A Thesis

entitled

What are the Impacts of Anthropogenic Nitrogen Deposition on Biological Soil Crust Communities of the Colorado Plateau and the Oak Openings Regions?

by

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Biological Soil Crusts (BSCs) provide a number of vital ecosystem services including increased soil stability, nitrogen (N) fixation, and increased water retention. Anthropogenic N deposition is predicted to alleviate N limitation in ecosystems that support BSCs, and some researchers have predicted that N deposition will lead to BSCs being out-competed for space, while others have found that water availability can control the effects of N deposition. The objective of my study was to determine the effects of added N in BSC soils from two endpoints of a precipitation and N deposition gradient, and then determine the fate of added N in arid BSC soils. BSC soils were sampled from Arches National Park in Moab, UT, an arid ecosystem with low N deposition, and from Kitty Todd Nature Preserve in NW Ohio, a temperate ecosystem with high N deposition. I used an existing N deposition field experiment in Arches National Park to test the effects of N deposition on BSC function in arid land soils, by measuring nutrient pool sizes (N), BSC respiration, soil stability, carbohydrate concentrations (a proxy for BSC exopolysaccharides), extracellular enzyme activities, microbial biomass C and N,
potential leaching losses, and potential denitrifying enzyme activity. I hypothesized
added N would not be used by microbes due to water limitation in the soil, and that added
N would leach from these soils following the infrequent and intense rainfall events in the
region. I then used an incubation experiment to test the effects of increasing soil moisture
on potential denitrifying enzyme activity, N mineralization rates, nitrification rates,
microbial biomass C and N, carbohydrate concentrations, and respiration rates in BSC
soils of the Oak Openings and Colorado Plateau regions. For the Arches soils I predicted
that increasing soil moisture to a moderate level would increase microbial activity and
that increasing soils moisture to a high level would depress microbial activity in both soil
textures, due to anaerobiosis. For the Kitty Todd soils I hypothesized that increasing soil
moisture in BSCs would not result in changes to nutrient cycling, except for increases in
N immobilization, as a previous study showed that the BSCs in these soils are able to
immobilize deposited N. I determined that added N had no effect on BSCs or microbial N
cycling, and that soil texture was the main driver of crust development and N cycling in
the Arches BSC soils. For the Kitty Todd crust soils I determined that BSCs in these soils
were able to prevent NO$_3^-$ (nitrate) from leaching but excess N was likely lost from these
soils as NO (nitric oxide), N$_2$O (nitrous oxide), and N$_2$ (dinitrogen) produced through
denitrification. The results of my study suggest that BSCs in Arches National Park are
not damaged by N deposition and that crusts have the potential to limit N leaching losses
in all three soils.
For my dad and mom, Don and Kathy Collier.

Thank you for all of your support and guidance as I continue to pursue my passions!
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List of Abbreviations

BSCs .......................... Biological soil crusts
ca. .......................... Circa
DEA ............................ Denitrification enzyme activity
DIN ............................ Dissolved inorganic nitrogen
EPS ............................. Exopolysaccharides
IRGA .......................... Infrared gas analyzer
LAP ............................. Leucine amino peptidase
MANOVA ....................... Multivariate analysis of variance
MBC ............................ Microbial biomass carbon
MBN ............................ Microbial biomass nitrogen
MC ............................. Methylcoumarin
MUB ............................ Modified universal buffer
MUF ............................. 4-methylumbelliferone
NAG ............................ β-1,4-N acetyl-glucosaminidase
PAHBAH ....................... Para-hydroxybenzoic acid hydrazide
PCR ............................ Polymerase chain reaction
PEROX .......................... Peroxidase
PHENOX ........................ Phenol oxidase
TDN ............................ Total Dissolved Nitrogen
TOC-V_{cpn}  .................... Shimadzu total organic carbon analyzer
TRS ............................ Total reducing sugar
USA ............................. United States of America
USGS .......................... United States Geological Survey
UT ............................... Utah
WHC ............................ Water holding capacity
List of Symbols

C.................................Carbon
CaCl ................................Calcium chloride

HCl...............................Hydrochloric acid

K₂SO₄...............................Potassium sulfate
KNO₃..............................Potassium nitrate

N........................................Nitrogen
N₂............................Dinitrogen
NO............................Nitric Oxide
N₂O............................Nitrous oxide
Na₃C₆H₅O₇..............Trisodium citrate
NaOH..............................Sodium Hydroxide
NH₄⁺..........................Ammonium
NO₃............................Nitrate

VCl₃..............................Vanadium chloride
Chapter 1

Introduction

Biological Soil Crusts (BSCs) are an assemblage of specialized organisms that stabilize sparsely vegetated soils (Belnap et al., 1999; Neher et al., 2003; Bowker et al., 2005; Veluci et al., 2006). Most BSCs form on or near the surface of soils and are found on every continent in all climate regions (Belnap et al., 1999; Belnap and Lange, 2001; Neher et al., 2003; Veluci et al., 2006). BSCs are dominant in environments that are nitrogen (N) limited and do not support many higher plants (Belnap et al., 1999; Belnap and Lange, 2001; Neher et al., 2003; Veluci et al., 2006). Arid and semi-arid climates commonly have N deficits due to water limitations, low plant growth and low organic matter accumulation (Belnap et al., 1999; Katlenecker et al., 1999; Neher et al., 2002; Bowker et al., 2005; Schwinning et al., 2005). This allows crusts to colonize areas between plants (Belnap et al., 1999; Katlenecker et al., 1999; Neher et al., 2002; Bowker et al., 2005). Temperate regions are also home to BSCs even though conditions are more favorable for vascular plants, by colonizing xeric patches with low organic matter accumulation, and limited water and nutrient availability (Neher et al., 2002; Veluci et al., 2006).
BSC’s provide a number of ecosystem services, with soil stabilization being one of the most important to land managers (Belnap et al., 1999; Bowker et al., 2005). Crust microbes, such as cyanobacteria, promote soil stability by exuding exopolysaccharides (EPS) that bind soil particles together (Reynolds et al., 2001), reducing wind erosion responsible for soil and nutrient losses (Reynolds et al, 2001; Bowker et al., 2005). Besides stabilizing soils, ecosystem services provided by BSCs include N fixation and increased water retention (Belnap et al, 1999; Katlenecker et al, 1999; Veluci et al, 2006). N fixation occurs during BSC growing seasons, between autumn and spring, and is a key N supply in N limited environments (Belnap et al., 1999; Schwinning et al., 2006). Such ecosystems can support a large BSC community, and some native plants, like Indian ricegrass and galleta in the Colorado Plateau, have evolved to rely on these seasonal N inputs for most of their N requirement (Schwinning et al, 2005). BSCs increase water retention in soils by holding water in soil aggregates created from EPS (Belnap et al, 1999; Veluci et al, 2006) and decrease the rate that water flows through the soil (Belnap et al., 1999). BSCs also provide cover from sunlight that slows evaporation from soil surfaces (Belnap et al., 1999; Neher et al., 2003; Veluci et al., 2006). These services make crusts particularly important components of systems that are N and water limited (Belnap et al., 1999; Veluci et al, 2006).

BSCs have been lost or severely impacted in many parts of their historic range, due to anthropogenic activities like overgrazing and surface disturbances from recreation (Belnap et al, 1999; Katlenecker et al 1999; Bowker et al, 2005). When land managers first noted a decrease in productivity in lands historically dominated by BSCs, they determined that the loss in BSC cover was responsible (Bowker et al, 2005). This resulted
in efforts to re-establish crust cover and to find ways to enhance BSC recovery (Bowker et al, 2005). For example, soil texture is important to the rate of BSC recovery from disturbance. Bertrand et al. (2014) found that finer texture soils promoted faster recovery, as coarser soils experienced more erosion, and had lower crust cover, C storage, and EPS concentrations. Bowker and Belnap (2008) determined that fine textured soils favored a wider variety of BSCs while coarser soils favored fewer types due to decreased water holding capacity and faster erosion. These findings indicated that, along with water and N deficiency, soil texture is important for crust establishment and persistence. Nonetheless, there is concern that other anthropogenic disturbances, including increased N deposition (Belnap et al, 1999; Evans & Belnap, 1999), will threaten ongoing BSC recovery.

