

THESIS

SUBALPINE FOREST ECOSYSTEM RESPONSES TO LONG-TERM NITROGEN LOADING AT
LOCH VALE WATERSHED, COLORADO, USA

Submitted by

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ABSTRACT

SUBALPINE FOREST ECOSYSTEM RESPONSES TO LONG-TERM NITROGEN LOADING AT LOCH VALE WATERSHED, COLORADO, USA

This thesis presents the results of a long-term N fertilization experiment in the Loch Vale watershed of Rocky Mountain National Park and presents a conceptual model hypothesizing environmental controls on ecosystem responses to N fertilization. The experiment consisted of annual 25 kg N ha⁻¹ application of NH₄NO₃ fertilizer to three 30 x 30 m subalpine forest plots from 1996 to 2017. Soil, plant, and lysimeter samples were collected over the course of the experiment to address three research goals. First, determine whether increased N input leads simply to increased N output, or whether observable ecosystem effects result. Second, describe changes in the ecosystem responses over time and determine the controls that govern those changes. Third, identify results of the experiment that may generalize to other coniferous forests around the world. The results show that N fertilization causes ecosystem effects in Loch Vale which vary over time and space due to the influence of hypothesized controlling factors such as soil moisture, pH, and plant uptake of N. The results of the Loch Vale experiment suggest that impacts of excess N on boreal forests may include increased plant growth where soil moisture is adequate, but when coupled with drought may lead to increased rates of nitrification, N mineralization, NO₃⁻ leaching, and soil acidification.

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CHAPTER 1 – INTRODUCTION TO A LONG-TERM NITROGEN FERTILIZATION EXPERIMENT AT LOCH

Excess reactive nitrogen (N) generated by agriculture, industry, and urbanization results in chronic N additions to surrounding ecosystems, via transport through the atmosphere and subsequent deposition in precipitation (Galloway et al., 1995). Although N is an essential nutrient for all life, excess N negatively impacts ecosystems, leading to algal blooms and fish die off, declines in biodiversity, and impaired water quality (Vitousek et al., 1997). Historically the highest levels of pollution from N deposition have been recorded near the world's large population centers (Galloway et al., 2004), but ammonia emissions from industrial agriculture contribute increasingly to N deposition (Li et al., 2016). Elevated N deposition has long been recorded at high elevations in the Rocky Mountains (Baron & Denning, 1993) and linked to urban and agricultural sources along the Front Range (Benedict et al., 2013; Thompson et al., 2015).

This thesis addresses the effects of excess N from deposition on subalpine forested soils in the Loch Vale watershed, an instrumental catchment in Rocky Mountain National Park (RMNP). Long-term research began at Loch Vale in 1982, studying ecosystem processes and changing biogeochemical fluxes in response to variability in climate and atmospheric deposition (Baron, 1992). A National Atmospheric Deposition Program (NADP) site was installed above the confluence of Andrew's Creek and Icy Brook in 1983, and NADP data indicate that LVWS receives an annual average 3.3 kg ha^{-1} N deposition from the atmosphere (Morris et al., 2019). Much of this N deposition originates in the South

Platte basin from human activities including animal agriculture and fossil-fuel burning (Burns, 2003; Benedict et al., 2018). Deposition of N in the western mountains of the USA generally ramped up or remained elevated through the 1980s and 1990s (Clow et al., 2003). Since 1994 average N deposition at Loch Vale has remained steady (Morris et al., 2019) at over twice the concentration of the estimated critical load ($1.5 \text{ kg ha}^{-1} \text{ yr}^{-1}$; Baron, 2006).

Having surpassed the critical load, N deposition has had measurable effects on organisms and ecosystems at Loch Vale. Diatom assemblages in high elevation lakes have shifted (Baron et al., 2000; Wolfe et al., 2001) and eutrophic conditions have led to declines in diatoms and increases in green algae (Oleksy et al., in revision). Rates of nitrification and mineralization in soils were elevated over low-deposition subalpine forests West of the continental divide, as were concentrations of nitrate (NO_3^-) and ammonium (NH_4^+ ; Rueth & Baron, 2002). Changes at Loch Vale have been largely consistent with trends in N deposition responses across the western US (Fenn et al., 2003).

To study the impacts of added N on forests and forest soil ecosystems, a N-fertilization experiment was conducted in the subalpine forest at Loch Vale from 1996 to 2017 (Rueth et al. 2003). The experiment was designed to test whether additional N could push Loch Vale subalpine forest soils into N saturation, while applying N in amounts consistent with those observed in atmospheric deposition at other locations within the US (Boot et al., 2016). Over the course of the experiment, 54 kg of pelletized NH_4NO_3 fertilizer was added to each of the experimental plots. Fertilization caused elevated soil NO_3^- and NH_4^+ concentrations (Rueth et al., 2003), declines in soil pH, soil C, and soil microbial

biomass (Boot et al., 2016) as well as reductions in the number of soil fauna (Shaw et al., 2019).

The Loch Vale experiment is one of several long-term N fertilization studies on forested ecosystems begun in the late 20th century to determine the effects of increasing N deposition (Table 1.1). The Loch Vale experiment is rare for being set in a subalpine environment and for applying a somewhat lower rate of NH_4NO_3 fertilizer. Research at the Cary Institute, Harvard Forest, and Nitrex G2 site were each conducted at lower elevation, although the boreal mixed-conifer forest at Nitrex was dominated by spruce trees, one of the co-dominant tree types at Loch Vale. The rate of N application at Loch Vale was half that of the low-N treatment at Harvard forest (Frey et al., 2014) and one-fourth of the application level in the first years of the Cary Institute study (Lovett & Goodale, 2011). Ambient atmospheric deposition at Loch Vale is approximately one-third of those sites, and a lower dose of experimental N has proved sufficient to test ecosystem response (Reuth et al., 2003, Boot et al., 2016). The rate of application at Nitrex G2 was closest to the Loch Vale rate, reaching 28.9 kg ha^{-1} in drier years, as the annual application rate was proportional to each year's precipitation amount (Moldan et al., 2018). The hypotheses and results of these long-term experiments will be discussed in the next chapter to provide context to the work at Loch Vale.

Table 1.1. Comparison of four long-term forest N fertilization experimental setups.

	Cary Institute	Harvard Forest	Loch Vale	Nitrex G2
Location	Hudson Valley, New York	Petersham, Massachusetts	Rocky Mountain National Park, Colorado	Gårdsjön, Sweden
Forest Type	Mixed-oak	Mixed-oak; pine	Spruce-fir	Spruce and other conifers
Time of Fertilizer Application	1996-2006	1988-present	1996-2017	1991-present
N Fertilizer Type	NH ₄ NO ₃	NH ₄ NO ₃	NH ₄ NO ₃	NH ₄ NO ₃
Amount of N Applied Annually	100 kg ha ⁻¹ until 1999, then 50 kg ha ⁻¹ until 2006	50 kg ha ⁻¹ ; 150 kg ha ⁻¹	25 kg ha ⁻¹	28.9-51.6 kg ha ⁻¹ , proportional to annual precipitation
Approximate Annual Ambient N Deposition	9 kg ha ⁻¹	8 - 10 kg ha ⁻¹	3.3 kg ha ⁻¹	9 kg ha ⁻¹
Reference	Lovett & Goodale, 2011	Frey et al., 2014	Reuth et al., 2003	Moldan et al., 2018

Site Description: Loch Vale Watershed

Loch Vale watershed is located on the eastern slope of the continental divide in Rocky Mountain National Park between 3,110 m and 4,009 m elevation. Old-growth subalpine forest covers 6% of the 660 ha catchment, compared with 80% exposed rock and talus, 11% alpine tundra, 2% glaciers and lakes, and 1% wetlands (Baron, 1992). Forests occur below 3,330 m along and on the slopes above Icy Brook and Andrew's Creek, the major stream channels in Loch Vale, and on the banks of The Loch, the lake which drains

the watershed. Most of the forested slopes have a northeast aspect, and a slope angle of approximately 20 degrees (Reuth & Baron, 2002). The forest was never logged, having been designated a National Park in 1915, and previously provided summer hunting and foraging grounds for the Ute and Arapaho peoples (Law, 2015).

The climate at Loch Vale is classified as subarctic (or boreal) under the Köppen system, due to long, cold winters and short, mild summers. The mean annual air temperature is 1.4 degrees C (Heath & Baron, 2014), and approximately 80% of the annual 105 cm precipitation occurs as snow (Baron & Denning, 1993). This precipitation regime produces a distinct mountain hydrography, characterized by high stream flows during the snowmelt period (usually May-June) when half of stream water may consist of meltwater, followed by a monsoonal rain season and then baseflow (Mast et al., 1995).

The climate supports a mixed-conifer forest biome, co-dominated by fir (*Abies lasiocarpa*) and spruce (*Picea engelmannii*). Although other conifer trees and aspen occur to a small extent within LVWS, none appear within the stands we studied. The ground cover consists largely of two species of *Vaccinium*, with other shrubs occasionally present including two species of *Ribes*. Some of the upland areas are grass-covered, while low sites near drainages provide enough moisture to support marsh marigolds (*Caltha leptosepala*) and sedges (*Carex spp*).

The soils in Loch Vale watershed developed from till and colluvium produced by glaciation of Precambrian granite, biotite gneiss, and schist (Lovering & Goddard, 1950). Weathering, steep slopes, high unvegetated ground cover, and high runoff rates lead to relatively high ratios of calcium to other cations and high rates of NO_3^- runoff (Clow & Sueker, 2000). High runoff corresponds to lower pH, and the soils at Loch Vale average pH

4.75 across all tundra, meadow, and forest covers, leading to cation depletion (Oropeza, 2008). The forest soils are classified as cryic spodosols, with rocky, skeletal sandy-loam textures, an organic horizon and shallow E horizon, and clay content of up to 18% (Soil Survey Staff, 2019). Present soil characteristics show the leaching of clay and organic material from the organic horizon to illuvial deposits high in sesquioxide metals in the mineral soil (Table 1.2).

Table 1.2. Soil profile of loamy-skeletal, isotic Typic Haplocryod in the subalpine forest at Loch Vale developed by the US Department of Agriculture, Natural Resources Conservation Service.

Approximate Depth (cm)	Horizon	Description
0 - 7.5	Oe	Accumulation of coarse organic matter due to Udic moisture regime (and productive forest) and cryic temperature regime (resulting in slow decomposition).
7.5 – 8	E	Zone of eluviation delineating leaching horizon (Oe) from depositional horizons (Bs ₁ , Bs ₂).
8 – 12	Bs ₁	Accumulation of illuvial organic matter, clays and sesquioxides (iron, aluminum) from organic horizon.
12 – 20	Bs ₂	
20 - 40+	BC	Lightly weathered parent material at shallow depth.

A major feature of spodosols like the Loch Vale soil is the organic horizon, the layer of partially decomposed organic particulate mixed with soil minerals that occurs below the forest duff and has distinctive chemistry from the mineral soil below (Table 1.3). The organic horizon has a much higher cation exchange capacity, base cation content, and percent C than the mineral horizon per unit of soil, and the soil samples collected in this work came from that horizon. The mineral soil shows greater base cation depletion and a

slightly lower pH. Acidity in the Loch Vale soils results primarily from high runoff leading to cation loss, but one-third of acidity results from organic acids derived from litter (Oropeza, 2008).

Table 1.3. Soil chemistry characteristic mean values and (standard error) in organic and mineral horizons reported in Oropeza (2008).

Horizon	Cation Exchange Capacity (cmol kg ⁻¹)	Base Cations (meq 100 g ⁻¹)	pH	C %
Organic	30.0 (1.3)	27.3 (1.5)	4.9 (0.1)	29.0 (1.5)
Mineral	19.3 (0.7)	8.3 (0.8)	4.7 (0.1)	6.9 (0.6)

Experimental Design and Goals

To test the impact of excess N and N saturation, 25 kg ha⁻¹ N was applied as dry NH₄NO₃ from 1996 to 2017 to three 30 X 30 m plots within the subalpine forest. Each treatment plot was paired with a control plot which received only ambient N addition of approximately 3.3 kg N ha⁻¹ yr⁻¹. Nitrogen was applied monthly from June through October each year in 2.5 kg/ha increments, with an overwinter application of 7.5 kg ha⁻¹ after snowfall (Rueth et al., 2003). The annual rate of 25 kg ha⁻¹ N was chosen to correlate with observed deposition rates in some parts of the United States, and to push the ecosystem deeper into N saturation (Boot et al., 2016). Five tension lysimeters (SoilMoisture Inc.) were installed in the rooting zone (10 cm) in each of the six plots to sample soil-water solution, each placed within the inner 15 X 15 m of the plots to minimize edge-effects (Rueth et al., 2003).

Three research goals of the N fertilization experiment drove the design of this research. First, determine whether increased N input leads simply to increased N output, or

whether observable ecosystem effects result. Second, describe changes in the ecosystem responses over time and determine the controls that govern those changes. Third, identify results of the experiment that may generalize to other coniferous forests around the world. These goals are reflected in the hypotheses and objectives presented in the following original research chapters.

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CHAPTER 2 – ECOSYSTEM RESPONSE TO LONG-TERM FERTILIZATION

Long-term nitrogen (N) fertilization experiments in forests have identified ecosystem components and processes that are sensitive to increased N availability and quantified the extent of N-driven impacts to N leaching, forest biomass, and soils. Increases in nitrate (NO_3^-) leaching, which pass N on to downstream ecosystems, were first hypothesized to occur after N limitation in forest plants had been relieved (Aber et al., 1989). Subsequent studies have found that N additions to forests ultimately lead to N leaching (Magill et al., 2000; Lovett & Goodale 2011), though not always immediately (Högberg et al., 2006). Forest biomass and foliar N increase as plant N-limitation is relieved, but ultimately tree mortality is hypothesized to result as new-growth tissues become depleted in micronutrients and low foliar C:N ratios reduce frost hardiness (Aber et al., 1998). Increased tree mortality has been observed in long-term studies, though the exact causes can be difficult to determine (Wallace et al., 2007; Frey et al., 2014).

In soils, microbial processes that depend on available N, like N mineralization and nitrification, often increase under N fertilization, even though soil microbes are often assumed not to be N limited (Bowman & Steltzer, 1998; Magill et al., 2000; Chen & Högberg, 2006). While microbial processes may respond positively to increased N, microbial communities of bacteria and fungi often shrink under fertilization possibly resulting from changes to mycorrhizal associations, substrate quality or increased soil acidity (Wallenstein et al., 2006; Boot et al., 2016). These microbial processes can provide important controls on N export to downstream aquatic systems (Lovett & Goodale 2011) where excess N may result in eutrophication. Declines in soil pH also result from increased

NO_3^- leaching causing depletion of soil base cations and micronutrients like calcium (Ca^{2+}) as well as acidifying cations like aluminum (Al^{3+} ; Nilsson et al., 1988). Soil carbon (C), a critical ecosystem component and the largest terrestrial C sink, often increases in forests under elevated N, though not in boreal conifer forests (Janssens et al., 2010).

A long-term N fertilization study was carried out in the Loch Vale watershed of Rocky Mountain National Park, USA, to determine the effects of elevated N in the subalpine forest, a less-studied biome. Loch Vale is sensitive to N addition, as evidenced by changes to soil, plant, and lake chemistry and biota due to present levels of atmospheric N deposition (Baron et al., 2000; Wolfe et al., 2001) so this work examined how rates of N application higher than presently observed but within the bounds of ambient N deposition in the USA would impact the forest ecosystem. This work investigates NO_3^- and cation leaching, forest biomass and stoichiometry, soil microbial processes, pH, and C over the course of a 20-year N fertilization experiment in Loch Vale to determine the ecosystem response to N addition and improve understanding of how elevated N may impact conifer forests more broadly.

Goals, questions, and hypotheses

The goal of this research was to explore the ecosystem consequences of long-term fertilization by evaluating soil solution, microbial process, and foliar chemistry data collected from 1996-2015, soil and fine-root data collected in 2018, and forest biomass data collected in 2019. The soil solution measurements consisted of NO_3^- and NH_4^+ concentrations, concentrations of the major cations K^+ , Mg^{2+} , Ca^{2+} , Na^+ , and Al^{3+} , and dissolved organic carbon (DOC). The microbial processes addressed were nitrification, or the microbial conversion of NH_4^+ to NO_3^- , and N mineralization, the microbial production of NH_4^+ and NO_3^- through the decomposition of organic matter. Foliar chemistry data

consisted of C:N ratios of live-collected spruce needles. Soils data include gravimetric moisture, extractable organic C (EOC) and extractable organic N (EIN), percent C, and roots were sampled for dry mass and C:N ratio. Forest data included aboveground biomass calculated from tree diameter.

These data were collected to address questions regarding how N-loading impacts the subalpine forest in Loch Vale. My research asks the following three main questions.

- 1) Does increased N input lead simply lead to increased N output?
- 2) Do ecosystem responses change over time, and if so, why?
- 3) Can results from this experiment be extrapolated to other coniferous forests?

Original hypotheses were developed based on these questions early in the experiment. The following two hypotheses were tested in this research.

Hypothesis one (H1): soil solution N, indicating leaching, will increase significantly after fertilization.

Hypothesis two (H2): increased N availability will lead to increased forest and understory production, and greater soil N mineralization.

In pursuing these questions and testing these hypotheses I have developed a new conceptual model of N fertilization impacts at Loch Vale. The model integrates observed responses to long-term N addition on the ecosystem processes and stocks of C and N measured at Loch Vale and hypothesizes environments control mechanisms that mediate how N effects manifest in the ecosystem. Elaboration on this model forms the framework for discussion of N impacts at Loch Vale in this thesis.

Methods

Nitrogen additions

Nitrogen fertilizer was applied to three 30 X 30 m subalpine forest plots, each paired with an adjacent control plot. Applications of 25 kg ha⁻¹ N as dry NH₄NO₃ were made from 1996 to 2017 in monthly increments of 2.5 kg ha⁻¹ N from June to October, with an overwinter application of 7.5 kg ha⁻¹ N after snowfall (Rueth et al. 2003). The annual rate of 25 kg N ha⁻¹ was chosen to correlate with observed deposition rates in some parts of the United States, and to push the ecosystem into N saturation (Boot et al., 2016). Five tension lysimeters (SoilMoisture Inc.) were installed in the rooting zone (10 cm) in each of the six plots to sample soil-water solution, each placed within the inner 15 X 15 m of the plots to minimize edge-effects (Rueth et al., 2003).

Lysimeter sampling

To assess the soil solution lysimeter sampling occurred annually from 1996 through 1999, and then again in 2005, 2010, and 2015. Sampling began in May, with weekly sampling until lysimeters dried out. Lysimeters dried out at different times over the summer depending on placement in the plots, ranging from June to August. Rueth et al. (2003) described the lysimeter sampling methods, recounted here. Hand pumps removed residual water and placed tension on each lysimeter hose. After 24 hours soil solutions were pumped from the lysimeters and filtered immediately in the field through a 0.2-µm membrane filter (Supor-Gelman; Pall, Ann Arbor, Michigan USA). Sample volumes ranged from over 150 mL in May less than 10 mL August.

Chemical analyses on refrigerated samples quantified sample concentrations of major cations, NO₃⁻, total dissolved N (TDN), and dissolved organic C (DOC). The cations

Na⁺, K⁺, Mg⁺², Ca⁺², and Al⁺³ were analyzed in each sampling year from 1996 - 2010 using ion chromatography performed on a Thermo Fisher Integriion (Thermo Fisher Scientific, Waltham, Massachusetts, USA). NO₃⁻ and NH₄⁺ concentrations were measured in each sampling year 1996 - 2015 on an Alpkem auto-analyzer (Alpkem, Perstorp Analytical Company, Wilsonville, Oregon, USA, 3500 Series). Total dissolved N was measured in 2005, 2010, and 2015 only, using a Shimadzu TOC-L model with Total Nitrogen Module (Shimadzu Scientific Instruments, Columbia, Maryland, USA). DOC concentrations were measured in 1997 - 1999, 2005, 2010, and 2015 by removing inorganic C with phosphoric acid, then converting organic C to CO₂ with a sodium persulfate digest, then measuring the oxidized C using an infrared detector (OI Analytical, College Station, Texas USA, Model 700 TOC Analyzer).

Forest tree measurements

A forest inventory was conducted over the summer of 2019 on each of the six plots according to Forest Service methods (USFS, 2019). The inventory included species, live/dead status, and diameter at 1.4 m above ground (DBH) for each tree (n = 368). Periodic blowdowns have resulted in dead and down trees in all plots, and these were counted and DBH estimated or measured if possible. The majority of spruce encountered showed signs of infection by bark beetles (*Dendroctonus rufipennis*), though there were few dead trees. Fir made up most of the trees in all plots.

In order to compare the forest stands in each plot by a metric relevant to belowground C, allometric biomass equations were used to calculate aboveground tree biomass using measured DBH for *Abies lasiocarpa*

$$\ln(\text{aboveground biomass [kg]}) = -2.3123 + 2.3482 \ln(\text{DBH [cm]})$$

(2.1)

and *Picea engelmannii*

$$\ln(\text{aboveground biomass [kg]}) = -3.0300 + 2.5567 \ln(\text{DBH [cm]})$$

(2.2)

using generalized biomass equations for North America (Chojnacky et al., 2014).

Aboveground biomass was calculated for each tree greater than 1.4 m in the inventory, and trees were separated for analysis into young and mature trees using 10 cm DBH as the cut-off.

Live foliage was sampled in in summer 1996, 1997, 2005, 2010, and 2015 from five canopy spruce in each plot (n = 30). Needles were collected from south-facing aspects at approximately 6 m and rinsed with deionized water in the field. After transport to the laboratory and drying at 25 degrees C they were ground to homogenous powder. A LECO Tru-Spec CN analyzer was used to measure percent C and N in each sample (Leco Corp., St. Joseph, MI, USA).

Soil sampling

Net rates of nitrification of NH_4^+ and mineralization of organic N were determined by the in-situ buried-bag technique (Binkley & Hart, 1989). In each of the years 1997 - 1999, 2005, 2010, and 2015, six to 15 cores were collected over the summer from the organic horizon of each plot, sufficient to fill quart-sized gas-permeable polyethylene bags. These bags were buried below the organic horizon for one month. The same number of

adjacent organic horizon samples were sampled to establish initial NO_3^- and NH_4^+ concentrations. Soils were homogenized and sieved in the field (2 mm) and 10 g extractions made in 100 mL of 2 mol L⁻¹ KCl. The samples were returned to the laboratory, shaken for ~45 minutes and stored at 10° C overnight before filtering with 41 Whatman filters (Whatman, Clifton, New Jersey, USA) and entering freezer storage. An Alpchem auto-analyzer was used to determine the concentrations of NO_3^- and NH_4^+ (Alpchem, Perstorp Analytical Company, Wilsonville, Oregon, USA, 3500 Series). Net nitrification rate was calculated by taking the difference in NO_3^- between incubated and initial extracts, while mineralization was calculated as the difference in NO_3^- and NH_4^+ summed.

Soil organic horizons and fine roots were sampled during the summer of 2018. Soils were collected for biogeochemical analyses in August, to coincide with season-high levels of soil respiration observed in a previous study (Allen, 2017). Twelve soil cores per plot were collected from the organic horizon (7.5 cm deep) after brushing off the litter layer (usually the top 1 cm or less). Samples were sieved through a 2 mm soil sieve, then partitioned for soil extractions and extracted within 6 hours of sampling. Samples were shaken for one hour, stored overnight at 10° C and filtered through Whatman 42 filter paper (Whatman, Clifton, New Jersey, USA). For extractable organic C (EOC) and total extractable N (TEN) measurement samples were extracted in a 1:10 ratio of soil weight to purified water volume (n = 71), filtered, and analyzed using a TOC-L analyzer with Total N Module (Shimadzu TOC-V_{CPN}; Shimadzu Scientific Instruments, Wood Dale, IL, USA). To analyze acidity half of the soils were subsampled in a 5:1 ratio soil to DI water extraction (n = 36) and analyzed with a Broadly-James electrode (Broadley-James Corp., Irvine, California, USA). Half the samples were also extracted in a 1:10 ratio of soil to 2 mol L⁻¹ KCl

solution ($n = 36$) and filtered to analyze extractable NO_3^- and NH_4^+ content. NH_4^+ was measured using segmented flow analysis and NO_3^- using flow injection analysis on an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station TX, USA). Measurements of extractable NO_3^- and NH_4^+ were used to calculate extractable inorganic N (EIN).

Soils were also sampled once each in July, August, and September of 2018 for measurement of bulk density, soil moisture, and sampling of fine roots, three cores per plot each time. Bulk density and moisture were calculated by weighing the cores on the collection day and then drying at 105°C to a constant weight and re-weighing them. Gravimetric soil moisture was calculated by dividing the mass of water from the dry mass of soil, and bulk density by dividing the dry mass of soil by the soil core volume. Continuous measurements of volumetric water content were also made over water years 2015 and 2016 using iButton moisture sensors (iBottonLink Technology) at ~ 10 cm depth. The sensors measured moisture every 10 minutes and logged every hour. Root cores were collected using a 0.5 m soil probe (19 mm diameter), sampling as deeply as possible. The float method, which separates buoyant organic materials from denser mineral materials in water and collects them using a series of fine sieves (Amato & Pardo, 1994), isolated roots from the soil. Roots were dried at 60°C to a constant weight. To determine the C and N content of soils and roots dried samples were ground with a mortar and pestle to a homogenous powder and analyzed on a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA).

Data analysis

A mixed-effects modeling approach was used to determine effects of N fertilization on measured quantities. Repeated measures of lysimeter solutes, foliar C:N ratio, rates of nitrification and N mineralization, soil pH and soil C were made over several years, so the mixed models of those measures tested the null hypothesis that control and fertilized plot means were equal in each year measured. The models treated each measured quantity as a response variable dependent on fixed effects of treatment, year, and treatment-year interaction, and random effects of plot and month of measurement. Mixed-effects models were fit using the lme4 package for R (Bates et al., 2014; R Core Team, 2019) and interrogated with type III, two-way ANOVA (implemented in the car package). Type III ANOVA of model results identifies significant differences in response variables by treatment, year, and treatment-by-year interaction (Fox et al., 2019). The emmeans package (Lenth, 2019) was used to estimate the least-squares means of each model factor and perform the Tukey's honestly significant differences test (HSD).

