THESIS

THE EFFECTS OF LONG TERM NITROGREN 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

Spring 2016

Master's Committee:

Advisor: A. Scott Denning
Co-Advisor: Jill Baron
Mike Ryan
Gillian Bowser

ABSTRACT

EFFECTS OF NITROGREN 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 CO_2 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 N saturated subalpine forests, and (2) try to understand the impacts of N additions on carbon flows in the ecosystem. A LiCor LI-820 infrared gas analyzer (IRGA) was used to quantify soil respiration rates. We compared soil CO_2 respiration from fertilized forest plots (30 x 30 m) with CO_2 respiration from control forests plots (30 x 30 m) that receive only ambient nitrogen deposition (3-5 kg/ $N/ha^{-1}/yr^{-1}$) during the 2013-growing season. Our results show that mean soil respiration measurements were not significantly different in the control plots (3.14 $\mu mol\ m^{-2}\ sec^{-1}$) than in the fertilized plots (3.02 $\mu mol\ m^{-2}\ sec^{-1}$).

Experiments with a very simple ecosystem model suggest that the negligible response of soil respiration in the fertilized vs control plots is a result of nitrogen saturation due to atmospheric deposition of N.

Interestingly, the Q_{10} function used to estimate the temperature sensitivity was 2.06ºC for every 8.1 $\mu mol\ m^{-2}\ sec^{-1}$ (R_0) in the control plots compared to 1.73ºC for every 6.3 $\mu mol\ m^{-2}\ sec^{-1}$ (R_0) in the fertilized plots, indicating that the temperature function is

slightly higher in the control plots. Treatment was insignificant in influencing soil respiration (p-value greater than 0.5). The Simple Ecosystem Model was used to simulate the output from land-atmosphere interaction with the sensitivity of Net primary production (NPP) and respiration in response to nutrients. Our results suggests the effects of nitrogen fertilization on soil respiration in Rocky Mountain National Park insignificantly influence changes in soil respiration to ambient atmospheric N deposition in subalpine forests.

ACKNOWLEDGEMENTS

As I embarked on this journey to pursue graduate education 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 ii

ACKNOWLEDGEMENTS iv

INTRODUCTION 5

METHODS 10

RESULTS 20

CONCLUSION 27

REFERENCES 29

1. Introduction

Since the Industrial Revolution, nitrogen (N) deposition has increased at an alarming rate (Hoberg, 2007). Nitrogen deposition is defined as the input of reactive nitrogen species from the atmosphere to the biosphere. The pollutants that contribute to nitrogen deposition derive mainly from nitrogen oxides (NO_X) and ammonia (NH_3) emissions and impact terrestrial ecosystems (Fenn et al. 2003). Due to anthropogenic activities atmospheric nitrogen fixed annually through human activities now exceeds that fixed via all natural processes combined (Neff et al. 2002). Nitrogen is deposited in ecosystems in gaseous, dissolved and particulate forms. 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 delivers nutrients in water droplets onto plant surfaces (Chaplin et al 2010). The form of nitrogen deposition determines its ecosystem consequences (Vitousek et al. 1997). Anthropogenic activities are the main causes of nitrogen deposition (Gruber and Galloway 2008). Due to high use of fertilizer agricultural systems are often nitrogen saturated and release substantial quantities of nitrogen to ecosystems. Some boreal and temperate forests increase their carbon sequestration in response to nitrogen deposition (Magnani et al. 2007).

The Colorado Front Range population growth, land use change and agricultural practices have increased pollution and specifically nitrogen deposition. The main sources of pollutants are power plants, vehicles, agriculture fertilizers and livestock (Baron et al. 2004). In the Rocky Mountain region, upslope winds from the east are transporting and depositing nitrogen. During weather events, reactive nitrogen is transported by wind, combined with moisture in the air and then deposited by precipitation (Wolyn and Mckee 1994, Markowski and Richardson 2010). Many studies have highlighted the effects of anthropogenic nitrogen deposition in ecosystems

(Agren et al. 1988; Asner et al. 1997; Currie et al. 1999), but most importantly in the Colorado Front Range, Baron et al. (2000) have found that slight increases in nitrogen deposition led to changes in ecosystem properties. 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).

Nitrogen deposition may cause soil to respire more CO_2 (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 CO_2 content of the atmosphere, if they are not balanced by simultaneous changes in other components of the carbon cycle (Pan et al. 2010). Annual emissions of anthropogenic CO_2 in the atmosphere has increased at an alarming rate due to the combustion of fossil fuels (Freidlingstein et al. 2010). The increase in CO_2 alters the carbon sinks such as the land, oceans, and the atmosphere. One of the main causes of global warming is increased carbon dioxide CO_2 in the atmosphere. The atmospheric concentration of CO_2 has increased from its preindustrial levels, about 280 parts per million (ppm), to 400 ppm (Mauna Loa Observatory). Nadelhoffer et al. 1999 found that nitrogen deposition makes a small contribution to carbon sequestration in temperate forests, but there is still uncertainty whether elevated nitrogen deposition is the main cause.

The global flux of CO_2 from soils is approximately 75 x 10^{15} gC/yr (Schlesinger, 1997). Waldrop et. al, 2004 found nitrogen deposition alters carbon cycling in soils and influences changes in microbial activity. In our study we are focusing on the effects of nitrogen fertilization on forest soil respiration in Rocky Mountain National Park. The role of soil in biogeochemical cycles is an important area of uncertainty in ecosystem ecology. One of the main reasons for this uncertainty is that we have a limited understanding of belowground microbial activity and how

this activity is linked to soil processes. 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 2003).

In this study our goal is 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. We therefore expect that 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. Chapter 2 describes the experimental site, methods, and calculations. Chapter 3 presents results of the measurements and some numerical experiments with a simple ecosystem model. Chapter 4 discusses the results, summarizes our conclusions, and offers some ideas for future work.

