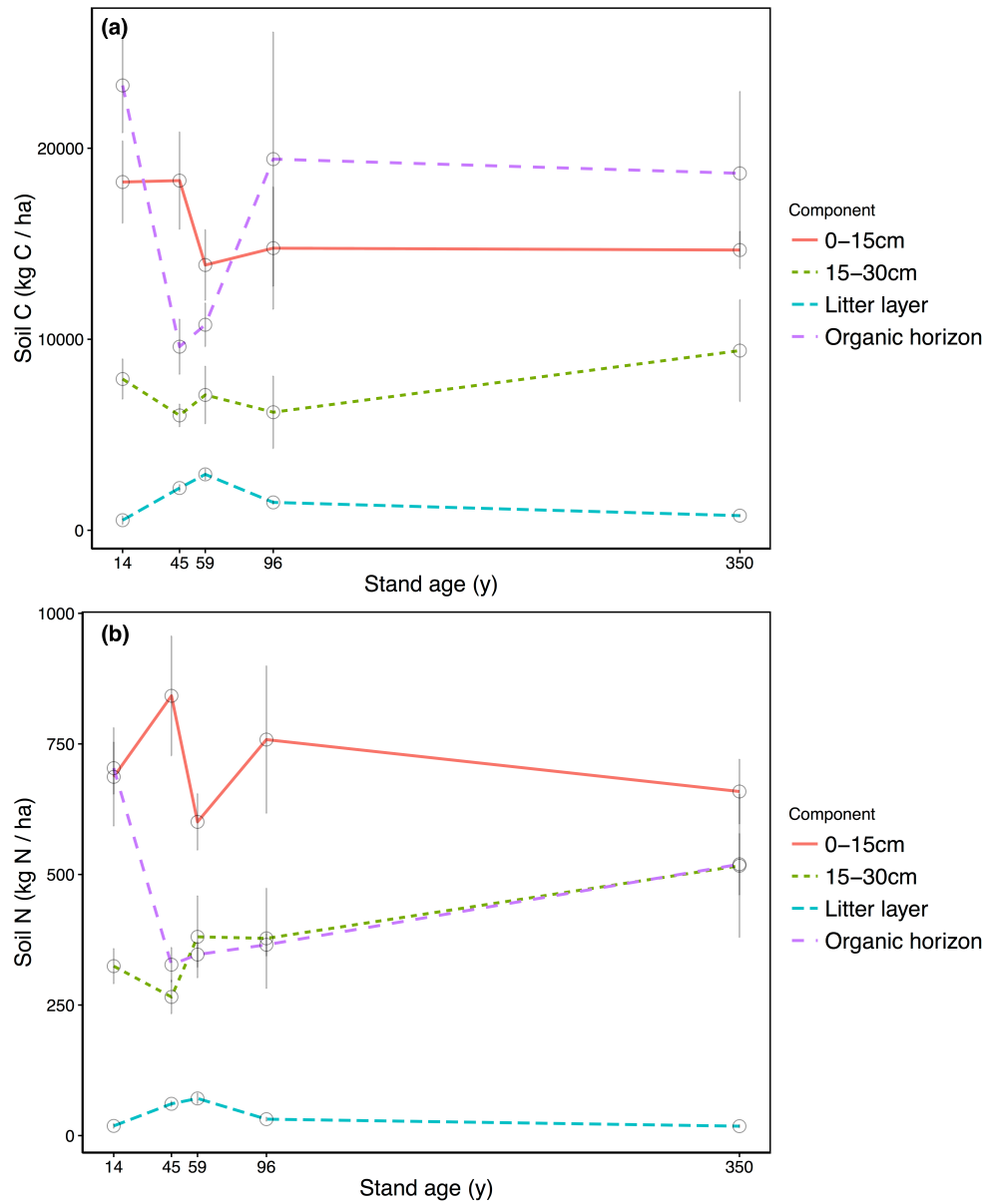


Figure 2. Total soil C (a) and N (b) pools and trajectories.

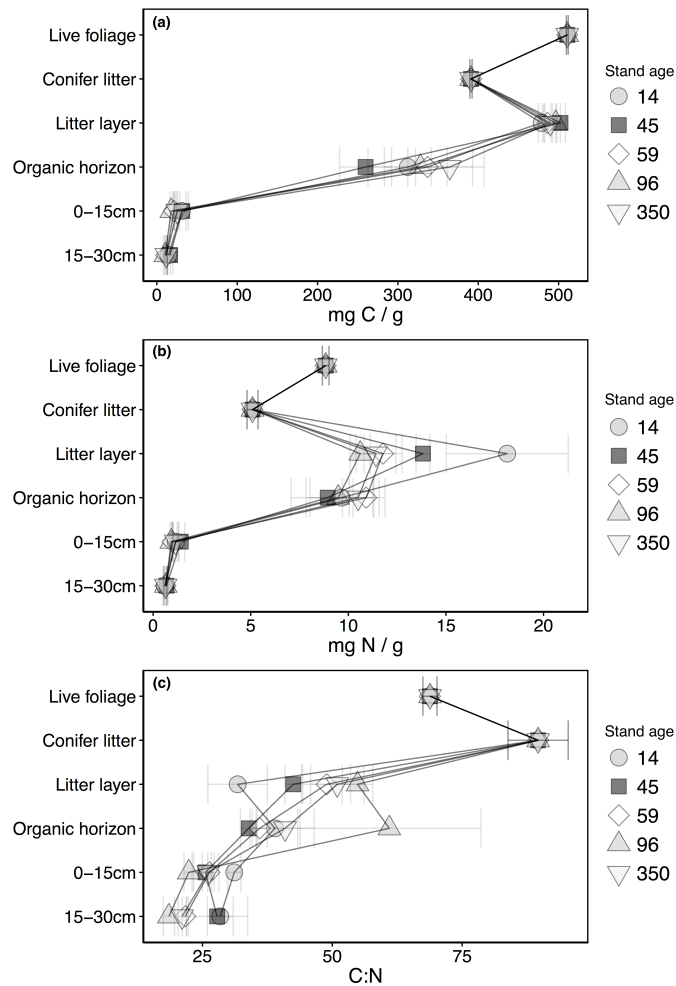


Figure 3. C (a) concentration changes with depth across soil components. C concentration increased significantly from the conifer litter inputs to the litter layer, and then declined steadily with depth. N concentrations (b) increased in the litter layer and organic horizons, and then decline d with depth. C:N (c) steadily declined with increasing depth. Live foliage C and N concentrations were not included in analyses comparing concentrations between components.