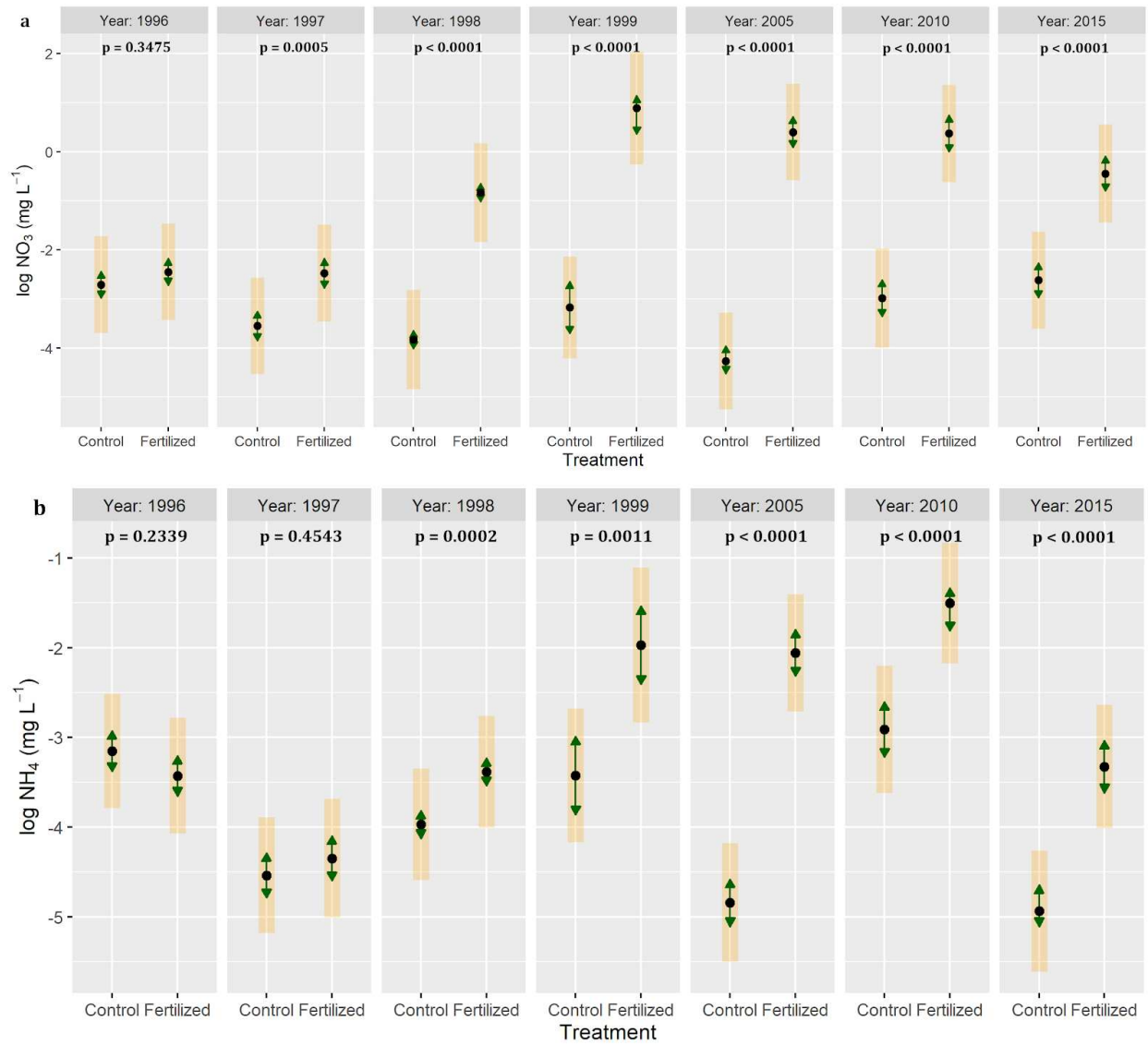
Tree biomass, fine root C:N ratio, and soil moisture were each measured in a single year, so the models tested the null hypothesis that control and fertilized means were the same in each plot. These models treated measured quantities as response variables dependent on treatment, plot, and treatment-plot interaction. These fixed-effects models were fit using base R and analyzed with type III ANOVA and least-squares means as above. All data required natural log transformation to meet assumptions of normality and homogeneity of variance, except for pH, and assumptions were examined using Levene's test implemented with the car package.

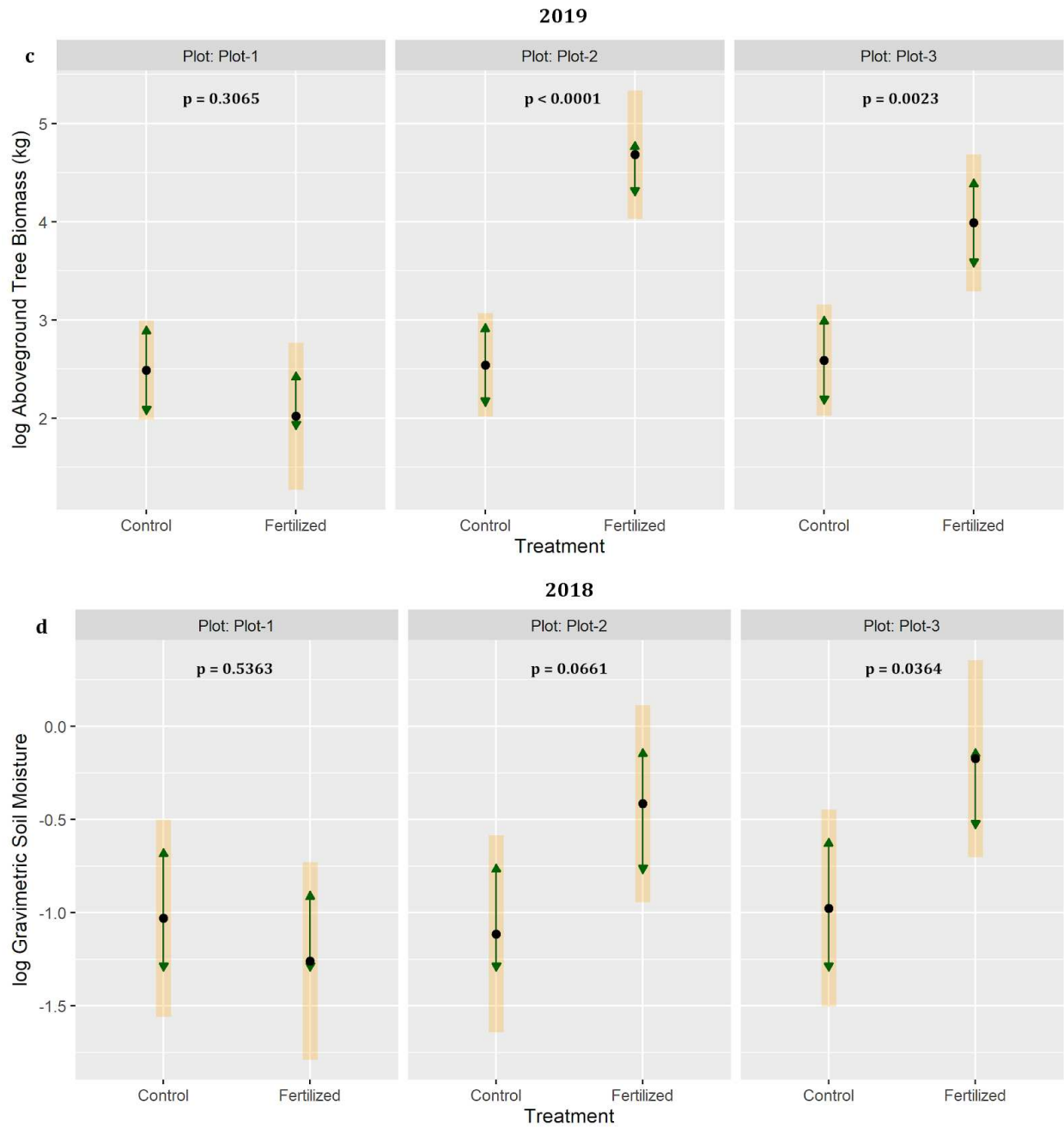
Treatment effects on response variables were compared using log response ratios, implemented with SingleCaseES (Pustejovsky & Swan, 2019). The log response ratio takes the mean of the natural log of the treatment observations divided by the mean of the control observations to produce a normalized and scaled measure of effect size (Hedges et al., 1999). This means that a log response ratio of zero represents equal mean measurements of a response variable between fertilized and control plots, while a ratio of one indicates a 2.71 (or e) times greater mean measure in the fertilized plots versus the controls. A response ratio of two indicates a 5.44 (or $2e$) times greater mean measure in the fertilized plots. A negative response ratio indicates a higher mean measure in the controls.

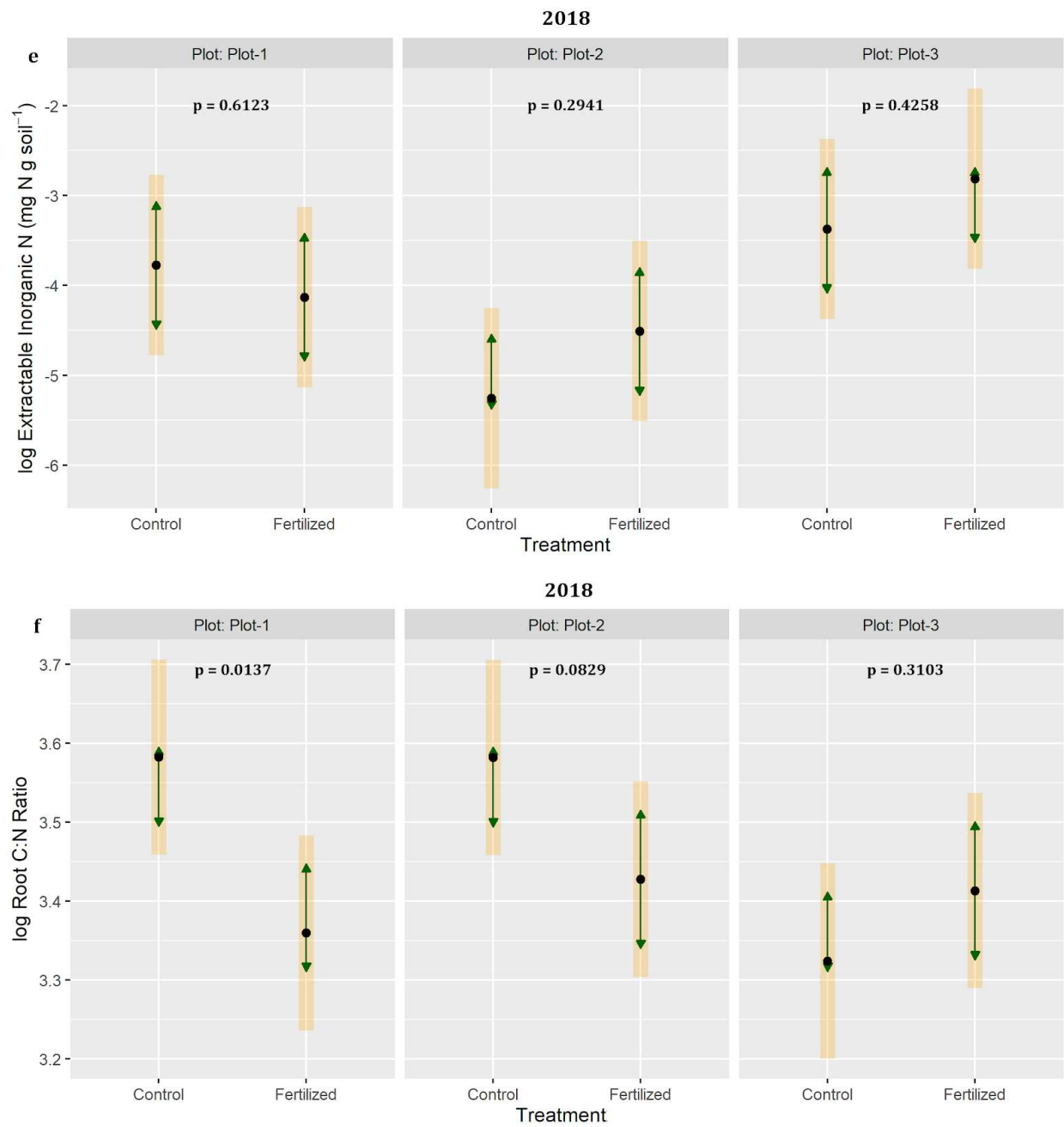
Results

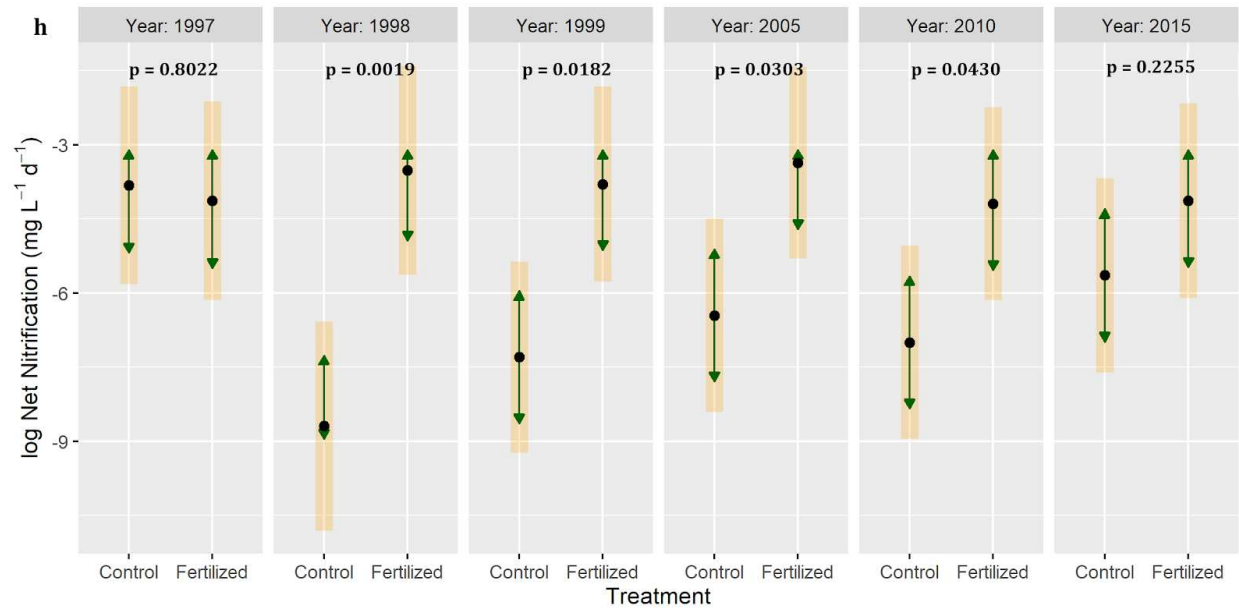
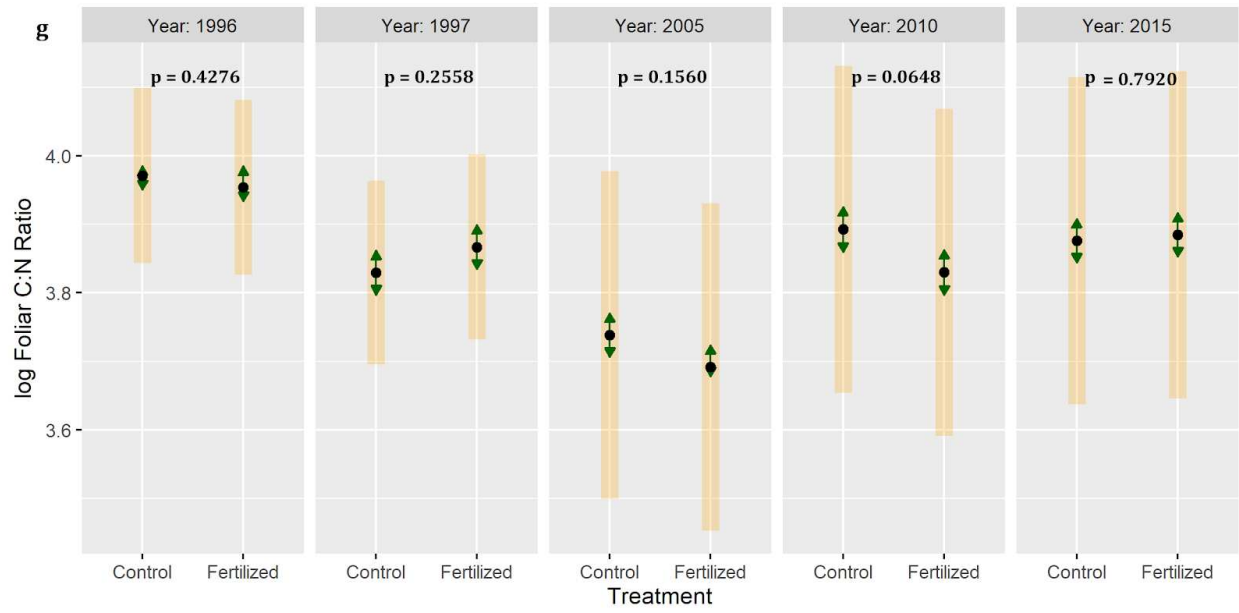
Soil solution N

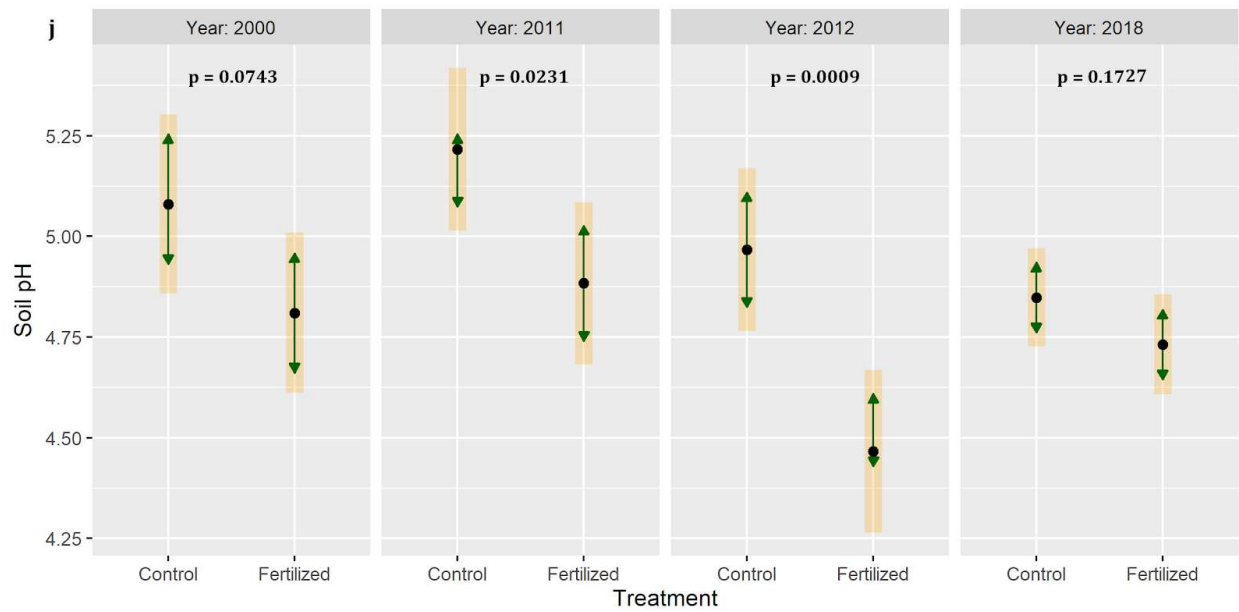
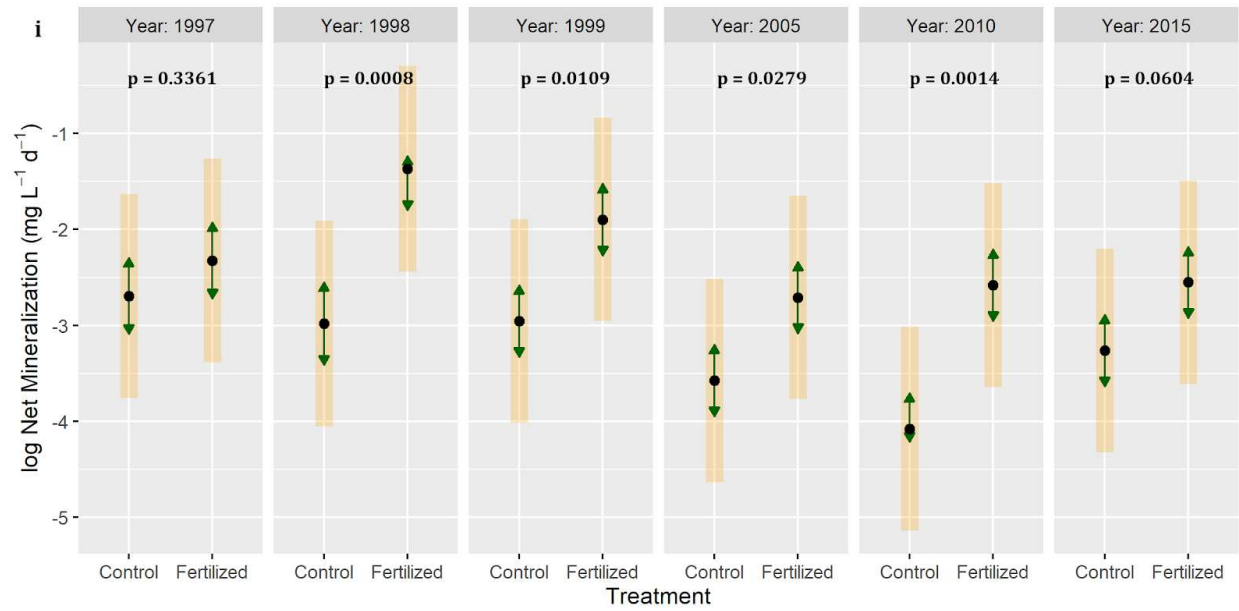
Lysimeter NO_3^- and NH_4^+ were significantly elevated in the fertilized plots versus the controls in most years (Figure 2.1a and b). Both analytes reached a maximum difference between fertilized and control plots in 2005, when NO_3^- was three orders of magnitude higher in the fertilized plots, and NH_4^+ one order of magnitude higher (Table 2.1). The response ratios peaked in 1999 (NO_3^-) or 2005 (NH_4^+), before narrowing by 2015 (Figure 2.2a). The effect of fertilization was significant for NO_3^- in all years after 1996 (Figure 2.1a) and for NH_4^+ in all years after 1997 (Figure 2.1b).