2. Methods

2.1 Site Description

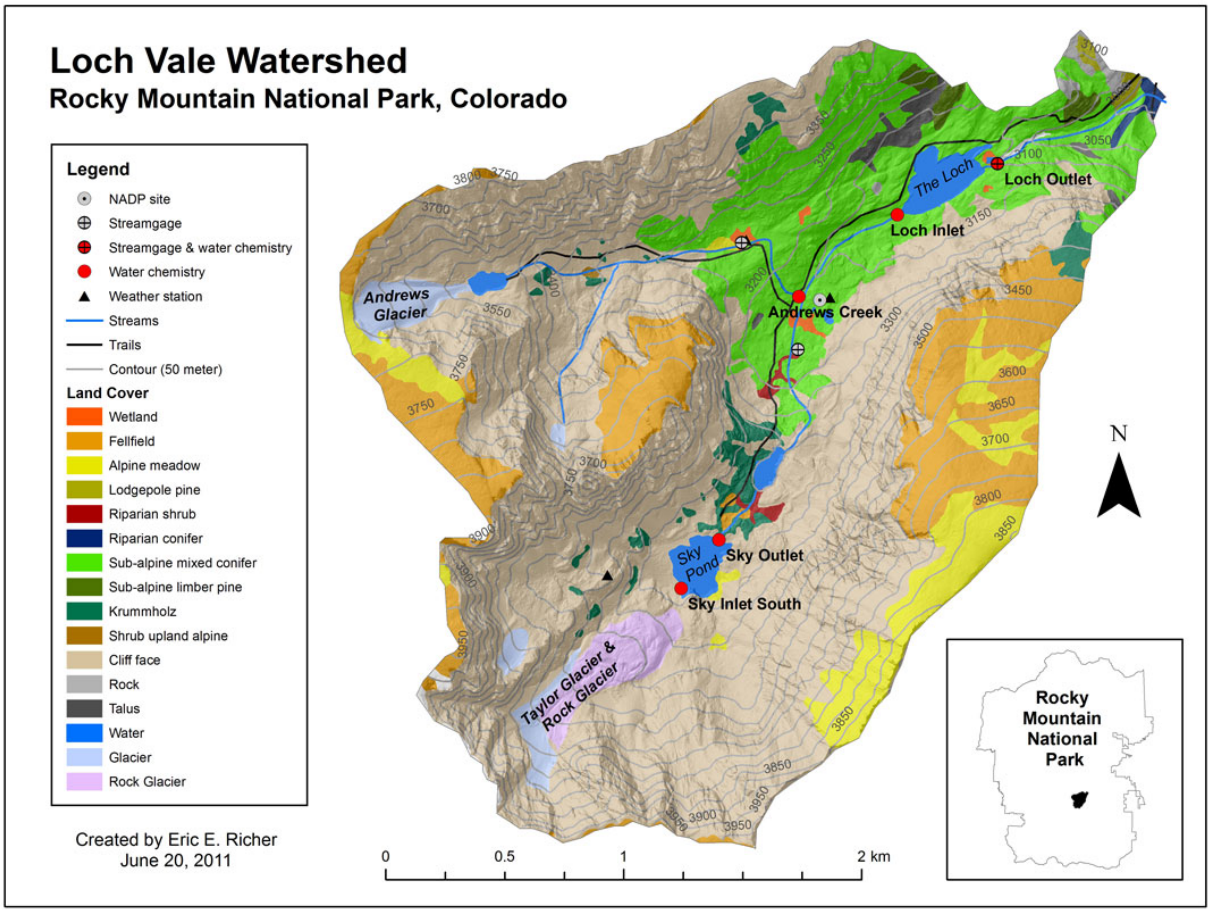


Figure 1. Loch Vale Watershed experimental plots including land cover.

This study was conducted in three Engelmann spruce (*Picea engelmannii*) 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 only during the growing season from mid July-October. 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. 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

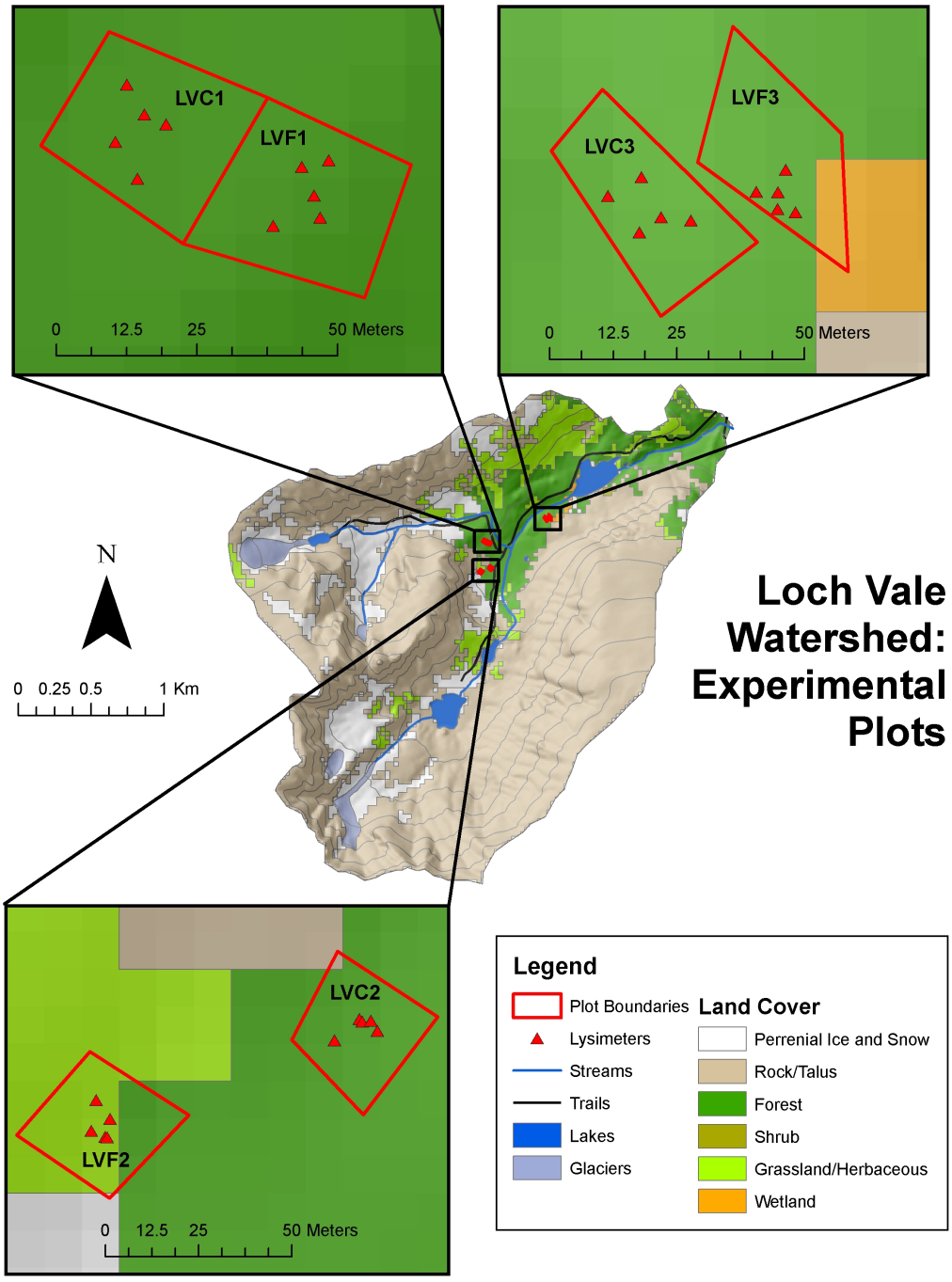


Figure 2. Plots shown in pairs of fertilized and control treatments in Rocky Mountain National Park. Lysimeters shown were not used in this study.

