EPIPHYTIC LICHENS, NITROGEN DEPOSITION AND CLIMATE IN THE US NORTHERN ROCKY MOUNTAIN STATES

by

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ABSTRACT

Forested ecosystems in the NW Interior Mountains (NWIM) of the United States are jeopardized by degrading air quality and changes in climate regimes. Monitoring and tracking changes in air quality and climate through instrumentation alone can be an expensive and challenging task. Biomonitors offer a cost-effective way to maximize monitoring resolution. This thesis explored the utility of lichens as biomonitors across three sections of the NWIM. First, in the Wind River Range, WY, nitrogen concentrations (%N) in lichen thalli were calibrated with measurements of canopy throughfall N deposition. A strong correlation verified % N as a useful metric to estimate N deposition. Nitrogen deposition in the Boulder drainage, closest in proximity to large natural gas drilling operations, was clearly elevated above estimated background conditions and measurements from other drainages. Degraded lichen communities were observed at deposition levels of 4.0 kg N $ha^{-1}year^{-1}$. The second study used lichen community composition, elemental analysis, and lichen functional groups to analyze the importance of nitrogen deposition and climate on lichen communities along the northern Rocky Mountains. Temperature and relative humidity were the most important climatic influences on community structure. A nitrogen pollution signal was independent of climate. The relationship between % N in lichen thalli and throughfall N (study 1) was used to estimate N deposition along the northern Rocky Mountains. Eutroph (N-tolerant) and oligotroph (N-sensitive) functional group indices were correlated with both N deposition and climate. Elevated N deposition (twice background conditions) was most notable around Bozeman, MT and Pinedale, WY. The final study stratified plots across the NWIM by latitude and longitude and found precipitation, dew point, and temperature were important variables to lichen community composition. Eutroph and oligotroph distributions were partially accounted for by climate; unfortunately no N data were available for comparison to lichen communities. The models created can be utilized for monitoring changes in lichen communities over time and to predict N deposition and climate conditions for new plots. Additionally, these models can be used to address management and conservation questions related to individual lichen species, lichen communities, forest health, air quality, and climate conditions.

CHAPTER ONE

INTRODUCTION

Change in climate patterns and increases in nitrogen (N) deposition from air pollution can adversely affect forest health in the Western United States (Baron et al. 2011; Beem et al. 2010; EPA 1997; Fenn et al. 2003; Saros et al. 2010). Monitoring current conditions, tracking changes, and predicting ecosystem responses to changes in climate and air pollution can be an expensive and challenging task. The use of biological indicators (bioindicators) provides an economical and practical means to maximize monitoring resolution, especially in remote areas and across vast landscapes (Jovan 2008).

Study Area – Geographic Locations

The study areas in this thesis geographically expand in size with each chapter. The first study takes place in the Wind River Range (WRR), in western WY (Bridger-Teton National Forest). Rapid expansion of natural gas drilling in close proximity to the WRR has warranted concern about the potential effects of enhanced N deposition to the Forest, especially the Class I Bridger Wilderness. The second study includes the WRR and stretches north along the Rocky Mountains. This study area spans the EPA designated (level III) Middle Rockies Ecoregion. EPA ecoregions divide North America into unique areas based on similarly functioning ecosystems and habitat patterns (Omernik 1995; 2004). The Middle Rockies ecoregion includes parts of Montana, Idaho,

and Wyoming. The final study expands west and north to include three EPA ecoregions: The Canadian Rockies, Middle Rockies, and Idaho Batholith, which are collectively referred to in this thesis as the NW Interior Mountains.

Climate Change

Over the last century, average annual temperatures in the NW Interior Mountains have increased by 0.74 °C (1.33 °F), while annual precipitation has decreased between 0.79 and 2.84 cm (EPA 1997; NOAA National Climatic Data Centers Climate, Services, and Monitoring Division—web accessed March 2012). Spring is beginning earlier in the year and the growing season is lengthening (Ault et al. 2011). Decrease in precipitation or the transition of precipitation from snow to rain will result in less snow water equivalent (SWE) stored in the snowpack and thus lower stream flows in the summer. Rising summer temperatures in the northern Rocky Mountains has been linked to treeline encroachment on alpine vegetation (Hessel and Baker 1997), increased water stress—due to decreased SWE, and more intense fire seasons (EPA 1997).

Nitrogen Deposition and Critical Loads

Anthropogenic emissions of reactive nitrogen (Nr) have become the dominant source of N deposition to many ecosystems (Bobbink et al. 2010; Galloway et al. 2008; Howarth 2008). In N-limited environments, such as high alpine zones of the NW Interior Mountains, even slight increases in N deposition can alter the way an ecosystem functions. Alterations include stimulation of plant and algal growth, and increased

competition within biotic communities—favoring invasive species and decreasing richness of sensitive species (Bobbink et al. 2010; Baron 2006; Baron et al. 2011; Beem et al. 2010; Howarth 2008).

Nr species exist in gaseous and particulate forms or in aqueous solution and are deposited as dry or wet deposition, respectively. Reduced Nr is predominantly from ammonia (NH_3) and ammonium (NH_4^+); oxidized Nr includes nitric oxide (NO), nitrous oxide (N_2O), nitrite (NO_2^-), nitrate (NO_3^-), and nitric acid (HNO_3) (Horii et al. 2006).

It is widely believed that current levels of Nr deposition in the Rocky Mountains are impacting or will impact the sensitive ecosystems in this region (Baron 2006; Baron et al. 2011; Beem et al. 2010; NPS 2010; Saros et al. 2010; Yellowstone Center for Resources 2011). Nr deposition (reduced + oxidized, dry + wet) has recently been estimated between 0.5 and 5 kg N ha^{-1} $year^{-1}$ (Burns 2003; Grenon and Story 2009; Grenon et al. 2010), while historic loads are thought to be < 1.0 kg N ha^{-1} $year^{-1}$ (Sverdrup et al. 2012).

Critical loads (CLO) are used to quantify the threshold deposition level below which no harmful effect will occur to an ecosystem component according to current knowledge (UBA, 2004). Thus CLO estimates will vary depending on the ecosystem component of interest. Total N CLO estimates for sensitive terrestrial ecosystem components in the Rocky Mountains range from 2.5 to 4.0 kg ha^{-1} $year^{-1}$ (Baron et al. 1994; Bowman et al. 2011; Rueth and Baron 2002).

Lichens

Lichens are composite life-forms that include two to three different organisms. The majority of the lichen thallus (body) consists of a fungus; this portion is called the mycobiont. The other organism is either a green algae or blue green bacterium (known as the photobiont). Some lichen species are comprised of all three organisms.

The diversity of lichens in North America is estimated to be around 3,600 species (Brodo et al. 2001). In many forests, lichens are important in nutrient cycling, food webs, and provide habitat for insects and microorganisms along with nesting materials for birds and squirrels (McCune et al 2007, Brodo et al. 2001). Lichens are also used by humans for emergency food, dyes, and pharmaceuticals (Brodo et al. 2001). Lichens also have a long history of use as indicators of air pollution with records dating back from the mid 1800's (Nylander 1866).

Lichens are poikilohydric organisms that rely on liquid water or water vapor to hydrate and become metabolically active (Lange et al. 1986). Each lichen species has an optimal range of moisture, temperature, light, and nutrient availability for maximum growth (Sillett et al. 2000). Epiphytic lichens absorb the bulk of their nutrients from the air, lack a waxy cuticle and stomata (which, in plants, helps slow the absorption of pollutants), and have no roots (in which elemental transfer can take place). Because of these characteristics, lichens are highly sensitive to changes in habitat structure, climate, and air pollution, especially fertilizing and acidifying nitrogen and sulfur containing pollutants. Lichen bioindication has been used successfully in numerous studies to

evaluate forest health and environmental conditions (Fenn et al. 2007; Fenn et al. 2008; Geiser and Neitlich 2007; Jovan and McCune 2005; Rogers and Ryel 2009).

In this thesis, three biomonitoring techniques (McCune and Geiser 2009) were employed: analysis of elemental concentrations in lichen thalli (Fenn et al. 2008; Fenn et al 2007; Geiser and Neitlich 2007); analysis of lichen community composition (Geiser and Neitlich 2007; Jovan and McCune 2005; McCune et al 2010); and eutroph (N-tolerant) and oligotroph (N-sensitive) functional group indices (Jovan and Riddell –*in review*; McCune and Geiser 2009).

Intent

Chapter 2 was located in the Wind River Range (WRR), in western WY (Bridger-Teton National Forest) in close proximity to extensive natural gas drilling project areas. The objectives of this study were to:

- Test the hypothesis (H_A) that thalli nitrogen concentrations from two widely distributed epiphytic macrolichens, *Letharia vulpina* L. (Hue) and *Usnea lapponica* Vainio, would be correlated with annual nitrogen throughfall deposition (kg ha^{-1} $year^{-1}$), and thus provide an economical tool to continue monitoring and further identify areas of elevated N deposition in the WRR.
- Quantify Nr deposition from ammonium, nitrate, and total dissolved inorganic N (DIN = $NH_4^+ + NO_3^-$) on the west side of the WRR, and
- Spatially assess local patterns of Nr deposition in the WRR.

Chapter 3 explored lichen communities along the northern Rocky Mountains. The objectives of this study were to:

- Gain knowledge of epiphytic macrolichen community richness and diversity in the northern Rocky Mountains.
- Investigate important site, climate, and N deposition variables associated with gradients in lichen community composition at the subregional level.
- Test the hypothesis that eutroph and oligotroph indices or individual species can be used to indicate climate and pollution conditions.
- Spatially evaluate patterns of N deposition in the study area.

The final chapter expanded exploration of lichen communities to the NW Interior Mountain States. The objectives were to:

- Gain knowledge of epiphytic macrolichen community richness and diversity across the NW Interior Mountains.
- Determine the important forest and climate variables associated with gradients in lichen community composition at the regional level.
- Test the hypothesis that eutroph and oligotroph indices or individual species can be used to indicate climate and pollution conditions.
- Evaluate the ability of the model to predict climate conditions for new plots.

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CHAPTER TWO

USING EPIPHYTIC LICHENS TO MONITOR NITROGEN DEPOSITION NEAR NATURAL GAS DRILLING OPERATIONS IN THE WIND RIVER RANGE, WY

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Abstract

Rapid expansion of natural gas drilling in Sublette County, Wyoming (1999-present) has raised concerns about the potential ecological effects of enhanced atmospheric nitrogen (N) deposition to the Wind River Range (WRR) including the Class I Bridger Wilderness. We sampled annual throughfall (TF) N deposition and lichen thalli N concentrations under forest canopies in four different drainages of the WRR. Measurements of TF N deposition and N concentrationsin lichen thalli were highest at plots closest to drilling operations (< 30 km). N concentrationsin lichens decreased exponentially with distance from drilling activity. Highest TF N deposition, 4.1 kg ha^{-1} $year^{-1}$, coincided with clear evidence of damage to lichen thalli. This deposition value is above estimated preindustrial deposition conditions (0.9 kg N ha^{-1} $year^{-1}$) and regional critical loads (a deposition value below which ecosystem harm is prevented) of N deposition for sensitive ecosystem components. N concentrations in *U. lapponica* were strongly correlated (r = 0.96) with TF N deposition, demonstrating that elemental analysis of lichen material can be used to estimate TF N deposition. N concentrations below 1.35% in *U. lapponica* and 1.12% in *L. vulpina* were associated with 0.9 kg N ha^{-1} $year^{-1}$. Additional lichen sampling in the Bridger Wilderness is recommended to further quantify and monitor spatial patterns of N deposition and to define areas of elevated N deposition.

Keywords: oil and gas development; lichens; throughfall nitrogen deposition; critical loads; monitoring; wilderness

Introduction

Anthropogenic emissions of reactive nitrogen (Nr) have become the dominant sources of N deposition in many ecosystems (Bobbink et al. 2010; Galloway et al. 2008; Howarth 2008). In N-limited environments, such as high alpine zones of the Northern Rocky Mountains, even slight increases in N deposition can alter ecosystem functions. Alterations include stimulation of plant and algal growth, and increased competition within biotic communities—favoring invasive species and decreasing richness of

sensitive species (Bobbink et al. 2010; Baron 2006; Baron et al. 2011; Beem et al. 2010; Howarth 2008).

Nr species exist in gaseous and particulate forms or in aqueous solution and are deposited as dry or wet deposition, respectively. Reduced Nr is predominantly from ammonia (NH_3) and ammonium (NH_4^+); oxidized Nr includes nitric oxide (NO), nitrous oxide (N_2O), nitrite (NO_2^-), nitrate (NO_3^-), and nitric acid (HNO_3) (Horii et al. 2006).

It is widely believed that current levels of Nr deposition in the Rocky Mountains are impacting or will impact the sensitive ecosystems in this region (Baron 2006; Baron et al. 2011; Beem et al. 2010; NPS 2010; Saros et al. 2010; Yellowstone Center for Resources 2011). Critical loads (CLOs) are a quantitative measure (typically kg ha^{-1} $year^{-1}$ for N deposition) used to protect sensitive ecosystem components from negative impacts of pollution (Nilsson and Grennfelt 1988).

Exceedence of CLOs for N deposition have been linked to ecosystem eutrophication or acidification depending on the level, duration, and type of N deposition (Baron et al. 2011; Bobbink et al. 2010; Fenn et al. 2003). Since little is known about biotic recovery from N-enrichment, it is important to study these systems before enrichment occurs and biotic communities shift or decline in species diversity (Schlesinger 2009).

Over the last decade, total inorganic Nr deposition (reduced + oxidized) in the northern Rocky Mountains has been estimated between 0.5 and 5 kg N ha^{-1} $year^{-1}$ (Burns 2003; Grenon et al. 2010; Yellowstone Center for Resources 2011), while historic levels (prior to 1950) are estimated to be less than 1.0 kg N ha^{-1} $year^{-1}$ (Sverdrup et al. 2012).

In recent years, Sublette County, WY (population = 8,100) has become notorious for air quality issues. Between January and March 2011, Sublette County experienced multiple days which exceeded the National Ambient Air Quality Standard (NAAQS) 8-hr standard for ozone (75 ppb), with hourly spikes measuring up to 166 ppb (WYDEQ 2011a; WYDEQ 2011b). In the last decade, energy development has increased exponentially. In 1998, fewer than 650 producing gas wells (PGW) and producing oil wells (POW) existed on public lands in Sublette County. As of December 2011, there were 4,184 active PGWs and POWs (BLM, Pinedale office *per. comm.*). These numbers do not include drilling activity on private lands.

The rapid expansion of oil and gas development concurrent with ozone violations has heightened concern about the quantity and the effects of Nr, specifically oxidized Nr deposition, to the surrounding Bridger-Teton National Forest (BTNF). Of particular concern is the Bridger (Class I) Wilderness located in the Wind River Range (WRR), parts of which are less than 30 km from drilling activity (Grenon et al. 2010).

In order to accurately assess the quantity of Nr deposition in forested landscapes, it is important to consider measurements of canopy throughfall (TF). We know of no other study which has measured Nr deposition of canopy TF within close proximity of a large energy development operation. Currently there are only a few deposition monitoring programs active on the west side of the WRR (within NF boundaries). The National Atmospheric Deposition Program (NADP) has measured average annual wet N deposition (2005-2010) at two sites in the WRR at 0.9 and 1.19 kg ha^{-1} (data available for down load at http://nadp.sws.uiuc.edu/NTN/ntnData.aspx -web accessed Jan. 2012). Dry

deposition in the Rocky Mountains has been estimated to account for 15 - 30 % of total N deposition (Beem et al. 2010; Ingersoll et al. 2008). The Clean Air Status and Trends Network (CASTNet) is co-located with one NADP site to measure dry N deposition. The average annual dry N deposition (2000-2009) was 0.45 kg ha^{-1}. Long-term lake monitoring by the BTNF (Svalberg and Porwoll 2002) has measured increased ammonium and nitrate concentrations in high alpine lake chemistry between 1984 and 2010 (Grenon et al. 2010). Measurements of bulk precipitation (rain and snow) at two of the monitored lakes found increased NH_4^+ and NO_3^- over time. Annually averaged N deposition from bulk precipitation sites increased from 1.6 and 2.1 kg $ha^{-1}year^{-1}$ (1987-1991) to 2.76 and 3.35 kg $ha^{-1}year^{-1}$ (2004-2008) respectively (Grenon et al. 2010; Svalberg and Porwoll 2008). In addition The Interagency Monitoring of Protected Visual Environments (IMPROVE) network operates monitors which measure ambient aerosols and tracks trends in visibility.

The jagged high-surface area profiles of forest canopies intercept airflow and trap ambient pollution as deposition (Fowler et al. 1999). During dry spells, deposition can accumulate on tree leaves and stems. When precipitation events occur, accrued dry deposition is delivered to the forest floor (Cape et al. 2010; Fenn et al. 2009). By deploying numerous TF monitors we hoped to gain an improved spatial and quantitative understanding of oxidized and reduced Nr deposition along the west flank of the WRR.