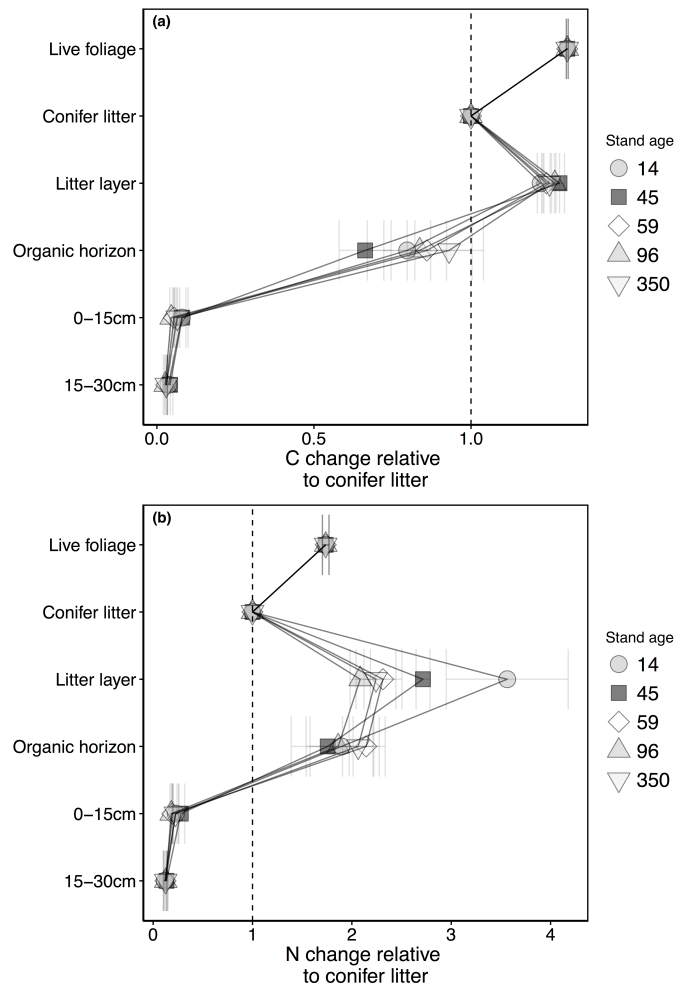


Figure 4. The C concentration change (a) relative to the conifer litter inputs. A change >1 indicates that the concentration has increased relative to conifer litter. For C, this increase was only present in the litter layer; below this layer the C concentration was significantly less than that in the conifer litter. N concentrations (b) were significantly increased relative to conifer litter in the litter layer and the organic horizons, indicating accumulation and preferential retention of N in these layers relative to C.

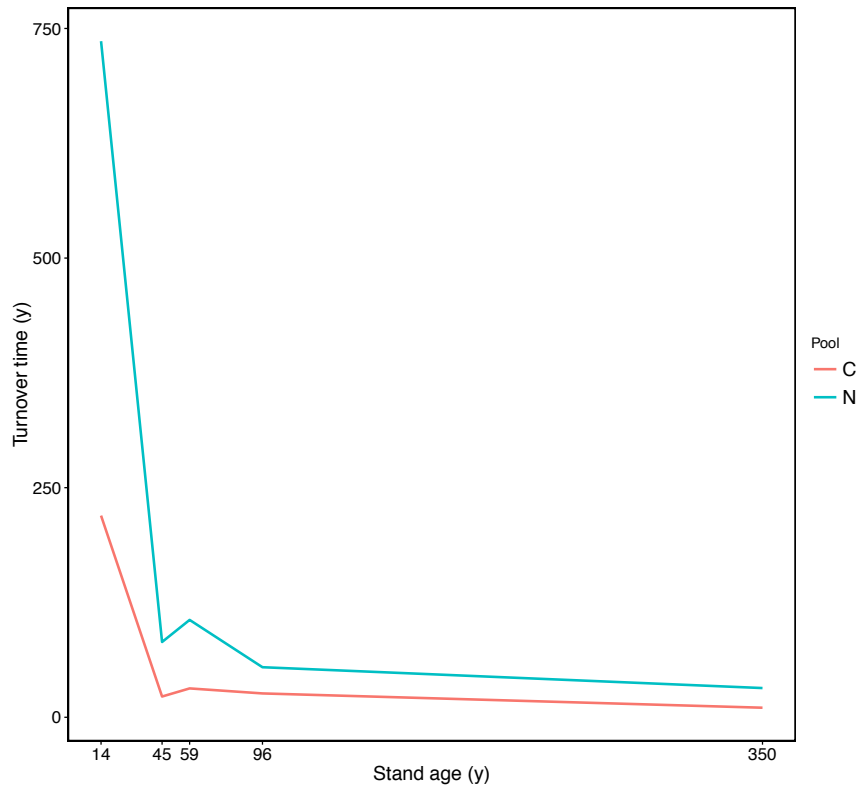


Figure 5. Turnover times of C and N were estimated using the input flux of C or N from litter, and a combined pool of the litter layer plus the organic horizon.