A total of six 30 x 30 m plots were established in June 1996. Pairs of adjacent plots (Figure 2) are fertilized (LVF1, LVF2, LVF3) and control (LVC1, LVC2, LVC3). Fertilized plots received 25 kg N ha^{-1} yr^{-1} as ammonium nitrate (NH_4NO_3) 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 (Fig 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. Due to the rocky terrain 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.

Figure 3. PVC collar in LVC1 plot.

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

Soil carbon dioxide fluxes were estimated by measuring the rate of CO_2 build up in a chamber created by capping the PVC collars with a foam lid (Fig 4). Measurements of CO_2 in the chambers was done using a LiCor LI820 infrared gas analyzer (IRGA). The LiCor was

connected to a Campbell Scientific data logger, which was programmed to record CO_2 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 roughly calibrated before each measurement using 400 ppm CO_2 as the ambient air standard. To make sure these flux measurements are not biased by CO_2 concentration gradients between the chamber and the air, we scrubbed the concentration within the chamber down to just below ambient CO_2 concentration using a second airflow tube to pump the air from the chamber through a soda lime trap. Soil respiration then causes the concentration to build up again and we measure the rate of change in concentration close to ambient levels. Linear regressions (concentration versus time) were used to determine rates of CO_2 flux. Soil CO_2 flux in μmol m^{-2} sec^{-1} was obtained by taking the slope of the line of CO_2 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 moles m^{-2} s^{-1} (see section 2.5 below). The rate of CO_2 build up was almost perfectly linear. Air temperature was measured at one location near the three pairs of plots and soil temperature and moisture was measured near each collar. 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 [CO_2] is above Lower Boundary Sampling Level

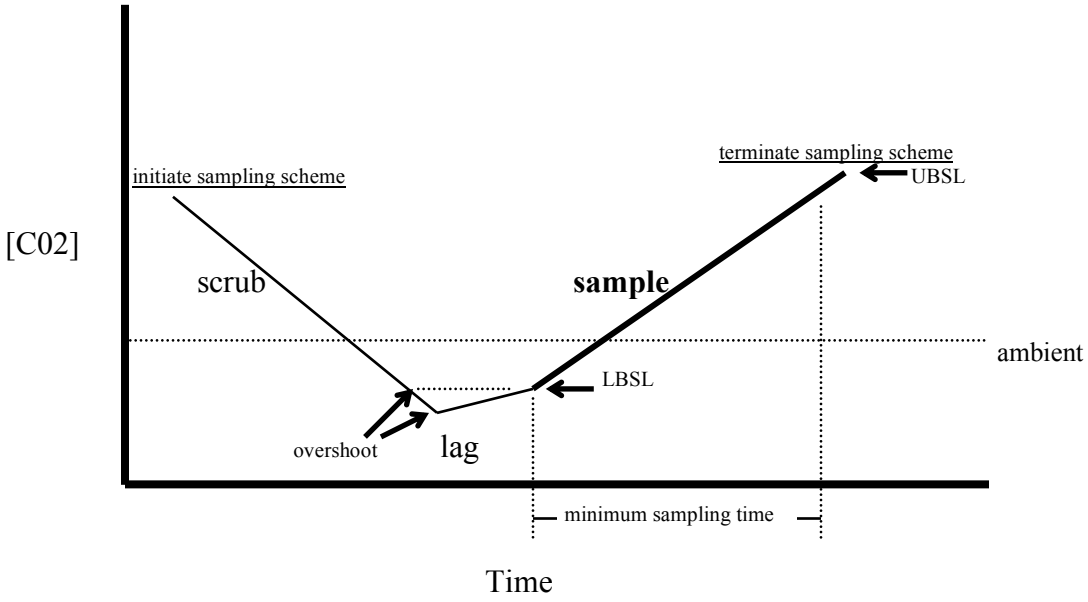
4) Datalogger records a sample every two seconds during the Sampling Time for flux calculations.

5) Sampling record terminates.

6) Datalogger sends new data to storage module.

Figure 4. Using a Li-Cor LI82 to measure soil respiration in Rocky Mountain National Park.

Graphical depiction of chamber CO_2 (ppm) concentration over time during full sampling cycle



2.5 Flux Calculations

- System + tubing = 50 cm^3 = 0.05 L
- Collar area (m^2): 10" diameter = 506.7075 cm^2 = 0.050671 m^2
- Chamber Volume = 5.365 cm^3 (inside LiCor)

(Collar Area (in cm^3) multiplied by the **Collar Depth)** plus **Chamber Volume** plus **System** plus **Tubing** (in cm^3)

Collar 5 cm deep: (506.7075 * 5) + 5365 + 50 = 7948.5 cm^3 or 0.0079485 m^3.

Flux = increase in CO_2/ time * volume / surface area

- The increase in CO_2 / time = slope

Conversion of CO_2 increase to soil respiration

Flux = slope µmol CO_2 mol Air^{-1} s^{-1} (slope of regression of CO_2 ppm versus time)

*(10^3 L/m^3) * (system volume in m^3) * (1 mol/22.414 L) * (Pressure in kPa/101.32 kPa) * (From Licor gas analyzer/Standard pressure of 101.32 kPa) *(273 °K/Air temperature in °K) 1/ 0.050671 m^2).

F = Flux

S = Slope

V_s = Volume of System

P = Ambient pressure

P_o = Standard Pressure (Sea level)

T_f = 273.15 K

T = Temperature from National Atmospheric Deposition Program National Trends Network (NADP/NTN) monitors

A_c = Area of Collar

$$F = S * V * P / P_o * T_f / T$$

$$(22.4 \text{ mol/L}) A_c$$

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

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 will use the Q_{10} relationship/function developed by (van't Hoff 1898)

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

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 ºC 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 Soil moisture and temperature measurements

Soil temperature and moisture were taken at each collar while soil respiration was being measured. Soil Temperature was initially measured using a Penetration Thermocouple Probes "T" style 304 stainless steel handle. We inserted the temperature probe 10 cm in depth in soil, but it did not work. Instead we used hourly measurements of air temperature, which were taken at the National Atmospheric Deposition Program National Trends Network (NADP/NTN) monitors 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).

2.8 Simple Ecosystem Model: To test simple hypotheses about how nitrogen deposition might affect soil respiration, we developed a model that describes the flow of carbon from photosynthesis through a set of plant and soil pools.

