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# Investigating Feedbacks From Soil Trace Gas Fluxes of Carbon Dioxide and Nitrogen Oxides to Anthropogenic Nitrogen Deposition and Climate Change

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Plant Biology

by

Jennifer Rae Eberwein

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Dissertation Committee:
Dr. Darrel Jenerette, Chairperson
Dr. Emma Aronson
Dr. Edith Allen

The Dissertation of Jennifer Rae Eberwein is approved:

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Committee Chairperson

University of California, Riverside

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Dedication

I dedicate this work to my daughter, Charlotte Eberwein, who reminds me to wonder at the world around me. She is my constant motivation to be a better person and my inspiration to want to make the world a better place.

ABSTRACT OF THE DISSERTATION

Investigating Feedbacks From Soil Trace Gas Fluxes of Carbon Dioxide and Nitrogen Oxides to Anthropogenic Nitrogen Deposition and Climate Change

by

Jennifer Rae Eberwein

Doctor of Philosophy, Graduate Program in Plant Biology
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Dr. Darrel Jenerette, Chairperson

Human alteration of the carbon (C) and nitrogen (N) cycles is having profound detrimental impacts on natural systems. This dissertation research focused on investigating soil feedbacks to multiple anthropogenic drivers to explore unifying mechanisms applicable to global biogeochemical modeling. In Chapter 1 and 2, I investigate how changes in C, N, and temperature regulate soil $CO_2$ production ($R_{soil}$) through changes to the Michaelis-Menten parameters (i.e. $V_{max}$ and kM) using soils from three contrasting ecosystems in southern California in Chapter 1 and in subtropical evergreen forests of southern China in Chapter 2. Overall, the response of $R_{soil}$ to N addition was generally dependent on C:N stoichiometry, consistent with predictions from the dynamic microbial carbon-use efficiency hypothesis. Furthermore, I show the first empirical results from whole soil measurements demonstrating temperature sensitivity of both $V_{max}$ and kM, showing strong support for substrate regulation of $R_{soil}$ temperature sensitivity across diverse soils. Results from Chapters 1 and 2 demonstrate great potential

for Michaelis-Menten kinetics in describing $R_{soil}$ responses to N and temperature, with implications for understanding microbial physiology and broad applicability to global biogeochemical modeling. Chapter 3 extends this research by exploring N trace gas emissions and evaluating variation in the microbial community composition along an N deposition gradient in the Colorado Desert. Results from Chapter 3 present soil N fluxes that were considerably higher than expected, demonstrating a need for greater appreciation of arid systems in global N budgets. While short term effects of experimental N addition resulted in inconsistent responses in the microbial community composition, long-term N deposition resulted in a distinct differentiation of the microbial community, with possible implications for N cycling. Microbial communities associated with nitrification, identified from 16S rRNA sequencing and qPCR of *amoA*, demonstrate a shift from archaeal dominance at the low deposition site to more bacterial dominance at the high deposition site. Overall, results from this dissertation contribute to mechanistic understanding of soil feedbacks to climate change and nitrogen deposition, which is necessary for predicting future changes and developing strategies for mitigation of anthropogenic global change drivers.

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# Introduction to the Dissertation

The Earth is over 4.5 billion years old, and humans have only existed on it for around 200,000 years. Yet, arguably, no other species has elicited such massive changes to the entire globe. The greatest impacts threatening terrestrial ecosystems include land use change, climate change, nitrogen deposition, biotic exchange and carbon dioxide (Sala et al., 2000). All of these influences involve the carbon and nitrogen cycles. Life depends on biogeochemical cycling of matter through biotic and abiotic factors between organic and inorganic forms (Schlesinger and Bernhardt, 2013). The carbon (C) and the nitrogen (N) cycles are among the most important of these processes. These cycles are highly interconnected, and pass through similar phases in their respective development. A variety of anthropogenic influences are impacting the natural course of these biogeochemical cycles. Human beings are experiencing exponential growth unchecked by any of the usual natural limitations (Gerland et al., 2014), which is quickly surpassing the availability of resources. While it is understood that the C and N cycles are being both altered by anthropogenic manipulation and that that alteration is significantly impacting ecosystem functioning, the extent of that impact is as yet unclear as well as the mechanisms regulating potential interaction. This information is vital to both predict future scenarios and for the development of mitigation strategies.

Due to fossil fuel combustion, anthropogenic emissions of $CO_2$ have added over 2000 Gt of cumulative $CO_2$ to the atmosphere between 1750 and 2011, which has resulted in an increase in global temperature of about 0.85 °C (IPCC 2014), and a 17 cm

rise in sea level over the last decade (Church and White, 2006). As a result, 1 in 6 species faces extinction due to global climate change (Urban, 2015). Much research is focused on plant C sequestration as a means for mitigation and for understanding future risks, but soils store approximately 1500 Pg C, with estimates ranging from 504-3000, which is more than plants and the atmosphere combined (Scharlemann et al., 2014). Therefore, soils represent a great potential for either mitigating climate change or exacerbating it, depending on the direction of future soil C stores. If rising temperatures increase microbial activity and decomposition, then soils could create a positive feedback to climate change (Bond-Lamberty and Thomson, 2010). However, improved microbial physiological representation in Earth System Models (ESMs) through inclusion of dynamics in microbial carbon-use efficiency (CUE) indicates that microbial acclimation will allow soils to serve as a sink under a future warming climate (Allison, 2014; Wieder et al., 2013). However, more research is necessary to demonstrate the validity of these models (Schimel 2013). Current ESMs employ a fixed CUE, despite a growing body of literature in support of dynamic CUE (Manzoni et al., 2012). This highlights the need for better understanding of microbial physiology for accurate representation in ESMs, to inform mitigation strategies and so that accurate predictions can be made to inform policy makers on what limits need to be set.

Fossil fuel use also contributes substantially to nitrogen pollution of natural systems. In unaltered environments, nitrogen is often a limiting resource (Elser et al., 2007; Lebauer and Treseder, 2008). Human activities have altered the nitrogen cycle to such an extent that anthropogenic inputs of nitrogen are double that of natural processes

(Vitousek et al., 1997). Anthropogenic nitrogen deposition results from fossil fuel combustion in industry and transportation and from agricultural activities. Through the Haber-Bosch process, humans are able to convert $N_2$ to $NH_3$ for use as a fertilizer. Overuse of fertilizers has had widespread impact on both terrestrial and aquatic ecosystems. In the western United States the largest sources of nitrogen deposition are transportation, agriculture and industry, resulting in 1-4 kg N $ha^{-1}$ $yr^{-1}$ deposition rates. This number can be as high as 30-90 kg N $ha^{-1}$ $yr^{-1}$ near urban and agricultural areas (Fenn et al., 2003b). This dramatic increase can result in severe consequences to organisms that have adapted to environments that are nutrient poor.

Furthermore, many ecosystems are subjected to multiple global change drivers acting in concert, and the cumulative effects of these alterations in the C and N cycles are as of yet unclear (Bardgett et al., 2008; Gärdenäs et al., 2011). C budgets are strongly influenced by vegetation. Nitrogen deposition has been demonstrated to cause shifts in species compositions, invasive species success, biodiversity loss and increased risk of fire (Bobbink et al., 2010; Fenn et al., 2003a; Vitousek et al., 1997). These N-induced ecosystem changes have the potential to impact C cycles. The direction of C change is difficult to predict. Any gains from plant productivity have to be balanced by respiratory losses. N enrichment has the potential to increase C sinks through aboveground biomass accumulation, but alterations in respiration due to species composition, biomass partitioning and litter quality and quantity indicate that the opposite could also occur. Furthermore, variability in precipitation patterns are increased due to climate change (Dore, 2005), and C sequestration in plant productivity can be offset by respiratory losses

under hotter and drier conditions (Angert et al., 2005; Piao et al., 2008). Furthermore, in ecosystems other than forests, there is greater potential for long-term C sequestration in soils than in vegetation (Scharlemann et al., 2014). N is also limiting to microbial growth, and could therefore result in accelerated decomposition, with feedbacks to climate change. Lastly, N fluxes can also feedback to climate change. $N_2O$ is a greenhouse gas with 300 times the global warming potential as $CO_2$ (Forster et al., 2007), and increased $N_2O$ production due to fertilization from N deposition could offset any positive benefits of N on plant C sequestration (Liu and Greaver, 2009).

To address these knowledge gaps, I explored the soil feedbacks of trace gas emissions of $CO_2$, $NO_x$ and $N_2O$ across a diverse set of environments to test unifying mechanisms regulating soil feedbacks to the anthropogenic influences of N deposition and climate change. In Chapters 1 and 2, I examine the response of soil $CO_2$ production to temperature and N availability, using substrate-based kinetics as a unifying mechanism for representation of global change drivers on soil respiration dynamics. Chapter 3 extends this research by exploring N trace gas emissions and evaluating the potential role of microbial community variation on soil trace gas emissions with molecular analysis.

Chapter 1: Carbon availability regulates soil respiration response to nitrogen and temperature

Abstract

The response of soil $CO_2$ fluxes ($R_{soil}$) to interactions between carbon (C) and nitrogen (N) availability or C and temperature conditions is not well understood, but may increasingly affect future C storage under the combined anthropogenic impacts of N deposition and climate change. Here we addressed this uncertainty through a series of laboratory incubation experiments using soils from three contrasting ecosystems to investigate how changes in C, N, and temperature regulate $R_{soil}$ through changes to the Michaelis-Menten parameters (i.e. $V_{max}$ and $K_m$). Results of this study demonstrate that $R_{soil}$ response to N enrichment and changes in temperature are dependent on the C availability of soil substrates. N addition influenced $R_{soil}$ through both the maximum rate ($V_{max}$) and the half saturation constant ($K_m$). The increase in $K_m$ corresponded to a decrease in $R_{soil}$ when C was limited. Alternatively, when C was abundant, N enrichment increased $R_{soil}$, which corresponded to an increase in $V_{max}$. Regulation of temperature sensitivity through $V_{max}$ and $K_m$ was also dependent on C availability. Both $V_{max}$ and $K_m$ demonstrated positive temperature responses, supporting the hypothesis of a canceling effect at low C concentrations. While temperature sensitivity was influenced by both C quantity and C complexity, our results suggested that C quantity is a stronger predictor. Despite strong differences in climate, vegetation, and management of our soils, C-N and C-temperature interactions were markedly similar between sites, highlighting the

importance of C availability in the regulation of $R_{soil}$ and justifying the use of Michaelis-Menten kinetics in biogeochemical modeling.

## Introduction

Soil organic matter represents the largest terrestrial pool for carbon (C) storage (Schlesinger and Bernhardt, 2013). Losses from this pool to the atmosphere are regulated by $CO_2$ production from microbial respiration (soil respiration; $R_{soil}$). Two global change drivers, N deposition and climate change, present great potential for altering loss from the soil C pool. However, limited understanding of $R_{soil}$ response to interactions between C, N and temperature inhibit our ability to predict future C storage under the combined anthropogenic impacts of N deposition and climate change.

Describing $R_{soil}$ in response to substrate availability can be useful for modeling purposes. This is commonly accomplished through Michaelis-Menten kinetics, which describes $R_{soil}$ as a saturating function of substrate concentration with the parameters $V_{max}$ and $K_m$. $V_{max}$ represents maximum respiration rate and $K_m$ is the half-saturation constant, which relates to the inverse of an enzyme's affinity for its substrate. While Michaelis-Menten kinetics are being applied to models of $R_{soil}$ (Davidson et al., 2012; Oikawa et al., 2014), experimental evidence of how $V_{max}$ and $K_m$ change in response to N and temperature in whole soils is lacking. Most studies applying Michaelis-Menten kinetics to $R_{soil}$ have focused on isolated soil enzymes (Allison et al., 2010; German et al., 2012a; Stone et al., 2012). Whole soils present additional constraints to $R_{soil}$ such as variability in microbial activity and microsite moisture and substrate availability (Castellano et al.,

2012; German et al., 2011; Schmidt et al., 2011). Thus, investigation of changes in Michaelis-Menten parameters in bulk soil due to changes in N and temperature may be useful to clarify $R_{soil}$ responses to N deposition and climate change.

Fossil fuel combustion and agricultural activities have resulted in inputs of N into many ecosystems that exceed biological fixation (Galloway et al., 2008). N addition influences $R_{soil}$ in complex ways, with both increases (Fog, 1988; Ramirez et al., 2010; Zhou et al., 2013) and decreases observed (Craine et al., 2007; Janssens et al., 2010; Liu and Greaver, 2010; Ramirez et al., 2010). The mechanisms responsible for these divergent responses are not well established. Microbial N mining is one hypothesis that describes how microbes "mine" recalcitrant C at the expense of labile C to relieve N limitation (Craine et al., 2007). This could explain the observed negative response of $R_{soil}$ to N addition, but does not account for the positive response that has been seen in many studies. Recently, modeling work has linked the N enrichment response of $R_{soil}$ to microbial physiology through C and N stoichiometry (Manzoni et al., 2008; Parton et al., 2007; Schimel and Weintraub, 2003). These studies suggest microbes adjust respiration rates based on external C and N availability to meet internal stoichiometric requirements. Thus the divergent responses of $R_{soil}$ to N enrichment may be explained by changes in soil C availability.

Concerns about $R_{soil}$ response to climate change have increased interest in the temperature sensitivity of $R_{soil}$. A positive feedback in the climate system could occur if warming stimulates additional soil C losses (Bond-Lamberty and Thomson, 2010). Temperature sensitivity is commonly described by $Q_{10}$, the factor of increase in a process

associated with a 10°C rise in temperature (Lloyd and Taylor, 1994). Despite numerous studies, the relationship between C availability and $Q_{10}$-value are not well resolved. Some studies demonstrate that $Q_{10}$-value should be inversely related to C complexity where more recalcitrant substrates require greater activation energy and therefore demonstrate a higher response to temperature than more labile substrates with lower activation energy requirements (Bosatta and Ågren, 1999; Fierer et al., 2005). Alternatively, C quantity may be more important in mediating temperature response through the individual $Q_{10}$-values of the Michaelis-Menten parameters $V_{max}$ and $K_m$ (Davidson et al., 2012; Davidson and Janssens, 2006; Gershenson et al., 2009). When substrate concentration is low, the individual $Q_{10}$-values of $V_{max}$ and $K_m$ can cancel, decreasing the overall "apparent" $Q_{10}$ of $R_{soil}$ (see Equation 3). Alternatively, when substrate concentration is abundant, the $Q_{10}$ of $R_{soil}$ is dependent on $V_{max}$ alone. Although recent modeling studies demonstrate this canceling effect at low substrate concentration (Davidson et al., 2012; Oikawa et al., 2014), there is a lack of studies that have experimentally measured the $Q_{10}$ of $V_{max}$ or $K_m$ in whole soils. Furthermore, if the $Q_{10}$-values of $V_{max}$ and $K_m$ are not equal, it would affect the magnitude of the canceling effect at low substrate concentrations. Thus, whether C complexity or C quantity would have a greater influence on the regulation of $R_{soil}$ temperature sensitivity is unclear.

To address these uncertainties regarding the interactive effects of C, N and temperature on $R_{soil}$ we present a series of soil incubation experiments using soils from ecosystems in southern California that span a range of climate conditions and nutrient availability. We hypothesize that 1) $R_{soil}$ response to both N enrichment and temperature

change is regulated by C availability, which can be described through Michaelis-Menten kinetics and 2) the role of C availability in the regulation of $R_{soil}$ response to changes in N and temperature is consistent across soil types, representing common mechanisms across diverse conditions. Tests of these hypotheses evaluate environmental influences on $R_{soil}$ Michaelis-Menten kinetics and validate their use in biogeochemical models.

## Methods

### *Study sites*

Soil samples were collected from three sites in southern California: a desert, a subalpine coniferous forest, and a fallow agricultural field, to represent a range in soil characteristics from arid and semi-arid environments (Table 1.1). The desert (DS) soils were collected from Boyd Deep Canyon Desert Research Center, which is part of the UC Reserve system located near Palm Springs, CA at the base of the Santa Rosa Mountain Range. The dominant vegetation at DS is *Larrea tridentata* (creosote bush). Soils are sandy texture, from alluvial fan deposits, and classified as sandy-skeletal, mixed, hyperthermic Typic Torriorthents (Carrizo). The subalpine (SA) coniferous forest is located at approximately 2500 m elevation in the Santa Rosa Mountain Range of southern California. The dominant vegetation is *Pinus jeffreyi* (Jeffrey pine) and soils are classified as coarse-loamy, mixed, mesic Ultic Haploxerolls (Crouch series). Samples were collected from DS and SA in July 2011. The agricultural (AG) soils were collected from the UC Desert Research and Extension Center in the Imperial Valley near El Centro, CA. Soils are silty clay and classified as Imperial - fine, smectitic, calcareous,

hyperthermic Vertic Torrifluvents. The field had been fallow for approximately one year prior to collection of soil samples in November 2011. Soil textures ranged from coarse (DS) to fine textured (AG). Climatic conditions ranged from soil temperatures at DS and AG frequently above 50°C with very little snow or frost to SA soils, where soil temperatures do not exceed 25°C and approximately half of precipitation is received as snow (http://soils.usda.gov/technical/classification/osd/index.html Accessed Nov/14/2013).

Table 1.1: Characteristics of study sites

| Site | Latitude/ Longitude | Elevation (m) | MAT (°C) | MAP (mm) | Vegetation | Soil texture | C:N |
|---|---|---|---|---|---|---|---|
| DS | N 33° 39.10' W 116° 22.48' | 289 | 24 | 138 | Desert scrub | Stony sand | 12±1.5[b] |
| SA | N 33° 31.42' W 116° 27.18' | 2489 | 10 | 521 | Coniferous forest | Sandy loam | 25±1.8[a] |
| AG | N 32° 48.76' W 115° 26.67' | - 50 | 22.7 | 76 | Agricultural (fallow) | Silty clay | 27±1.6[a] |

* Letters represent significant difference at $p<0.05$

At each of the study sites, five field replicates were collected with a hand trowel from the top 0-15 cm of the soil mineral layer under the canopy of the dominant vegetation (where applicable). Samples were air dried in the lab, sieved to 2 mm and homogenized. Samples were not composited so that replicates represent field variability. Total soil C and N contents were determined on a combustion elemental analyzer with an Eager 200 CE Instrument. Water holding capacity (WHC) was determined by the

gravimetric water content of soil placed in a filter funnel and saturated with deionized water, then allowed to drain for two hours. To prepare for incubation, sieved soils of 50g dry weight from each field replicate were placed in 237ml glass jars and brought to 40% WHC with deionized water. A dynamic closed system similar to Yuste et al. (2007) was used to measure $R_{soil}$ with a Li-7000 infrared gas analyzer (Licor Biosciences).

*120 day incubation*

To investigate $R_{soil}$ response to C and N interactions four treatments were used: control (Con; water addition only), nitrogen (Nit; nitrogen addition at 100 µg g-1 soil), dextrose (Dex; 90g/L) and both nitrogen and dextrose (N+D) additions. The dextrose treatments translate to carbon additions of 27, 12.6 and 19.8 mg $g^{-1}$ soil for AG, DS and SA, respectively. Levels of dextrose addition were designed to reach saturating conditions for all soils (Jenerette and Chatterjee, 2012), and N amounts were chosen to represent a moderate amount of N addition (Ramirez et al. 2010). After the one time addition of treatments, soils were maintained at 40% WHC with DI water in an incubator at 25°C for approximately 120 days. $CO_2$ flux was measured on Day 1, 7, 10, 17, 22, 29, 59, 94 & 122 for AG, Day 1, 7, 10, 17, 22, 59, 94 & 122 for DS and Day 1, 4, 8, 14, 21, 29, 94 & 120 for SA after treatment additions. For every day of analysis, the soils were brought to 13, 19, 25 and 31°C with a circulating water bath to evaluate temperature sensitivities between treatments. $R_{soil}$ was calculated from the linear increase in $CO_2$ concentration over time as follows (adapted from Licor 8100 Manual):

$$F_c = \frac{VP_0\left(1 - \frac{W_0}{1000}\right)}{RS\,(T_0 + 273.15)} \times \frac{\partial C'}{\partial t'}, \qquad [1]$$

where $F_c$ is the soil $CO_2$ efflux rate (μmol $g^{-1}$ soil $s^{-1}$), V is volume ($cm^3$), $P_0$ is the initial pressure (kPa), $W_0$ is the initial water vapor mole fraction (mmol mol-1), R is the ideal gas constant (8314 $cm^2$ kPa $K^{-1}$ $mol^{-1}$), S is soil weight(g), $T_0$ is initial air temperature (°C), and $\partial C'/\partial t'$ is change in water-corrected $CO_2$ mole fraction derived from linear regression (μmol $mol^{-1}$). For all flux calculations, $R^2$ values for linear regressions were greater than or equal to 0.99 for measurements taken 24 hours after treatment additions and greater or equal to 0.94 for fluxes measured thereafter.

Temperature sensitivity was calculated from the increase in $R_{soil}$ between 13 and 31°C as follows:

$$Q_{10} = \left(\frac{R2}{R1}\right)^{10/(T2-T1)}, \quad [2]$$

where $Q_{10}$ is temperature sensitivity and R2 and R1 are soil $CO_2$ flux at temperatures T2 and T1, respectively.

*Michaelis-Menten Model*

To quantify $R_{soil}$ substrate dependent kinetics, dextrose was added at 0, 1.5, 3, 7.5 and 15 mg dextrose $g^{-1}$ soil with and without N addition at 200 μg $NH_4NO_3$ $g^{-1}$ soil. Soils were maintained at 40% WHC, and $CO_2$ flux was measured approximately 24 hours after additions at 13-31°C as described above. Parameters were fit by non-linear regression (Matlab 2012a) for each of the field replicated samples and averaged to the Michaelis-Menten model:

$$R_{soil} = \frac{V_{max} * [S]}{K_m + [S]}, \quad [3]$$

where $R_{soil}$ is a function of substrate concentration [S] (mg dextrose $g^{-1}$ soil), $V_{max}$ is maximum respiration rate (μmol $g^{-1}$ soil $s^{-1}$) and $K_m$ is the half-saturation constant (mg dextrose $g^{-1}$ soil), which relates to the inverse of an enzyme's affinity for its substrate. Fits to the Michaelis-Menten model produced an average $R^2$ of 0.91 ± 0.07. $Q_{10}$ was calculated by the increase in $V_{max}$ or $K_m$ between 13 and 31°C as described in Equation 2.