N deposition is one of the greatest potential threats to biological soil crusts (BSCs) because even a small increase in N is predicted to alter nutrient cycling in N limited environments dominated by BSCs (Belnap et al., 1999; Fenn et al., 2003; Galloway et al, 2003). Anthropogenic N sources, which include fertilizer consumption and production, fossil-fuel emissions, and biomass burning, have more than doubled the amount of N entering terrestrial systems (Smil, 1990; Matson et al., 2002; Galloway et al, 2003; Fenn et al, 2003). Increasing N is a major threat to biodiversity and historic patterns of nutrient cycling in areas where plant and soil communities are adapted to low N conditions (Belnap et al, 1999; Fenn et al, 2003). N deposition is occurring around the globe but rates vary depending on the number of N producing industries upwind of a location (Smil, 1990; Matson et al, 2002; Galloway et al, 2003; Fenn et al, 2005). BSCs in the western United States are subject to low but increasing levels of N deposition, while BSCs in much of the eastern USA experience N deposition levels at least an order
of magnitude larger (Fenn et al., 2003; Veluci et al., 2006; Zhang et al., 2012). This increase across the continental USA creates a gradient of N deposition over BSC covered lands.

It is important to determine how BSCs will respond to increasing N deposition levels because BSCs regulate nutrient cycling important to establishing native plant communities in N limited ecosystems (Belnap et al., 1999; Katlenecker et al., 1999; Fenn et al., 2003; Neher et al., 2003; Hall et al., 2011). Previous research suggests that N deposition has the potential to damage recovering crust communities in the western United States. Belnap et al (1999) identified N deposition as a major regional threat to recovering BSC communities of the Colorado Plateau. Fenn et al. (2003) found that lichens, a component of mature crusts, are vulnerable to even low levels of deposition (Belnap et al., 1999; Katlenecker et al, 1999). However, most N deposition studies have focused on forested areas, where common effects of increased N deposition (Galloway et al, 2003), include increased N losses due to oversaturation, soil acidification, and loss of biodiversity (Magill et al., 1997; Uehara and Fillman, 1981; Huenneke et al., 1990; Tilman, 1997; Matson et al, 2002). N deposition may eliminate the competitive advantage of plant and soil communities thriving in N limited systems (Belnap et al, 1999; Fenn et al, 2003), permitting fast growing, often invasive, species (Belnap et al., 1999; Schwinning et al., 2005), to out-compete BSCs and native plants for space and light, decreasing BSC productivity and both BSC and plant diversity (Belnap et al, 1999; Bowker et al., 2005; Fenn et al, 2003).

The prediction that crusts will lose the competitive advantage they have in low N conditions with N deposition is still up for debate, and the outcome may be influenced by
moisture availability. The global distribution of BSCs makes it possible to study how crusts respond to increasing N deposition across both soil moisture and N deposition gradients. Schwinning et al (2005) found that water limitation eliminated the effects of added N on the bunchgrass community of the Colorado Plateau, where both water and N limitations prevented invasive species growth (Schwinning et al., 2005). Baez et al. (2007) and Whitford and Gutierez (1989) found that effects of N deposition on species dominance and plant biomass were stronger when accompanied by precipitation in a Chihuahuan desert mixed grassland that is limited by both N and water. N that was deposited in periods of rainfall was immobilized by plant and microbial communities at greater rates than N deposited during droughts (Baez et al., 2007). N deposition is an order of magnitude greater and water availability also is much higher in the sand barrens ecosystem in Northwest Ohio than in the arid regions of the SW USA. In Northwest Ohio, Veluci et al. (2006) concluded that the BSCs are able to tolerate high levels of N deposition by immobilizing and storing excess N in biomass, thus preventing N accumulation in the N deficient soils (Veluci et al, 2006).

Some studies have shown that immobilization is not the only fate for deposited N, even in N limited ecosystems (Hall et al., 2008; Turnbull et al., 2010; Hall et al., 2011). Nitrification, denitrification, and leaching are three other fates for added N (Hall et al., 2008; Turnbull et al., 2010; Hall et al., 2011). Relatively high precipitation can create an anaerobic environment favorable for denitrification inside soil aggregates, but could ultimately inhibit nitrification, and subsequent denitrification. In contrast, more moderate conditions promote denitrification by allowing nitrification to continue because oxygen is more available (Duncan et al., 2013; Morse et al., 2015 a,b). However, relatively low
precipitation can inhibit both nitrification and denitrification. If denitrification does occur in N limited ecosystems, then greenhouse gas emissions from the soil could increase, with some of the N leaving as nitrous oxide (N₂O). Another pathway for N to leave the soil is through leaching losses, which can occur with infrequent and intense rainfall events that can leach NO₃⁻ and NH₄⁺ (Hall et al., 2011). Thus, the effects of precipitation on losses of added N vary according to many factors.

To learn how crusts respond to added N and how much of the N is retained it is necessary to determine the fates of N in BSCs at different precipitation levels. Studying crusts that are limited by both N and water availability in the arid SW USA versus those that have much higher N and water availability in temperate NW Ohio will allow me to compare the fates of deposited N in contrasting environments.

1.1 Objectives and Hypotheses:

The main objectives of my research were to determine the effects of added N in crust soils from two endpoints of a precipitation and N deposition gradient, and to determine the fate of added N in arid BSC soils. To achieve these objectives I took advantage of an existing field N addition experiment on crust soils in Arches National Park (Moab, UT), and conducted a lab N and moisture addition experiment on these, and BSC soils from Kitty Todd Nature Preserve in the Oak Openings region of northwest Ohio. The field experiment was used to determine the effects of added N on Arches National Park crust soils, and to determine the fate of added N in these arid crust soils. The lab incubation experiment was used to determine how different soil moisture levels change the effects of added N in crust soils from Arches National Park and Kitty Todd Nature Preserve. By using the field experiment to determine the fate of added N in the
Arches crust soils I will address a knowledge gap that has already been addressed in temperate, Northwest Ohio crust soils (Veluci et al. 2006) but not in the arid crust soils of Arches National Park.

My first objective for the field experiment in Arches National Park was to determine the effects of added N in an arid BSC containing ecosystem with low N deposition levels. To achieve this objective, soil aggregate stability, soil respiration, microbial biomass, enzyme activities, and carbohydrate concentrations (a proxy for EPS) were measured on crust soils sampled from plots with and without added N in two different textured soils in Arches National Park. I hypothesized that N deposition would have no effect on BSC respiration, soil stability, enzyme activities, which can be used to assess microbial nutrient acquisition, microbial biomass, and carbohydrate concentrations in the Arches soils because crust and microbes are limited by both water and N in this ecosystem and would not be able to use added N without additional water.