Epiphytic lichens live in and under most forest canopies, receive their nutrients primarily from the atmosphere, lack regulatory structures such as stomata and a cuticle, and are sensitive to acidifying and fertilizing pollutants (Munzi et al. 2010). Because of

these characteristics, epiphytic lichens have been used successfully in numerous air quality studies in the western US (Blett et al. 2003; Fenn et al. 2008; Geiser and Neitlich 2007; Jovan 2008; Jovan and Carlberg 2007) and globally (Frati et al. 2007, 2008; Munzi et al. 2010; Sparrius 2007; van Herk 1999). Biological indicators (bioindicators) such as lichens can provide an economical and practical means to maximize monitoring resolution, especially in remote areas (Fenn et al. 2008; Geiser and Neitlich 2007; Jovan 2008). It is important to evaluate different forms of Nr in lichen studies because oxidized and reduced forms of N have varying eutrophication and acidifying effects on lichen physiology and will influence lichen community response (Jovan et al. –*in review*; Munzi et al. 2010; Riddell et al. 2008; van Herk et al. 2003; van Dobben & ter Braak 1999).

The objectives of this study were to:

- Test the hypothesis (H_A) that nitrogen thalli concentrations from two widely distributed epiphytic macrolichens, *Letharia vulpina* L. (Hue) and *Usnea lapponica* Vainio, would be correlated with annual nitrogen throughfall deposition (kg ha^{-1} $year^{-1}$), providing an economical tool to continue monitoring and further identify areas of elevated N deposition in the WRR.
- Quantify Nr deposition from ammonium, nitrate, and total dissolved inorganic N (DIN = $NH_4^+ + NO_3^-$) on the west side of the WRR.
- And spatially assess patterns of Nr deposition.

To accomplish these goals, 121 passive monitors were installed in four different drainages to measure annual TF and bulk Nr deposition. Samples of *U. lapponica* and *L.*

vulpina (when available) were collected from each plot and analyzed for % N concentrations in lichen thalli. Regression analysis was used to assess the relationship between lichen thalli N concentrations and TF DIN.

Methods

Study Area

The Bridger-Teton National Forest (BTNF) in the Greater Yellowstone Area is the second largest National Forest in the contiguous United States, encompassing over 1.38 million hectares of land in western Wyoming. Designated Class I and II wilderness areas make up 0.5 million hectares of the BTNF. The Wind River Range (WRR) is a 160 km stretch of stout, granitic mountains that run northwest to southeast along the Continental Divide. The study area was located on the west-side of the WRR in and around the Bridger Wilderness on the BTNF (Figure 2.1). The Bridger Wilderness spans 173,374 hectares and includes the tallest peak in Wyoming (Gannett Peak 4,209 m), large glaciers, and over 1,000 high alpine lakes. Common tree species include *Pinus albicaulis, Pinus contorta*, *Pinus flexilis, Pseudotsuga menziesii, Picea engelmannii*, *Abies lasiocarpa,* and *Populus tremuloides,* which eventually opens to a treeless alpine habitat around 3,100 m. The exposed foothills of the WRR are speckled with shrubby species of *Salix, Juniperus*, *Artemisia*, and *Chrysothamnus*.

Study Design

In the summer of 2010, nine temporary 0.378 hectare circular plots were established in the WRR just outside the Bridger Wilderness. At each plot, passive ion

exchange resin-filled collectors (IER) were installed to measure throughfall (TF) and bulk deposition. In addition, lichen thalli from *Letharia vulpina* and *Usnea lapponica* were collected for elemental analysis (Geiser 2004).

Plot installation followed the Forest Inventory and Analysis (FIA) protocol for design and methodology of "off-grid" plots so that the collected lichen data could be combined with FIA data in future studies (FIA P3 Field Guide 2011). Plot locations were selected based on: the presence of collectable lichen thalli from at least one targeted lichen species, the probability of capturing different levels of Nr pollutants, available access for hauling bulky heavy equipment into non-motorized areas, canopy dominance by either *Pseudotsuga menziesii* or *Picea engelmannii* (Douglas-fir and Engelmann spruce respectively), stand age greater than 50 years, absence of recent fire, and low density of beetle-killed trees. We also chose plot locations to minimize presence of hardwoods (angiosperm tree species), because hardwood and conifer canopies differ in structure and TF chemistry (Cronan and Reiners 1983).

Most drainages on the west side of the WRR are oriented in a NE to SW direction. Two to three plots were installed in four different drainages: the Green River, Pine Creek, Boulder, and Big Sandy [ordered from north to south] (Figure 2.1).

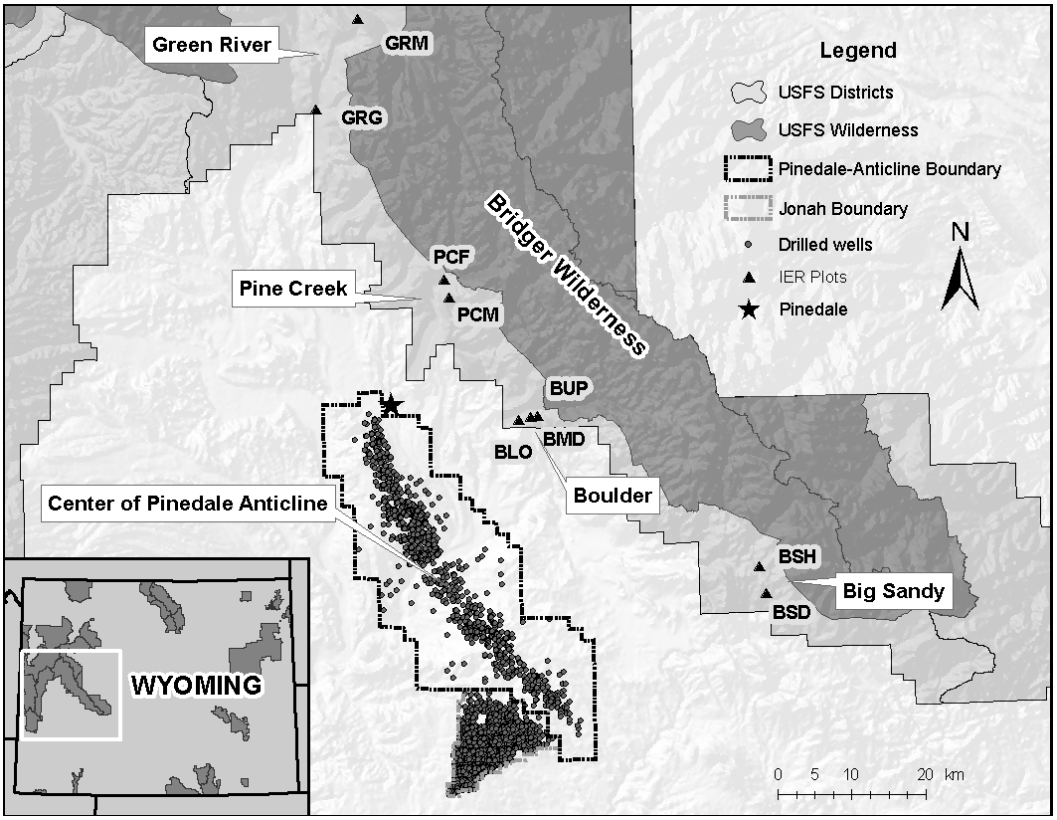


Fig. 2.1 The Wind River Range, plot locations and nearby oil and gas wells from the Pinedale Anticline and Jonah project areas. Center of the Pinedale Anticline approximates where distance measures were taken from. Green River, Pine Creek, Boulder, and Big Sandy represent the four drainages in which this study took place. Three-letter acronyms for plots are: GRM = Green River Moose Creek, GRG = Green River Gypsum Creek, PCF = Pine Creek Fremont Lake, PCM = Pine Creek Mulligan Park, BLO = Boulder low, BMD = Boulder middle, BUP = Boulder up, BSH = Big Sandy High, BSD = Big Sandy Dutch Joe Guard Station.

Field Data Collection

On each IER plot, nine collectors were placed under Douglas-fir or Engelmann spruce trees to measure TF deposition (total n = 81, Figure 2.2). One tree species was used per plot. Four collectors were installed near each TF plot, under open skies to measure bulk deposition (total n = 40). A control (capped resin tube) was attached to one

of the fence posts at each bulk and throughfall plot. Installation protocols followed Fenn et al. (2007) and Fenn and Poth (2004).



Fig. 2.2 Volunteers help install TF collectors near Boulder Lake (site—BLO). Each collector had an ion-exchange resin tube (IER) attached to the bottom of a 21 cm diameter funnel. IER tubes had a small opening at the bottom to allow precipitation to pass freely through. On top of each funnel was a snow tube (0.914 m long) topped with a pointy metal crown to keep birds from perching on the tube perimeters. Deer netting (used for gardening with 1.9 by 1.9 cm squares) was placed over the snow tube opening to keep birds and small mammals from fataly entering the tubes while still allowing snow and rain to freely fall into the collectors. The whole collecting unit was suspended above ground, strapped between two steel fencing posts.

Precipitation that fell into the collectors (TF and Bulk) was funneled through an IER tube where mixed-bed polystyrene anion and cation exchange resin beads (Amberlite MB150 Rohm and Haas) captured positive and negative ions as they passed through. Each IER tube had the capacity to collect up to 416 kg N ha^{-1} (Fenn and Poth 2004). IER tubes were left on the collectors for one year, June 2010 to June 2011. The tubes were then detached from the funnels and shipped to the USFS Pacific Southwest Research

Station in Riverside, CA for extraction and analysis of ammonium (NH_4^+), nitrate (NO_3^-), total dissolved inorganic nitrogen (DIN = NH_4^+, + NO_3^-), and phosphate (PO_4^{3-}).

At each TF plot, 2 – 4 separate samples of *Letharia vulpina* and *Usnea lapponica* thalli (~10 g/sample) were collected using powder-free nitrile gloves and placed into sterile air-tight bags (Whirl-Pak® polyethylene) for elemental analysis following Geiser (2004). Lichens were sampled on the same day the IER tubes were removed from the collectors. Samples were collected over the whole plot from a minimum of 6 substrates to ensure that a representative sample of the population (age and location) was collected. If only one species was present, two to three samples of that species were collected. At all plots, latitude, longitude, and elevation were measured using a hand held GPS unit (Garmin eTrex®; 1 degree resolution). Linear distance between each plot and the center of the Pinedale Anticline (PA) natural gas field (Figure 2.1) was measured using ArcGIS (ArcGIS 9.3.1; ERSI 2009).

Lab Protocol and Analysis

Lichen samples were hand-cleaned of debris using powder-free nitrile gloves and sent to the University of Minnesota Research Analytical Laboratory, St Paul, MN for total ash and N analyses. At the laboratory, each sample was dried (65 °C), ground (stainless steel grinder with 20 mesh sieve), mixed, and then sub-sampled. Total nitrogen content was measured with a LECO Nitrogen Analyzer, Model No. FP-528. To determine % ash, subsamples of lichen material were dry ashed at 485 °C for 10-12 hours (for more details see: http://ral.cfans.umn.edu/plant.htm). Laboratory accuracy and precision was assessed every 10th sample with reference materials of known N values

from LECO standard reference material. Blanks with 0.0 N content were also run for calibration purposes.

Ions were extracted from IER tubes (Fenn and Poth 2004) with 1*M* KI (Potassium iodide—two extractions per tube) using an analysis procedure modified from Simkin et al. (2004). Phosphate, nitrate and sulfate ion concentrations in the extracts were analyzed with a Dionex high performance ion chromatograph (Thermo Fisher Scientific, Sunnyvale, CA, USA) and ammonium with a TRAACS 800 Autoanalyzer (Tarrytown, NY, USA). Concentrations were converted to loading amounts in kg ha^{-1} $year^{-1}$. Extracted ions from the control tubes were used for zero-calibration purposes in the lab. Contaminated samples from bird droppings were identified by high ammonium coupled with high phosphate ion concentrations and were deleted from further analysis.

Statistical Procedures

Lichen N concentrations were tested for within-plot variability between the same and between the two different species with two-sided paired t-tests. Within and between plot variation for both lichen concentrations and IER TF measurements were evaluated with the coefficient of variation (CV) and standard error (SE). Pearson's correlation was used to test relationships between variables. Simple linear regression (SLR) was used to test if N concentrations in one lichen species could be used to predict the other (R Development Core Team 2011). To increase sample size for comparison of lichen thalli concentrations, plots from the surrounding area were included. SLR was also used to approximate the relationships between lichen N concentrations and TF deposition kg ha^{-1} $year^{-1}$ for NO_3^-–N, NH_4^+–N and DIN.

Results

Variation Among Samples

Lab accuracy was acceptable with an average deviation of 0.03% N for material of known value with a slight bias for underestimation (< 0.001%). Lab precision tested with reference materials had a mean CV and SE of 0.023 and 0.009 for N. Remeasurements of lichen samples had a mean CV of 0.023 for % N (n=5 for % N).

Within plots, N concentrations in *U. lapponica* and *L. vulpina* were not different among paired samples of the same species and as a result were averaged. However, N concentrations between the two species were different and could not be averaged (n=12, p = <0.001 (% N)). The variation in *U. lapponica* N concentrations between plots was almost seven times the variation measured within plots (Table 2.1).

The variation of TF DIN measurements between plots was twice the variation measured within plots (Table 2.1). Within—plot TF DIN variation was expected due to natural differences in canopy density and processes (e.g. uptake of N). We did not use bulk sample data in any analyses because contamination resulted in insufficient replication of valid samples.

Table 2.1 Coefficient of variation (CV) for within and between plot measurements of lichen thalli. Usnea = *U. lapponica* and Letharia = *L. vulpina*.

	Within plot CV ±	*Between plot CV* ±	*Between vs. Within*
Usnea N	0.03	0.23	6.93
Letharia N	0.04	0.15	3.98
TF total N	0.32	0.78	2.42
TF NH_4^+–N	0.39	0.80	2.06
TF NO_3^-–N	0.52	0.92	1.76

N Concentrations in Lichen Thalli

U. lapponica and *L. vulpina* are fruticose epiphytes with overlapping distributions in the northern Rocky Mountains. Dry weight concentrations of N measured in *U. lapponica* were consistently higher but correlated to paired samples of *L. vulpina* [r =0.91, p = <0.001, n=12] (Figure 2.3). Table 2.3 shows approximated linear relationships. The mean differences between actual and predicted values of N in *U. lapponica* and *L. vulpina* were calculated to be 0.09 % and 0.094 %.

In our study area, N concentrations in *U. lapponica* ranged from 1.19 to 2.42 % with concentrations in the Boulder drainage up to double the concentrations measured in the Green River drainage (Table 2.2). N concentration in *U. lapponica* were negatively correlated (r = -0.81 after log transformation) with distance from the middle of the PA oil and gas development (Figure 2.4).

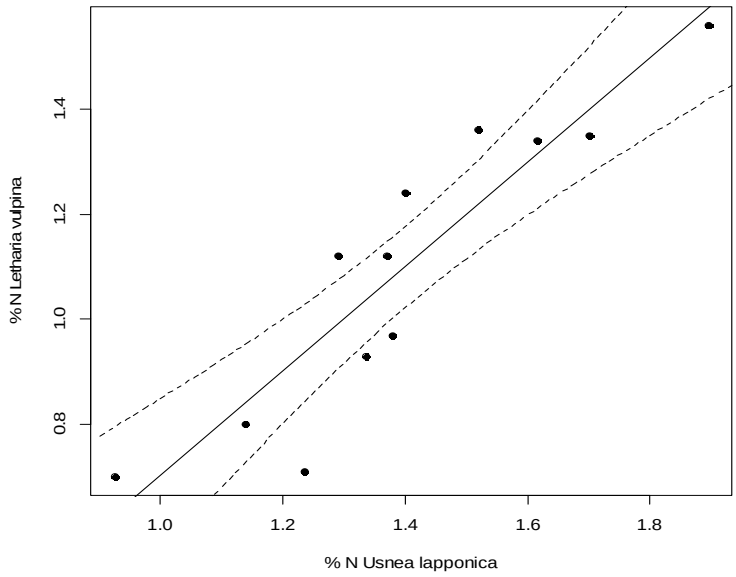


Fig 2.3 % N (dry weight) found in *Usnea lapponica* vs. % N (dry weight) found in, *Letharia vulpina*, shown as simple linear regression with 95% confidence bands (dotted lines. $r^2 = 0.83$, p = <0.001. In order to increase sample size plots from WY without IER monitors were included in the model.