*Carbon complexity*

To explore the effect of carbon complexity on $R_{soil}$, a recalcitrant, high complexity C source, tannin (gallotannin) was added to compare with dextrose additions (a labile, low complexity carbon source) consistent with previous research investigating the carbon complexity-temperature sensitivity relationship (Fierer et al., 2005). Tannin addition was performed similarly to dextrose additions with preliminary experiments used to determine saturating concentration of tannin. The five levels of tannin addition were 0, 7.5, 15, 30, and 60 mg $g^{-1}$ soil with and without nitrogen enrichment at 200 μg $NH_4NO_3$ $g^{-1}$ soil. Temperature sensitivity of $R_{max}$ was calculated according to equation 2 from the difference between 13 and 31°C.

*Statistics*

Analysis of Variance (ANOVA) was used to test for treatment effects for the 120 day incubation. There were significant interactions between treatment, study site and temperature, so one-way ANOVAs were used for each study site at each temperature for each day to compare treatments. Student's t-tests were used to determine if N enrichment

significantly increased $V_{max}$ or $K_m$. Two-way ANOVAS were used to test for differences in $Q_{10}$ between $V_{max}$ and $K_m$ and the effect of N enrichment on the $Q_{10}$ of $V_{max}$ or $K_m$. Additionally, two-way ANOVAs were used to compare $R_{max}$ from tannin addition to $V_{max}$ from dextrose addition, along with the N addition response of these parameters. Normality and homoscedacity were confirmed for all analyses using the Shapiro-Wilks and Bartlett's test (F test for t-tests), respectively. The Box-Cox family of transformations were used to fulfill assumptions of normality and homoscedasticity where appropriate (Box and Cox, 1964). For all t-tests, the Welsh test was used in cases of unequal variance, and the Mann-Whitney test was used in cases of non-normality. When ANOVAs presented significant treatment effects, Tukey's Honest Significant Difference was used for post-hoc comparisons.

## Results

### *120-day incubation*

After a one-time addition of dextrose and/or ammonium nitrate, $R_{soil}$ demonstrated an initial large pulse of activity. On day 1 of the incubation, dextrose increased $R_{soil}$ relative to the control by an average of 554, 414, and 234% for AG, DS, and SA, respectively (Figure 1.1). There was a decrease for N addition compared with the control for only SA of 21% ($p = 0.003$). However, all soils demonstrated greater fluxes in the N+D treatment versus Dex alone ($p < 0.001$), with the combined treatment increasing fluxes by 54, 49, and 73% for AG, DS, and SA, respectively. Cumulative fluxes for the 120 day incubation, calculated by trapezoidal integration, revealed that 44,

67 and 65% of the added C was lost through respiration in the Dex treatment and 63, 64, and 69% in the N+D treatment (for AG, DS and SA, respectively). Although the treatment effect persisted for different lengths of time depending on the origin of the soil (Figure 1.2), cumulative effects from the 120 day incubation mirrored the responses on day 1. Therefore, all comparisons were made on results from day1 where the treatment effect was most pronounced.

There was a significant interaction between treatment and temperature effects on $R_{soil}$ ($p = 0.011$), with the magnitude of the response for N+D versus Dex increasing with higher temperature (Figure 1.1). Furthermore, interactions between N and dextrose drove changes in $Q_{10}$-values (Table 1.2). Treatment effects on $Q_{10}$-values were mainly associated with dextrose addition, with inconclusive effects from N. The exception was in the DS soil, where the N+D addition increased $Q_{10}$-values by an average of 39% over Dex alone ($p<0.001$).

Table 1.2: $Q_{10}$ of $R_{soil}$ on Day 1 of the 120 day incubation

| Study site | Con | Nit | Dex | N+D |
|---|---|---|---|---|
| DS | $1.78 \pm 0.21^{a}$ | $1.55 \pm 0.18^{ab}$ | $1.89 \pm 0.17^{ab}$ | $2.63 \pm 0.31^{c}$ |
| AG | $1.95 \pm 0.070^{a}$ | $1.87 \pm 0.037^{abc}$ | $1.77 \pm 0.080^{b}$ | $1.80 \pm 0.13^{bc}$ |
| SA | $1.55 \pm 0.070^{a}$ | $1.46 \pm 0.15^{b}$ | $1.86 \pm 0.046^{b}$ | $2.15 \pm 0.34^{b}$ |

* Letters represent significant difference between treatments at $p<0.05$

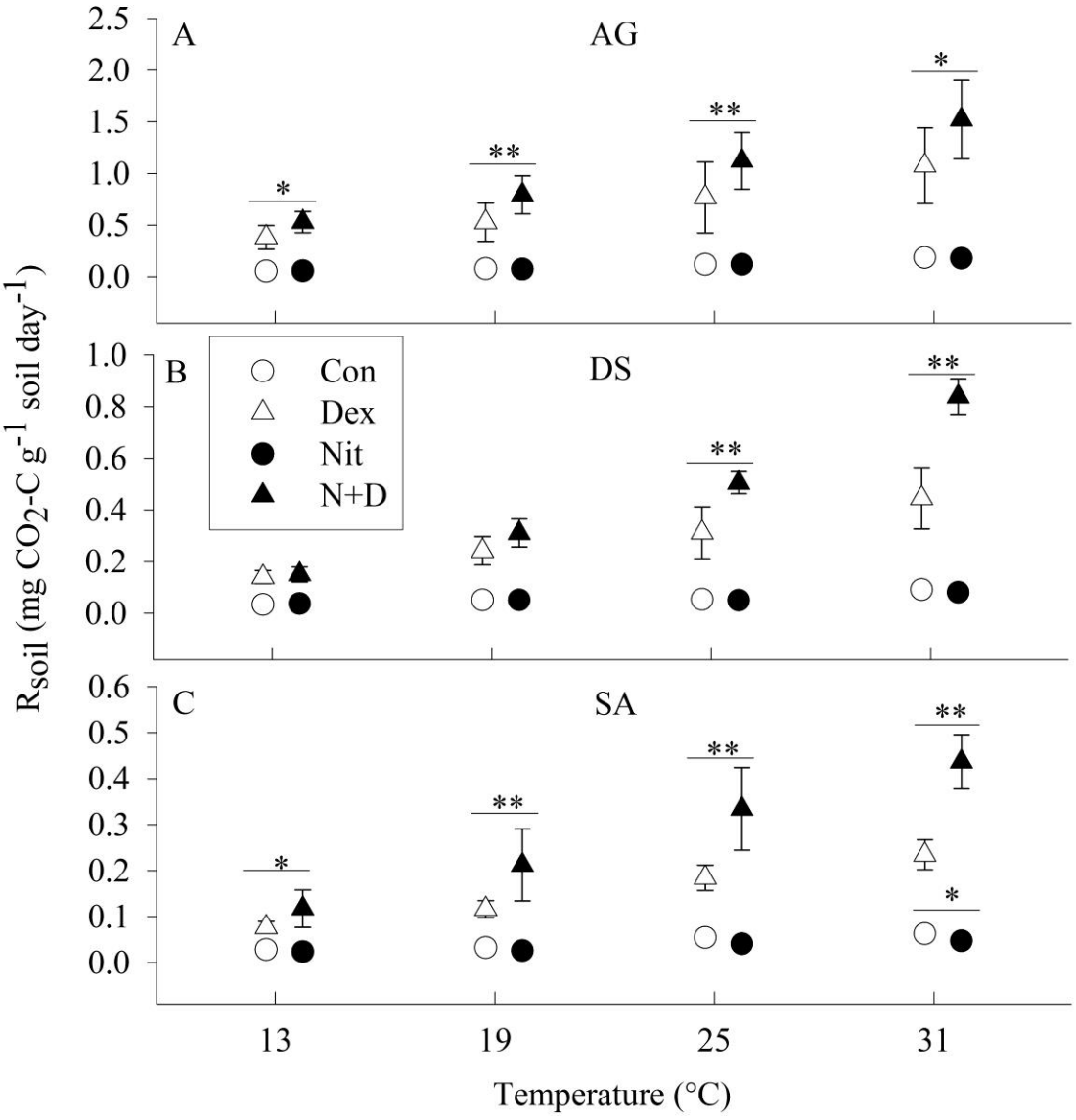


Figure 1.1: Soil respiration ($R_{soil}$) measured one day after carbon and/or nitrogen additions for an agricultural (a; AG), a desert (b; DS), and a subalpine (c; SA) soil. Treatments are Con, control, Nit, nitrogen (100 µg $NH_4NO_3$ g-1 soil), Dex, dextrose (90g/L), and N+D, both nitrogen and dextrose. ** represents significance at $p<0.05$ and * represents marginal significance at $p<0.1$ (ANOVA, Tukey HSD) for with versus without nitrogen, indicated by the two points under the line. Error bars are ±1SD (n=5). Note the difference in scale for the y-axis between study sites.

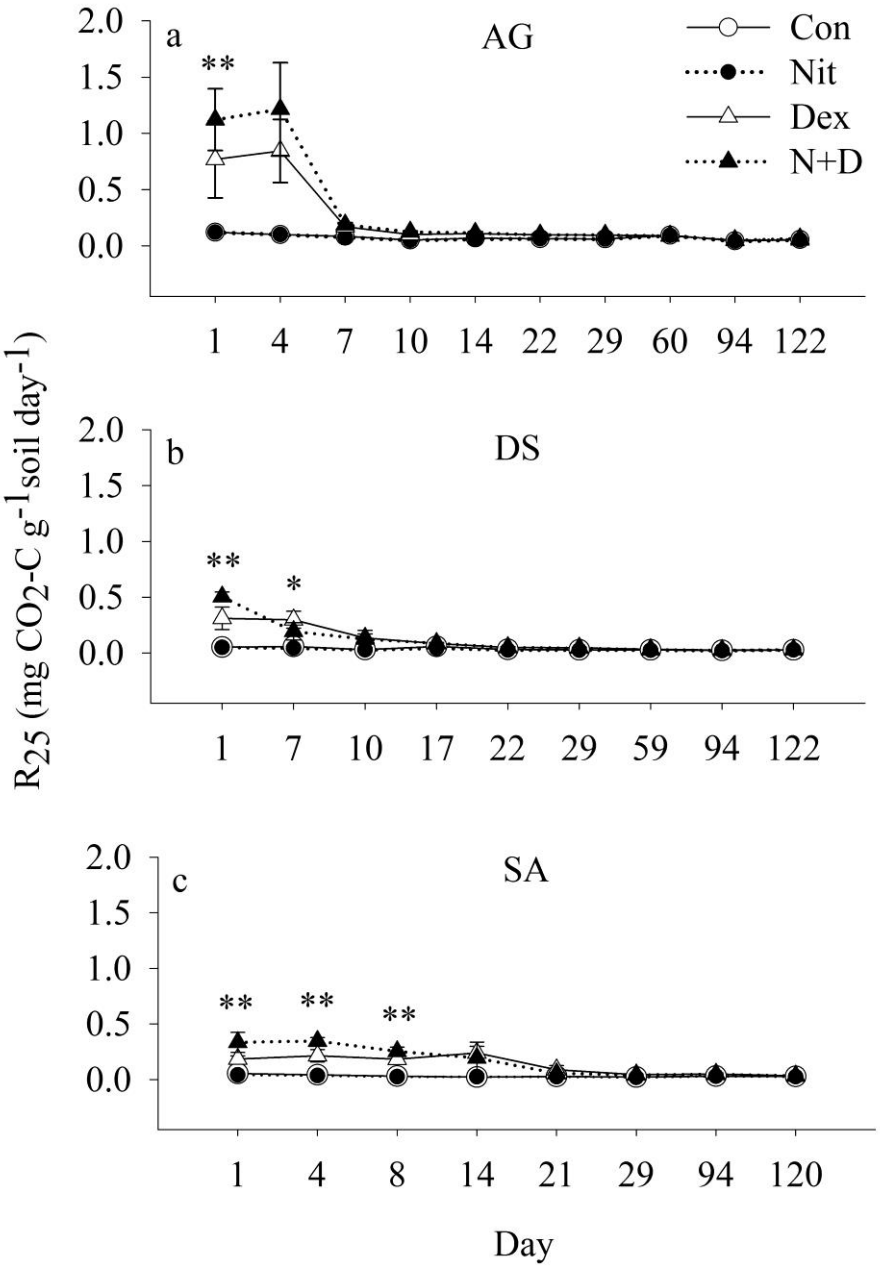


Figure 1.2: Soil respiration at 25°C ($R_{25}$) measured over approximately 120 days after a one time addition of carbon and/or nitrogen for an agricultural (a; AG), a desert (b; DS), and a subalpine (c; SA) soil. Treatments are Con, control, Nit, nitrogen (100 µg $NH_4NO_3$ g-1 soil), Dex, dextrose (90g/L), and N+D, both nitrogen and dextrose. ** represents significance at $p<0.05$ and * represents marginal significance at $p<0.1$ for Dex compared to N+D (ANOVA, Tukey HSD). Dextrose effect was significant ($p<0.05$) until Day 29 for AG and DS and for the entire incubation for SA (not shown). Error bars are ±1SD (n=5).

*Michaelis-Menten Model*

Determination of Michaelis-Menten parameters with and without N additions allowed for investigation of interactions between C, N and temperature in regulation of $R_{soil}$ (Figure 1.3). N addition substantially increased $V_{max}$ and $K_m$ ($p<0.05$) for AG and SA but not for DS ($p>0.3$), although the trend was consistent (Figure 1.3A). Temperature also increased $V_{max}$ ($p<0.001$) and $K_m$ ($p<0.05$) for all soils, with the exception of the $K_m$ of DS ($p=0.56$). The $Q_{10}$-values of $V_{max}$ were significantly greater than the $Q_{10}$-values of $K_m$ for AG and DS ($p<0.01$), but not for SA ($p=0.24$; Figure 1.3B). Furthermore, the effect of N on the $Q_{10}$-value of $V_{max}$ and $K_m$ was marginally significant for all soils ($p = 0.07$ and 0.06 for $V_{max}$ and $K_m$, respectively).

*Carbon complexity*

Finally, we evaluated the role of C complexity in the regulation of N and temperature sensitivity of $R_{soil}$. Due to inhibition of $R_{soil}$ at high levels of tannin concentrations, attempts to fit to the Michaelis-Menten model were poor (greater than 35% of $R^2$ were less than 0.85). Therefore, $R_{max}$, maximum soil respiration corresponding to saturating substrate concentration, was used in place of $V_{max}$ in C complexity analyses. $R_{max}$ occurred at 30, 15 and 7.5 mg $g^{-1}$ tannin for AG, DS and SA, respectively. In general, $R_{max}$ was similar under the tannin and dextrose treatments (Figure 1.4A). However, in SA with N additions $R_{max}$ was greater with dextrose compared to tannin ($p<0.001$). For the AG soil, $R_{max}$ was greater in the tannin treatment without N, but with N addition $R_{max}$ was greater with the dextrose treatment ($p<0.001$).

Similarly, C complexity did not exert a strong influence over $Q_{10}$-value of $R_{soil}$, where the $R_{max}$ $Q_{10}$-values of the tannin treatment were equal to or less than the $Q_{10}$-values for the dextrose treatment (Figure 1.4B).

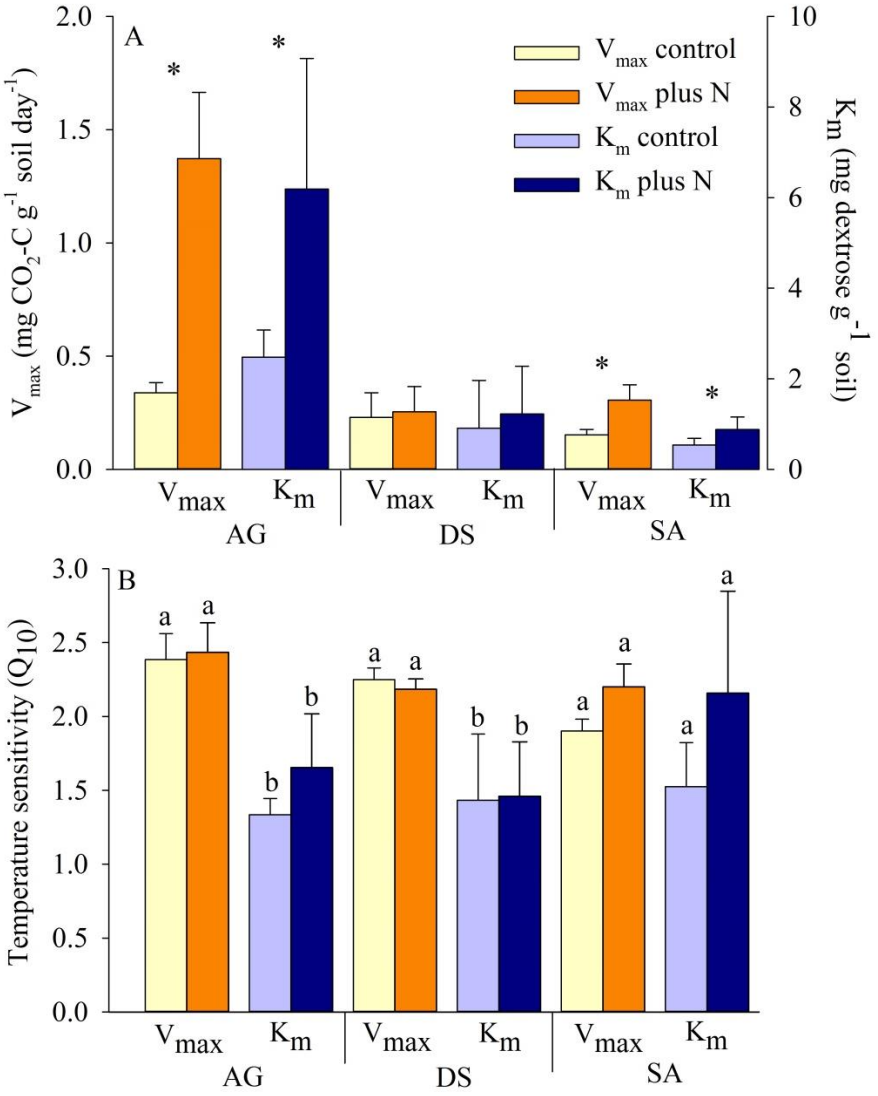


Figure 1.3: Michaelis-Menten parameters ($V_{max}$ and $K_m$) at 19°C (a) and temperature sensitivity ($Q_{10}$) of the Michaelis-Menten parameters (b) for whole soils with and without nitrogen addition at 200 μg $g^{-1}$ soil. Note the different units for $K_m$ represented by the right y-axis. Asterisk (*) indicates significant difference at $p<0.05$ (t-test) for with versus without nitrogen. Letters represent significance ($p<0.05$) between measurements for each soil (ANOVA, Tukey HSD). Error bars are 1SD (n=5).

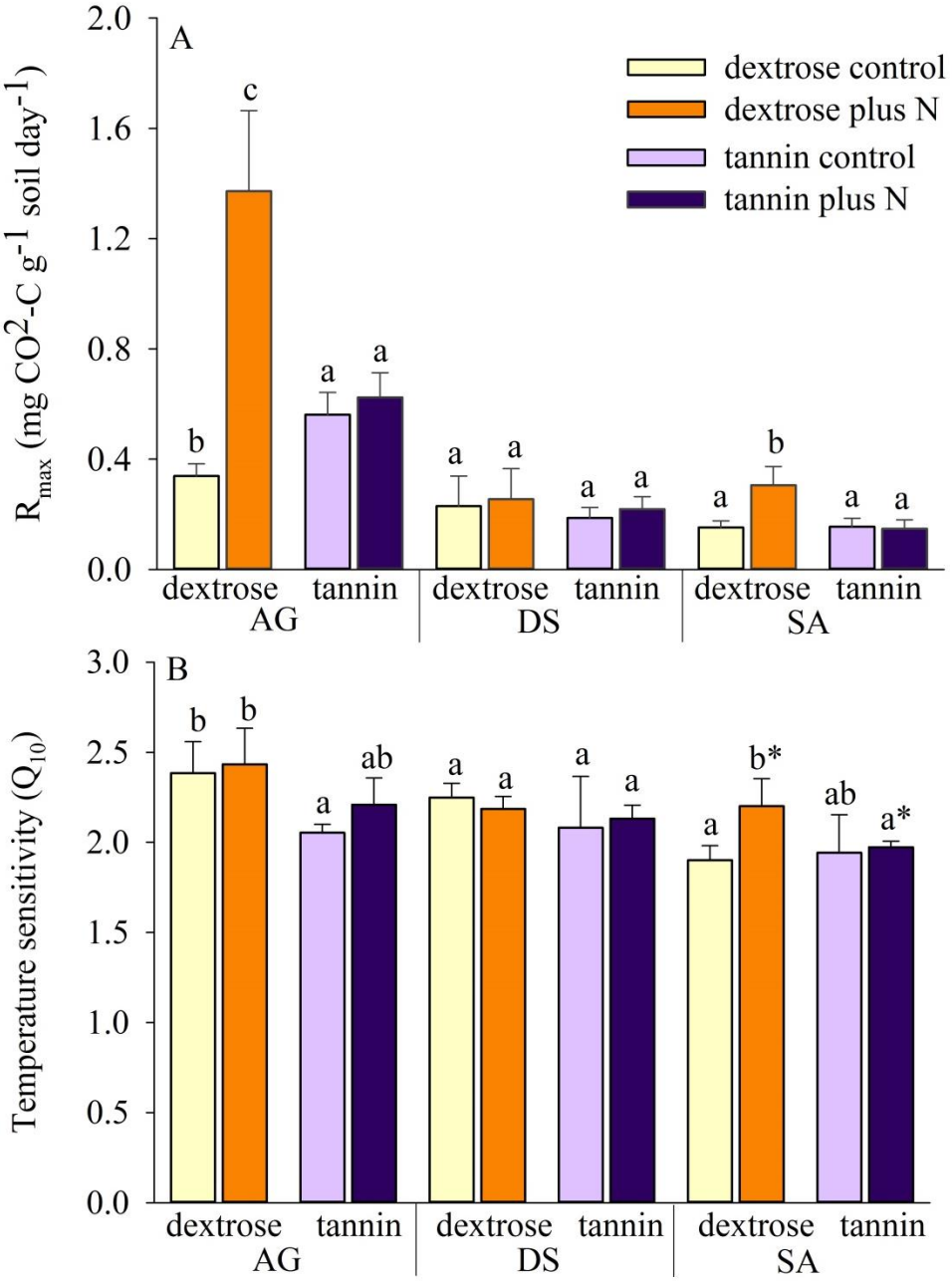


Figure 1.4: Comparison of maximum respiration rate at 19°C (a; $R_{max}$) and temperature sensitivity (b;$Q_{10}$) of $R_{max}$ between a labile carbon source (dextrose) and a more recalcitrant carbon (tannin) with and without nitrogen enrichment at 200 µg $g^{-1}$ soil. Letters represent significance ($p<0.05$) between treatments for each soil (ANOVA, Tukey HSD). Error bars are 1SD (n=5).