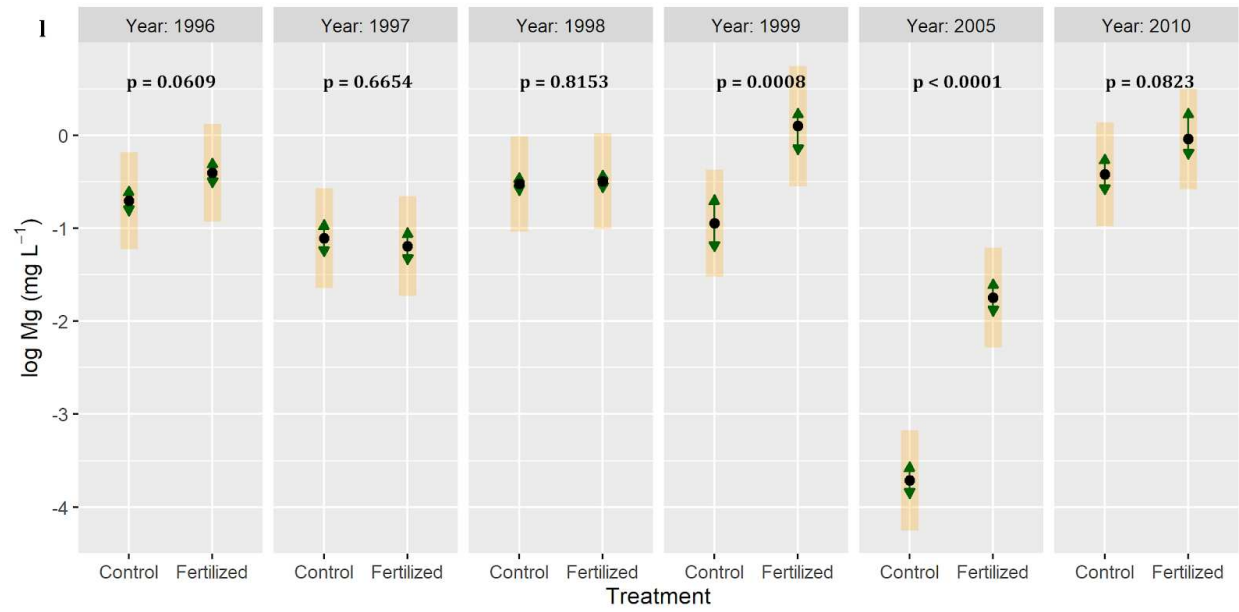
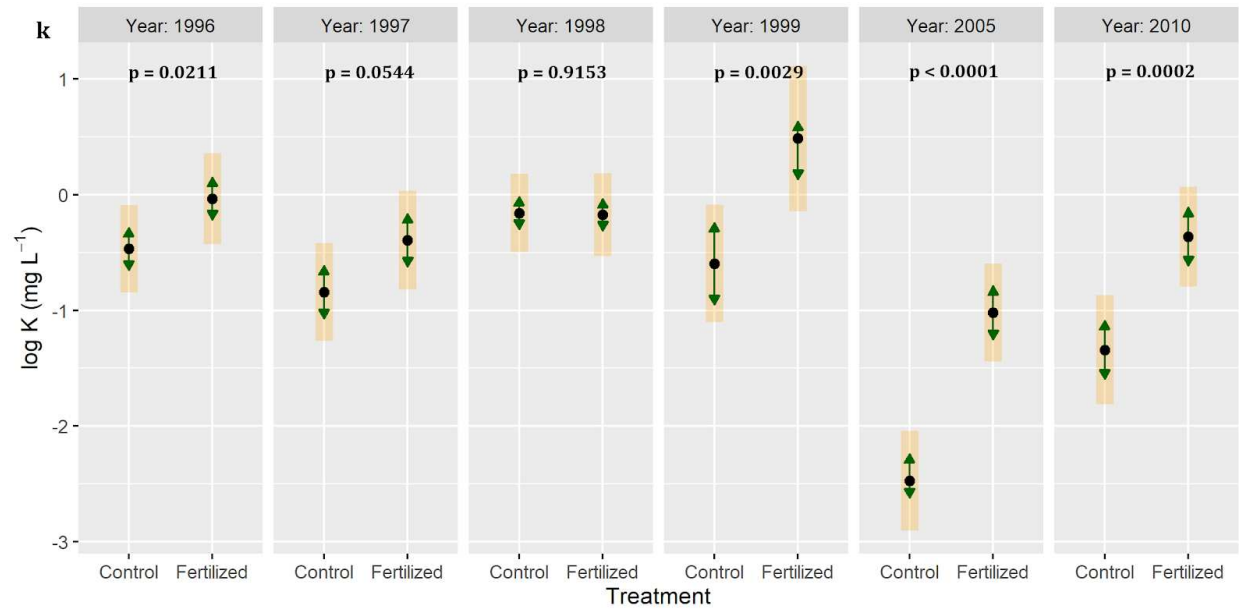


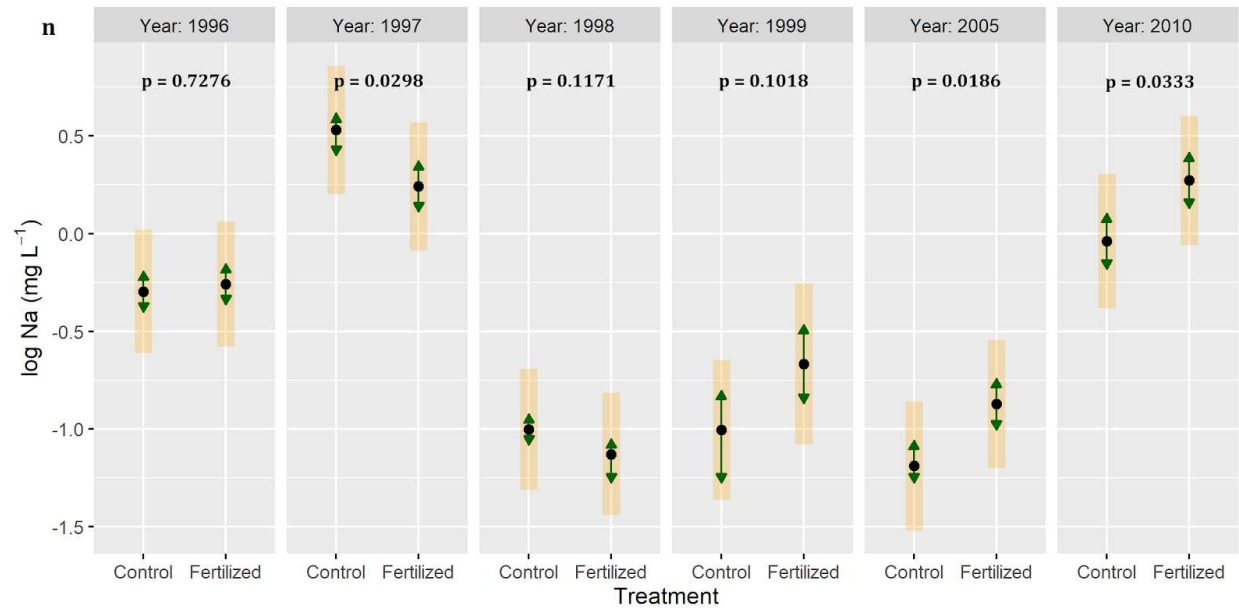
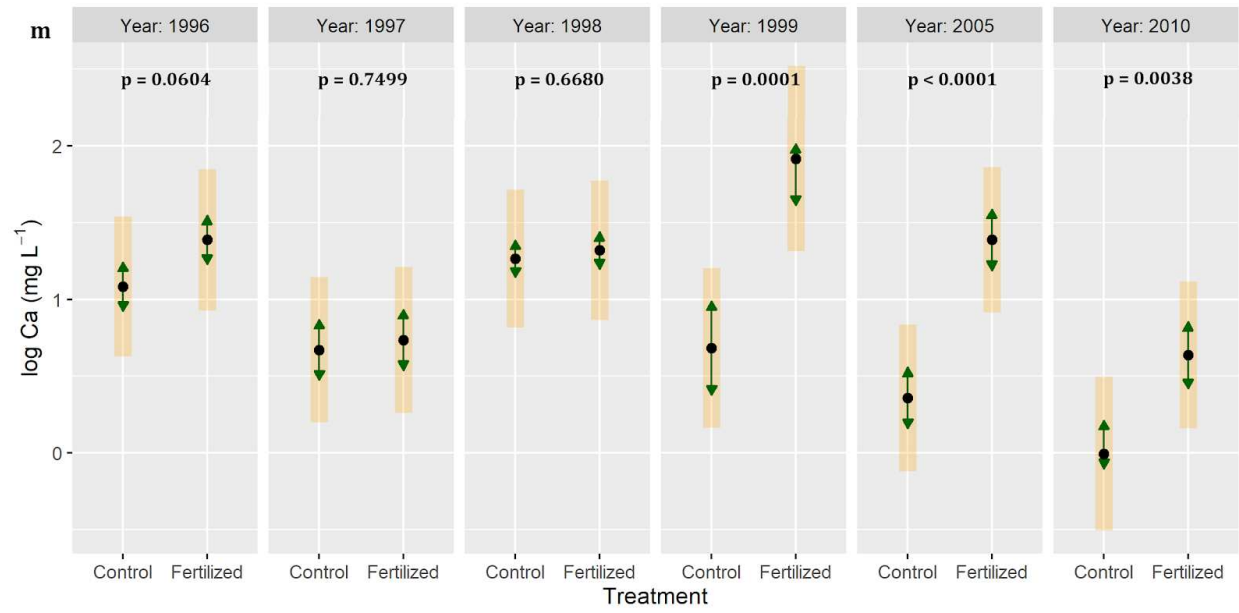


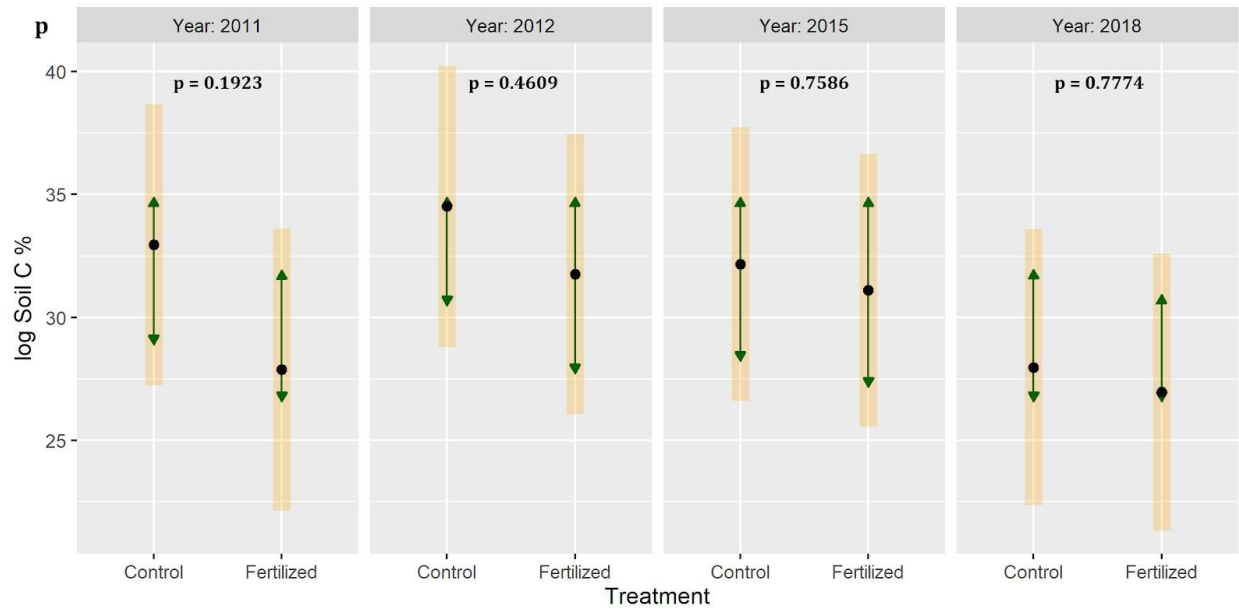
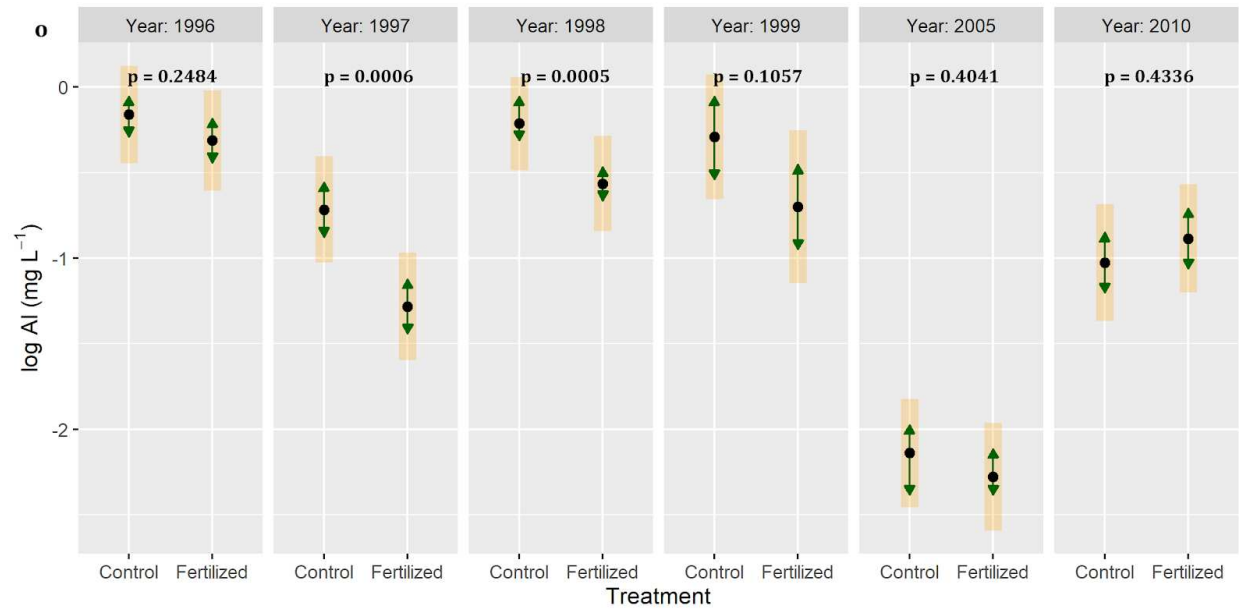












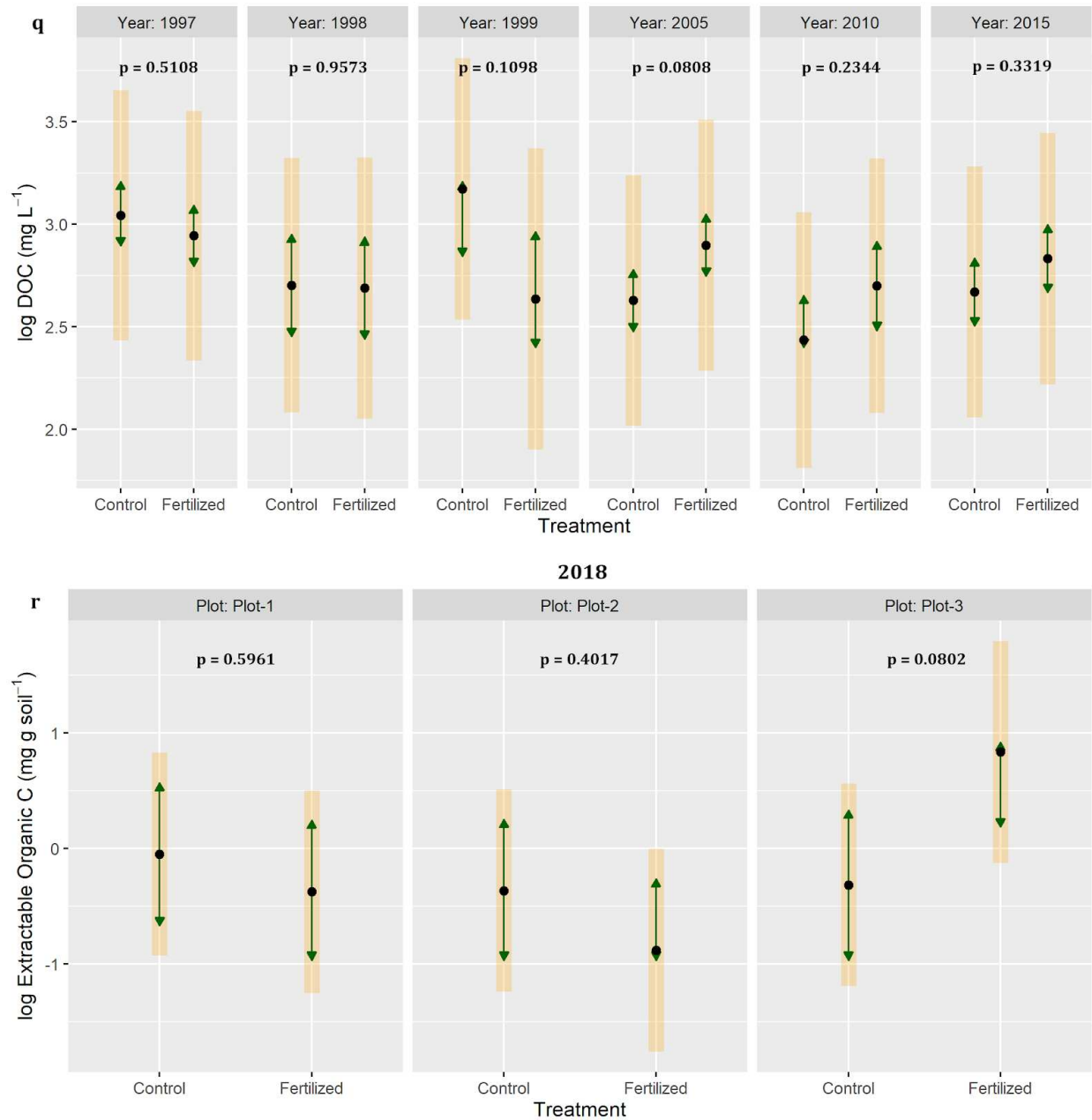


Figure 2.1 a - r. Mixed-model estimates of least-squares means for measured quantities in the Loch Vale experimental plots. The black dots represent the means, the orange rectangles represent 95% confidence intervals, and the green arrows represent Tukey's HSD. Non-overlapping Tukey arrows indicate statistically significant differences between means. P values test the null hypothesis that the control and fertilized measures are equal in that year or plot.

Table 2.1. Untransformed data showing median (M), range (R), and sample size (n) for all measured quantities by treatment and year.

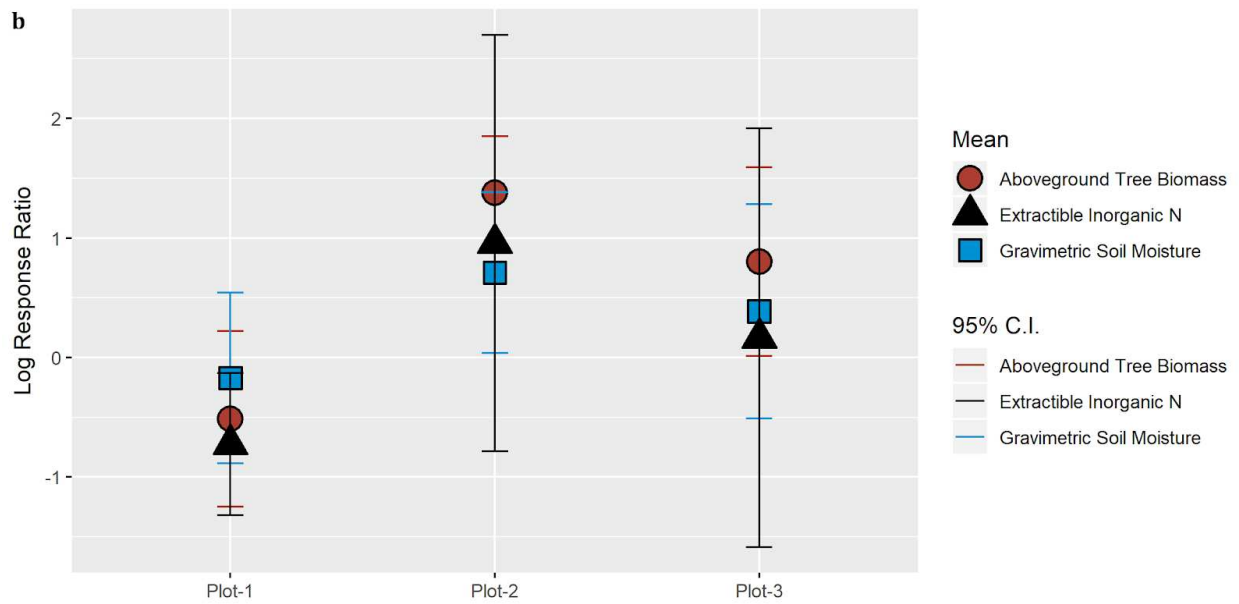
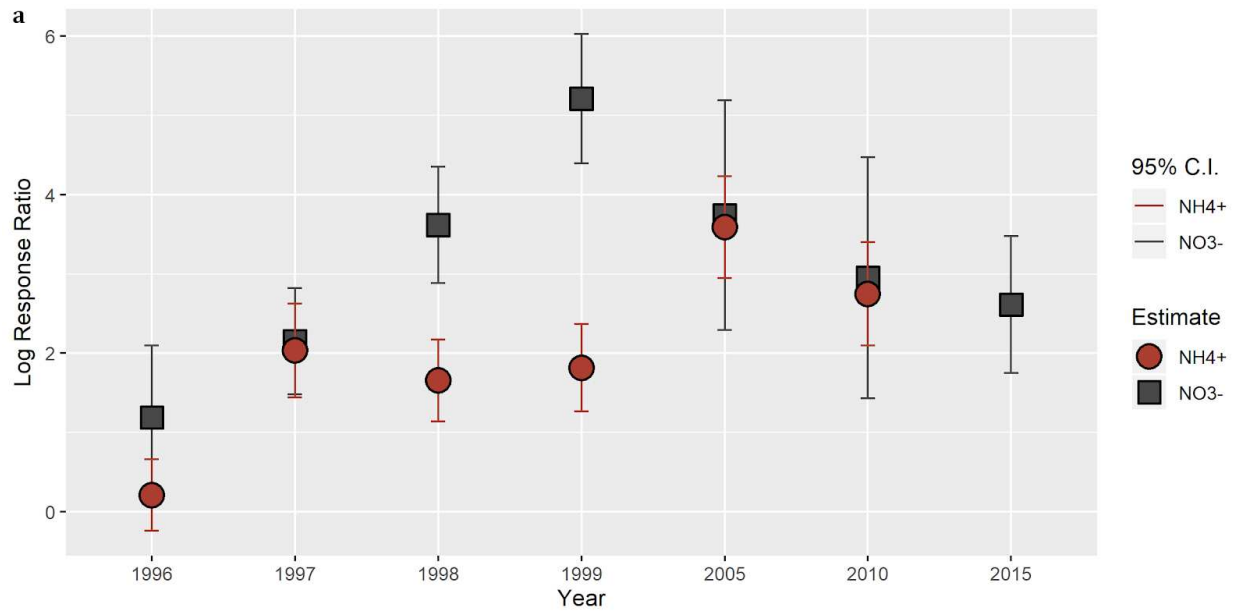
		Nitrification			Mineralization			DOC		
		(mg L ⁻¹ d ⁻¹)			(mg L ⁻¹ d ⁻¹)			(mg L ⁻¹)		
Year	Treatment	M	R	n	M	R	n	M	R	n
1997	Control	1.8E-02	1.7E-01	26	4.8E-02	6.6E-01	26	1.8E+01	8.8E+01	59
	Fertilized	5.6E-03	5.8E-01	26	8.6E-02	8.0E-01	26	2.0E+01	9.8E+01	46
1998	Control	1.0E-05	4.7E-02	18	6.7E-02	2.9E-01	18	1.4E+01	7.6E+01	21
	Fertilized	1.3E-02	5.4E-01	18	3.1E-01	1.7E+00	18	1.7E+01	1.5E+02	17
1999	Control	1.2E-03	2.3E-01	45	5.3E-02	1.3E+00	45	1.9E+01	4.5E+01	17
	Fertilized	1.7E-04	7.7E-01	46	1.3E-01	1.4E+00	46	8.0E+00	2.1E+01	8
2005	Control	3.2E-04	1.8E-01	45	1.6E-02	3.3E-01	45	1.5E+01	6.0E+01	51
	Fertilized	2.3E-02	3.7E-01	43	5.7E-02	4.4E-01	43	1.6E+01	1.7E+02	58
2010	Control	8.9E-04	7.6E-02	44	2.4E-02	2.6E-01	44	1.1E+01	3.9E+01	25
	Fertilized	1.5E-02	5.0E-01	45	9.3E-02	1.3E+00	45	1.6E+01	7.5E+01	27
2015	Control	1.1E-03	1.6E-01	45	5.9E-02	3.6E-01	45	1.6E+01	6.0E+01	45
	Fertilized	5.6E-03	6.6E-01	42	8.9E-02	1.3E+00	42	2.3E+01	8.0E+01	40

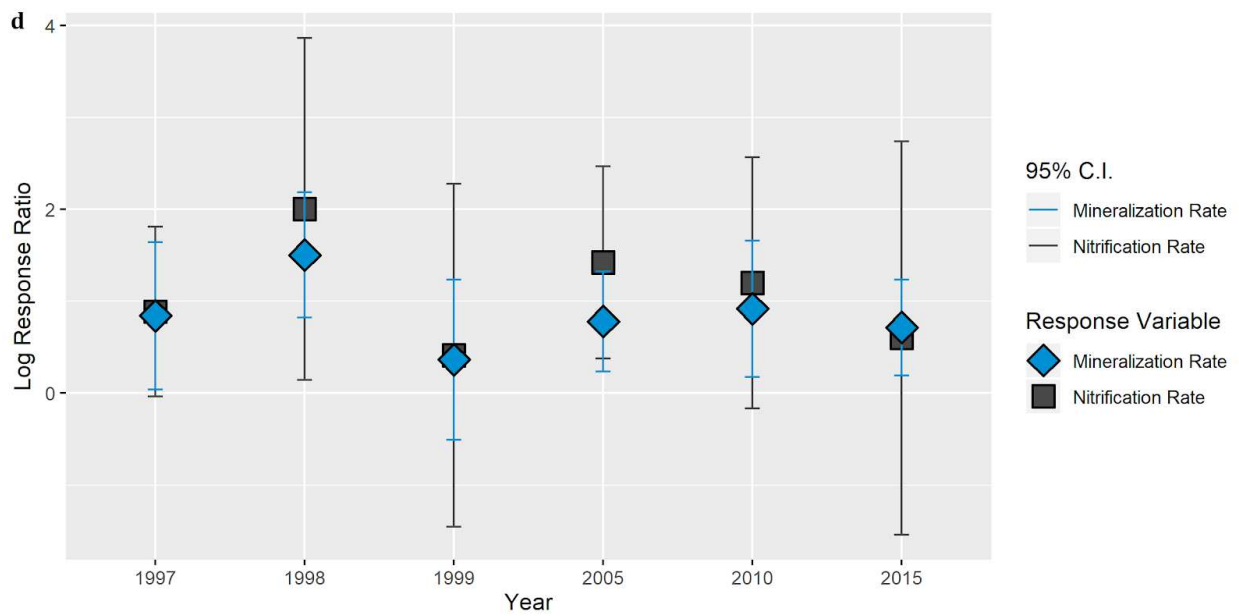
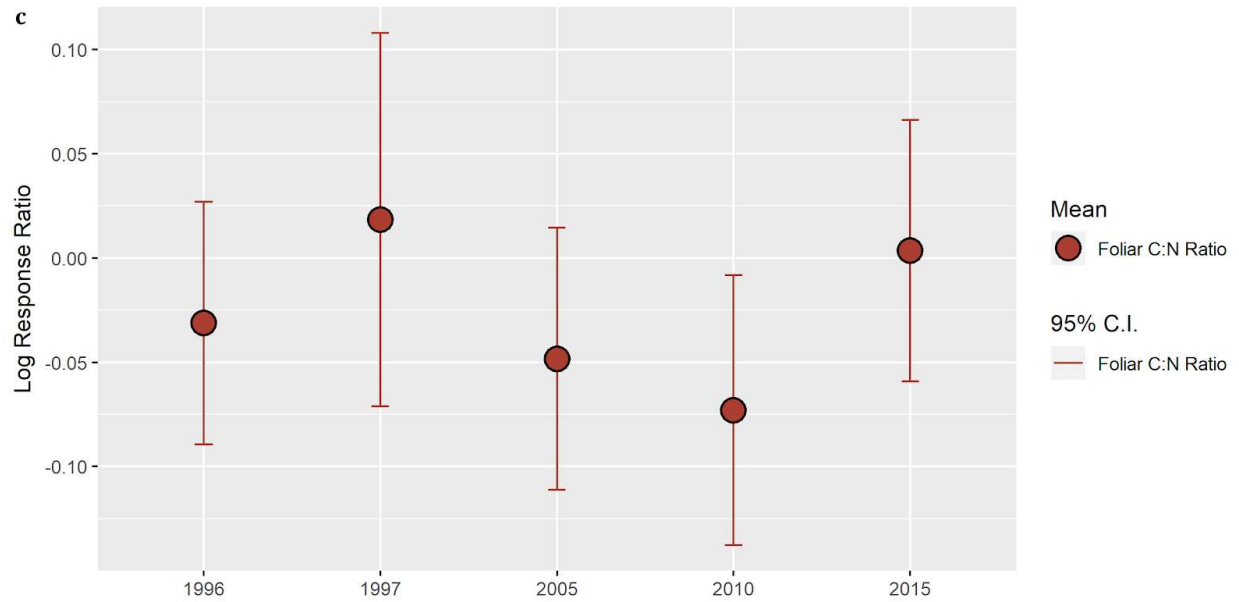
		NO ₃ ⁻			NH ₄ ⁺			Foliar C:N Ratio		
		(mg L ⁻¹)			(mg L ⁻¹)					
Year	Treatment	M	R	n	M	R	n	M	R	n
1996	Control	4.0E-02	5.9E+00	78	5.0E-02	4.9E-01	78	5.2E+01	2.4E+01	34
	Fertilized	5.0E-02	1.4E+01	60	6.0E-02	1.0E+00	60	5.2E+01	2.5E+01	39
1997	Control	2.0E-02	6.6E-01	65	1.0E-02	1.3E-01	65	5.1E+01	2.1E+01	16
	Fertilized	1.5E-01	4.4E+00	40	5.0E-02	1.6E+00	40	5.0E+01	2.1E+01	14
1998	Control	1.6E-02	3.3E+00	156	3.0E-02	2.0E-01	156	-	-	0
	Fertilized	6.0E-01	3.1E+01	144	5.0E-02	5.0E+00	144	-	-	0
1999	Control	3.2E-02	8.2E-02	26	3.0E-02	1.4E-01	26	-	-	0
	Fertilized	3.0E+00	3.5E+01	15	1.3E-01	8.2E-01	15	-	-	0
2005	Control	7.0E-03	4.7E+00	47	2.0E-02	5.0E-02	47	4.3E+01	1.5E+01	15
	Fertilized	2.6E+00	7.0E+01	57	1.6E-01	1.3E+01	57	3.9E+01	1.0E+01	15
2010	Control	2.6E-02	5.2E+00	33	6.0E-02	2.2E-01	33	5.1E+01	1.8E+01	14
	Fertilized	1.3E+00	5.3E+01	45	1.6E-01	1.3E+01	45	4.6E+01	1.0E+01	14
2015	Control	5.6E-02	7.9E-01	45	1.0E-02	2.7E-01	45	4.9E+01	1.9E+01	15
	Fertilized	7.8E-01	3.0E+01	40	6.0E-02	5.1E+00	40	4.8E+01	8.7E+00	15

