

THESIS

THE EFFECTS OF LONG TERM NITROGEN FERTILIZATION ON FOREST SOIL
RESPIRATION IN A SUBALPINE ECOSYSTEM IN
ROCKY MOUNTAIN NATIONAL PARK

Submitted by

Jordan Allen

Graduate Degree Program in Ecology

Impartial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2016

Master's Committee:

Advisor: A. Scott Denning

Co-Advisor: Jill Baron

Mike Ryan

Gillian Bowser

Copyright by Jordan Allen 2016

All Rights Reserved

ABSTRACT

EFFECTS OF NITROGEN FERTILIZATION ON FOREST SOIL RESPIRATION IN A SUBALPINE ECOSYSTEM IN ROCKY MOUNTAIN NATIONAL PARK

Anthropogenic activities contribute to increased levels of nitrogen deposition and elevated CO₂ concentrations in terrestrial ecosystems. The response of soil respiration to nitrogen fertilization in an on going 18- year field nitrogen amendment study was conducted from July 2014 to October 2014. The focus of this study was to determine the effects of nitrogen fertilization on soil carbon cycling, via respiration. Our objectives were to (1) test the hypothesis that N additions would increase soil respiration in Rocky Mountain National Park, and (2) understand the impacts of N additions on carbon flows in subalpine forests. A LiCor LI-820 infrared gas analyzer (IRGA) was used to quantify soil respiration rates. We compared soil respiration from fertilized forest plots (30 x 30 m) with soil respiration from control forest plots (30 x 30 m) that receive only ambient nitrogen deposition (3-5 kg/ N/ha⁻¹/yr⁻¹) during the 2014-growing season. Our results shows that mean soil respiration measurements were not significantly different in the control plots (3.14 μmol m⁻² sec⁻¹) than in the fertilized plots (3.02 μmol m⁻² sec⁻¹).

Treatment was insignificant in influencing soil respiration (p-value greater than 0.5), allowing us to reject our primary hypothesis: that nitrogen additions would lead to an increase in soil respiration. Our results confirm previous research in these plots Advani (2004). The statistically identical soil respiration rates between the control and fertilized plots may result from nitrogen saturation due to elevated levels of ambient N deposition,

microbial suppression due to very high levels of N additions in the fertilized plots, or some combination of the two.

ACKNOWLEDGEMENTS

As I embarked on this journey to complete a graduate degree at Colorado State University, my goals were to develop as a scholar, work with a collaborative/interdisciplinary research group, ultimately finish and pursue a career in science. I really appreciate the guidance and mentorship from my committee Dr. A. Scott Denning, Dr. Jill Baron, Dr. Mike Ryan and Dr. Gillian Bowser. I am grateful for your support. I also, would like to thank Daniel Bowker for helping me throughout my field season. Most importantly, I would like to thank my family and friends for their continuous support throughout my pursuit of continuing my education. Lastly, the National Science Foundation under Award Number 0425247 Center for Multiscale Modeling of Atmospheric Processes (CMMAP) funded this research.

TABLE OF CONTENTS

ABSTRACT	2
ACKNOWLEDGEMENTS	4
INTRODUCTION	6
METHODS	10
RESULTS	18
DISCUSSION	21
REFERENCES	41

1. Introduction

1.1 Nitrogen Deposition

The Colorado Front Range population growth, land use change and agricultural practices have increased nitrogen deposition through emissions from power plants, vehicles, agricultural fertilizers and livestock (Baron et al., 2004). Deposition occurs by three processes: (1) Wet deposition from precipitation, which delivers dissolved nutrients; (2) Dust or aerosols by sediments known as dry deposition; and (3) Cloud-water deposition as water droplets onto plant surfaces (Chapin et al., 2011).

Many human activities increase the conversion of mostly inert N₂ in the atmosphere to reactive N, and this reactive N is then deposited into terrestrial ecosystems. Since the Industrial Revolution, nitrogen (N) deposition the input of reactive nitrogen species from the atmosphere to the biosphere has increased at a rapid rate (Galloway et al., 2003). The chemicals in nitrogen deposition are derived mainly from nitrogen oxides (NO_x) and ammonia (NH₃) emissions. Human activities now fix more atmospheric nitrogen into reactive compounds than all reactive N created by natural processes (Neff et al. 2002).

1.2 Nitrogen Deposition Impacts

Nitrogen deposition impacts terrestrial ecosystems in many ways (Agren et al., 1988; Asner et al., 1997; Currie et al., 1999). Productivity increases leaf area index (LAI) and photosynthesis, lower belowground flux, soil carbon storage, and decrease decomposition. There are many potential mechanisms N deposition may alter for example biological systems such as lakes, trees, and microbes in pristine mountain systems are influenced by excess nitrogen deposition (Williams et al., 2000; & Friedland et al., 1991). Most importantly in the Colorado Front Range, Baron et al., (2000) found that slight increases in nitrogen deposition (3-5 kg N y⁻¹)

led to changes in ecosystem properties C: N ratios in soils and lake uptake of nitrogen. Long-term consequences of excess N deposition can move forests from N - limited state towards N saturation (Aber et al.1995).

1.3 Nitrogen depositions and the Carbon cycle

Nitrogen deposition may cause soil to respire more CO₂ (Janssens et al., 2010). The rate of carbon storage has to increase or change at a dramatic rate for change. Small changes in large pools of carbon can have a dramatic impact on the CO₂ content of the atmosphere, if they are not balanced by simultaneous changes in other components of the carbon cycle (Pan et al., 2010). Literature suggests that the impact of nitrogen deposition on carbon sequestration in forests is very uncertain and may vary. Even though Magnani et al., (2007), found boreal and temperate forests increase their carbon sequestration in response to nitrogen deposition. Sutton et al., (2008) found that net primary production (NEP) was not driven overwhelmingly by nitrogen deposition. Studies have shown that nitrogen deposition alters carbon cycling in soils, influences changes in microbial activity (Waldrop et al., 2004) and makes a small contribution to carbon sequestration in temperate forests, but there is still uncertainty whether elevated nitrogen deposition is the main cause (Nadelhoffer et al., 1999). The long term effects of N deposition on soil respiration will help us understand the impacts of forest ecosystem response to N deposition. To predict this response we will investigate how forest soils process additional nitrogen inputs.

Photosynthesis requires a catalytic enzyme called **ribulose biphosphate** carboxylase/oxygenase (rubisco) (Bonan, 2008). At the chloroplast level, the maximum rate of photosynthesis is directly proportional to the concentration of rubisco, and therefore to leaf N content. Under N-limited conditions, we therefore expect additions of bioavailable N to cause elevated rates of photosynthesis. Greater photosynthesis should lead to increased production of

leaves, stems, and roots, which should in turn increase the rate of dead plant material through enhanced litter production both above and below ground. Microbial decomposition should therefore be enhanced as litter pools accumulate, leading to greater microbial respiration. In addition, root respiration should respond to N additions as a consequence of greater plant production and increased root biomass.

Past studies have shown that increased forest growth and carbon accumulation responses under low to moderate Nitrogen additions (Vitousek et al., 1991, Berg et al., 1999 & Franklin et al., 2003). However, some studies have shown that microbial activity may be suppressed by high levels of reactive N loading, leading to decreased soil respiration (Bowden et al., 2004; Burton et al., & 2004; Frey et al., 2014).

1.4 Soil Respiration

Soil respiration is the one of the most important components of ecosystem respiration. It is linked to photosynthesis, litter fall and plant metabolism because of belowground activity by both autotrophic and heterotrophic activity (Ryan 1991). Autotrophic respiration is defined as root growth and rhizo-microbial respiration. Heterotrophic is defined as litter, labile soil organic matter and stable soil organic matter. It is still very much uncertain why some soil organic matter persists for a long period of time and some decomposes fast (Schmidt et al., 2001). Soil respiration is primarily an indicator of plant response, because flux of C from any response of the soil C pools is small in comparison to the flux from the autotrophic pool and material recently produced by plants (Giardina & Ryan, 2002; Giardina et al., 2004; Ryan et al., 2004; Ryan & Law, 2005). There is limited understanding of belowground microbial activity and how this activity is linked to soil processes. Total belowground carbon allocation can be estimated using a

carbon balance approach, which measures root respiration, soil respiration, erosion, leaching and any change in carbon storage (Giardina et al. 2002).

1.5 The effects of N fertilization on Soil Respiration

In this study we hypothesized that N fertilization to subalpine forest soils in Rocky Mountain National Park would respire more carbon than non-fertilized soils only receiving ambient N deposition. Assuming that microbial biomass growth is accompanied by an increase in respiration, we expect soil to be N limited, microbial activity would be stimulated and an increase of soil CO₂ efflux would occur following monthly N fertilization. If the soils are not N limited we did not expect microbial activity to increase and soil respiration to respond to N fertilization.