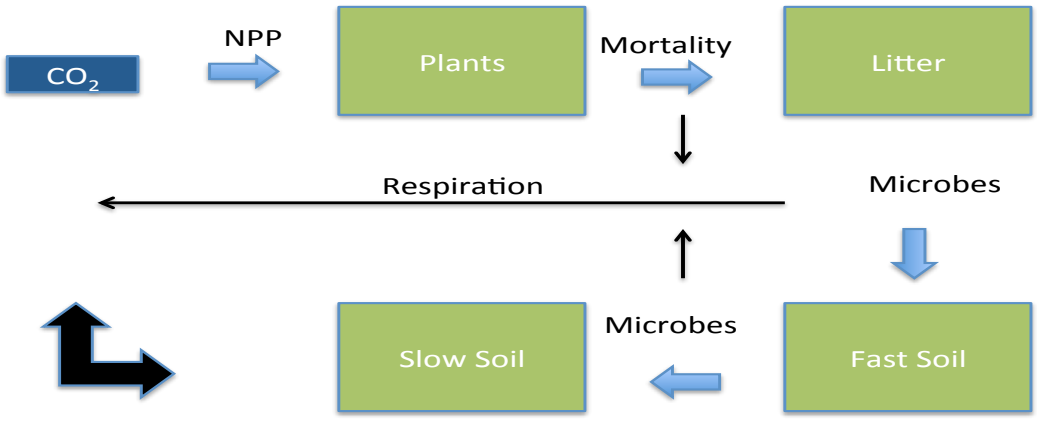


Figure 5. Four box numerical model of carbon stocks and flows in an ecosystem.

We used four boxes to represent storage of carbon in organic matter (Figure 5). Carbon transfers among the pools are represented as a "cascade" from one pool to the next. Photosynthesis converts CO_2 to plants (P), and is limited by nitrogen. Plant mortality (death) converts plant carbon to "litter," (L) which is slowly decomposed by microbes to produce fast-turnover soil organic matter (F). Fast soil is decomposed more slowly by other microbes to produce slowly-decomposing soil organic matter (S), which will eventually decompose back to CO_2. The model was implemented in R, and the code is presented in the Appendix.

$$\frac{dP}{dt} = NPP - mort \qquad 1$$

$$\frac{dL}{dt} = mort - \frac{L}{\tau_L} \qquad 2$$

$$\frac{dF}{dt} = (1-\varepsilon)\frac{L}{\tau_L} - \frac{F}{\tau_L} \qquad 3$$

$$\frac{dS}{dt} = (1-\varepsilon)\frac{F}{\tau_F} - \frac{S}{\tau_S} \qquad 4$$

Losses from each pool are represented as sources of carbon to the next pool. Carbon turnover from litter, fast soil organic matter and slow soil organic matter is represented using turnover times ($\tau_L, \tau_F,$ and τ_S). Microbial transformations from litter to fast soil, and from fast to slow soil organic matter are incomplete due to respiration losses. We represent the metabolic efficiency of these microbial transfers as ε, meaning that a fraction ε of the carbon lost from the litter and fast soil pools is respired away as CO_2, with the remaining fraction (1- ε) being transferred to the next pool in the cascade.

To investigate the effects of nitrogen fertilization on soil respiration, we experimented with the simple ecosystem carbon model described in Section 2.8

Ecosystem net primary production was represented using a logistic function

$$NPP = g\left(\frac{1-P}{K}\right)P \qquad 5$$

where g is the rate of intrinsic growth (unitless), P is the carbon stored in plants (kg C m^{-2}), and K is the "*carrying capacity*" of the ecosystem (kg C m^{-2}).

We represent the effect of nitrogen fertilization by modifying the carrying capacity K as follows

$$K = K_0(1 + N\ m_N) \qquad 6$$

Here K_0 is a reference carrying capacity, and N represents the effect of added nitrogen. For convenience, we also introduce a nitrogen "multiplier" m_N which can be used to adjust the relative strength of the nitrogen effect.

Mortality is represented as

$$mort = \frac{g}{2}P \qquad 7$$

meaning that the rate of transfer of carbon to the litter pool is half the rate of growth of plant carbon.

We represented a hypothetical effect of nitrogen on soil respiration as

$$R = \left[\varepsilon\left(\frac{L}{\tau_L} + \frac{F}{\tau_F}\right) + \frac{S}{\tau_S}\right](1 + N\ m_R) \qquad 8$$

For the simulations shown here, we use ε = 0.8, τ_L = 2 yr, τ_F = 20 yr, and τ_S = 500 yr. The model was initialized by setting the time derivatives on the left-hand sides of equations (1) – (4) to zero and solving for steady-state values of the carbon pools *P, L, F,* and *S*.

We created two sets of experiments because we wanted to predict the response of respiration and

NPP to nutrient additions.

$K = K_0 (1 + N \times multiplier)$

N= 0.20 Nutrient concentration

1. Let nutrients increase over a 20-year period.
   - Hold the npp/resp multiplier at 0.
   - Increase npp and resp multiplier.
   - Decrease npp and increase resp multiplier
2. Hold nutrients constant over a 20 year period
   - Hold the npp/resp multiplier at 0.
   - Increase npp and resp multiplier.
   - Decrease npp and increase resp multiplier

2.9 Statistical Analysis

Linear regression analysis was performed using ***R*** statistical software version 3.0.1(R Core Team. 2012). I analyzed respiration as a function of treatment, soil moisture, soil temperature and interactions across data collected from all plots during the field season. 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 Temperature and Moisture

The comparison of control and fertilized plots respiration rates in response to temperature.

Soil respiration, temperature, and moisture were statistically identical between fertilized and control plots over the entire summer (Fig 6). During our field season the mean soil temperature for the control plots were 11.09°C and the fertilized plots was approximately 11.15°C. The flux measurements were a slightly higher in the control plots at approximately 3.14 μmol m^{-2} sec^{-1} compared to 3.02 μmol m^{-2} sec^{-1} in the fertilized plots. Soil moisture was very similar in both the fertilized and control plots 0.29 and 0.28 percent.