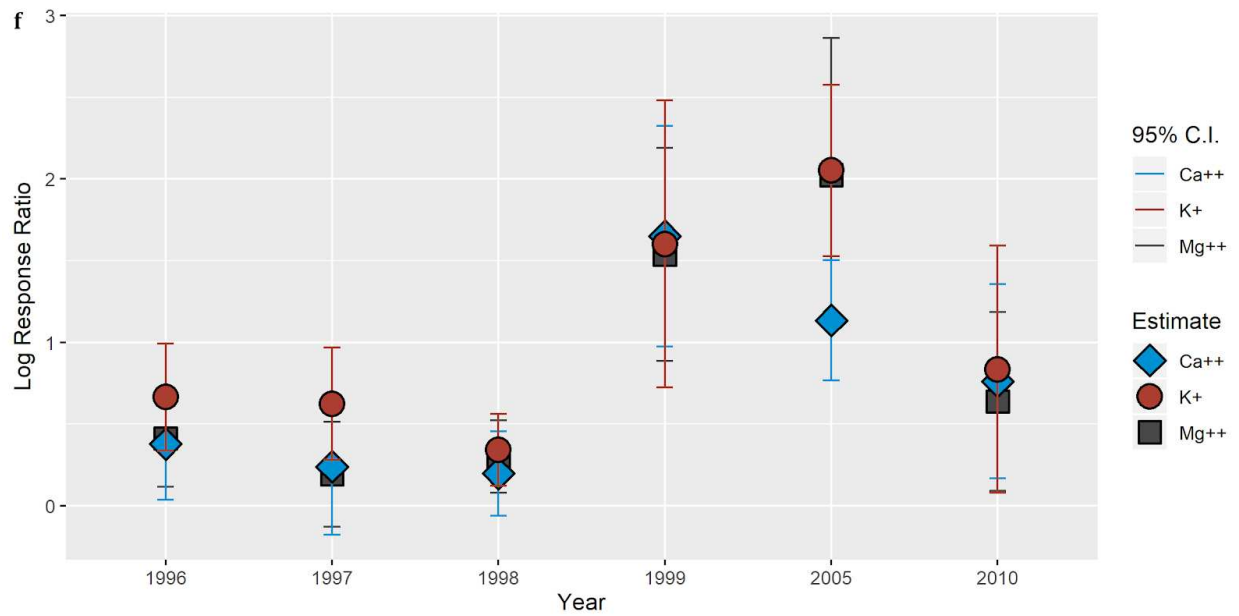
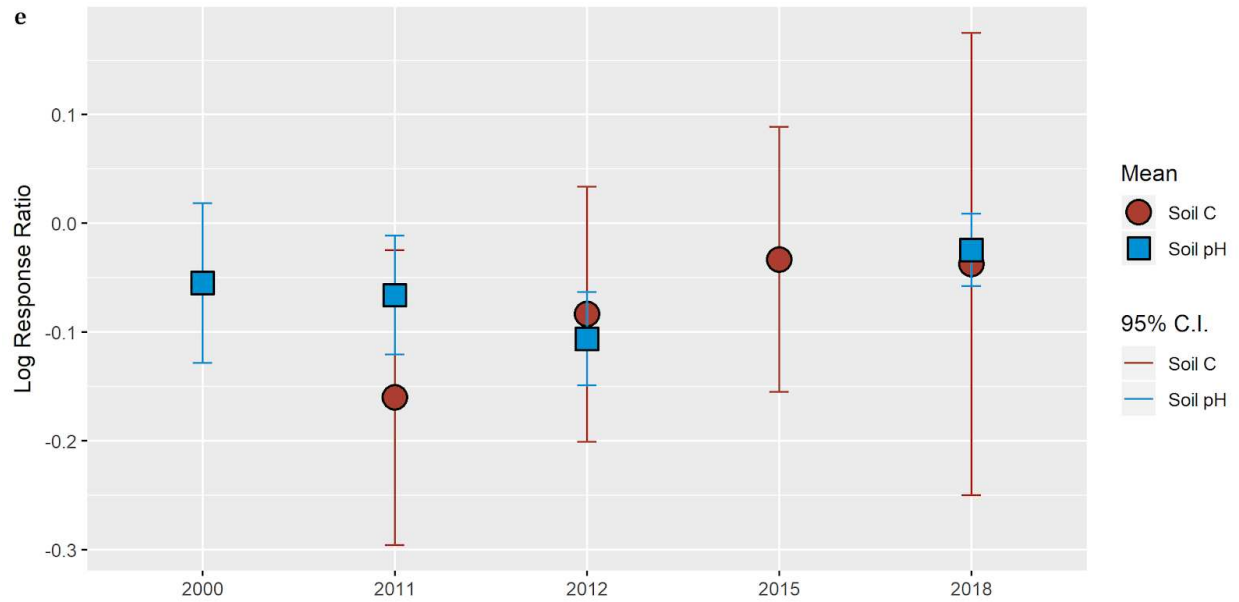
		Na ⁺			K ⁺			Mg ⁺²		
		(mg L ⁻¹)			(mg L ⁻¹)			(mg L ⁻¹)		
Year	Treatment	M	R	n	M	R	n	M	R	n
1996	Control	6.4E-01	5.0E+00	78	6.9E-01	4.4E+00	78	4.6E-01	2.4E+00	78
	Fertilized	6.7E-01	4.4E+00	64	1.1E+00	9.3E+00	64	5.5E-01	4.4E+00	64
1997	Control	2.0E+00	2.8E+00	46	3.8E-01	1.6E+00	46	2.8E-01	1.3E+00	46
	Fertilized	1.8E+00	7.5E+00	45	8.3E-01	4.2E+00	45	2.7E-01	1.6E+00	45
1998	Control	3.4E-01	1.6E+00	153	7.5E-01	3.4E+00	153	6.0E-01	2.3E+00	153
	Fertilized	3.6E-01	1.3E+00	117	9.6E-01	7.5E+00	117	6.1E-01	6.2E+00	117
1999	Control	2.7E-01	9.2E-01	26	4.3E-01	2.3E+00	26	3.1E-01	9.2E-01	26
	Fertilized	4.4E-01	8.6E-01	15	1.7E+00	2.1E+01	15	8.2E-01	6.3E+00	15
2005	Control	2.6E-01	9.4E-01	44	5.0E-02	8.4E-01	44	2.0E-02	1.1E+00	44
	Fertilized	3.3E-01	2.6E+00	50	4.9E-01	6.9E+00	50	2.3E-01	4.6E+00	50
2010	Control	6.3E-01	2.6E+00	33	1.3E-01	4.4E+00	33	5.6E-01	3.5E+00	33
	Fertilized	1.1E+00	4.3E+00	44	7.2E-01	1.4E+01	44	8.3E-01	1.2E+01	44

		Ca⁺²			Al⁺³		
		(mg L⁻¹)			(mg L⁻¹)		
Year	Treatment	M	R	n	M	R	n
1996	Control	2.9E+00	1.8E+01	78	7.8E-01	1.8E+00	78
	Fertilized	4.0E+00	3.8E+01	64	6.7E-01	3.6E+00	64
1997	Control	1.7E+00	1.8E+01	46	4.6E-01	1.6E+00	46
	Fertilized	2.1E+00	1.3E+01	45	2.4E-01	3.4E+00	45
1998	Control	3.9E+00	2.9E+01	153	8.6E-01	2.8E+00	153
	Fertilized	4.4E+00	3.7E+01	117	6.8E-01	2.0E+00	117
1999	Control	2.0E+00	5.8E+00	26	7.4E-01	1.1E+00	26
	Fertilized	6.9E+00	5.5E+01	15	5.2E-01	1.1E+00	15
2005	Control	1.5E+00	8.6E+00	44	5.0E-02	1.0E+00	44
	Fertilized	5.5E+00	2.7E+01	50	5.0E-02	7.8E-01	50
2010	Control	9.0E-01	1.1E+01	33	3.8E-01	7.9E+00	33
	Fertilized	1.6E+00	2.7E+01	44	4.5E-01	3.5E+00	44

		Soil pH			Soil C %		
Year	Treatment	M	R	n	M	R	n
2000	Control	5.3E+00	6.9E-01	5	-	-	-
	Fertilized	4.9E+00	9.0E-01	7	-	-	-
2011	Control	5.2E+00	5.0E-01	6	3.3E+01	3.1E+01	29
	Fertilized	4.9E+00	7.0E-01	6	2.8E+01	3.2E+01	28
2012	Control	4.9E+00	6.0E-01	6	3.7E+01	2.7E+01	29
	Fertilized	4.4E+00	4.0E-01	6	3.3E+01	3.8E+01	30
2015	Control	-	-	-	3.3E+01	3.3E+01	45
	Fertilized	-	-	-	3.3E+01	4.1E+01	45
2018	Control	4.8E+00	7.7E-01	19	2.9E+01	4.0E+01	36
	Fertilized	4.7E+00	9.5E-01	18	2.7E+01	5.7E+01	35







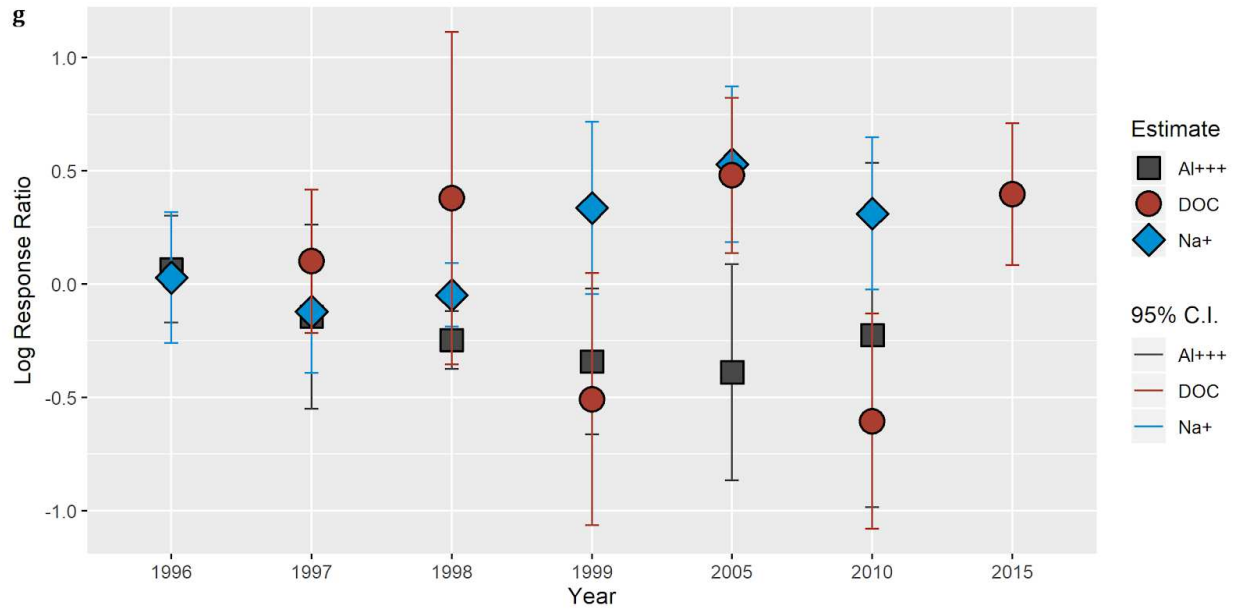


Figure 2.2 a - g. Log response ratios of measures made in the Loch Vale experimental plots, with 95% confidence intervals. A log response ratio of 1 indicates the mean measurement was e times greater in the fertilized plots than the controls in that year.

Forest biomass, soil moisture, and extractable N

Aboveground tree biomass responded differently to fertilization in the different plots, with no effect in plot one ($p = 0.3065$) but a significant effect in plots two ($p < 0.0001$) and three ($p = 0.0023$; Figure 2.1c). Tree biomass was lower in the fertilized unit of plot one than the control (1,8714 kg versus 7,113 kg), higher in plots two (16,007 kg versus 6,193 kg) and three (10,927 kg versus 7,555; Table 2.2). The response ratio was -0.5 in plot one, 1.4 in plot two, and 0.8 in plot three (Figure 2.2b).

Table 2.2 Aboveground tree biomass calculated from DBH measurements of *n* trees by treatment and plot, and dry-weight fine root biomass median (*M*) and range (*R*) separated by flotation from *n* soil cores. Tree biomass calculated from species-specific allometric equations in Chojnacky et al., 2014.

	Treatment	Above-ground tree biomass (kg)		Aboveground young tree biomass (kg)		Fine Root Biomass (mg g soil ⁻¹)			Fine Root C:N Ratio			Soil Moisture (gravimetric)		
		Total	n	Total (%)	n	M	R	n	M	R	n	M	R	n
Plot 1	Control	7,113	86	195 (2.7)	43	35	50	9	36	15	9	0.34	0.60	6
	Fertilized	1,871	39	177 (9.5)	28	40	85	9	29	13	9	0.26	0.65	6
Plot 2	Control	6,193	79	211 (3.5)	41	30	40	9	33	28	9	0.34	0.56	6
	Fertilized	16,007	51	79 (0.5)	10	25	45	9	28	22	9	0.74	1.1	6
Plot 3	Control	7,555	68	173 (2.3)	39	25	55	9	28	11	9	0.32	1.6	6
	Fertilized	10,927	45	107 (1.0)	14	25	60	9	30	20	9	0.84	0.78	6

Soil moisture varied between plots, and like tree biomass was lower in fertilized plot one than control (0.26 versus 0.34), higher in fertilized and control plots two (0.74 versus 0.34) and three (0.84 versus 0.32; Table 2.2). The difference between fertilized and control plots was not significant in plot one ($p = 0.5363$), was significant at $\alpha = 0.10$ in plot 2 ($p = 0.0661$) and was significant at $\alpha = 0.05$ in plot 3 ($p = 0.0364$; Figure 2.1d). The response ratio was -0.2 in plot one, 0.7 in plot two, and 0.4 in plot 3 (Figure 2.2b).

Extractable inorganic N (EIN) was not significantly different between treatments, although there were differences at the plot level (Figure 2.1e). Plot two had the lowest concentrations of EIN, 0.005 mg N g soil⁻¹ in the control plot versus 0.010 mg N g soil⁻¹ in the fertilized plot ($p = 0.2941$; Table 2.3; Figure 2.1e). The response ratio for EIN in plot one was -0.72, in plot two was 0.96, and in plot three was 0.17. The response ratios of

aboveground tree biomass, soil moisture, and EIN are thus all lowest in plot one and highest in plot two (Figure 2.2b).

The percentage of tree biomass made up of young trees (< 10cm DBH) was consistently 2-3 percent in the control plots but varied more in the fertilized plots. In the controls, the biomass of young trees was 195 kg (3% of the total tree biomass) in plot one, 211 kg (3%) in plot two, and 68 kg (2%) in plot three. In the fertilized plots, the young tree biomass was 177 kg (9% of the total tree biomass) in plot one, 79 kg (0.5%) in plot two, and 45 kg (1%) in plot three (Table 2.2).

The fine root biomass was not significantly different between any fertilized and control plots, but there was a significant difference in fine root C:N ratio in plot one. The median fine root C:N ratio in control plot one was 36, compared to 29 in fertilized plot one ($p = 0.0137$; Figure 2.1f). In the other plots, median fine root C:N ratio ranged from 28 to 33, and median fine root biomass ranged from 25 to 40 mg g soil⁻¹ (Table 2.2).

Root and foliar C:N ratios

Differences in fine root biomass between plots were generally small, although here plot one also showed a negative response to fertilization. The median fine root biomass in the fertilized unit of plot one was lower (0.94 g) than the control (1.46 g; Table 2.2). Fine root biomass difference tracked with aboveground biomass difference in plot one, where the fertilized plot soil was drier than the control, while fine root biomass lagged aboveground production in the other plots.

The median C:N ratio in foliage declined from 52 in control and fertilized plots in 1996 to 43 in control and 39 in fertilized plots in 2005 (Table 2.1). Differences between fertilized and control plots were not significant at $\alpha = 0.05$ in any year but were significant

at $\alpha = 0.10$ in 2010 ($p = 0.0648$; Figure 2.1g), when the median control plot ratio was 51 and the fertilized plot ratio was 46 (Table 2.1). The response ratio confidence intervals for foliar C:N ratio overlapped zero in all years except 2010, when the ratio was -0.07 (Figure 2.2c).

Nitrification and N mineralization

Net nitrification was significantly higher in the fertilized plots versus controls in each year from 1998 ($p = 0.0019$) to 2010 ($p = 0.0430$; Figure 2.1h). The response ratio for nitrification peaked in 1998 at 2.0 before declining in response to increased nitrification in the control plots (Figure 2.2d). In 1998 net nitrification was essentially zero in the control plots, compared to $0.013 \text{ mg L}^{-1} \text{ d}^{-1}$ in the fertilized plots (Table 2.1). By 2015 the median nitrification rate was still an order of magnitude higher in the fertilized plots ($0.78 \text{ mg L}^{-1} \text{ d}^{-1}$) than the controls ($0.056 \text{ mg L}^{-1} \text{ d}^{-1}$) but the difference was not significant ($p = 0.2255$; Figure 2.1h).

Net N mineralization was also significantly elevated in fertilized plots from 1998 ($p = 0.0008$) to 2010 ($p = 0.0014$; Figure 2.1i) at $\alpha = 0.05$. The response ratio for N mineralization peaked in 1998, at 1.5, and showed greater interannual variability than nitrification (Figure 2.2d). In 1998 median net N mineralization was $0.067 \text{ mg L}^{-1} \text{ d}^{-1}$ in the control plots and $0.31 \text{ mg L}^{-1} \text{ d}^{-1}$ in fertilized plots, while by 2015 the control plot median was $0.059 \text{ mg L}^{-1} \text{ d}^{-1}$ and the fertilized plot median was $0.089 \text{ mg L}^{-1} \text{ d}^{-1}$ (Table 2.1), the narrowed difference reflected in a higher p value ($p = 0.0604$; Figure 2.1i).

Acidity and soluble cations

Median soil pH declined in the control plots from 2000 (5.3) to 2018 (4.8). The lowest fertilized plot pH was measured in 2012 (4.4), and pH had bounced back to 4.8 by 2018 (Table 2.1). Fertilized plot soil pH was lower than controls in all years, with significant differences in 2011 ($p = 0.0231$) and 2012 ($p = 0.0009$; Figure 2.1j). The strongest response ratio for soil pH was also in 2012, -0.11, and by 2018 was essentially zero (-0.02; Figure 2.2e).

In lysimeter samples the concentrations of K^+ and Mg^{2+} were generally similar in order of magnitude and higher in the fertilized plots than controls (Table 2.1). Both cations dropped in concentration in 2005, rebounding in 2010 (Figure 2.1k and l). The median concentration of K^+ in the control plots was 0.69 mg L^{-1} in 1996, compared to 1.1 mg L^{-1} in the fertilized plots ($p = 0.0211$). Those concentrations fell to 0.05 mg L^{-1} in the control versus 0.49 mg L^{-1} in the fertilized in 2005 ($p < 0.0001$) before climbing to 0.13 mg L^{-1} in the control versus 0.72 mg L^{-1} in the fertilized ($p = 0.0002$). The highest response ratios corresponded with the 2005 drop, 2.1 for K^+ and 2.0 for Mg^{2+} (Figure 2.2f).

The concentrations of Ca^{2+} were higher in the lysimeter samples than the other major cations (besides NH_4^+) and although there was no abrupt drop in Ca^{2+} like there was for K^+ and Mg^{2+} , concentrations declined over time from 1998 to 2010 (Table 2.1). The median sample concentration of Ca^{2+} in the control plots was 3.9 mg L^{-1} in 1998 compared to 4.4 mg L^{-1} in the fertilized plots ($p = 0.6680$), gradually declining to 0.9 mg L^{-1} in the controls in 2010 compared to 1.6 mg L^{-1} in the fertilized plots ($p = 0.0038$; Figure 2.1m). The concentrations in the fertilized plots were higher than controls after 1998, with the highest response ratio, 1.7, in 1999 (Figure 2.2f).

The response of lysimeter Na^+ and Al^{3+} concentrations were weaker than the other cations, although the measured concentrations were generally the same order of magnitude as K^+ and Mg^{2+} (Table 2.1). Na^+ exhibited high interannual variability, including a large drop between 1997 and 1998 and a jump up between 2005 and 2010 (Figure 2.2g). The differences between fertilized and control plot Na^+ concentrations were usually not significant, though one exception was 2010, when the median control plot concentration was 0.63 mg L^{-1} versus 1.1 mg L^{-1} in the fertilized plot ($p = 0.0333$; Figure 2.1n). Al^{3+} concentrations were sometimes lower in the fertilized plots than controls, such as in 1997 when the median control concentration was 0.46 mg L^{-1} versus 0.24 mg L^{-1} in the fertilized plots ($p = 0.0006$; Figure 2.1o). In most years any differences between the fertilized and control plots were not significant, however. Like K^+ and Mg^{2+} , Al^{3+} concentrations dropped in 2005 and rebounded in 2010 (Figure 2.2g).

Soil C % and soluble organic C

Soil C varied only a small amount from 2011 to 2018 (Figure 2.1p). The response ratio was highest in 2011 at -0.16 (Figure 2.2e) when the C percentage in control plots was 33 and in the fertilized plots 28 (Table 2.1). The response ratio was essentially zero by 2015 (Figure 2.2e), when the C percentage was 33 in both control and fertilized plots (Table 2.1). The lysimeter sample concentrations of DOC varied more than soil C, but generally without significant differences between fertilized and control plots (Figure 2.2g). The largest treatment difference occurred in 2005 when the median control plot DOC concentration was 15 mg L^{-1} compared to 16 mg L^{-1} in the fertilized plots ($p = 0.0808$; Table 2.1; Figure 2.1q). The EOC measurements were similar between plots (Table 2.3) but

elevated in fertilized plot three, 2.5 mg L⁻¹, versus control, 0.75 mg L⁻¹ (p = 0.0802; Figure 2.1r).

Table 2.3. Untransformed data for soil extracts by treatment and plot, including median (M), range (R), and sample size (n).

	Treatment	Plot 1			Plot 2			Plot 3		
		M	R	n	M	R	n	M	R	n
Soil EIN (mg N g soil ⁻¹)	Control	3.9E-02	5.7E-02	6	4.5E-03	4.1E-02	6	2.4E-02	4.2E-01	6
	Fertilized	1.6E-02	1.2E-02	6	9.5E-03	1.1E-01	6	4.1E-02	4.3E-01	5
Soil EOC (mg g soil ⁻¹)	Control	1.4E+00	2.2E+00	6	6.5E-01	1.3E+01	6	7.5E-01	3.2E+00	6
	Fertilized	6.2E-01	1.6E+00	6	4.6E-01	7.4E-01	6	2.5E+00	2.9E+00	5

Interannual variability

Many response variables exhibited temporal variability between years (Figure 2.1). For example, control and fertilized plot means for Ca²⁺ were significantly different from each other in 1999, but they were also significantly different from what they had been in 1998, producing significant p values for both treatment and year (Figure 2.1m). Variance in annual weather may have contributed to variance in system response. The average annual temperature was largely steady over the course of the experiment (Figure 2.3). However there were differences in annual precipitation and streamflow at the Loch outlet between years over the course of the experiment.