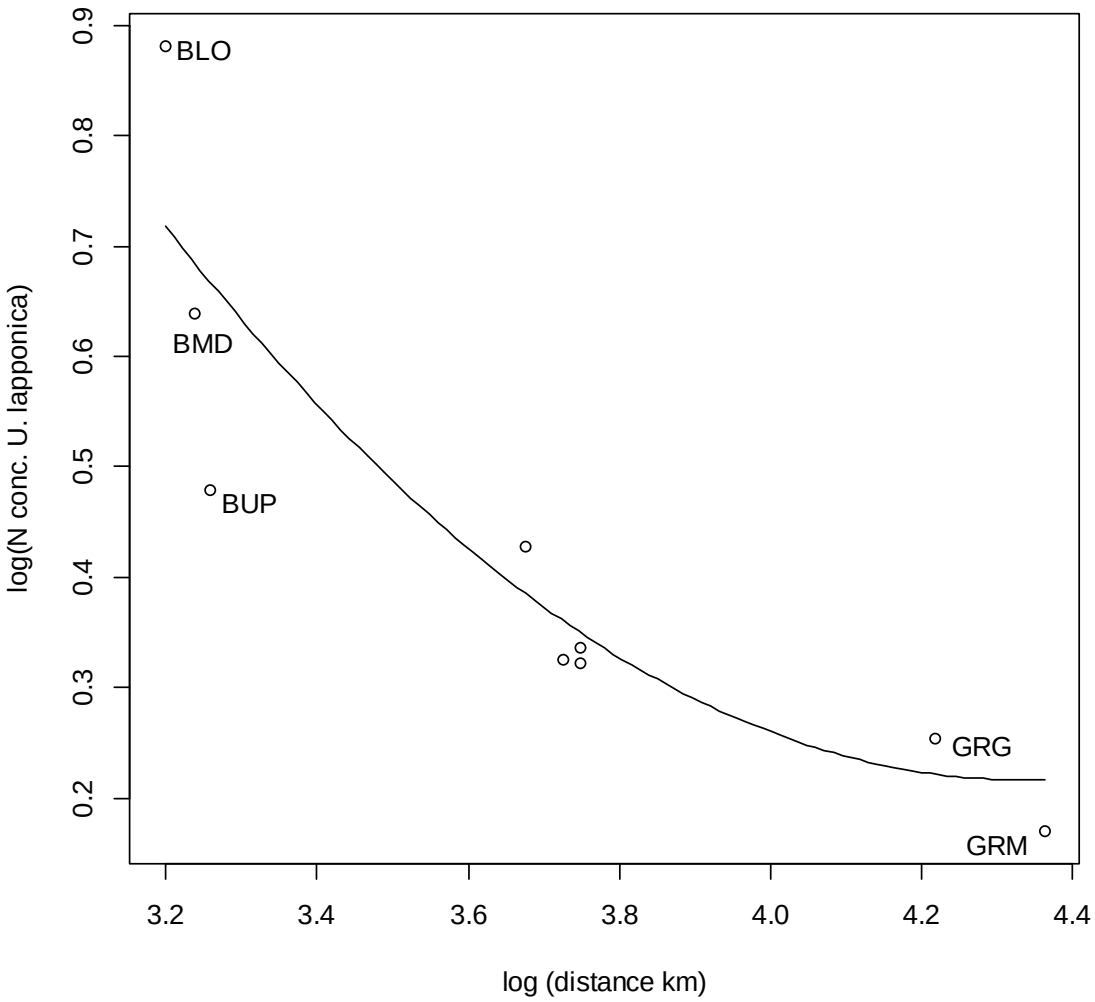


Fig. 2.4 Log transformation of both nitrogen concentrations in *U. lapponica* and distance from the center of the Pinedale Anticline natural project area. $r^2 = 0.76$, $p = 0.006$. Curve $= 7.54 - 3.38x + 0.39x^2$. GRM = Green River Moose Creek, GRG = Green River Gypsum Creek, BLO = Boulder low, BMD = Boulder middle, BUP = Boulder up.

Throughfall Measurements

Annual DIN in TF samplers ranged from 0.78 to 4.13 (kg N ha^{-1}) respectively. The plots with the highest measured annual NO_3^-–N and NH_4^+–N were located in the Boulder drainage. A maximum 5-fold increase between DIN measurements and an 11-

fold increase in NO_3^-–N was found between the Boulder drainage and other drainages (Figure 2.5). The plot with the lowest measured DIN deposition was located in the Big Sandy drainage (Figure 2.5; Table 2.2).

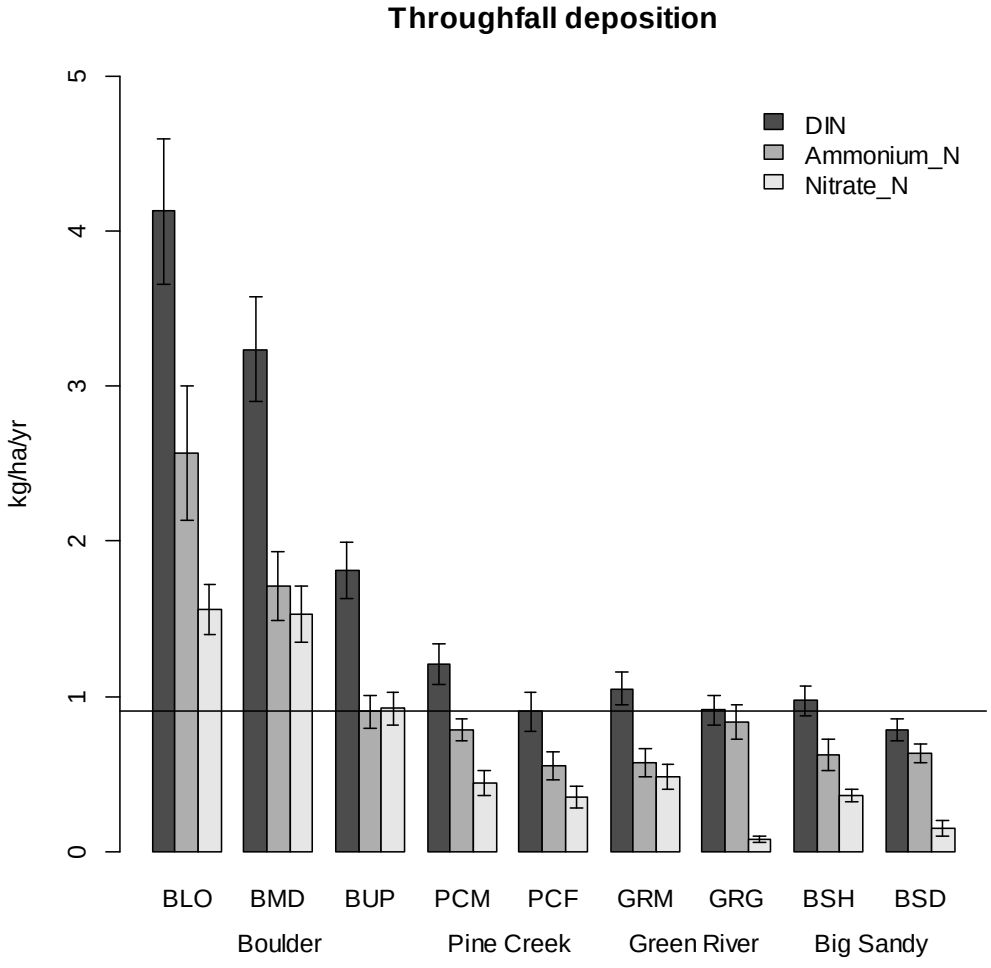


Fig. 2.5 Annual throughfall N deposition from four drainages in the Wind River Range. DIN refers to total dissolved inorganic N (ammonium N plus nitrate N). The horizontal line represents background levels of N deposition in the Northern Rockies as estimated by Sverdrup et al. (2012).

Table 2.2 Measures of throughfall N deposition (kg N ha^{-1} $year^{-1}$) and lichen N concentrations (% dry weight) in the Wind River Range. Elev = elevation, Dist. = distance to the middle of the PA gas field, DIN = dissolved inorganic nitrogen. SE = standard error. *Usn. = U. lapponica* and *Let. = L. vulpina.*

Plot	***Drainage***	***Elev. (m)***	***Dist. (km)***	***DIN***	***SE DIN***	***% N Usn.***	***% N Let.***
BLO	Boulder	2248	24.5	4.13	0.47	2.42	--
BMD	Boulder	2241	25.5	3.24	0.34	1.90	1.56
BUP	Boulder	2256	26.0	1.81	0.18	1.62	1.34
PCM	Pine Creek	2713	39.5	1.21	0.11	1.54	--
GRM	Green River	2661	78.5	1.01	0.13	1.19	--
BSH	Big Sandy	2800	41.5	0.97	0.10	1.39	--
GRG	Green River	2409	68.0	0.91	0.10	1.29	1.12
PCF	Pine Creek	2288	42.5	0.90	0.13	1.40	1.24
BSD	Big Sandy	2706	42.5	0.78	0.07	1.38	0.97

Lichen N vs. Throughfall N Deposition

Throughfall DIN deposition correlated exceptionally well with the concentrations of N in both *U. lapponica* and *L. vulpina* ($r = 0.96$, $r = 0.91$ respectively; Figure 2.6, Table 2.3). Though both reduced N and oxidized N were important in total TF N measurements, NH_4^+–N typically accounted for a larger portion of the total N measured and was a better fit model than NO_3^- –N with *U. lapponica* N concentrations (Figure 2.6). Preferential uptake for ammonium over nitrate in canopy TF and lichen thalli was not known for our study. The correlations between lichen N concentrations in *U. lapponica* and NH_4^+ and NO_3^-were $r = 0.96$ and $r = 0.85$ respectively. TF DIN decreased with distance from the middle of the PA oil and gas project areas ($r = -0.79$, $p = 0.01$ after log transformation). NO_3^- –N was more strongly correlated than NH_4^+–N with distance to project areas (-0.74 and -0.69 respectively after log transformations).

No relationship was found between % ash and any TF or concentration variables. Both tree species measured overlapping TF N deposition, which suggests that differences

between TF in Engelmann Spruce and Douglas-fir canopies in our study was minor. Due to the small sample size further investigation is warranted. It should be noted that sample size was higher with *U. lapponica* (n= 9) than *L. vulpina* (n= 5).

Table 2.3 Regression models and approximated equations. * = Note, since linear regressions are not inverse relationships (due to differences in horizontal and vertical errors) one equation could not be used to solve for both lichen species.

Model	*Regression equation*	R^2	*n*	*p-value*
TF DIN (y) vs. *U. lapponica* % N (x)	$y = 3.05x - 3.12$	0.91	9	<0.001
TF NH_4^+–N (y) vs. *U. lapponica* % N (x)	$y - 1.73x - 1.70$	0.92	9	<0.001
TF NO_3^-–N (y) vs. *U. lapponica* % N (x)	$y = 1.31x - 1.39$	0.75	9	<0.01
TF DIN (y) vs. *L. vulpina* % N (x)	$y = 4.26x - 3.77$	0.78	5	<0.05
TF NH_4^+–N (y) vs. *L. vulpina* % N (x)	$y = 1.70x - 1.19$	0.57	5	0.087
TF NO_3^-–N (y) vs. *L. vulpina* % N (x)	$y = 2.56x - 2.59$	0.84	5	<0.05
L. vulpina% N (y) vs *U. lapponica* % N (x)	$y = 0.99x - 0.29$	0.83	12	<0.001
U. lapponica % N (y) vs *L.* vulpina% N (x)*	$y = 0.48 + 0.84x$	0.83	12	<0.001

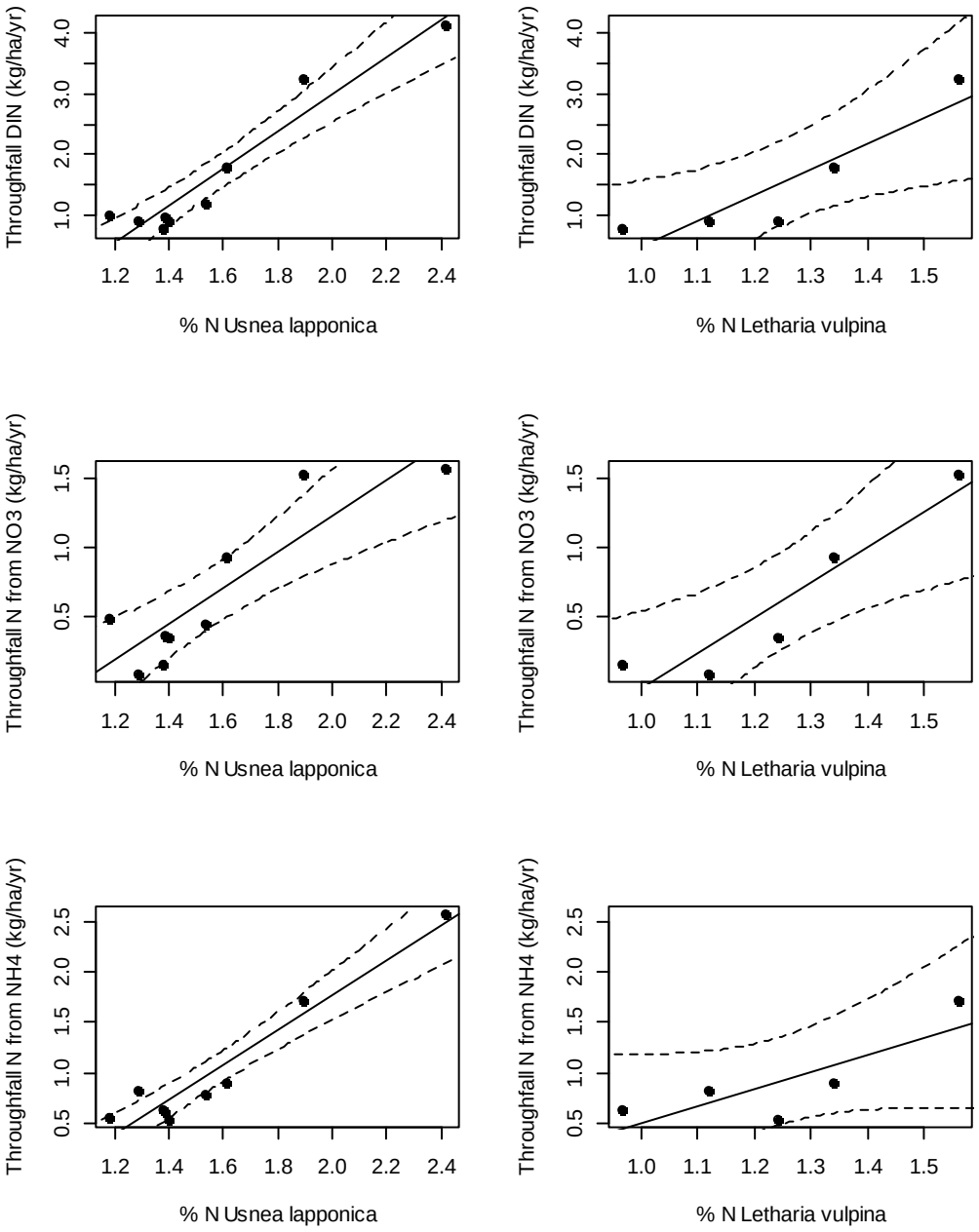


Fig 2.6 Simple linear regressions of % N in lichens vs. Throughfall (TF) dissolved inorganic N, TF NO_3^-–N, and TF NH_4^+–N deposition (kg ha^{-1} $year^{-1}$). Dotted lines represent 95% confidence bands.

Discussion

TF DIN Deposition

Five of the nine plots measured TF DIN deposition less than 1.0 kg ha^{-1} $year^{-1}$, reflecting background conditions (0.9 kg N ha^{-1} $year^{-1}$). In comparison, many parts of the United States and Europe receive N deposition loads above 10 kg ha^{-1} $year^{-1}$ (Fenn and Poth 2004; Fenn et al. 2008; Matejko et al. 2009; Mitchell et al. 2005; Pardo 2011). The WRR is a pristine N-limited environment with deposition levels close to pre-industrial background levels and is likely to respond to small increases in Nr deposition (Baron et al. 2011; Bobbink et al. 2010). TF DIN measurements may underestimate deposition since N can be absorbed by canopy processes as it filters through (Cape et al. 2010; Fenn et al 2008). Also we did not measure organic N, which in Rocky Mountain National Park, was found to account for 17% of the total wet N deposition (Beem et al. 2010).

Nr deposition in the Boulder drainage was elevated above all other drainages in the study area (1.8 – 4.13 kg N ha^{-1} $year^{-1}$). N concentrations of lichen thalli from the Boulder drainage were up to 2 times higher than all other lichen samples. N concentrations in *U. lapponica* and TF DIN were negatively correlated with distance to oil and gas project areas. The Boulder drainage is the closest measured drainage to the Jonah and Pinedale Anticline oil and gas project areas. In addition this drainage is bowl-shaped and has the tendency to trap air masses. Analysis of IMPROVE and meteorological data show high nitrate impact days in the Boulder drainage are associated with stagnant air days (Scott Copeland *per comm*.).

Possible Nr Sources

In 2010, annual NO_x emissions from oil and gas production and compressor stations in Sublette County were estimated at 4,315.6 tons (WDEQ: http://deq.state.wy.us/aqd/Actual%20Emissions.asp web accessed Jan. 2012). The amount of NH_3 associated with catalytic converter slip from oil and gas activities is unknown. Cattle grazing on USFS land and fertilizer use on private lands may contribute to local NH_3 inputs in Sublette County. Nitrate isotope analysis of ice cores from the Fremont Glacier in the Bridger Wilderness (1940 to early 1990's) indicated that vehicle emissions were the main source of NO_x in the WRR prior to energy development (Naftz et al. 2011).

Measurements from two DEQ monitoring stations centrally located within Sublette County indicate up to 50% of the wind flow comes from the NW and NNW. Though occurring less often, winds from SW and WSW are associated with higher NO_x and O_3 concentrations (WYDEQ 2011a; WYDEQ 2011b). Most of the oil and gas activity in Sublette County occurs S and SW of these two monitoring stations.

Salt Lake City, UT is a large urban center located just over 300 km to the SW of Sublette County. Annual NO_x emissions from Salt Lake County were estimated to be 38,106 tons in 2005 (UT DEQ 2009). Logan, UT and the Snake River Plain, ID , located W and SW of Sublette County, are areas of concentrated NH_y pollution (EPA: http://www.epa.gov/AMD/EcoExposure/ESRP.html web acessed Jan. 2012; NADP 2011) influenced by intense agricultural and feedlot operations (Strait et al. 2008). Most animal waste from feedlots deposit locally, but up to 30% of ammonia can be volatilized

into the atmosphere and transported long distances as NH_4^+, NH_4NO_3, N_2O, and ammonium sulfate ($(NH_4)_2SO_4$) (Howarth et al. 2002; Twigg et al. 2011; van Herk et al. 2003). Air masses that move through the Snake River Plain have been associated with poor air quality days in Hells Canyon (Geiser et al. 2008).