Our goal was to understand the effects of long-term nitrogen deposition on forest soil respiration in Rocky Mountain National Park. Primary production by forest ecosystems is usually nitrogen limited, meaning that adding nitrogen causes an increase in photosynthesis (LeBauer et al., 2008). We therefore expect N deposition of anthropogenic reactive nitrogen in Rocky Mountain National Park will lead to increased photosynthesis and greater carbon storage in biomass and litter. Over time, increased carbon storage in litter and soils should lead to increased respiration from forest soils. To test this hypothesis, we measured soil respiration in forest plots that had been fertilized for 18 years, and compared the results to control plots that received only ambient nitrogen deposition. This particular study is important because we want to assess potential impacts of N fertilization on subalpine forests and compare results found in 2003 and 2004. This is not the first time these plots were used to measure the response of soil respiration impacted by N fertilization. Advani (2004) found no significant difference in soil respiration in response to N fertilization between fertilized and control plots. Nitrogen

fertilization studies have shown increases, reduction and no response in soil respiration (Bowden et al., 2000, Kan et al., 2003, Magill et al., 2004, and Frey et al., 2014). It is still misunderstood if high, moderate and low level nitrogen fertilization cause more carbon to be stored. Root respiration is altered by N concentrations (Burton et al., 1996 Ryan et al., 1996). Microbial respiration could decrease in response to N additions, but foliar mass and litter inputs are likely to increase (Ryan et al., 1996). There have been many studies that have studied short-term effects of N fertilization on soil respiration. (Abner et al., 1998, Fauci et al., 2004, Micks et al., 2004). It is important for scientists to move this research forward to better understand long-term effects of N deposition on soil respiration to be able to predict impacts of soil carbon fluxes and storage. The purpose of this study is to (1.) Compare soil respiration rates and seasonal patterns in long term nitrogen fertilized plots and unfertilized control plots in subalpine spruce forests. (2.) To quantify and compare the effects of soil temperature and soil moisture on soil respiration in nitrogen fertilized plots and unfertilized control plots. (3.) Understand if nitrogen additions change carbon cycling: does more nitrogen cause increase carbon storage and therefore increase soil respiration? It is important to understand how soil respiration is altered because small changes in respiration rates may change atmospheric concentrations of CO₂ and rates of carbon sequestration. This research will help us better understand Nitrogen fertilization on soil carbon storage and accurately predict the net response of terrestrial ecosystems to N deposition.

We revisited the treated and untreated plots of Advani (2004) to determine the effects of an additional 11 years of N additions to the ecosystem. Experimental methods are presented in section 2 below. Our results are presented in section 3, and discussed in section 4.

2. Methods

2.1 Site Description

This study was conducted in three old-growth Engelmann spruce (*Picea engelmannii*) and subalpine fir stands in the Loch Vale watershed (LVWS) in Rocky Mountain National Park, Colorado, USA (40.3333° N, 105.7089° W). The area is remote, and accessible year round. The elevation varies slightly across the study sites ranging from 3000 – 3200 m. The terrain is very rocky and the organic soil is very thin. A more thorough site description is found in Rueth et al. (2003). The average annual precipitation is 100 cm, and approximately 70% accumulates in a seasonal snowpack between November and April (Baron, 1992).

2.2 Plot Establishment

The forest fertilization studies were initiated to ask: 1) if additional N would lead to nitrogen saturation; and 2) what would be the effects on tree and shrub foliage chemistry, soil microbial activity, soil chemistry, and soil leachates. A total of six 30 x 30 m plots were established in June 1996. Pairs of adjacent plots are fertilized (LVF1, LVF2, LVF3) and control (LVC1, LVC2, LVC3) (Figure 2.). Fertilized plots received 25 kg N ha⁻¹ yr⁻¹ starting in 1997 and continuing to the present as ammonium nitrate (NH₄NO₃) pellets, while control plots received only ambient atmospheric nitrogen deposition of 3 to 5 kg N ha⁻¹ yr⁻¹ (Rueth et al., 2003). Plots were accessed in the summer using hiking trails.

2.3 Procedure

We installed seven PVC collars (Figure 3) in each plot using a knife to cut into the soil and a rubber mallet to pound each collar into the ground. The average depth of the collars in the soil was approximately 2-4 cm. To avoid bias each collar was randomly placed in the 30 x 30 m plot.

The collar is 1.8 cm thick and 25.4 cm in diameter. We waited a week after the installation of the collars to start measurements, hoping to avoid influencing respiration by disturbing soils.

Carbon dioxide flux measurements began in July 2014 and continued through October. Measurements were conducted at last once per month from July through October for a total of 195 flux measurements. Our experimental design is similar to methods found in Norman et al. (1997) and is described briefly below.

Soil carbon dioxide fluxes were estimated by measuring the rate of CO₂ build up in a chamber created by capping the PVC collars with a foam lid (Fig 4). Measurements of CO₂ in the chambers were collected using a LiCor LI820 infrared gas analyzer (IRGA). The LiCor was connected to a Campbell Scientific data logger, which was programmed to record CO₂ concentrations every two seconds in air pumped in a tube from the IRGA to the chamber open only to the soil in the collar. The IRGA was calibrated before each measurement using 400 ppm CO₂ as the ambient air standard. To make sure these flux measurements are not biased by CO₂ concentration gradients between the chamber and the air, we scrubbed the concentration within the chamber down to just below ambient CO₂ concentration using a second airflow tube to pump the air from the chamber through a soda lime trap. Soil respiration then caused the concentration to build up again and we measured the rate of change in concentration close to ambient levels (Figure 5). Linear regressions (concentration versus time) were used to determine rates of CO₂ flux. Soil CO₂ flux in $\mu\text{mol m}^{-2} \text{sec}^{-1}$ was obtained by taking the slope of the line of CO₂ concentration (parts per million) as a function of time, and multiplying it by the volume of the chamber divided by its surface area, correcting for temperature and pressure to obtain respiration flux in $\text{moles m}^{-2} \text{s}^{-1}$ (see section 2.5 below). The rate of CO₂ build up was almost perfectly linear. Soil moisture measurements were taken at each collar while soil respiration was being

measured. We intended to collect weekly soil temperature measurements using a Penetration Thermocouple Probes “T” style 304 with a stainless steel handle, but it did not work. Instead we used hourly measurements of air temperature, which were taken at the USGS Main Loch Vale Weather Station a few hundred meters away from the experimental plots. Soil moisture was measured with a hand held Hydrosense time domain reflectometer (TDR) probe that measured soil moisture in percent volumetric water content (VWC). Measurements were conducted during the growing season between the dates of July 21 and October 20 in 2014.

2.4 Full sampling cycle using LiCOR and soil collar

- 1) User initiates start – air flows through soda lime trap
- 2) Scrubbing of [CO₂] ppm until it is below Lower Boundary Sampling Level
- 3) Air flows through IRGA for duration of user determined Lag Time and until [CO₂] is above Lower Boundary Sampling Level
- 4) Datalogger records [CO₂] every two seconds during the Sampling Time for flux calculations.
- 5) Sampling record terminates.
- 6) Datalogger sends new data to storage module.

2.5 Flux Calculations

$$F = \frac{S * V * P / P_o * T_f / T}{(22.4 \text{ mol/L}) A_c}$$

Where **F** is flux in $\mu\text{mol m}^{-2} \text{sec}^{-1}$, **S** is slope in ppm/s, **V** is system volume in liters, **P** is ambient pressure in kPa, **P_o** is sea level pressure (101.32 kPa), **T_f** 273.15 K is freezing point, **T** is ambient temperature in Kelvin and **A_c** is Area of the collar in m².

2.6 Relationship between soil respiration and temperature

Past studies have shown an exponential relationship between soil respiration and temperature (Reich and Schlesinger 1992). We used the Q_{10} relationship/function developed by (van't Hoff 1898)

$$R = R_0 Q_{10}^{\frac{T-T_0}{10}} \quad (1) .$$

This function is used to predict or simulate the temperature response of soil respiration. For most biological systems the Q_{10} parameter is approximately 2, meaning the respiration rate doubles for every 10°C increase in temperature (Lloyd and Taylor 1994). We used linear regression to estimate the overall seasonal dependence of respiration on temperature. Each respiration rate was paired with a temperature measurement, and care was taken to sequence field measurements so that each chamber was sampled at different times of day (different temperatures). At the end of the field season, we used a linear regression model to fit the coefficients R_0 and Q_{10} in equation 1.

2.7 Statistical Analysis

Linear regression analysis was performed using **R** statistical software version 3.0.1 (R Core Team. 2012) (See Table 1.). I analyzed respiration as a function of treatment, soil moisture, air temperature and interactions across data collected from all plots during the field season (See Table 2.). The effect of soil moisture and temperature on soil carbon flux was evaluated using these factors as covariates to the effect of fertilization.