Discussion

Results from this study confirm that C availability is an important regulator in the response of $R_{soil}$ to both N enrichment and changes in temperature, and these processes are well described through Michaelis-Menten kinetics (Figure 1.5). Increases in both $V_{max}$ and $K_m$ due to N enrichment resulted in increased $R_{soil}$ when there was abundant available C and a decrease or no change in $R_{soil}$ when C was limiting. Furthermore, all soils demonstrated positive temperature sensitivity in both parameters $V_{max}$ and $K_m$, mediating the response of apparent $Q_{10}$ to C availability. C complexity may also have played a lesser role in determining the $Q_{10}$-value of the AG soil, and other factors such as physical protection in the soil are likely important. Finally, the interaction between N and temperature sensitivity of $R_{soil}$ showed inconsistent results, which may mirror the response of $R_{soil}$ to C:N availability.

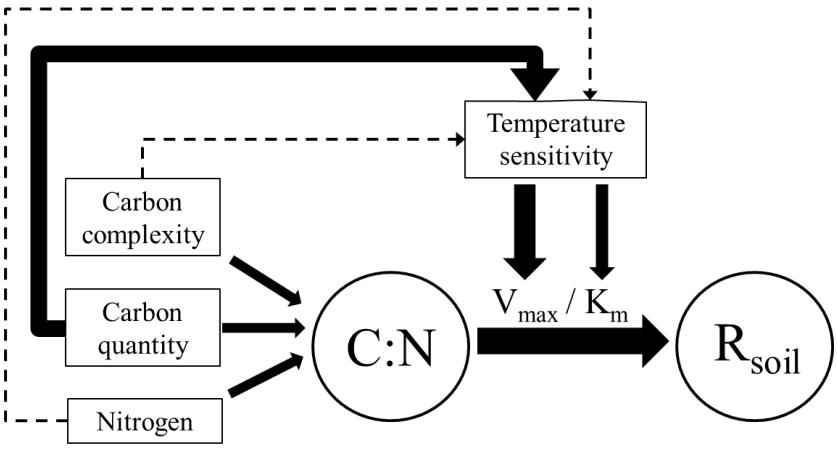


Figure 1.5: Conceptual diagram of the influence of carbon, nitrogen and temperature on soil respiration. Width of arrow represents the strength of the relationship. Dashed lines represent minimal or variable relationships.

*$R_{soil}$ response to N is dependent on C availability*

Observations from both the 120-day incubations and the Michaelis-Menten experiments were consistent with the hypothesis that $R_{soil}$ response to N enrichment would depend on C availability. There was an increase in $R_{soil}$ when N was added with dextrose (a labile C source), but a decrease or no response when N was added alone (Figure 1.1). Michaelis-Menten kinetics mediated these results through increases in both $V_{max}$ and $K_m$ following N enrichment (Figure 1.3A).

The response of $R_{soil}$ to N enrichment at high C concentrations can be explained by relief of both C and N co-limitation (Liang et al., 2015). However, the results at low C concentrations are more difficult to explain. Microbial N mining may account for the decrease in $R_{soil}$ from N addition, however, it did not appear to occur under saturating C conditions. N mining is an extension of the concept of priming (i.e. – changes in soil C mineralization due to added substrates). Less than 70% of added dextrose was respired over the course of the 120 incubation, making it unlikely substrate additions caused changes in soil organic matter turnover. Such a lack of priming is usually expected in cases of high substrate addition (Blagodatskaya and Kuzyakov, 2014).

Alternatively, there is a growing body of evidence demonstrating microbial ability to adjust carbon-use efficiency (CUE) in response to environmental conditions (Blagodatskaya et al., 2014; Keiblinger et al., 2010; Thiet et al., 2006). According to the dynamic CUE hypothesis, microbes can conserve or exhaust C through respiration, based on C and N availability. This would generate a divergent response in $R_{soil}$ to N addition depending on C availability, as seen in this study. The observed changes from the

Michaelis-Menten experiment also support this dynamic CUE hypothesis. If we assume microbial uptake of C is equal between treatments, $K_m$ can represent the balance between substrate addition versus respiratory loss at sub-saturating C conditions. The increase in $K_m$ with N addition (Figure 1.3A) results in lower $R_{soil}$ per unit of dextrose addition, a trend seen across soils (Figure 1.1). Additionally, effects from C addition persisted for 60 days and longer while N effects were no longer detectable within 7 days or less (Figure 1.2), indicating greater conservation of N compared to C within microbial biomass (Manzoni and Porporato, 2009), further emphasizing the importance of a mechanism in microbial physiology for efficient use of external resources. While this study was not designed to account for all possible avenues of C transformation, the results are supportive of the dynamic CUE hypothesis to explain the $R_{soil}$ response to N addition. As most climate models employ a static CUE (Manzoni et al., 2012) and soil C:N alone is not a good predictor of CUE (Hartman and Richardson, 2013), understanding the relationship between substrate C:N and CUE represents a clear research need. Regardless of the mechanism responsible, the results from this study present a similar response across three highly diverse ecosystems of a consistent C:N stoichiometry effect on $R_{soil}$. Furthermore, Michaelis-Menten kinetics may provide the means for better representation of C and N interactions, a large source of uncertainty in current climate models (Piao et al., 2013).

*C quantity is a greater predictor of $Q_{10}$-value than C complexity*

The temperature response of $R_{soil}$ also responded strongly to C availability. However, our results challenge the generality of either the C quantity or C quality -$Q_{10}$ hypotheses, and suggest temperature sensitivity results from interactions between C quality, C quantity and site specific factors influencing $R_{soil}$. Despite the strong theoretical justification of activation energy requirements, (Bosatta and Ågren, 1999; Fierer et al., 2005), the C quality – $Q_{10}$ relationship was not supported by comparisons of dextrose and tannin additions. The $Q_{10}$ of $R_{max}$ with dextrose addition was either greater or not different from the $Q_{10}$-value of tannin (Figure 1.4B). However, a decrease in $Q_{10}$-value with labile C addition for the AG soil was observed (Table 1.2), in support of the C quality-$Q_{10}$ hypothesis. Conversely, there was strong support of interactions between C quantity and apparent temperature sensitivity. We show experimentally a consistent finding of temperature dependence in both $K_m$ and $V_{max}$ across all sites (Figure 1.3B) demonstrating potential for the carbon-dependent canceling effect on $R_{soil}$ temperature sensitivity. Canceling of the $Q_{10}$-values of $V_{max}$ and $K_m$ when C concentration is low would result in a reduction in the "apparent" $Q_{10}$ of $R_{soil}$ (Davidson et al., 2006; Davidson and Janssens, 2006), which was seen in the SA and DS soils as an increase in $Q_{10}$ following labile C addition (Table 1.2). However, the $Q_{10}$-values of $V_{max}$ and $K_m$ are not always equal (Figure 1.3B), which may explain why temperature sensitivities are still observed at low C concentrations. These findings highlight the growing importance of coupled Arrhenius-Michaelis-Menten modeling frameworks (Davidson et al. 2012;

Oikawa et al. 2014) that simultaneously evaluate both temperature and substrate availability kinetics.

While this study did not directly test effects of physico-chemical protection in the regulation of $Q_{10}$-value, indirect evidence was found through comparisons between C sources and across soils. Physico-chemical stabilization of soil C can be accomplished through physical protection within soil aggregates (Conant et al., 2011). Tannin additions resulted in poor fits to the Michaelis-Menten model due to inhibition of $R_{soil}$ at high concentration, which was more pronounced at higher temperatures. Tannin has been shown to bind both substrates and exoenzymes in soil (Benoit and Starkey, 1968; Fierer et al., 2001; Kraus et al., 2004). This effect may have been enhanced with higher tannin concentrations and higher temperatures, therefore masking a temperature response. Additionally, higher temperatures can increase adsorption of substrates to soil particles (Conant et al., 2011; Thornley and Cannell, 2001). The AG soil had higher clay content than the other soils, which could make adsorption of substrates a greater factor and may explain the difference in temperature response compared to the DS and SA soils. Therefore, C complexity, C quantity and physical stabilization are all necessary considerations when investigating the temperature response of $R_{soil}$.

*N showed inconsistent effects on $Q_{10}$*

The temperature sensitivity of $R_{soil}$ had variable responses to N addition with both increases and decreases observed (Table 1.2). N addition also resulted in a general increase in the $Q_{10}$-values of $V_{max}$ and $K_m$, similar to the response of $R_{soil}$. Other studies

have demonstrated an inconsistent response of $Q_{10}$ to N addition (Jin et al., 2010; Peng et al., 2010; Tu et al., 2011). The parallel response patterns of $Q_{10}$-value and $R_{soil}$, with both increases and decreases seen with N addition depending on C:N availability, may explain these inconsistent results (Table 1.2), which is also supported by the increase in the Q10-values of $V_{max}$ and $K_m$ following N addition (Figure 1.3B). The responses of $R_{soil}$ temperature sensitivity to N additions suggest more complex responses to combined global changes of increasing temperatures and nitrogen deposition than would be expected from either alone.

*Differences between study sites*

While the general response of $R_{soil}$ to N and temperature increase was consistent across soils, there were a few notable variations. The AG soil demonstrated a decrease in $Q_{10}$-value with dextrose addition while the SA and DS soils demonstrated an increase in $Q_{10}$-value with dextrose addition (Table 1.2). This might be explained by the greater clay content in the AG soil accounting for greater physico-chemical stabilization of substrates. Furthermore, the C content of the AG soil was higher (Figure 1.6) with sources of C inputs differing between sites depending on the dominant vegetation (Jenerette and Chatterjee, 2012). This resulted in peak $R_{soil}$ fluxes from AG three times the peak rates from the wildland soils from DS and SA, but treatment effects were seen for a longer time period in the wildland soils (Figure 1.2). The increase of $R_{soil}$ in the N+D addition over Dex persisted for one day in AG versus approximately one week in the soils from SA and DS. Despite these site-specific differences, the general $R_{soil}$ response to

temperature change and N enrichment were remarkably similar between soils regardless of considerable differences in climate and nutrient availability (Table 1.1).

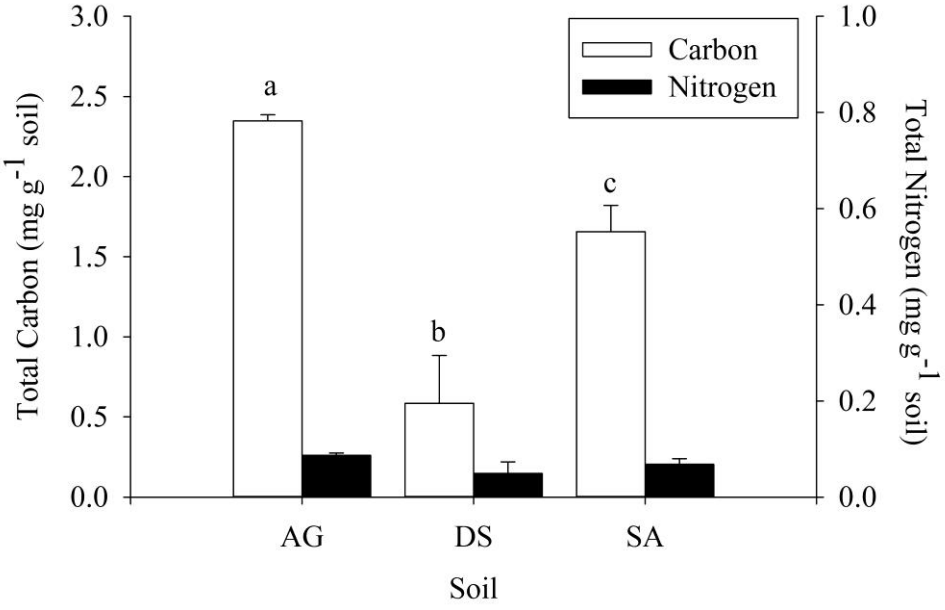


Figure 1.6: Soil total carbon and nitrogen values. Note the secondary y-axis. Letters represent significance at $p<0.05$. Error bars represent 1SD (n=5).

## Conclusions

Results from this study emphasize the importance of C availability as a major driver of $R_{soil}$, regulating the response of $R_{soil}$ to both N enrichment and temperature change. These findings substantiate the use of Michaelis-Menten kinetics in modeling of the temperature sensitivity of $R_{soil}$ and demonstrate its potential for use in modeling C-N interactions. Especially useful would be continued work on resolving connections between CUE and the Michaelis-Menten parameters for better representation of the influence of C- N interactions on $R_{soil}$ in biogeochemical models. Furthermore,

Michaelis-Menten kinetics may provide additional opportunities in biogeochemical modeling efforts to reconcile other $R_{soil}$ drivers, such as multiple nutrient limitations or water-driven pulse responses. Study of the interacting factors regulating $R_{soil}$ has important global relevance for understanding greenhouse forcing and is necessary for accurate predictions of soil feedbacks to C stocks under multiple global change drivers.

Chapter 2: Michaelis-Menten kinetics and soil respiration feedbacks to nitrogen deposition and climate change in subtropical forests

Abstract

China experiences some of the highest rates of anthropogenic nitrogen deposition globally, with further increases projected. Understanding of soil feedbacks to the combined anthropogenic influences of climate change and nitrogen deposition in these systems is critical to improve predictive abilities for future climate scenarios. Here we used a Michaelis-Menten substrate-based kinetics framework to explore how soil $CO_2$ production ($R_{soil}$) responds to changes in available soil nitrogen (N) and temperature. Results from field and laboratory experiments demonstrated a general decrease in $R_{soil}$ with increasing soil available N that was variably dependent on carbon (C) availability. Alternatively, the temperature sensitivity of $R_{soil}$ was strongly influenced by labile C additions. Both the field and the lab measurements demonstrate a consistent decrease in the Michaelis-Menten parameter kM with increasing soil available N, indicating an increase in the efficiency of soil $CO_2$ production with increasing N. Furthermore, these results provide evidence of interactions between N additions and temperature sensitivity with implications for future C storage under combined anthropogenic global change drivers.

Introduction

Developing a mechanistic and predictive understanding of soil carbon (C) pool responses to interactive global change drivers remains an important research goal (Gärdenäs et al., 2011; Zaehle and Dalmonech, 2011). In many regions soil C dynamics are influenced simultaneously by increased climate warming and nitrogen (N) deposition. With strong theoretical justification from thermodynamics and enzymatic processes (Arrhenius, 1889; Wallenstein et al., 2011), warming should lead to increased rates of soil $CO_2$ production (soil respiration; $R_{soil}$), however, a broad range of responses have been observed (Davidson et al., 2006b). The effects of N enrichment are even more complex with conflicting theoretical predictions and empirical evidence showing increases, decreases and no response of $R_{soil}$ to increased N availability (Fog, 1988; Liu and Greaver, 2009; Janssens et al., 2010; Ramirez et al., 2010; Zhou et al., 2013). The interactions between warming and nitrogen availability on soil C emissions are likely equally complex and may create a positive climate change feedback in a future warming climate. Resolving these uncertainties in $R_{soil}$ responses to both warming and N addition is important for defining soil functioning, ecosystem dynamics, and the repercussions of multiple interacting global change drivers.

Substrate-based mechanisms for describing soil biogeochemical processes can be a valuable tool for modeling and predicting soil feedbacks to anthropogenic global change drivers (Davidson et al., 2012; Niu et al., 2016). This approach is hierarchical in that the effects of warming and nitrogen availability are conceptualized as influencing parameters in a substrate dependent model. A commonly-used model of substrate-based

kinetics is the Michaelis-Menten model, which describes a saturating function of substrate concentration, with parameters $V_{max}$, the maximum reaction velocity, and kM, the half-saturation constant, which corresponds to the substrate concentration [S] when $V_{max}/2$, as follows:

$$R = \frac{Vmax*[S]}{kM+[S]}. \quad [1]$$

The role of substrate-based limitations has been considered for assessments of $R_{soil}$ temperature sensitivity, but there are competing hypotheses (Conant et al., 2011). The inverse carbon-quality hypothesis predicts that low quality, recalcitrant substrates will require higher activation energy, and therefore be more temperature sensitive than high quality, labile substrates (Bosatta and Ågren, 1999; Fierer et al., 2005). Alternatively, the availability of C may be a greater regulator of temperature sensitivity of $R_{soil}$ via the temperature sensitivities of the Michaelis-Menten parameters, $V_{max}$ and kM. If both are temperature sensitive, but the importance of the kM temperature sensitivity is less when substrate is abundant, this can create a canceling effect that is more apparent at low substrate concentrations (Davidson et al., 2012; Oikawa et al., 2014). Therefore, it is not clear whether carbon quality or availability is more important in regulation of the temperature sensitivity of $R_{soil}$. Furthermore, few studies have empirically tested the temperature sensitivity of $V_{max}$ and kM in soil.

How soil biogeochemical processes are influenced by N deposition, and the divergent responses of $CO_2$ emissions to N availability are yet to be resolved (Suddick and Davidson, 2012). Through an N limitation perspective, relief of N limitation should increase soil microbial activity and lead to a positive response of soil $CO_2$. However, this

cannot explain the suppressive influence of N on soil $CO_2$ emission seen in many studies (Fog, 1988; Ramirez et al., 2012; Sun et al., 2014). Alternatively, the N mining hypothesis describes the process where microbes "mine" recalcitrant carbon for N (Craine et al., 2007). Through this process N addition alleviates the need for mining of recalcitrant carbon, thereby decreasing $R_{soil}$. Another promising hypothesis incorporates the divergent response of N addition on $R_{soil}$ through dynamic carbon-use efficiency (CUE) of soil microorganisms in order to adjust metabolic activity to better fit inconsistent resource availability (Manzoni et al., 2008; Schimel and Weintraub, 2003; Sinsabaugh et al., 2013). This hypothesis predicts that the influence of N addition on $R_{soil}$ is dependent on the stoichiometry between C and N. When C is limiting compared to N, microbes will increase their CUE, thereby decreasing $R_{soil}$. Alternatively, when carbon is abundant compared to nitrogen, decreased CUE results in increased $R_{soil}$. When both resources are abundant, metabolic activity and growth are at a maximum. Initial laboratory tests have demonstrated $R_{soil}$ dynamics consistent with dynamic CUE in arid and semi-arid environments (Eberwein et al., 2015a; Ramirez et al., 2010), but not in a mesic system or from field experiments.

In addressing this uncertainty, we examined how soils from a subtropical moist forest in Guangdong Province, China respond to increasing N availability. This region in the highly industrialized Pearl River Delta has experienced rapid land use change and the urbanized areas, including Guangzhou, have become a large source of nitrogen deposition to the outlying forest ecosystems (Huang et al., 2012). We conducted field and laboratory experiments to evaluate how nitrogen deposition may influence $R_{soil}$ in

this region and potential interactions with warming. We asked: 1) How does soil N availability influence the response of soil respiration to labile carbon addition? 2) How does labile carbon addition affect the temperature sensitivity of soil respiration, and 3) Does nitrogen deposition influence this temperature response? We hypothesized that N availability would influence $R_{soil}$ based on C:N stoichiometry, following the dynamic CUE hypothesis and that temperature sensitivity would be mediated through the Michaelis-Menten parameters, $V_{max}$ and kM.

## Methods

### *Study Sites*

The study was located in Guangdong Province of South China. The area is under the influence of a subtropical monsoon climate with alternating wet and dry seasons, and has undergone rapid urban expansion since the 1970s. Three sites were selected along an urban to rural gradient in Guangdong Province in southern China to capture an anthropogenic nitrogen deposition gradient (Huang et al., 2012). The urban to rural gradient started in the urban site of Pu Gang in the South China Botanic Gardens, then the suburban site of Lou Gang, and ended in the Shimentai Nature Reserve in Yingde County. All sites were located in late successional evergreen broadleaf forests with at least 50 years since disturbance. All soils were latosolic red. Published nitrogen deposition data from these sites reveal higher than expected nitrogen deposition in the rural site (Table 2.1). Increased canopy interception of nitrogen deposition in urban sites may explain the gradient in soil available nitrogen seen in this study (Figure2.1; Huang et

al., 2012). Additionally, soils were collected from nitrogen addition plots in a mixed legume and native forest at Heshan National Field Research Station of Forest Ecosystems in Heshan County, Guangdong Province. The plots consisted of three replicates each of a control (no additions; Low N), medium nitrogen addition (50 kg N $ha^{-1}$ $yr^{-1}$; Mid N), and high nitrogen addition (100 kg N $ha^{-1}$ $y^{-1}$; High N) for a total of nine plots. Nitrogen was applied as ammonium nitrate in 10L of water with a backpack sprayer once a month for two years prior to analysis (Zhang et al., 2012). Additional site characteristics are summarized in Table 2.1.

Table 2.1: Site characteristics

| Site | Lat/Long | MAT (C) | MAP (mm) | N deposition (kg N $ha^{-1}$ $y^{-1}$) | Soil pH | Dominant species |
|---|---|---|---|---|---|---|
| Pu Gang[a] (urban) | E113°48', N23°37' | 21.5 | 1700 | 30-43 | 3.71±0.1 | *Schima superba*, *Psychotria rubra and Lophatherum gracile*. |
| Luo Gang[a] (suburban) | E113°18', N23°06' | 21.5 | 1738 | 30-43 | 3.8±0.2 | *Cryptocarya concinna* |
| Shimentai[b] (rural) | E113°05', N24°22' | 20.8 | 2364 | 34.1 | 3.55 | *Cryptocarya concinna*, *Schima superba*, *Machilus chinensis*, *Castanea henryi (Skan)* Rehd., and *Engelhardtia roxburghiana* |
| Heshan[c] (N addition plots) | E112°50', N22°30' | 21.7 | 1295 | 43.1±3.9 | 3.7-3.8 | *Acacia auriculiformis* |

[a]Huang et al., 2012; [b]Zhang et al., 2015; [c]Zhang et al., 2012

*Field measurements*

Plots were established at the three sites along the nitrogen deposition gradient to compare *in situ* $R_{soil}$ responses to glucose additions. For these experiments, soil collars measuring 20cm in diameter and 5cm in height were set 24 hours before sampling in a

plot measuring approximately 25 $m^2$ at each site along the gradient. Treatments included a control (water only) and four levels of glucose concentration: 5, 15, 30 and 90 g/L. Each treatment was replicated four times for a total of 20 samples per site. 200 mL of water or glucose solution were applied to each collar, and soil respiration was measured with a portable infrared gas analyzer (LI-8100, Licor Biosciences) at 1, 8 and 24 hours after addition of water or glucose solution.