Reduced vs. Oxidized Nr Deposition

Oxidized forms of N (NO_x, N_2O, HNO_3) are associated with fossil fuel emissions whereas reduced forms (NH_y) typically come from agriculture (fertilizer application and animal waste) and fertilizer production (Fenn et al. 2003; Howarth 2008; Bobbink et al. 2010). Nr can be difficult to measure since molecules rapidly undergo multiple chemical transformations in the atmosphere, producing products with differing rates of deposition velocity (Twigg et al. 2011). For example, dry deposition from NO_x almost always occurs in the form of HNO_3 (Horii et al. 2006).

Ammonium and nitrate concentrations in TF measurements were correlated ($r=0.88$, $p = 0.002$), which suggests they have deposited together from ammonium nitrate (Boonpragob et al. 1989). Ammonium nitrates are found in fertilizers and explosives, but can also be formed in the atmosphere from the reaction of: $NH_3 + HNO_3 \rightarrow NH_4NO_3$ (Twigg et al. 2011), and while neither NH_3 nor HNO_3 are prone to long distance transport individually, combined as a particulate, they have the ability to travel hundreds of kilometers (Rubasinghege et al. 2011; Sheppard et al. 2011; Twigg et al. 2011).

If long-distance transport of NH_4NO_3 or other N compounds (NH_4^+, N_2O, NO_x) was the main sources of Nr deposition in the WRR study area, we would not expect such high loading of Nr in one drainage (Boulder), but rather a more uniform distribution of N

deposition. Two high elevation plots in different drainages (BSD and GRG) measured 4 and 10 times more NH_4^+–N than NO_3^-–N, further suggesting that long-distance transport of NH_4NO_3 or NO_3^- is not driving Nr deposition on the west flank of the WRR. Two IMPROVE sites located in the study area (BRID1 up the Pine Creek drainage and BOLA1 in the Boulder drainage) have also measured low levels of NH_4NO_3 (IMPROVE: http://vista.cira.colostate.edu/improve/). It is plausible that NO_x and NH_3 trapped by inversions in the Boulder drainage form HNO_3, a molecule with a high deposition velocity. Unfortunately, no ambient measurements of HNO_3 or NH_3 exist within the Boulder drainage.

Critical Loads

The critical load (CLO) of N deposition varies among different ecosystem components (Pardo et al 2011). In the Rocky mountains, Nr wet deposition above 1.5 kg ha^{-1} $year^{-1}$ has been linked to changes in species composition of diatom communities (Baron 2006; Baron et al. 2011; Saros et al. 2010), while wet + dry N deposition above 4.0 kg ha^{-1} $year^{-1}$ has been associated with episodic freshwater acidification and changes in mineralization, nitrification, and soil chemistry of subalpine forests (Baron et al. 1994; Baron et al 2011; Bowman et al. 2011; Fenn et al. 2008; Rueth and Baron et al. 2002; Saros et al. 2010; Williams and Tonnesson 2000). In addition to CLOs, empirical threshold values for elemental concentrations in lichens can be calculated to help gauge whether elemental concentrations fall within normal background levels or are considered elevated. For instance, in the Pacific Northwest, 97.5% quantiles (of the distribution of N values in lichens at clean sites) were used to estimate threshold N concentrations of 1.03

in *L. vulpina* (n=535) and 0.75 in *Usnea spp.* (n=40) (Fenn et al. 2008; The USFS National Lichen and Air Quality Database web accessed Jan. 2012: http://gis.nacse.org/lichenair/index.php?page=cleansite).

No CLOs have been ascribed to epiphytic macrolichen communities in the northern Rocky Mountains. In northwestern Forested Mountains of Oregon and Washington, CLO estimates, sensitive to precipitation regimes, range from 2.3 – 9.5 kg N ha^{-1} $year^{-1}$ (Geiser et al. 2010). A parallel study implemented in mixed conifer forests of California found a CLO of 3.1 kg N ha^{-1} $year^{-1}$ related to a known threshold value for N concentration in *L.vulpina* (1.03%). Noticeable decline in sensitive lichen species was also observed above this CLO (Fenn et al. 2008).

CLO models should account for forest type and climate, as these factors affect TF N deposition and N concentrations in lichen thalli. Different forest-types take up different amounts of N as it filters through the canopy (Bobbink et al. 2010; Cape et al. 2010, Gaige et al. 2007) and N concentrations in lichen thalli can be influenced by precipitation, temperature, and growth rates (Boonpragob and Nash 1989; Geiser et al. 2010; Jovan and Carlberg 2007). When the highest concentration value for *L. vulpina* (1.56) from the BMD plot is used in the mixed conifer forest model (Fenn et al. 2008), 7.76 kg N ha^{-1} $year^{-1}$ (± 2.4) is estimated, which is well above measured N deposition at this site (3.24 kg ha^{-1} $year^{-1}$ ±0.34).

Though we could not define a CLO, clear evidence of degraded lichen communities was observed at approximately 4.0 kg N ha^{-1} $year^{-1}$, including necrotic and bleached thalli and deformed and stunted growth. Thus, chronic levels of N deposition

under 4.0 kg N ha^{-1} $year^{-1}$ are likely to have negative impacts on lichen communities. The degree to which nitrogen deposition affects lichen depends on the sensitivity of the species along with the type, rate, and duration of N exposure (Gaio-Oliveira et al. 2005; Munzi et al. 2010; Sheppard et al. 2011). We were not able to differentiate whether damage was from reduced or oxidized Nr, both, or some other factor such as ozone. Further studies and manipulative experiments are needed to help solidify any CLO estimations.

Lichen Thresholds

A strong linear correlation between TF DIN and N concentrations in *U. lapponica* ($r^2 = 0.91$) allowed us to predict TF DIN from *U. lapponica* N concentrations. A relationship between *L. vulpina* and TF DIN also existed ($r^2 = 0.78$), but more samples are needed to validate this model. Nr measured at most plots in the WRR was near or below 1.0 kg $ha^{-1}year^{-1}$. These assumed background or "non-polluted" conditions are associated with 1.35% and 1.12 % N in *U. lapponica* and *L. vulpina* respectively. Both concentration estimates are above PNW designated thresholds. N concentrations in lichen thalli were matched to lower TF DIN in the WRR compared to studies along the West Coast (Fenn et al. 2007; Fenn et al. 2008). The PNW receives greater amounts of precipitation per year than the interior west; it is likely that soluble elements such as N and S are less concentrated in lichen thalli in the PNW due to more frequent leaching events (Boonpragob et al 1989; Geiser et al. 2010; Jovan and Carlberg 2007)

Conclusions

This study documented baseline canopy TF DIN deposition across the western front of the WRR. Nr deposition in the Boulder drainage is elevated, a 2 to 5-fold increase above other drainages was measured. Lichen communities were impacted in the Boulder drainage when Nr inputs were above 4.0 kg N ha^{-1} $year^{-1}$. We could not separate community response to reduced vs. oxidized Nr. Pine Creek and Green River drainages also measured TF DIN above estimated background conditions. Both reduced and oxidized forms of Nr were important components of DIN at most plots. Local sources are likely contributing to Nr deposition in the Boulder drainage. Measurements of ambient NH_3, HNO_3, and NO_2 in the Boulder drainage could help us better understand sources of Nr deposition.

Percent N concentrations in *Usnea lapponica* and *Letharia vulpina* thalli was correlated with N deposition measured by canopy throughfall monitors. More samples are needed to solidify the relationship with *L. vulpina*, but *U. lapponica* can be used as a cost effective means (~ $22.00/ sample for N lab analysis) to expand N monitoring in the WRR with special focus on the Class I Bridger Wilderness. N concentrations of about 1.3% in *U. lapponica* and 1.1 % in *L. vulpina* were the upper threshold of background (pre-industrial) conditions.

Species and ecosystem response to N varies with the quantity, type, and duration of N deposition (Sheppard et al. 2011). N pollution in Sublette County is liable to continue with the ongoing extraction of natural gas. It is important to continue monitoring N deposition in the WRR.

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CHAPTER THREE

EPIPHYTIC LICHEN INDICATION OF NITROGEN DEPOSITION AND CLIMATE CONDITIONS IN THE NORTHERN ROCKY MOUNTAINS

Contribution of Authors and Co-Authors

Manuscript in Chapter Three

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Contributions: Helped formulate and implement the study design, collected and analyzed the data, and wrote the manuscript.

Co-Author: David W. Roberts

Contributions: Helped formulate the study design and conduct data analysis, wrote R code for analysis, provided comments at all stages, and edited the manuscript.

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Contributions: Helped formulate the study design, provided comments at all stages, and edited the manuscript.

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Abstract

Climate change and nitrogen (N) deposition can negatively impact ecological communities in the northern Rocky Mountains (Idaho, Montana, and Wyoming). Epiphytic macrolichen bioindication is an economical tool that can maximize monitoring resolution of climate and pollution impacts. We used non-metric multidimensional scaling (NMDS) of lichen communities and generalized additive models (GAMs) to analyze important climate and pollution gradients. Temperature extremes and relative humidity were the variables most strongly related to lichen community composition as portrayed in the NMDS ordination. However, a nitrogen pollution signal was also identified independent of climate variables. We found marked differences in the distribution of eutroph (N-tolerant) and oligotroph (N-sensitive) lichen species. Eutrophic species from the genera *Physcia*, *Xanthomendoza,* and *Xanthoria* were associated with N deposition, low precipitation, and temperature extremes. Oligotrophic species were typically restricted to moist temperate regions. Accordingly, future use of oligotroph and eutroph indices for N indication should include considerations of climate conditions. Small increases in N deposition can alter the sensitive N–limited environments of the northern Rocky Mountains. Estimated N deposition ranged from <0.5 to 4.26 kg N ha^{-1} $year^{-1}$. We found clear evidence of degraded lichen communities at 4.0 kg N ha^{-1} $year^{-1}$ which indicates that critical loads (CLOs) of < 4.0 kg N ha^{-1} $year^{-1}$ are needed to protect sensitive biota in our study area. N deposition was highest around Bozeman, MT (Gallatin National Forest) and Pinedale, WY (Bridger-Teton National Forest). This research has practical applications for management and conservation purposes. The resulting model can serve to track changes in climate and N pollution related to lichen communities over time, identify probable sensitive or impacted habitats, and to provide key information for lichen conservation in northern Rocky Mountain conifer forests.

Keywords: Climate change; nitrogen deposition; monitoring; lichen; Rocky Mountains; critical load

Introduction

Change in climate patterns and increases in nitrogen (N) deposition from air pollution can negatively affect forest health in the Northern Rocky Mountains (Baron et al. 2011; Beem et al. 2010; EPA 1997; Saros et al. 2010). Assessing current conditions, monitoring changes, and predicting ecosystem responses to climate and air pollution can

be an expensive and challenging task. The use of biological indicators (bioindicators) provides an economical and practical means to maximize monitoring resolution, especially in remote areas and across vast landscapes (Jovan 2008).

Epiphytic lichens absorb the bulk of their nutrients from the air, lack a waxy cuticle and stomata (which, in plants, helps slow the absorption of pollutants), and have no roots (in which elemental transfer can take place). Because of these characteristics, lichens are highly sensitive to changes in habitat structure, climate, and air pollution, especially nitrogen- and sulfur-containing fertilizing and acidifying pollutants, and have been used successfully as bioindicators in numerous studies to evaluate forest health (Blett et al. 2003; Fenn et al. 2007; Fenn et al. 2008; Geiser and Neitlich 2007; Jovan and McCune 2005; Jovan and Carlberg 2007; Rogers and Ryel 2009; van Herk et al. 2003).

In forests of the northern Rocky Mountain states (Montana, Idaho, and Wyoming), total inorganic N deposition (reduced + oxidized, dry + wet) has recently been estimated between 0.5 and 5 kg N ha^{-1} $year^{-1}$ (Burns 2003; Grenon et al. –*in prep*; Grenon and Story 2009; Grenon et al. 2010), while historic loads are thought to be < 1.0 kg N ha^{-1} $year^{-1}$ (Sverdrup et al. 2012). Critical loads (CLO) are used to quantify the threshold deposition level below which no harmful effect will occur to an ecosystem component according to current knowledge (UBA, 2004). Thus CLO estimates will vary depending on the ecosystem component of interest. CLOs for diatom communities in sensitive Rocky Mountains lakes are estimated to be 1.5 kg wet N ha^{-1} $year^{-1}$ (Baron 2006; Baron et al. 2011; Saros et al. 2010). Total N CLO estimates for sensitive terrestrial ecosystem components in the Rocky Mountains range from 2.5 to 4.0 kg ha^{-1}

year^{-1} (Baron et al. 1994; Bowman et al. 2011; Rueth and Baron 2002). Baron et al. (2011) suggest current air quality standards are not stringent enough to protect sensitive ecosystems from the effects of atmospheric N.

Over the last century, average annual temperatures in the Northern Rocky Mountains have increased by 0.74 °C (1.33 °F), while annual precipitation has decreased between 0.79 and 2.84 cm (EPA 1997; NOAA National Climatic Data Centers Climate, Services, and Monitoring Division—web accessed March 2012). Decrease in precipitation or the transition of precipitation from snow to rain will result in less snow water equivalent (SWE) stored in the snowpack and thus lower stream flows in the summer. Rising summer temperatures in the northern Rocky Mountains has been linked to treeline encroachment on alpine vegetation (Hessel and Baker 1997), increased water stress—due to decreased SWE, and more intense fire seasons (EPA 1997). In the northern Rocky Mountains, little is known about biotic recovery from pollution and response to climate change, so it is important to establish baseline knowledge and study these systems before shifts occur (Schlesinger 2009).

The relationship of lichen community composition to environmental factors including site characteristics, climate, and air quality is largely unexplored in the northern Rocky Mountains. The scale of our study is likely to influence which environmental gradients are seen as important (Huston 1999; Jovan 2008; Will-Wolf et al. 2006). Small-scale studies, for example—forest-level, often identify tree or forest characteristics as the most important factors in determining patterns in lichen communities (Lesica et al. 1991; Nascimbene et al. 2009; Sillett et al. 2000). In contrast, studies that span broad

areas typically relate climate (temperature and moisture) and sometimes pollution gradients to patterns in lichen community composition (Jovan and McCune 2004, 2005; McCune et al. 2010; Neitlich et al. 1999).

We sought to answer the following questions: 1) what is the diversity and species richness of epiphytic macrolichens in northern Rocky Mountain conifer forests? 2) Can lichen indices or individual lichen species be used as indicators of climate and pollution conditions? 3) What are the important site, climate, and N deposition variables associated with gradients in lichen community composition? 4) What is the quantity and spatial distribution of N deposition in the study area?

To answer these questions we installed sixty-eight plots following the National Forest Inventory and Analysis (FIA) protocols (FIA P3 Field Guide 2011). Lichen communities were sampled for epiphytic macrolichen species and abundance. Lichen thalli from two species were analyzed for concentrations (% dry weight) of N and ash. Nonmetric multi-dimensional scaling (NMDS) is an unconstrained ordination technique and was used to model variability among lichen communities. Generalized additive models (GAMs) were used to fit environmental gradients to the ordination coordinates to assess variable importance and to assign a fitted value ('scores') to plots along the fitted gradients. These scores can be used to track changes in revisited plots over time and to predict environmental conditions of newly added plots. Lichen species were assessed for indicator value along environmental gradients by using weighted averages from species abundances.

Methods

Study Area and Plot Locations

This study is located primarily within the Environmental Protection Agency (EPA) designated Middle Rockies (Level III) ecoregion (Figure 3.1). EPA ecoregions divide North America into unique areas based on similarly functioning ecosystems and habitat patterns (Omernik 1995; 2004). The Middle Rockies ecoregion includes parts of Montana, Idaho, and Wyoming. The summers tend to be dry and the winters cold to severe. The majority of the precipitation falls as snow especially at higher elevations.