My second objective for the field experiment was to determine the fate of the added N. To achieve this objective I measured N pool sizes, dissolved N concentrations, microbial biomass, and potential denitrifying enzyme activities (DEA). Potential nitrification rates in these soils were also measured in my incubation experiment. I predicted that added N would build up in the soil until precipitation leached it from the system. I predicted this would occur because BSCs and microbes are limited by both water and N, and because rainfall in Arches is infrequent and intense, causing accumulated N to leach from the system faster than crusts and microbes can use it for growth or for nitrification and denitrification (Hall et al., 2011).
My third objective was to determine how changes in soil moisture levels at different ends of the east-west N deposition and precipitation gradients in the continental USA alter the effects of added N in crust soils. In order to achieve this objective I used a lab incubation experiment to determine the effects of added N at different soil moisture levels on nutrient cycling in crust containing soils of Arches National Park and Kitty Todd Nature Preserve in the Oak Openings region of NW Ohio. This allowed me to mechanistically determine how crusts found at two ends of the N deposition and precipitation gradient respond to added N at different soil moisture levels. For the Arches soils I hypothesized that as soil moisture was increased to a moderate level N deposition would result in increased N mineralization, increased carbon (C) immobilization, and increased rates of nitrification and denitrification, because of the alleviation of water limitation allowing microbes to use the added N, increasing their demand for C, and resulting in the mineralization, nitrification, and denitrification of excess N. At the highest soil moisture level I predicted that N mineralization and C immobilization rates would decrease, along with decreases in nitrification and denitrification rates because the soil would be too wet for aerobic activity, limiting N immobilization, mineralization, and potential nitrification and denitrification rates (due to NO$_3^-$ limitation). I hypothesized added N would have no impact on the Oak Openings BSCs and microbial activity, except for increased N immobilization rates, because even though these soils are in xeric microsites, they are not limited by water availability as strongly as the Arches BSCs, and because Veluci et al. (2006) found that these crusts are able to immobilize added N.
Chapter 2

Methods

2.1 Sites

Crust soils were sampled from two locations. The first was Arches National Park in Moab, UT where crusts are widespread in the high desert ecosystem. The second location was Kitty Todd Nature Preserve in the Oak Openings region of NW Ohio where crusts can be found in sand barren ecosystems. These two locations were at different ends of the North American continental precipitation gradient: the Arches crusts in an arid environment that received an annual average of 21.5 cm of rain, while the NW Ohio crusts received 85 cm of rain annually (Neher et al., 2002; Veluci et al., 2006; USDA, 2011). The Arches BSCs experienced relatively low but increasing deposition levels, approximately 1-4 kg-N ha\(^{-1}\) yr\(^{-1}\) (Fenn et al, 2003; Driscoll et al, 2001; Dentener et al. 2006; Zhang et al., 2012). N deposition in the Oak Openings region was an order of magnitude greater, approximately 13 kg-N ha\(^{-1}\) yr\(^{-1}\), due to the number of upwind, industrial N producing sites (Veluci et al., 2006; Dentener et al. 2006, Zhang et al., 2012). The presence of crusts in these two ecosystems with different levels of precipitation and N deposition was a good contrast to address the question of how N deposition will affect
BSCs and their associated microbial communities in BSC soils experiencing different soil moisture levels.

Soils in both Arches sites were well-drained sandy soils classified as coarse-loamy, mixed, superactive, mesic Ustic Haplocalcids (Web Soil Survey, 2013; USDA, 2011). These soils were alkaline with a pH ranging from 7.5-8.2, with soil C ranging from 0.3-1.6% and N from 0.02-0.04% (USDA, 2011). The plant community of these sites consisted of grasses, shrubs, and cacti (Belnap et al., 1999; Schwinning et al., 2005; USDA, 2011). The most common plant species found in the USGS experimental plots was a grass, *Achnatherum hymenoides*, and other recorded species included *Ambrosia sp.*, *Aristida sp.*, *Bromus tectorum*, *Chenopodia sp.*, *Cryptantha sp.*, *Ephedra sp.*, *Eriogonum sp.*, *Festuca ovina*, *Pleuraphis jamesii*, *Krascheninnikovia lanata*, *Machaeranthera canescens*, *Mentzelia sp.*, *Opuntia sp.*, *Phacelia sp.*, *Descurainia pinnata*, *Plantago ovata*, *Salsola tragus*, and *Spiranthes romanzoffiana*.

The Oak Openings region crusts were found in a midwest sand barrens ecosystem, with well-drained sandy soils classified as mixed, mesic Aquic Udipsamments (Brewer and Vankat, 2004; Web Soil Survey, 2013). The Kitty Todd sandy soils were similar in texture to the fine textured soils sampled in Arches National Park (Brewer and Vankat, 2004; USDA, 2011; Web Soil Survey, 2013). These soils were slightly acidic with a pH ranging from 5.9-6.7 and have an organic matter content that ranged from 2.5-4.5% (Marinis, unpublished data). The plant community of the barrens consisted of grasses including *Carex rugosperma* and *Aristida purpureascens*, cacti including *Opuntia humifusa*, forbs including *Krigia virginica*, and *Solidago nemoralis*,
and shrubs, the most common being *Prunus pumila var. cuneata* (Gardner and Haase, 2009).

Soils for the incubation experiment were collected from 15 sites with biological soil crusts from three soil types; 5 coarse and 5 fine textured Arches National Park sites and 5 Kitty Todd Preserve sites. For the field experiment at Arches National Park I sampled crust soils from 20 experimental plots at 2 sites, approximately 2 miles apart, at 615195E 4295951N and 618158E 4292856N, that were maintained by the United States Geological Survey (USGS). 10 of the sampled plots (5 coarse and 5 fine textured soils) did not receive added N and 10 (5 course and 5 fine textured soils) received 8 kg-N ha\(^{-1}\) yr\(^{-1}\) in two 4 kg-N ha\(^{-1}\) doses in late winter/early spring and late summer/early fall. Added N was applied to the plots as a solution of ammonium-nitrate in DI water sprayed over a 2.25 m\(^2\) area to minimize edge effects that could occur by fertilizing only an area the exact size of the 1 m\(^2\) plots. The plots were spaced approximately 3 m from outer boundary to outer boundary. The Oak Openings soils were sampled from 5 dunes in Kitty Todd Nature Preserve at 267182E 4611111N, 267366E 4611266N, 267755E 4611394N, 267883E 4611153N, and 267774E 4611235N. These sites did not receive experimentally added N, but experienced N deposition levels of ca. 13 kg-N ha\(^{-1}\) yr\(^{-1}\).

2.2 Sample Collection

For the field experiment in Arches National Park, approximately 35 g of soil was collected to a depth of 5 cm using 2.5 cm soil corers from each of the 20 experimental plots. For the incubation experiment, 180 g of soil, total, was collected to a depth of 5 cm using 2.5 cm soil corers from an area outside of the USGS experimental plots, so that soils were exposed only to natural N deposition levels. 180 g of Oak Openings crust soils
were collected from each dune to a depth of 5 cm using a 2.5 cm soil corer. Crusts were
only exposed to natural deposition levels and all cores were collected within 30 cm at
each site. All soils were sieved using a 2 mm mesh before incubation to remove rocks
and large organic matter particles and then stored at 4 °C to delay microbial activity for
up to three days before being weighed into incubation jars and adjusted to the desired soil
WHC for the incubation experiment

2.3 Field Sampling at Arches National Park

The objectives of my work with the field experiment at Arches National Park
were to determine if added N has an effect on BSC activity and the fate of added N in
arid crust soils. In order to accomplish these objectives, measurements of soil stability,
crust respiration, carbohydrate concentrations, enzyme activities, microbial biomass,
potential denitrification, N pool sizes, and dissolved N were taken on BSC soils collected
from Arches National Park that had no added N or 8 kg-N ha\(^{-1}\) yr\(^{-1}\). Samples were
collected for laboratory measurements of extracellular enzyme activities, microbial
biomass C and N, and carbohydrate concentrations on 28 September 2013, 6 March 2014,
8 September 2014, and 11 September 2014. The first three sampling dates occurred
approximately six months after N was added to the plots and the fourth sampling date
occurred two days after N was added to the plots to determine if there were any pulse
effects immediately following N addition.

2.3.1 Soil Aggregate Stability

To determine if added N affects BSC structure, soil aggregate stability was
measured as an in situ measure of crust development. Stability was used as a measure of
crust development because more active crusts exude more EPS which results in increased
soil stability (Belnap et al., 1999). Soil stability was measured on 15 November 2013 using a soil stability test developed by Herrick et al. (2009). Soil aggregates 2-3 mm thick and 6-8 mm in diameter were collected from the surface using a small spatula provided in the Jornada Experimental Range soil stability test kit (Synergy Resource Solutions, Inc., Belgrade, MT). Once the samples were allowed to air dry, they were lowered into compartments filled with deionized water and were visually checked after 5 seconds, 30 seconds, and 5 minutes. After five minutes, the samples were dipped in the water, without touching the bottom, five times and a stability class value of 1-6, with 6 being the most stable, was assigned based on how quickly and how much the sample dissolved.