*Incubation experiments*

Soils were collected from the three sites along the urban to rural gradient as well as the nine nitrogen addition plots at Heshan Station to perform laboratory incubations to explore the response of soil respiration to glucose addition under controlled temperature and moisture conditions. Five field replicates were collected from each plot. Each field replicate was composed of approximately ten soil cores collected from 0-15 cm of the mineral soil layer. The soils were brought back to the lab and air dried for 3 to 5 days at room temperature. The soils were then sieved to 2mm and homogenized. Before drying, an aliquot from each field replicate was placed in the freezer for nitrate and ammonium analysis. An additional aliquot from the dried and sieved samples was used for total organic C and total N analysis. Soil chemical analysis was performed by the Key Laboratory of Vegetation Restoration and Management for Degraded Ecosystems at the South China Botanical Gardens, Chinese Academy of Sciences using the copper cadmium reduction-diazotization coupling method for nitrate, potassium chloride leaching-indophenol blue method for ammonium, Kjeldahl method for total organic

carbon and external heating using potassium dichromate oxidation method for total nitrogen (Liu et al., 1996). Water holding capacity (100%) was determined by the gravimetric water content of soil placed in a filter funnel and saturated with deionized water, then allowed to drain for two hours.

The same five treatments were applied to the incubation experiments as were used in the field: control (water only), 5, 15, 30 and 90 g/L of glucose. In total, the three sites along the deposition gradient included five treatments and five field replicates for a total of seventy-five samples. For the N addition plots at Heshan station, there were five glucose treatments, three nitrogen levels, and three replicates (one for each plot) for a total of forty-five samples. For each replicate, 50g of soil was placed in a 200ml Erlenmeyer flask with a vented rubber stopper. The glucose solutions (and water in the case of the control) were used to bring the soils to 40% WHC, and then they were incubated at 25°C for 24 hours. After the incubation period, soil respiration was measured with an infrared gas analyzer (LI-6262 Licor Biosciences) at 13, 19, 25, and 31°C. Fluxes were calculated from the linear portion of the curve generally between 30 and 90 seconds after the jar was sealed, and adjusted for chamber volume, soil weight and chamber temperature. The $CO_2$ flux measurements were then fit to the Michaelis-Menten model with nonlinear least squares regression to determine $V_{max}$ and kM using Matlab (2015b). Temperature sensitivity (Q10) was determined by the following equation:

$$Q10 = {R2}/{R1}^{\frac{10}{T2-T1}}, \quad [2]$$

where R1 and R2 are soil respiration rates measured at temperatures T1 and T2, respectively.

*Statistical Analysis*

Statistical significance for treatment effects were determined by n-way Analysis of Variance (ANOVA) in RStudio (R version 3.2.5). Assumptions of normality and homoscedacity were tested with the Shapiro-Wilks and Bartlett's tests, respectively. When these assumptions were not met, the Box-Cox family of transformations was used. Linear regressions for the relationship between kM and available soil nitrogen were performed in Matlab (2015b).

Results

Soils from the urban to rural sites spanned a range of available N ($NH_4^+$ plus $NO_3^-$) from 14.7 to 71.4 mg $kg^{-1}$ (Figure 2.1a). While total N availability was significantly different ($p<0.05$) between the urban and rural sites and the rural and suburban sites, the urban and suburban sites were not significantly different. For simplicity, the urban site will hereafter be referred to as "Low N", the suburban site as "Mid N" and the rural site as "High N". The Heshan Station N addition plots ranged from 30.3 to 49.6 in soil total available N (Figure 2.1b). While there were no significant differences between plots for ammonium or nitrate individually, total N for the low addition plot was significantly less than the mid N plot, but not significantly different

from the high plot (at $p<0.05$). However, the "Low N", "Mid N" and "High N" labels will continue to refer to the experimental N addition for the N addition plots.

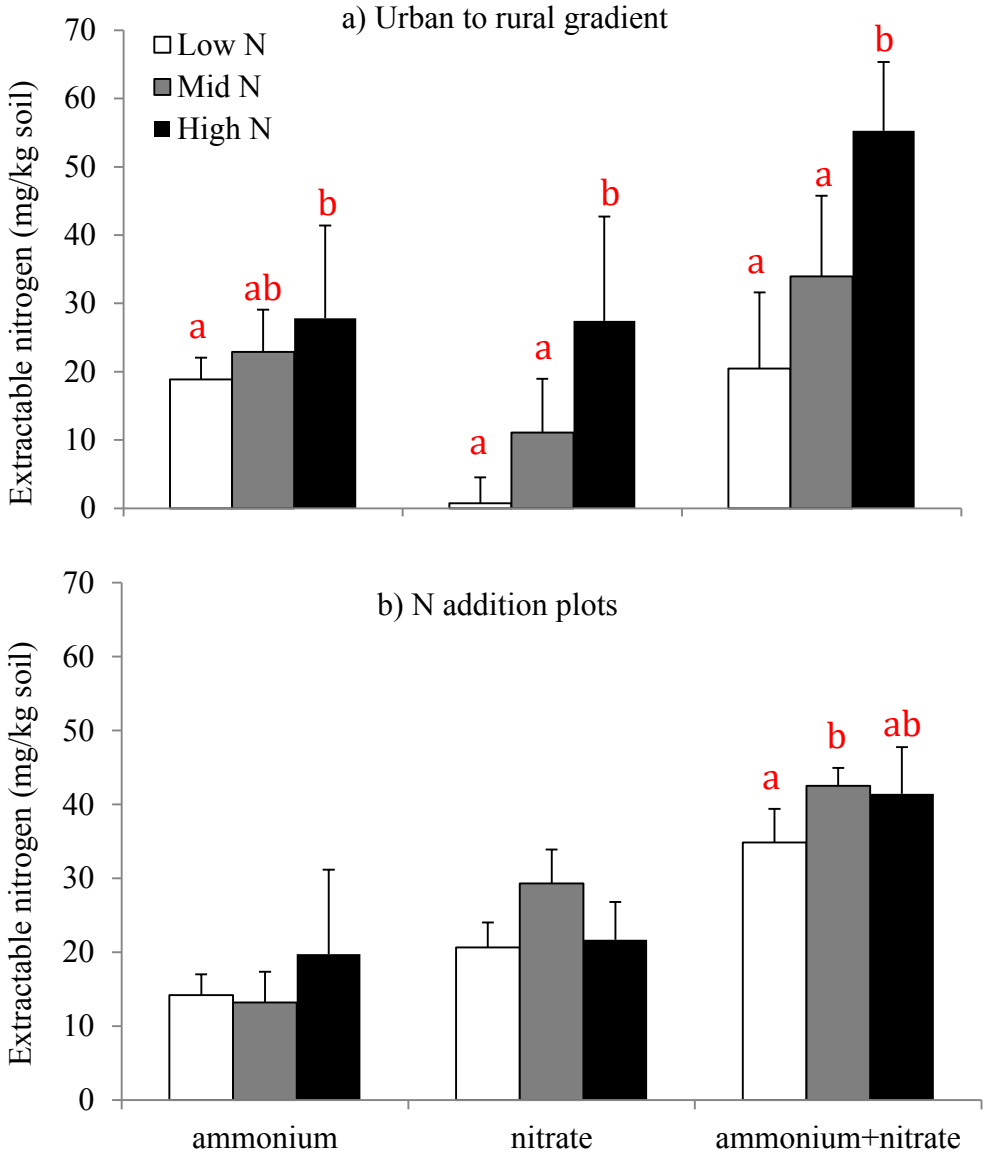


Figure 2.1: Soil KCl extractable nitrogen in the urban to rural gradient (a) and the nitrogen addition plots (b). Error bars represent standard deviation. Letters represent significance at $p<0.05$.

For all measurements, soil $CO_2$ production generally decreased with increasing soil extractable N levels (Figure 2.2). Field measurements along the urban-to-rural gradient showed decreasing $CO_2$ production with increasing soil available N (Figure 2.2a,d). The laboratory incubation results for both the urban-to-rural gradient and the N addition plots showed decreased $CO_2$ production in the mid N compared to the low N. However, $CO_2$ production in the high N soils were all higher than the mid N soils and not significantly different (Figure 2.2b,c,e) or greater than (Figure 2.2f) the low N soils. In general, the response of $R_{soil}$ to soil N was the same at both ambient soil C concentrations (Figure 2.2a-c) and at saturating C concentrations ($V_{max}$; Figure 2.2d-f).

The Michaelis-Menten parameter kM also demonstrated a general pattern of decline with increasing N across all soils (Figure 2.3). Results of the field experiment for the urban to rural gradient demonstrated the decrease in $R_{soil}$ with increasing soil N, evident at all sampling times (Figure 2.3a). $R_{soil}$ was measured at 1, 8 and 24 hours after glucose additions for fitting the Michaelis-Menten parameters $V_{max}$ and kM, which both increased with time since wetting (Figure 2.2d,2.3a). The laboratory incubation experiment with the soils from the urban-to-rural gradient further allowed for manipulation of temperature to understand the temperature sensitivity of kM. Generally, kM declined from the low N to the high N soils across all temperatures (Figure 2.3b). Furthermore, in the laboratory incubation of soils from the N addition plots, kM was negatively related with measured soil N (Figure 2.3c) at 25 and 31°C ($p<0.05$). Also apparent from the temperature manipulations, kM was highly temperature dependent and this relationship was non-linear.

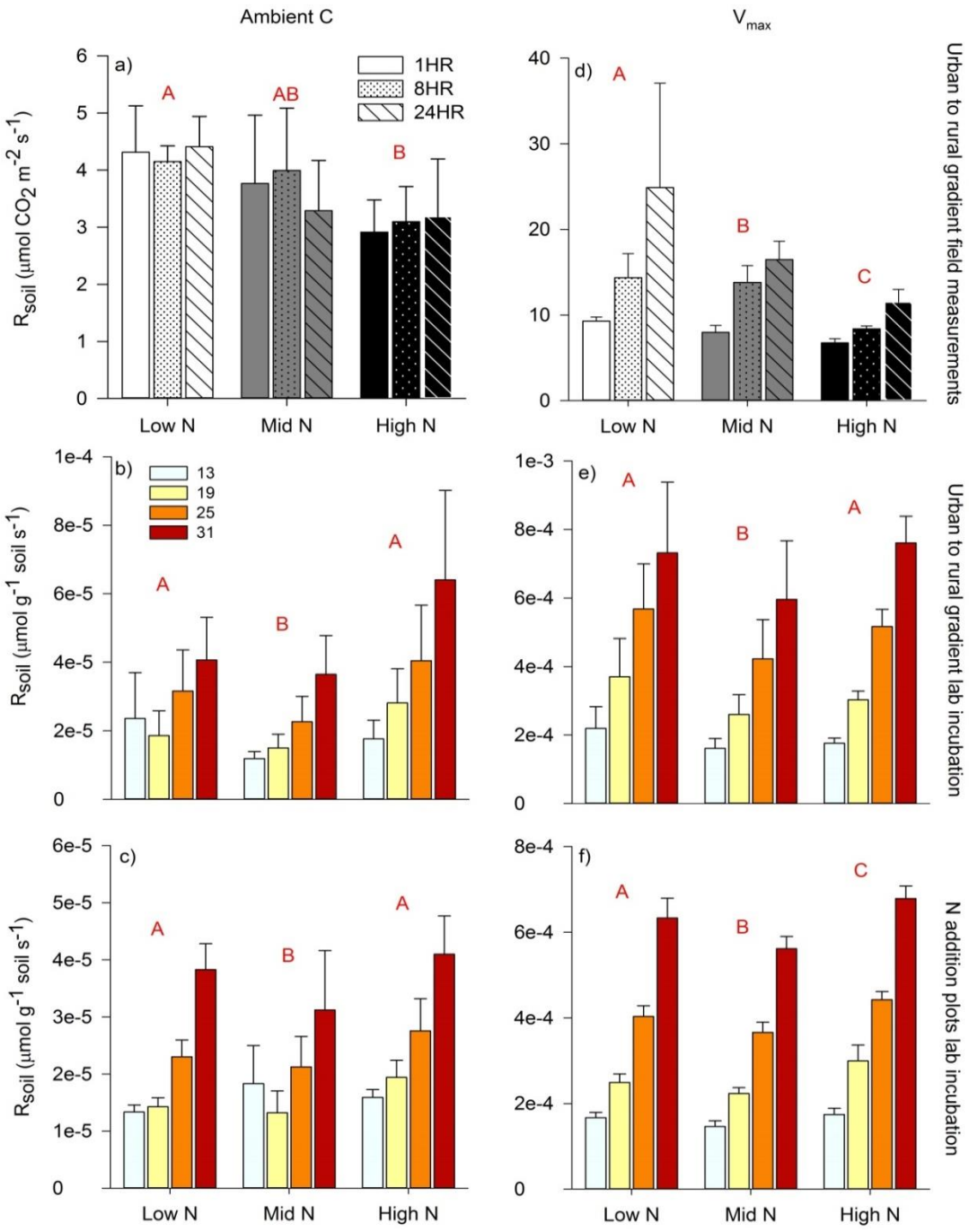


Figure 2.2: Soil $CO_2$ production ($R_{soil}$) for ambient soil carbon (a-c) compared to saturating carbon ($V_{max}$; d-f) for the field measurements along the urban to rural gradient (a,d), the laboratory incubation of soils from the urban to rural gradient (b, e), and the laboratory incubation of soils from the nitrogen addition plots. Legends indicate time since treatment addition (a, d) or temperature in $^{\circ}C$ (b, c, e, f). Error bars represent standard deviation. Letters represent significance at $p<0.05$.

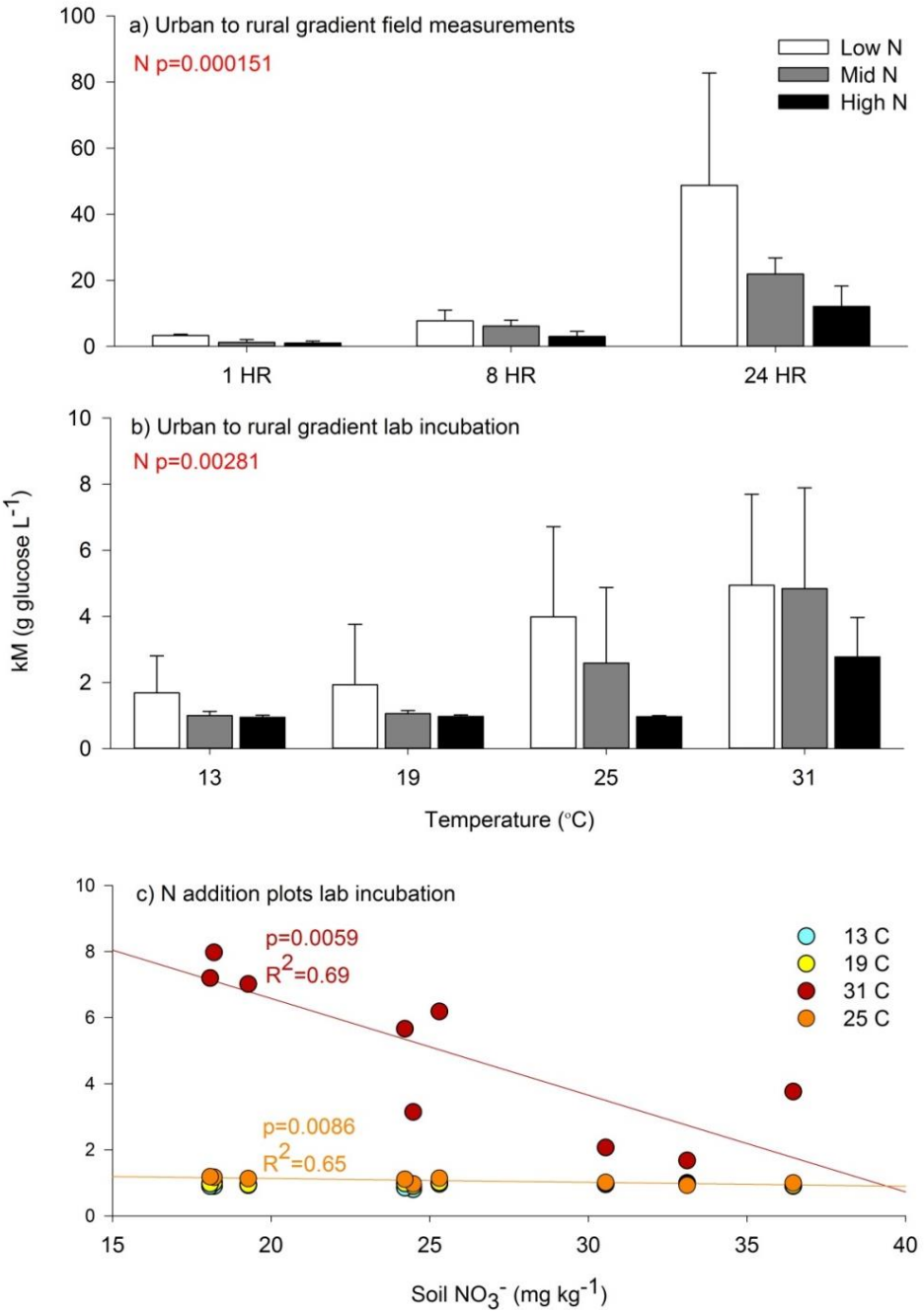


Figure 2.3: The Michaelis-Menten half-saturation constant (kM) for the field measurements along the urban to rural gradient (a), the laboratory incubation of soils from the urban to rural gradient (b), and the laboratory incubation of soils from the nitrogen addition plots (c). Error bars represent standard deviation. P value represents nitrogen effect for all timepoints (a) or temperatures (b) combined at $p<0.05$. In (c) lines represent significant linear regressions.

Additionally, the laboratory incubations allowed for exploration of relationships between availability of labile carbon and the temperature sensitivity of $R_{soil}$ (Figure 2.4). The incubation results from the urban-to-rural gradient soils showed a significant increase in the $Q_{10}$ of $V_{max}$ with increasing N ($p<0.05$), but no relationship between soil N and the $Q_{10}$ of kM (Figure 2.4a). N also increased $R_{soil}$ $Q_{10}$ in the high N compared to the low and mid N treatments for all C concentrations (Figure 2.4b). Alternatively, soils from the nitrogen addition plots did not exhibit differences in $Q_{10}$ of $V_{max}$ or kM (Figure 2.4c). For both the urban-to-rural gradient and the N addition plots, there was a divergent response of $R_{soil}$ Q10 to glucose addition, with a general decrease from ambient carbon to 5 g/L glucose, followed by an asymptotic increase from 5 g/L through 90 g/L glucose (Figure 2.4b,d).

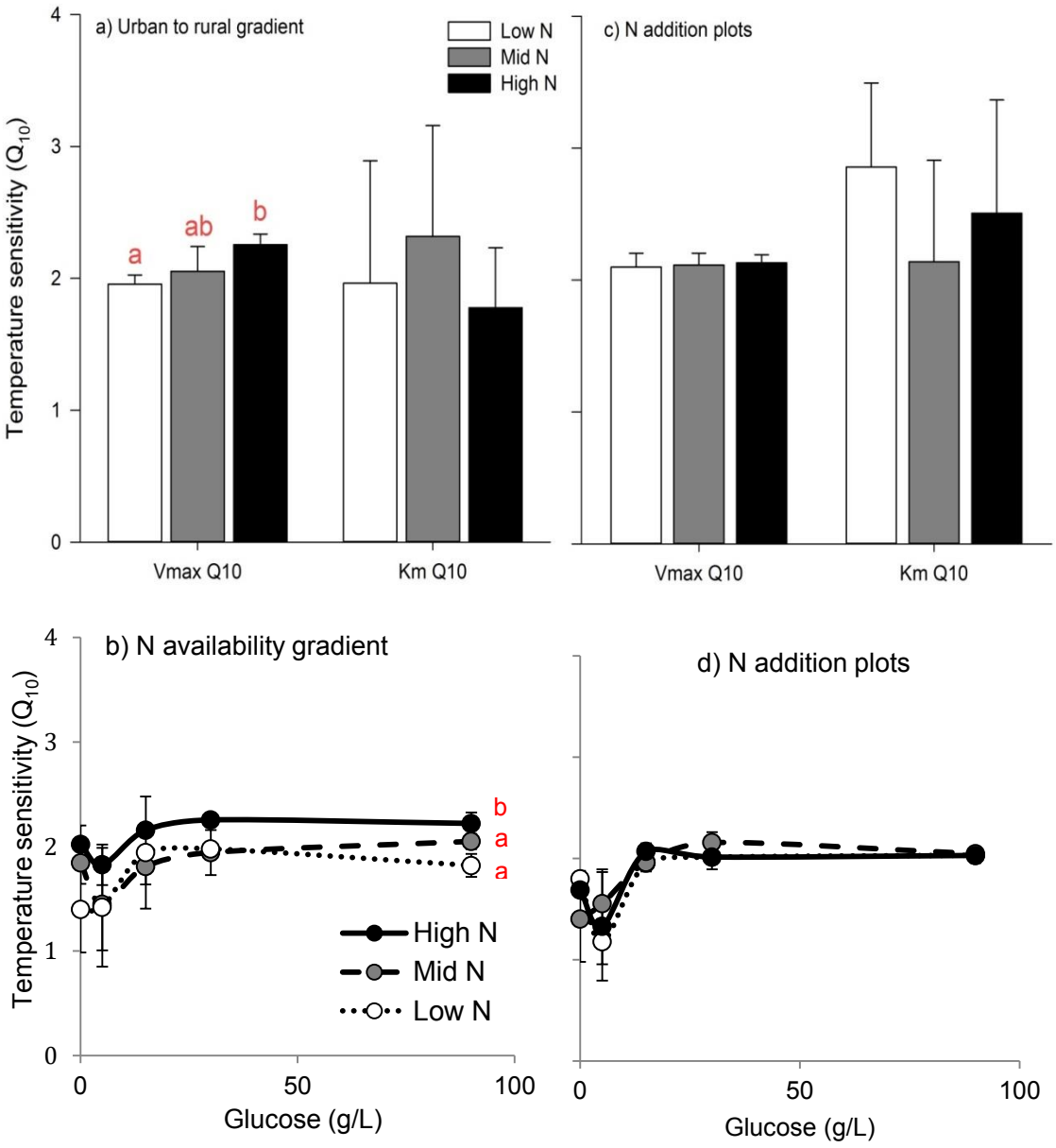


Figure 2.4: Temperature sensitivity ($Q_{10}$) of the Michaelis-Menten parameters $V_{max}$ and kM (a,c), and Q10 versus glucose addition (b,d). Error bars represent standard deviation. Letters represent significant difference at $p<0.05$.