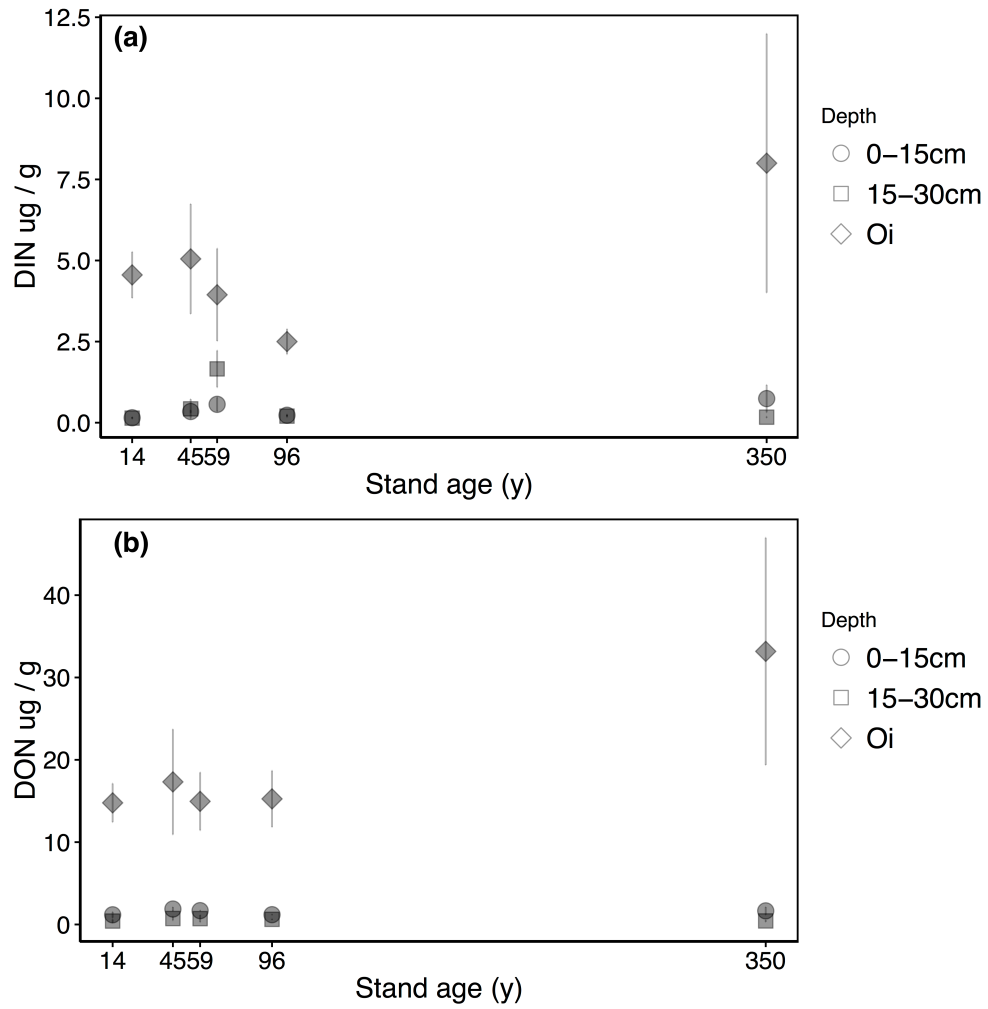


Figure 6. DIN (a) and DON (b) concentrations across sites. DON dominated the dissolved N pools in all sites.

Table 1. Comparison of the biomass N increment for given age intervals, and the subsequent change in soil N pools and N supplied from turnover of the litter layer and organic horizon. Only the biomass N sink from the period between 14-45 years appears balanced by removal from the soil N pool, while turnover alone appear insufficient to meet the biomass N sink demand between 14-45 years and 59-96 years, indicative of transactional N limitation.

Age interval years	Biomass N accumulation rate kg N ha ⁻¹ year ⁻¹	Soil N pool rate of change kg N ha ⁻¹ year ⁻¹	Litter layer + organic horizon N turnover kg N ha ⁻¹ year ⁻¹
0-14	4.5	1	8.9
14-45	11	-7.5	2.9
45-59	-4	-6.8	4.3
59-96	8.6	0.3	5.6
96-350	3.5	1.2	12

References

- National Atmospheric Deposition Program*. (2016). . Available at: <http://nadp.sws.uiuc.edu/nadpdata/annualReq.asp?site=MT05>. Last accessed 01 April 2016.
- Berg, B. & McClaugherty, C. (2014). Decomposition as a Process: Some Main Features. In: *Plant Litter*. Springer Berlin Heidelberg, pp. 11–34.
- Binkley, D. (2003). Seven decades of stand development in mixed and pure stands of conifers and nitrogen-fixing red alder. *Can. J. For. Res.*, 33, 2274–2279.
- Bormann, F.H., Likens, G.E., Fisher, D.W. & Pierce, R.S. (1968). Nutrient Loss Accelerated by Clear-Cutting of a Forest Ecosystem. *Science*, 159, 882–884.
- Covington, W.W. (1981). Changes in forest floor organic matter and nutrient content following clear cutting in northern hardwoods. *Ecology*, 62, 41–48.
- Davidson, E.A., Hart, S.C. & Firestone, M.K. (1992). Internal Cycling of Nitrate in Soils of a Mature Coniferous Forest. *Ecology*, 73, 1148–1156.
- Foote, J.A., Boutton, T.W. & Scott, D.A. (2015). Soil C and N storage and microbial biomass in US southern pine forests: Influence of forest management. *For. Ecol. Manag.*, 355, 48–57.
- Giesen, T.W., Perakis, S.S. & Cromack, K. (2008). Four centuries of soil carbon and nitrogen change after stand-replacing fire in a forest landscape in the western Cascade Range of Oregon. *Can. J. For. Res.*, 38, 2455–2464.
- Hedin, L.O., Armesto, J.J. & Johnson, A.H. (1995). Patterns of Nutrient Loss from Unpolluted, Old-Growth Temperate Forests: Evaluation of Biogeochemical Theory. *Ecology*, 76, 493–509.
- Hendrickson, O.Q. & Burgess, D. (1989). Nitrogen-fixing plants in a cut-over lodgepole pine stand of southern British Columbia. *Can. J. For. Res.*, 19, 936–939.
- Hooker, T.D. & Compton, J.E. (2003). Forest Ecosystem Carbon and Nitrogen Accumulation During the First Century After Agricultural Abandonment. *Ecol. Appl.*, 13, 299–313.
- Johnson, D.W. & Curtis, P.S. (2001). Effects of forest management on soil C and N storage: meta analysis. *For. Ecol. Manag.*, 140, 227–238.
- Jurgensen, M.F., Graham, R.T., Larsen, M.J. & Harvey, A.E. (1992). Clear-cutting,

woody residue removal, and nonsymbiotic nitrogen fixation in forest soils of the Inland Pacific Northwest. *Can. J. For. Res.*, 22, 1172–1178.

Jurgensen, M.F., Harvey, A.E., Graham, R.T., Page-Dumroese, D.S., Tonn, J.R., Larsen, M.J., *et al.* (1997). Review article: Impacts of timber harvesting on soil organic matter, nitrogen, productivity, and health of inland northwest forests. *For. Sci.*, 43, 234–251.

Kira, T. & Shidei, T. (1967). Primary production and turnover of organic matter in different forest ecosystems of the western Pacific. *Japan Journ. Ecol.* 17, 70–87.

Knops, J.M.H., Bradley, K.L. & Wedin, D.A. (2002). Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecol. Lett.*, 5, 454–466.

Law, B.E., Sun, O.J., Campbell, J., Van Tuyl, S. & Thornton, P.E. (2003). Changes in carbon storage and fluxes in a chronosequence of ponderosa pine. *Glob. Change Biol.*, 9, 510–524.