### 2.3.2 Biological Soil Crust Respiration

Another parameter I used to test the hypothesis that added N will not affect crust activity due to crusts being limited by both N and water availability was *in situ* crust respiration, an integrative measure of biological activity. Crust respiration was measured in the field at Arches National Park using a Li-8100A Automated Soil CO\textsubscript{2} Soil Flux System (LI-COR Biosciences, Lincoln, Nebraska, USA) on 28 September 2013 and 11 March 2014. One PVC soil collar, 20 cm in diameter and 15 cm tall, was placed 3 cm deep in each plot in spring 2013. If any plants were found growing in the collars, they were removed to avoid measuring plant respiration (plant removal was noted). Once plants were removed, and soils were allowed at least two days to recover, the Li-8100A was placed on the collar and CO\textsubscript{2} flux was measured over two minutes. Soil moisture and soil temperature were also measured over two minutes at each site using a soil temperature thermistor probe and an ECH\textsubscript{2}O volumetric water content dielectric
permittivity sensor (LI-COR Biosciences, Lincoln, Nebraska, USA) attached to the Li-8100A. Crust respiration rates were reported as μmol CO₂ m⁻² s⁻¹.

2.3.3 Extracellular Enzyme Assays

Extracellular enzyme assays were conducted to determine the effects of N deposition on microbial nutrient cycling in the Arches field experiment. High throughput fluorometric and colorimetric enzyme assays were conducted based on methods from Saiya-Cork et al (2002) and German et al (2011). These assays were used to measure the activities of β-1,4-N acetyl-glucosaminidase (NAG) leucine amino peptidase (LAP), phenol oxidase (PHENOX), and peroxidase (PEROX). NAG, which hydrolyzes N-acetyl glucosamine from chitin-derived oligomers, and LAP, which hydrolyzes leucine and other amino acid residues from peptides, are N acquiring enzymes. PHENOX and PEROX catalyze oxidative reactions that degrade lignin, and excess N has been found to suppress lignin degrading enzyme production (Waldrop et al., 2004; Sinsabaugh et al., 2008; Sinsabaugh, 2010; Rinkes et al., 2011, 2013; Bach et al., 2013).

Soil slurries were prepared using soil from the USGS Arches sites and a modified universal buffer (MUB) adjusted to a pH of 7.5. Slurries were homogenized over two 30-second intervals using a BioSpec Tissue Tearer (BioSpec Products, Bartlesville, OK).

The hydrolytic enzyme assays (NAG and LAP) are fluorometric and the oxidative enzyme assays (PHENOX and PEROX) are colorimetric. Hydrolytic enzyme assays were conducted with black, 96-well microplates while oxidative enzyme assays were conducted with clear, 96-well microplates. Assay wells contained slurry and substrate (4-MUF-N-acetyl-β-D-glucosaminide for NAG, 7-amido-4-methylcoumarin (hydrochloride) for LAP, and L-3,4-dihydroxyphenylalanine for PHENOX and PEROX). Blank wells
(slurry and buffer), quench standard wells (slurry and standard, 4-methylumbelliferone (MUF) for NAG or 7-amino-4-methylcoumarin (MC) for LAP), negative control wells (buffer and substrate) and reference standard wells (buffer and standard) were included with all assays. PEROX plates also receive hydrogen peroxide in each well. Fluorometric and colorimetric microplates were incubated at 20 °C in the dark for 2-4 and 2-3 hours respectively and then read on a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA) at 360 nm excitation and 460 nm emission (hydrolytic) and 460 mm (oxidative) wavelengths. Hydrolytic enzyme activities were expressed as nmol hr⁻¹ g dry soil⁻¹. Oxidative enzyme activities were expressed as μmol hr⁻¹ g dry soil⁻¹, and net PEROX activities were calculated as the difference between PHENOX and PEROX activities.

2.3.4 Microbial Biomass

The effects of added N on microbes associated with BSCs was determined in part by measuring microbial biomass. Microbial biomass was measured using methods described by Scott-Denton et al (2006) modified from Brookes et al (1985). Freshly collected soils were extracted by mixing 5 g of soil with 15 mL of 0.5 M potassium sulfate (K₂SO₄) and shaking on an orbital shaker table for 1 hour, then vacuum filtering through 2 μm glass fiber filters. Extracts were then frozen until analysis. A second set of samples was fumigated by adding 2 mL of ethanol-free chloroform to 5 g of soil in 125 mL Erlenmeyer flasks. The flasks were sealed for 24 hours and then vented for 30 minutes in a fume hood at which time they were extracted in the same way as the non-fumigated K₂SO₄ extracts and then frozen until analysis.
Both sets of extracts were analyzed for total dissolved N (TDN) and dissolved organic C (DOC) using a Shimadzu total organic carbon (TOC-VCPN) analyzer equipped with a total nitrogen analyzer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). DOC and TDN concentrations in the non-fumigated K$_2$SO$_4$ extracts were subtracted from the fumigated values to determine microbial biomass C and N in $\mu$g C or N g dry soil$^{-1}$. Three blanks from each extraction were used to account for any C or N that may have been contaminating the K$_2$SO$_4$ or glassware.

2.3.5 Carbohydrate Analysis

Carbohydrate concentrations were measured to determine if added N was influencing BSC production of EPS. A total reducing sugar assay (TRS) based on Lever (1973), and modified for microplates by Fursova et al (2012), was used as a proxy measurement for EPS concentration. Pereira et al. (2009) reported that approximately 75% of cyanobacterial EPS are composed of monosaccharides with glucose being the most common. The non-fumigated K$_2$SO$_4$ extracts used for measuring microbial biomass were used in this assay. Para-hydroxybenzoic acid hydrazide (PAHBAH) and hydrochloric acid (HCl) (Reagent A) and sodium hydroxide (NaOH) plus calcium chloride (CaCl) plus trisodium citrate (Na$_3$C$_6$H$_5$O$_7$) (Reagent B) were combined with the K$_2$SO$_4$ extracts in quadruplicates into a 96-well polymerase chain reaction (PCR) microplate. The PCR plate was heated at 100 °C in a heating block for 6 minutes and then put in an ice water bath for 5 minutes. The solution was pipetted into a clear, 96-well microplate and read at 410 nm on a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA). TRS concentrations were determined relative to glucose standards and were reported in $\mu$g glucose equivalents g dry soil$^{-1}$. 
2.3.6 Ion Exchange Resin Membrane Strips

To test the hypothesis that added N was leaving the system through leaching due to infrequent and intense rainfall, potential leaching of dissolved inorganic N was measured using cation and anion resin strips. One cation and one anion ion exchange resin strip (6 cm x 1.5 cm) were buried vertically to a depth of 6 cm in each of the 20 experimental plots. After six months, the strips were removed on 8 September 2014 and extracted by adding 35 mL of KCl per strip in 50 mL centrifuge tubes containing strips that were washed with nanopure to remove soil particles. The cups were shaken on an orbital shaker table for one hour, and the extracts were decanted and frozen until analysis (Jasrotia and McSwiney, 2012).

Resin extracts were analyzed for available ammonium using a colorimetric ammonium assay developed by Rhine et al. (1998). Sample or standard was pipetted in triplicates into a clear 96-well microplate with citrate reagent, 2-phenylphenol-nitroprusside reagent, buffered hypochlorite reagent, and nanopure. The plates were then incubated at 20 °C for two hours before being read with a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA) at 660 nm. Three blanks were run to account for any ammonium in the KCl and for any contamination issues. Results were expressed as μg-NH$_4^+$-N cm$^{-2}$ resin strip.