Discussion

Our findings demonstrate in mesic subtropical forests there are complex interactions between C availability, warming, and N availability, on $R_{soil}$, a key ecosystem process with potential climate feedbacks. Our findings provide initial experimental support of the key model assumptions using whole soil community dynamics for an integrated hypothesis of coupled temperature and substrate dependent $R_{soil}$ models that have been increasingly proposed (Davidson et al. 2012; Oikawa et al. 2014). This framework helps resolve much of the apparent complexity in $R_{soil}$ soil warming experiments finding a broad range of $R_{soil}$ responses to temperature (Conant et al., 2011; Davidson and Janssens, 2006). Further, our results show patterns of decreasing $R_{soil}$ responses to N availability consistent with general global patterns in forest soils (Janssens et al., 2010; Sun et al., 2014; Zhou et al., 2013) and provides needed experimental evidence of mechanisms for the influence of N on $R_{soil}$ in the context of temperature and C substrate sensitivities. Overall, our results support a dynamic CUE mechanism for the response of $R_{soil}$ to N availability (Eberwein et al., 2015a; Manzoni et al., 2012). The implications of this mechanism for ecosystem C balance and soil feedbacks to climate changes may be widespread and increasing in response to global trajectories of higher N deposition.

First-order decay of C inputs is a fundamental property of the C cycle that is shared across all ecosystems, presenting a useful tool for modeling global C budgets and predicting C storage under future climate scenarios (Luo et al., 2015). Substrate-based kinetics allow for a mechanistic approach to predict $R_{soil}$ response to warming (Davidson et al., 2012). However, there are few empirical estimates of the kM of soil $CO_2$

production (Eberwein et al., 2015a; Moorhead and Sinsabaugh, 2006). Here, we show, in the first field experimental evaluation of $R_{soil}$ substrate sensitivity, consistent support for Michaelis-Menten kinetic responses for decomposition of a labile C source. Furthermore, the lab incubations demonstrate positive temperature sensitivity for both $V_{max}$ and kM (Figure 2.4), which both demonstrate a non-linear relationship with temperature, similar to Arrhenius kinetics. The positive temperature response of both $V_{max}$ and kM supports the potential for a canceling effect at low substrate concentrations (Davidson et al., 2006). This is supported by the increase in $Q_{10}$ with increasing C seen in this study (Figure 2.4b, d). However, the C availability influence on temperature sensitivity produced a divergent response in almost every soil. There was an initial decrease in $Q_{10}$ with initial C addition and then an increase with subsequent increasing C addition (Figure 2.4b,d). These results appear to support that both the C quality and the C availability hypotheses are working together. Integrating both of these hypotheses into a cohesive framework should substantially improve predictions of C storage under future climate scenarios.

A key uncertainty in C substrate and temperature sensitivity models is the additional inclusion of the effects from other resources. With globally increasing rates of N availability, an evaluation of a potential N effect on $R_{soil}$ may be important for predicting soil climate feedbacks. As found across previous studies (Eberwein et al., 2015a; Fog, 1988; Liu and Greaver, 2010), we likewise observed variable influences of N availability. In general N availability reduced $R_{soil}$ emissions at both ambient and saturating substrate concentrations, consistent with the nitrogen mining hypothesis (Figure 2.2). However,

we observed some evidence of N limitation that, when alleviated, increased $R_{soil}$ capacity. Our findings were consistent with the variable CUE hypothesis, which predicts increasing N leads to greater conservation of available C through decreased respiration losses (Manzoni et al., 2012). Both the urban-to-rural gradient and experimental N addition plots showed shifts in soil $CO_2$ production consistent with the variable CUE hypothesis predicting decreased respiration with N addition. Furthermore, in the N addition plots, differences in $V_{max}$ associated with available soil N resulted in greater $V_{max}$ in the high N soil compared to the control, but lower $V_{max}$ in the mid N plot compared to the control (Figure 2.2f). This pattern corresponds to a shift from N limitation in the low N soil, to balanced C:N availability, and then C limitation in the highest available N soil. These changes reflect shifts in CUE regulating $R_{soil}$ as previously observed from laboratory incubations from dryland ecosystems (Eberwein et al., 2015a; Liang et al., 2015) and that we extend to tests from more mesic environments, including field experiments.

Across all experiments in this study, increasing soil N resulted in a reduction in the Michaelis-Menten parameter, kM (Figure 2.3) and we interpret this as a further indicator of dynamic CUE influence on $R_{soil}$. In traditional enzyme assays, kM represents the inverse of an enzyme's affinity for its substrate (German et al., 2012b; Stone et al., 2012). In the context of this study, kM allows for an estimation of efficiency as it represents the balance between $CO_2$ production for a particular amount of substrate addition. The decrease in kM suggests a more efficient use of labile C with increasing N, contrary to the expected view for CUE. However, application of kM to CUE in this study would require that microbial biomass and extracellular enzyme production remain

constant across treatments as $CO_2$ production was the only parameter measured. While we only measured $CO_2$ production, many studies have shown decreases in microbial biomass with N additions (Gao et al., 2014; Janssens et al., 2010; Liu and Greaver, 2010; Sirulnik et al., 2007; Treseder, 2008). Alternatively, the empirical kM estimates from this study could be interpreted in the more traditional sense as a shift in enzyme specificity. N addition can result in shifts in extracellular enzyme production that are more targeted at C decomposition and therefore more efficient rather than enzymes directed at both C and N acquisition (Carreiro et al., 2000; Stone et al., 2012). Regardless, the consistent decrease in kM with N addition across all soils in this study presents a useful tool for modeling $R_{soil}$ dynamics under anthropogenic nitrogen deposition. These results also highlight the potential for relationships between $R_{soil}$, kM and CUE, indicating that further work resolving these relationships could be beneficial for informing global C models.

Importantly, we also found that N availability can influence the sensitivity of $R_{soil}$ to both temperature and C substrate availability. These results present a potential for interaction between N deposition and climate change. N addition caused a significant increase in the temperature sensitivity of $R_{soil}$ in the urban to rural gradient (Figure 2.4a,b). The N influence on temperature sensitivity of the Michaelis-Menten parameters was limited to $V_{max}$ $Q_{10}$ and not kM $Q_{10}$. This could result in a dampening of the canceling effect and therefore greater temperature sensitivity at lower substrate concentrations under N deposition, which is supported by results from the urban to rural gradient demonstrating greater $Q_{10}$ at the high N site (Figure 2.4b). Furthermore, the

influence of N on kM in the N addition plots was magnified at higher temperatures. A decrease in kM would result in an increase in $R_{soil}$ at less than saturating substrate concentrations. As C is usually not saturating in most natural settings the decrease in $V_{max}$ could be of less significance than the decrease in kM in terms of annual C emissions.

The N and C cycles are closely connected through many ecological processes, including photosynthesis, respiration (both autotrophic and heterotrophic) and decomposition. Despite considerable research on the subject, the influence of N deposition on C sequestration remains unclear. A fertilization effect from N deposition can result in a greater C sink through plant biomass (Lebauer and Treseder, 2008), but this flux may be offset by soil C losses. As soils represent the greatest terrestrial C sink (Scharlemann et al., 2014), accurate representation of this process is crucial to make reliable predictions under multiple global change drivers. The dynamic CUE hypothesis allows for a mechanistic representation of $R_{soil}$ response to N enrichment under diverse environmental conditions providing a valuable framework for modeling efforts. However, CUE is difficult to estimate empirically (Manzoni et al., 2012). The use of substrate-based kinetics could bridge that gap, providing a simpler method for estimation of CUE in the field, given better resolution between Michaelis-Menten kinetics and CUE relationships.

In conclusion, these results indicate that soil N availability can have a significant effect on soil $CO_2$ production, independent of C availability. These results highlight the benefit of substrate-based kinetics for improving understanding of soil biogeochemical

processes, particularly $R_{soil}$ temperature and N sensitivity. Although increasing soil N can decrease $R_{soil}$, interactions between N and temperature could offset this response and result in greater loss of soil C stores. Anthropogenic activities have increased N inputs to terrestrial systems by about 46Tg N $y^{-1}$, with about one third being deposited to forests (Galloway et al., 2008). China experiences some of the highest rates of N deposition globally, with further increases projected (Liu et al., 2011). Developing a mechanistic understanding of soil feedbacks to the combined anthropogenic influences of climate change and nitrogen deposition in these systems is critical to develop predictive abilities for future climate scenarios, and substrate-based kinetics provide a valuable tool to help accomplish that goal.

Chapter 3: Investigating the microbial community composition responsible for unexpectedly high wetting-induced pulses of $NO_x$ and $N_2O$ fluxes from desert soils under contrasting nitrogen deposition

Abstract

The importance of soil nitrogenous and carbon emissions is well accepted in terms of local and global ecological relevance. However, considerable knowledge gaps remain concerning the mechanisms regulating their production, particularly in arid systems. This study aimed to connect desert soil trace gas emissions of nitrogen oxides ($NO_x$ and $N_2O$) and $CO_2$, with compositional changes in the microbial community. We quantified real-time soil trace gas emissions at two sites in the Colorado Desert experiencing contrasting anthropogenic nitrogen deposition loads (<5 and 15 kg N $ha^{-1}$ $y^{-1}$). Measurements were made through 48 hours following water (to simulate a 2 cm rain event) and nitrogen additions (at 30 kg $NH_4NO_3$ $ha^{-1}$). In conjunction with flux measurements, soil samples were collected for 16S rRNA gene sequencing to characterize the soil microbial community and qPCR of the *amoA* gene in ammonia oxidizing archaea and bacteria throughout the wetting induced pulse. $NO_x$ emissions reached as high as 345 ng $NO_x$-N $m^{-2}$ $s^{-1}$ and remained elevated past 24 hours post-wetting. $N_2O$ fluxes reached as high as 1700 ng $N_2O$-N $m^{-2}$ $s^{-1}$, well above most published emissions, but returned to near pre-wetting conditions within 12 hours. Similarly, $CO_2$ produced a large, but short-lived pulse at 15 minutes post-wetting. Results from the 16S and *amoA* analysis indicate distinct differences in the microbial

community composition between the high and low nitrogen deposition sites, but short term changes from experimental N addition were variable. The microbial community associated with nitrification in the low deposition site is associated with higher archaea abundance compared to bacterial dominance in the high deposition site. These results suggest that gaseous N export, particularly $N_2O$ emission, is a greater form of nitrogen loss in this system than is currently assumed. Experimental nitrogen additions and anthropogenic nitrogen deposition show potential for shifting soil microbial community composition, with implications for soil nitrogenous emissions. Furthermore, the high $N_2O$ and $CO_2$ fluxes at 15 minutes post-wetting indicate either an abiotic mechanism or a remarkable ability for soil microorganisms to recover from extreme water stress. As aridlands cover over one third of the Earth's land surface, understanding the mechanisms that contribute to such high soil nitrogenous emissions in these systems is of important global relevance.

## Introduction

Human activities, primarily fertilizer use and fossil fuel combustion, cause inputs of nitrogen (N) into natural ecosystems that have surpassed biological fixation (Galloway et al., 2008). The resulting atmospheric deposition of N from anthropogenic sources has resulted in detrimental ecological effects worldwide, such as species composition shifts, soil acidification, and eutrophication of aquatic systems (Bobbink et al., 2010; Fenn et al., 2003a; Vitousek et al., 1997). The effects of N deposition on soil trace gas emissions are less well characterized (Suddick and Davidson, 2012), especially in arid environments.

$NO_x$, $N_2O$ and $CO_2$ emissions, in particular, are important contributors to air quality and terrestrial feedbacks to climate change (Butterbach-Bahl et al., 2011; Schimel, 1995). While deserts are generally thought to have low potential for soil trace gas emissions, recent evidence suggests pulses of $CO_2$ from deserts can be large (Huxman et al., 2004; Jenerette and Chatterjee, 2012). The corresponding emissions of $NO_x$ and $N_2O$ from deserts are not well characterized but may also be larger than expected (Mccalley and Sparks, 2008). Considering that drylands cover over 40% of the terrestrial land surface (Lal, 2004; Safriel et al., 2005), understanding soil feedbacks in response to anthropogenic drivers, such as N deposition, in dryland ecosystems has important global relevance.

Soil microorganisms produce nitric oxide (NO) and nitrous oxide ($N_2O$) predominantly through the processes of nitrification and denitrification (Firestone and Davidson, 1989; Meixner and Yang, 2006). NO produced by soils is quickly oxidized in the atmosphere to form $NO_2$ ($NO + NO_2$ are collectively termed $NO_x$). While $NO_x$ is not a potent greenhouse gas, it directly contributes to the production of ozone, which is responsible for warming both the upper and lower atmosphere and represents a health concern for people and plants (Isaksen et al., 2009). $N_2O$ is a greenhouse gas, with roughly 300 times the warming potential of $CO_2$ and contributes to ozone depletion in the upper atmosphere where it serves as protection from harmful UV radiation (Forster et al., 2007). Nitrification and denitrification are generally limited by N availability and therefore increased N due to deposition should result in higher N trace gas emissions from these processes (Brown et al., 2012; Fenn et al., 1996; Venterea et al., 2004). While

the effects of N deposition on soil emissions have been studied in many ecosystems, very few studies have been performed in deserts (Aronson et al., 2012; Liu and Greaver, 2009). The processes that lead to $NO_x$ and $N_2O$ production are complex, and the sources of each gas are not well resolved (Baggs, 2011; Butterbach-Bahl et al., 2011; Giles et al., 2012). Quantification of the genes involved in N cycling processes may help clarify the sources of soil $NO_x$ and $N_2O$. The key enzyme that regulates the oxidation of ammonia in the first step of nitrification is ammonia monooxygenase, encoded by the gene *amoA* in autotrophic ammonia-oxidizing bacteria or archaea under aerobic conditions (Medinets et al., 2015). Describing the effect of N deposition on N trace gas emissions in desert soils is inhibited by limited study on the factors regulating $NO_x$ and $N_2O$ emissions in these systems, but is necessary for understanding soil feedbacks to global change drivers.

The principal driver of climate change is $CO_2$, with the largest terrestrial C pool contained in soils. Soil $CO_2$ production ($R_{soil}$), resulting from the combination of microbial and root respiration regulates losses from this C pool back to the atmosphere, with potential feedbacks to climate change. In desert soils, soil C and N accumulate during the dry season when microbial and plant activity is limited by water. A precipitation event results in a large pulse of activity, making both C and N available simultaneously, which can have synergistic effects on $R_{soil}$ (Eberwein et al., 2015b). Whether this is driven by changes in microbial activity, community structure or abiotic processes is not clear. Furthermore, these wetting-induced pulses in $R_{soil}$ contribute significantly to the annual budget in arid systems (Austin et al., 2004; Ma et al., 2012),

and whether N deposition can influence the magnitude or direction of these pulses is not well resolved.

Diversity and community structure effects on ecosystem properties have been well studied in plants, but similar characterization of the soil microbial community is still in its infancy (Madsen, 2011). The processes producing $NO_x$, $N_2O$ and $CO_2$ are all regulated by soil microorganisms, and therefore could be influenced by changes in microbial structure and diversity. Although respiration is a ubiquitous process in soil microorganisms, different taxa have different rates of growth, activity, and carbon allocation patterns. Recent data from laboratory soil pulse studies have shown regulation of the $R_{soil}$ pulse response through sequential resuscitation of different microbial communities (Placella et al., 2012). Unlike C respiration, nitrification is limited to a small subset of soil microorganisms, and changes in these populations are more likely to influence rates of nitrification. This is not the case with denitrification, which serves as a facultative alternative to respiration in a wide variety of soil microorganisms under anaerobic conditions. Exploration of soil microbial community responses to wetting in a field setting is needed to better understand pathways and regulation of $NO_x$, $N_2O$ and $CO_2$ pulse dynamics.

Current evidence indicates that N deposition can induce substantial changes in certain microbial populations, and this effect may interact with soil pulse trajectories. Decreases in fungal to bacterial ratios have been reported as plant succession progresses and are attributed to increasing soil N availability (Gurevitch et al., 2006; Klein et al., 1996). Experimental additions of N can alter fungal to bacterial ratios over much shorter

time periods (de Vries et al., 2006). In southern California, research shows changes in mycorrhizal communities due to N deposition, associated with effects on invasive species success (Egerton-Warburton et al., 2001; Egerton-Warburton and Allen, 2000; Sigüenza et al., 2006a, 2006b). Likewise, fast-growing copiotrophic bacteria have the advantage over the slow-growing oligotrophic species in a variety of ecosystems when N is abundant (Fierer et al., 2012). While many studies have demonstrated N deposition affects microbial activity and diversity, these have generally been restricted to subsets of the microbial community. More research is necessary to characterize the overall soil microbial community response to N deposition and to connect these differences to wetting-induced pulses of soil gaseous emissions. Here, we address this knowledge gap by combining measurements of soil trace gas emissions of $NO_x$, $N_2O$ and $CO_2$ with molecular characterization of the microbial community along an anthropogenic N deposition gradient in the Colorado Desert of southern California.

In addition to the overall goal of quantifying soil trace gas emissions response to nitrogen deposition in a desert ecosystem, the addition of molecular analysis will address aspects of the following questions: 1) How does N deposition affect microbial community dynamics? 2) Can changes in microbial community dynamics describe changes in soil trace gas emissions? Increased availability of N due to deposition is expected to increase the abundance of taxa associated with nitrification, and to shift microbial communities towards those taxa capable of rapid growth. Furthermore, this shift in microbial community composition along with N fertilization of microbial C and

N metabolism should result in greater pulses of $NO_x$, $N_2O$ and $CO_2$ in response to nitrogen addition and in the high deposition site.

## Methods

### *Study sites*

Two sites were selected based on the CMAQ model (Community Multiscale Air Quality; Fenn et al., 2010) to represent the extremes of N deposition in the deserts along the N deposition gradient generated by the Los Angeles metropolitan area. The low deposition site was located in Boyd Deep Canyon Desert Research Center (BDC), a part of the University of California Natural Reserve System (UCNRS) near Palm Desert, CA receiving approximately 5 kg N $ha^{-1}$ $y^{-1}$ modeled deposition. The high deposition site was located in the Oasis de los Osos (Oasis) another site in the UCNRS located at the north face of San Jacinto Mountain receiving approximately 15 kg N $ha^{-1}$ $y^{-1}$ modeled deposition. These sites are located on the interface between the Mojave and Colorado Deserts, with hot summers and occasional precipitation, which occurs primarily in the winter. Creosote bush (*Larrea tridentata*) scrub is the dominant vegetation at both sites. Mean annual minimum and maximum temperatures are 10-39 °C and 14-31.4 °C for BDC and Oasis, respectively. Mean precipitation is 145 mm/yr and 139 mm/yr for BDC and Oasis, respectively (http://deepcanyon.ucnrs.org; http://www.wrcc.dri.edu). Soils are classified as Carizo stony sand at BDC, with 96, 1.5 and 2.5 % sand, silt and clay, and soil pH of 8.2 (http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx). At Oasis, the soils are classified as Tujunga gravelly loamy sand with 80.5, 17, and 2.5 % sand, silt

and clay and a pH of 6.7 (http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx). Vegetation patterns at the two sites were markedly different, consistent with patterns that would be expected from increased N deposition (Allen et al., 2009; Fenn et al., 2003a). At Oasis shrubs were larger in stature with interspaces populated with invasive grasses compared to BDC where the shrubs were smaller in stature with sparsely populated interspaces dominated by species of the *Cactaceae* family.

*Nitrogen deposition*

Atmospheric N deposition was measured with ion-exchange resins for bulk deposition collected in the interspaces (n=5) as well as throughfall deposition measured under the canopy of *Larrea tridentata* (n=8) following Fenn and Poth, 2004. Four inch resin tubes were filled with mixed bed resin composed of equal parts cation and anion beads. Resin tubes are connected to an 8 inch diameter PVC funnel suspended above the ground on a pole. Deposition collectors were left out over three approximately six-month exposure periods from May 2014 to December 2016. Capped resin tubes were left out over the same time periods to serve as a blank. Deposition rates were calculated for the area covered by the funnel over the time of the exposure period for a deposition rate in kg N $ha^{-1}$ $y^{-1}$ for each of the three exposure periods, and the three exposure periods were averaged together for an average annual deposition rate.

*Soil nitrogen*

To compare soil chemical properties with N deposition, soil samples were collected in January 2014 and July 2014 with 0-10 cm soil cores. The soil core was homogenized and 2.5 g were combined in a 1:10 soil weight-to-solution volume ratio with 2M KCl. The extracts were placed on ice for transport back to the lab were they were shaken for 1 hour at room temperature, centrifuged , filtered through Whatman no. 40 filter paper, and then frozen until analysis on an AQ2 Discrete Analyzer (Seal Analytical Inc., Mequon, Wisconsin) in the University of California Riverside (UCR) Environmental Science Research Laboratory (ESRL). The soil samples were additionally processed for total C and N. The soils were dried at 105°C to constant weight, ball-milled and analyzed by combustion analysis on the Flash EA1112 (Thermo Fisher Scientific, Inc.) in the UCR ESRL.

*Treatment additions*

Field experiments were conducted mid-summer just prior to the rainy season at both the high and the low N deposition sites to correspond with peak soil N accumulation and prolonged water limitation (Padgett et al., 1999), in July 2014. Experiments were conducted within two weeks between sites to minimize climatic variation. Two treatments were applied to the soil, a water addition (W; to simulate a 2 cm rain event) or a water plus N treatment (N; 30 kg $NH_4NO_3$ $ha^{-1}$ in a 2 cm rain event, equivalent to 10.5 kg N $ha^{-1}$). The experimental design consisted of six replicate plots for each treatment at both site. Measurements were conducted under the canopy of a creosote bush (*Larrea*

*tridentata*) with paired plots of adjacent shrubs receiving either a W or a N treatment. Soil collars constructed of PVC were driven 10 cm into the ground for treatment additions, for a total of three collars under each shrub. One collar (30 cm diameter by 15 cm height) was used for soil sample collection and monitoring of soil temperature and moisture, and the other two collars were used for trace gas measurements. Soil temperature was recorded at 0-5 cm with a ProCheck Sensor equipped with GS3 probe (Decagon Device, Inc Pullman. WA) and soil moisture was measured from 0-10 cm with a handheld water content probe (HCS620 HydroSense, Campbell Scientific, Inc., Logan, CO) calibrated for our soils. The treatments were applied only to the inside of the collar to allow for more precise wetting and to ensure that conditions were the same for the trace gas measurements as the soils for DNA extraction.