Law, B.E. & Waring, R.H. (2015). Carbon implications of current and future effects of drought, fire and management on Pacific Northwest forests. *For. Ecol. Manag.*, Carbon, water and nutrient cycling in managed forests, 355, 4–14.

Le Quéré, C., Moriarty, R., Andrew, R.M., Peters, G.P., Ciais, P., Friedlingstein, P., *et al.* (2015). Global carbon budget 2014.

McLauchlan, K.K., Higuera, P.E., Gavin, D.G., Perakis, S.S., Mack, M.C., Alexander, H., *et al.* (2014). Reconstructing Disturbances and Their Biogeochemical Consequences over Multiple Timescales. *BioScience*, bit017.

Menge, D.N.L., Pacala, S.W. & Hedin, L.O. (2009). Emergence and Maintenance of Nutrient Limitation over Multiple Timescales in Terrestrial Ecosystems. *Am. Nat.*, 173, 164–175.

Nave, L.E., Vance, E.D., Swanston, C.W. & Curtis, P.S. (2010). Harvest impacts on soil carbon storage in temperate forests. *For. Ecol. Manag.*, 259, 857–866.

Noormets, A., Epron, D., Domec, J.C., McNulty, S.G., Fox, T., Sun, G., *et al.* (2015). Effects of forest management on productivity and carbon sequestration: A review and hypothesis. *For. Ecol. Manag.*, Carbon, water and nutrient cycling in managed forests, 355, 124–140.

Perakis, S. & Hedin, L.O. (2002). Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. *Nature*, 415, 416.

- Perakis, S.S., Sinkhorn, E.R. & Compton, J.E. (2011). $\delta^{15}\text{N}$ constraints on long-term nitrogen balances in temperate forests. *Oecologia*, 167, 793–807.
- Perakis, S.S., Tepley, A.J. & Compton, J.E. (2015). Disturbance and Topography Shape Nitrogen Availability and $\delta^{15}\text{N}$ over Long-Term Forest Succession. *Ecosystems*, 1–16.
- Pickett, S.T.A. & White, P.S. (1985). Natural disturbance and patch dynamics. In: *Academic Press*.
- Pregitzer, K.S. & Euskirchen, E.S. (2004). Carbon cycling and storage in world forests: biome patterns related to forest age. *Glob. Change Biol.*, 10, 2052–2077.
- Prescott, C.E. (1997). Effects of clearcutting and alternative silvicultural systems on rates of decomposition and nitrogen mineralization in a coastal montane coniferous forest. *For. Ecol. Manag.*, 95, 253–260.
- Rastetter, E.B., Yanai, R.D., Thomas, R.Q., Vadeboncoeur, M.A., Fahey, T.J., Fisk, M.C., *et al.* (2013). Recovery from disturbance requires resynchronization of ecosystem nutrient cycles. *Ecol. Appl.*, 23, 621–642.
- Ryan, M.G., Binkley, D. & Fownes, J.H. (1997). Age-Related Decline in Forest Productivity: Pattern and Process. In: *Advances in Ecological Research* (ed. Fitter, M.B. and A.H.). Academic Press, pp. 213–262.
- Seymour, R.S., White, A.S. & deMaynadier, P.G. (2002). Natural disturbance regimes in northeastern North America—evaluating silvicultural systems using natural scales and frequencies. *For. Ecol. Manag.*, Forest Ecology in the next Millennium : Putting the long view into Practice, 155, 357–367.
- Sollins, P., Grier, C.C., McCorison, F.M., Cromack, K., Fogel, R. & Fredriksen, R.L. (1980). The Internal Element Cycles of an Old-Growth Douglas-Fir Ecosystem in Western Oregon. *Ecol. Monogr.*, 50, 261–285.
- Stark, J.M., Hart, S.C. & others. (1997). High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature*, 385, 61–64.
- Sun, O.J., Campbell, J., Law, B.E. & Wolf, V. (2004). Dynamics of carbon stocks in soils and detritus across chronosequences of different forest types in the Pacific Northwest, USA. *Glob. Change Biol.*, 10, 1470–1481.
- Thomas, R.Q., Brookshire, E.N.J. & Gerber, S. (2015). Nitrogen limitation on land: how can it occur in Earth system models? *Glob. Change Biol.*, 21, 1777–1793.

- Vergutz, L., Manzoni, S., Porporato, A., Novais, R.F. & Jackson, R.B. (2012). Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecol. Monogr.*, 82, 205–220.
- Vitousek, P.M. & Howarth, R.W. (1991). Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, 13, 87–115.
- Vitousek, P.M. & Matson, P.A. (1984). Mechanisms of Nitrogen Retention in Forest Ecosystems: A Field Experiment. *Science*, 225, 51–52.
- Vitousek, P.M., Porder, S., Houlton, B.Z. & Chadwick, O.A. (2010). Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecol. Appl.*, 20, 5–15.
- Vitousek, P.M. & Reiners, W.A. (1975). Ecosystem Succession and Nutrient Retention: A Hypothesis. *BioScience*, 25, 376–381.
- Waring, R.H. & Franklin, J.F. (1979). Evergreen coniferous forests of the Pacific Northwest. *Science*, 204, 1380–1386.
- Western Regional Climate Center. (2016). *Swan Lake, Montana*. Available at: <http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?mt8087>. Last accessed: 01 April 2016.
- Yanai, R.D., Currie, W.S. & Goodale, C.L. (2003). Soil Carbon Dynamics after Forest Harvest: An Ecosystem Paradigm Reconsidered. *Ecosystems*, 6, 197–212.

CHAPTER FOUR

CONCLUSIONS

The coupled tree-soil system appears to have a strong capacity to retain nitrogen (N) across our N-limited forest chrono-sequence. We find that uptake is a vital means by which trees can retain N, and that foliar resorption plays an increasing role in N retention through forest succession in our sites. At the ecosystem scale, our chrono-sequence appears to be exceptionally deficient in N capital. We also find that sink-driven and transactional N limitation result in the preferential retention of N over C across more than 300 years of succession. Preferential retention and slow N turnover ultimately lead to N pools that change little following stand-replacing disturbance through succession, as we find net accumulation of N only in tree biomass.