Nitrate was measured using a colorimetric nitrate assay method developed by Doane and Horwath (2003). Sample or standard was pipetted in a clear, 96-well microplate in triplicates. Vanadium chloride (VCl$_3$) solution (sulfanilamide, N-(1-naphtyl)-ethylenediamine dihydrochloride, and vanadium (III) chloride) was added to each well. Microplates were incubated at 20°C for 3 to 5 hours before being read at 540
nm on a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA). Three blanks were run to account for any nitrate in the KCl and for any contamination issues. Results were expressed as μg-NO₃⁻-N cm⁻² resin strip.

2.3.7 Denitrifying Enzyme Activity

Potential denitrifying enzyme activities were measured to determine if added N was increasing the pool of microbial denitrifying enzymes, which could indicate if N was leaving the plots through denitrification. A denitrification enzyme assay (DEA) developed by Smith and Tiedje (1979) and modified by Wang and McGill (2012) was used. 5 g of soil was added to a Half Pint Wide Mouth Canning Jar (Jarden Co.) with a septa installed in the lid, plus 10 mL of DEA media consisting of 0.72 g L⁻¹ potassium nitrate (KNO₃), 0.5 g L⁻¹ glucose, and 0.125 g L⁻¹ chloramphenicol (Wang and McGill, 2012). Jars were then sealed and evacuated using a vacuum manifold and flushed with N₂ gas three times to create anaerobic conditions before being brought to atmospheric pressure again with N₂ gas. 10 mL of acetylene gas was then added to each jar. Jars were then placed on a shaker table and 7 mL of gas was sampled from jar headspaces and injected into evacuated vaccutainers, 90 minutes after the addition of acetylene. Samples were analyzed on a gas chromatograph to measure the concentration of nitrous oxide in ppm (N₂O) and converted to μg-N g dry soil⁻¹ hour⁻¹.

2.3.8 N Pool Sizes

Taking a one-time snapshot of soil N concentrations in plots with and without added N could indicate if added N was accumulating in the soil or if it was being lost through another pathway. Ammonium and nitrate pool sizes were measured using the 0.5
M K₂SO₄ extracts as described above. Extractable dissolved inorganic N concentrations (DIN; NH₄⁺ plus NO₃⁻) were reported as µg N g dry soil⁻¹.

2.4 Field Sampling in Northwest Ohio

Field sampling in Northwest Ohio was conducted to determine the potential leaching of N in the five Kitty Todd sites that were sampled for the incubation experiment. One cation and one anion ion exchange resin strip (6 cm x 1.5 cm) were buried vertically to a depth of 6 cm in each of the five Kitty Todd Nature Preserve sample sites for six months. The resin strips were extracted and measured as described above in the field experiment at Arches National Park.

2.5 Incubation Experiment

To achieve my third objective, determining how changes in soil moisture levels at different ends of the North American N deposition and precipitation gradient would alter the effects of added N in crust soils, I conducted a lab incubation experiment. 22 g of soil from the 5 sites in each of the 3 soil types (2 from Moab and 1 from northwest Ohio) described above were incubated in Half Pint Wide Mouth Canning Jars (Jarden Co.) with septa in the lids for one week in the dark at 20 °C. Soils were incubated at three soil moisture levels: 20%, 45%, and 70% water holding capacity (WHC), to simulate low, moderate, and high soil moisture, with either no added N or with added N. WHC, g H₂O per g dry soil, was determined by subtracting the mass of dry soil from the mass of saturated soil and dividing that result by the mass of dry soil. The resulting value represented the mass of water in the soil at 100% soil WHC, and was used to determine how many grams of DI water to add to the soils to reach the desired incubation WHC. 5 µg-N/g soil of ammonium-nitrate was added to half of the jars, in solution with DI water,
to simulate the conditions in the N treated plots at Arches National Park. This concentration was equivalent to what was applied when the Arches plots were fertilized. Before N addition, soils were pre-incubated for 8 days at 20 °C to allow them to equilibrate to their adjusted soil moisture levels. 10 g of crust soil were taken from each jar on incubation days 0 and 7 to measure potential DEA, potential N mineralization and nitrification rates, microbial biomass C and N, and carbohydrate concentrations.

2.5.1 Nutrient Mineralization and Nitrification

Nutrient mineralization rates and nitrification were measured to determine how increasing soil moisture changed N cycling in each soil type with and without added N. On days 0 and 7, 2.5 g of soil were destructively harvested from each of the incubation jars and extracted with 12.5 mL of K₂SO₄ before and after chloroform fumigation for measuring microbial biomass as described above.

Nitrogen mineralization rates were expressed as the change in extractable DIN between days 0 and 7 of the incubation. NH₄⁺ and NO₃⁻ concentrations were measured in 0.5 M K₂SO₄ extracts as described above. The initial DIN concentration (day 0) was subtracted from the final concentration (day 7) and then divided by 7 to determine the rate of mineralization, μg-N g dry soil⁻¹ day⁻¹. Potential nitrification rates were measured by determining nitrate accumulation over the incubation as μg NO₃⁻-N g dry soil⁻¹ day⁻¹.

2.5.2 Denitrifying Enzyme Activity

Potential DEA was measured as described above on days 0 and 7 of the incubation.

2.5.3 Respiration Rates
Respiration (C mineralization) rates were measured daily using a Li-820 Infrared Gas Analyzer (IRGA) (LI-COR Biosciences, Lincoln, Nebraska). Lids were left loosely covering the jars to allow gas exchange but limit evaporation. Lids were removed from the jars each day to vent at ambient conditions and then sealed and incubated for 3 hours in the dark at 20 °C. 2 mL of gas drawn from the jar headspace through a septa in the lid and was injected into the IRGA. Peak heights were converted to the concentration of CO₂ using known standards of 2500 and 5000 ppm. Each sample was run twice and the values for each sample were averaged and converted to μg-C g dry soil⁻¹ day⁻¹. Empty jars at ambient conditions were used as blanks.

2.5.4 Microbial Biomass

Microbial Biomass C and N were measured on days 0 and 7 as described above.

2.5.5 Carbohydrate Analysis

TRS concentrations were measured on days 0 and 7 as described above.

2.6 Statistical Analysis

Statistical analysis were conducted using R (version 3.1.1, www.r-project.org). Data from the field experiment were analyzed with a two-way multivariate analysis of variance (MANOVA), with texture and N addition as factors. DEA, MBN, TRS, and respiration data from the incubation experiment were analyzed with a three-way MANOVA, with N addition, soil moisture level, and incubation day as factors followed by Tukey’s multiple comparison test to determine significant differences and any interaction effects for specific pairwise comparisons. Nitrification and N mineralization data from the incubation experiment were analyzed with a two-way MANOVA with N addition and soil moisture level as factors. Tukey’s multiple comparison test was used
after the MANOVA to determine significant differences within factors and interaction effects. Differences between factors were considered statistically significant if the P value was less than 0.05.
Chapter 3

Results

3.1 Arches National Park Field Study

3.1.1 Inorganic Nutrients

Inorganic N pool sizes, measured in soils collected from the USGS experimental plots, were not significantly different between plots with added N and plots without added N (Figure 3-1). On 8 September 2014 and 11 September 2014 (Figure 3-1) there was a statistically insignificant trend in increasing inorganic N concentrations in both textures due to added N. On 28 September 2013 and 6 March 2014 N concentrations did not show this trend in either soil texture (Figure 3-1).

3.1.2 Total Reducing Sugar Concentrations

TRS concentrations measured in the USGS experimental plots were not significantly different between unfertilized and fertilized plots (not shown). TRS concentrations were significantly higher in fine textured soils on 8 September 2014 (Figure 3-2) but there was no significant interaction between texture and treatment.