3. Results

3.1 Soil Respiration

Plot mean soil CO₂ ranged between 2.1-to 3.1- $\mu\text{mol m}^{-2} \text{sec}^{-1}$ over the field season (Table 1). Rates are consistent with those measures during the first time these plots measured soil respiration Advani (2004). Mean soil respiration rates were highest during the first few months of measurements and lowest at the end of the growing season (Figure 6). There was no difference in respiration values between any of the plots or between fertilized and control (Table 1-2, Figure 6-8). Mean Respiration from fertilized Plot 1 and 3 soils did not increase significantly over control levels following fertilization in either plots (Figure 6 & 8) except in Plot 2 the control plot respiration rates were higher than the fertilized plots, but not significantly different (Table 1, Figure 7). Throughout the study period, fertilized soils respiration rates in Plot 2 were generally greater than the control soils. This may be due to the location of the plots and vegetation in the plots. Soil respiration, temperature, and moisture were statistically similar between fertilized and control plots over the entire summer (Table 2, Figure 9). During our field season the mean air temperature for the control plots was 11.09°C and the fertilized plots was approximately 11.15°C. The flux measurements were similar in the control plots at approximately 3.14 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ compared to 3.02 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ in the fertilized plots. Soil moisture was also similar in both the fertilized and control plots, at 0.29% and 0.28%, respectively (Figure 9).

We used linear regression to fit a base respiration rate (R₀) and temperature sensitivity (Q₁₀) to the respiration and soil temperature data from fertilized and unfertilized plots separately using Equation 1 (Figure 10). As temperatures increased the control plot respiration rates were slightly higher than the fertilized plots as seen by the two fitted lines

in Figure 10 (Red=Fertilized, Blue=Control). The base respiration rate R_0 was slightly higher ($8.2 \mu\text{mol m}^{-2} \text{sec}^{-1}$) in the control vs. the fertilized plots ($6.3 \mu\text{mol m}^{-2} \text{sec}^{-1}$). The temperature sensitivity parameter Q_{10} was higher (2.06) in the control plots compared to the fertilized plots (1.73). Soil respiration rates were higher in drier soils in both the control and fertilized plots (Figure 10).

4. Discussion

4.1 Long-term effects of N fertilization on soil respiration

We hypothesized that excess N fertilization would lead to subalpine forest soils in Rocky Mountain National Park to respire more carbon than non-fertilized soils only receiving ambient N deposition. Overall our results do not support our hypothesis. Even though Plot 2 average fertilized respiration rates are slightly higher than the control it is not significantly different. Long-term levels of ambient N deposition (3-5 kg N ha⁻¹ yr⁻¹) in the Rocky Mountain Region and additional N fertilization (25 kg N ha⁻¹ yr⁻¹) in the form of ammonium nitrate suggest that soils maybe N saturated even when fertilizer is applied.

The Statistical analysis was done using a linear regression model performed using **R** statistical software version 3.0.1(R Core Team. 2012)(See Table 1.). We used a t-test to evaluate differences in respiration between treatment, moisture and temperature. To compare treatment effects at each plot we compared each respiration measurement to treatments. The measurements were not significantly different, p-value was greater than 0.5. For statistical significance at the 95% confidence level, we need $p < 0.05$. This falsified our hypothesis that fertilized soils would respire more carbon than control soils.

We also used a t-test to evaluate differences in respiration between temperatures. The measurements show that temperature is a major factor in respiration rates. The p-value: $< 2.2e-16$. Next we used a t-test to evaluate differences in respiration between moisture. The measurements show that moisture was a major factor in respiration rates. The p-value: $7.299e-15$. Lastly, we used a multiple regressions model results to show that temperature and moisture are significant in soil respiration measurements because the p-value is < 0.001 . The effect of temperature on respiration was highly significant ($p = 1.81e-$

11), but adding moisture did not improve the model ($p = 3.78e-07$). Temperature alone explained 40% of variance in respiration; neither treatment nor moisture improves this (See Table 2 for results). There was a significant response of respiration at Loch Vale in both control and fertilized plots when either soil temperature or moisture was analyzed as a covariate. It is important to note, however, that seasonal cycles of plant growth (phenology) are also primary drivers of seasonality in respiration. It may be that the strong statistical relationships among soil respiration, temperature, and soil moisture are simply the result of correlated seasonal cycles in these variables. (See Figure 13)

4.2 Past Studies

Nitrogen fertilization at high levels have decreased microbial and fungal activities used very high levels of inorganic nitrogen for example Burton et al., (2004) added 30 kg N/ha/yr, Bowden et al., (2004) added 50 and 150 kg N/ha/yr, and Frey et al., (2014) added 50 and 150 kg N/ha/yr. In our study we used approximately 25 kg N/ha/yr, in our fertilized plots and control plots received ambient deposition 3 to 5 kg N/ha/yr. We have also documented similar respiration rates in these plots Advani (2004). The main reasons or mechanisms for why respiration rates are similar in both the fertilized and control plots in the Loch Vale watershed has not been identified. However, we have two ideas: (1) the ecosystem may already be saturated with N due to atmospheric deposition. Ambient N deposition has been high for decades, elevated levels of NO_3 and NH_4 in lakes and streams suggest N demand in forest is weak (Baron et al., 2000). This can be explained by, excess N, which can lead to nitrogen fertilization of vegetation, soils and water. In addition, short growing seasons at high elevation limit the amount of time plants have to absorb nitrogen during the year. Also granitic bedrock and shallow soils found in Rocky Mountain National

Park don't provide much chemical buffering (Nanus et al., 2009). (2) The mechanism of suppressed microbial activity may exist at our sites. There were decreases in soil C in our plots, which are driven by increased microbial degradation of soil organic matter and reduced inputs to soil organic matter in the form of microbial biomass (Boot et al., 2015). Microbial decomposition rates are influenced by soil pH, litter quality and nutrient availability, microbial activity and may cause for a reduction of soil respiration following long term nitrogen fertilization.

At our sites there is no evidence of respiration increasing more in fertilized soils compared to control soils. Soil respiration rates in subalpine spruce forests in Rocky Mountain National Park were similar in both the fertilized and control soils. Long-term nitrogen fertilization did not significantly change soil respiration rates. Soil temperature and moisture dependence was not different between control and fertilized plots. Also, our multiple regressions model results show that temperature and moisture are significant in soil respiration measurements because the p-value is < 0.001 . Measured insignificant changes in soil respiration rates at this site in response to N fertilization Advani (2004) are consistent with reduced microbial activity there were no changes in the C degrading enzyme activity due to N fertilization (Boot et al., 2015).

There are studies that support our findings that N fertilization doesn't significantly increase soil respiration (Magill et. al., 2004, Bowden et al., 2001, Allison et al., 2008). However, some studies have even shown a decrease in soil respiration in response to N fertilization (Kowalenko et al., 1976, Bowden et al., 2004, Olsson et al., 2005). A decrease in belowground carbon allocation can lead to a decrease in root respiration in forests with N fertilization (Giardina et al., 2004). We conclude from our testing sites the effects of

nitrogen fertilization in Rocky Mountain National Park suggests long-term nitrogen fertilization insignificantly decrease soil respiration in fertilized plots.

4.3 Comparison of 2004 and 2014 soil respiration measurements

Advani (2004) found that there was no significant difference between treatments in Loch Vale plots. In 2004 and 2014 show respiration rates are highest in July and lowest toward the end of the growing season in October. In 2004 mean respiration rates ranged between 2.5 and 3.97 ($\mu\text{mol m}^{-2} \text{sec}^{-1}$). In 2014 the mean respiration rates ranged between 2.1 and 3.1 ($\mu\text{mol m}^{-2} \text{sec}^{-1}$). However, the role of nitrogen deposition may be able to control how fast plants and microbes are decomposing organic matter in the Rocky Mountain Region (Bobbink et al 2010). Previous research in this subalpine forest ecosystem microbial community properties and soil carbon is altered by nitrogen fertilization. Fertilized soils had lower %C than controls soils and fertilized soils had lower microbial biomass C compared to controls soils (Boot et. al 2015). This supports our findings that increase in nitrogen in subalpine forests influences soil respiration, even though it may not be significantly different. The similarities between both sampling seasons 2004 and 2014 response to N shows that soil respiration did not respond significantly to N fertilization. Our respiration measurements in the summer of 2014 supports previous findings of insignificant response of soil respiration to N additions in 2004, which may support that these soils are N saturated. Research that can contribute to our understanding of soil organic matter turnover rates and how they are affected by added nitrogen can be used to provide insight into the correlations between nitrogen fertilization and soil respiration.