*Trace gas fluxes*

Soil trace gas fluxes were measured before treatment additions (as described above) to serve as a control ("Pre"), and then at 15minutes and 12, and 24 hours after additions. For gas flux measurements, the soil collars were fitted with a custom made PVC chamber top with a mixing fan mounted on the inside and reflective tape covering the outside of the chamber. A 30 by 15cm collar was used for N trace gas measurements. Air was pulled from the chamber top at approximately 1 L $min^{-1}$ and routed sequentially to analyzers for measurement of soil emissions of $N_2O$, then $NO_x$. $N_2O$ emissions were measured with cavity-enhanced laser absorption spectroscopy with a tunable diode laser (Model 908-0014, Los Gatos Research, Inc., Mountain View, CA). The outflow of the

$N_2O$ analyzer was routed to a portable $NO_x$ monitor in combination with a molybdenum convertor (Model 410 and Model 401, 2B Technologies, Boulder CO), where the quantitative depletion of ozone was measured using UV absorbance. Soil N fluxes were calculated using the rate of increase in $NO_x$ or $N_2O$ concentration, and calculated by linear regression to determine rates of change, using approximately 3 minutes of data to maximize linearity of gas production (Davidson et al., 1991). The third collar, measuring 5cm by15cm was used for $R_{soil}$ analysis with a portable infrared gas analyzer (LI-8100, Licor Biosciences, Lincoln NE), and flux calculations were generated from the LI-8100 software (version 3.1.0).

*16S rRNA sequencing*

Soil samples for molecular analysis were collected concurrently with trace gas measurements and also at 2hr and 48 hours after treatment additions. Cores were taken from the top 10cm of soil and placed in sterile containers. The soil samples were immediately placed on dry ice and then frozen at -20°C until extraction. Microbial DNA was extracted using the PowerSoil DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) following manufacture's guidelines on 0.25 g of soil (±0.025 g). The V3-V4 region of the 16S rRNA gene was amplified in PCR reactions using primers S-D-Bact-0341-b-S-17 127 and S-D-Bact-0785-a-A-21 (Klindworth et al., 2013) by combining 2.5 µL of DNA template, 5 µL each of 1 µM forward and reverse primers, and 12.5 µL KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Inc., Wilmington, MA, USA). The thermocycler conditions were: 95 °C for 3 minutes, then 25 cycles of 95 °C

for 30 seconds, 55 °C for 30 seconds, 72°C for 30 seconds, and an extension step for 5 minutes at 72 °C. This was followed by a clean-up using Agencourt AMPure XP Beads (Beckman Coulter Genomics, Danvers, MA, USA) and then a second round of PCR to attach dual indices to each sample with the Nextera XT Index Kit (Illumina Inc., San Diego, CA, USA) using 5 µL DNA, 5 µL each of 1 µM forward and reverse index primers, 25 µL KAPA HiFi HotStart ReadyMix, and 10 µL PCR grade water. The thermocycler conditions were: 95 °C for 3 minutes, followed by 8 cycles of 95°C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and an extension step for 5 minutes at 72 °C. The indexed amplicons then went through another clean-up step (same as described above). Finally, the samples were quantified with the Quant-it PicoGreen® dsDNA assay kit (Life Technologies Inc., Grand Island, NY, USA) to be pooled in equimolar concentrations and then sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the UCR Genomics Core Facility.

*qPCR*

The same soil extracts, as described above, were quantified with the Quant-it PicoGreen® dsDNA assay kit (Life Technologies Inc., Grand Island, NY, USA), and diluted to 1 ng DNA µL-1 for quantitative polymerase chain reaction (qPCR) of the *amoA* gene from bacteria and archaea following Beman et al., 2008. For bacterial *amoA*, we used the AmoA1F/amoA2R primer set (Rotthauwe et al., 1997) and for archaea we used the Arch-amoAF/ArchamoAR primers (Francis et al., 2005). Twenty-five µL reactions were used for qPCR of *amoA*, consisting of SYBR Premix F (Epicentre

BioTechnologies, Madison, WI, USA), 2 mM MgCl2, 1.25 units AmpliTaq polymerase (Life Technologies Corporation, Carlsbad, CA, USA), 40 ng μL-1 BSA (Life Technologies Corporation, Carlsbad, CA, USA), 0.4 μM forward and reverse primer, and 1 ng μL-1 DNA. The reaction mix was the same for bacteria and archaea, except for the exclusion of $MgCl_2$ for qPCR of bacterial *amoA*. The protocol for amplification of AOB *amo*A fragments was 5 minutes at 95°C, followed by 40 cycles of 45 seconds at 95 °C, 30 seconds at 56°C and 60 seconds at 72°C and a detection step for 7 seconds at 81°C. The protocol for amplification of AOA *amo*A fragments was 4 minutes at 95°C, followed by 40 cycles of 30 seconds at 95 °C, 45 seconds at 53 °C and 60 seconds at 72 °C. Both protocols were followed by a melt curve analysis from 65°C to 95°C by 0.5°C increments every 5 seconds. Standards were constructed at Blue Heron Biotechnologies where the sequence of interest was cloned into the standard pUCminusMCS vector. Sequences were selected based on well-known archaeal and bacteria ammonia oxidizing organisms (*amo*A gene of *Nitrosomonas europaea* ATCC 19718 and crenarchaeota genomic fragment 54d9). Standard curves were generated from serial dilutions ($10^4$ to $10^9$ and $10^2$ to $10^8$ copies for AOB and AOA, respectively) of *amoA* standards and produced efficiencies of 76-86% for AOA ($R^2$ 0.96-1.00) and 75-86% for AOB ($R^2$ 0.99-1.00), respectively. Gene abundances were calculated per g of dry soil. To account for the large variation between the W plots versus the N plots in the pre-treatment samples, response ratios were calculated from the abundance at the specified timepoint divided by the abundance for the pre timepoint.

*16S rRNA Sequence Analysis*

Demultiplexed sequences from the Illumina Miseq were processed using default parameters in Quantitative Insights into Microbial Ecology (QIIME; Caporaso et al., 2010). Forward and reverse reads were joined (allowing for maximum 20% divergence in the overlap region) using the default QIIME parameters for quality control, which require greater than 75% consecutive high-quality base calls, Phred scores greater than three, total sequence length greater than 75 bases and exclude reads with more than three low-quality base calls in a row, or any ambiguous calls (Bokulich et al., 2013). Then operational taxonomic units (OTUs) were assigned with the open reference OTU picking approach in which sequences were first grouped with UCLUST (Edgar, 2010) at 97% similarity and assigned taxonomy with the 13_8 version of the Greengenes database (McDonald et al., 2012). Then, reads that had no sequence matches in the database were clustered de novo. The phylogenetic tree containing the aligned sequences was produced using FastTree (Price et al., 2009). Three samples were removed due to low read counts, leaving 140 samples and resulting in 10,711,813 total reads. Before diversity analysis sequences were rarified to an even depth of 24,650.

Beta diversity was visualized using non-metric multidimensional scaling (NMDS) of the weighted UniFrac distance calculated in QIIME, which incorporates information about phylogenetic distance between pairs of samples using the phylogenetic tree (Lozupone and Knight, 2005). The NMDS ordination was graphed in R (R version 3.2.1; R Core Team 2015) using ggplot2 (Wickham, 2009) and the 'stat_ellipse' function with 95% confidence intervals. Differences in overall microbial community composition

across site and treatments were performed with permutational multivariate ANOVA (perMANOVA) implemented using the Vegan function 'adonis2' in R (999 permutations; McArdle and Anderson, 2001). Shannon diversity was calculated in R using the estimate_richness function in the phyloseq package.

The total relative abundance of organism capable of ammonia oxidation (i.e.: nitrifiers) was compiled by summing all occurrences of OTUs in the order *Nitrosomonadale*s and the phylum *Nitrospir*a from the *Bacteria* with the class *Thaumarcheota* of the *Archaea* for a net relative abundance (Madigan and Martinko, 2006). Response ratios were calculated for Shannon diversity and nitrifier relative abundance as described above.

*Statistical analysis*

ANOVA with post hoc Tukey's honestly significant difference test (HSD) was used to compare soil gas fluxes, soil N, N deposition, Shannon diversity metrics, nitrifier relative abundance, nitrifier response ratios, *amoA* gene abundances and *amoA* response ratios, between sites, across treatments (where applicable) and over time since wetting (where applicable). The boxcox family of transformations were used to fulfill assumptions of normality and homoscedacity where necessary (Box and Cox, 1964). Statistical analysis was performed in R version 3.2.1 (R Development Core Team 2015).

## Results

### *Nitrogen deposition*

The expected N deposition amounts based on the CMAQ model were largely supported by empirical estimates (Figure 3.1a). Both bulk deposition measured in the interspaces as well as throughfall deposition measured under the canopy of *Larrea tridentata* were significantly higher at Oasis than BDC ($p<0.0001$). Furthermore, deposition was significantly higher under the canopy than in the interspaces at both sites ($p<0.0001$). N Deposition was composed of roughly equal parts $NH_4^+$ and $NO_3^-$ at both sites ($p=0.322$). Soil KCl extractable N analysis also confirmed this pattern in January 2014, but not July 2014 (Figure 3.1b). However, soil $NO_3^-$ was significantly greater than soil $NH_4^+$ at both sites for both measurement times ($p<0.0001$). Total C was 1.14±0.58 and 0.81±0.34, and total N was 0.18±0.051and 0.15±0.029 for BDC and Oasis, respectively, which were not significantly different between sites ($p>0.05$; not shown).

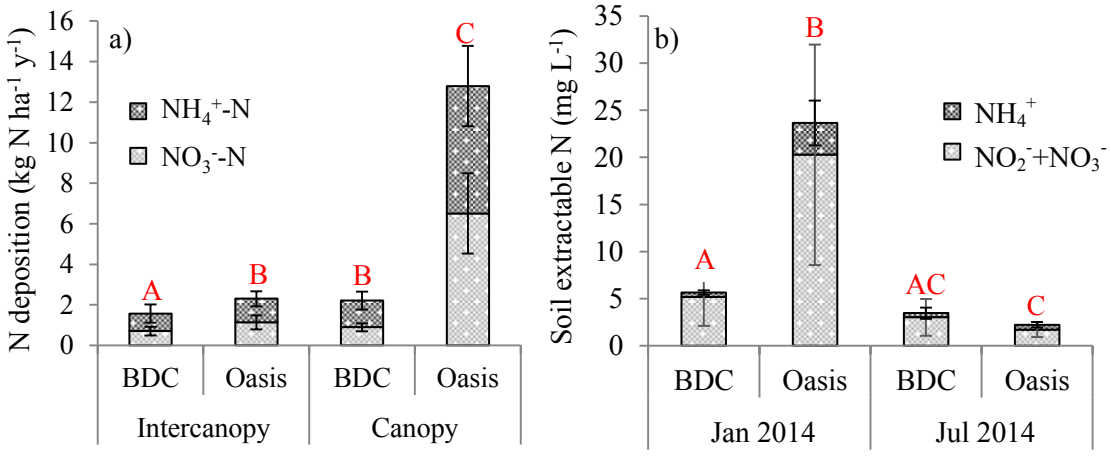


Figure 3.1: Atmospheric nitrogen (N) deposition (a) and soil KCl extractable N (b). Measured ammonium ($NH_4^+$) is shown in dark grey and nitrate ($NO_3^-$; a) or nitrate plus nitrite ($NO_3^-+NO_2^-$; b) is shown in light grey. Capital letters represent significant difference at $p<0.05$ for total N. Error bars represent standard deviation.

*Soil trace gas fluxes*

Soil $NO_x$ production responded strongly to experimental wetting at both the low (BDC) and high (Oasis) N deposition sites (Figure 3.2), reaching as high as 345 ng $NO_x$-N $m^{-2}$ $s^{-1}$. At both sites, time since wetting significantly influenced $NO_x$ fluxes ($p<0.0001$), which peaked at 12 hours after wetting and remained elevated through 24 hours post-wetting. Furthermore, N addition increased $NO_x$ fluxes at both sites ($p=0.028$ and 0.0029 for BDC and Oasis, respectively). However, fluxes were overall greater at BDC, the low N deposition site ($p<0.0001$).

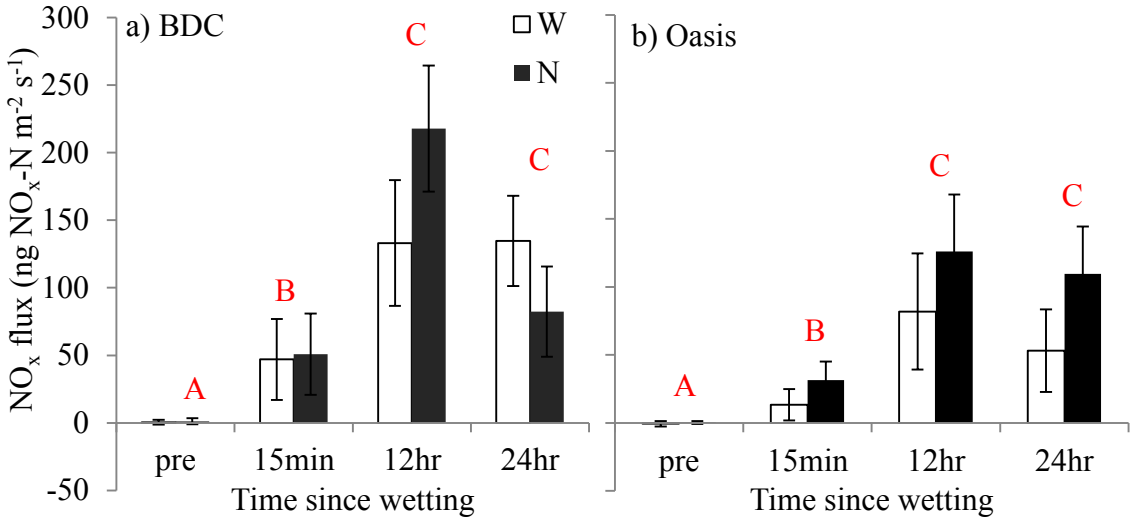


Figure 3.2: $NO_x$ fluxes following experimental (to simulate a 2 cm rain event: W) and nitrogen (30 kg $NH_4NO_3$ $ha^{-1}$ in a 2cm rain event; N) additions at a low (a; BDC) and a high nitrogen deposition (b; Oasis) site in the Colorado Desert. Error bars represent standard deviation. Letters represent significant difference between timepoints within s site at $p<0.05$.

Soil $N_2O$ production also demonstrated a strong wetting-induced pulse at both sites, reaching as high as 1725 ng $N_2O$-N $m^{-2}$ $s^{-1}$ (Figure 3.3). Time was also significant for $N_2O$ fluxes at both sites ($p<0.0001$), with a very large pulse at 15 min, which

decreased dramatically by 12 hours, but remained elevated over pre-wetting fluxes at 12 and 24 hours for Oasis ($p<0.05$) and was not significantly different from pre-wetting fluxes for BDC at 24 hours ($p=0.13$). $N_2O$ fluxes were also greater at the low N deposition site ($p<0.0001$). However, unlike $NO_x$, N addition did not influence soil $N_2O$ production ($p=0.61$).

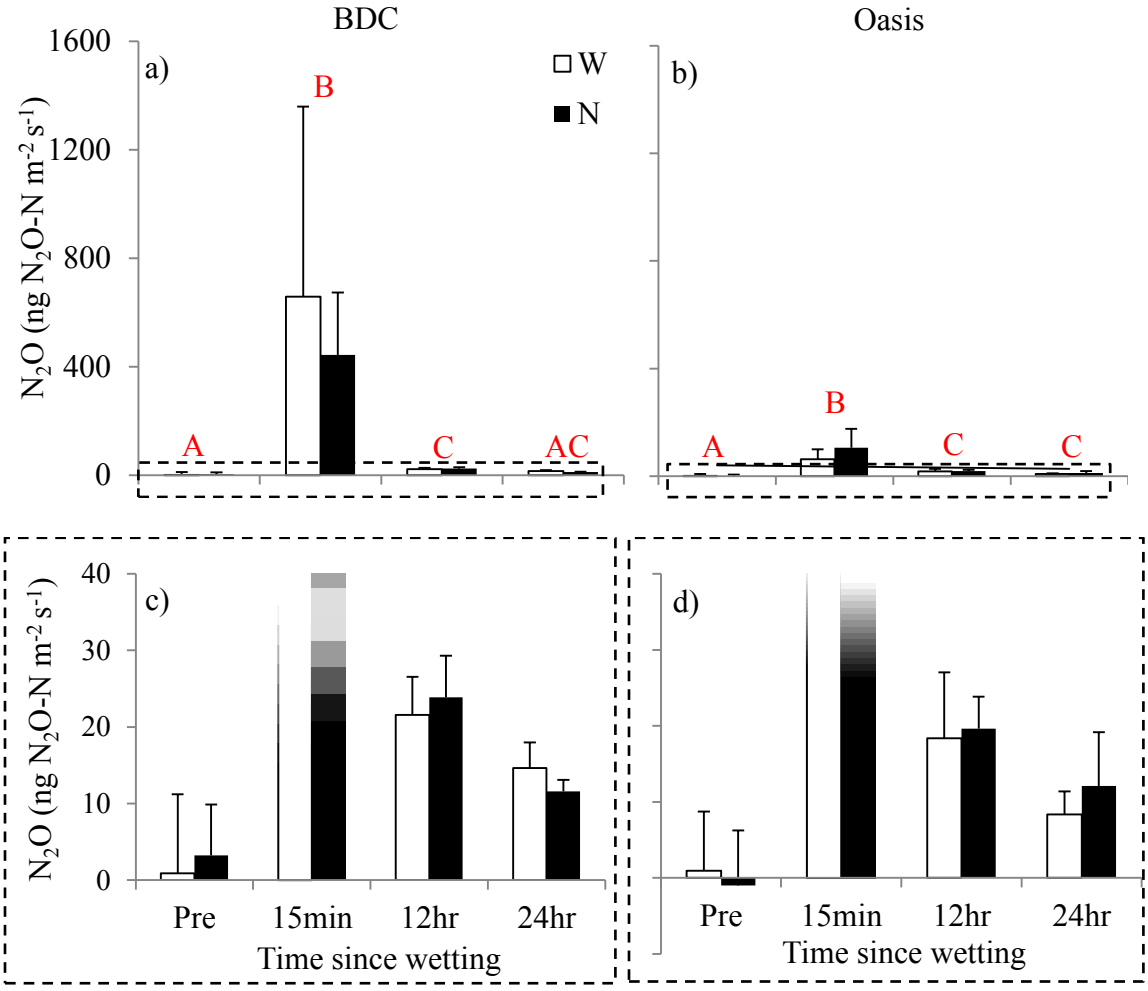


Figure 3.3: $N_2O$ fluxes at a low (a,c; BDC) versus a high (b,d; Oasis) nitrogen deposition site in the Colorado Desert following experimental water (to simulate a 2 cm rain event; W) and nitrogen (30 kg $NH_4NO_3$ $ha^{-1}$ in a 2cm rain event; N) additions. Bottom panels (c, d) represent enlargement, so that lower fluxes could be differentiated. Error bars represent standard deviation. Letters represent significance between timepoints within a site at $p<0.05$.

Patterns in $R_{soil}$ were very similar to $N_2O$ (Figure 3.4), being significantly affected by time since wetting ($p<0.0001$) and demonstrating peak fluxes at 15 minutes after wetting that also remained slightly elevated compared to pre-wetting samples through 24 hours at both sites. Similar to $N_2O$, N addition did not influence $R_{soil}$ ($p=0.96$). However, unlike $NO_x$ and $N_2O$, there was no difference in $R_{soil}$ between sites ($p=0.60$).

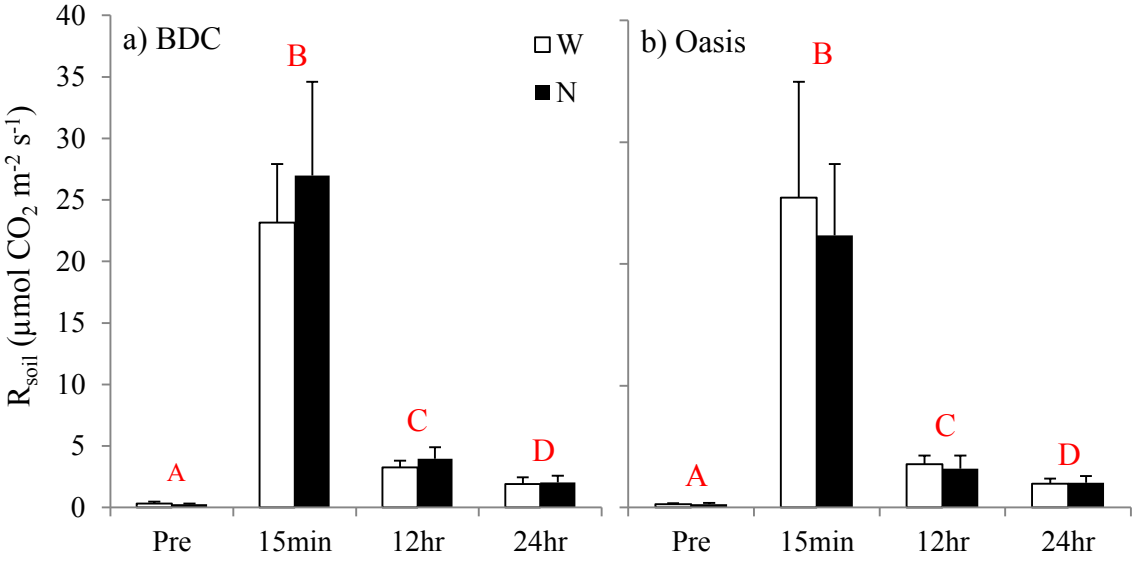


Figure 3.4: Soil $CO_2$ production ($R_{soil}$) at a low (a; BDC) versus a high (b; Oasis) nitrogen deposition site in the Colorado Desert following experimental water (to simulate a 2 cm rain event; W) and nitrogen (30 kg $NH_4NO_3$ $ha^{-1}$ in a 2cm rain event; N) additions. Error bars represent standard deviation. Letters represent significance between timepoints within a site at $p<0.05$.