3.1.3 Microbial Biomass
Figure 3-1: Extractable DIN (NH$_4^+$ + NO$_3^-$) concentrations (μg N/g dry soil) in the Arches National Park experimental plots. No statistically significant differences were found due to N addition on any of the four sampling dates. Error bars show standard error of the mean.

Microbial biomass C concentrations were not significantly different between the fertilized and unfertilized plots (data not shown), but there was a statistically insignificant trend toward higher microbial biomass C concentrations in the fine textured soils on 28 September 2013, 8 September 2014 and 11 September 2014.

Differences in microbial biomass N concentrations were not statistically significant between the fertilized and unfertilized experimental plots (Figure 3-3). On 28
September 2013 (Figure 3-3) microbial biomass N concentrations were significantly lower in the fine textured soils textured soils, with no significant interaction with N addition (P < 0.05).

3.1.4 Extracellular Enzyme Activities

NAG activities measured in the experimental plots had no significant differences due to N addition or soil texture (data not shown). The data from the four sampling dates
Figure 2-3: Microbial Biomass N (μg N/g dry soil) in the Arches National Park experimental plots. Error bars show standard error of the mean. Letters indicate statistically significant differences.

suggested a statistically insignificant trend of higher NAG activities in fine texture soils (data not shown).

There were no statistically significant differences due to N addition or the interaction effect between N addition and soil texture in LAP activities in the Arches
experimental plots (Figure 3-4). LAP activities were typically higher in the fine textured soils, and significantly so on 6 March 2014 (P < 0.05) (Figure 3-4).

Figure 3-4: LAP activities (nmol/hr/g dry soil) in the Arches National Park experimental plots. Error bars show standard error of the mean. Letters indicate statistically significant differences.

There were no statistically significant differences in PHENOX or PEROX activities due to N addition in the experimental plots at the time of sampling (data not shown). PHENOX activities were typically higher in the fine textured plots except on 11
September 2014 when PHENOX activities were consistent across plots. PHENOX activities appeared to be higher in the fine textured plots on 8 September 2014 due to one outlier measurement. PEROX activities were typically higher in the fine textured soils, and significantly so on 28-September 2013 (P < 0.05).

Figure 3-5: Resin NO$_3^-$ (μg NO$_3^-$ - N/cm$^2$ day) in the Arches National Park experimental plots. Error bars show standard error of the mean. Letters indicate statistically significant differences.

3.1.5 Denitrifying Enzyme Assay
Denitrification potential was measured using the DEA on 6 March 2014, 8 September 2014, and 11 September 2014, but was not detected in any of the samples from the experimental plots.

![Soil Aggregate Stability Graph](image)

Figure 3-6: Soil aggregate stability in the Arches National Park experimental plots on 15-November-2013. Error bars show standard error of the mean. Letters indicate statistically significant differences.

### 3.1.6 Resin Nitrate

Ion exchange resin strips accumulated significantly more dissolved NO$_3^-$ (P < 0.05) in the fertilized plots than in the unfertilized plots (Figure 3-5). There was a
significant interaction effect between N addition and soil texture with resin NO$_3^-$ significantly higher in the fertilized coarse texture plots than in the unfertilized coarse texture plots (Figure 3-5). There was no statistically significant difference or apparent trend in the concentration of resin NO$_3^-$ between unfertilized and fertilized fine textured soils (Figure 3-5).

There were no significant differences in ammonium availability between the coarse and fine textures and the fertilized and unfertilized plots (Data not shown). Ammonium availability was less than 0.05 kg-NH$_4^+$ ha$^{-1}$ year$^{-1}$ in the coarse and fine fertilized and unfertilized plots showing that adding N did not result in significant differences between the fertilized and unfertilized plots in either soil texture.

### 3.1.7 Soil Aggregate Stability

Soil aggregate stability was not significantly affected ($P < 0.05$) by soil texture or N addition (Figure 3-6). There was a statistically insignificant trend toward soil stability being highest in the unfertilized fine textured soil (Figure 3-6).

### 3.1.8 Biological Soil Crust Respiration

Field BSC respiration rates were significantly higher ($P < 0.05$) in fine textured soils on 28 September 2013 and 6 March 2014 (Figure 3-7). N addition had no statistically significant effect on soil respiration rates (Figure 3-7).

### 3.2 Kitty Todd Preserve, Northwest Ohio Field Sampling

#### 3.2.1 Resin Nitrate

Ion exchange resin strips were placed in the five dunes that were sampled for Kitty Todd crust soil analysis to measure dissolved NO$_3^-$. An average 0.957 kg-NO$_3^-$ /ha/yr was determined to be available in the Kitty Todd crust soils, less than the ca. 13
kg-N ha\(^{-1}\) yr\(^{-1}\) that is deposited in the region. Resin ammonium was not measured because the cation strips placed in the five dunes were lost from the field.

Figure 3-7: BSC respiration in the Arches National Park experimental plots. Error bars show standard error of the mean. Letters indicate statistically significant differences.

3.3 Incubation Experiment

3.3.1 Denitrifying Enzyme Assay
As in the field experiment, there was no detectable denitrifying enzyme activity in either Arches soil on Day 0 or 7 (not shown). However, there were detectable potential denitrifying enzyme activities in the Kitty Todd samples after seven days (Figure 3-8).

Figure 3-8: Potential denitrification (µg N/g dry soil/hour) measured on days 0 and 7 of the incubation experiment in the Kitty Todd soils. Error bars show standard error of the mean. Letters indicate statistically significant differences.

There were no statistically significant differences in potential denitrification due to soil moisture (Figure 3-8) or added N, and there were no statistically significant interaction
effects. There was a statistically insignificant increase in potential denitrification from 20% and 45% WHC to 70% WHC (Figure 3-8).

![Figure 3-8: Lab incubation N mineralization rates (μg N/g dry soil/day) in Kitty Todd and coarse and fine textured Arches soils at 20%, 45%, and 70% soil moisture level with and without added N. Error bars show standard error of the mean. Letters indicate statistically significant differences.](image)

**3.3.2 Nitrogen Mineralization Rates**

N mineralization rates were significantly higher (P < 0.05) in the coarse Arches soils than in the Kitty Todd or Fine Arches soils. In the Kitty Todd soils, there were no significant effects of added N or changing soil moisture level (Figure 3-9), but there was...
a statistically insignificant trend of decreasing N mineralization rates with increasing soil moisture (Figure 3-9).

In the coarse textured Arches soils, there was no significant difference in N mineralization rates due to N addition and there was no significant interaction between soil moisture and N addition. However, N mineralization rates in coarse textured soils were significantly higher at 70% soil WHC than at 20% or 45% soil WHC, and showed a statistically insignificant decrease with added N (Figure 3-9).

In the fine textured Arches soils, N mineralization rates decreased significantly due to N addition, but there were no significant effects due to soil moisture and no significant interaction (Figure 3-9). While there was no statistically significant effect due to soil moisture, there was a statistically insignificant trend of increasing N mineralization from 20% to 45% soil WHC and a decrease in N mineralization from 45% to 70% WHC (Figure 3-9).

3.3.3 Nitrification Rates

Nitrification rates were significantly higher in the coarse Arches soils than in the Kitty Todd and fine textured Arches soils (P < 0.05). In the Kitty Todd soils there was a statistically significant increase (P < 0.05) in nitrification rates with added N (Figure 3-10). Nitrification rates in the Kitty Todd soils were not significantly affected by soil moisture and there was no statistically significant interaction between the factors. There was a statistically insignificant trend of a decrease in nitrification rates with increasing soil moisture (Figure 3-10).

In the coarse Arches soils there was a statistically significant increase in nitrification rates (P < 0.05) from 20% soil WHC to 70% soil WHC (Figure 3-10).
were no statistically significant differences in nitrification rates due to added N and no significant interaction effect between N addition and soil moisture level.

Figure 3-10: Lab incubation nitrification rates (NO$_3^-$ - N/g dry soil) in two soils types at 20%, 45%, and 70% soil moisture level. Error bars show standard error of the mean. Letters indicate statistically significant differences.