Our findings for individual trees have focused on two common Pacific Northwest species for root morphology and foliar resorption, parameters that are critical to our results. Future work should consider further investigating the relationships of root morphology and resorption efficiency with tree size to assess the extent to which our findings may apply to other species and forest types. Our chrono-sequence displays many of the C and N dynamics seen elsewhere, and we expect a good proxy of substituting space for time. Our inclusion of old-growth forest stands is unique among many harvest-related chrono-sequences that concern only rotation-length aged stands, but we believe it is vital in assessing long-term trajectories. However, our results indicating that soil carbon has not yet returned to the levels we observed early in succession may indicate

that even older yet sites are necessary to approximate “steady state” conditions, given the evident recalcitrance at which N accumulation appears to occur in our sites, which constrains ecosystem C accumulation. Nonetheless, we have identified important roles for both individual trees and forest ecosystems in a highly retentive N cycle.

For individual trees, scaling relationships between N uptake by roots and aboveground biomass indicated that uptake fluxes continue to increase as aboveground biomass increases. Our uptake estimates required inputs of fine root biomass and root morphology. In our sites, we found little evidence to suggest that root morphology changed systematically or directionally with increasing tree size. Allometric and theoretical estimates for individual trees predict that fine root biomass allocation will decrease proportionately to aboveground biomass, but total increases in fine root biomass will continue with increasing tree size. Given this, coupled with the lack of any strategic trend in root morphology changes that could enable increases in uptake efficiency without fine root biomass increases, the increases in fine root biomass were sufficient to allow total N uptake for trees to continue increasing. To our knowledge, few studies have attempted to link tree age or size with fine root morphology. Many studies concern root morphological changes as they relate to nutrient availability or root order, which could indicate uptake efficiency shifts. However, we did not find a clear relationship between nutrient availability (DIN or N:P) and changes in fine root morphology. It is possible that as DIN and N:P were similar across all sites respectively, individual trees were experiencing similar levels of nutrient stress and therefore displayed little change in root morphology as a result. However, we observed a large range in root morphology among

individual trees, though no differences were related to tree species. Root morphology is known to be highly heterogeneous even within an individual root system, so it is likely that our measures are indicative of this natural variation. It is fortunate that we found very similar levels of N availability in the study sites, as it could be difficult to disentangle the effects of tree size from N availability on root morphology, were a definitive trend to exist. Regardless, our findings suggest that trees should have an increasing uptake capacity due to increases in fine root biomass that accompany tree growth, without the need for strategic increases in uptake efficiency with tree age. Ultimately, our conclusion that N uptake rates continue to increase with increasing tree size support recent observations and theoretical predictions that tree growth rates should also continue to increase with tree size. Additionally, our simulations indicate that trees are able to sustain this increasing growth as they age without the need for increases in growth efficiency.

Foliar N resorption in our study sites also did not change systematically with tree size. Likewise, foliar N concentration did not change directionally across the range of tree sizes that we measured. Numerous others have reported declines in foliar N concentration with tree age, but for our sites this did not appear to be evident. Foliar N concentration is almost uniformly coupled to resorption efficiency, so the absence of a trend between resorption efficiency and tree size may be related to the similar absence with foliar N concentration. The invariance in both N resorption efficiency and N concentration could be specific to the two species we measured for foliar N resorption, but there is not a large body of evidence regarding resorption efficiency for *A. grandis* or *T. plicata* in the literature from which to draw many conclusions on the specificity of our

results. Nonetheless, this led us to conclude that in our sites N resorption efficiency tracks changes in foliar biomass. At the stand level, this indicated that foliar resorption does constitute an increasing proportion of N flux to a stand over time as foliar biomass increases exponentially with increases in tree diameter. However, our estimates indicate that N uptake by roots remains the dominant flux of N supplied to trees over the 350 years spanned by our chrono-sequence. Many prior chrono-sequence studies indicate the opposite trend: that N flux budgets in aging forests are increasingly dominated by recycled fluxes of N. Such estimates are not (to our knowledge) based on root-and tree-level estimates of uptake, but rather stand-level resorption estimates, and indirect methods of estimating N uptake. In this regard, we have contributed a novel approach from which to assess the relative contributions of N uptake v. foliar N resorption.

Our work has identified a keystone role for individual large trees in stand-level N cycling. Our estimates predict that large trees that represent less than 10% of the stem count in a stand can account for on average 25% of the uptake flux of the total stand. These results suggest that mortality of large trees may have significant impacts on stand level N cycling, especially by inducing large losses of N as single large trees die or are removed from the landscape via harvesting. Prior work had identified the potential for large losses of DIN upon tree harvesting, and others have shown that loss of several individuals can be sufficient to enable detection of DIN losses. Our work is novel in that it is based on the estimated uptake by large individual trees and thus constrains the potential magnitude of such losses at the individual level. This finding has implications

for both natural gap-phase dynamics in forest ecosystems and forest management practices that include the removal of even small numbers of individual trees.

We are also able to infer from our measures of available DIN and tree uptake of DIN that mean residence times must decrease in association with increasing uptake (which corresponds to increasing stand age in our sites) as DIN was very similar across our chrono-sequence sites. This finding implies that mean residence time of nitrogen must decrease in older and larger stands, which agrees well with prior work investigating the rates of N cycling processes in young v. mature forests. These studies showed experimentally that gross N mineralization was greater in older undisturbed forests and that N turnover may be faster, though difficult to detect due to rapid re-uptake of DIN by microbes. Our findings, constructed from a plant-centric uptake context, support these assertions, and imply that the enhanced N cycling found in older forests supports the increasing demand for DIN by larger trees indicated in our work.

Given the multitude of effects that stand-replacing disturbance can impose on the recovery of ecosystem C and N pools, we expected to find variability in the expression of sink-driven vs. transactional N limitation at various stages through succession. In ecosystem N pools, we expected that a system highly retentive for N would display accumulation of N across the chrono-sequence depending on the balance of plant demand and N inputs to the system. We observed an increasing sink for N in tree biomass, but we did not observe overall accumulation of N from the youngest to oldest sites in soils. Estimates of N turnover in the litter layer and organic horizons and N demand by the tree

biomass sink indicated two strong periods of transactional N limitation where N turnover rates are insufficient to meet plant demand.