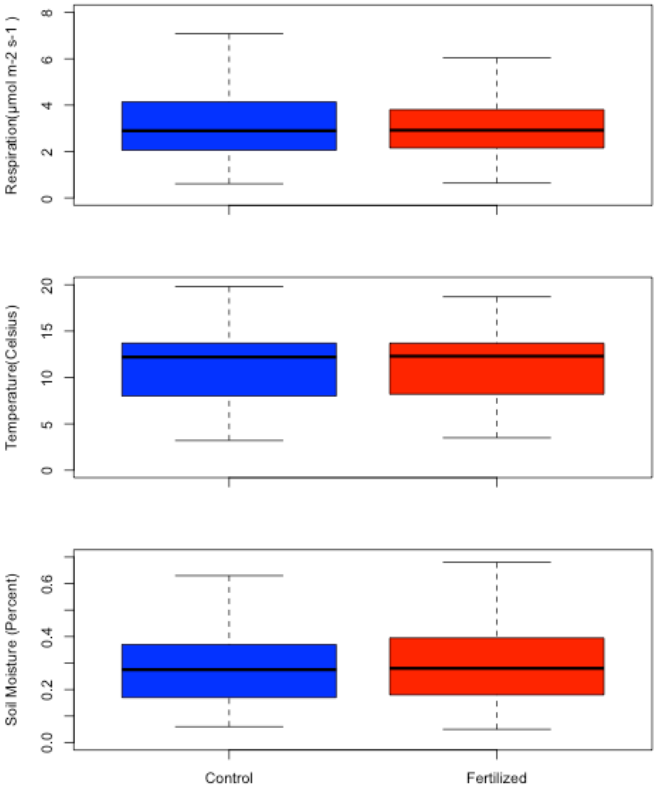


Figure 6. Comparison of soil respiration, temperature and moisture between treatments. Blue = Control, Red = Fertilized

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 (Fig 7).

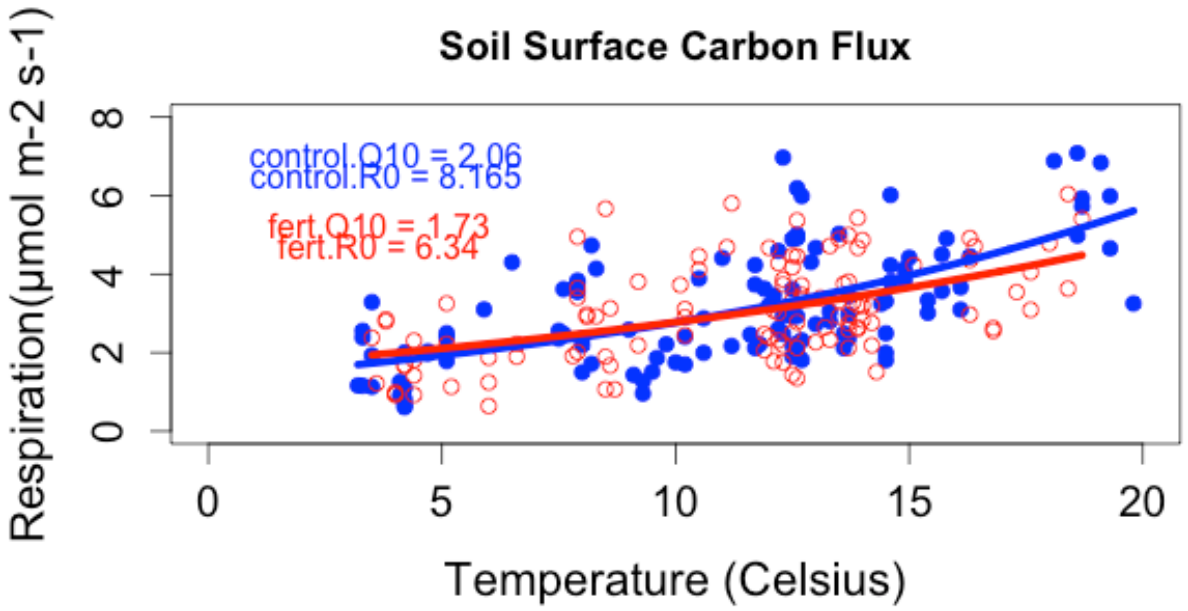


Figure 7. Soil respiration (Y-axis) as a function of soil temperature (X-axis) at 2-4 cm depth in a subalpine forest in Rocky Mountain National Park from July 2014 to October 2014. Fitted exponential lines represent the Control (blue) and Fertilized (red).

Fertilized plots respiration rates are slightly higher than control plots at lower temperatures. As temperatures increase the control plots respiration rates are slightly higher than the fertilized plots as seen by the two fitted lines (Red=Fertilized, Blue=Control). For most biological systems the Q10 parameter is approximately 2. The respiration rate doubles for every 10ºC (R0).

The base respiration rate R0 was slightly higher 8.2 µmol m^{-2} sec^{-1} in the control vs. the fertilized plots 6.3 µmol m^{-2} sec^{-1}. The temperature sensitivity parameter Q10 was higher (2.06) in the control plots compared to the fertilized plots (1.73).

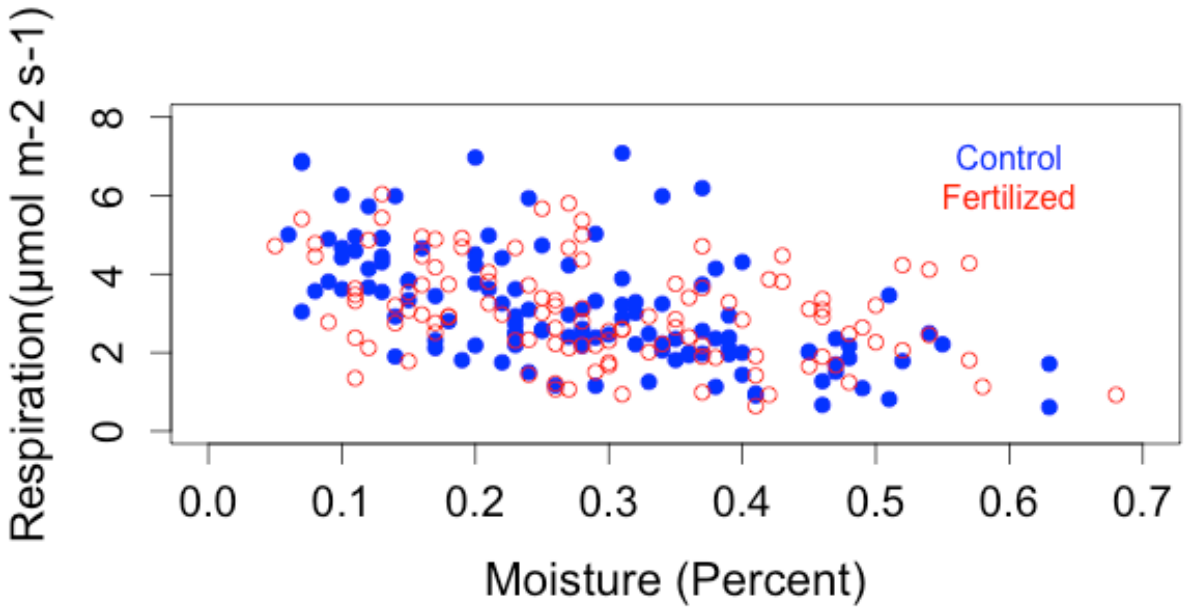


Figure 8. Soil respiration (Y-axis) as a function of soil moisture (X-axis) at 10 cm depth in a subalpine forest in Rocky Mountain National Park from July 2014 to October 2014.