Sixty-eight 0.378 ha circular plots were established in the summers of 2008 (n=5), 2010 (n= 7), and 2011 (n= 56). Plot design and methodology followed the Forest Inventory and Analysis (FIA) protocol for "off-grid" plots so that the data could be incorporated into the FIA framework and used for future studies (FIA P3 Field Guide 2011). All plots were located on United States Forest Service (USFS) public lands in mountainous conifer forests. Plots were stratified by elevation, slope, aspect, and expected pollution gradients. Annual precipitation across plots ranged from 32 to 154 cm, elevations ranged from1108 to 3109 m and estimated N deposition ranged from <0.5 to > 4.0 kg ha^{-1} $year^{-1}$. Plot locations clustered around urban and agricultural centers in order to capture a pollution gradient. Plot locations were also chosen to minimize presence of hardwoods (< 5%), because hardwood stands are known to support different lichen communities than conifer stands (Martin and Novak 1999, Rogers and Ryel 2008). The dominant tree species were *Pinus contorta*, *Pseudotsuga menziesii, Picea engelmannii*, and *Abies lasiocarpa.*

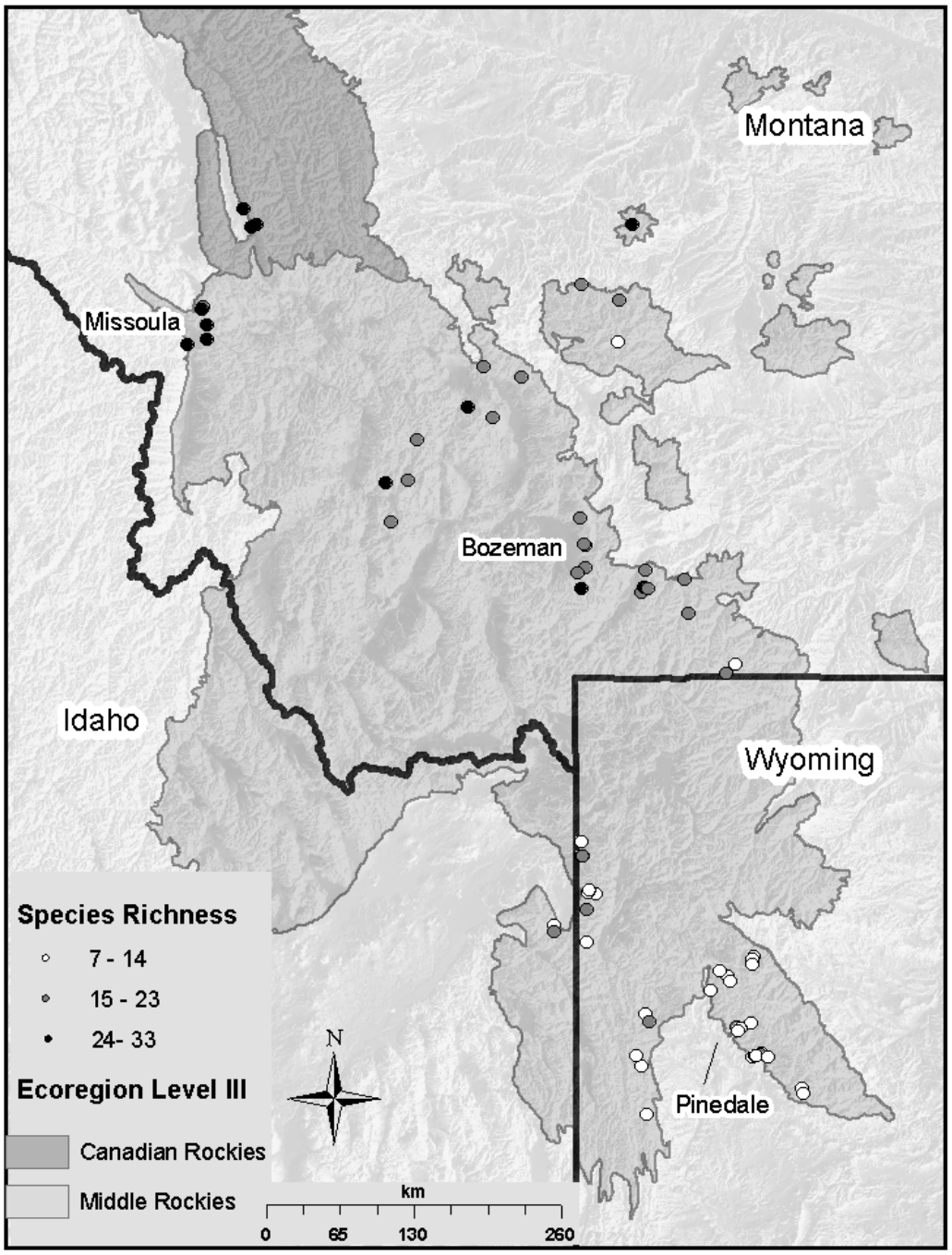


Fig. 3.1 Shows the range of species richness per plot. Plots in the Canadian Rockies ecoregion were installed because CMAQ (an N deposition model) indicated elevated N deposition.

Lichen Data

At each plot, separate samples of *Letharia vulpina* L. (Hue) and *Usnea lapponica* Vainio thalli (10 g/sample) were collected with powder-free nitrile gloves into sterile air-tight bags (Whirl-Pak® polyethylene) for elemental analysis following Geiser (2004). Each sample contains multiple thalli collected over the whole plot from a minimum of 6 substrates to ensure that a representative sample of the population (age and location) was collected. If only one species was present, two to three samples of that species were collected.

To record the composition of lichen communities, epiphytic macrolichens were collected over the whole plot from woody substrates above 0.5m. Sampling occurred for a minimum of 45 minutes and a maximum of 2 hours (FIA P3 Field Guide 2011). Each species was placed into a separate packet and sent to a certified lichenologist for identification. Taxonomic identification followed McCune and Geiser (2009) and McCune and Goward (1995). For each collected species, a corresponding abundance rating was recorded, where 1 = rare (≤ 3 thalli), 2 = uncommon (4 – 10 thalli), 3 = common (> 10 thalli, and species occupies less than half the available substrate), and 4 = abundant [>10 thalli, and species occupies over half the available substrate] (FIA P3 Field Guide 2011). In forests of Montana, McCune and Lesica (1992) found that accuracy with visual abundance ratings for epiphytic lichens were comparable to methods using continuous coverage.

Site Data

Site data collected at each plot included: latitude, longitude, elevation, aspect, slope, percent basal area of live trees, percent basal area of hardwood trees, tree size class, percent gap disturbance, shrub percent cover, and stand age. Slope was measured with a clinometer, tree age was measured by averaging the counted tree-rings for the three largest live trees on the plot, and basal area measurements were collected using a 20 factor prism to count trees based on diameter at breast height (DBH) at plot center and four subplots –measured in the cardinal directions 2/3 of the distance to the plot perimeter. A heat load index and direct incident radiation were calculated from aspect (McCune and Keon 2002):

eq.2.3.1

$$Heat\ load\ index = \frac{1 - \cos(\theta - 45)}{2}$$

Where θ = aspect in degrees.

eq.2.3.2

Direct incident radiation

$$= -1.467 + 1.582 * COS(A) * COS(B) - 1.5 * COS(C) * SIN(B) * SIN(A) - 0.262 * SIN(A) * SIN(B) + 0.607 * SIN(C) * SIN(B)$$

Where A = latitude, B = slope (degrees), and C = folded aspect (McCune and Keon 2002). We considered a total of 13 site, 12 air quality, and 7 climate variables in our modeling efforts.

Climate and Air Quality Data

The climate dataset was constructed from gridded data downloaded from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) (Daly and Taylor 2000) and extracted in ArcGIS (ArcGIS 9.3.1; ERSI 2009). PRISM employs point data and a digital elevation model (800m resolution) in algorithms to give continuous spatial information on climate data (4km resolution).

We considered: annual averages of precipitation, maximum temperature (Tmax), minimum temperature (Tmin), dew point, relative humidity (RH), relative humidity from mean annual maximum temperatures (RHmax) and relative humidity from mean annual minimum temperatures (RHmin). Climate variables represent annual averages from 1971-2000 except for dew point and RH (1999-2009).

Nine nitrogen deposition variables (kg ha^{-1} $year^{-1}$) were extracted from the Community Multiscale Air Quality model [CMAQ; 12 km grid resolution] (Byun and Schere 2006) in ArcGIS (ArcGIS 9.3.1; ERSI 2009): total nitrogen (includes wet and dry forms of reduced and oxidized N), total dry N, dry reduced N, dry oxidized N, total wet N, wet reduced N, wet oxidized N, total oxidized N, and total reduced N.

Percent N in *U. lapponica* thalli was used to estimate annual throughfall (TF) N deposition under forest canopies. Equation 2.3.1 was derived from co-located TF measurements at 9 off-grid plots in the southern part of our study area (Grenon et al. –*in prep*):

eq.2.4.1

$$TF\ N\ kg\ ha^{-1}\ year^{-1} = 3.05(\%\ N\ U.\ lapponica) - 3.12$$

Where TF = dissolved inorganic N measurements from ammonium and nitrate in canopy throughfall, an estimate of total N deposition. Adjusted $r^2 = 0.91$.

Eutroph and Oligotroph Indices

Sensitivity and response to N pollution differs among lichen species. As a result lichens are often placed into three categories—eutrophs, mesotrophs, and oligotrophs (McCune and Geiser 2009; Jovan 2008; Jovan and Riddell –*in review*). Eutrophs (also called nitrophytes, nitrophiles) tend to flourish in N–enriched environments, mesotrophs show little change across the deposition range in our study area, and oligotrophs (also called acidophytes, acidophiles) typically decline or vanish under high N deposition. Nitrogen pollution has been found to cause shifts in lichen communities—from oligotroph- to eutroph-dominated over relatively short time frames (< 8 years) (Larsen Vilsholm et al. 2009). Gadsdon et al. (2010) suggest proportion of lichen functional groups be used over count measures in lichen and N pollution studies. We created two indices, the percent of eutroph and oligotroph species present per plot— to see whether patterns existed with N deposition and other environmental variables. Species designations were based on published classifications from the Western United States (McCune and Geiser 2009; The United States Forest Service National Lichen & Air Quality Database and Clearinghouse: http://gis.nacse.org/lichenair/index.php?page=cleansite, web accessed- Feb. 2012; Jovan 2008, Jovan and Ridell—*in review*; McCune et al. 2010; Neitlich et al. 1999).

Lab Protocol and Analysis

Lichen samples were hand-cleaned of debris, insects, and other lichens with powder-free nitrile gloves and sent to the University of Minnesota Research Analytical Laboratory, St Paul, MN for total ash and N analyses. At the laboratory, each sample (n=201) was dried (65 °C), ground (stainless steel grinder with 20 mesh sieve), and then sub-sampled. Total nitrogen content was measured with a LECO Nitrogen Analyzer, Model No. FP-528. To determine percent ash, subsamples of lichen material were dry ashed at 485 °C for 10-12 hours (for more details see: http://ral.cfans.umn.edu/plant.htm). Laboratory accuracy and precision was assessed every 10^{th} sample with reference materials of known N values from the National Institute of Standards and Technology (NIST). Blanks with 0.00 N content were also run for calibration purposes.

Statistical Procedures

Relationships among continuous variables were evaluated with pair-wise scatter plots and Pearson correlation coefficient (R Development Core Team 2011). Simple linear regression was used to quantify the relationship between N concentrations in *L. vulpina* and *U. lapponica* thalli in order to estimate missing values in the dataset. We calculated species richness per plot (α-diversity) and across the whole study area [γ-diversity] (Whittaker 1972). All statistical analyses were completed with R.2.9.2 (R Development Core Team 2010).

Nonmetric Multidimensional Scaling (NMDS; Kruskal 1964) is a non-parametric, unconstrained ordination technique commonly used to detect gradients in community composition (McCune and Grace 2002, Minchin 1987). First, we created a matrix of

dissimilarities of the lichen communities using the Bray-Curtis (Bray and Curtis 1957) dissimilarity index. This matrix was subjected to NMDS (package = 'vegan'; Oksanen et al. 2011), and the coordinates of points in the NMDS were plotted to portray communities in multidimensional space. Ordination plot coordinates for each dimension are denoted as NMDS 1, NMDS 2, and NMDS 3.

Through an iterative process, NMDS seeks to minimize "stress", which is the square root of the sum of the squared differences between the ranked distance measures in the dissimilarity matrix and the ranked distance measure in the ordination space divided by the differences in the ordination squared (Kruskal and Wish 1978). We compared ordination solutions from 400 runs of random starts to maximize the probability of finding the best global solution (McCune and Grace 2002). Final ordination selection was based on the solution with lowest stress (reported as a percent) and highest correlation between matrix dissimilarities and ordination distances (package = 'labdsv', Roberts 2010).

Generalized additive models (GAMs) (Hastie and Tibshirani 1986, 1990; package = 'mgcv', Wood 2011) were fit to the ordination coordinates to identify important site, climatic, and air quality gradients (function surf, package = 'vegan', Oksanen et al. 2011). GAMs have the unique ability to fit flexible surfaces or lines to represent complex (nonlinear) relationships through the use of smoothing algorithms (Hastie and Tibshirani 1986, Hastie and Tibshirani 1987; Wood 2011). We used individual smoothing splines to allow flexible fits of the environmental variables along community gradients (the axes of the ordination).

GAMs are a data-driven compromise between parsimony and goodness - of -fit with a goal to minimize residual deviance and maximize deviance explained (D^2), selecting the appropriate model degrees of freedom of the smoothing with a penalty function.

eq.2.7.1

$$D^2 = \frac{null\ deviance - residual\ deviance}{null\ deviance}$$

Where D^2 = Deviance explained (in Gaussian models $D^2 = r^2$). The specific GAM function we used also estimates adjusted r^2 which incorporates penalties for actual degrees of freedom used. To avoid over fitting variables we used a gamma of 1.4 which increases the penalty for degrees of freedom used (Wood 2006). Models were fit with Gaussian or Poisson distributions as appropriate.

Latitude, longitude, and elevation were overwhelmingly important in ordination space. From a physiological standpoint, however, these variables are not directly tied to lichen community composition. To better interpret the ordination from an ecological perspective, we modeled (GAMs) latitude, longitude, and elevation as a function of climate variables. The best models were selected based on results from AIC and ANOVA (using Chi square test).

All plots were assigned a fitted value or 'score' along individual fitted GAM surfaces for each climate or pollution gradient of interest (package = 'vegan'; Oksanen et al. 2011). Lichen species were identified as indicators for climatic and pollution conditions based on weighted averages from species abundance codes to calculate a weighted mean (package = 'vegan', Oksanen et al. 2011).

Results

Lichen Diversity

A total of 88 different species of epiphytic macrolichens was collected from 68 plots in the Northern Rockies (γ diversity). Lichen species richness within plots ranged from 7 to 33, with a mean richness (α diversity) of 16.79. Of the 88 species, 55 occurred fewer than 10 times and 24 were found once (Appendix A). The two target species analyzed for thalli concentrations—*L.vulpina* and *U. lapponica*—were found on 66 and 63 of the plots respectively. The most diverse genera were *Bryoria*, *Cetraria*, *Hypogymnia*, *Melanelia*, *Phaeophyscia*, *Physcia*, *Usnea*, and *Xanthoria* (Appendix A).

Geographically, plots with high species richness (≥20) were located in northern latitudes at mid to low elevations while plots with low species richness (≤10) were located at the highest elevations and in southern latitudes (Figure 3.1).

N Concentrations in *L. vulpina* and *U. lapponica*

Lab accuracy and precision for lichen thalli concentration were acceptable with an average standard deviation of 0.03% N for NIST certified peach and apple leaves. Underestimation of samples averaged < 0.001%. Nitrogen concentrations from lichen thalli ranged from 0.70 – 2.13 % in *L. vulpina* and 0.93 – 2.42 % in *U. lapponica*. *L. vulpina* and *U. lapponica* were not available for thalli collections at all plots (missing plots n=9 and n=14 respectively). A strong linear relationship was measured between % N concentrations in paired samples of the two lichens ($r = 0.85$, $p = <0.001$). Linear equations were employed to approximate missing values of *L. vulpina* and *U. lapponica*

N concentrations (Figure 3.2). When compared, the mean difference between actual and predicted % N values of *L. vulpina* and *U. lapponica* were 0.10 % N and 0.11% N respectively (n = 45, CV 0.007 and 0.057). From here on we report only % N from *L. vulpina* since the two species showed correlated response patterns, and *L. vulpina* required fewer predicted values (with lower error) and is more widely used in other studies (Fenn et al 2008; Jovan and Carlberg 2007; Nascimbene et al. 2008).

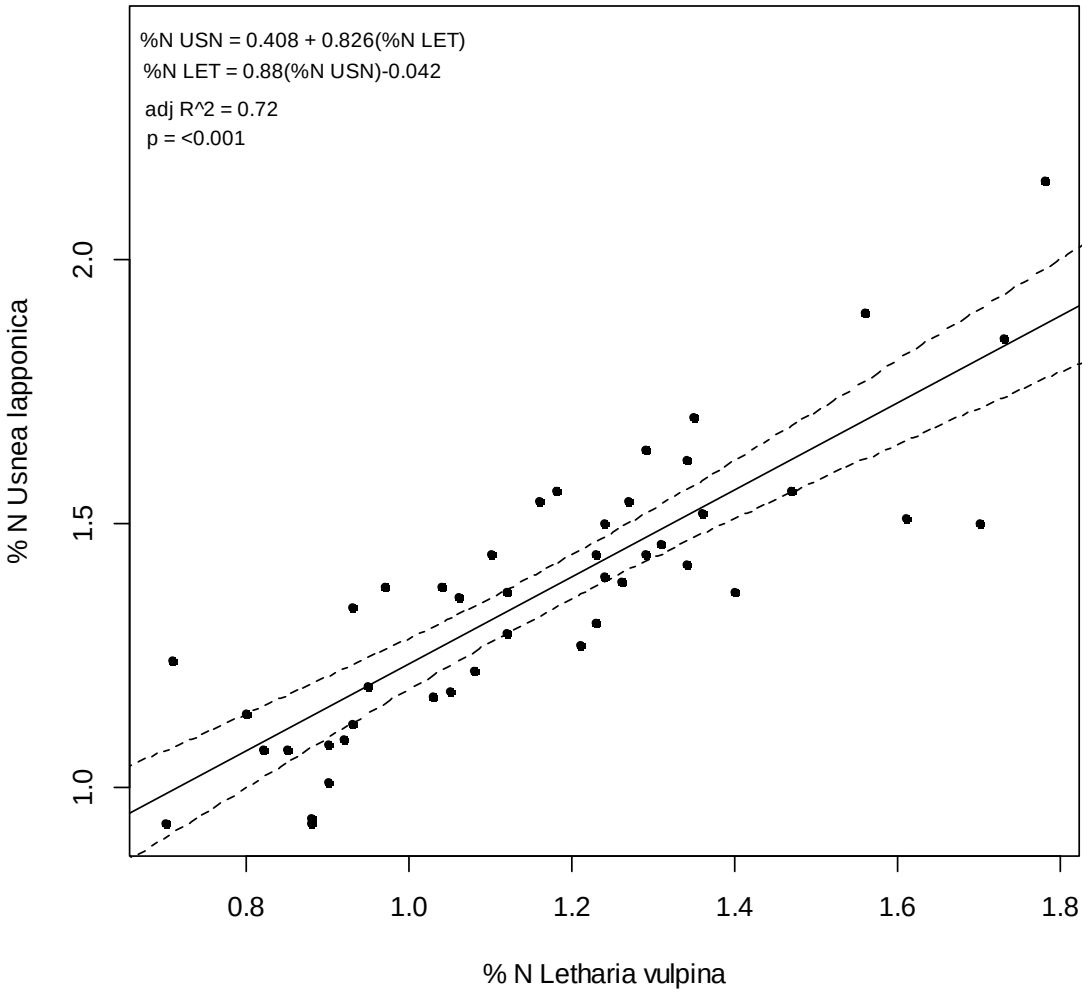


Fig. 3.2 Shows the relationship between % N measured in paired samples of *L. vulpina* and *U. lapponica* (n=45). The dotted lines show 95% confidence bands, adj R^2 = the adjusted r^2 value.