Within the soil components, we found evidence to show how transactional N limitation may be expressed. We concluded this by examining the relative concentrations of C and N in the various soil components relative to conifer litter inputs. N concentrations remained elevated relative to the N concentrations of litter inputs until the transfer to the mineral soil, indicating that N was accumulating in a temporary N sink via immobilization, the slow turnover of which indicated transactional N limitation. Our findings support the idea that N sequestration into plant biomass represents a long-term sink that may sustain ecosystem-level N limitation but also reveal how stability in overall soil N pools emerges from the dynamic exchanges of N among active-cycling pools. Despite C inputs that appeared to outpace those of N given the increases in ecosystem C:N through time, we found that processing of these inputs resulted in preferential retention of N. Across all sites there was a strong trend in decreasing C:N with increasing depth from the litter layer, to the organic horizon, and to the mineral soil depths indicating preferential retention of N with this progression. Strong preferential retention of N over long time scales as indicated across our chrono-sequence ultimately resulted in slow turnover as N is tightly held, preventing rapid perturbation and N loss following disturbance.

Overall, we found that trees individually and collectively, and their tightly coupled interactions with the soil environment were highly retentive for N in our sites. A strong uptake sink by individual trees and efficient recycling via foliar N resorption prevented N

loss from trees. Our findings hold both fundamental and applied importance for the study of trees and forests. Fundamentally, we have shown that N uptake and resorption align with theoretical predictions from metabolic scaling that have to this point not been tested via the root- and leaf- scale. These findings relate basic properties of tree physiology vital to the growth and functioning of trees to easily measureable components of tree biomass allocation. Understanding the relationships between tree and forest size and nutrient retention may be especially important in predicting the response of the future forest C sink, as both N uptake and recycling efficiency are implicated in sustaining growth responses to elevated CO₂ (Finzi *et al.* 2007; Norby *et al.* 2010). Whether this implies that large statured, older forests will remain the most responsive to rising atmospheric CO₂ is uncertain, and will be an important direction for future research. Our findings may hold particular importance as large trees appear particularly susceptible to mortality from drought events (Bennett *et al.* 2015) and may be disappearing from forests globally (Lindenmayer *et al.* 2012). Forest management activities that include the removal of even small numbers of large trees may need to consider the long-term implications for ecosystem C and N retention.

The recovery of clear-cut forests, and the associated changes in N availability and limitation following management activities are vital considerations in assessing the trajectory of terrestrial C storage. While N accumulation may be difficult to detect or may occur over time scales longer than our study chrono-sequence, evidence for N retention suggests that net N loss following stand-replacing disturbance is unlikely in areas similar to our study sites with minimal external inputs and severe deficits in N capital. Nitrogen

pool dynamics in our sites match well with other forest chrono-sequence studies, and together may indicate that N-limited forests do possess a strong capacity to retain N and will continue to contribute significantly to the terrestrial C sink.

References

Bennett, A.C., McDowell, N.G., Allen, C.D. & Anderson-Teixeira, K.J. (2015). Larger trees suffer most during drought in forests worldwide. *Nat. Plants*, 1, 15139.

Finzi, A.C., Norby, R.J., Calfapietra, C., Gallet-Budynnek, A., Gielen, B., Holmes, W.E., *et al.* (2007). Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO₂. *Proc. Natl. Acad. Sci.*, 104, 14014–14019.

Lindenmayer, D.B., Laurance, W.F. & Franklin, J.F. (2012). Global Decline in Large Old Trees. *Science*, 338, 1305–1306.

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E. & McMurtrie, R.E. (2010). CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proc. Natl. Acad. Sci.*, 107, 19368–19373.

REFERENCES CITED

Aerts, R. (1996). Nutrient Resorption from Senescing Leaves of Perennials: Are there General Patterns? *J. Ecol.*, 84, 597–608.

Ayres, H.B. (1900). The Lewis and Clark Forest Reserve, Montana. In: *21st Annual Report of the USGS, 1899-1900*. US Government Printing Office, Washington, D.C., pp. 47–78.

Balster, N.J. & Marshall, J.D. (2000). Decreased needle longevity of fertilized Douglas-fir and grand fir in the northern Rockies. *Tree Physiol.*, 20, 1191–1197.

Bennett, A.C., McDowell, N.G., Allen, C.D. & Anderson-Teixeira, K.J. (2015). Larger trees suffer most during drought in forests worldwide. *Nat. Plants*, 1, 15139.

Berg, B. & McClaugherty, C. (2014). Decomposition as a Process: Some Main Features. In: *Plant Litter*. Springer Berlin Heidelberg, pp. 11–34.

Binkley, D. (2003). Seven decades of stand development in mixed and pure stands of conifers and nitrogen-fixing red alder. *Can. J. For. Res.*, 33, 2274–2279.

Blasius, D. & Oberwinkler, F. (1989). Succession of mycorrhizae: a matter of tree age or stand age? In: *Annales Des Sciences Forestieres*. EDP Sciences, p. 758s–761s.

Bond-Lamberty, B., Gower, S.T., Wang, C., Cyr, P. & Veldhuis, H. (2006). Nitrogen dynamics of a boreal black spruce wildfire chronosequence. *Biogeochemistry*, 81, 1–16.

Bormann, F.H., Likens, G.E., Fisher, D.W. & Pierce, R.S. (1968). Nutrient Loss Accelerated by Clear-Cutting of a Forest Ecosystem. *Science*, 159, 882–884.

Chojnacky, D.C., Heath, L.S. & Jenkins, J.C. (2014). Updated generalized biomass equations for North American tree species. *Forestry*, 87, 129–151.

Cleveland, C.C., Houlton, B.Z., Smith, W.K., Marklein, A.R., Reed, S.C., Parton, W., *et al.* (2013). Patterns of new versus recycled primary production in the terrestrial biosphere. *Proc. Natl. Acad. Sci.*, 110, 12733–12737.

Covington, W.W. (1981). Changes in forest floor organic matter and nutrient content following clear cutting in northern hardwoods. *Ecology*, 62, 41–48.

Crowther, T.W., Glick, H.B., Covey, K.R., Bettigole, C., Maynard, D.S., Thomas, S.M., *et al.* (2015). Mapping tree density at a global scale. *Nature*, 525, 201–205.

Davidson, E.A., Hart, S.C. & Firestone, M.K. (1992). Internal Cycling of Nitrate in Soils of a Mature Coniferous Forest. *Ecology*, 73, 1148–1156.