*16S rRNA sequencing*

The results of the 16S rRNA sequencing analysis demonstrate a clear separation in microbial community composition between the two study sites (Figure 3.5; $p= 0.001$). The effect of N addition was much less dramatic and only BDC showed significant

changes in microbial community composition (p= 0.048 and 0.12, for BDC and Oasis, respectively). The influence of time since wetting on microbial community composition was also minimal, again only showing significant differences at BDC (p=0.42 and 0.008 for Oasis and BDC, respectively). Specifically, NMDS ordination of the weighted UniFrac distances reveal contrasting patterns in the microbial community response to wetting in the W compared to the N treatments. For the W treatments, the Pre samples were distinct from the other timepoints, versus in the N treatments the 48 hour samples group separately (Figure 3.6a,b). Although time was not significant for the Oasis samples, the same general pattern is supported (Figure 3.6c,d).

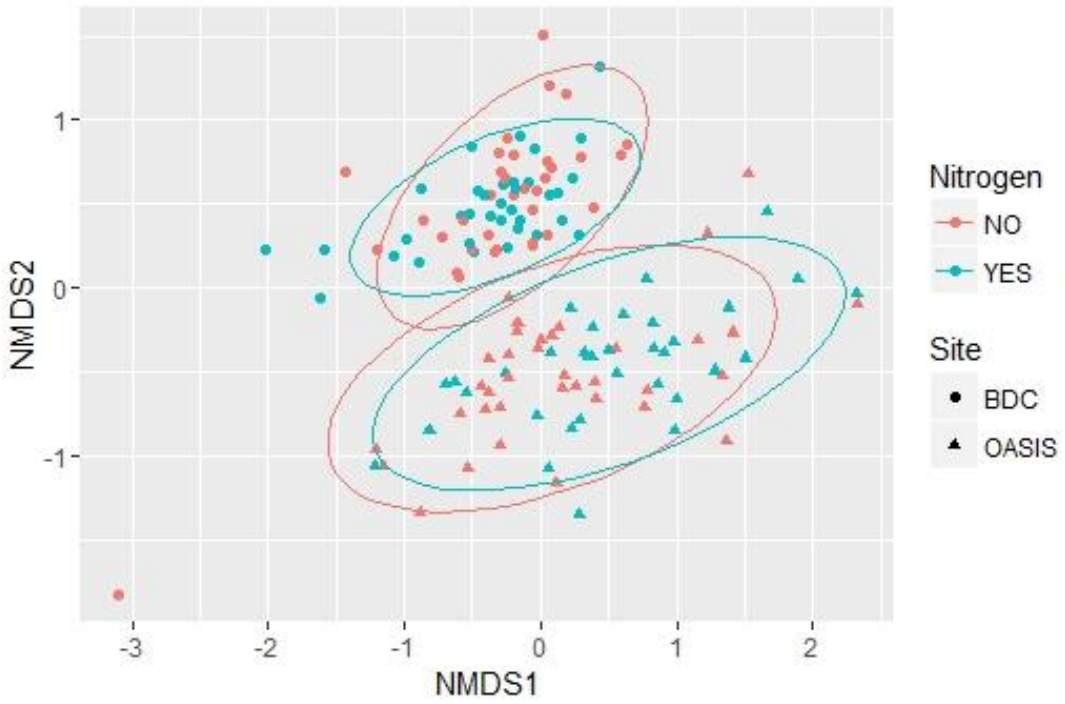


Figure 3.5: NMDS of the weighted UniFrac distances for a low (BDC; circles) versus a high (Oasis, triangles) nitrogen deposition site in the Colorado Desert following experimental water (W; to simulate a 2 cm rain event; red points) and nitrogen (N; 30 kg $NH_4NO_3$ $ha^{-1}$in a 2cm rain event; blue points) additions. Ellipses represent 95% confidence intervals per treatment for each site.

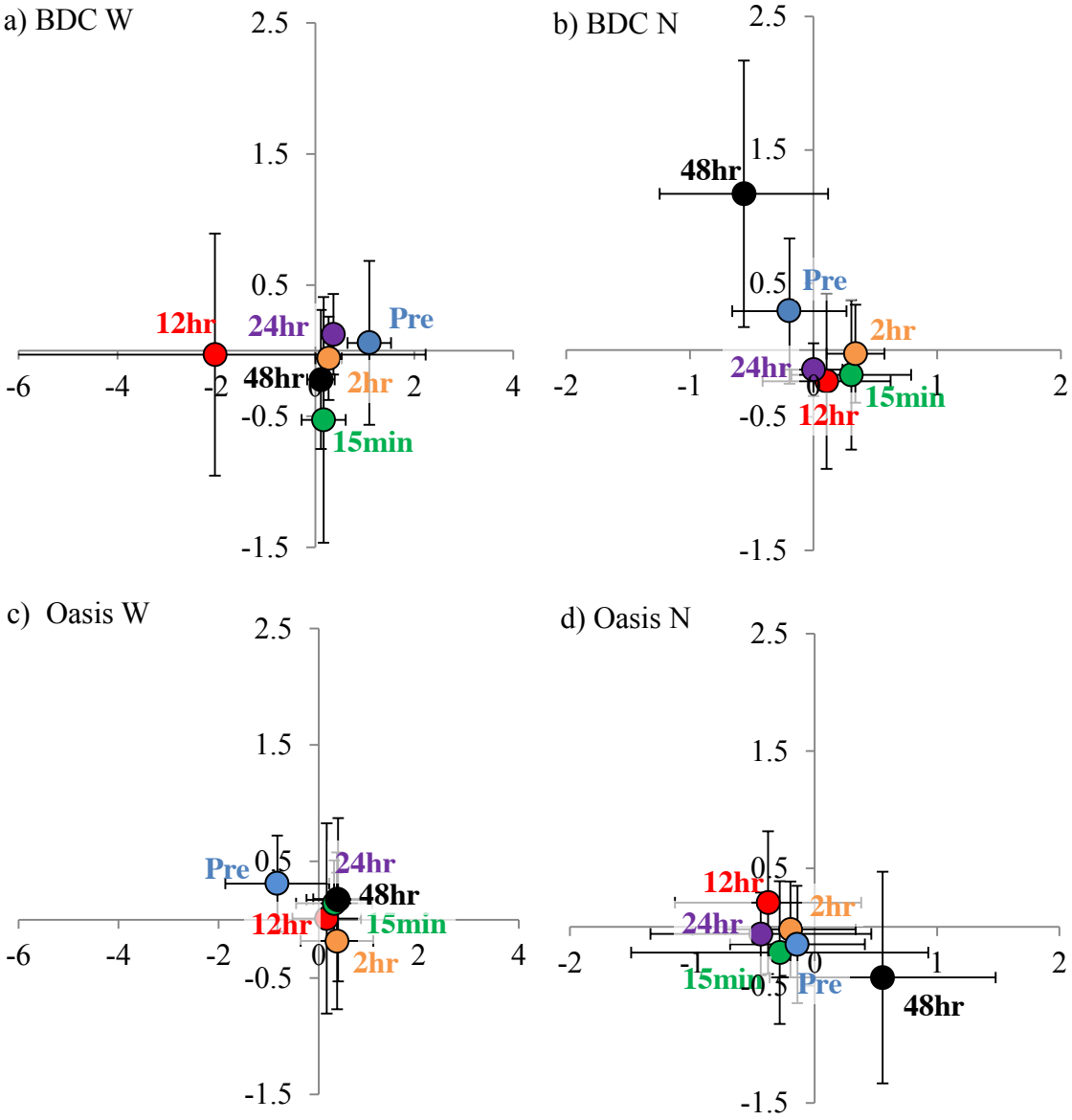


Figure 3.6: NMDS of the weighted UniFrac distance averaged by time point for a low (a,b; BDC) versus a high (c,d; Oasis) nitrogen deposition site in the Colorado Desert following experimental water (a,c; to simulate a 2 cm rain event; W) and nitrogen (b, d; 30 kg $NH_4NO_3$ $ha^{-1}$in a 2cm rain event; N) additions. Error bars represent ±1 standard deviation.

Shannon diversity was also influenced by site and N addition. Averaged Shannon diversity was 6.97±0.20 at BDC and 6.88±0.27 at Oasis (p=0.012). At both sites,

diversity was greater at 48 hours than the pre samples ($p<0.0001$ and $p=0.0022$ for BDC and Oasis, respectively). However, large differences between the W and N plots pre-wetting reflect a high amount of variation in these sites that could artificially skew the effects of treatment and time on diversity. Therefore, response ratios were used to correct for this variation. Shannon diversity response ratios were generally greater at Oasis ($p=0.022$), but were lower under N addition ($p<0.0001$; Figure 3.7). Furthermore, time did not significantly influence Shannon diversity response ratios ($p=0.34$), with one exception. Linear regression demonstrated a significant increase in Shannon diversity with time for the N treatment at BDC ($p=0.001$, adjusted $R^2=0.25$).

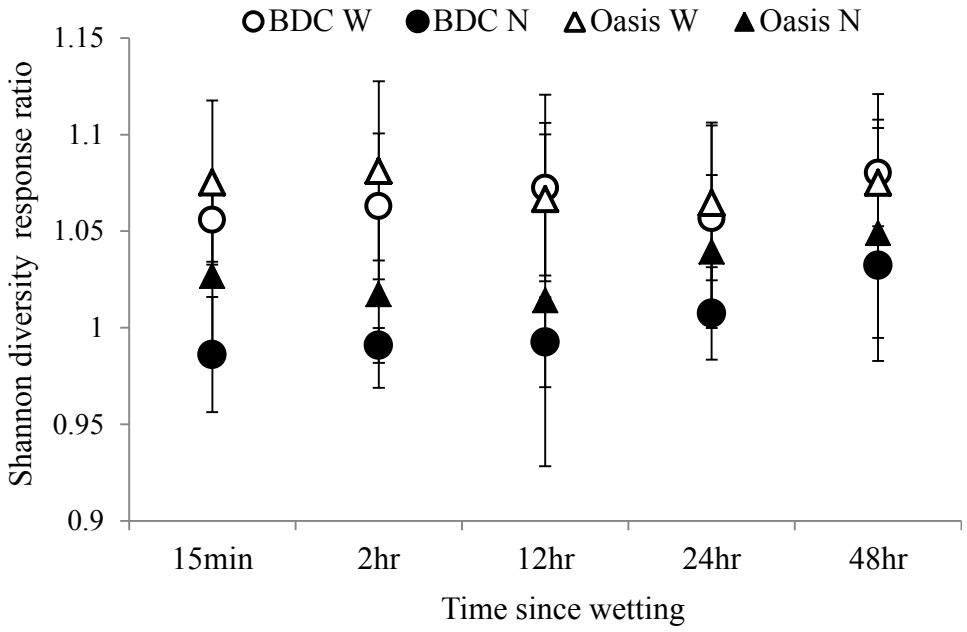


Figure 3.7: Shannon diversity response ratios for the 16S rRNA sequences from a low (BDC; circles) versus a high (Oasis; triangles) nitrogen deposition site in the Colorado Desert following experimental water (W; to simulate a 2 cm rain event; black points) and nitrogen (N; 30 kg $NH_4NO_3$ $ha^{-1}$in a 2cm rain event; white points) additions. Error bars represent ±1 SD. Response ratios were calculated from the Shannon diversity at the specified timepoint divided by the pre-wetting Shannon diversity for that sample.

*Shifts in taxa associated with Nitrification*

Quantification of archaeal and bacterial ammonia oxidation genes also revealed site differences in the abundance of microorganisms capable of nitrification (Figure 3.8). Overall *amoA* gene abundance of ammonia oxidizing bacteria (AOB) were approximately 30% greater at Oasis than BDC ($p=0.00075$) and demonstrated a greater response ratio at Oasis than BDC ($p=0.0080$; Figure 3.8a). While response ratios reached up to 38 at BDC and 109 at Oasis, neither time since wetting nor treatment significantly influenced AOB *amoA* gene abundance ($p=0.52$ and 0.20 for time and treatment, respectively) or response ratios ($p= 0.57$ and 0.23 for time and treatment, respectively; Figure 3.8a). Alternatively, *amoA* gene abundance of ammonia oxidizing archaea (AOA) demonstrated a very strong response to wetting, with significant increases from pre to 48 hours ($p=0.0069$; Figure 3.8c). While AOA *amoA* gene abundance was 159% greater at BDC ($p=0.0050$), the AOA *amoA* response ratio was greater at Oasis ($p=0.0026$). Similar to AOB, there was no effect of experimental N addition on the abundance of AOA *amoA* genes ($p=0.81$) nor the AOA response ratio ($p=0.19$).

Patterns in the relative abundance of 16S rRNA sequences associated with a potential for nitrification (i.e.: nitrifiers) were also influenced by site and experimental N addition. Overall nitrifier relative abundance was 24% greater at Oasis than BDC ($p=0.0045$), but the response ratio was not significantly different between sites ($p=0.12$). Nitrifier relative abundance did not change with time since wetting ($p=0.48$), nor was the nitrifier response ratio influenced by time ($p=0.33$). However, the response ratio was

significantly affected by experimental N addition (p=0.0057), demonstrating a general decrease with N addition compared to water alone (Figure 3.8c).

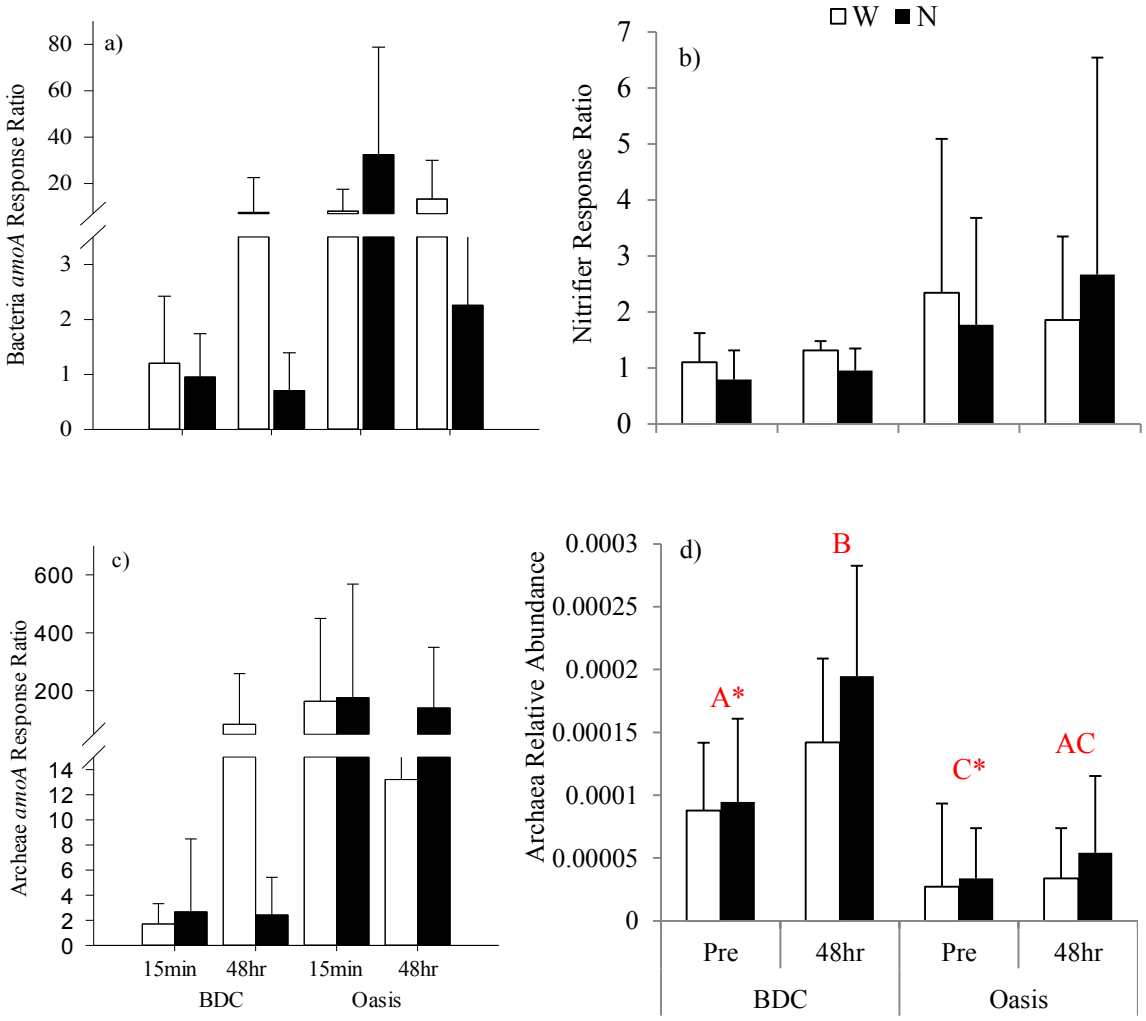


Figure 3.8: Shifts in microbial groups associated with nitrification at low (BDC) versus high (Oasis) nitrogen deposition sites in response to wetting (W; to simulate a 2cm rain event) and nitrogen (N; 30 kg NH4NO3 in a 2cm rain event). Panels a and c show qPCR of bacterial (a) and archaeal (c) *amoA* response ratios. Panels b and d show Nitrifier relative abundance response ratio (b) and archaea relative abundance. Response ratio was calculated from amoA gene abundance (a,c) or relative abundance (b) at the specified time point divided by the pre-wetting value for that sample. Error bars represent ±1 standard deviation. Capital letters represent significance at $p<0.05$, except for marginal significance for BDC versus Oasis pre-wetting ($p=0.09$) noted with an asterisk (*).

Relative abundance of archaea 16S rRNA (Figure 3.8d) was on average 163% greater at BDC than Oasis ($p<0.0001$) and was significantly affected by time since wetting ($p=0.019$). However, there was a marginally significant site by time interaction ($p=0.089$). While there was a general increase in relative abundance of archaea with experimental N addition, this was not a significant effect ($p=0.29$). Note that response ratios could not be calculated because of a high occurrence of zeroes in the pre-wetting samples.

Discussion

The results from this study present nitrogenous soil emissions that were substantially higher than expected from desert soils at both the low and high deposition sites, but these fluxes exhibited complex responses to N additions. Long-term effects from N addition (assessed between sites differing in N deposition), were associated with reduced soil N emissions while short term effects from experimental N additions were associated with an increase in soil $NO_x$ emissions. These differences in soil trace gas emission responses to the temporal scale of N addition were linked with differences in microbial community composition. Large differences in community composition were observed between sites, but the influences of short term N addition on community composition shifts were variable. Furthermore, the very rapid nature of the observed trace gas fluxes, especially $N_2O$, present the possibility for a mechanism that is separated from active microbial metabolism as an important, but overlooked source for soil N trace gas fluxes in arid environments.

All trace gas fluxes responded strongly to water additions, demonstrating a clear wetting-induced pulse response. However, contrary to our predictions, nitrogenous fluxes were overall higher at the low N deposition site. While atmospheric N deposition measurements support the N deposition gradient between the two sites (Figure 3.1a), soil analysis at the time of soil trace gas flux measurements showed no significant difference in soil extractable ammonium and nitrate between the high and the low deposition sites (Figure 3.1b). The method of experimental wetting may not have captured well the full effects of N deposition, as dry deposition is first intercepted by the canopy before it is washed through to the soil in a precipitation event (Fenn et al., 2009). As we only applied water to the soil surface, we may have omitted the flush of nitrogen deposition that would have naturally occurred in a precipitation event. Alternatively, plant uptake could differ between the sites, influencing N availability. While the soil N at the time of sampling did not parallel measured atmospheric N deposition inputs, large differences between the high and low N deposition sites were observed.

Surprisingly, gaseous nitrogen export may be a greater form of nitrogen loss in desert ecosystems than is currently assumed. $N_2O$ fluxes, in particular were remarkably high reaching up to 1700 ng $N_2O$-N $m^{-2}$ $s^{-1}$ (Figure 3.3), among the highest fluxes measured globally in any system (Figure 3.9). Desert soils are not typically considered substantial sources of $N_2O$ emissions (Meixner and Yang, 2006), and our findings challenge this expectation. More real-time field measurements are necessary to understand if this is a phenomenon occurring widely across arid systems, and if other systems are being underestimated as well. High desert $N_2O$ emissions can influence

ecosystem N cycling, global N budgets, and desert contributions to greenhouse gas concentration. As $N_2O$ represents 300 times the warming potential of $CO_2$ (Forster et al., 2007), understanding the potential for such high $N_2O$ emissions in arid systems is important for predicting future climate change.

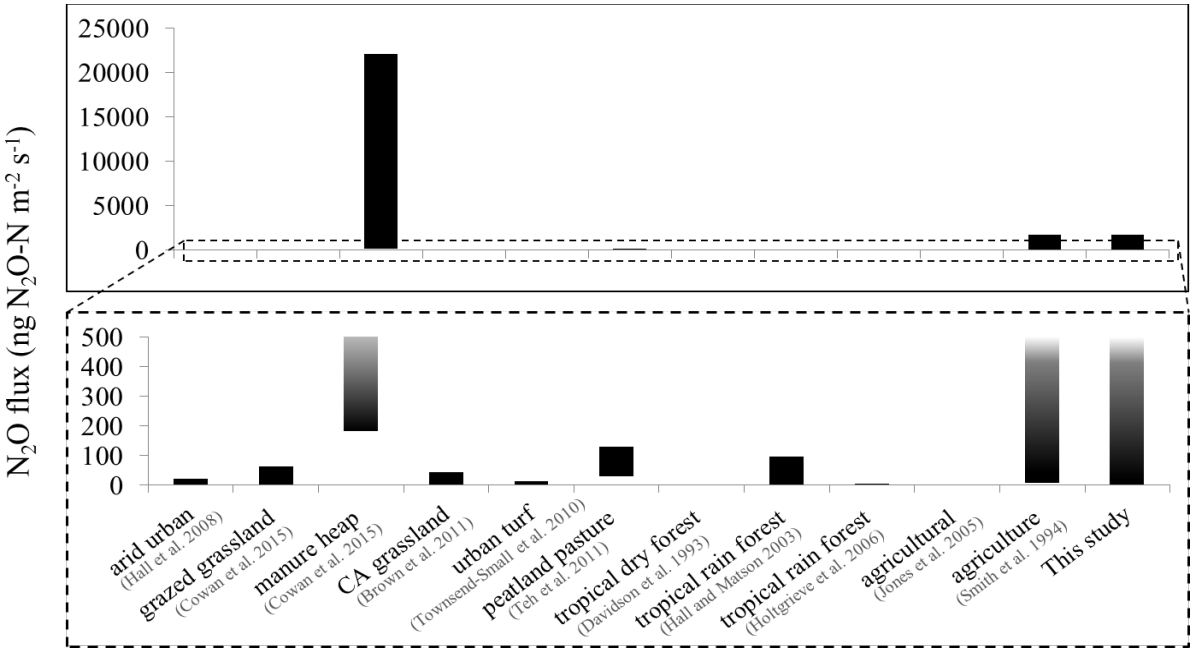


Figure 3.9: Comparison of literature $N_2O$ emissions. The reported ranges are shown. Bottom panel represents an enlargement, so that smaller fluxes could be differentiated.