In the fine textured Arches soils there were no statistically significant differences due to added N or soil moisture level. There was a statistically insignificant increase in nitrification rates from 20% soil moisture to 45% soil moisture and then a decrease from 45% to 70% soil WHC (data not shown).
3.3.4 Microbial Biomass

MBC concentrations were significantly higher in in the fine textured Arches soils than in the coarse Arches and Kitty Todd soils, and were significantly higher in the Kitty Todd soils than in the coarse textured Arches soils on both day 0 and day 7 (P < 0.05). MBC concentrations in all soil types only significantly differed due to incubation day, with concentrations increasing significantly from day 0 to day 7 in all three soils (P < 0.05) (data not shown).

MBN concentrations were significantly higher in the fine textured Arches soils than the Kitty Todd and coarse Arches soils, and significantly higher in the Kitty Todd soils than the coarse Arches soils on day 0 (P < 0.05). On day 7 MBN concentrations in the Kitty Todd and fine textured Arches soils were significantly higher than in the coarse Arches soils (P < 0.05). In the Kitty Todd samples, MBN increased significantly due to incubation day (Data not shown) but there were no significant effects due to added N or soil moisture level and there were no significant interactions (Figure 3-11). There was a statistically insignificant trend of MBN increasing from 20% to 45% soil WHC and then decreasing from 45% to 70% soil WHC (Figure 3-11).

The coarse Arches soils experienced no significant differences in MBN due to incubation day, soil moisture level, or N addition. There was a statistically insignificant trend of MBN increasing from 20% to 45% and from 45% to 70% soil WHC (Figure 3-11). There were also no significant differences in the MBN concentrations of the fine textured Arches soils due to incubation day, soil moisture level, or N addition, and there were no significant interaction effects between the three factors. As with the coarse
Arches soil, there was a statistically insignificant trend of MBN concentrations increasing from 20% to 45% WHC then decreasing from 45% to 70% WHC (Figure 3-11).

Figure 3-11: Lab Incubation microbial biomass N concentrations (μg N/g dry soil) in Kitty Todd and coarse and fine texture Arches soils incubated at 20%, 45%, and 70% soil WHC with and without added N. Error bars show standard error of the mean. Letters indicate statistically significant differences.

### 3.3.5 Total Reducing Sugar Concentrations

On day 0 TRS concentrations in the Kitty Todd soils were significantly higher (P < 0.05) than in the coarse texture Arches soils, and TRS concentrations in the fine
textured Arches soils were significantly higher than the coarse Arches soils. On day 7 TRS concentrations were significantly higher (P < 0.05) in the coarse Arches soils and

![Bar charts showing TRS concentrations in Kitty Todd, Coarse Arches, and Fine Arches soils at day 0 and day 7.](image)

Figure 3-12: Lab incubation TRS concentrations at low, moderate, and high WHC on day 0 and day 7. Error bars show standard error of the mean. Letters indicate statistically significant differences.

the Kitty Todd soils than in the fine textured Arches soils. TRS concentrations in the Kitty Todd and fine textured Arches soils decreased significantly (P < 0.05) from day 0 to day 7 (Figure 3-12). N addition and soil moisture did not have statistically significant effects on any of the three soils. In contrast to the fine textured Arches and Kitty Todd
soils, TRS concentrations significantly increased in the coarse Arches soil from day 0 to day 7 (Figure 3-12).

3.3.6 Respiration Rates

Respiration rates were significantly higher in the Kitty Todd soils than the coarse and fine Arches soils. In the Kitty Todd soils respiration rates increased significantly (P < 0.05) on day 1 from 20% to 70% soil WHC, day 5 from 20% to 45% and 20% to 70% soil moisture, day 6 from 20% to 70% soil WHC, and on day 7 from 20% to 45% and 20% to 70% soil moisture (data not shown). Differences due to N addition and the interaction effect between the two factors were not statistically significant in the Kitty Todd soils, but there was a statistically insignificant decrease due added N on day 7 (Data not shown).

In the coarse Arches soils respiration rates increased significantly from 20% to 45% soil WHC on days 2, 3, 4, 5, and 7 (data not shown). There were significant increases in respiration from 20% to 70% WHC on days 4, 5, and 7. Respiration rates on day 4 increased significantly with added N (data not shown).

There was a statistically significant increase in respiration from 20% to 45% WHC on days 3, 4, 5, 6, and 7 in the fine textured Arches soils (Data not shown). Respiration rates in these soils also increased significantly from 20% to 70% WHC on days 0, 1, 3, 4, 5, and 7 (Data not shown). There was no statistically significant change in respiration rates from 45% to 70% soil moisture.
Chapter 4

Discussion

4.1 Effects of N on Arches BSCs

One of my central questions was how N deposition would affect BSCs and their associated microbial communities in Arches National Park, an arid, N limited ecosystem. Researchers in the Colorado Plateau region predicted that increasing N deposition would alter historical N cycling patterns, which could subsequently encourage invasive, exotic plants and exclude BSC communities, eventually leading to decreased productivity and soil stability (Belnap et al., 1999; Bowker et al., 2005; Fenn et al., 2003; Schwinning et al., 2005). In the field experiment at Arches National Park, we observed no effects of added N on inorganic N concentrations, MBC and MBN, TRS concentrations, extracellular enzyme activities, soil aggregate stability, or BSC respiration in either soil texture. In the incubation experiment no effects of added N were observed in the Arches crust soils except for increases in soil respiration in the coarse textured soils. There was no effect of added N on soil stability, BSC respiration, and TRS concentrations. Therefore, we must conclude that predictions regarding negative effects of N deposition on BSC communities are unsupported.
4.2 Fate of Added N in Arches BSC Soils

Determining the fate of added N in arid Arches National Park crust soils was another objective of my study. Added N did not result in increased N immobilization in either the field or incubation experiment. N immobilization rates appeared to be significantly higher in the fine textured Arches soils with added N than without added N but the change during the incubation was relatively small compared to the background (N initial was ca. 12.3 μg/g dry soil and N final was ca. 11.5 μg/g dry soil), and there was no increase in MBN, suggesting the apparent N immobilization in the fine Arches soils was questionable. Furthermore, nitrification rates in the fine textured soils were not significantly different due to added N. In the coarse textured Arches soils increasing soil moisture levels significantly increased nitrification rates in the incubation experiment, indicating that water is an especially important N cycling control.

Unlike nitrification, denitrification was not an important N cycling mechanism in the Arches soils. Interestingly, there were no detectable denitrifying enzymes even when the soils were incubated with added N at 70% soil moisture for one week. This indicates that denitrifying bacteria are not present or not present in great enough numbers to denitrify added N in these soils (Groffman et al., 1999; Hall et al., 2008; Turnbull et al., 2010), thus NO$_3^-$ in these soils was not being anaerobically denitrified into N$_2$O or NO$_x$ (Groffman et al., 1999). However, potential leaching losses were significantly higher in the coarse textured plots with added N than without added N. Extrapolated to one year this would be a difference of 123.595 μg NO$_3^-$-N/cm$^2$, or the equivalent of 12.359 kg-NO$_3^-$/ha. This difference in resin NO$_3^-$ accounted for the more than the concentration of N added to the plots as fertilizer. These results suggest that added N was nitrified and
leached from the soil before organisms could use it due to limitations imposed by water availability and the pulsed nature of rainfall in this system (Schwinning et al., 2005; Hall et al., 2011).