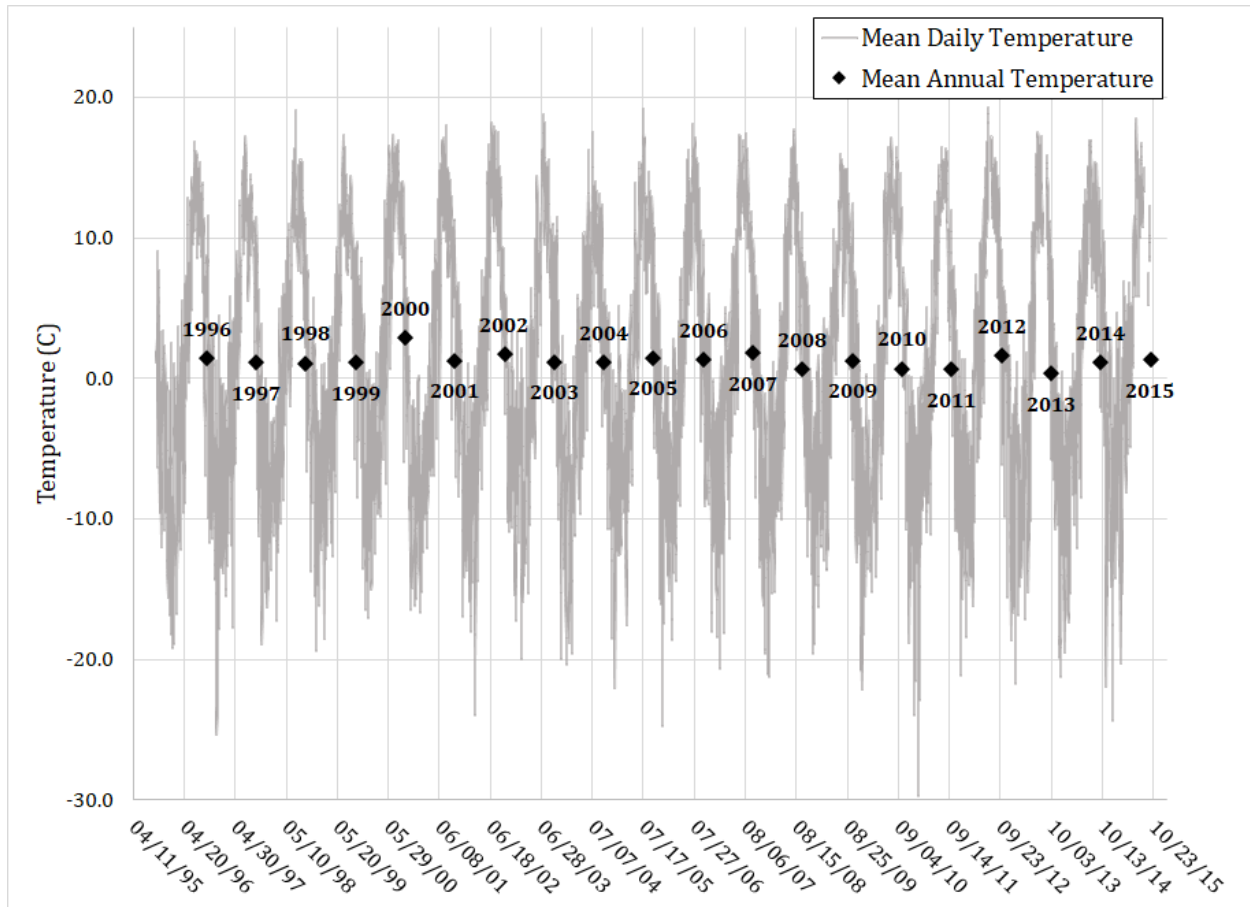


Figure 2.3. Mean daily temperature and mean annual temperature at Loch Vale from 1996 - 2015. Data from 2m temperature sensor at the USGS Loch Vale weather station, located < 3 km from all experimental plots.

Overall, the second decade of the experiment was wetter than the first in terms of annual precipitation and trends in mean daily streamflow (Figure 2.4). Linear regressions were used to examine whether differences in precipitation or streamflow correlated with lysimeter or other measurements, and whether those correlations differed between treatments. No relationships emerged between streamflow and any measured quantities, but annual snowpack correlated weakly with median annual values of some analytes.

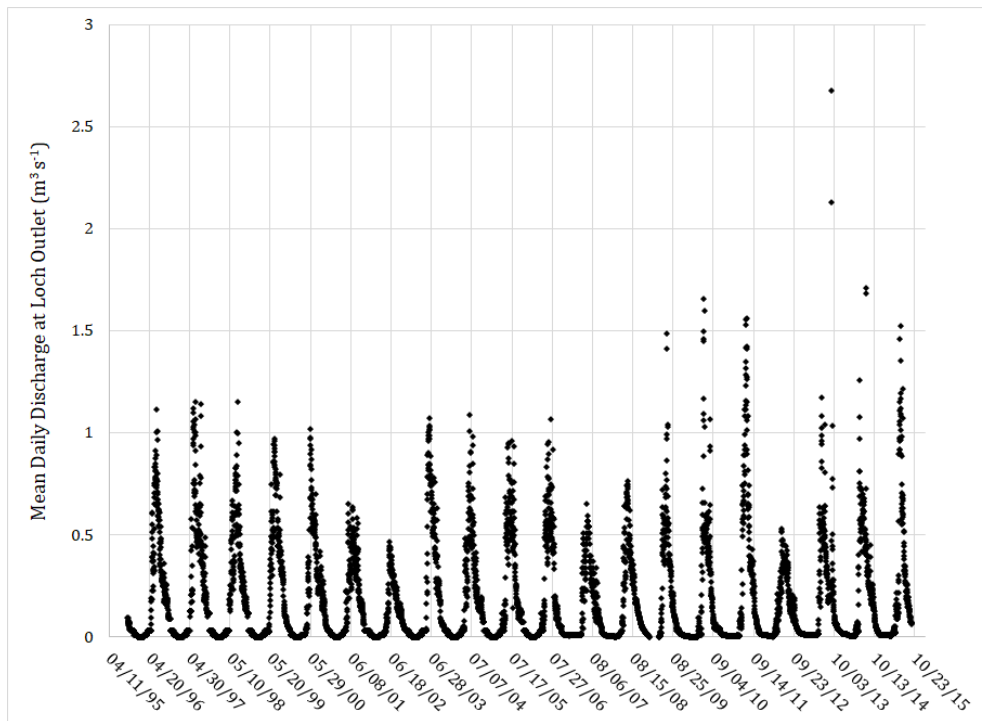
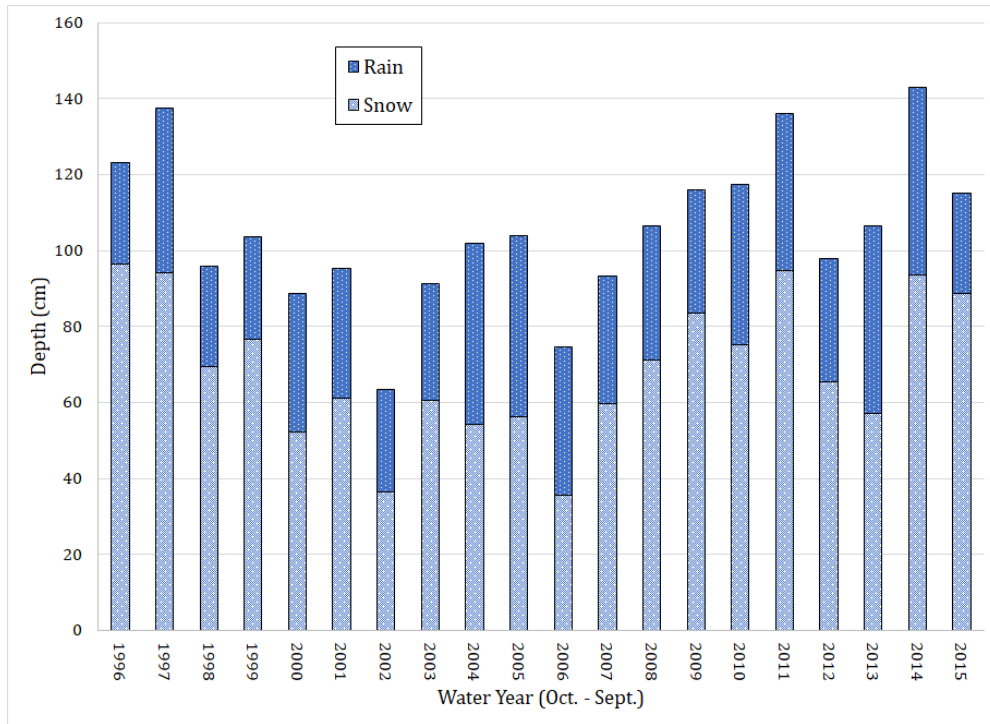
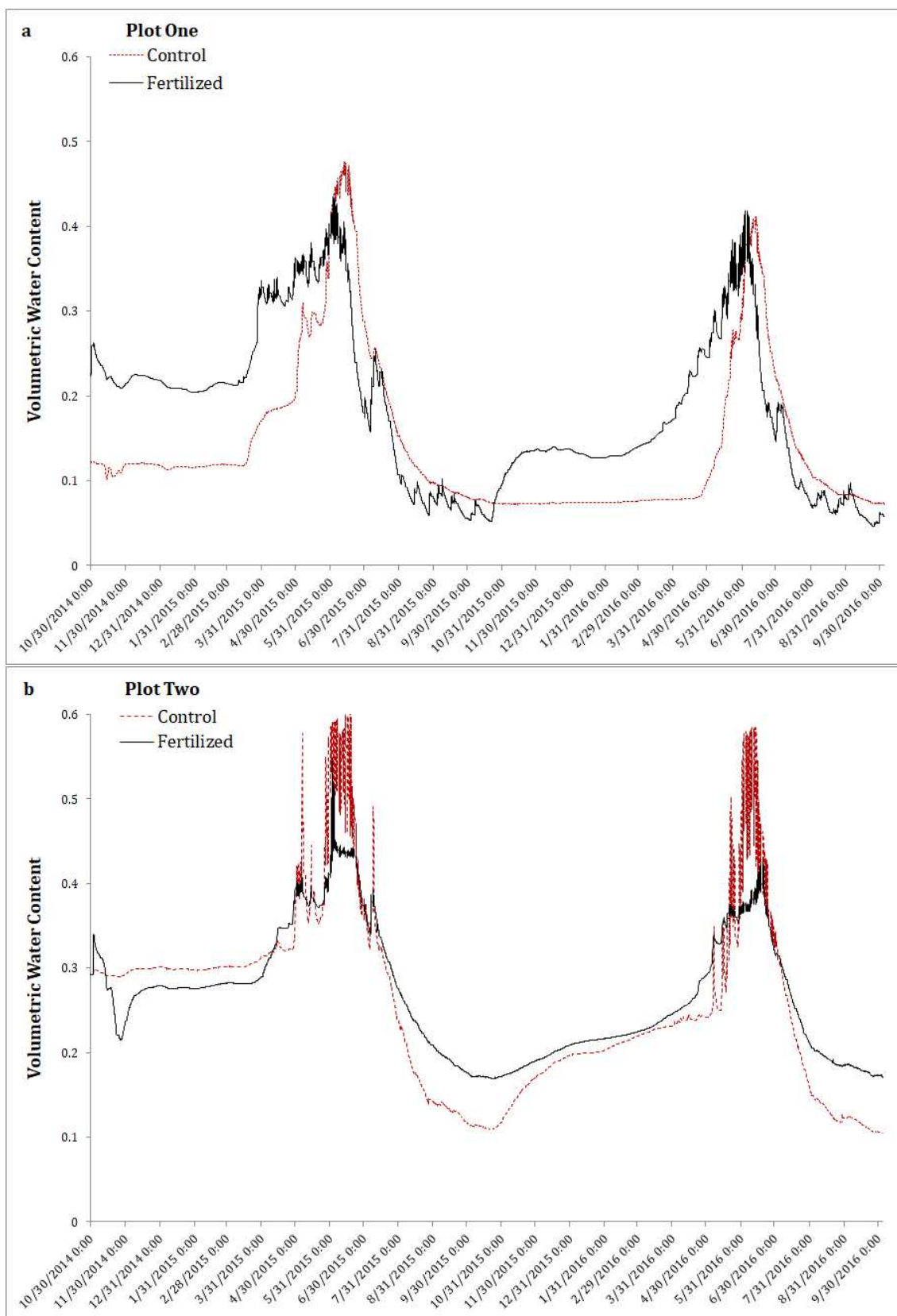


Figure 2.4. Annual precipitation depth at Loch Vale of snow and rain from weighing precipitation gauge at the USGS Loch Vale weather station, located < 2 km from experimental plots, from water years 1996 - 2015 (a). Mean daily discharge measured at the Loch Outlet, the lowest point in the Loch Vale watershed, 1996 - 2015 (b).

Several lysimeter analyte concentrations were lowest in 2005, a low snowpack year (Figure 2.4). Median NO_3^- concentration correlated negatively with annual snowpack depth (slope = -0.06, $R^2 = 0.49$) in the fertilized plots, but had a flatter slope in the control plots ($R^2 = 0.45$). Median Ca^{2+} concentration also correlated negatively with snowpack in the fertilized plots, but snowpack explained much less of the variance (slope = -0.05, $R^2 = 0.18$). Again the control plot median concentrations had no slope, and snowpack explained 1% of the variance. Nitrification and mineralization rates and other lysimeter concentrations did not correlate in regressions with snowpack over time.

The continuous soil data from 2015 and 2016 found consistent patterns of volumetric soil moisture between plots (Figure 2.5). In plot one, the fertilized soil was wetter than the control in winter, but drier in the summer. The 2018 measurements of gravimetric soil moisture also found that the fertilized soil in plot one was drier than the control over the summer (Table 2.2). In plot two the control soil was wetter than the fertilized soil during May and into June, but drier from late June through September. In plot three the fertilized soil was consistently wetter than the control all year. These findings are consistent with 2018 measurements as well, which found that the fertilized soils were wetter than controls measured July - September.



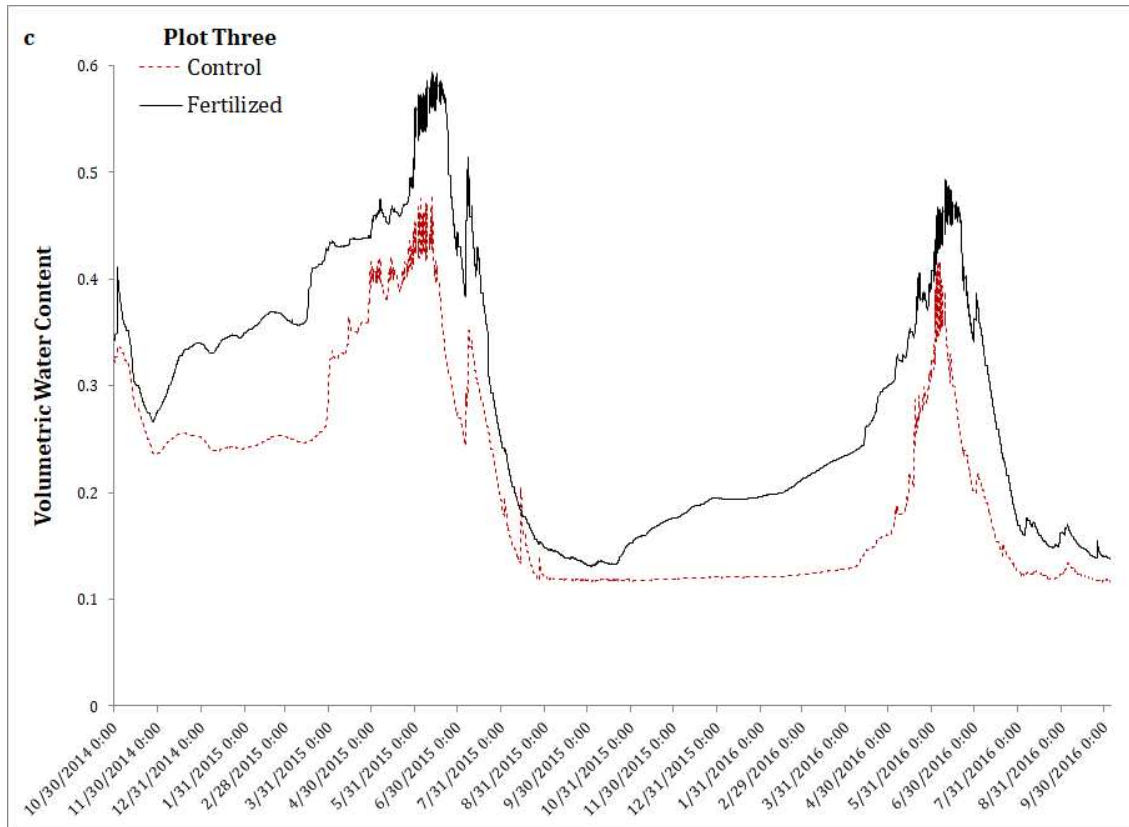


Figure 2.5. Hourly volumetric soil moisture over water years 2015 and 2016 in plots one (a), two (b), and three (c).

Discussion

Conceptual model of N effects

Figure 2.5 presents a new conceptual figure I developed to represent the effects of elevated N observed in the Loch Vale watershed subalpine forest ecosystem over the course of the long-term experiment. The curves represent simplified responses of the ecosystem processes and C and N pools listed down the right side of the figure. The line type used to illustrate the curve corresponds to new hypotheses about environmental controls that mediate the influence of N fertilization on a particular ecosystem response. The controls, listed in the figure key, are available soil moisture, plant N uptake, and soil pH. The ecosystem responses are nitrate leaching (or soil solution N), aboveground biomass, fine-root biomass, root and foliar C:N ratio, nitrification and N mineralization rates, cation leaching (or soil solution cation concentration), and soil C and N. These responses and their controls are each discussed below.

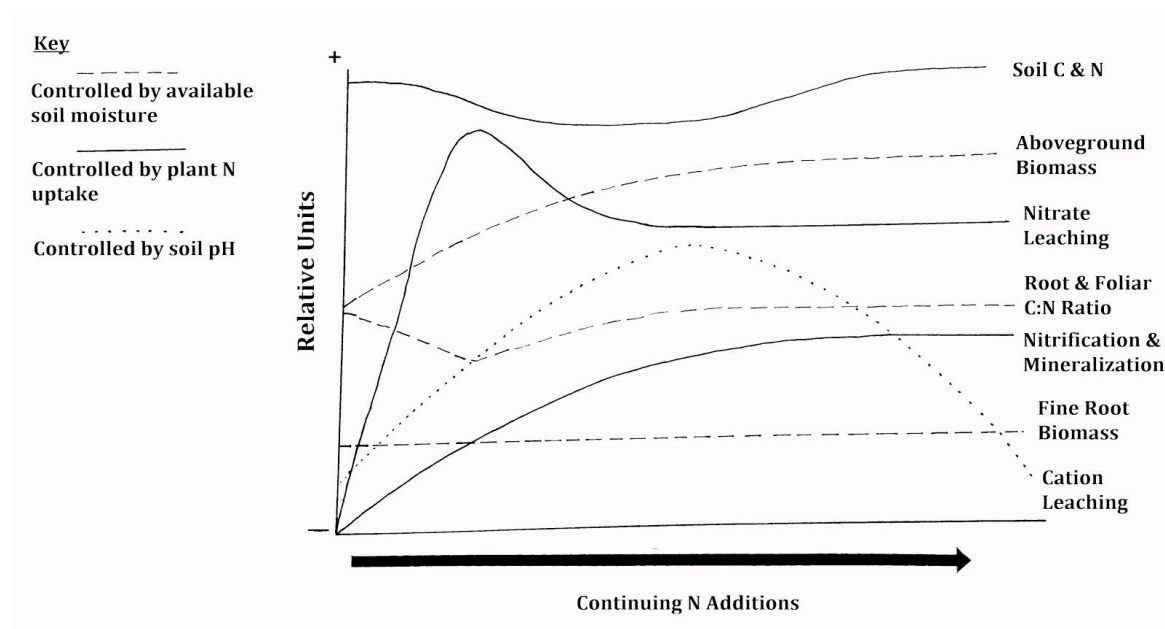


Figure 2.5. A conceptual model of N effects observed at Loch Vale under continuous N fertilizer application and hypothesized control variables mediating N effects.

Soil solution N

Soil solution N was measured to address whether N fertilization increases N losses via leaching. Both NH_4^+ and NO_3^- were consistently elevated over the course of the experiment (Figure 2.1a and b), supporting the hypothesis that elevated N input leads to higher N leaching (H1), if increased N in soil solution is assumed to produce a proportional increase in N losses from a high-runoff environment. Elevated N leaching is generally observed in long-term experiments (Magill et al., 2000; Högberg et al., 2006; Lovett & Goodale 2011) and in environments with thin soils and high runoff like Loch Vale where N leaching may increase immediately after application (Moldan et al., 2018). However both N species became less concentrated in the soil solution over time, while N fertilizer application remained constant. This implies that the relationship between N application and N leaching is dynamic rather than simply proportional and therefore additional N is available in the ecosystem to impact other ecosystem processes and components. The conceptual model in Figure 2.5 presents the hypothesis that soil solution N depends on plant N demand, discussed in the next section.

Forest biomass, soil moisture, and extractable N

Forest biomass was measured to assess whether N fertilization stimulated forest growth, storing additional N in biomass. It did, with the combined aboveground biomass of fertilized plots exceeding controls by 7,944 kg (Table 2.2), meaning that over the course of the experiment a cumulative 55 g m^{-2} dose of N produced 160 g tree biomass per gram N added. Although these results seem to support the hypothesis that N fertilization increases forest biomass production, that conclusion is complicated by heterogeneity within the experimental plots. Plot one seems to have responded negatively to N fertilization, in

contrast to plots two and three (Figure 2.1c), and this plot-level heterogeneity may reveal important relationships between forest production, N, and soil moisture.

In plot one where the fertilized plot aboveground tree biomass was 5,292 kg lower than control, both soil moisture (Figure 2.1d, Figure 2.5a) and soil EIN (Figure 2.1e) were lower as well. In plot two, where the effect of fertilization on tree biomass was strongest, the fertilized plot also had the highest soil moisture and EIN. The positive effect of fertilization in plot three also coincided with higher soil moisture and EIN. A strong relationship between aboveground tree biomass, available soil N, and soil moisture has been observed in boreal forest studies, where conifers responded to N fertilization only when soil moisture was sufficient (Högberg et al., 2006; Moldan et al., 2018). The heterogeneity in fertilization response observed at Loch Vale may reveal colimitation on plant growth between soil moisture and N. The conceptual model in Figure 2.5 hypothesizes the soil solution N depends on plant N demand, but plant N demand, and biomass growth, depends on available soil moisture.

Note that soil EIN is lower where biomass response is lower, and higher where it's higher (Figure 2.2b). It might be expected that more soil N should be found where less plant growth, and therefore N uptake, is occurring. However differences in forest stands within the plots, resulting from disturbance history, may account for this result. In plot one, young trees represent 9% of the aboveground tree biomass, 9 times as high a percentage as plot three, and 18 times as high as plot two (Table 2.2). A preponderance of young trees within plot one may be a legacy of a blowdown event during the winter of 2011-2012. This event is estimated to have redistributed 18,000 kg C from woody biomass in 0.1 ha area of Loch Vale (Wohl, 2014), encompassing the fertilized portion of plot one. Regrowth of young

trees with lower N and water demands than older, larger trees could then be expected, and match the 2019 observations. The legacy of this event may be the heterogeneity currently observed in forest biomass and N biogeochemistry within the Loch Vale plots.

Another possibility is that EIN is higher where N cycling is stronger. Under this supposition, higher N uptake by plants leads to higher rates of growth and senescence of dead tissues, leading to decomposition and accumulation of soil EIN. If soil N were then the result of turnover, rather than representing 'leftover' N not used by plants, the rate of N mineralization should be higher in the plots with higher EIN. The opposite appears to be true in the Loch Vale plots, where in 2015 there was essentially no N mineralization response in plots two and three, in contrast to plot one, where mineralization was elevated in the fertilized plot (Table 2.1).

Belowground, no effect of N fertilization on biomass production was evident (Table 2.2). This adds a further wrinkle to the hypothesis of increased forest biomass under N fertilization, as apparently the trees allocated assimilated N toward aboveground components at a greater rate than belowground. This result is not surprising given previous work on productivity allocation that found trees in conditions of N abundance direct resources away from root biomass and towards leaves and photosynthesis (Nadelhoffer, 2000). Although no effect of N on mass of fine roots was evident in this study, effects on the stoichiometry of roots and foliage provide clues to timing and spatial variability of N impacts on the forest ecosystem.

Root and foliar C:N ratios

Because foliar C:N ratio changed over the years of the experiment, and root C:N ratio varied between plots, these measurements can help understand how the effects of N

fertilization varied in space and time. As the growth rate of a plant increases, the C:N ratio of plant tissues decreases due to the larger number of proteins (composed of N-containing amino acids) required for growth (Ågren, 2004). In plot one the C:N ratio of the fertilized plot was significantly lower than the control (Figure 2.1f), indicating increased growth rate consistent with the observations of more young trees and less EIN available in the soil. As plants grow their N requirement to maintain a constant growth rate increases with increased biomass, so for a N-limited plant to maintain a given growth rate as its biomass increases, the supply of N must grow as well (Dickson, 1989). Since the rate of N application at Loch Vale was constant, a discrete period of plant growth response to N should be expected, and foliar C:N ratios should increase as the size of plants increases and the growth rate declines (Elser et al., 2010). The lower foliar C:N ratio in fertilized plots in 2010 (Figure 2.1g) may therefore indicate a period of higher growth response to N fertilization that had an outsize impact on aboveground tree biomass in fertilized compared with control plots two and three observed in 2019. It's unclear what would have caused such a delayed response but worth noting that the years 2009 through 2011 each had greater annual precipitation than the previous 11 years (Figure 2.4), possibly another indication of colimitation of tree growth, and therefore tissue C:N ratios, by N and soil moisture.