$NO_x$ emissions were also considerable, reaching as high as 345 ng $NO_x$-N $m^{-2}$ $s^{-1}$ (Figure 3.2). The $NO_x$ emissions produced by these desert soils were comparable to or greater than fluxes measured in most other ecosystems (Figure 3.10). Furthermore, pulsed $NO_x$ emissions resulted in more prolonged production following wet-up compared to $N_2O$ emissions. Soil $NO_x$ emissions can contribute to local ozone production (Oikawa et al., 2015), with serious implications for local air quality, and may perpetuate the

detrimental effects of N deposition further downwind from the source. The potential for high $NO_x$ emissions in arid systems and the capacity for anthropogenic N deposition to increase these emissions may have important implications for local air quality in these systems.

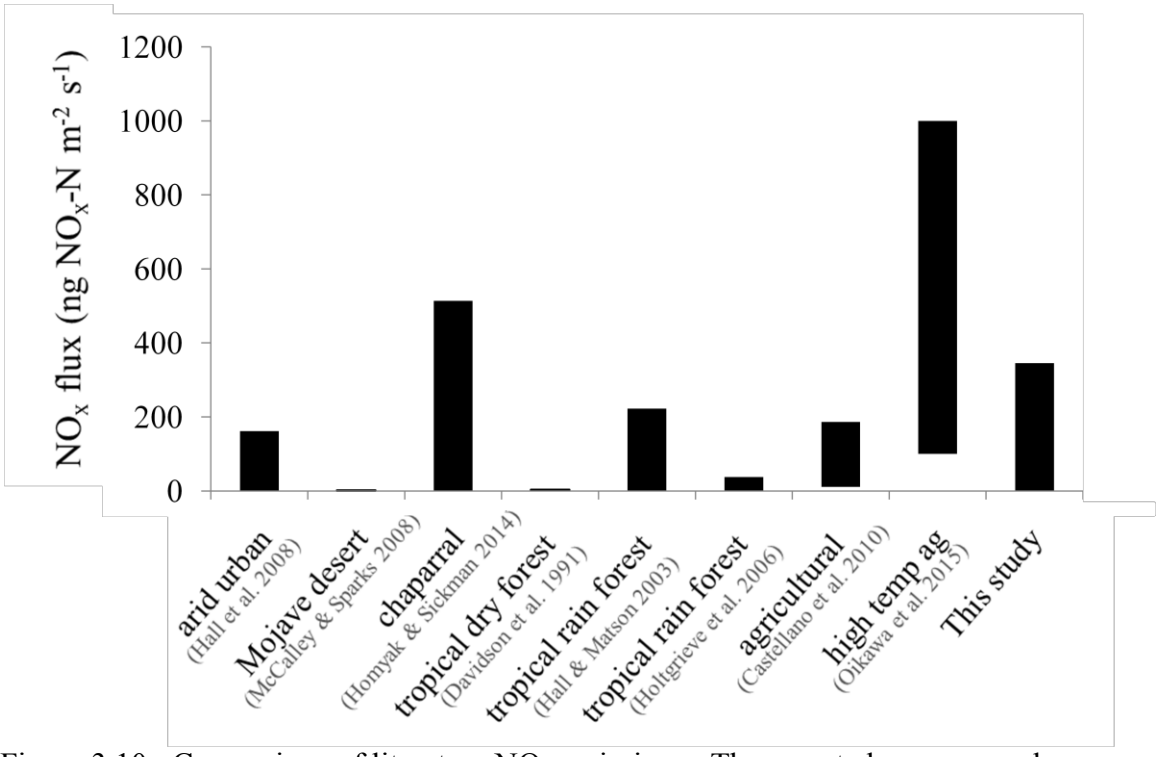


Figure 3.10: Comparison of literature $NO_x$ emissions. The reported ranges are shown.

The patterns in wetting-induced pulses were very different for $NO_x$ compared to $N_2O$ and $CO_2$. $NO_x$ fluxes did not peak until 12 hours post-wetting and remained elevated through 24hours. Alternatively, $N_2O$ and $CO_2$ fluxes both demonstrated a substantial pulse at 15 minutes post-wetting, which was back to near pre-wetting conditions by 12 hours. Furthermore, $NO_x$ fluxes increased with N addition, suggesting fertilization of N metabolism, but $N_2O$ and $CO_2$ fluxes showed no response. Several studies have found evidence suggestive of abiotic pathways for pulsed gaseous fluxes and

the differences between abiotic and living pathways may in part explain differences in pulse trajectories among trace gasses. Microbial metabolism of C substrates depends on the activity of extracellular enzymes. These extracellular enzymes can be preserved in the soil during dry periods and activated as the initial wetting from a precipitation event removes separation of the enzymes from their substrates (Blankinship et al., 2014; Schimel and Schaeffer, 2012). This could explain the immediate pulse in $CO_2$ emissions, but the enzymes for nitrification and denitrification are primarily intracellular, requiring a functional membrane for generating proton gradients (Madigan and Martinko, 2006). Recent evidence indicates that nitrification intermediates (i.e.: nitrite and hydroxylamine) might be isolated in soils during dry down, which could result in chemodenitrification upon wetting (Heil et al., 2016; Homyak et al., 2016; Zhu-Barker et al., 2015). Furthermore, there is evidence that nitrification and denitrification potentials are higher in summer (Parker and Schimel, 2011; Sullivan et al., 2012), and this may be the result of abiotic transformations, especially at high temperatures (McCalley and Sparks, 2009). Therefore, the immediate pulse in N and C fluxes following a precipitation event could be the result of biological activities that become separated from active microbial metabolism due to rapid dry down. Nevertheless, the delay in $NO_x$ peak emissions following wetting suggests that the pulse trajectories regulating $N_2O$ and $CO_2$ emissions are different for these trace gasses, and thorough understanding of these pulse trajectories is crucial for global accounting and development of accurate biogeochemical models.

On the other hand, ordination of the 16s sequencing data suggests that there might be shifts in the microbial community composition immediately after wetting (Figure 3.6).

While a dormant microbial population should take hours to days to return to active metabolism (Blagodatskaya and Kuzyakov, 2013), recent work suggests that certain taxa are able to respond within minutes of wetting proceeded by prolonged drought (Placella et al., 2012). Furthermore, previous experiments have found $CO_2$ emissions respond to labile C additions rapidly following wetting, suggestive of a microbial pathway for C emissions (Crum, 2016; Jenerette and Chatterjee, 2012). It is possible that shifts in 16S rRNA genes, as seen here, can result from degradation of relic non-living microorganisms due to cell rupture with wetting (Borken and Matzner, 2009; Carini et al., 2016; Moyano et al., 2013). However, here we saw a general increase in microbial diversity with wetting (Figure 3.7). Furthermore, at the high deposition site, the abundance of genes for ammonia oxidizing archaea and bacteria increased dramatically at 15 minutes post-wetting (Figure 3.8a,c). While changes in microbial DNA relative abundance does not directly represent which taxa are actually actively metabolizing (Blagodatskaya and Kuzyakov, 2013), it does indicate that some soil microorganisms might be able to respond quickly to a wetting event proceeded by extended drought.

We saw distinct differences in the microbial community composition between these two sites (Figure 3.5), which could be attributed to the long-term influence of N deposition. The high deposition site, Oasis, demonstrated higher abundance of AOB ($p=0.00075$) and higher nitrifier relative abundance ($p=0.0045$), where the low deposition site had significantly greater AOA gene abundance ($p=0.005$) and higher relative abundance of total archaea ($p<0.0001$; Figure 3.8d). While we saw clear shifts in microbial community composition with higher N deposition, it was not clear if those

shifts would be responsible for changes in soil trace gas fluxes. N fluxes were generally greater at the low N deposition site (Figure 3.2 and 3.3). A shift to a more copiotrophic community could result in microorganisms that are not able to respond as strongly under N limitation (Koch, 2001; Leff et al., 2015; Ramirez et al., 2012). In this study, we were not able to identify clear shifts in phyla that have been associated with a copiotrophic (i.e.: Proteobacteria or Firmicutes) or oligotrophic (i.e.: Acidobacteria or Actinobacteria) life strategy (Fierer et al., 2007; Trivedi et al., 2013). These are broad phylogenetic classes that include a diverse array of taxa. However, we did see greater abundance of archaea at BDC, which are associated with oligotrophic conditions.

Furthermore, the influence of N addition on the pulse response differed between sites. While we saw a strong positive response to wetting at both sites in terms of both trace gas emissions, and microbial community composition, the influence of N on this pulse response in the microbial community composition produced contrasting effects at BDC compared to Oasis. At BDC, the inclusion of N dampened the nitrifier pulse response compared to water alone (Figure 3.8). Alternatively, at Oasis N addition resulted in equal to or greater responses from nitrifying organisms as water alone (Figure 3.8). This indicates that the microbial community is primed to respond to higher levels of N availability consistent with higher levels of atmospheric N deposition at this site. The greater 16S beta diversity at BDC over Oasis and decrease in diversity response ratio with experimental N addition (Figure 3.5) supports that N can have a detrimental effect on some microbial communities. Furthermore, N addition caused a shift in microbial community composition at BDC ($p=0.048$), but not Oasis. This could be because the

microbial community composition had already been shifted by N deposition. As pulses can represent a significant portion of annual budgets from dryland systems, the potential for interactions between N deposition and wetting-induced pulses requires further study.

Results from this study present N fluxes from the desert that are orders of magnitude higher than expected (Figure 3.9 and 3.10). While short term effects of experimental N addition resulted in inconsistent responses in the microbial community composition, long-term N deposition resulted in a distinct differentiation of the microbial community, with possible implications for N cycling. Such high N fluxes need to be accounted for in global models for accurate predictions of future climate scenarios and for a complete understanding of N biogeochemistry.

## Synthesis and Future Directions

This dissertation research focused on investigating soil feedbacks to multiple anthropogenic global change drivers across a diverse array of environments from the arid and semi-arid environments of southern California's Mediterranean climate to the subtropical monsoonal evergreen forests in southern China. I examined mechanisms in soil biogeochemistry and microbial physiology that were universal across these diverse systems in order to inform Earth System Models (ESM) with broad applicability for understanding global carbon (C) and nitrogen (N) cycling. Mechanistic understanding of soil feedbacks is necessary for predicting future changes as well as developing strategies for C sequestration and mitigation of anthropogenic global change drivers (Trivedi et al., 2013; Woodward et al., 2009). In Chapters 1 and 2, I examined soil respiration ($R_{soil}$) responses to substrates, temperature, and N availability, demonstrating strong support for use of Michaelis-Menten kinetics as a unifying mechanism for representation of global change drivers on $R_{soil}$ dynamics. Chapter 3 extended this research by exploring N trace gas emissions and evaluating the potential role of microbial community variation on soil trace gas emissions through molecular analysis, demonstrating a need for greater appreciation of arid systems in global C and N budgets.

Temperature sensitivity of $R_{soil}$ is a large source of uncertainty in global C models (Conant et al., 2011). Recently, improvements to modeling efforts have been accomplished through incorporation of substrate-based kinetics, notably through the incorporation of temperature sensitivity of the Michaelis-Menten parameters $V_{max}$ and

kM (Davidson et al., 2012). However, there are very few empirical estimates to support use of this analysis (Moorhead and Sinsabaugh, 2006), particularly in the highly heterogeneous environment of bulk soils. In this work, I found wide agreement across diverse systems for the justification of temperature sensitivity of the Michaelis-Menten parameters, $V_{max}$ and kM, for soil decomposition of a labile C source in bulk soil. Furthermore, the relationship between these parameters and temperature is not linear, but exponential similar to Arrhenius kinetics, which is useful information for modeling efforts. The incorporation of temperature sensitivity through the Michaelis-Menten parameters helps to explain the wide range of estimates of $R_{soil}$ temperature sensitivity in the literature by describing a canceling effect of apparent temperature sensitivity as a result of substrate availability (Davidson et al., 2006). I also show evidence of this canceling effect in Chapters 1 and 2 as temperature sensitivity increased with increasing substrate availability.

However, there are two highly compelling hypotheses regarding the influence of substrate on temperature sensitivity of $R_{soil}$. The other hypothesis describes the inverse relationship between carbon quality and temperature sensitivity, which predicts that recalcitrant substrates should require higher activation energy and therefore demonstrate greater temperature sensitivity than more labile substrates (Bosatta and Ågren, 1999; Fierer et al., 2005). My work also demonstrates evidence of this hypothesis. In Chapter 2, evidence of the inverse carbon quality hypothesis is seen as a general decrease in temperature sensitivity of $R_{soil}$ with the initial addition of a labile substrate compared to the ambient, more recalcitrant substrate pool. Alternatively, in Chapter 1, I found labile

carbon demonstrated greater temperature sensitivity than the more recalcitrant substrate, tannin. However, tannin has a high potential for physical stabilization in the soil (Benoit and Starkey, 1968; Fierer et al., 2001; Kraus et al., 2004). These results highlight the need for further study on a mechanism that can incorporate both C quality and availability with physico-chemical stabilization parameters into a signal unifying framework. Globally, soils store approximately 1500 Pg C, with estimates range from 504-3000, which is more than plants and the atmosphere combined (Scharlemann et al., 2014). The only way to predict if soil C will become a sink or a source for C in a future warming climate, is to have a reliable means of estimating temperature sensitivity of losses from the soil C pool under a future warmer climate. While the Michaelis-Menten framework is not the final solution, it demonstrates a great improvement and a means for consolidating many varied observations. My results further justify its usefulness in biogeochemical modeling efforts and ESMs, with the first attempt in the literature at empirical estimation of the Michaelis-Menten parameters and their temperature sensitivity in bulk soils.

I also found strong support for use of substrate-based kinetics in understanding potential soil feedbacks to the influences of anthropogenic nitrogen deposition. Nitrogen deposition has been shown to have a suite of detrimental effects on ecological systems (Bobbink et al., 2010; Fenn et al., 2003a; Vitousek et al., 1997), but the influence on biogeochemical cycling is less well characterized (Suddick and Davidson, 2012). Even less is known about how multiple global change drivers can interact to influence soil feedbacks (Bardgett et al., 2008; Gärdenäs et al., 2011). Increased $CO_2$ from

anthropogenic sources can increase plant productivity, but productivity is eventually limited by N availability (Luo et al., 2004). This progressive nitrogen limitation can be alleviated by anthropogenic nitrogen deposition. However, the influence of N deposition on plant productivity has to be balanced by respiratory and decomposition losses (Lal, 2013). N has also been shown to limit plant litter decomposition through inhibition of ligninolytic enzymes (Carreiro et al., 2000; Knorr et al., 2005), further contributing to C sequestration. This indicates that there are important interactions between these anthropogenic global change drivers, which is not surprising given the tight coupling of C and N in biogeochemical cycles and within living organisms. However, the responses of soil respiration to N deposition are less consistent (Craine et al., 2007; Fog, 1988; Liu and Greaver, 2010; Ramirez et al., 2010; Zhou et al., 2013), and a unifying mechanism for these varying responses is necessary for accurate representation in ESMs.

In general, my results support the research suggesting that N deposition can increase C sequestration. I saw a decrease in soil respiration under many conditions. In the subtropical moist forests of southern China, increasing soil N resulted in a general decrease in soil respiration in both field and laboratory measurements. However, in southern California, the response of $R_{soil}$ to N enrichment was dependent on C availability, causing no change or a decrease compared to the control when C was limited, but an increase in $R_{soil}$ when N was added with a labile C source, compared to C alone. These differing responses can be explained in the context of dynamic microbial carbon-use efficiency (Manzoni and Porporato, 2009). This hypothesis predicts that the influence of N addition on $R_{soil}$ is dependent on the stoichiometry between C and N.

When C is limiting compared to N, microbes will conserve C through an increase in CUE, thereby decreasing $R_{soil}$. Alternatively, when carbon is abundant compared to nitrogen, decoupling of catabolism and anabolism results in decreased CUE and therefore increased $R_{soil}$. When both resources are abundant, catabolism is again coupled to anabolism and metabolic activity and growth are at a maximum. In Chapter 1, I saw this divergent response of $R_{soil}$ to N dependent on C availability, indicating the coupling-decoupling of catabolism and anabolism. In the moist subtropical forests of China, there was a consistent decrease in $R_{soil}$ with increasing soil N across the urban to rural gradient, consistent with an increase in CUE, but the recoupling of catabolism and anabolism when both resources are abundant did not occur for the urban to rural transect. China is experiencing much higher rates of N deposition than southern California, which may result in a permanent decoupling of catabolism and anabolism, possibly due to toxicity from high N or inhibition of certain microbial groups (Koch, 2001; Ramirez et al., 2012), as was seen in Chapter 3. However, there was some evidence of the divergent response in N addition plots in Chapter 2, demonstrating a similar divergent response to soil N availability as was seen in the laboratory incubation of Chapter 1. Overall, these results indicate that the dynamic CUE hypothesis has great potential for describing the influence of C-N interactions on $R_{soil}$ in a diverse set of environments.

Furthermore, I present a potential relationship between Michaelis-Menten kinetics and CUE as a potential means to bridge the methodology gaps in CUE measurements. Current global models employ a fixed CUE (Manzoni et al., 2012), but using a dynamic CUE can greatly improve modeling of global C (Allison, 2014; Wieder et al., 2013).

However, there is a lack of empirical evidence to support this hypothesis (Schimel, 2013), likely because CUE is difficult to estimate empirically (Manzoni et al., 2012). The use of substrate-based kinetics could bridge that gap, providing a simpler method for estimation of CUE in both lab and field settings. This highlights the need for research directed at explicit comparison of the relationships between Michaelis-Menten kinetics and CUE relationships.

Furthermore, understanding of whether results from laboratory incubation can be applied to field settings is necessary to prove their applicability to global modelling efforts. In China I saw the same general decrease in $R_{soil}$ with increasing soil N in both laboratory incubations and field measurements. In Chapter 3, I extended the laboratory incubation for the desert site in Chapter 1 to field measurements in comparison with a high deposition site and under experimental N additions. In the laboratory incubation of the desert soil, I show that N addition influences $R_{soil}$ based on C availability. In the field experiment, I predicted that the pulse from a simulated precipitation event would result in a flush of C and N from accumulation during the dry season, resulting in a similarly synergistic response as was observed in the incubation. However, there was no significant difference in the field $R_{soil}$ measurements between the high and low deposition sites, nor was there an effect from experimental N additions. In the laboratory incubation in Chapter 1, the complex C, tannin, also didn't elicit the same response as the labile C source, dextrose. Therefore, the complexity of the C source seems to be another important parameter that can influence the response of $R_{soil}$ to N deposition in natural field settings. However, the rapid pulse in $CO_2$ emissions from the desert sites were

similar in magnitude to the highest fluxes observed in the subtropical forests in Chapter 2. Therefore, while N deposition may not be a major driving factor influencing $R_{soil}$ dynamics in desert systems, the pulsed emissions from desert systems may have an important role in accounting of global C budgets.

Interactions between the effects of temperature and N on $R_{soil}$ suggest more complex responses to combined global changes of increasing temperatures and nitrogen deposition than would be expected from either alone. In Chapter 1, I show that N addition resulted in a general increase in the temperature sensitivity of $V_{max}$ and $K_m$ and a divergent response in $R_{soil}$, with both increases and decreases seen, indicating that the temperature sensitivity of $R_{soil}$ may also be influenced by C:N stoichiometry and microbial CUE. In Chapter 2, N addition caused a significant increase in the temperature sensitivity of $R_{soil}$ in the urban to rural gradient, but the N influence on temperature sensitivity of the Michaelis-Menten parameters was limited to $V_{max}$ and not kM. This could result in a dampening of the canceling effect and therefore greater temperature sensitivity at lower substrate concentrations under N deposition, which is supported by results from the urban to rural gradient demonstrating greater $Q_{10}$ at the high N site. Furthermore, the influence of N on kM in the N addition plots was magnified at higher temperatures. Therefore, emergent properties are likely to occur due to the combined influences of climate change and nitrogen deposition, and more research is needed to better quantify these interactions and identify the regulating mechanisms. Furthermore, potential influences of N deposition on nitrogenous emissions can have strong feedbacks to climate change (Liu and Greaver, 2009).

In Chapter 3, I show large pulses of $N_2O$ and $NO_x$ soil emissions that were substantially higher than expected from desert soils at both the low and high deposition sites, but these fluxes exhibited complex responses to N additions. Long-term effects from N addition, which were assessed as between sites differing in N deposition, were associated with reduced soil N emissions while short term effects from experimental N additions were associated with an increase in soil $NO_x$ emissions. Furthermore, the very rapid nature of the observed trace gas fluxes, especially $N_2O$, present the possibility for a mechanism that is separated from active microbial metabolism as a source for soil N trace gas fluxes in arid environments or the remarkable ability of soil microorganisms to rapidly recover from prolonged drought conditions. Therefore, it is not clear how N deposition will influence soil nitrogenous fluxes, but it is obvious from these results that desert soils present a greater potential source for N emissions than is currently appreciated in ESMs and global N budgets. These results highlight the need for greater study of soil trace gas fluxes in desert ecosystems. It is not currently clear if this is a widespread phenomenon seen across many deserts. Nor is it clear if the cumulative annual emissions compare to other systems. Low vegetative productivity and biomass make the deserts often overlooked, in combination with extreme conditions that can be problematic for scientific equipment but the very high fluxes seen at both desert sites indicate that it is likely that desert N fluxes are important contributors to global N budgets.

Furthermore, the results from Chapter 3 demonstrates the potential for N deposition to influence the microbial community composition and the microbial

community's response to wetting. Large differences in community composition were observed between sites, but the influences of short term N addition on community composition shifts were variable. This shift in microbial community, particularly the decreases in microbial diversity, associated with N addition, can help to explain the decreases in $R_{soil}$ observed in Chapter 1 and 2, indicating an adverse effect of N addition on particular microbial communities, which has also been seen in other studies (Eisenlord et al., 2013; Leff et al., 2015; Ramirez et al., 2012). Furthermore, integration of the changes in microbial community composition into the dynamic CUE framework is a potential topic for future research, which could further improve on the mechanistic understanding of N deposition effects on $R_{soil}$.

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