Estimated N Deposition and CMAQ N

Nitrogen deposition estimated from *U. lapponica* concentrations ranged from <0.5 to 4.26 kg ha^{-1} $year^{-1}$, and modeled N deposition from CMAQ ranged between 1.83 and 3.45 kg ha^{-1} $year^{-1}$ (Table 31); these two N estimates lacked spatial correlation (Figure 3.3). The lowest predicted N deposition value from CMAQ was 1.83 kg N ha^{-1} $year^{-1}$ whereas 5 throughfall N deposition measurements co-located with our plots (in WY), measured ≤ 1.0 kg N ha^{-1} $year^{-1}$ at 5 plots (Grenon –*in review*).

Table 3.1 Comparison of N deposition estimated by CMAQ to N deposition based on % N *U. lapponica* (calibrated to throughfall measurements). Total N = oxidized + reduced (both wet and dry). r = Pearson correlation statistic.

Nitrogen variable	***CMAQ***	***estimated N***	***CMAQ vs. estimated N***	
	kg ha^{-1} $year^{-1}$		**r-value**	**p-value**
Total N	1.83 – 3.45	<0.5 – 4.26	<0.00	0.28
Oxidized N	0.81 – 1.9	<0.5 – 1.78	-0.13	0.04
Reduced N	0.68 – 2.16	<0.5 – 2.49	0.05	0.66

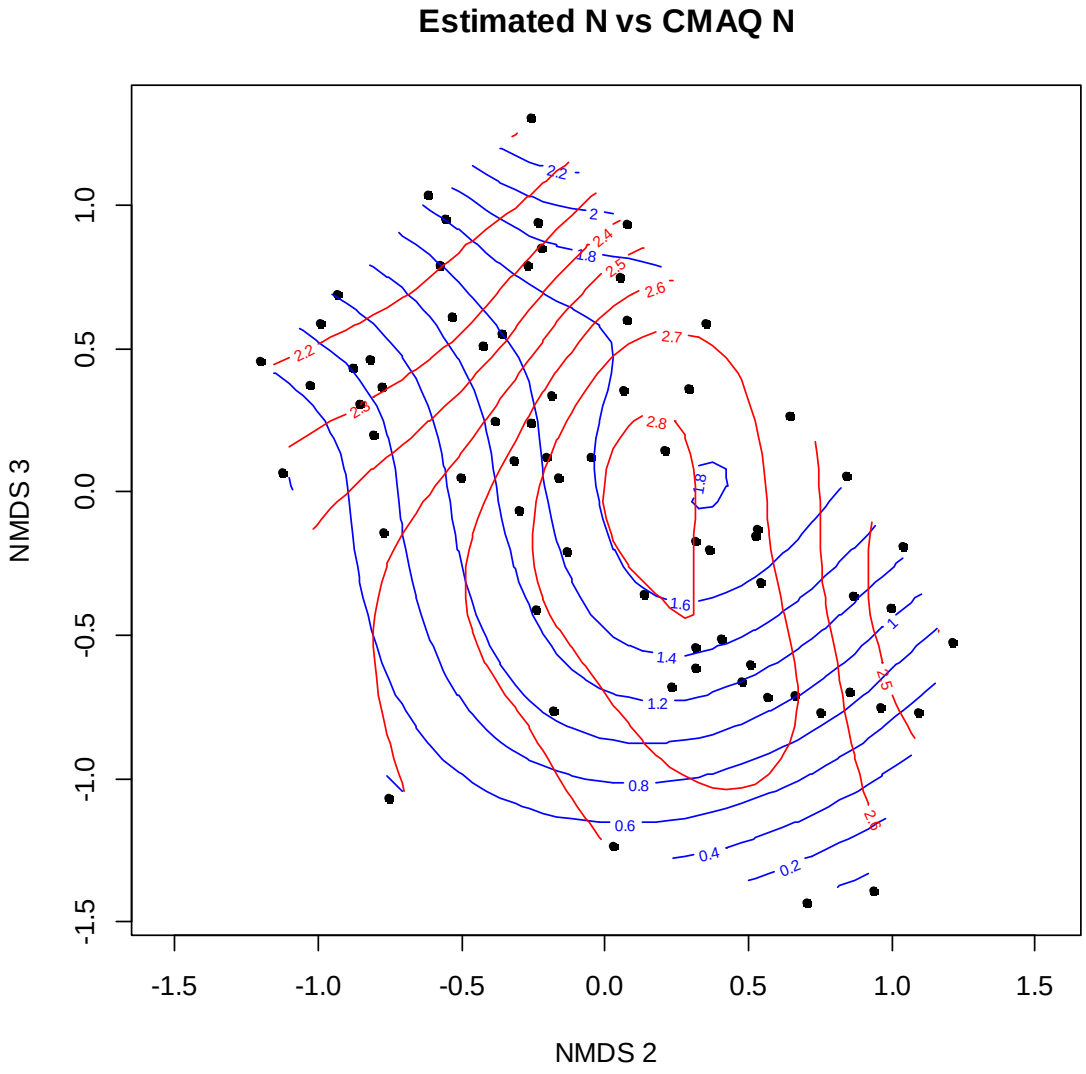


Fig. 3.3 NMDS ordination (axes 2 and 3) with two fitted GAM surfaces: blue lines= estimated N deposition kg ha^{-1} $year^{-1}$ from lichen thalli concentrations and red lines = N deposition from CMAQ. Note the two different predictions of N deposition occur along two different community gradients (represented by cross-hatched lines). N deposition includes total annual estimates (wet, dry, oxidized, and reduced).

Climate Variable Correlations

Latitude and longitude were correlated with elevation, temperature, and moisture gradients (Figure 3.4). The strongest correlations existed between elevation and Tmax ($r = -0.87$, $p = <0.001$) and between dew point and Tmin ($r = 0.82$, $p = <0.001$; Figure 3.4). Many climate variables were interrelated (e.g. mean dew point and RH). Site variables showed no strong correlations.

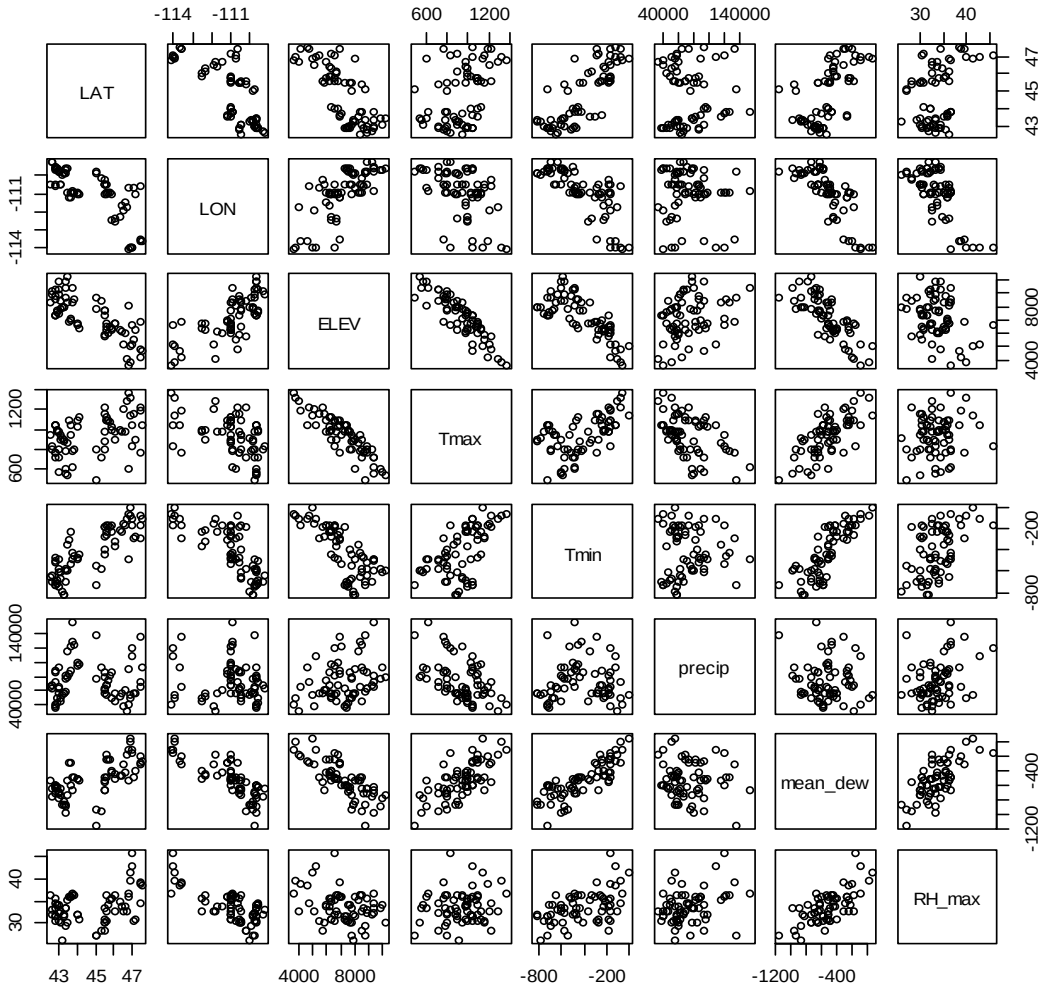


Fig. 3.4 Paired plots show correlations between topographic variables latitude (LAT), longitude (LON), elevation (ELEV) and climatic variables Tmax, Tmin, precipitation (precip), dew point (mean_dew), and RHmax.

Eutroph and Oligotroph Indices

Table 3.2 lists lichen species included in the eutroph and oligotroph indices. Many species included in the literature-based indices were also found to be individually associated with high or low nitrogen concentrations in lichen thalli (Table 3.2).

Table 3.2 Eutroph and oligotroph species based on literature. Bold names represent species that were also associated with high† and low ¤ %N in lichen thalli (based on weighted averages). * = species not mentioned in the literature, but included from personal observation.

category	***Lichen species***
Eutroph	*Candelaria pacifica, Melanohalea exasperatula,* ***M. subolivacea****†, Parmelia sulcata, Phaeophyscia nigricans,* ***P. orbicularis****†, Physconia americana, P. enteroxantha, P. isidiigera, P. perisidiosa,* ***Physcia adscendens****†,* ***P. aipolia****†,* ***P. biziana****†,* ***P. dimidata****†, P. stellaris, P. tenella, Xanthoria candelaria,* ***Xanthomendoza fallax****,* ***X. fulva****†, X. galericulata,* ***X. montana*****†, X. oregana, Xanthoria parietina, X. polycarpa*
Oligotroph	***Ahtiana sphaerosporella****¤,* ***Alectoria imshaugii****¤,* ***A. sarmentosa****¤,* ***Bryoria fremontii****¤, B. fucellata, B.glabra, B. pseudofuscescens,* ***B.simplicior*****¤, Cetraria Canadensis,* ***C.merrillii****¤, C orbata,* ***C platyphylla****¤,* ***Esslingeriana idahoensis****¤, Hypogymnia apinata, H. imshaugii,* ***H. metaphysodes****¤,* ***Melanohalea exasperata****¤, Nodobryoria abbreviata,* ***N. oregana****¤, Parmeliopsis ambigua,* ***P.hyperopta*****¤, Usnea filipendula.*

Percent eutroph richness and percent oligotroh richness were negatively correlated (r = -0.74; Figure 3.5) and exemplified opposing correlations with climate and N variables (Table 3.3). Percent eutroph richness decreased with northern latitudes and western longitudes. Positive correlations existed among % eutroph richness, % ash and % N in *L.vulpina* (Table 3.3). Total species richness decreased with elevation and increased with latitude, temperature and average dew point.

Table 3.3 Correlations between selected variables and total species richness (sp. rich), percent of total richness as oligotrophs (% Oli.rich), and percent of total richness as eutrophs (% Eu.rich) found per plot.

Variable	Pearson's correlations (r)		
	Sp.rich	%Oli rich	% Eu rich
Latitude	0.80	0.61	-0.63
Longitude	-0.74	-0.60	0.45
Elevation	-0.82	-0.31	0.32
% ash	-0.48	-0.56	0.45
% N *Letharia*	-0.04	-0.43	0.40
RH maxtmp	0.40	0.44	-0.18
Tmin.	0.71	0.38	-0.37
Dew point	0.67	0.32	-0.23
Precipitation	-0.24	0.23	-0.06
Sp. rich	1.00	0.45	-0.47
% oli rich	0.45	1.00	-0.74
% eu rich	-0.47	-0.74	1.00

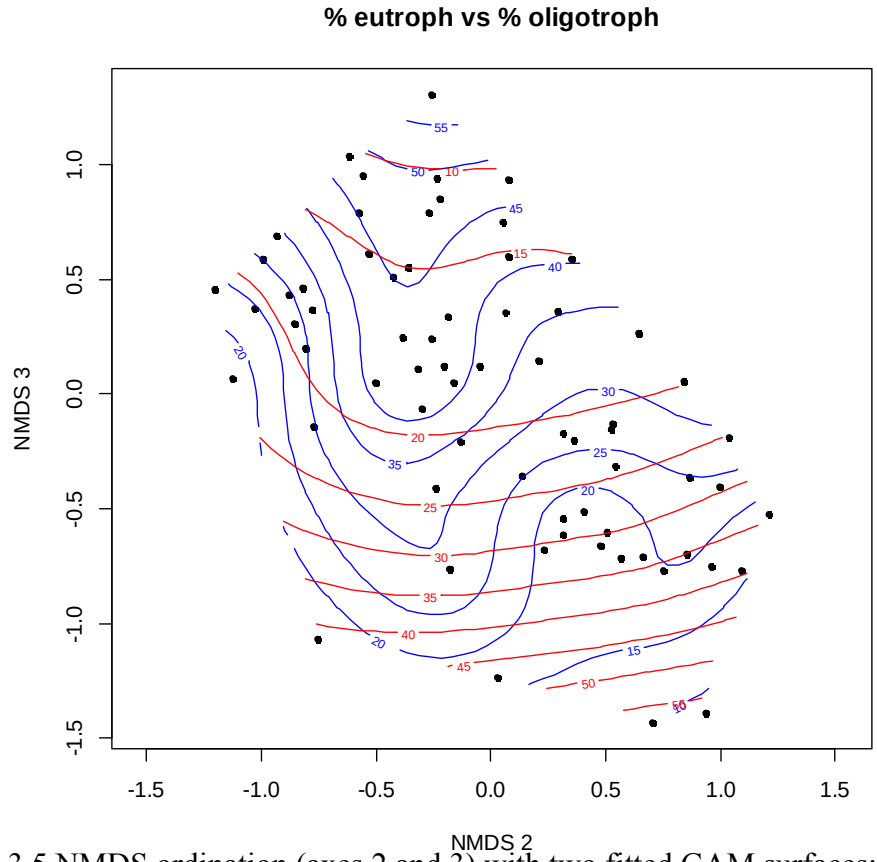


Fig. 3.5 NMDS ordination (axes 2 and 3) with two fitted GAM surfaces: blue lines = % eutroph richness and red lines = % oligotroph richness. Percent oligotroph richness increases towards the bottom right whereas % eutroph richness increases towards the top of the figure.

Indicator Species—Weighted Scores

Similar species were associated with low % N from *L. vulpina*, high precipitation, moderate temperatures, and high RH max (Table 3.4). The strongest indicators of elevated N deposition were members of the genera *Physcia* and *Xanthomendoza*, but these species also were associated with low precipitation and temperature extremes (Table 3.4).

Table 3.4 Lists of lichen species associated with environmental variables represent the weighted averages (package = 'vegan' Oksanen et al. 2011). Superscript numbers = the order of association with gradients with the top 5 species in bold, kg ha^{-1} $year^{-1}$= estimated from % N in *U. lapponica*. Rare species which occurred < 4 times were not included.