4.3 Role of Water in Arches BSC N Cycling

Another question was how changing soil WHC influenced the effects of added N in the Arches crust soils. Data from my incubation experiment showed that increasing soil moisture from 20% to 70% WHC in coarse textured Arches soils significantly increased nitrification rates. This results in NH$_4^+$ being nitrified to NO$_3^-$ under wet conditions which could then lead to NO$_3^-$ leaching from the soil, further supporting the field observations that added N was leached from the coarse soils before microbes could use it. The coarse Arches soils also experienced a significant increase in N mineralization rates from 20% and 45% soil WHC to 70% WHC. Thus, given time under wetter conditions microbes were able to increase N cycling rates, but in situ it is likely that under wetter conditions added N in coarse soils would leach out of the system. These results suggest that water availability does limit microbial processing of added N in the coarse crust soils, but such a large amount of water is required to ameliorate this limitation that excess N is either directly leached from the soil or nitrified and then leached from the soil before it can negatively affect crusts or change N cycling patterns (Hall et al., 2008; Turnbull et al., 2010; Hall et al., 2011). This is likely due to the coarse soil crusts not being as strongly established as their fine textured counterparts, which limits their ability to retain water and nutrients (Belnap et al., 1999; Bowker et al., 2005; Bertrand et al., 2014).
Unlike the coarse soils, the fine textured Arches soils did not experience any differences in microbial N cycling in the lab experiment due to changes in soil moisture. This was surprising because increasing soil moisture in coarse textured soil resulted in increased nitrification and N mineralization rates. These results indicate that the limitations imposed by water availability on the coarse textured Arches soils were not as strong in the fine textured Arches soils. This is likely because fine textured soils have smaller aggregates that crusts can bind together more easily, enabling them to hold onto more water than the larger aggregates in coarse textured soils (Bertrand et al., 2014).

4.4 Role of Soil Texture in Arches BSCs

Bowker and Belnap (2008) and Bertrand et al. (2014) found that soil texture had a significant impact on crust establishment and nutrient cycling in BSCs. Textural differences in Arches field plots supported these studies, with crusts on fine textured soils appearing to provide stronger ecosystem services. Statistically insignificant trends in soil stability and significant differences in BSC field respiration and incubation experiment TRS concentrations between soil textures indicated that the fine textured soils promoted crust development. Significantly higher LAP, PEROX, and soil respiration rates in fine textured soils suggested that microbial communities were more active in the fine textured plots than the coarse. The field resin results and the incubation experiment also revealed that added N was nitrified and leached from the soil in the coarse textured soils, while there was no increase in leaching losses with added N in the fine textured soils. The difference in crust establishment due to soil texture may allow the fine textured soil crusts to prevent added N from leaching out of the system (Belnap et al., 1999; Katlenecker et al., 1999, Reynolds et al., 2001; Bowker et al., 2005). By increasing soil stability and
preventing leaching losses of soil nutrients, recovering crusts in fine textured soils may provide land managers with a tool to help restore degraded lands on the Colorado Plateau to more productive ecosystems (Reynolds et al., 2001; Bowker et al., 2005).

In summary, my results indicate that soil texture is the most important factor controlling crust development and leaching of added N on the Colorado Plateau. Maturing fine textured crust soils in Arches appear to inhibit deposited N from leaching from the soil and impacting crust establishment or soil N cycling, whereas still recovering crusts in the coarse textured Arches soils are unable to prevent deposited N from leaching from the soil (Belnap et al., 1999, Katlenecker et al., 1999, Reynolds et al., 2001; Fenn et al., 2003; Bowker et al., 2005; Bowker and Belnap, 2008; Zhang et al., 2012; Bertrand et al., 2014).

4.5 Effects of Added N and Water in Kitty Todd, NW Ohio Crust Soils

BSCs from Kitty Todd Nature Preserve were used in the incubation experiment to compare how crusts from an environment that experiences natural N deposition levels an order of magnitude greater than Moab, UT, and average annual rainfall four to five times greater, reacted to added N. Resin strips were placed in the five sampled dunes in NW Ohio for six months in order to determine potential N leaching losses. The equivalent of less than 1 kg N per year of dissolved NO$_3^-$ was resin-available in these soils each year, indicating that most atmospherically deposited N was not leaching from these soils.

Like the fine textured Arches BSCs, the NW Ohio BSCs appear to be able to prevent added N from saturating the system and leaching from the soil. Nitrification rates in the NW Ohio soils were significantly higher with added N, but based on the resin strip data, much of the nitrified N was not leaching from the soil. The Kitty Todd crust soils
were the only soils to have detectable levels of denitrification. Notably, rates at 20% and 45% soil WHC were nearly as high as those at 70% WHC in jars with added N. Furthermore, higher nitrification rates at less-than-saturated soil moisture increases NO$_3^-$ availability for denitrification, potentially enhancing it in the field under moderate moisture conditions (Groffman et al., 1999; Bardgett, 2005). I observed no other effects of added N on microbial N cycling. These results suggest that the Kitty Todd crusts and the associated microbial communities are able to retain enough water for bacteria to nitrify and denitrify excess N out of the soil, preventing it from accumulating and saturating or leaching from these soils.

These results highlight the importance of crusts as mediators of atmospheric N deposition in NW Ohio. This finding generally agrees with those of Velucì et al. (2006), but the method by which crusts prevent leaching was not the same. Velucì et al. (2006) concluded that leaching did not occur because plants and microbes incorporated the additional N into their biomass, whereas my results showed no increase in microbial N immobilization rates or biomass N with added N. The results of my study suggest that these crust communities are able to nitrify and subsequently denitrify the added N and release it from the soil as gases, even with soil moisture as low as 20% WHC or possibly lower. Unfortunately, N$_2$O is a potent greenhouse gas and its release from the soil is not a desirable outcome for land managers (Groffman et al., 1999; Hall et al., 2008). These results highlight the importance of crusts in the Oak Openings region preventing deposited N from leaching from the soil, but they also show that these BSC soils may not completely prevent negative effects from deposited N, as even at low soil moisture levels denitrification reduces added N and releases greenhouse gases from the soil.
Chapter 5

Conclusions

The results of my experiments and analyses of field samples indicate that soil texture is the most important factor determining the extent of crust establishment and how nutrients are cycled in Arches National Park crust soils. Added N had no significant effects on the development of crusts and microbial cycling of N in either coarse or fine textured soils. Even when water limitation was alleviated added N had no effect on N cycling, and limited effects on respiration. However, soil texture affected soil aggregate stability, BSC Respiration, nutrient leaching, and microbial N cycling (MBN, N mineralization, nitrification, and LAP activities). My results support previous findings that crusts better support ecosystem services, such as soil stabilization and nutrient retention, in finer textured soils.

Crust soils in NW Ohio were studied to determine how crusts that experience N deposition levels an order of magnitude greater than in Arches National Park and approximately five times as much rain respond to added N. These crusts were also more developed than those from both Arches National Park. My results suggest that these crusts may be able to denitrify added N from the soil before it impacts microbial activity or N mineralization, thereby preventing leaching losses of NO₃⁻ from these soils.
Unfortunately, denitrifying added N results in increased greenhouse gas emissions of N\textsubscript{2}O. This leads me to conclude that protecting crusts in these temperate, sand barren ecosystems is important tool for land managers to limit leaching losses connected with N deposition, but new strategies need to be developed and/or implemented to prevent the deposited N from being lost as a greenhouse gas.

The results from my study of the NW Ohio crust soils also highlight the importance of restoring crusts to their historic lands in the Colorado Plateau. The crusts from NW Ohio are more mature, with better-developed moss and lichen assemblages than the crusts in the experimental plots in Arches National Park. The greater maturity of these crusts might explain why these BSCs are able to prevent leaching losses of deposited N, which is an important difference from the coarse textured Arches soils where added N appears to be leaching from the soils. My results also showed that denitrification did not occur in either Arches soil, even after incubating at a high WHC for one week, suggesting that if these crust soils are able to prevent leaching losses of N as they mature they may not experience the same greenhouse gas emissions observed in the NW Ohio soils. This leads me to conclude that protecting and promoting the restoration of crusts over their historic range on the Colorado Plateau could be key in regulating the influx of deposited N to a historically N limited system.
References


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