Has N deposition fertilized control plots? Due to increases in N deposition or fertilization N demand is met. There was no significant additional respiration response to fertilization. Even though the control plots are not receiving ammonium nitrate pellets, they are receiving ambient nitrogen deposition. The possibility that the soils may be saturated or microbial activity is suppressed is that control and fertilized soils have adjusted to maximum net primary production as excess N additions have been added over time.

Our findings suggest long-term nitrogen fertilization does not significantly affect soil respiration in fertilized and control plots. Soil respiration in both the control and fertilized plots followed a similar seasonal pattern, with the highest rates occurring in July the warm and wet growing season and the lowest rates in October. We also found significant effects of both soil temperature and moisture on soil respiration, but this was due to a strong correlation between temperature and moisture ($P < 0.001$). Microbial activity is affected by the changes in the availability of soil moisture (Orchard et al., 1983). We expected fertilization to affect carbon dynamics within the plots since old growth forest are sensitive to increase in nitrogen (Hedin et al., 1995). The lack of significant soil respiration response from fertilized plots and control plots shows our difficulty to determine how much N saturation or microbial suppression affect our results in the Loch Vale Watershed.

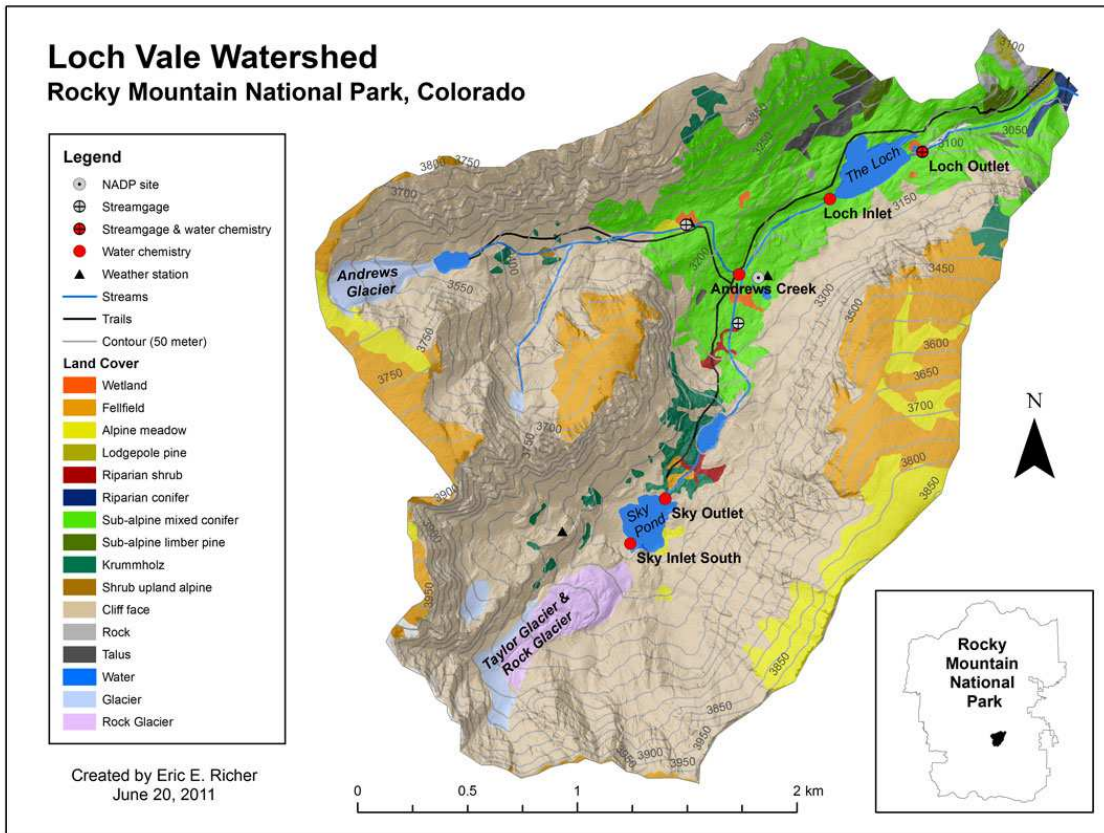


Figure 1. The location of Loch Vale watershed forest nitrogen experimental plots in Rocky Mountain National Park.

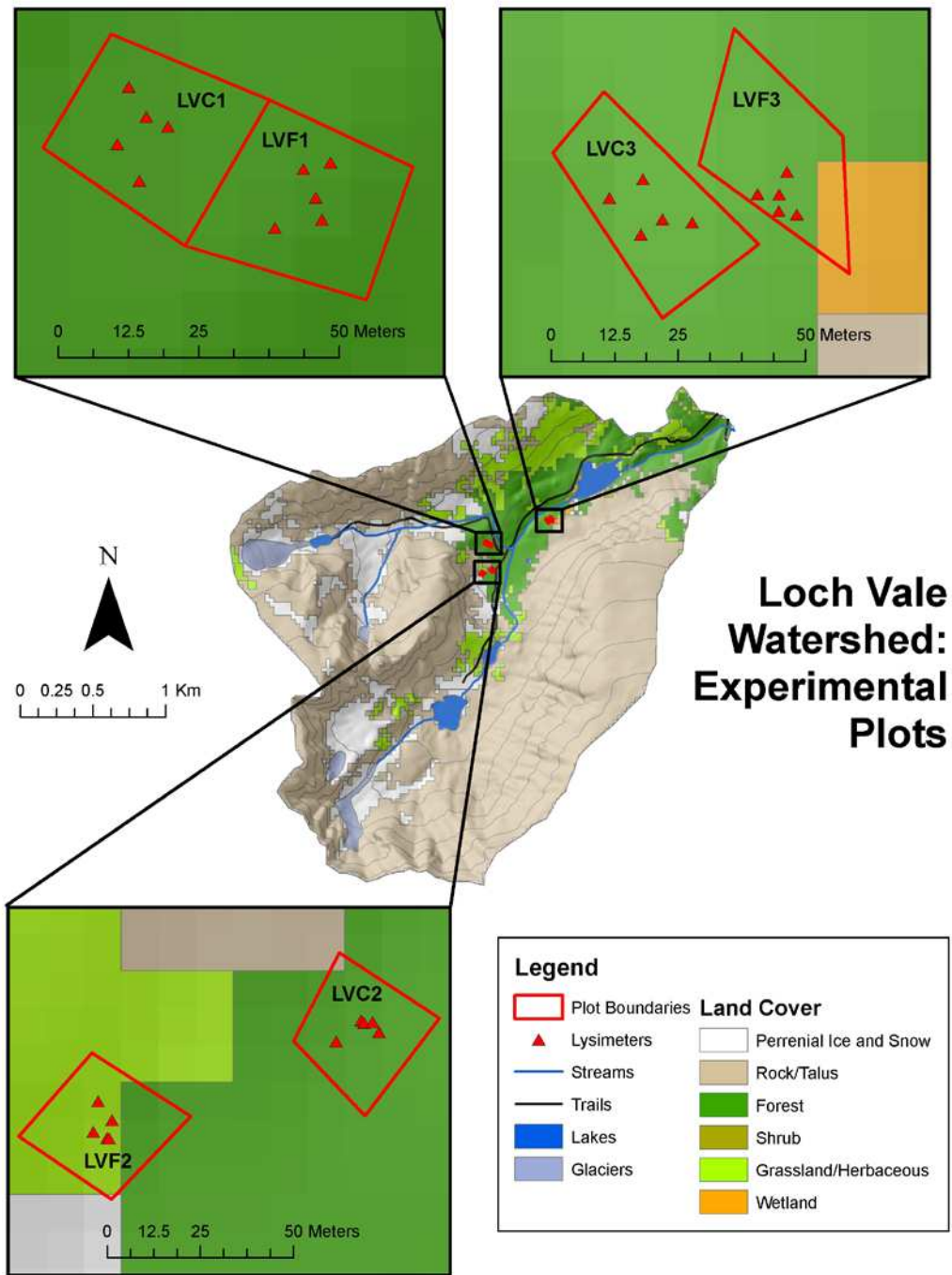


Figure 2. Plots shown in pairs of fertilized and control treatments in Rocky Mountain National Park. The lysimeters shown with red triangles were not used in this study.



Figure 3. Seven PVC collars such as this one were randomly located in each plot.



Figure 4. The author using a Li-Cor LI82 to measure soil respiration in Loch Vale watershed, Rocky Mountain National Park.