Variable	***Indicator species***
> 1.5 kg ha^{-1} $year^{-1}$ **> 1.25** % N *L.vulpina*	*Cetraria pinastrii*[9], *Melanohalea subolivacea*[7], *Phaeophyscia orbicularis*[8], *Physcia aipolia*[10], ***P. biziana***[3], ***P. dimidata***[1], *Usnea hirta*[6], ***Xanthomendoza fallax***[2], ***X. fulva***[5], ***X. montana***[4]
<1.5 kg ha^{-1} $year^{-1}$ **<1.25** % N *L.vulpina*	***Ahtiana sphaerosporella***[3], ***Alectoria imshaugii***[2], *A.sarmentosa*[7], ***Bryoria capillaris***[1], *B. fremontii*[11], *B. simplicior*[9], *Cetraria platyphylla*[8], ***Esslingeriana idahoensis***[4], *Hypogymnia metaphysodes*[10], *Melanohalea exasperata*[6], *Nodobryoria oregana*[12], ***Parmeliopsis hyperopta***[5]
Precipitation >80 cm	***Ahtiana sphaerosporella***[1], ***Alectoria imshaugii***[2], *A.sarmentosa*[8], ***Bryoria capillaris***[5], *B. fremontii*[6], *B. pseudofuscescens*[7], *B. simplicior*[9], ***Nodobryoria oregana***[4], ***Parmeliopsis hyperopta***[3], *Xanthoria polycarpa*[10]
Precipitation <65 cm	*Bryoria furcellata*[10], *Melanohalea exasperata*[8], *Physcia aipolia*[9], ***P. biziana***[1], ***P. caesia***[5], *P. dimidata*[6], *Usnea hirta*[7], ***Xanthomendoza fallax***[3], ***X. montana***[4], ***Xanthoria candelaria***[2]
Tmax >10.8 °C	***Cetraria Canadensis***[4], *C. platyphylla*[6], ***Esslingeriana idahoensis***[3], *Parmelia hygrophila*[8], ***Physcia aipolia***[2], ***P. dimidata***[5], *Usnea hirta*[7], ***Xanthoria candelaria***[1]
Tmin < - 4.5 °C	***Bryoria lanestris***[2], *Candelaria pacifica*[8], *Melanohalea elegantula*[10], ***M. exasperata***[3], *M. subelegantula*[6], *Physcia adscendens*[7], ***P. biziana***[4], ***P. tenella***[5], ***Xanthomendoza fallax***[1], *X. montana*[9],
RH max >36	*Ahtiana sphaerosporella*[7], ***Alectoria imshaugii***[1], *A.sarmentosa*[8], ***Bryoria capillaris***[4], ***Cetraria merrellii***[5], *C. orbata*[9], ***C. platyphylla***[2], ***Esslingeriana idahoensis***[3], *Nodobryoria oregana*[6]

NMDS Community Analysis
with Fitted GAM Surfaces

We found a three-dimensional solution with a stress of 12.4 was a good approximation of the full dimensional space (r = 0.937). The dissimilarity matrix used in the ordination included all lichen species in our dataset. The removal of rare species did not enhance our understanding of the model and detracted from the ability to track changes in climate and pollution gradients.

Latitude, longitude, and elevation were overwhelmingly the most important gradients isolated by the ordination. These variables offer little in terms of direct ecological interpretation, but were strongly correlated to climate gradients (Figure 3.4). GAMs indicated that temperature, RHmax, and dew point were the most important explanatory variables (Table 3.5).

Table 3.5 GAMs created to increase ecological interpretability of latitude (LAT), longitude (LON), and elevation (ELEV). Variables represent annual means: Tmin = minimum temperature, Tmax =maximum temperature, RH max = relative humidity derived from maximum temperature and dew = mean dew point. D^2 = deviance explained, adj. r^2 = the adjusted r^2, gamma = 1.4 model family = Gaussian, s = individual smoothing splines, and ~ = "as a function of". Model selection was based on AIC and ANOVA.

Variable	GAMs	D^2	adj. r^2
latititude	LAT~ s(Tmin) + s(RH max) + s(dew)	80.9	0.77
longitude	LON~ s(Tmin) + s(Tmax) + s(RH max)	91.3	0.89
elevation	ELEV~ s(Tmin) + s(Tmax) + s(RH max)	92.1	0.91

Climate variables from Table 3.5 were fit independently to the ordination axes to assess their importance in the ordination space. The most important explanatory variable found, Tmin was included in a GAM. Other variables were then tested for significance

(as residuals of Tmin) and added to the model—in order of effect size. The resulting model (*eq.3.7.1*) modeled out the importance of latitude, longitude, and elevation in the ordination and was used to test remaining variables for significance.

eq.3.7.1

$$Model\ 1 - gam(var \sim s(Tmin) + s(Tmax) + s(RH\ max))$$

Where gam = generalized additive model function (package = 'mgcv', Wood 2011), var = the variable to be tested (i.e. precipitation) after Tmin, Tmax, and RHmax were considered (Figure 3.6).

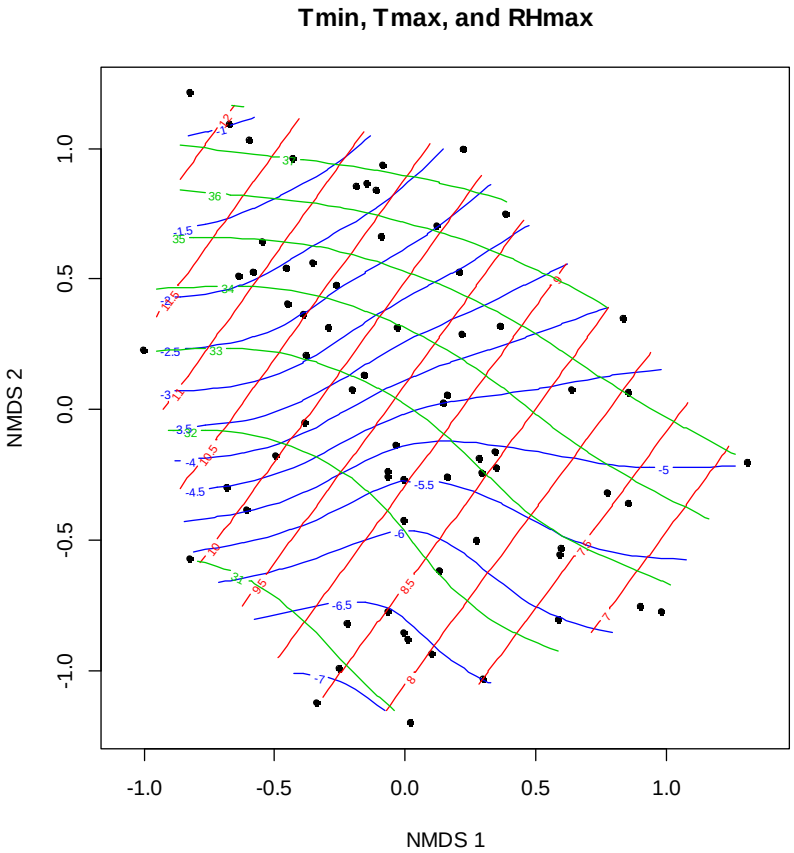


Fig 3.6 NMDS ordination (axes 1 and 2) with fitted GAM surfaces: blue lines = Tmin, red lines = Tmax, and green lines = RH max. The cross-hatch patterns show that variables are not correlated and are important along different community gradients in the ordination.

The residuals of Model 1 were then fit to the coordinates of the ordination (Table 3.6).

eq.3.7.2

$$Model\ 2 = gam(residuals(model1) \sim s(NMDS\ 1) + s(NMDS\ 2) + s(NMDS\ 3)$$

NMDS 1, 2, and 3 represent the ordination coordinates. After accounting for climate, only a few variables were significant along community gradients in ordination space. Precipitation was significant along axis 1 and longitude was significant along axis 1 and 2. Percent oligotroph and eutroph indices were negatively correlated with one another along axis 3 (Figure 3.5). Percent N in *L.vulpina* was significant along axis 3 and had a positive relationship with percent eutroph richness along this axis (Table 3.6; figure 3.7). Latitude was important along axis 3. CMAQ and site variables were not significant with the exception of percent shrubs, but the r^2 value was low and we will not discuss it further. Pearson's correlations between explanatory variables and axes were used to signify a positive or negative relationship (Table 3.6).

Table 3.6 Shows the adj. r^2 value for variables when fitted independently to the ordination (adj. r^2 A) and when fit with *eq. 3.7.1* and *eq. 3.7.2* (adj. r^2 B). NMDS axes and D^2 = deviance explained values are from eq. 3.5.1. p-values: * = <0.05, ** = <0.01, *** = <0.001. + / - = positive or negative correlation (Pearson's) with ordination surface respectively.

Variable	***adj. r^2A***	***adj. r^2B***	***D^2***	***Importance along NMDS axes***		
				1	**2**	**3**
Latitude	0.87	0.28	32.2	…	…	*** -
Longitude	0.76	0.12	15.7	* -	* -	…
Elevation	0.75	0.17	22.0	…	…	…
% N *L. vulpina*	0.28	0.24	32.1	…	…	* +

Table 3.6 Continued						
Precipitation	0.41	0.21	28.8	** +	…	…
% eutroph richness	0.77	0.44	49.5	…	** -	*** +
% oligotroph richness	0.70	0.35	40.2	* -	…	*** -
Tmin	0.64	…	…	…	…	…
Tmax	0.58	…	…	…	…	…
RH max	0.35	…	…	…	…	…

Distribution of N Deposition

The five highest N deposition estimates (2.28 – 4.26 kg ha^{-1} $year^{-1}$) were from Pinedale, WY and Bozeman, MT (The Bridger-Teton and Gallatin National Forests respectively). Two other plots had estimated N deposition greater than 2.0 kg ha^{-1} $year^{-1}$, one on the Lewis and Clark NF and one on the Carabou-Targhee NF (Figure 3.8).

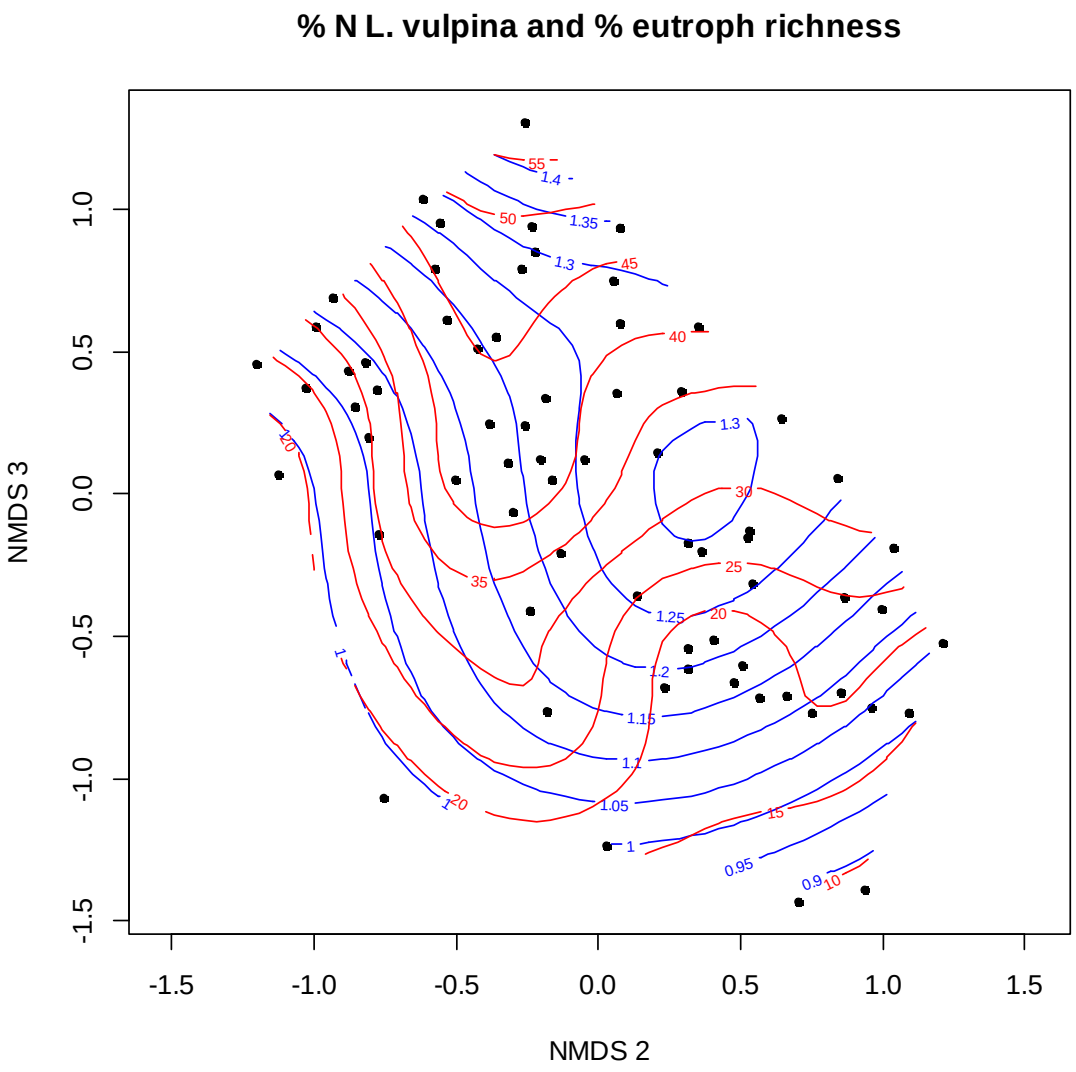


Fig. 3.7 NMDS ordination (axes 2 and 3) with fitted GAM surfaces: blue lines = % N *L. vulpina*, red lines = % eutroph richness.

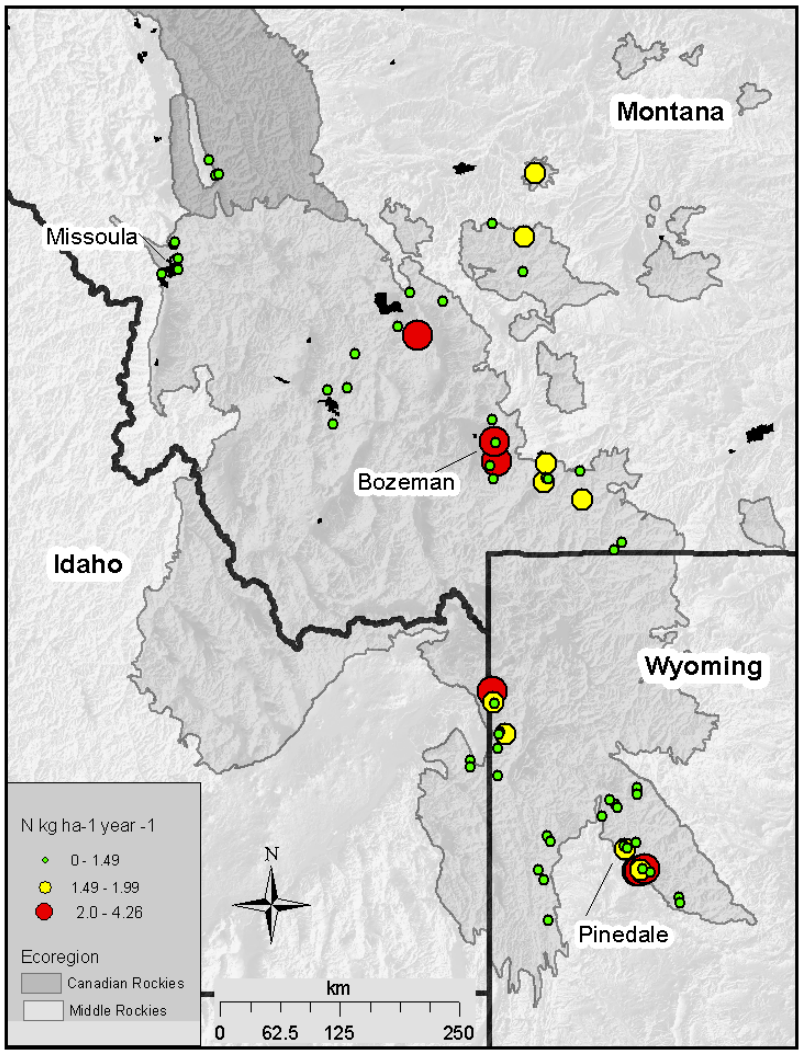


Fig. 3.8 Graduated symbols show increasing amounts of estimated N deposition. The large red circles represent areas where deposition is estimated > 2.0 kg N ha^{-1} $year^{-1}$ twice the estimated background conditions. There are 3 overlapping red circles near Pinedale, WY.

Environmental differences existed between the plots with highest ('polluted' = twice the estimated background conditions) and lowest ('clean') estimated N deposition (> 2.0 and < 0.5 kg $ha^{-1}year^{-1}$ respectively). Welch's two sample t-test revealed polluted

plots had higher % ash in lichen thalli and percent eutroph richness and lower relative humidity than clean plots (figure 3.9).