Nitrification and N mineralization

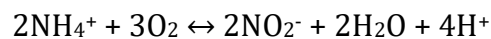
Net N Mineralization and nitrification measurements addressed both driving questions behind this research, does increased N input lead simply to increased N losses, and how do ecosystem responses to N change over time? Mineralization in the fertilized plots was elevated over controls by 1998, lasting until 2015 (Figure 2.1i), largely

supporting H2, that increased N would lead to increased N mineralization. Aber et al. (1998) hypothesized that increases in mineralization would take years to materialize and would result as a secondary response to N fertilization, coming after senescent plant materials became more labile. At Loch Vale, elevated mineralization was apparent after two years of fertilization, and foliar C:N ratios did not indicate increased lability of plant matter until much later. Possibly unmeasured changes in belowground plant tissues fed an increase in N mineralization, or microorganisms accomplished a higher mineralization rate through increasing decomposition of essentially unchanged soil organic matter (SOM). Fertilization experiments in the boreal forest of Sweden found increasing N mineralization resulted from an increased soil microbial biomass (Tahovská et al., 2020). Previous work at Loch Vale associated fertilization with a smaller soil microbial biomass from 2011 - 2013, but also found a loss of soil C in fertilized plots (Boot et al., 2016). An increase in SOM decomposition would explain both the decline in soil C and the increase in net N mineralization. Although the explanation for increased N mineralization is not clear, the fact of it demonstrates that even though N additions led to increased soluble N and probable leaching, ecological effects still resulted from N fertilization.

Rates of net nitrification in the fertilized plots changed little from 1997 - 2015, but in the control plots nitrification rates increased from essentially zero in 1998 to almost the same level as in the fertilized plots (Figure 2.1h). In acidic forest soils nitrification is driven by ammonia-oxidizing archaea (Hu et al., 2014), fungal nitrifiers (Zhang et al., 2014), or low-pH specialist autotrophic bacteria like *Nitrospira spp.*, populations of which respond positively to nitrogen fertilization (Stephen et al., 1998; Tahovská et al., 2020). The rates of nitrification observed early in the experiment suggest that a microbial community of

nitrifiers was already present in the Loch Vale soil, so the increasing rate in the control plots is more likely a response to increased available NH_4^+ . The gradual elevation of control plot nitrification then could have resulted from the steady annual supply of $\sim 3 \text{ kg ha}^{-1} \text{ N}$ from atmospheric deposition to Loch Vale (Morris et al., 2019). This implies that a significant portion of added N is stored within the ecosystem as logically there's no way for N additions to result in mounting increases in nitrification over time unless a portion of that N is retained year-to-year. Therefore the conceptual model of Figure 2.5 hypothesizes that N stored in the ecosystem, largely in plant matter, exerts a control over the rates of microbial N mineralization and nitrification under N fertilization.

Increased rates of nitrification may lower soil pH (Bolan et al., 1991), as the biologically mediated nitrification reaction produces acidity at a rate of 2 mol H^+ per mol NH_4^+ :



Uptake of NO_3^- by plants generates HCO_3^- which may help balance soil acidity, but NO_3^- leaches readily from soils, and may bind with soil cations before leaching, reducing the acid neutralizing capacity of the soil at the same time soil acidity is increasing (Nilsson et al., 1988). It is therefore possible that elevated nitrification rates contributed to soil acidity at Loch Vale, discussed in the next section.

Acidity and soluble cations

The linked processes of soil acidification and cation solubilization showed how ecosystem responses to N fertilization can change over time even under a constant rate of application. Consistent with H1, we observed increased NO_3^- in lysimeter samples,

indicating greater leaching of NO_3^- under N fertilization (Figure 2.1a). When NO_3^- leaches deeper into soils it brings with it soluble cations, increasing acidity in the rooting zone (Bolan et al., 1991). N fertilization led to lower soil pH at Loch Vale (Figure 2.1j), as well as increased concentrations of the soil cations K^+ , Mg^{2+} , and Ca^{2+} in fertilized plots (Figure 2.2f). As lysimeter NO_3^- concentration began to decline after peaking in 1999, so did concentrations of those cations and by 2018 soil pH had recovered somewhat in the fertilized plots.

A long-term N fertilization experiment in Sweden found that N addition caused soil pH to drop below the threshold at which toxic Al^{3+} solubilizes out of soil minerals and becomes increasingly plant-available (Nilsson et al., 1988). Soil acidity at Loch Vale apparently rebounded before reaching that threshold (approximately 4.5, per Reuss & Johnson, 1986) and lysimeter Al^{3+} remained unresponsive to N fertilization (Figure 2.1o). If plant uptake was responsible for the decline in soluble NO_3^- after 1999 then a cascade of effects on soil acidity and cation solubility may have resulted, ultimately avoiding the Al-toxicity observed in the Swedish study.

Soil C % and soluble organic C

Soil C response to fertilization at Loch Vale was generally weak, with little apparent effect on organic horizon soil C (Figure 2.1p), DOC (Figure 2.1q), or EOC (Figure 2.1r). This suggests that increasing N storage within the ecosystem probably doesn't result from greater N storage in SOM. Since both forest biomass and soil inorganic N increased with fertilization, the N storage necessary, for example, to feed increasing rates of nitrification may derive from those sources, rather than SOM. The next chapter will delve more deeply

into the question of what controls soil C, but since ultimately plant production produces the organic matter that becomes soil C, the Loch Vale model hypothesizes plant N uptake as a major control.

Comparability of Loch Vale forests to other coniferous forests

The results of the Loch Vale N experiment will be most useful if applied beyond the Rocky Mountain subalpine forest. Boreal forests cover 9% of the Earth's land surface and store 10% of all terrestrial C (Kasischke, 2000), and are similar to subalpine forests in many respects. Boreal forests are characterized by cold climates and conifer trees growing in acidic, often highly organic soils (Deluca & Boisvenue, 2012), like the subalpine soils at Loch Vale. N deposition in Swedish boreal forests ranges from 3 – 14 kg N ha⁻¹ y⁻¹ (Swedish Enviro. Mon., 2011), in many cases higher than the estimated critical load of ~ 6 kg N ha⁻¹ y⁻¹ (Nordin et al., 2005). N deposition has been increasing in eastern Russia and Canada (Ackerman et al., 2018), where decreases in forest biomass have already occurred due to drought (Ma et al., 2012).

Based on the results of this research synthesized in Figure 2.5, I can present hypotheses for how elevated N may affect boreal forest ecosystems and what environmental factors may control those impacts. Where soil moisture is not a limiting factor, increased N may result in increased forest production, but under drought forest biomass will not be able to take up excess N. Increased rates of nitrate leaching should be expected, but leaching does not mean that N simply moves through the ecosystem without triggering a biological response. Rates of mineralization and nitrification will likely increase simultaneously with leaching, as will acidification and resulting cation depletion. Results from Loch Vale show that Al³⁺ leaching and toxicity does not inevitably result from

this process, but as has long been established, the degree of acidification and loss of buffering capacity control the risk of Al^{3+} mobilization.

Summary

This research asked whether increased N at Loch Vale led to increased N export. Although lysimeter NO_3^- and NH_4^+ concentrations were elevated in fertilized plots immediately, increases in tree biomass, nitrification, and N mineralization were all observed as well. In addition NO_3^- leaching may have contributed to observed declines in soil pH and cation leaching. Increased solubility of N in the soil solution was not evidence that N was simply lost from the system at a higher rate under N fertilization, as numerous ecological effects of elevated N occurred.

The research also sought to identify changes in response to N over time and drivers of those changes. Figure 2.5 summarizes the observed changes and the hypothesized control mechanisms that determine how elevated N can impact ecosystem processes and components. High NO_3^- and NH_4^+ solubility increased rapidly but leveled off after ~ 4 years, while foliar C:N ratios suggest that a response in tree biomass growth did not occur until several years into the experiment. I hypothesized that plant growth is co-limited by available N and soil moisture, so N leaching losses may occur more in years where plant growth is constrained by water. Microbial process of nitrification and N mineralization remained high for most of the experiment, suggesting they may be N limited.

Finally, this research sought to extend the findings from Loch Vale to conifer forests elsewhere in the world and considered how elevated N may impact boreal forests. The extent of N impacts on those forests will depend largely on the ability of plants to assimilate added N, and this will depend in part on access to soil moisture. Where drought

is already impacting boreal forests, N leaching and increased nitrification and N mineralization may increase. Declines in soil pH from leaching should lead to losses of base cations, and potentially, though not necessarily, to Al^{3+} mobilization.

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CHAPTER 3 – MODELING THE SOIL C RESPONSE TO LONG-TERM N FERTILIZATION

Soil organic matter is a critical ecosystem component (Schmidt et al., 2011; Six & Paustian, 2014) and soils act as a crucial carbon sink (Schlesinger & Bernhardt, 2013) yet the impact of anthropogenic nitrogen pollution on soil C is not well understood. Uncertainty arises because N influences both production and decomposition of plant biomass, and N is essential to both anabolically sequestering and catabolically releasing C (Ciais et al., 2013). Forests, for example, store more C in biomass and soils under elevated N conditions today, but whether this will continue in the long term and how much capacity for C accumulation remains are open questions (de Vries et al., 2014). The large body of work on this subject indicates that the C response of terrestrial ecosystem soils to elevated N is spatially and temporally dynamic (Janssens et al., 2010; Zhou et al., 2014), characterized by biome-specific responses (Liu & Greaver, 2010) or responses that depend on degree of N saturation in the ecosystem (Chen et al., 2015). Although the fluxes of C into and out of soils are well known (eg. heterotrophic respiration, leaching, root exudation) the magnitude and direction of changes in C pools under elevated N are difficult to predict (Chang et al., 2019). In spite of this uncertainty, more is known about the stabilization of soil C than ever before, so strategies to assimilate streams of both data and uncertainty from multiple sources may provide a path toward better prediction of soil C responses to elevated N.

The amount of C stored in soil organic matter (SOM) in a given soil under elevated N depends on both above- and belowground factors. N fertilization often produces an

increase in aboveground plant biomass (although not always, e. g. Magill et al., 2000) but may lead to no change or a decline in fine root or root biomass (Phillips & Fahey, 2007; Lee & Jose, 2003; Haynes & Gower, 1995) and roots are thought to contribute disproportionately to stable soil C (Rasse et al., 2005). Soil pH is often decreased under elevated N (Nilsson et al., 1988; Sitaula et al., 1995; Boot et al., 2016), and changes in pH are linked to respiration rates of soil C (Sitaula et al., 1995; Anderson & Nilsson, 2001). In order to better understand which environmental factors control the effect of elevated N on soil C at a given site, a modeling approach may be used to simplify real-world complexity and identify important drivers.

Bayesian modeling has been used in recent years to integrate diverse datasets and quantify error from multiple sources in field- or landscape-scale ecosystem science research. It has been used to better understand ecosystem function, including the partitioning of soil respiration sources (Ogle & Pendall, 2015), the effect of changing land-cover on soil C (Cable et al., 2009), and the metabolism of lotic systems (Appling et al., 2018). Researchers have applied Bayesian models to structural questions, like the impacts of N deposition on indicator species (Oishi, 2019), as well as interactions between disturbance and other drivers on community and ecosystem structure (Hradsky et al., 2017; Bell et al., 2020).

To better understand the impact of elevated N on soil C at Loch Vale, a high elevation catchment in Rocky Mountain National Park, USA, soil C and a variety of covariates were measured at the end of a 20-year N fertilization experiment in Loch Vale watershed. The goal was to combine the effect of N fertilization treatment with important cofactors influencing soil C to elucidate what environmental variables mediate the

response of soil C to N addition. Plot-level characteristics of soil biogeochemistry, forest community structure, N-condition, and organic inputs were considered in a Bayesian regression model framework to test predictions of soil C and determine the most telling covariates.

Research goals

Prior work that measured soil C in the experimental N fertilization plots at Loch Vale found that N fertilization resulted in an 11% loss of organic-horizon C in treated plots (Boot et al., 2016), an unusual finding among forests generally. However, boreal forests, typified by conifer species and cold temperatures, respond relatively weakly to N fertilization in the organic horizon (Liu & Greaver, 2010). With this work we sought to determine whether the losses of soil C observed earlier in the experiment persisted to the experiment's conclusion and identify drivers of organic soil C loss/storage that could produce changes in soil C response over time.

Data were collected on soil C from the organic horizon and eleven covariates representing forest measurements, soil core samples, and lysimeter soil solution measurements, that could help explain the soil C response to N fertilization in a linear model. The forest covariates include aboveground biomass, foliar C:N ratio, and root C:N ratio. The soil core covariates were soil N percent, extractable organic C (EOC) and extractable inorganic N (EIN), pH of a soil water extract, and net rates of microbial nitrification and mineralization. Lysimeter soil solution covariates were dissolved organic C (DOC) and nitrate (NO_3^-). These covariates were modeled as predictors of soil C and

tested to determine which were most likely to control soil C response to elevated N at Loch Vale.

Methods

Soil and fine root biogeochemistry and physical properties

Soils and fine roots were collected during the summer of 2018 to determine the impact of N addition on forest biogeochemistry at the end of the long-term experiment we sampled. Soils were collected for biogeochemical analyses in August, to coincide with season-high levels of soil respiration observed in a previous study (Allen, 2017). We collected soil cores from the organic horizon (7.5 cm deep) after brushing off the litter layer (usually the top 1 cm or less) and pounding a steel soil ring (152 mm diameter) into the organic horizon with a rubber mallet. Twelve samples came from each of the three control and three fertilized plots. Samples were sieved through a 2 mm soil sieve, then partitioned for soil extractions. We partitioned and extracted soils within 6 hours of sampling and shook samples for one hour. After overnight storage at 10 degrees C samples were filtered through Whatman 42 filter paper (Whatman, Clifton, New Jersey, USA). All subsamples were extracted in a 1:10 ratio of soil weight to purified water volume (n = 71) and filtered to measure extractable organic C (EOC) and total extractable N (TEN), using a TOC-L analyzer with Total N Module (Shimadzu TOC-V_{CPN}; Shimadzu Scientific Instruments, Wood Dale, IL, USA). To analyze acidity we subsampled half of the soils in a 5:1 ratio soil to DI water extraction (n = 36) and measured pH of the unfiltered solution using an ion-specific probe. Half the samples were subsampled in a 1:10 ratio of soil to 2 molar KCl solution (n = 36) and filtered to analyze NO₃⁻ and NH₄⁺ content. We measured NH₄⁺ using

segmented flow analysis and NO_3^- using flow injection analysis on an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station TX, USA).

Soils were sampled throughout the 2018 snow-free season for measurements of bulk density, soil moisture, and fine root properties. Three cores per plot were collected to measure soil properties, and three cores per plot to measure root properties, in each of the months July, August, and September. Bulk density and moisture were calculated by weighing the cores on the collection day and then drying at 105°C to a constant weight and re-weighing them. Gravimetric soil moisture was determined by dividing the mass of water from the dry mass of soil, and bulk density by dividing the dry mass of soil by the soil core volume. Root cores were collected using a 0.5 m soil probe (19 mm diameter), sampling as deeply as possible. The float method, which separates buoyant organic materials from denser mineral materials in water and collects them using a series of fine sieves (Amato & Pardo, 1994), isolated roots from the soil. Roots were dried at 60°C to a constant weight. To determine the C and N content of soils and roots we also dried subsamples of soils at 60°C , ground them with a mortar and pestle to a homogenous powder, and analyzed on a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA).

Rates of net nitrification and mineralization, as well as C and N percent, were measured in all plots in 2015. We collected 14 cores of 6.5 cm diameter from the organic horizon of each plot and buried them in the mineral horizon in gas-permeable polyethylene bags for one month (Binkley & Hart, 1989). Each was paired with an unburied core to determine initial NO_3^- and NH_4^+ concentrations. Soils were sieved and homogenized in the field through a 2-mm sieve and 10 g of sample were extracted into 2 mol L^{-1} KCl solution. These were shaken for one hour in the laboratory and stored overnight at 10°C

degrees C before filtering through Whatman filters (Whatman, Clifton, New Jersey, USA) and analyzing with an Alpkem auto analyzer (O.I. Analytical, College Station TX, USA). Net rates of nitrification and mineralization came from taking the difference of the buried bag samples and the initial samples. The remaining soil samples were dried at 60 degrees C to a constant weight, ground them, and analyzed for C and N content using a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA).

Lysimeter samples of NO_3^- and DOC

Lysimeter samples were collected and analyzed for NO_3^- and dissolved organic carbon (DOC) concentrations. The lysimeter sampling methods, first described in Reuth et al., (2003), consisted of using hand pumps to generate tension on lysimeters and clamps to maintain tension for 24 hours, preferentially drawing soil solution into the lysimeter cup. Samples were pumped from the cups and filtered through 0.2- μm membrane filters (Supor-Gelman; Pall, Ann Arbor, Michigan USA). Sample volumes ranged from over 150 mL in May less than 10 mL August, limiting analyses on late-season samples. Dissolved organic C concentration were measured in the years 1997 - 1999, 2005, 2010, and 2015 by removing inorganic C with phosphoric acid, then converting organic C to CO_2 with a sodium persulfate digest, then measuring the oxidized C using an infrared detector (OI Analytical, College Station, Texas USA, Model 700 TOC Analyzer). Nitrate concentrations were measured in the same years on an Alpkem auto-analyzer (Alpkem, Perstorp Analytical Company, Wilsonville, Oregon, USA, 3500 Series).

Forest tree measurements

A forest inventory was conducted over the summer of 2019 on each of the six plots according to Forest Service methods (USFS, 2019). The inventory included species, live/dead status, and diameter at 1.4 m above ground (DBH) for each tree (n = 484). Periodic blowdowns have resulted in dead and down trees in all plots, and these were counted and DBH estimated or measured if possible. The majority of spruce encountered showed signs of infection by bark beetles (*Dendroctonus rufipennis*), although most of the spruce were still alive. Fir made up the majority of trees in all plots.

In order to compare the forest stands in each plot by a metric relevant to belowground C, the following allometric biomass equations were used to calculate aboveground tree biomass using measured DBH for *Abies lasiocarpa*

$$\ln(\text{aboveground biomass [kg]}) = -2.3123 + 2.3482 \ln(\text{DBH [cm]})$$

(3.1)

and *Picea engelmannii*

$$\ln(\text{aboveground biomass [kg]}) = -3.0300 + 2.5567 \ln(\text{DBH [cm]})$$

(3.2)

using generalized biomass equations for North America (Chojnacky et al., 2014).

Aboveground biomass was calculated for each tree greater than 1.4 m in the inventory.

Live foliage was sampled in September 2015 from five canopy spruce in each plot (n = 30). Needles were collected from south-facing aspects and rinsed them with deionized water in the field. After transport to the laboratory and drying at 25 degrees C needles

were ground to homogenous powder. A LECO Tru-Spec CN analyzer measured percent C and N in each sample (Leco Corp., St. Joseph, MI, USA).

Data exploration

Data exploration involved visualization and initial tests for both treatment- and plot-level effects. We developed effects size estimates and confidence intervals for soil C and covariates using the log response ratio, implemented in the SingleCaseES package for R (Pustejovsky & Swan, 2019; R Core Team, 2019). These analyses contributed to the selection of covariates for testing in a Bayesian hierarchical model.

Bayesian hierarchical regression modeling

A Bayesian model combines three main components to estimate the probability of making particular observations if the model accurately describes the system which produces the phenomena observed (Hobbs & Hooten, 2015). The **prior distributions** (or ‘priors’) leverage previously observed data to constrain modeled parameter estimates toward the plausible (Muth et al., 2014). The **likelihood function** links the data to the model by estimating the likelihood of the observed data in the response variable given all possible values of the model parameters (Hobbs & Hooten, 2015). The **posterior probability** (or ‘joint’) joins the priors and likelihood together to produce the target estimates of the model. These estimates, called the posterior probability distribution, may be checked against observed values to demonstrate the relative effectiveness of the model at describing the underlying system (Muth et al., 2014). The following models produced the joint distribution from conditional probabilities nested hierarchically (for example, the effect of treatment within each plot), so the model is hierarchical (Hobbs & Hooten, 2015).

The `rstanarm` package was used to fit the models to conduct convergence and posterior predictive checks (Gabry & Goodrich, 2017).

Within the Bayesian framework multiple regression models were used to infer relationships between covariates observed at either the plot or sample level and the response variable soil C. The regression model treated soil N and the fertilization treatment as sample-level predictor variables with one value for every value of soil C:

$$h(x_{ij}^N, x_{ij}^{fert}, \alpha_j, \beta) = \alpha_j + \beta_1 x_{ij}^N + \beta_2 x_{ij}^{fert} \quad (3.3)$$

where x^N is soil N, x^{fert} is treatment, α is the intercept parameter, β is the slope parameter, i is the i th sample and j is the j th plot. The treatment took a value of 0 for control samples and 1 for fertilized samples. The model examined all other covariates as plot-level predictors, using plot-level means:

$$g(w_j, \beta^w, \alpha^w) = \alpha^w + \beta^w w_j \quad (3.4)$$

Where w is a plot-level covariate. We used the data collected in 2015 to determine an informed prior on soil N:

$$\times \text{normal}(\beta_1 \mid 0.287, 0.297) \quad (3.5)$$

where β_1 is the slope parameter on soil N.

The posterior probability that the model parameters have been correctly fit, given the vector of soil C observations is proportional to the likelihood of those observations given the regression models and the priors:

$$\begin{aligned}
 & [\alpha_j, \beta, \sigma_j, \alpha^w, \beta^w, \sigma^w \mid \mathbf{y}_{ij}] \propto \\
 & \prod_{i=1}^{71} \prod_{j=1}^3 \text{lognormal}(\mathbf{y}_{ij} \mid \ln(h(x_{ij}^N, x_{ij}^{fert}, \alpha_j, \beta)), \sigma_j) \\
 & \times \text{normal}(\alpha_j \mid g(w_j, \beta^w, \alpha^w), \sigma^w)
 \end{aligned}
 \tag{3.6}$$

where $\mathbf{y}$ is the vector of soil C and σ is unmodeled variance.

The Bayesian approach requires a method of approximating the integral of the posterior distribution, which makes it a true probability density function that may be interpreted as the probability that a model explains a process, given the observed data. Typically a Markov chain Monte Carlo (MCMC) algorithm estimates the posterior distributions using three chains of 25,000 iterations after burn-in. The MCMC algorithm normalized the likelihood function to equal one and weights it by the information contained in the prior (Hobbs & Hooten, 2015).

The models were checked for convergence used model checking procedures developed for Bayesian regressions and described in Muth et al. (2014), The Gelman-Rubin ($\hat{R}$) diagnostics assess convergence of the chains, or the sequences of values drawn

randomly from the posterior (Brooks & Gelman, 1998). All $\hat{R}$ values equaled 1.0, failing to find evidence of non-convergence. All effective sample sizes were greater than 2,000. Posterior predictive draws were made to demonstrate how well models could reproduce the true distribution of observed soil C.

Fifteen different models were fit by varying the covariate predictors in order to find which models best estimated soil C, and therefore which covariates were most useful for predicting soil C. This required a model comparison process, implemented using the *loo* package (Vehari et al., 2019). Leave-one-out cross-validation (*loo*), is a method that blinds the model to part of the data and then scores the model's ability to estimate the left-out data. This was computed using Pareto-smoothed importance sampling, shown to develop importance weights and standard error terms that are comparable between models (Vehari et al., 2017). All Pareto *k* constants were less than 0.7, and most were less than 0.5. The strongest models were selected by comparing estimated mean square prediction error (ELPD) and standard error (SE) to determine the most effective combination of covariates. In order to only test models that could elucidate drivers of C storage and loss in our system, we constrained our selection of covariates to biologically plausible factors affecting soil C pools.

One model was tested with out-of-sample validation. Using a set of data collected from the same plots and by the same methods in 2012 (analyzed in Boot et al., 2016) the model was tested for its ability to predict out-of-sample data. Due to differences in which model parameters were measured between this and the earlier study, only one model could be tested, however the model used the most effective components in the study.

Results

In contrast to previous research done in the same plots, soil C here exhibited a null response to fertilizer treatment when considered in the aggregate. Disaggregated to the plot level however, soil C responded variably to the N additions. In Plot one, median soil C was -22% in the fertilized unit versus the control, -6% in Plot two, and +26% in Plot three (Table 3.1). When considered as a log response ratio, the 95% confidence intervals for soil C response in all three plots overlapped zero (Figure 3.1a). This suggests differences in soil C resulted not from N fertilizer treatment but rather as an expression of heterogeneity in soil C-stabilizing factors within the plots.

Table 3.1. Untransformed data for all measured quantities or calculated ratios of measured quantities by treatment and plot, including median (M), range (R), and sample size (n).