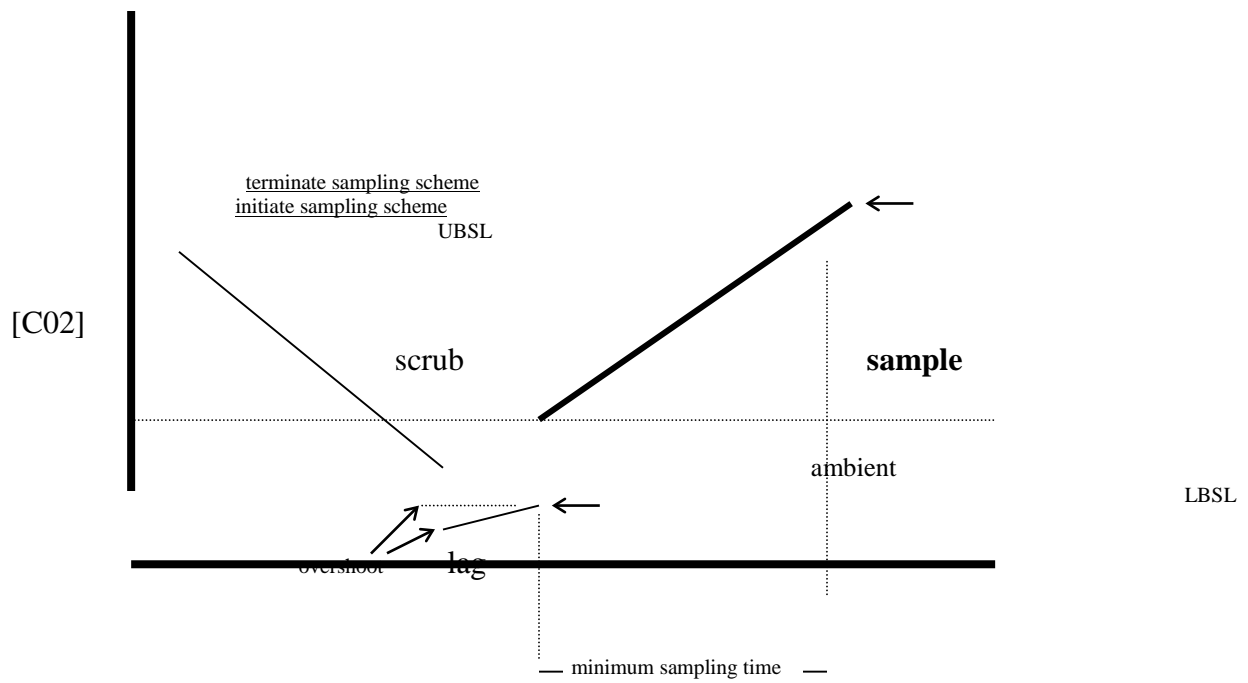


Figure 5. Graphical depiction of chamber CO₂ (ppm) concentration over time during full sampling cycle. LBSL and UBSL are lower boundary and upper boundary sampling level respectively.

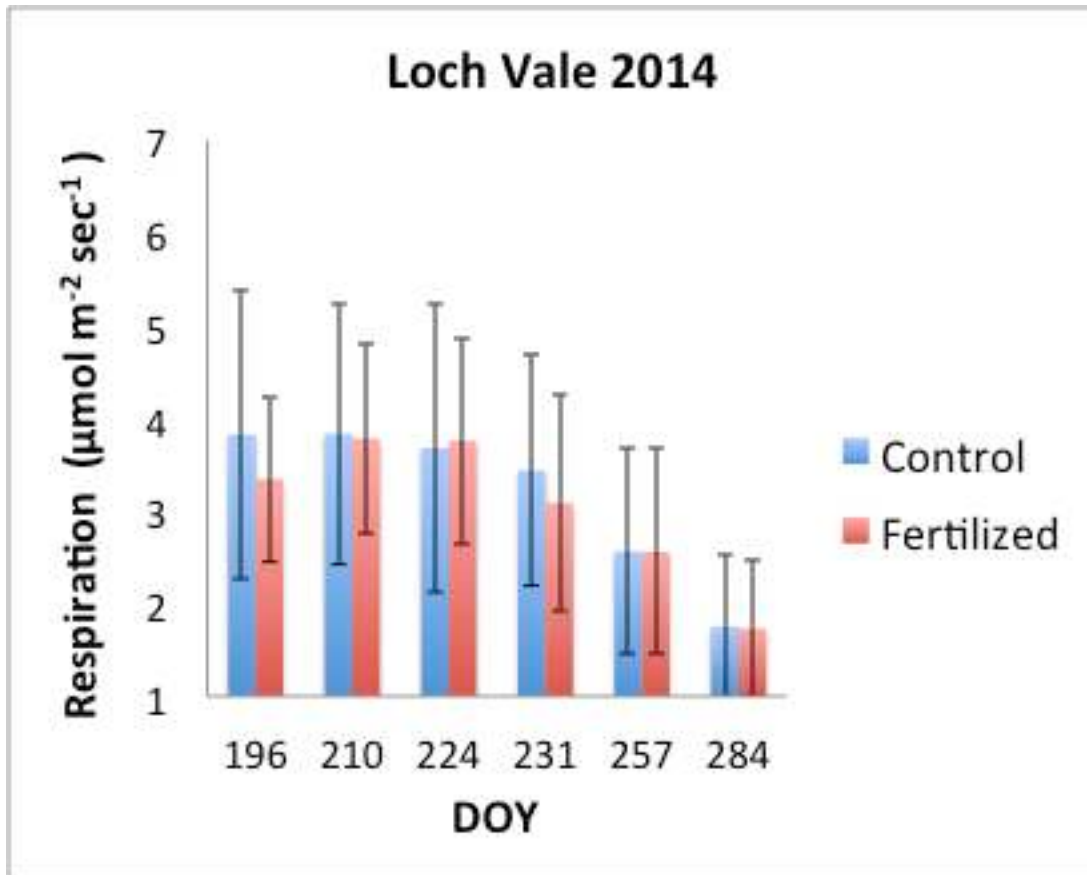


Figure 6. Loch Vale overall average respiration measurements throughout the 2014-sampling season. Respiration was measured in $\mu\text{mol m}^{-2} \text{sec}^{-1}$. DOY is Day of Year.

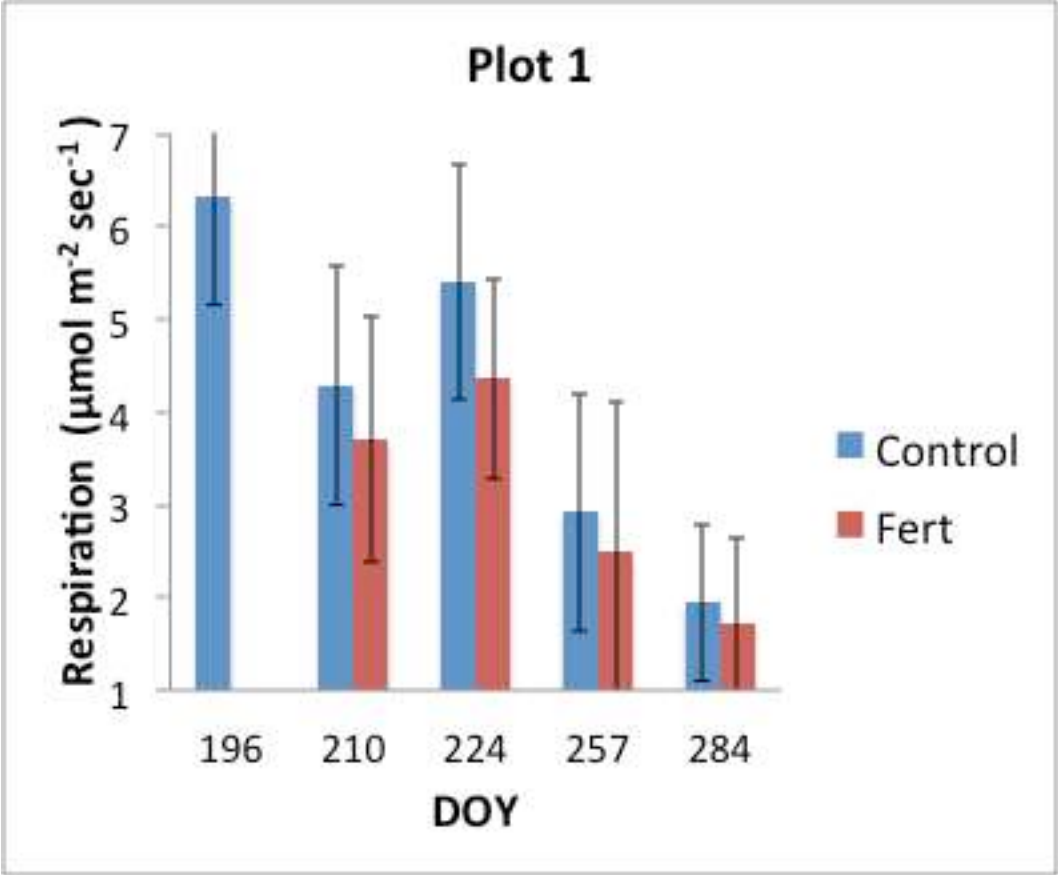


Figure 7 - Plot 1 average respiration measurements by Day of Year (DOY).