Soil respiration rates were higher in soil that is less moist in both the control and fertilized plots (Fig 8). These subalpine soil are usually quite wet. It appears that microbial activity may be oxygen – limited because higher respiration rates occur in less moist soils compared to wetter soils (Scott-Denton et al. 2003).

3.2 Statistical Analysis

The difference in soil respiration measurements between treatments was not significantly different. We used a t-test to evaluate differences in respiration between

control and fertilized plots. The measurements were not significantly different, p-value was greater than 0.5. For statistical significance at the 95% confidence level, we need $p < 0.05$.

1. Formula lm (flux ~ treatment)

Residuals:

Min	1Q	Median	3Q	Max
-2.5295	-0.9700	-0.1823	0.8266	3.9415

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	3.1412	0.1268	24.771	<2e-16 ***
treatment Fertilized	-0.1169	0.1817	-0.643	0.521

Residual standard error: 1.377 on 228 degrees of freedom
Multiple R-squared: 0.001811, Adjusted R-squared: -0.002567
F-statistic: 0.4137 on 1 and 228 DF, **p-value: 0.5207**

We 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.

Formula 1.2: lm (flux ~ temp)

Residuals:

Min	1Q	Median	3Q	Max
-2.1898	-0.8569	-0.1390	0.8380	3.6595

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.94044	0.21302	4.415	1.56e-05 ***
temp	0.19270	0.01795	10.734	< 2e-16 ***

Residual standard error: 1.124 on 228 degrees of freedom
Multiple R-squared: 0.3357, Adjusted R-squared: 0.3328
F-statistic: 115.2 on 1 and 228 DF, p-value: < 2.2e-16

1.3 Formula lm (flux ~ moisture)

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.

Residuals:

Min	1Q	Median	3Q	Max
-2.6130	-0.7688	-0.2234	0.6961	4.1089

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	4.5053	0.1881	23.946	< 2e-16 ***
Moisture	-4.9399	0.5927	-8.335	7.3e-15 ***

Residual standard error: 1.207 on 228 degrees of freedom
Multiple R-squared: 0.2336, Adjusted R-squared: 0.2302
F-statistic: 69.48 on 1 and 228 DF, p-value: 7.299e-15

In our multiple regressions model results 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. This falsified our hypothesis.

1.4 Formula: lm (flux ~ treatment + temp + moisture)

Residuals:

Min	1Q	Median	3Q	Max
-2.4539	-0.7741	-0.0826	0.6819	3.4046

Coefficients:

	Estimate	Std.Error	t value	Pr(>|t|)
(Intercept)	2.28644	0.32479	7.040	2.29e-11 ***
treatmentFertilized	-0.07891	0.14070	-0.561	0.575
temp	0.15282	0.01864	8.199	1.81e-14 ***
moisture	-3.00327	0.57376	-5.234	3.78e-07 ***

Residual standard error: 1.064 on 226 degrees of freedom
Multiple R-squared: 0.4094, Adjusted R-squared: 0.4016
F-statistic: 52.23 on 3 and 226 DF, **p-value: < 2.2e-16**

3.3 Simple Ecosystem Model

We expected fertilized soils to increase net primary production (NPP), which would increase decomposing litter and soil C, leading to increased respiration. This suggestion would support our hypothesis that nitrogen fertilized plots would respire more carbon, but there was no effect of fertilization. One possibility is that nitrogen deposition in the control plots are so high that N demand is met. Another possibility is that the effects of fertilization are only temporary, with rising NPP and respiration reaching a new equilibrium over time.

Ultimately we wanted our Simple Ecosystem Model to test how respiration will increase as nutrient concentrations changed over time and if we kept nutrient concentrations constant over time. Our field results show that respiration wasn't significantly different in control and fertilized plots. We conducted two sets of experiments. The first set of experiments we held the Nutrient level constant over a 20 - year period to see how NPP and Respiration would respond. Our field flux measurements are insignificantly different, maybe NPP and respiration increase initially, but over 20 years the effect is gone. The second set of experiments we increase the Nutrient level over a 20 year period to see how net primary production (NPP) and respiration would respond. In the field respiration is maybe suppressed by nitrogen.