- Dighton, J. & Mason, P.A. (1985). Mycorrhizal dynamics during forest tree development. In: *Developmental Biology of Higher Fungi*, British Mycological Society Symposium. Cambridge University Press, Cambridge, pp. 117–139.
- Dixon, R.K., Solomon, A.M., Brown, S., Houghton, R.A., Trexler, M.C. & Wisniewski, J. (1994). Carbon Pools and Flux of Global Forest Ecosystems. *Science*, 263, 185–190.
- Dybzinski, R., Farrior, C.E. & Pacala, S.W. (2014). Increased forest carbon storage with increased atmospheric CO₂ despite nitrogen limitation: A game-theoretic allocation model for trees in competition for nitrogen and light. *Glob. Change Biol.*, n/a–n/a.
- Eis, S. (1987). Root systems of older immature hemlock, cedar, and Douglas-fir. *Can. J. For. Res.*, 17, 1348–1354.
- Enquist, B.J. & Niklas, K.J. (2002). Global Allocation Rules for Patterns of Biomass Partitioning in Seed Plants. *Science*, 295, 1517–1520.
- Enquist, B.J., West, G.B., Charnov, E.L. & Brown, J.H. (1999). Allometric scaling of production and life-history variation in vascular plants. *Nature*, 401, 907–911.
- Finzi, A.C., Norby, R.J., Calfapietra, C., Gallet-Budynek, A., Gielen, B., Holmes, W.E., *et al.* (2007). Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO₂. *Proc. Natl. Acad. Sci.*, 104, 14014–14019.
- Foot, J.A., Boutton, T.W. & Scott, D.A. (2015). Soil C and N storage and microbial biomass in US southern pine forests: Influence of forest management. *For. Ecol. Manag.*, Carbon, water and nutrient cycling in managed forests, 355, 48–57.
- Gerber, S. & Brookshire, E.N.J. (2014). Scaling of Physical Constraints at the Root-Soil Interface to Macroscopic Patterns of Nutrient Retention in Ecosystems. *Am. Nat.*, 183, 418–430.
- Giesen, T.W., Perakis, S.S. & Cromack, K. (2008). Four centuries of soil carbon and nitrogen change after stand-replacing fire in a forest landscape in the western Cascade Range of Oregon. *Can. J. For. Res.*, 38, 2455–2464.
- Gorham, E., Vitousek, P.M. & Reiners, W.A. (1979). The regulation of chemical budgets over the course of terrestrial ecosystem succession. *Annu. Rev. Ecol. Syst.*, 10, 53–84.
- Harlow, B.A., Duursma, R.A. & Marshall, J.D. (2005). Leaf longevity of western red cedar (*Thuja plicata*) increases with depth in the canopy. *Tree Physiol.*, 25, 557–562.

- Hedin, L.O., Armesto, J.J. & Johnson, A.H. (1995). Patterns of Nutrient Loss from Unpolluted, Old-Growth Temperate Forests: Evaluation of Biogeochemical Theory. *Ecology*, 76, 493–509.
- Hendrickson, O.Q. & Burgess, D. (1989). Nitrogen-fixing plants in a cut-over lodgepole pine stand of southern British Columbia. *Can. J. For. Res.*, 19, 936–939.
- Hooker, T.D. & Compton, J.E. (2003). Forest Ecosystem Carbon and Nitrogen Accumulation During the First Century After Agricultural Abandonment. *Ecol. Appl.*, 13, 299–313.
- Jackson, R.B., Mooney, H.A. & Schulze, E.-D. (1997). A global budget for fine root biomass, surface area, and nutrient contents. *Proc. Natl. Acad. Sci.*, 94, 7362–7366.
- Johnson, D.W. & Curtis, P.S. (2001). Effects of forest management on soil C and N storage: meta analysis. *For. Ecol. Manag.*, 140, 227–238.
- Jurgensen, M.F., Graham, R.T., Larsen, M.J. & Harvey, A.E. (1992). Clear-cutting, woody residue removal, and nonsymbiotic nitrogen fixation in forest soils of the Inland Pacific Northwest. *Can. J. For. Res.*, 22, 1172–1178.
- Jurgensen, M.F., Harvey, A.E., Graham, R.T., Page-Dumroese, D.S., Tonn, J.R., Larsen, M.J., *et al.* (1997). Review article: Impacts of timber harvesting on soil organic matter, nitrogen, productivity, and health of inland northwest forests. *For. Sci.*, 43, 234–251.
- Kira, T. & Shidei, T. (1967). Primary production and turnover of organic matter in different forest ecosystems of the western Pacific. *Japan Journ. Ecol.* 17, 70–87.
- Knops, J.M.H., Bradley, K.L. & Wedin, D.A. (2002). Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecol. Lett.*, 5, 454–466.
- Lambers, H., Iii, F.S.C. & Pons, T.L. (2008). Mineral Nutrition. In: *Plant Physiological Ecology*. Springer New York, pp. 255–320.
- Law, B.E., Sun, O.J., Campbell, J., Van Tuyl, S. & Thornton, P.E. (2003). Changes in carbon storage and fluxes in a chronosequence of ponderosa pine. *Glob. Change Biol.*, 9, 510–524.
- Law, B.E. & Waring, R.H. (2015). Carbon implications of current and future effects of drought, fire and management on Pacific Northwest forests. *For. Ecol. Manag.*, Carbon, water and nutrient cycling in managed forests, 355, 4–14.

Le Quéré, C., Moriarty, R., Andrew, R.M., Peters, G.P., Ciais, P., Friedlingstein, P., *et al.* (2015). Global carbon budget 2014.

Lesica, P., McCune, B., Cooper, S.V. & Hong, W.S. (1991). Differences in lichen and bryophyte communities between old-growth and managed second-growth forests in the Swan Valley, Montana. *Can. J. Bot.*, 69, 1745–1755.

Lindenmayer, D.B., Laurance, W.F. & Franklin, J.F. (2012). Global Decline in Large Old Trees. *Science*, 338, 1305–1306.

Lucash, M.S., Eissenstat, D.M., Joslin, J.D., McFarlane, K.J. & Yanai, R.D. (2007). Estimating nutrient uptake by mature tree roots under field conditions: challenges and opportunities. *Trees*, 21, 593–603.

Luyssaert, S., Schulze, E.-D., Börner, A., Knohl, A., Hessenmöller, D., Law, B.E., *et al.* (2008). Old-growth forests as global carbon sinks. *Nature*, 455, 213–215.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., *et al.* (2007). The human footprint in the carbon cycle of temperate and boreal forests. *Nature*, 447, 848–850.

McLauchlan, K.K., Higuera, P.E., Gavin, D.G., Perakis, S.S., Mack, M.C., Alexander, H., *et al.* (2014). Reconstructing Disturbances and Their Biogeochemical Consequences over Multiple Timescales. *BioScience*, bit017.