	Treatment	Plot 1			Plot 2			Plot 3		
		M	R	n	M	R	n	M	R	n
Soil C	Control	3.6E+01	3.1E+01	12	1.8E+01	2.5E+01	12	2.9E+01	3.6E+01	12
(%)	Fertilized	2.8E+01	3.6E+01	12	1.7E+01	3.1E+01	12	3.9E+01	5.4E+01	11
Soil N	Control	1.1E+00	9.8E-01	12	8.0E-01	1.3E+00	12	1.3E+00	1.3E+00	12
(%)	Fertilized	1.1E+00	1.6E+00	12	7.6E-01	1.3E+00	12	1.7E+00	1.8E+00	11
Soil pH	Control	4.7E+00	3.1E-01	7	5.0E+00	7.4E-01	6	4.8E+00	4.2E-01	6
	Fertilized	4.9E+00	6.4E-01	6	4.7E+00	4.7E-01	6	4.6E+00	6.3E-01	6
Soil C:N	Control	2.9E+01	1.3E+01	12	2.4E+01	1.7E+01	12	2.2E+01	9.1E+00	12
Ratio	Fertilized	2.4E+01	1.1E+01	12	2.2E+01	9.6E+00	12	2.3E+01	1.1E+01	11
Root C:N	Control	3.6E+01	1.5E+01	9	3.3E+01	2.8E+01	9	2.8E+01	1.1E+01	9
Ratio	Fertilized	2.9E+01	1.3E+01	9	2.8E+01	2.2E+01	9	3.0E+01	2.0E+01	9
Foliar C:N	Control	5.2E+01	1.0E+01	5	4.9E+01	1.8E+01	5	4.5E+01	1.2E+01	5
Ratio	Fertilized	5.0E+01	8.1E+00	5	5.0E+01	8.4E+00	5	4.8E+01	2.1E+00	5
Nitrification	Control	1.8E-03	1.1E-02	5	4.4E-03	1.4E-01	10	2.8E-02	9.5E-02	12
(mg L ⁻¹ d ⁻¹)	Fertilized	5.6E-02	3.9E-01	8	2.0E-02	1.6E-01	9	3.1E-02	6.1E-02	9
Mineralization	Control	4.4E-02	1.1E-01	12	5.5E-02	1.8E-01	15	9.2E-02	3.1E-01	15
(mg L ⁻¹ d ⁻¹)	Fertilized	1.3E-01	9.4E-01	13	8.2E-02	6.3E-01	14	9.8E-02	5.5E-01	10
Lysimeter NO₃⁻	Control	4.0E-02	1.0E-01	19	2.8E-01	7.3E-01	12	8.0E-02	6.9E-01	12
(mg L ⁻¹)	Fertilized	1.8E+00	8.8E+00	16	4.5E-01	7.9E-01	14	7.0E-01	2.9E+01	9
Soil EOC	Control	1.4E+00	2.2E+00	6	6.5E-01	1.3E+01	6	7.5E-01	3.2E+00	6
(mg L ⁻¹)	Fertilized	6.2E-01	1.6E+00	6	4.6E-01	7.4E-01	6	2.5E+00	2.9E+00	5
Soil Moisture	Control	3.4E-01	6.0E-01	6	3.4E-01	5.6E-01	6	3.2E-01	1.6E+00	6
(Gravimetric)	Fertilized	2.6E-01	6.5E-01	6	7.4E-01	1.1E+00	6	8.4E-01	7.8E-01	6

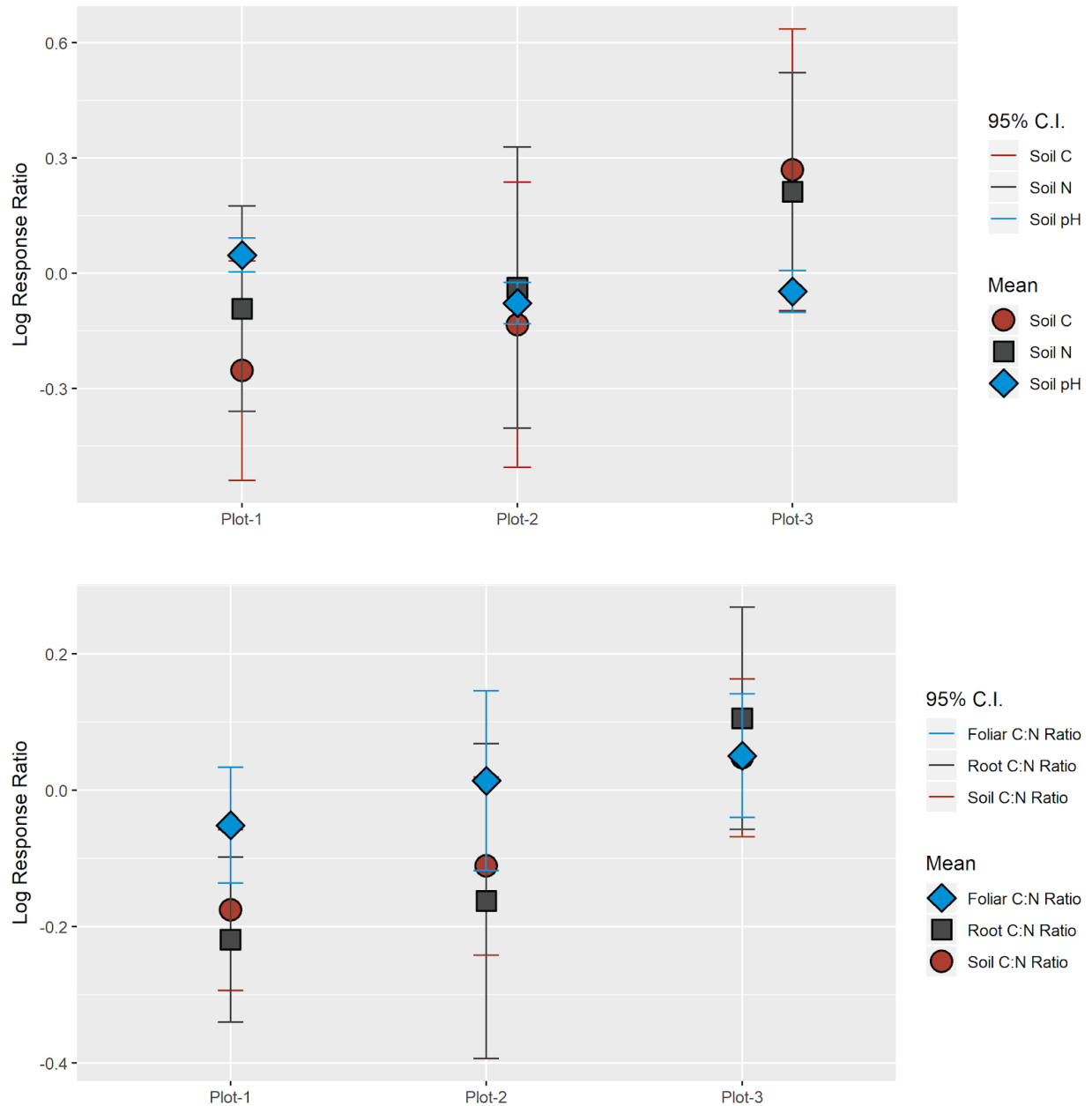


Figure 3.1. Log response ratios of soil C and N % and pH (a) and foliar, root, and soil C:N ratio (b) by plot, with 95% confidence intervals. A log response ratio of 1 indicates the mean measurement was e times greater in the fertilized unit than the controls in that plot.

Soil N and pH were reported to decline with fertilization in previous work (Boot et al., 2016), but the results presented here do not show similar responses. Median soil N exhibited null response in plot one, weakly negative response in plot two (-5% in the treatment plot versus the control), and positive response in plot three (+24%). Soil pH

response was even weaker and less variable: +4% in plot one, -6% in plot two, and -4% in plot three (Table 3.1). The confidence intervals on log response ratios of soil N and pH all overlapped zero, with the exception of soil pH in plot two, and the mean responses of C and N correlated positively (Figure 3.1a).

The C:N ratio of soils, roots, and foliage were similarly unresponsive at the treatment level but variable across plots. Plot one stood out as having response ratios for root and soil C:N ratio that did not overlap zero. The mean log responses for these variables were approximately -0.2 (Figure 3.1b). The soil C:N ratio in plot one was -17% in fertilized versus control units, while root C:N ratio was -19% (Table 3.1). Ratios of C:N generally responded more weakly in plots two and three compared to plot one, a pattern that repeated across other measures.

The effect of N fertilization on rates of net nitrification and mineralization in the soil as well as NO_3^- concentration in lysimeter samples was also considered. Nitrate was higher in the fertilized versus control units of plot one (1.8 mg L^{-1} versus 0.04 mg L^{-1}) and plot three (0.70 mg L^{-1} versus 0.08 mg L^{-1}) with response ratios of 3.9 in plot one and 3.4 in plot three (Figure 3.2a). Net nitrification rate responded positively in the fertilized units of plots one and two, $0.056 \text{ mg L}^{-1} \text{ d}^{-1}$ versus $0.002 \text{ mg L}^{-1} \text{ d}^{-1}$ in plot one, $0.020 \text{ mg L}^{-1} \text{ d}^{-1}$ versus $0.004 \text{ mg L}^{-1} \text{ d}^{-1}$ in plot two. Net nitrification did not respond in plot three, $0.031 \text{ mg L}^{-1} \text{ d}^{-1}$ fertilized versus $0.028 \text{ mg L}^{-1} \text{ d}^{-1}$ control (Table 3.1). Plot one did not overlap zero in the confidence interval of the response ratio (3.3), while the other plots did (Figure 3.2a). Net mineralization also responded positively in plot one, 0.013 in the fertilized unit versus 0.004 in the control. There was little response in plot two, 0.082 versus 0.055 , and no

response in plot three, 0.098 versus 0.092. The response ratio was 1.6 in plot one and 0.9 in plot two (Figure 3.3a). Here again plot one exhibited stronger responses to N fertilization than the other plots.

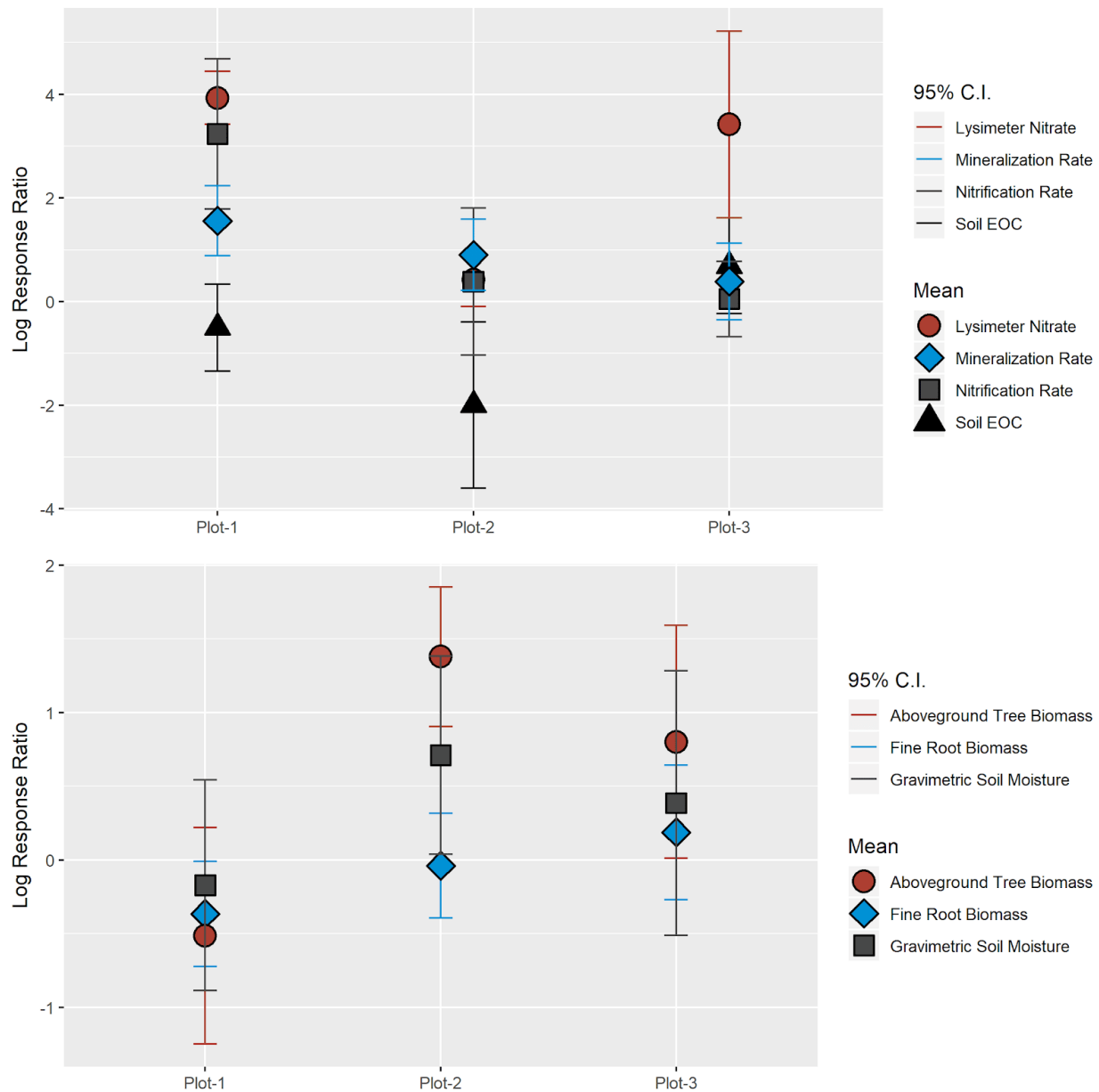


Figure 3.2. Log response ratios of lysimeter nitrate concentration, and nitrification and mineralization rates (a) and aboveground tree biomass and gravimetric soil moisture (b) by plot, with 95% confidence intervals. A log response ratio of 1 indicates the mean measurement was e times greater in the fertilized unit than the controls in that plot.

Nitrogen fertilization had little effect on soluble forms of organic C. Between 1997 and 2015, lysimeter DOC response ratios ranged between -0.61 and 0.40 (Figure 3.3). Although the confidence intervals did not overlap zero in 2005, 2010, or 2015, the effect direction oscillated between positive and negative, resulting in no net effect of N fertilization on lysimeter DOC concentration. Likewise, treatment had little apparent effect on soil EOC concentration except in plot 2 (Figure 3.2a).

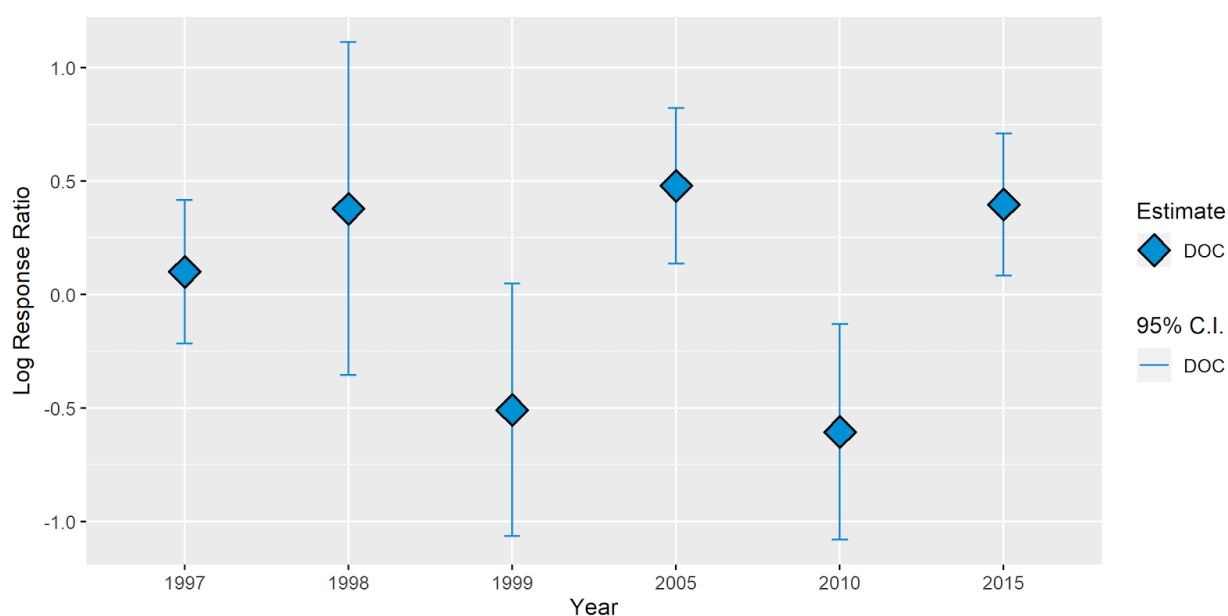


Figure 3.3. Log response ratios of lysimeter DOC concentrations by year, with 95% confidence intervals. A log response ratio of 1 indicates the mean measurement was e times greater in the fertilized plots than the controls in that year.

Soil moisture and aboveground tree biomass responded weakly in plot one compared to plots two and three. Tree biomass was lower in the fertilized unit of plot one (1,8714 kg versus 7,113 kg), higher in plots two (16,007 kg versus 6,193 kg) and three (10,927 kg versus 7,555, Table 3.2), with response ratios of 1.4 in plot two and 0.8 in plot three (Figure 3.2b). Soil moisture was similarly distributed, lower in plot one (0.26 versus 0.34), higher in plots two (0.74 versus 0.34) and three (0.84 versus 0.32, Table 3.1), with a

response ratio of 0.7 in plot two (Figure 3.2b). Fertilization corresponded with an order of magnitude greater aboveground tree biomass in plots two and three, where soil moisture was higher in the fertilized units than the controls (Table 3.2). In plot one, where soil moisture was about equal in fertilized and control units, tree biomass did not respond to N fertilization (Figure 3.3b).

Table 3.2 Aboveground tree biomass calculated from DBH measurements of *n* trees by treatment and plot, and dry-weight fine root biomass median (*M*) and range (*R*) separated by flotation from *n* soil cores. Tree biomass calculated from species-specific allometric equations in Chojnacky et al., 2014.

		Aboveground tree biomass (kg plot ⁻¹)		Aboveground sapling biomass (kg plot ⁻¹)		Fine Root Biomass (g)		
	Treatment	Total	N	Total	n	M	R	n
Plot 1	Control	7,113	86	195	43	1.46	1.65	9
	Fertilized	1,871	39	177	28	0.94	1.04	9
Plot 2	Control	6,193	79	211	41	1.32	1.81	9
	Fertilized	16,007	51	79	10	1.25	1.47	9
Plot 3	Control	7,555	68	173	39	1.23	1.79	9
	Fertilized	10,927	45	107	14	1.38	1.61	9

Differences in fine root biomass between plots were generally small, although plot one also showed a negative response to fertilization. While the response ratios in plots two and three overlapped zero, the plot one ratio was -0.37 (Figure 3.2a), reflecting the lower median fine root biomass in the fertilized subplot (0.94 g) versus the control (1.46 g; Table 3.2). Fine root biomass difference tracked with aboveground biomass difference in plot one, where the fertilized plot soil was drier than the control, while fine root biomass lagged aboveground production in the other plots.

As a starting point a Bayesian regression model was fit to test the effect on soil C of only two factors, treatment and a plot-level covariate on plot. As expected given the lack of response of soil C to the N fertilization treatment in our results, the model provided little information to explain the variance of soil C. Regressing the actual soil C data (y) against predicted or ‘replicated’ data (y_{rep}) such that $p(y_{rep} | y)$ showed low predictive ability (Figure 3.4a). The estimated effect of N fertilization treatment (natural log scale) on mean soil C was 3.1. The standard deviation associated with the plot parameter was 0.37, and the residual standard deviation was 0.53. A posterior-predictive check, wherein 100 sets of random draws were made from the model-predicted distribution of soil C, and compared to random draws from the actual distribution, showed the weakest agreement of any model we tested (Figure 3.4b).

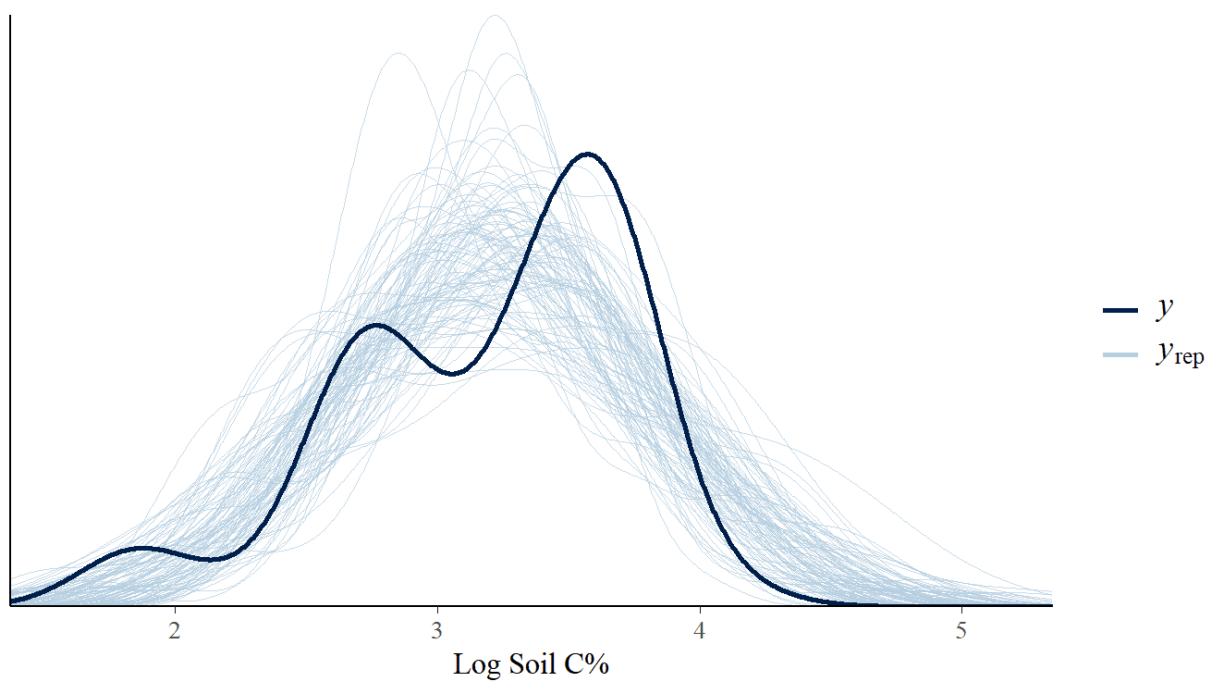
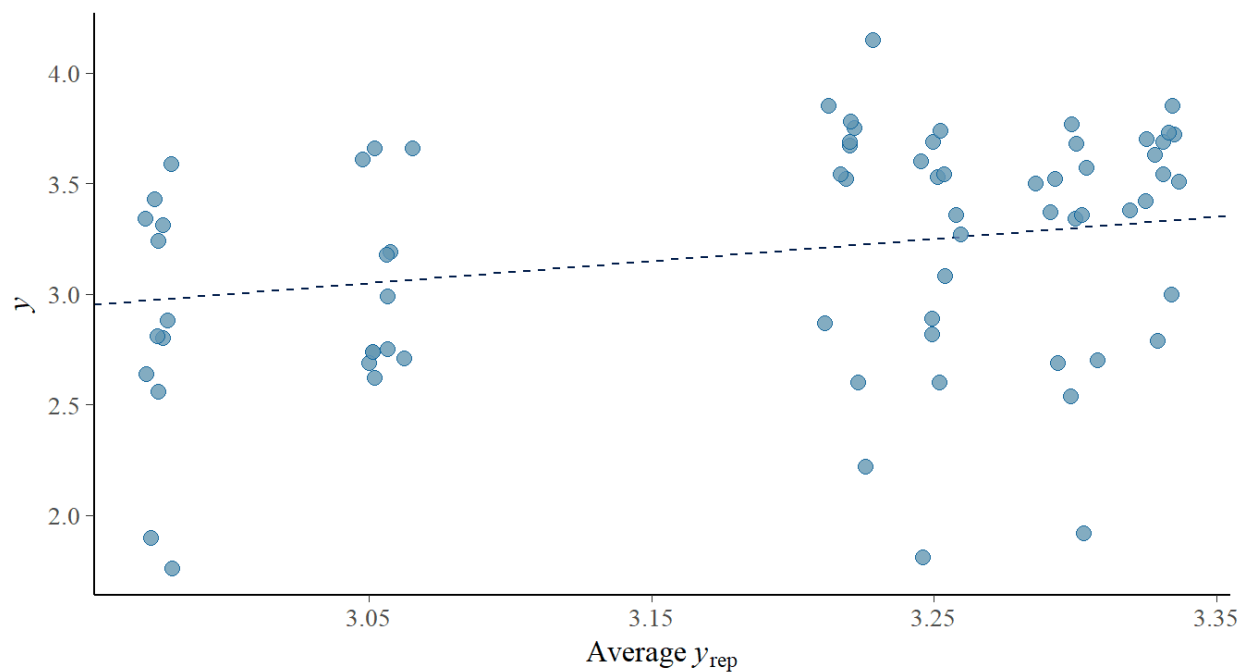


Figure 3.4. Scatterplot of observed soil C (y) over replicated soil C (y_{rep}) from the posterior predictive distribution (a) and posterior predictive check comparing 100 sets of random draws from y_{rep} , the replicated soil C data, with random draws from y the observed soil C data (b). Model: $\log \text{soil C} \sim \text{treatment} + (1 + \text{treatment} | \text{plot})$.

Adding a predictor variable to the model representing soil N greatly improved the predictive ability of the model. A model which used treatment and soil N at the sample level, and plot at the plot level, replicated the actual data with much better predictive power (Figure 3.5). The estimated effect of soil N (log) on mean soil C was 4.2 and treatment was 3.1. The standard deviation associated with the plot parameter was 0.19, and the residual standard deviation was 0.16, showing much less unmodeled variance than the previous model.