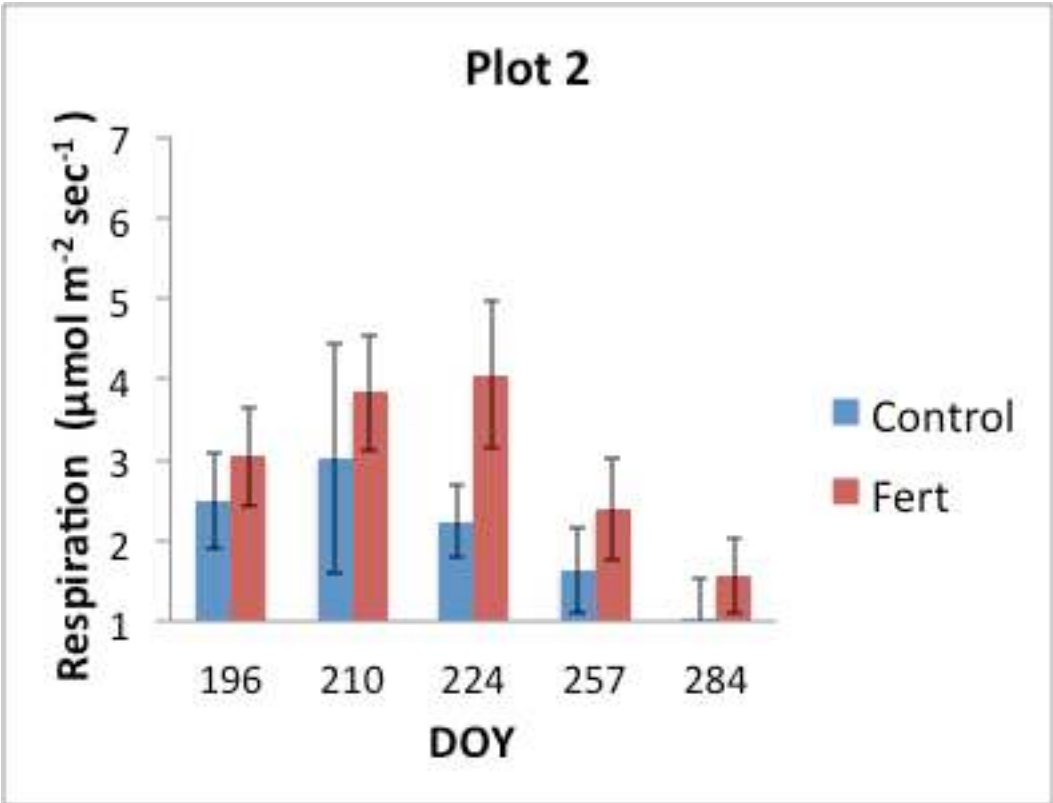


Figure 8. Plot 2 average respiration measurements by Day of Year (DOY).

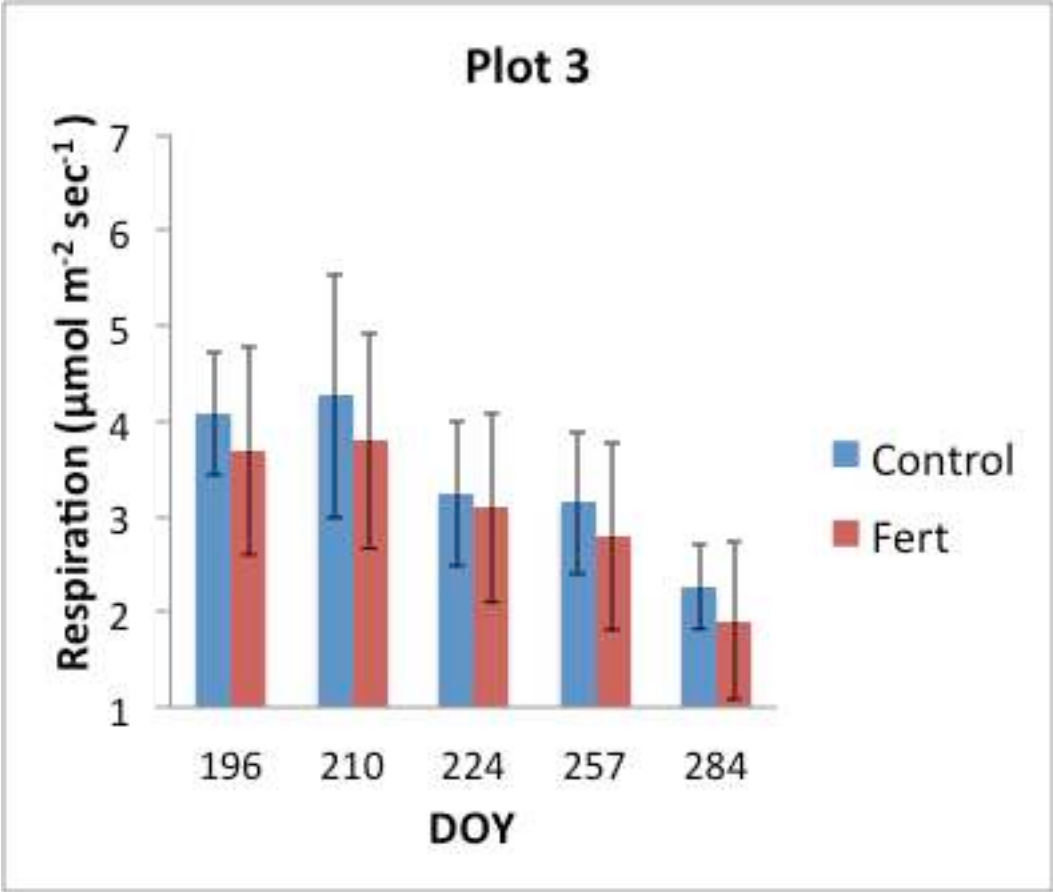


Figure 9. Plot 3 average respiration measurements by Day of Year (DOY).

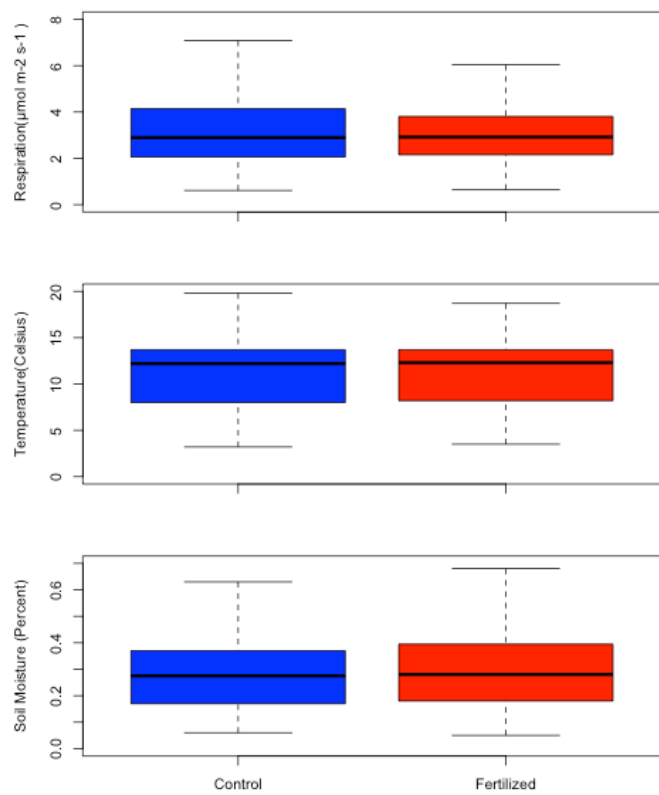


Figure 10. Comparison of soil respiration, temperature and moisture between treatments. Blue = Control, Red = Fertilized