Menge, D.N.L., Pacala, S.W. & Hedin, L.O. (2009). Emergence and Maintenance of Nutrient Limitation over Multiple Timescales in Terrestrial Ecosystems. *Am. Nat.*, 173, 164–175.

National Atmospheric Deposition Program. (2016). Available at: <http://nadp.sws.uiuc.edu/nadpdata/annualReq.asp?site=MT05>. Last accessed 01 April 2016.

Nave, L.E., Vance, E.D., Swanston, C.W. & Curtis, P.S. (2010). Harvest impacts on soil carbon storage in temperate forests. *For. Ecol. Manag.*, 259, 857–866.

Niklas, K.J. & Enquist, B.J. (2001). Invariant scaling relationships for interspecific plant biomass production rates and body size. *Proc. Natl. Acad. Sci.*, 98, 2922–2927.

- Noormets, A., Epron, D., Domec, J.C., McNulty, S.G., Fox, T., Sun, G., *et al.* (2015). Effects of forest management on productivity and carbon sequestration: A review and hypothesis. *For. Ecol. Manag.*, Carbon, water and nutrient cycling in managed forests, 355, 124–140.
- Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E. & McMurtrie, R.E. (2010). CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proc. Natl. Acad. Sci.*, 107, 19368–19373.
- Perakis, S. & Hedin, L.O. (2002). Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. *Nature*, 415, 416.
- Perakis, S.S., Sinkhorn, E.R. & Compton, J.E. (2011). $\delta^{15}\text{N}$ constraints on long-term nitrogen balances in temperate forests. *Oecologia*, 167, 793–807.
- Perakis, S.S., Tepley, A.J. & Compton, J.E. (2015). Disturbance and Topography Shape Nitrogen Availability and $\delta^{15}\text{N}$ over Long-Term Forest Succession. *Ecosystems*, 1–16.
- Pickett, S.T.A. & White, P.S. (1985). Natural disturbance and patch dynamics. In: *Academic Press*.
- Pregitzer, K.S. & Euskirchen, E.S. (2004). Carbon cycling and storage in world forests: biome patterns related to forest age. *Glob. Change Biol.*, 10, 2052–2077.
- Prescott, C.E. (1997). Effects of clearcutting and alternative silvicultural systems on rates
- Rastetter, E.B., Yanai, R.D., Thomas, R.Q., Vadeboncoeur, M.A., Fahey, T.J., Fisk, M.C., *et al.* (2013). Recovery from disturbance requires resynchronization of ecosystem nutrient cycles. *Ecol. Appl.*, 23, 621–642.
- Ryan, M.G., Binkley, D. & Fownes, J.H. (1997). Age-Related Decline in Forest Productivity: Pattern and Process. In: *Advances in Ecological Research* (ed. Fitter, M.B. and A.H.). Academic Press, pp. 213–262.
- Seymour, R.S., White, A.S. & deMaynadier, P.G. (2002). Natural disturbance regimes in northeastern North America—evaluating silvicultural systems using natural scales and frequencies. *For. Ecol. Manag.*, Forest Ecology in the next Millennium: Putting the long view into Practice, 155, 357–367.
- Soil Survey Staff. (2014). *Web Soil Survey*. Available at: <http://websoilsurvey.nrcs.usda.gov/>. Last accessed 01 April 2016.

- Sollins, P., Grier, C.C., McCorison, F.M., Cromack, K., Fogel, R. & Fredriksen, R.L. (1980). The Internal Element Cycles of an Old-Growth Douglas-Fir Ecosystem in Western Oregon. *Ecol. Monogr.*, 50, 261–285.
- Stark, J.M., Hart, S.C. & others. (1997). High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature*, 385, 61–64.
- Stephenson, N.L., Das, A.J., Condit, R., Russo, S.E., Baker, P.J., Beckman, N.G., *et al.* (2014). Rate of tree carbon accumulation increases continuously with tree size. *NATURE*, 507, 90–92.
- Sun, O.J., Campbell, J., Law, B.E. & Wolf, V. (2004). Dynamics of carbon stocks in soils and detritus across chronosequences of different forest types in the Pacific Northwest, USA. *Glob. Change Biol.*, 10, 1470–1481.
- Sun, Z., Liu, L., Peng, S., Peñuelas, J., Zeng, H. & Piao, S. (2016). Age-Related Modulation of the Nitrogen Resorption Efficiency Response to Growth Requirements and Soil Nitrogen Availability in a Temperate Pine Plantation. *Ecosystems*.
- Tang, J., Luyssaert, S., Richardson, A.D., Kutsch, W. & Janssens, I.A. (2014). Steeper declines in forest photosynthesis than respiration explain age-driven decreases in forest growth. *Proc. Natl. Acad. Sci.*, 111, 8856–8860.
- Thomas, R.Q., Brookshire, E.N.J. & Gerber, S. (2015). Nitrogen limitation on land: how can it occur in Earth system models? *Glob. Change Biol.*, 21, 1777–1793.
- Twieg, B.D., Durall, D.M. & Simard, S.W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytol.*, 176, 437–447.
- Van Heerwaarden, L.M., Toet, S. & Aerts, R. (2003). Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. *Oikos*, 101, 664–669.
- Vergutz, L., Manzoni, S., Porporato, A., Novais, R.F. & Jackson, R.B. (2012). Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecol. Monogr.*, 82, 205–220.
- Vitousek, P.M. & Reiners, W.A. (1975). Ecosystem Succession and Nutrient Retention: A Hypothesis. *BioScience*, 25, 376–381.
- Vitousek, P.M. & Matson, P.A. (1984). Mechanisms of Nitrogen Retention in Forest Ecosystems: A Field Experiment. *Science*, 225, 51–52.

Vitousek, P.M. & Howarth, R.W. (1991). Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, 13, 87–115.

Vogt, K.A., Moore, E.E., Vogt, D.J., Redlin, M.J. & Edmonds, R.L. (1983). Conifer fine root and mycorrhizal root biomass within the forest floors of Douglas-fir stands of different ages and site productivities. *Can. J. For. Res.*, 13, 429–437.

Waring, R.H. & Franklin, J.F. (1979). Evergreen coniferous forests of the Pacific Northwest. *Science*, 204, 1380–1386.

Western Regional Climate Center. (2016). *Swan Lake, Montana*. Available at: <http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?mt8087>. Last accessed: 01 April 2016.

Yanai, R.D., Currie, W.S. & Goodale, C.L. (2003). Soil Carbon Dynamics after Forest Harvest: An Ecosystem Paradigm Reconsidered. *Ecosystems*, 6, 197–212.