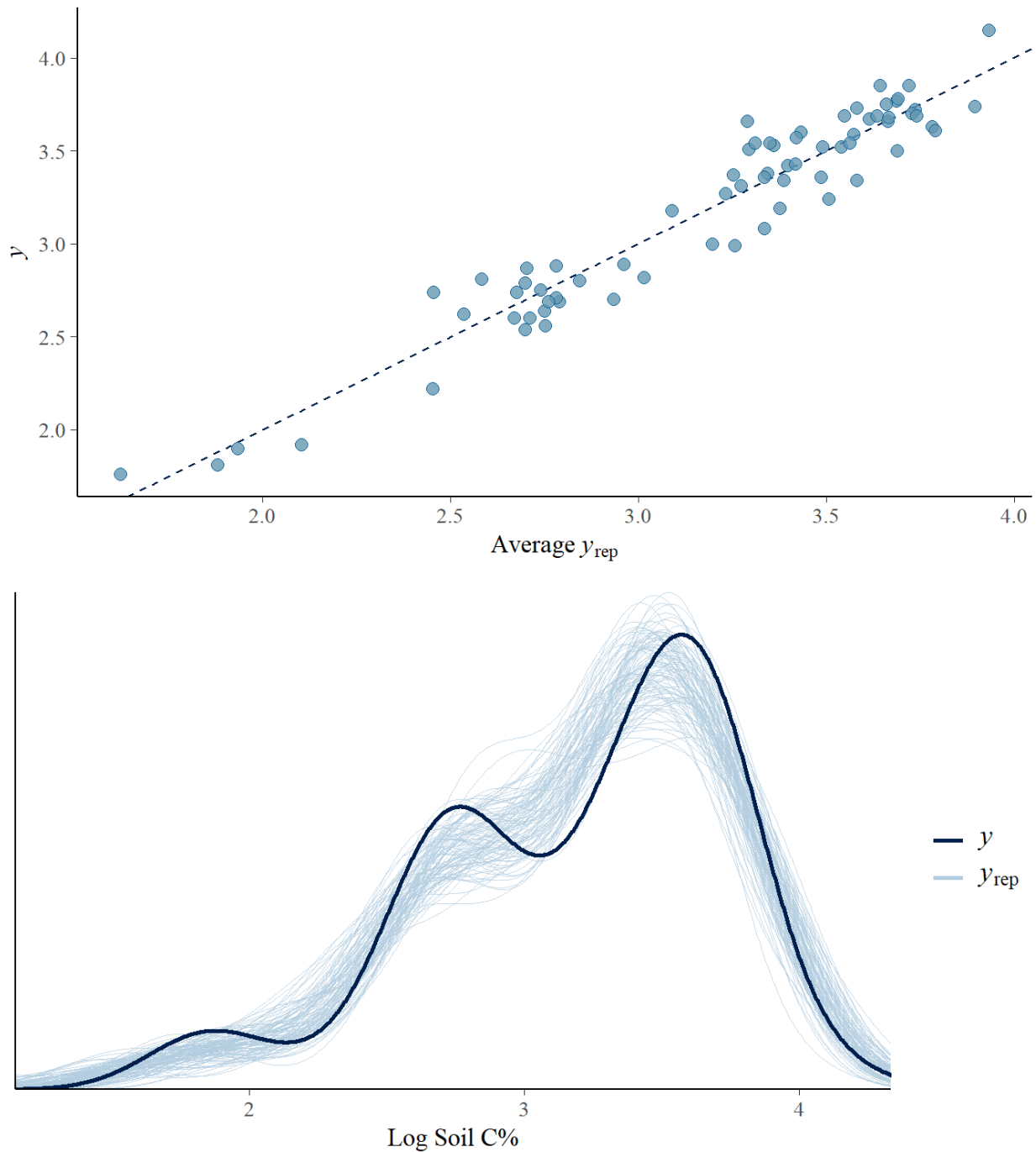


Figure 3.5. Scatterplot of observed soil C (y) over replicated soil C (y_{rep}) from the posterior predictive distribution (a) and posterior predictive check comparing 100 sets of random draws from y_{rep} , the replicated soil C data, with random draws from y , the observed soil C data (b). Model: $\log \text{soil C} \sim \text{treatment} + \log \text{soil N} + (1 + \log \text{soil N} \mid \text{plot})$.

A comparison of all models tested revealed information about the strength of covariates in predicting soil C at Loch Vale. The lowest-performing model only included

treatment and plot and had an ELPD difference of -87.9. The model that added soil N to treatment and plot had an ELPD difference of -0.9, indicating that treatment and plot-level variance together contributed little to predicting mean soil C, but that adding N greatly improved predictive ability. We then added several other covariates to the treatment, soil N, plot model, and each of them marginally improved predictive ability (Table 3.4). The highest-ranked model incorporated aboveground biomass and soil pH, while the model that included those but also added foliar C:N ratio and nitrification rate performed essentially just as well, but with a 0.3-higher SE, probably indicating collinearity between the predictors. The model that used plot, soil N, aboveground biomass, and pH but not treatment also performed as well, but with a 0.4-higher SE.

Table 3.4. Model rankings of the 20 Bayesian hierarchical regression models tested. Each model is identified by the covariates included, with parenthetical covariates acting on intercepts only. The models are ranked first by lowest score on the estimated least-squares means (ELPD) difference, then by lowest standard error (SE) difference, then by lowest number of components.

Model Covariates	ELPD difference	SE difference	No. of components
Treatment + Soil N + (Plot) + (Soil Moisture) + (pH)	0.0	0.0	5
Treatment + Soil N + (Plot) + (Root C:N)	-0.1	0.7	4
Treatment + Soil N + (Plot) + (EOC)	-0.2	0.4	4
Treatment + Soil N + (Plot) + (Root C:N) + (Soil Moisture)	-0.2	0.2	5
Treatment + Soil N + (Plot) + (NO ₃ ⁻)	-0.2	0.4	4
Treatment + Soil N + (Plot) + (Foliar C:N)	-0.2	0.4	4
Treatment + Soil N + (Plot) + (Aboveground BM) + (pH)	-0.2	0.4	5
Treatment + Soil N + (Plot) + (Foliar C:N) + (Aboveground BM) + (pH) + (Nitrification Rate)	-0.2	0.3	7
Treatment + Soil N + (Plot) + (Soil Moisture) + (pH) + (Root C:N)	-0.3	0.2	6
Treatment + Soil N + (Plot) + (Aboveground BM)	-0.3	0.4	4
Treatment + Soil N + (Plot) + (pH)	-0.4	0.4	4
Treatment + Soil N + (Plot) + (Soil Moisture)	-0.4	0.2	4
Treatment + Soil N + (Plot) + (Foliar C:N) + (Aboveground BM) + (pH)	-0.4	0.2	6
Treatment + Soil N + (Plot)	-2.1	1.9	3
Treatment + (Plot)	-89.9	8.7	2

The top-ranked model combined treatment and soil N predictors with plot-level predictors for soil pH, aboveground biomass, and plot. The model predicted soil C marginally better than the model that only used treatment, soil N, and plot (Figure 3.6). The estimated effect of soil N (log) on mean soil C was 4.2 and treatment was 3.1. The standard deviation associated with the plot parameter was 0.15, with soil pH was 0.10, and with aboveground tree biomass was 0.11. The residual standard deviation was 0.15, the lowest of any model tested (though by only 0.01) meaning it had the least unmodeled variance of any model, but by a small amount.

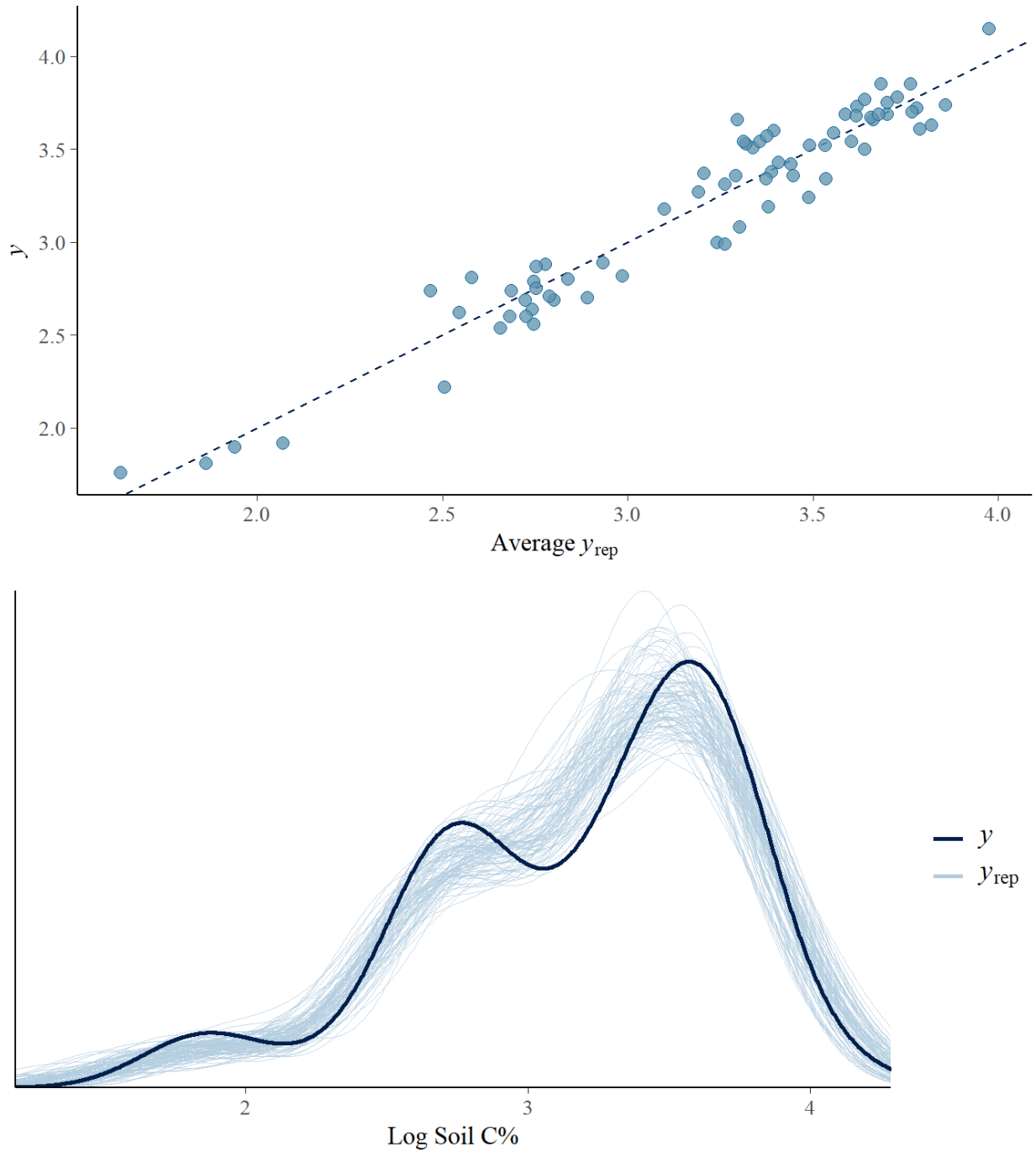


Figure 3.6. Scatterplot of observed soil C (y) over replicated soil C (y_{rep}) from the posterior predictive distribution (a) and posterior predictive check comparing 100 sets of random draws from y_{rep} , the replicated soil C data, with random draws from y the observed soil C data (b). Model: $\log \text{soil C} \sim \text{treatment} + \log \text{soil N} + (1 + \log \text{soil N} \mid \text{plot}) + (1 + \log \text{soil N} \mid \text{aboveground tree biomass}) + (1 + \log \text{soil N} \mid \text{soil pH})$.

Using a predictive model to estimate out-of-sample data demonstrates the flexibility necessary for the model to represent the system under varied conditions. The data collected at our sites in 2012 was used to compare the model's ability to predict out-of-sample data with training data (the data collected in 2015-2019). Due to limitations in what data were collected in 2012, the only model tested was the one that used treatment, soil N, and plot. Since such a model performed nearly as well at predicting soil C on the training data as the highest-ranked model, this test is an adequate representation of predictive ability. The estimated effect of soil N (log) on mean soil C was 4.0 and treatment was 3.0. The standard deviation associated with the plot parameter was 0.15, and the residual standard deviation was 0.16.

Out-of-sample datasets also help determine the inherent uncertainty in the model. The ELPD-difference of the model on out-of-sample data was 9.5 less than on the training data, meaning that the model better predicted out-of-sample data than training data. This is probably due to the significant and plot-consistent treatment effect observed in 2012 (see Boot et al., 2016), making the treatment variable more predictive on the 2012 dataset. Regression of $p(y_{rep} | y)$ showed high predictive ability, as did a posterior predictive check (Figure 3.7). Not surprisingly, the uncertainty in the modeled treatment effect is lower when the effect is more pronounced.

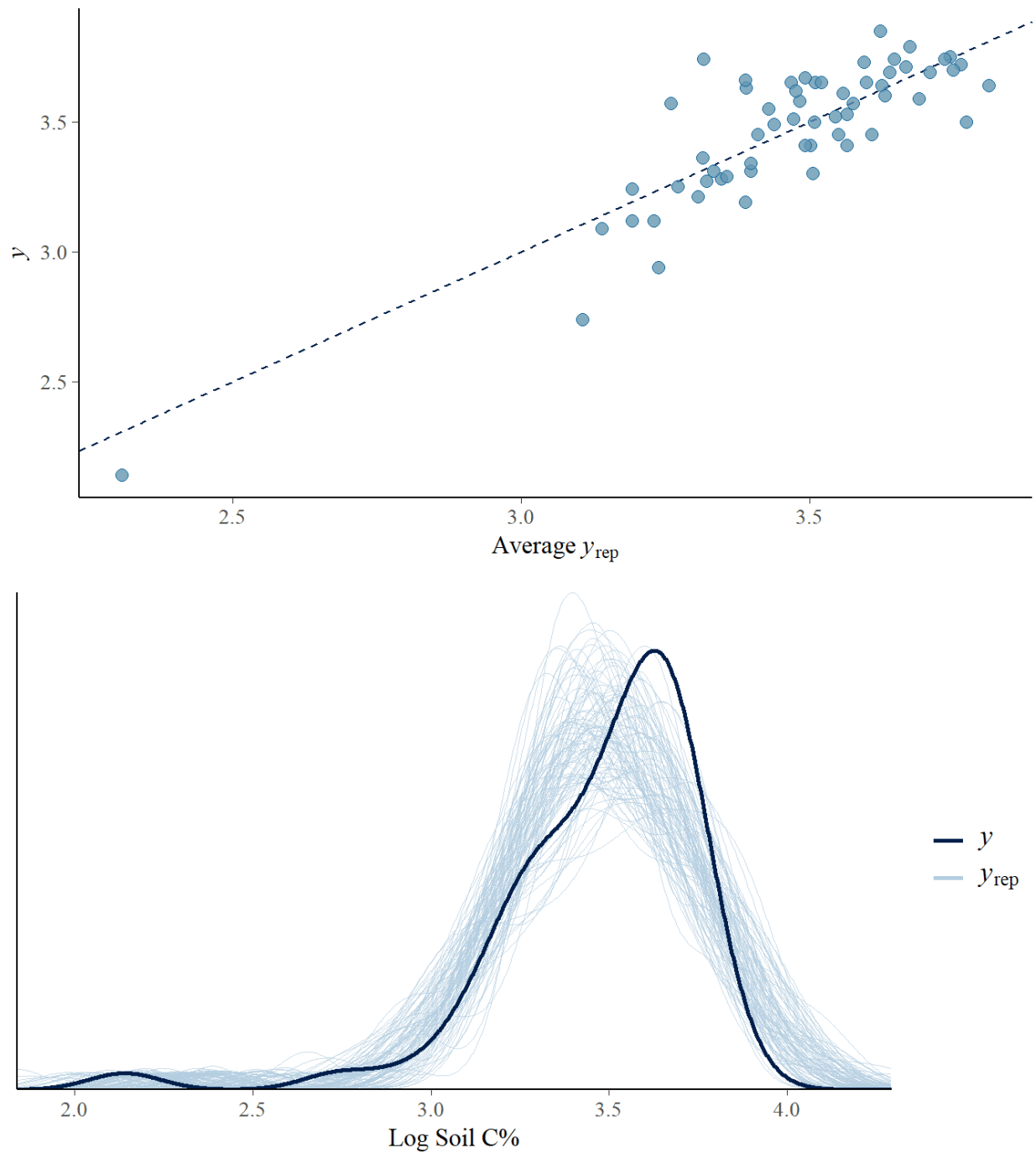


Figure 3.7. Scatterplot of observed soil C (y) over replicated soil C (y_{rep}) from the posterior predictive distribution (a) and posterior predictive check comparing 100 sets of random draws from y_{rep} , the replicated soil C data, with random draws from y the observed soil C data (b) using out-of-sample data collected in 2012 and analyzed in Boot et al., 2016. Model: $\log \text{soil C} \sim \text{treatment} + \log \text{soil N} + (1 + \log \text{soil N} \mid \text{plot})$.

Discussion

Global analyses of N fertilization experiments show a significant increase in forest soil C under elevated nitrogen due to increased aboveground C inputs and decreased C losses (Janssens et al., 2010; Liu & Greaver, 2010), or else an essentially flat response due to increases in both aboveground C input and soil respiration (Lu et al., 2011). The present study found essentially no difference between organic-horizon C between fertilized and control plots, but did find high variance in C response at the plot level (Figure 3.1a). Plot-level heterogeneity occurred across several covariates and can be used to explain some of the variance in soil C observed at Loch Vale.

Relationships between soil N and C

Soil N predicted soil C better than any of the plot-level covariates, although the relationship between soil C and N is complex and contingent. Where added N stimulates nitrification and consequent decline in soil pH, decomposition rates slow and C builds up in soil organic matter (SOM). But N can also alleviate growth limitation of the decomposer community, increasing C loss through respiration of SOM (Averill & Waring, 2018). Because SOM contains both C and N, patterns of soil C and N over heterogeneous space look similar at global (Post et al., 1985) and local scales (Smeglin et al., 2020). This may reveal a limit to the independence between soil C and N, violating the assumptions of single-level linear models. This study accounts for independence using a hierarchical approach which varies soil N at both the sample and plot levels to reduce correlation.

The relationship between soil C and N also varies depending on the level of N saturation in the ecosystem, with lower C:N ratios in soils where N input is greater (Aber et

al., 1998). We observed a treatment effect on C:N ratio only in plot one, where the control C:N ratio was 29, the highest of all our plots. If N fertilization alleviated N limitation on decomposition in that plot we would expect to see soil C and C:N ratio decline, as observed (Figure 3.1). We would also expect the observed increase in nitrification in that plot (Figure 3.3a). However, if the plant community in plot one was N limited, we would expect to see an increase in plant biomass associated with N fertilization, which did not occur (Figure 3.3b). Plant biomass in plot one may not be limited by N, as it seems to be in plots two and three.

Plant biomass contributions to soil C

We modeled plant exports to soil using measurements of foliar and fine root C:N ratios, fine root biomass, and aboveground tree biomass including live and dead trees and downed logs. Senescent foliage, fine roots, and dead wood undergo decomposition by soil organisms, which leads to loss of C through respiration as well as stabilization of C in microbial products and soil aggregates incorporated into SOM (Cotrufo et al., 2015). Root exudates were not measured but are thought to transfer a small proportion of fixed C to soils (Phillips et al., 2008) which may have a large effect on priming the decomposition of SOM (Keiluweit et al., 2015). Of the plant covariates measured, root C:N ratio and aboveground tree biomass contributed to the high-ranking models (Table 3.4).

Whether the C:N ratio of plant inputs contributes to the C or N content of SOM is a matter of some controversy in the literature (discussed in Liang et al., 2017), but inputs with a lower C:N ratio decompose more quickly (Hobbie, 2005). This study found fertilization produced a small response of root C:N ratio in plot one, and no response to root or foliar C:N in other plots (Figure 3.1). Both foliar and root C:N marginally improved

model predictions when combined with soil N, treatment, and plot, and the second best-performing model included root C:N ratio. Although other long-term N fertilization work has associated N addition within increase in fine root mass in the organic soil horizon (Carrara et al., 2018), we found no difference in fine root biomass between treatments or among plots. We did find that aboveground tree biomass responded relatively strongly to fertilization in plots two and three, with log response ratios > 1 (Figure 3.3b). In addition aboveground tree biomass contributed to the prediction of soil C well enough to figure into the final model (Table 3.4).

The response of tree biomass to fertilization in plots two and three suggests plant N limitation in those plots, in contrast to plot one. The response of soil moisture broadly followed the same pattern, with a smaller difference in soil moisture between the fertilized and control units of plot one, and a greater difference in plots two and three (Figure 3.3b). The median gravimetric soil moisture contents in all three control units were approximately equal (0.32 - 0.34), while the fertilized unit of plot one was drier (0.26) and the fertilized units in plots two and three were wetter (0.74, 0.84; Table 3.1). Possibly, moisture limitation in plot one prevented a biomass response to N fertilization, in contrast to the other plots. When both aboveground tree biomass and soil moisture were combined in one model they each explained the same amount of variance and showed high autocorrelation in trace plots, suggesting a strong link between soil moisture and tree biomass. A recent study of soil C drivers across spatially heterogeneous terrain in a broadleaf forest found soil moisture was the strongest C predictor tested, with soil C positively correlated with increasing number of dry days (Smeglin et al., 2020). Here soil moisture seems to positively correlate with higher soil C, however tight coupling between

tree biomass and soil moisture makes it impossible to say whether soil moisture as such predicts soil C, or whether a soil moisture effect on tree biomass actually drives soil C. The response of tree biomass to N fertilization seemed to depend on adequate soil moisture in the plot, and soil moisture and pH, which did not respond strongly to fertilization, best predicted soil C.

Soil C and N cycling pathways

The N saturation hypothesis predicts increased leaching of NO_3^- over time as plant uptake slows due to limitation by other nutrients or physical factors (Aber et al., 1998). Such increases have been observed in long-term N fertilization experiments of conifer forests in, for example, the northeast USA (Magill et al., 2000) and northern Sweden (Tamm et al., 1995). At Loch Vale soluble nitrate was elevated in two plots after 19 years of N fertilization (Figure 3.3a). The log response ratio in plot one, where we observed no plant biomass response to fertilization, was 3.9, while in plot three, where we did observe a plant biomass increase, it was 3.4. In plot two, which also exhibited a plant biomass increase, the log response ratio was 0.4, with error bars overlapping zero. The weak response in plot two may indicate a continued high level of plant N uptake, a high retention of N in the soil, as observed in other long-term studies (Aber et al., 1998), or losses via another pathway. We did not measure nitrous oxide flux in these soils but note that plot two had the highest median soil moisture (Table 3.1), making losses via denitrification more plausible.

An analysis of soil C in these plots in 2011-2013 found that fertilization accounted for an 11% loss of C from treatment plots versus controls, and hypothesized on potential C loss pathways, including increased leaching of DOC (Boot et al. 2016). Elevated DOC flux has explained loss of soil C from bog systems under N fertilization (Bragazza, 2006) as well

as from N-saturated forest systems (Chen et al., 2015). However, we found no clear trend in lysimeter DOC concentration in treatment plots to explain loss of C via the DOC leaching pathway (Figure 3.2). Previous studies at Loch Vale have also measured soil respiration and found no significant differences between treatment and control plots (Advani, 2005; Allen, 2017). Possibly, soil C loss occurred in one or a series of pulse events, measurable in DOC, soil respiration, or both, but primarily in years between sampling events. We found no evidence however that soil C loss at Loch Vale came as a result of increased DOC leaching.

Although lysimeter DOC varied little over time, heterogeneity in EOC concentrations between plots, and perhaps the large negative fertilization response in plot 2, contributed to explaining soil C in modeling. That negative response in a plot where soil C was stable under fertilization may suggest that stabilization of EOC preserved soil C, per the MEMS framework (Cotrufo et al., 2013). However, there was no strong response of EOC to fertilization in plot three, where the fertilized plot had much higher soil C than the control. Although EOC was explanatory in modeling, it was less so than other factors and did not contribute to the final soil C model (Table 3.4).

Aber et al. (1998) hypothesized that mineralization rates increase under N fertilization as added N is incorporated in SOM and then decomposed, and they found many examples where this occurred for a number of years before mineralization rates declined. However, the effect has not been consistently observed in conifer forests (Magill et al., 2000; Högberg et al., 2007). At Loch Vale elevated mineralization occurred with fertilization, particularly in plot one (Figure 3.3a) where the log response ratio was 1.6. This elevated mineralization corresponded with lower soil C and lower soil C:N ratio (Figure 3.1), tracking with the hypothesis that N mineralization results from the

mineralization of SOM with greater N-content. It is interesting to observe that elevated N mineralization was measured 19 years after N fertilization began, apparently making Loch Vale an outlier among N fertilization studies, in terms of the longevity of N mineralization effect. Plot two also had elevated N mineralization (log response ratio = 0.9), although the confidence intervals for lowered soil C and soil C:N ratio overlapped zero (Figure 3.1). Mineralization seemed to explain the same variance in the model as nitrification, although slightly less well, and neither factor contributed to the final model (Table 3.4).

The results of investigation into C and N pathways under N fertilization generally seemed to support the N saturation hypothesis. They diverged from previous studies, however, in some respects, including the timing of effects regarding N transformations and the presence of effect regarding soluble C. Ultimately, these transformations did not contribute as much predictive or explanatory power to the model as did soil N, aboveground tree biomass, and soil pH.

pH and soil C

The data show essentially no impact of fertilization on soil pH, with mean control pH of 4.8 compared to a mean treatment pH of 4.7. Heterogeneity was present in the pH data, with a range of plot-level means from 4.6 - 5.0 (Table 3.1), comparable to previous soil pH measurements made throughout the forested areas of the watershed (mean pH = 4.9; Oropeza, 2008). Despite the lack of fertilization effect, the variance in pH was predictive of variance in soil C and figured into the top model (Table 3.4).

Soil pH has been shown to drive carbon use efficiency (CUE) in soil microbial communities, as well as shape fungal community structure (Zhang et al., 2020). In turn, CUE is hypothesized as a major driver of SOM stabilization within a given soil matrix

(Cotrufo et al., 2013). The CAMP (Carbon, Acidity, Mineral Protection) framework predicts that when N fertilization results in a soil pH decline, soil C will increase due to suppression of decomposition and a build-up of particulate organic matter (POM). However when N fertilization does not impact soil pH, CAMP predicts that soil C should remain steady as increased decomposition of POM is balanced out by increased mineral stabilization of microbial products (Averill & Waring, 2018). Overall our findings of nil impacts to pH and soil C fit with the CAMP framework, although in plot two we did observe a 0.3 unit drop in pH of the fertilized unit versus the control, without a commensurate increase in soil C (Table 3.1).

Conclusion

This research addressed the impact of N fertilization on organic horizon soil C at Loch Vale after a twenty-year N fertilization experiment. Overall, N fertilization did not alter organic horizon soil C, in contrast to previous work at Loch Vale. Results of a soil C study in 2011-2012 indicated that soil C was lost in response to N fertilization, but this research suggests that those results captured the soil C response at a moment in time, and by 2018 differences in soil C between fertilized and control plots were no longer detectable. This dynamic response of soil C over time may have resulted from environmental controls on soil C which mediated the response to N fertilization. This study used a Bayesian linear modeling approach to identify possible environmental controls.

Soil N exerted the strongest control on soil C, but is not truly independent of soil C, as both derive from SOM. Among the other covariates measured, root C:N ratio, soil moisture, aboveground tree biomass, and soil pH exerted the strongest control on soil C, after soil N, as evidenced by the increased predictive ability of models employing those

covariates. Based on these findings I hypothesize that plant demand for N exerts an important control on soil C storage under N fertilization. Where tree roots have higher N per unit C, more C is stored in soils, and tree root C:N ratios are lower where soil moisture is higher. Thus, colimitation of water and N for tree growth may ultimately predict the fate of soil C at Loch Vale.

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