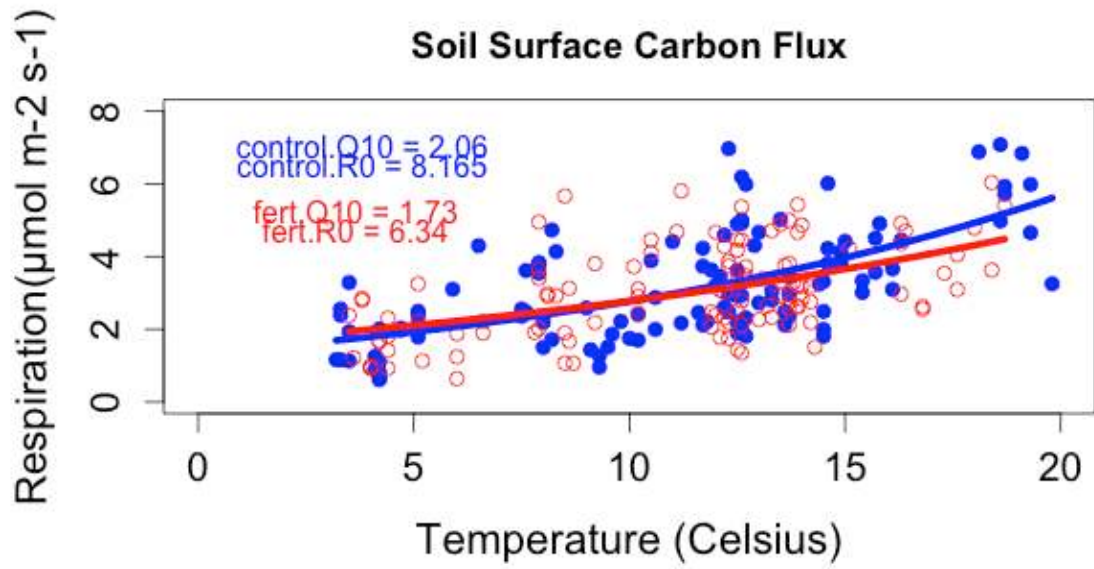


Figure 11. Soil respiration as a function of soil temperature at 2-4 cm depth in a subalpine forest in Loch Vale watershed, Rocky Mountain National Park, from July 2014 to October 2014. Fitted exponential lines represent the Control (blue) and Fertilized (red). Temperature is air temperature at the Loch Vale main weather station.

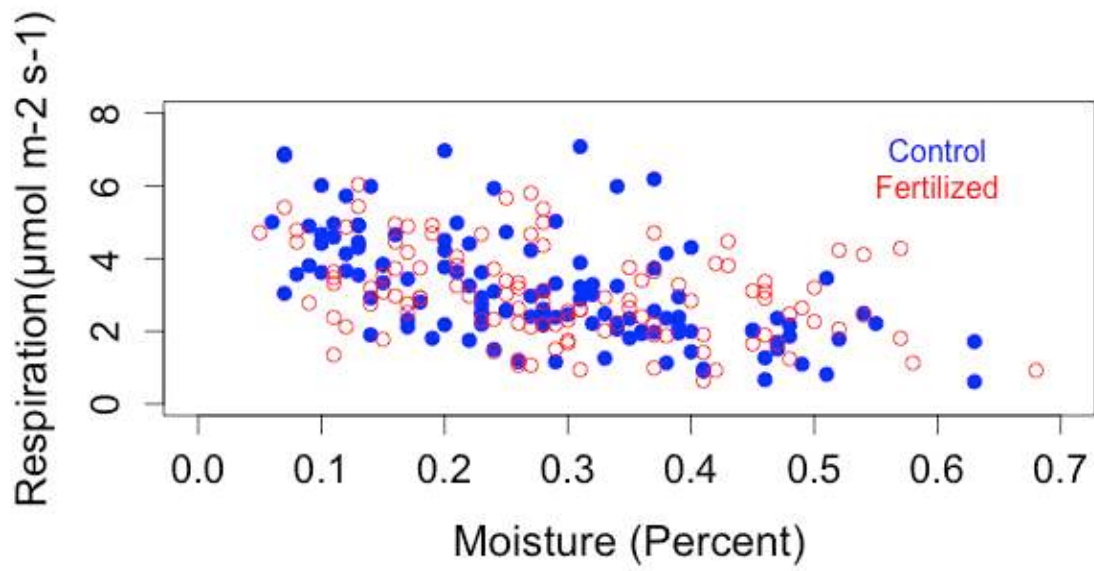


Figure 12. Soil respiration as a function of soil moisture at 10 cm depth in a subalpine forest in Loch Vale watershed in Rocky Mountain National Park from July 2014 to October 2014.

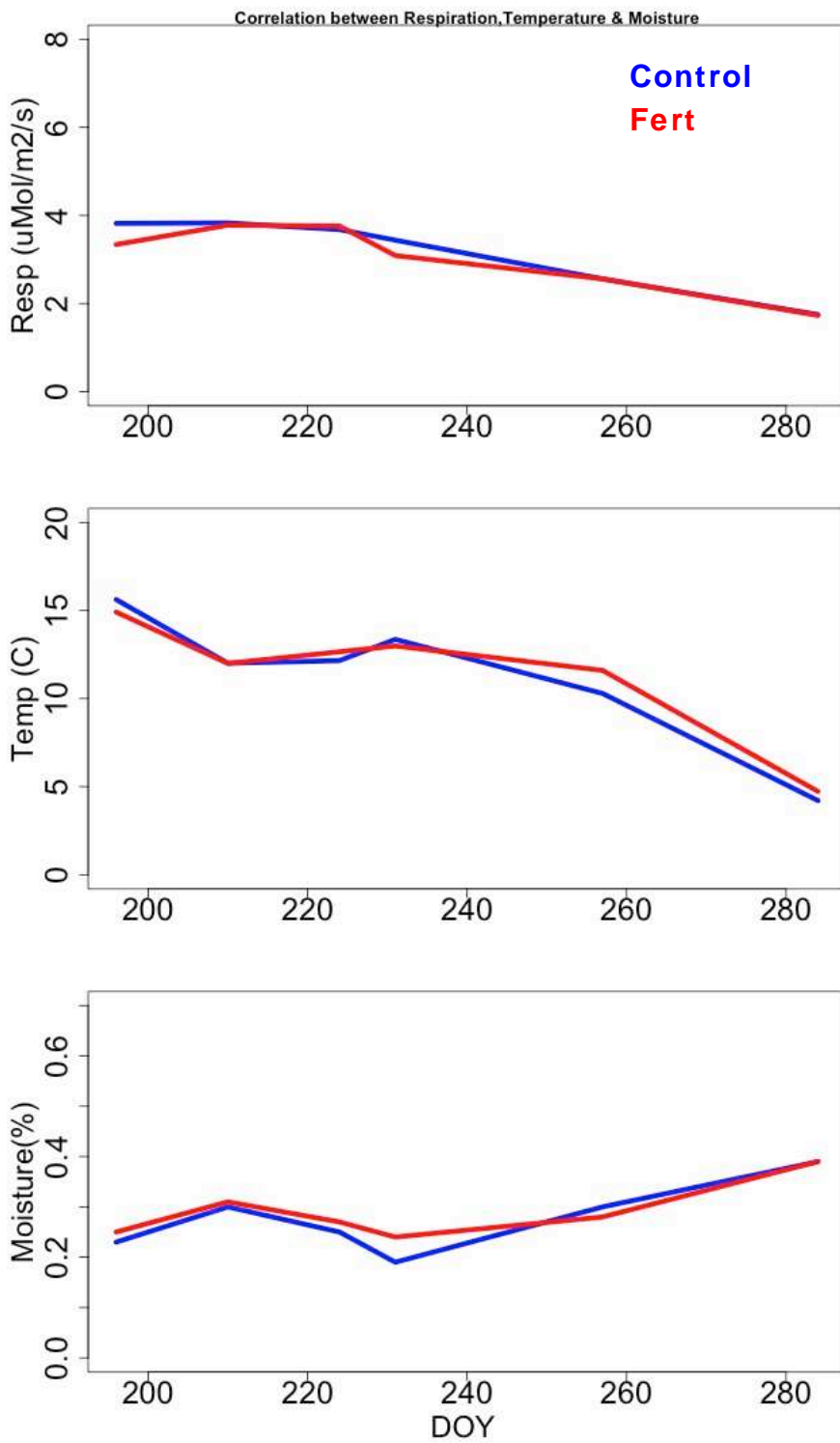


Figure 13. This graph is showing the seasonal pattern of soil respiration, temperature and moisture.

Table 1. Total number of samples, mean and standard deviations for respiration, in $\mu\text{mol m}^{-2} \text{sec}^{-1}$ for the control and fertilized plots.

Plot #	Total #of Control Measurements	Control Mean Respiration	Control Stdev	Total # of Fertilized Measurements	Fert Mean Respiration	Fert Stdev
1	29	4.2	0.2	28	3.1	0.3
2	33	2.1	0.4	35	3.0	0.2
3	35	3.4	0.3	35	3.1	0.1

Table 2. -Statistical results of linear model showing the significance of different variables.