Sensitivity of NPP and Respiration in response to Nutrients

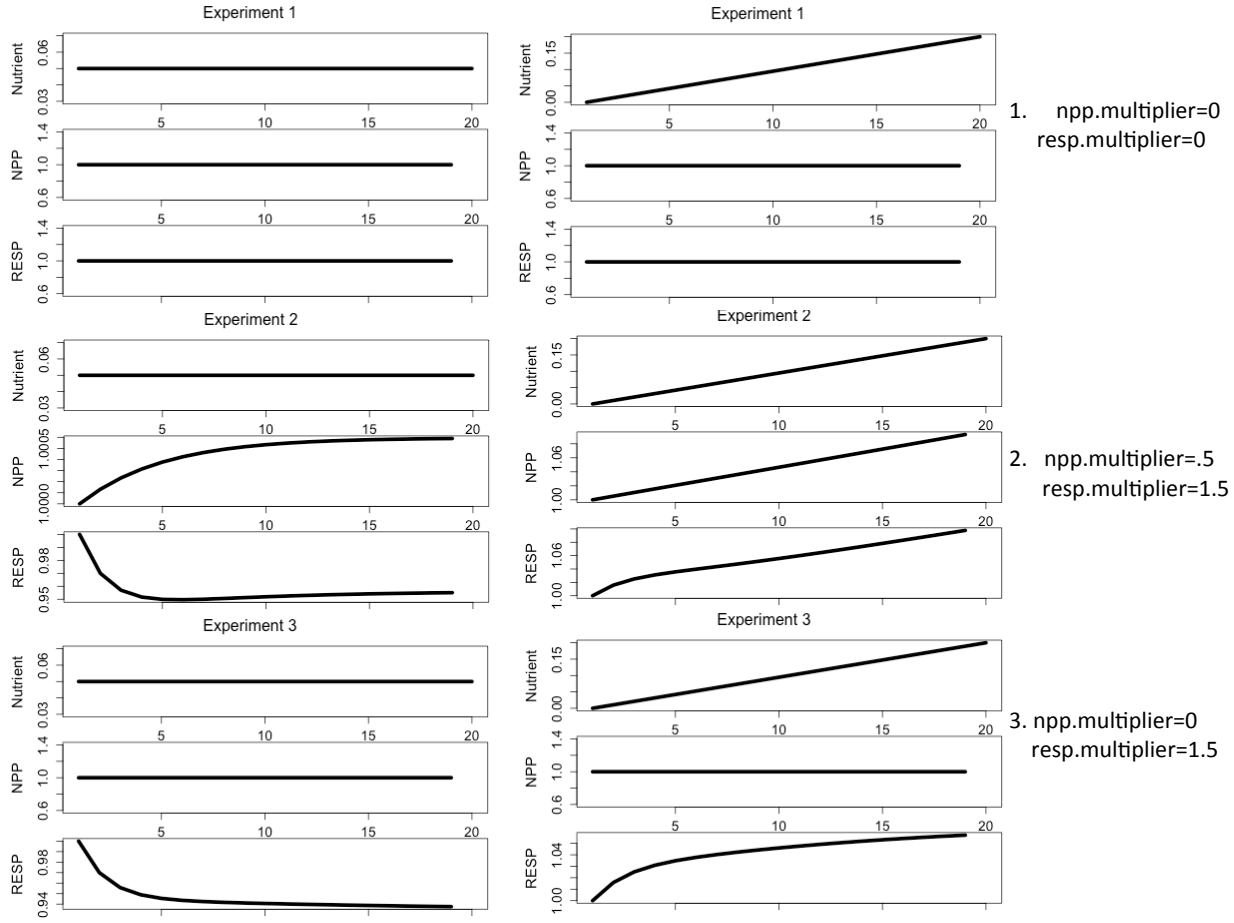


Figure 9. Is showing 2 sets of experiments. The first set is holding the nutrient level constant. The second set is increasing the level of nutrients over time.

Model runs show we can only replicate the change in respiration in two experiments. 1) No N effect on NPP in Experiment 3 with the increase in nutrients. 2) Experiment 2 in the second set is what we would expect in the fertilized plots, as more nutrients enter the system NPP and respiration should increase.

4. Conclusions

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. There are studies that support our findings that fertilization reduces soil respiration (Kowalenko et. al 1976, Bowden et. al 2004, Olsson et. al 2005). 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). 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 hypothesis that increase in nitrogen in subalpine forests influences soil respiration, even though it may not be significantly different. 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. Also temperature was a major factor in soil respiration rates. Our Simple ecosystem model was unable to explain lack of fertilization effect on soil respiration. We used a simple model to test the idea that a transient fertilization effect is now gone. The model results suggest this is not realistic. Nitrogen

saturated soils adjusts to maximum net primary production. Control plots are already saturated because of excess nitrogen deposition received over time.

Discussion

Nitrogen Fertilization

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). Which supports our findings that soil moisture and temperature plays a major factor on soil respiration. We expected fertilization to affect carbon dynamics within the plots since old growth forest are sensitive to increase in nitrogen (Hedin et al 1995). In 2003 Sanjay et. al, found a similar result during his master's thesis: "Soil Respiration responses to fertilization: A comparison of two forests with different nitrogen deposition histories". The lack of significant soil respiration response from fertilized plots suggests nitrogen demand has been saturated by chronic elevated nitrogen deposition.

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.

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.

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.

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

Hogber, P. (2007) Nitrogen impacts on forest carbon. Nature. 447, 781-782

Gruber, N., & Galloway, J. N. (2008). An Earth-system perspective of the global nitrogen cycle. *Nature*, *451*(7176), 293-296.

Janssens, I. A., Dieleman, W., Luyssaert, 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.

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.

Markowski, P., & Richardson, Y. (2011). *Mesoscale meteorology in midlatitudes* (Vol. 2). John Wiley & Sons.

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., Kjønaas, 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.

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, M. G. (1991). Effects of climate change on plant respiration. Ecological Applications, 1(2), 157-167.

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.

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.

Wolyn, P. G., & Mckee, T. B. (1994). The mountain-plains circulation east of a 2-km-high north-south barrier. *Monthly weather review*, *122*(7), 1490-1508.

Vant Hoff JH(1898) Lectures on theorectical and physical chemisty . In: Chemical Dynamics Part I. pp. 224-229. Edward Arnold, London

Calculate Flux and Fit Q10 coefficient

```
#Use soil respiration data to find the Q10

file.name <- file.choose()

#read.csv("/Users/jordan/LVWS.model/Loch Vale Spreadsheet
Data.csv")
#data <- read.csv("/Users/jordan/LVWS.model/Loch Vale
Spreadsheet Data.csv")
data <- read.csv(file.name)

control <- subset(data, Treatment != 'Fertilized')
fert <- subset(data, Treatment == 'Fertilized')

#Date = day of sample
#Treatment = Control or Fertilized
#Plot = location of plot
#Collar  = collar number
#Flux = Respiration measurement
#slope =
#temp      = temperature of soil
#moisture = moisture of soil
#rt  = reference temperature
#pressure  = pressure in KPa
#area     = area of collar in cm^2
#depth = average collar depth
#volume   = volume of chamber
#system   = air in tubing + system
#VolumeC  = Volume of collar =( Collar area * Collar Depth) +
chamber volume +System +Tubing
#Mol of Air in System = =VolumeC/22.414/1000
#pressure = atmospheric pressure

# Calculate respiration for all control data
pressure <- control$pressure * 1000 #(pressure in PA)
volume <- control$Volume/ 1000000 # convert cm^3/cm^3  to m^3
Ref <- control$ref  # kelvin temperature correction for
reference temperature of Licor
R <- 8.314 #J/(K *mol) universal gas constant
control.temp <- control$WST
area <-  control$Collar.area / 10000 # convert cm^2 to m^2
control.flux <- control$slope * pressure *  volume / (R *
(control.temp+273.15) * area)

# Calculate respiration for all fertilized data
pressure <- fert$pressure * 1000 #(pressure in PA)
volume <- fert$Volume/ 1000000 # convert cm^3/cm^3  to m^3
```

```
Ref <- fert$ref  # kelvin temperature correction for reference
temperature of Licor
R <- 8.314 #J/(K *mol) universal gas constant
fert.temp <- fert$WST
area <-  fert$Collar.area / 10000 # convert cm^2 to m^2
fert.flux <- fert$slope * pressure *  volume / (R *
(fert.temp+273.15) * area)


# Convert character dates with slashes to dates R recognizes
data$Date <- as.Date(data$Date, format='%m/%d/%y')
fert.obs <- data.frame(temp=fert.temp, resp=fert.flux)
control.obs <- data.frame(temp=control.temp, resp=control.flux)

# Fit the exponential equation to the control observations
fit <- nls(resp ~ R0 * Q10 ^ ((temp-25)/10), data=control.obs,
           start = list(R0=10, Q10=2), trace=TRUE)
control.R0 <- coef(fit)[1]
control.Q10 <- coef(fit)[2]

# Fit the exponential equation to the fertilized observations
fit <- nls(resp ~ R0 * Q10 ^ ((temp-25)/10), data=fert.obs,
           start = list(R0=10, Q10=2), trace=TRUE)
fert.R0 <- coef(fit)[1]
fert.Q10 <- coef(fit)[2]


# Before making plots, set the plot margins

old.par <- par(no.readonly=TRUE)  # remember the old parameters


bot <- 5
left <- 5
top <- 3
right <- 1
par(mar=c(bot,left,top,right))

par(mfrow=c(1,1))
#Make plot of control data


plot(control.obs$temp, control.obs$resp,
     main='Soil Surface Carbon Flux',
     pch=19, ylim=c(0,8), xlim=c(0,20), col='blue',
     xlab='Temperature (Celsius)', ylab='Respiration(μmol m-2 s-
1)')
```

```
# Overlay the fertilized data as circles
points(fert.obs$temp, fert.obs$resp, pch=1, col='red',
       ylim=c(0,8), xlim=c(0,20))

# Add the fitted curve of control data in blue to the plot
min.temp <- min(control.obs$temp)
max.temp <- max(control.obs$temp)
control.fit.temp <- seq(min.temp, max.temp,
                        (max.temp-
min.temp)/length(control.obs$temp))
control.fit.flux <- control.R0 * control.Q10 ^
((control.fit.temp -25)/10)
lines(control.fit.temp, control.fit.flux, col='blue',lwd=5)

# Add the fitted curve of fertilized data in red to the plot
min.temp <- min(fert.obs$temp)
max.temp <- max(fert.obs$temp)
fert.fit.temp <- seq(min.temp, max.temp,
                        (max.temp-
min.temp)/length(fert.obs$temp))
fert.fit.flux <- fert.R0 * fert.Q10 ^ ((fert.fit.temp -25)/10)
lines(fert.fit.temp, fert.fit.flux, col='red',lwd=5)

#Add text to plots
text(x=2, y=7, label="Control", col='blue')

text(x=2, y=6, label="Fertilized", col='red')
# Restore the old graphics parameters
par(old.par)
```

Ecosystem Carbon Model
Simple Land Script File

```
land <- function(resp.multiplier=0, npp.multiplier=0){

  # Parameters:
  longevity <- 2 # turnover time for live plants (default = 2
yr)
  tau.litter <- 2 # turnover time for dead plant material
(default = 2 yr)
  tau.fast <- 20   # turnover time of fast soil organic matter
(default = 20 yr)
  tau.slow <- 500   # turnover time of slow soil organic matter
(default = 500 yr)
  eff.microbes <- 0.80 # efficiency of microbial respiration
  plant.eq <- 50
  NPP.eq <- 6

  # Initialize a bunch of arrays for later plotting
  nYears <- 20
  NPP <- replicate(nYears,NA)
  resp.total <- replicate(nYears,NA)
  resp.litter <- replicate(nYears,NA)
  resp.fast <- replicate(nYears,NA)
  resp.slow <- replicate(nYears,NA)
  plant <- replicate(nYears,NA)
  litter <- replicate(nYears,NA)
  fast.soil <- replicate(nYears,NA)
  slow.soil <- replicate(nYears,NA)
  mortality <- replicate(nYears,0.)

  # Initialize mass of carbon (kg C) in plants, soil, and
passive pools
  # These are calculated as steady-state solutions to the
differential equations
  capacity <- plant.eq / (1-1/longevity)  # resource-limited
"carrying capacity"
  growth.rate  <- NPP.eq / (plant.eq*(1-plant.eq/capacity))
  death.rate <- growth.rate/longevity  # fractional death per
year

  # Initialize each pool (GtC) to be in equilibrium with NPP and
decay
  plant[1] <- plant.eq
  litter[1] <- tau.litter * death.rate * plant[1]
  fast.soil[1] <- tau.fast/tau.litter * (1-eff.microbes) *
litter[1]
```

```
  slow.soil[1] <- tau.slow/tau.fast * (1-eff.microbes) *
fast.soil[1]

  # Read and set up driver data for nutrients
  driver.data <- read.table('new.lvws.history.txt',
col.names=c('year','nutrient'))
  nutrient <- driver.data$nutrient

  # Integrate the model
  for (i in 1:(nYears-1)){

    # Adjust plant carrying capacity according to nutrients in
soil
    current.capacity <- capacity * (1 + nutrient[i] *
npp.multiplier)

    # If requested, enhance respiration according to nutrients
too
    resp.enhancement <- (1 + nutrient[i] * resp.multiplier)

    # Apply nutrient limitation & fertilization to get updated
NPP
    NPP[i] <- growth.rate * plant[i] * (1 -
plant[i]/(current.capacity))

    # Apply disturbance & mortality
    mortality[i] <- death.rate * plant[i]

    # Calculate respiration for each pool
    resp.litter[i] <- eff.microbes * litter[i]/tau.litter *
resp.enhancement
    resp.fast[i] <- eff.microbes * fast.soil[i]/tau.fast  *
resp.enhancement
    resp.slow[i] <- slow.soil[i]/tau.slow  * resp.enhancement
    resp.total[i] <- resp.litter[i] + resp.fast[i] +
resp.slow[i]

    # Update all the carbon pools in plants and soils
    plant[i+1] <- plant[i] + NPP[i] - mortality[i]
    litter[i+1] <- litter[i] + mortality[i] -
litter[i]/tau.litter * resp.enhancement
    fast.soil[i+1] <- fast.soil[i] + resp.enhancement * (
      (1.-eff.microbes) * litter[i]/tau.litter -
        fast.soil[i]/tau.fast)
    slow.soil[i+1] <- slow.soil[i] + resp.enhancement  * (
      (1.-eff.microbes) * fast.soil[i]/tau.fast -
        slow.soil[i]/tau.slow)
```

```
  }

  return(data.frame(plant=plant, litter=litter,
fast.soil=fast.soil,
                    slow.soil=slow.soil, NPP=NPP,
                    resp.litter=resp.litter,
resp.fast=resp.fast, resp.slow=resp.slow,
                    resp.total=resp.total, nutrient=nutrient))
}
```

 # Create the 20-year history of conditions in LVWS for use in the land model

```
 nitrogen.start <- 0.
 nitrogen.end <- .20
 step <- (nitrogen.end - nitrogen.start) / 19
 nitrogen <- seq(nitrogen.start, nitrogen.end, step)

#nitrogen <- replicate(20,1.05)
#nitrogen[1:5] <- seq(1.01,1.05,.01)

years <- 1996:2015

history <- data.frame(years=years, nitrogen=nitrogen)

write.table(history, file='new.lvws.history.txt', row.names=F,
col.names=F)
```