Formula	Variable	p-value	Significant
lm (flux ~ treatment)	Treatment	>0.52	No
lm (flux ~ temp)	Temperature	< 2.2e-16	Yes
lm (flux ~ moisture)	Moisture	< 7.2e-15	Yes
lm (flux ~ treatment + temp + moisture)	Treatment Temperature Moisture	< 3.8e-08	Yes

5. References

- Agren G & Bosatta E (1988) Nitrogen saturation of terrestrial ecosystems. *Environmental Pollution* 54: 185–197.
- Asner, G. P., Seastedt, T. R., & Townsend, A. R. (1997). The decoupling of terrestrial carbon and nitrogen cycles. *BioScience*, 226-234.
- Baron, J. S., Rueth, H. M., Wolfe, A. M., Nydick, K. R., Allstott, E. J., Minear, J. T., & Moraska, B. (2000). Ecosystem responses to nitrogen deposition in the Colorado Front Range. *Ecosystems*, 3(4), 352-368.
- Baron, J. S., Del Grosso, S., Ojima, D. S., Theobald, D. M., & Parton, W. J. (2004). Nitrogen emissions along the Colorado Front Range: response to population growth, land and water use change, and agriculture. *Ecosystems and land use change* (R. DeFries, G. Asner, and R. Houghton, eds.). American Geophysical Union, Washington, DC, 117-127.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., ... & De Vries, W. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological applications*, 20(1), 30-59.
- Bonan, G. B. (2008). Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *science*, 320(5882), 1444-1449.
- Boot, C. M., Hall, E. K., Denef, K., & Baron, J. S. (2016). Long-term reactive nitrogen loading alters soil carbon and microbial community properties in a subalpine forest ecosystem. *Soil Biology and Biochemistry*, 92, 211-220.
- Bowden, R. D., Davidson, E., Savage, K., Arabia, C., & Steudler, P. (2004). Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. *Forest Ecology and Management*, 196(1), 43-56.
- Bowden RD, Rullo G, Stevens GR et al. (2000) Soil fluxes of carbon dioxide, nitrous oxide, and methane at a productive temperate deciduous forest. *Journal of Environmental Quality*, 29, 268–276.
- Bowman, W. D., Turnbull, J., Gleixner, G., Neff, J. C., Lehman, S. J., & Townsend, A. R. (2002). Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature: International weekly journal of science*, 419(6910), 915-917.
- Burton AJ, Pregitzer KS, Zogg GP et al. (1996) Latitudinal variation in sugar maple fine root respiration. *Canadian Journal of Forest Research*, 26, 1761–1768.

- Chapin III, F. S., Matson, P. A., & Vitousek, P. (2011). *Principles of terrestrial ecosystem ecology*. Springer Science & Business Media.
- Fenn, M. E., Baron, J. S., Allen, E. B., Rueth, H. M., Nydick, K. R., Geiser, L., ... & Neitlich, P. (2003). Ecological effects of nitrogen deposition in the western United States. *BioScience*, 53(4), 404-420.
- Friedland, A. J., Miller, E. K., Battles, J. J., & Thorne, J. F. (1991). Nitrogen deposition, distribution and cycling in a subalpine spruce-fir forest in the Adirondacks, New York, USA. *Biogeochemistry*, 14(1), 31-55.
- Friedlingstein, P., Houghton, R., Marland, G., Hackler, J., Boden, T., Conway, T., Canadell, J., Raupach, G., Ciais, P., LeQuere, C., Update on Emissions CO2, *Nature Geoscience* 3, 811–812 2010
- Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B., & Cosby, B. J. (2003). The nitrogen cascade. *Bioscience*, 53(4), 341-356.
- Giardina CP, Binkley D, Ryan MG, Fownes JH, Senock RS. 2004. Belowground carbon cycling in a humid tropical forest decreases with fertilization. *Oecologia* 139: 545–550.
- Giardina CP, Ryan MG. 2002. Total belowground carbon allocation in a fast growing *Eucalyptus* plantation estimated using a carbon balance approach. *Ecosystems* 5: 487-499.
- Gruber, N., & Galloway, J. N. (2008). An Earth-system perspective of the global nitrogen cycle. *Nature*, 451(7176), 293-296.
- Hanson, P. J., Edwards, N. T., Garten, C. T., & Andrews, J. A. (2000). Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, 48(1), 115-146.
- Janssens, I. A., Dieleman, W., Luysaert, S., Subke, J. A., Reichstein, M., Ceulemans, R., ... & Law, B. E. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, 3(5), 315-322.
- Kane ES, Pregitzer KS, Burton AJ (2003) Soil respiration along environmental gradients in Olympic National Park. *Ecosystems*, 6, 326–335.
- Kowalenko, C. G., & Ivarson, K. C. (1978). Effect of moisture content, temperature and nitrogen fertilization on carbon dioxide evolution from field soils. *Soil Biology and Biochemistry*, 10(5), 417-423.
- LeBauer, D. S., & Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89(2), 371-379.

- Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., ... & Kowalski, A. S. (2007). The human footprint in the carbon cycle of temperate and boreal forests. *Nature*, 447(7146), 849-851.
- Nadelhoffer, K. J., Emmett, B. A., Gundersen, P., Kjønnaas, O. J., Koopmans, C. J., Schleppi, P., ... & Wright, R. F. (1999). Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature*, 398(6723), 145-148.
- Nanus, L., Williams, M. W., Campbell, D. H., Tonnessen, K. A., Blett, T., & Clow, D. W. (2009). Assessment of lake sensitivity to acidic deposition in national parks of the Rocky Mountains. *Ecological Applications*, 19(4), 961-973.
- Norman, J. M., Kucharik, C. J., Gower, S. T., Baldocchi, D. D., Crill, P. M., Rayment, M., ... & Striegl, R. G. (1997). A comparison of six methods for measuring soil-surface carbon dioxide fluxes. *Journal of Geophysical Research: Atmospheres* (1984–2012), 102(D24), 28771-28777.
- Orchard, V. A., & Cook, F. J. (1983). Relationship between soil respiration and soil moisture. *Soil Biology and Biochemistry*, 15(4), 447-453.
- Olsson, P., Linder, S., Giesler, R., & Högberg, P. (2005). Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. *Global Change Biology*, 11(10), 1745-1753.
- Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., ... & Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science*, 333(6045), 988-993.
- R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Rueth, H., Baron, J., Allstott, E., Responses of Engelmann spruce forests to nitrogen fertilization in the Colorado Rocky Mountains, *Ecological Applications* 13(3), pp. 664-673, 2003.
- Ryan MG, Hubbard RM, Pongracic S et al. (1996) Foliage, fineroot, woody tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. *Tree Physiology*, 16, 333-343.
- Ryan, M. G. (1991). Effects of climate change on plant respiration. *Ecological Applications*, 1(2), 157-167.
- Ryan MG, Binkley D, Fownes JH, Giardina CP, Senock RS. 2004. An experimental test of the causes of forest growth decline with stand age. *Ecological Monographs* 74: 393-414.

- Ryan MG, Law BE. 2005. Interpreting, measuring, and modeling soil respiration. *Biogeochemistry* **73**: 3-27.
- Schmidt, M., Torn, M., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I., Kleber, M., Kogel, I., Lemann, J., Manning, D., Nannipieri, P., Rasse, D., Weiner, S., Trumbore, S., Persistence of soil organic matter as an ecosystem property, *Nature*. Vol.478. 2001.
- Schlesinger, W. H., & Andrews, J. A. (2000). Soil respiration and the global carbon cycle. *Biogeochemistry*, 48(1), 7-20.
- Scott-Denton, L. E., Sparks, K. L., & Monson, R. K. (2003). Spatial and temporal controls of soil respiration rate in a high-elevation, subalpine forest. *Soil Biology and Biochemistry*, 35(4), 525-534.
- Sutton, M. A., Simpson, D., Levy, P. E., Smith, R. I., Reis, S., Van Oijen, M. and De Vries, W. (2008), Uncertainties in the relationship between atmospheric nitrogen deposition and forest carbon sequestration. *Global Change Biology*, 14: 2057–2063.
- Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: how can it occur?. *Biogeochemistry*, 13(2), 87-115.
- Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, 277(5325), 494-499.
- Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H. and Tilman, D. G. (1997), HUMAN ALTERATION OF THE GLOBAL NITROGEN CYCLE: SOURCES AND CONSEQUENCES. *Ecological Applications*, 7: 737–750.
- Waldrop, M. P., Zak, D. R., Sinsabaugh, R. L., Gallo, M., & Lauber, C. (2004). Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications*, 14(4), 1172-1177.
- Williams, Mark W., and Kathy A. Tonnessen. "Critical loads for inorganic nitrogen deposition in the Colorado Front Range, USA." *Ecological Applications* 10.6 (2000): 1648-1665.
- Vant Hoff, J.H. (1898) Lectures on theoretical and physical chemistry . In: *Chemical Dynamics Part I*. pp. 224-229. Edward Arnold, London