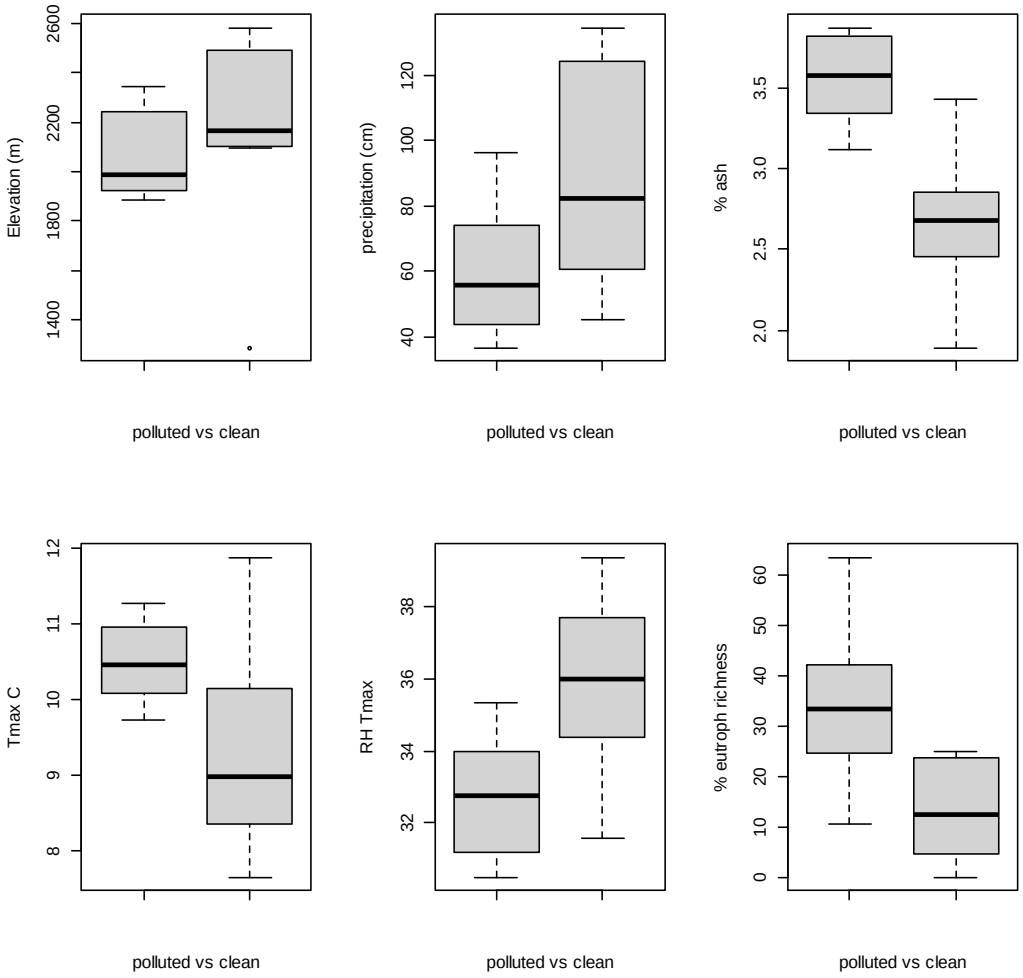


Fig. 3.9 Environmental differences between plots with highest (over twice the estimated background conditions) and lowest estimated N deposition (> 2.0 and < 0.5 kg $ha^{-1}year^{-1}$ respectively), n = 7.

Prediction Ability for New Plots

Sixty-five FIA plots from a 30 mile buffer were added into the ordination without changing the configuration (customized R function = addpoints; Roberts 2012).

Predicted values were adequate if the variable was important along ordination gradients, as is exemplified with percent eutroph richness (Table 3.7). Predicted values of marginally significant variables (e.g. precipitation) did not perform well, but values can still be used as scores for relative comparisons.

Table 3.7 Correlation (r) and linear model (adjusted r^2) between real values and predicted values for selected climate variables. n= 133 total plots (65 FIA plots + 68 plots from the original model). N deposition values, n=68 (no N values for FIA plots). Original r^2 represents the variable fit from the original ordination model (n=68).

	r	***Real vs. pred. r^2***	***Original r^2***
Elevation	0.59	0.34	0.75
Tmin	0.56	0.31	0.64
Precipitation	0.44	0.19	0.41
RH max	0.53	0.27	0.35
% N *Letharia*	0.60	0.35	0.28
% eutroph richness	0.80	0.64	0.77

Discussion

Lichen Communities

Epiphytic macrolichen communities in the northern Rocky Mountains were diverse, with a total of 88 species found. Species richness was consistently higher in the northwest corner of our study area, around Missoula and the Swan valley in MT and low throughout most of WY. Additional species-rich pockets were scattered around MT. The strong correlation between latitude and longitude and species richness is recurrent throughout the Rocky Mountains (McCune et al. 2010; Neitlich et al. 1999). As expected, species richness decreased with elevation. Higher elevations are naturally reduced in available habitat and exhibit climate extremes (Körner 2007).

An intensive lichen diversity study from Lewis and Clark Caverns in central Montana found 168 different species of lichens with only 54 (34%) occurring on bark (Salix 2004). The recorded 88 species of epiphytic macrolichens in our study greatly underestimates total lichen diversity and richness throughout the northern Rocky Mountains.

Eutroph and Oligotroph Indices and Species Scores

The literature–based oligotroph and eutroph indices were supported by the list of indicator species (scores) independently derived from % N *L. vulpina*. Percent eutroph richness was moderately related to gradients of N and climate. Mean eutroph richness was higher at the 12 most polluted vs 12 cleanest plots (39% vs 25%). In our model the importance of percent eutroph and percent oligotroph richness was greatly reduced after climate was accounted for. The xerophytic, photophilic, and temperature tolerant nature of eutrophic lichens is well documented in the literature (Davies et al. 2007; Jovan and McCune 2004; Neitlich et al. 2003). Our eutroph indices also followed this pattern of climatic preference. Percent eutroph richness was negatively correlated with overall species richness; we interpret this to be a result of eutroph adaptation to unfavorable climates.

We cannot fully discern the relationship between eutrophs, climate, and N deposition. Species scores associated with N were also associated with Tmax, Tmin, and low precipitation. Davies and others (2007) found species associated with low rainfall and minimum temperatures were typically the most pollution tolerant. The tolerant

nature of eutrophs is probably influenced by their small size and low cation-exchange capacity (CEC) (Gaio-Oliveria et al. 2001). Low CEC reduces the amount of N that can 1) enter the lichen thalli and 2) bind to cell walls. These mechanisms may protect eutroph species against the toxic effects of N pollution (Gaio-Oliveria et al. 2001; Godinho 2009) as well as increase survival in harsh climates.

Oligotrophs tend to be larger with higher surface area than eutrophs. We found oligotrophs exhibited opposite response patterns seen by eutrophs. Oligotrophs were associated with moist environments of moderate temperature. One oligotroph, *M. exasperata,* found only in the Wind River Range, was associated with cold temperatures and low precipitation. McCune et al. (2010) suggests that for a species to be considered as a clean air indicator, it should be present throughout 'clean' sections of the study area and largely absent from polluted sites. Most oligotrophs in our study were spatially restricted by climate conditions. Therefore temperature and moisture conditions should be considered, or held constant, when assessing oligotroph responses to other variables.

Indices derived from weighted-average species scores represent a regionally-tailored list. We suggest combining literature and score-based indices in future studies to gain general understanding of climate and N pollution patterns. We included *M. subolivacea* and *X. montana* in the literature-based index even though we could not find information on these species. Species scores independently highlighted these two species as indicators of high N. We strongly recommend that these two species should be added to future eutrophic indices in the northern Rocky Mountains.

Climate

Similar to other gradient models in the western U.S., temperature extremes and moisture (RHmax) were the strongest climate variables associated with lichen community composition (Geiser and Neitlich 2007; Jovan and McCune 2004; McCune et al. 2010). Temperature and moisture gradients were confounded with elevation (Körner 2007; McCune et al. 2010).

Each lichen species has an optimal range of moisture, temperature, light, and nutrient availability for maximum growth (Sillett et al. 2000). Moisture is an important limiting factor in lichen growth (Paterson et al. 1983). Lichens are poikilohydric organisms that become metabolically active when wet. Some species (mainly those with a cyanobacteria photobiont) need liquid water to metabolically reactivate; other species (those with a green algae photobiont) can reach this state through water vapor alone (Lange et al. 1986). We found only three species of cyanolichens (4 specimens) from two plots both located in the Swan Valley, MT. RH was more important than precipitation in our model, which suggests lichens in our study area rely in part on water vapor for metabolic activity. In climates with low RH, metabolic activity of lichens is often restricted to nighttime and early morning (Lange et al. 2007).

Warm temperatures between 15 °C and 20 °C have been found to maximize mycobiont growth (Hale 1983). We found species richness increased with temperature ($r = 0.71$). In the last two decades, the northern Rocky Mountains have experienced fewer cold days in the winter and a rise in summertime temperatures (National Climatic Data Center, web accessed March 2012).

Increasing temperatures expected from climate change may actually increase hospitable habitat conditions for lichen species in our study area, especially if days with extreme cold temperatures are reduced. Lange and Green (2005) suggest that the ability of lichens to adapt to changes in climate and other habitat conditions has greatly been underestimated. However, if increased temperatures occur, coupled with drought conditions (both decreases in precipitation and water vapor), we would expect a decline or shift in lichen communities either directly—extirpation of sensitive moisture dependent lichens—or indirectly though loss of habitat. Drought-stressed forests are more susceptible to wildland fires, disease, and bark beetle infestations (Dale et al 2001).

N Deposition

Tissue concentration of N in *L. vulpina* was significant in our model. Importance was not reduced after climate was accounted for which indicates independence from climate variables along community gradients. This is further supported by the overall lack of linear relationships between N and all other variables in the dataset except for mild correlations with precipitation and Tmax ($r = -0.3, 0.33$; $p = 0.014, 0.006$ respectively). Moderate correlations existed between N concentrations in *L. vulpina* and percent eutroph richness ($r = 0.4$; $p = <0.001$) and percent oligotroph richness ($r = -0.43$; $p = <0.001$).

Over the last two decades, increases in ammonium deposition have been wide-spread throughout the study area, while increases in nitrate deposition have occurred more sporadically— around Pinedale, WY and Helena, MT (Grenon and Story 2009; Grenon et al. 2010). Both ammonia (NH_3) and nitric acid (HNO_3) deposition can cause

shifts in lichen community composition (Frati et al. 2007; 2008; Jovan and McCune 2005; Jovan and Riddell –*in* review; Riddell et al. 2008; van Herk et al. 2003; van Dobben & ter Braak 1999). The rate of community shift depends on the sensitivity of species and, the type, rate, and duration of N exposure (Munzi et al. 2009; 2010; Sheppard et al. 2011). Unfortunately CMAQ data were too coarsely scaled to inform our model; therefore we were unable to differentiate between types of N deposition. The lack of spatial resolution indicates more on-the-ground studies will be needed to fine-tune estimates of N deposition distribution in the northern Rocky Mountains.

Our estimates of N deposition from lichen thalli ranged from <0.5 to 4.26 kg N ha^{-1} $year^{-1}$. Compared with other parts of the western U.S., the N gradient in our model is small (Fenn and Poth 2004, Fenn et al. 2008; Jovan and Riddell –*in review*). However, we consider our study area to be an N-limited environment (Baron et al. 2011; Beem et al. 2010) and in such systems even small increases in N-deposition can alter sensitive biotic communities (Baron 2006; Sheppard et al. 2011). Over half of our plots (n=41) exhibited estimated N deposition above pre-anthropogenic conditions (0.9 kg N ha^{-1} $year^{-1}$) in the northern Rocky Mountains (Sverdrup et al. 2012).

N deposition may initially act as fertilizer and increase algal cells in a lichen thalli (and thus increase net photosynthesis), but an excess of nitrogen can cause cellular damage to one or both symbionts (Kaupii 1980; Munzi et al. 2009), nutrient allocation can shift, and the breakdown of the symbiotic relationship may result (Nash 2008). How much N deposition is too much? The answer is somewhat species specific and climate sensitive. Estimates range from 2.5 – 7.1 kg N ha^{-1} $year^{-1}$ in the Northwestern Forested

Mountains level I ecoregion of the contiguous US (Fenn et al. 2008; Geiser et al. 2010; Bowman et al. 2011, Jovan and Carlberg 2007). We found clear evidence of damaged lichen communities (Sigal and Nash 1983) at > 4.0 kg N ha^{-1} $year^{-1}$, including necrotic and bleached thalli along with deformed and stunted growth. Chronic levels of N deposition less than 4.0 kg ha^{-1} $year^{-1}$ are likely affecting lichen communities.

Similar to other studies, we did not find a correlation between N deposition and species richness (Geiser and Neitlich 2007, Jovan and Riddell –*in review*). We did however find patterns between N deposition and percent eutroph and oligotroph richness (section 4.2).

Locations of N Deposition Concern

We focused our attention on three areas with N deposition double estimated background conditions (> 2.0 kg $ha^{-1}year^{-1}$):

- *Wyoming:* Measured N deposition from TF monitors was 3.24 and 4.13 kg $ha^{-1}year^{-1}$ at two plots in the Boulder drainage on the west flank of the Wind River Range in Sublette County (Grenon et al. –*in prep*). A third plot in the Boulder drainage (within the Bridger Wilderness Boundary) had an estimated N deposition at 2.28 kg $ha^{-1}year^{-1}$. Sublette County (pop = 8100) supports numerous oil and natural gas extraction operations. Other plots in Sublette County (n = 12), farther from oil and gas operations, measured N deposition between <0.5 and 1.1 kg $ha^{-1}year^{-1}$.
- *Idaho:* North of Driggs, N deposition (2.07 kg N $ha^{-1}year^{-1}$) may be elevated from localized agricultural practices or from the Snake River Plain. The Snake River

Plain in ID is known for concentrated NH_y pollution from intense agricultural and feedlot operations (EPA: http://www.epa.gov/AMD/EcoExposure/ESRP.html web acessed Jan. 2012; NADP 2011; Strait et al. 2008). Most animal waste from feedlots deposits locally, but up to 30% of ammonia can be volatized into the atmosphere and transported long distances as NH_4^+, NH_4NO_3, N_2O, and ammonium sulfate ($(NH_4)_2SO_4$) (Howarth et al. 2002; Twigg et al. 2011; van Herk et al. 2003).

- *Montana:* Elevated areas of N were mainly confined to urban centers—Bozeman (3.44 and 2.52 kg N $ha^{-1}year^{-1}$) and Helena (2.03 kg N $ha^{-1}year^{-1}$). Industrial emission sources near Bozeman and Helena emit approximately 740.12 and 1,789 tons of NO_x per year respectively (Grenon and Story 2009). Vehicle emissions and agricultural practices could also be contributing to elevated N deposition.

We expected Missoula, the second largest city in MT located in a valley with frequent inversions, to have higher estimated N deposition. Two significant factors could contribute to lower-than-expected N estimates. First, in 2009 the Smurfit-Stone Container Mill closed. This mill contributed 2,253 tons of NO_x per year, an estimated 78% of industrial NO_x emission in the valley (Grenon and Story 2009).

Second, Missoula receives higher rainfall and milder winters than much of the study area. We found a weak but negative correlation between precipitation and percent N in *L. vulpina*. Though it is widely accepted that high amounts of precipitation affect concentrations of soluble elements like N in lichen thalli (Boonpragob and Nash 1989; Geiser et al. 2010; Geiser et al. 2007; Jovan and Carlberg 2007), the relationship is less

clear in areas of intermittent precipitation or in cold climates where precipitation falls primarily as snow. In Portugal, Marques (2009) found mixed results between deposition of soluble elements and precipitation volumes (8 – 90 cm) in transplant studies. We sampled 5 sites in early spring (after many weeks of precipitation) and revisited these sites in the fall after a dry summer. We found no difference in % N or % ash between paired samples (paired t-test; n=10, p= 0.64, 0.41, stdev = 0.06 and 0.13 respectively). Our sample size was small and clustered to one region of the study area; further investigation is warranted.

In warm wet and humid environments, lichens spend more time metabolically active than their counterparts in harsh and arid environments (Lange et al. 2007). It seems plausible that longer hours of metabolic activity would consume more stored N in the lichen thalli, especially in relatively N-limited environments. It may be that more rainy days clean the air and reduce residence time of pollutants in the air. Grenon et al. (-*in prep*) found background conditions of N deposition in western Wyoming were associated with 1.35% N in *U. lapponica* and 1.12 % N in *L. vulpina*. Double background conditions (2.0 kg N ha^{-1} $year^{-1}$) were associated with 1.65 % N for *U. lapponica* and 1.35 % N for *L. vulpina*. However, manipulated experiments are needed to see what threshold of % N causes damage to the thalli of these two lichens.

Modeling Considerations

Our model allows for new or revisited plots to be added without altering the ordination space. Re-measured plots can be used to monitor shifts in lichen communities. New plots added to the ordination can be scored along existing climate and N deposition

gradient response surfaces. This model can be used to track individual species trends for conservation purposes. Currently the ability to add points is a customized function in R; we are working on making it available for public use (R core Development Team 2011).

GAMs assume explanatory variables are measured without error. We did not explicitly consider error associated with the data and so the fit of our model may contain bias (Chatterjee and Hadi 1988). Response curves reported could be skewed due to over, under, and clustered sampling in certain areas (Yee and Mitchell 1991). We did not remove plots from high density areas such as the Wind River Range because of the low number of total plots in the study. Sampling more plots and geographically dividing the study area would reduce the climate signal and allow other gradients to be evaluated. Installation of new plots in both remote and urban (non USFS lands) areas may expand the N deposition gradient. Actual measurements of reduced and oxidized N co-located with plots is important to understand how lichen communities respond to different forms of N.

We did not account for dispersal limitations of individual lichen species. Most lichens in our study area disperse by microscopic asexual and sexual structures via water, animals, or wind.

The model could adequately predict values for environmental variables as long as the variable was important along ordination gradients (e.g. percent eutroph richness). In the case of marginally significant variables such as precipitation, predicted values should be used as a score for comparison purposes rather than an absolute measure.

Conclusions

A diversity and abundance of epiphytic macrolichen species were found in conifer forests of the northern Rocky Mountains.

We combined three approaches to better understand the relationship between epiphytic macrolichens and environmental variables in the northern Rocky Mountains, including: analysis of lichen community composition, analysis of % N in lichen thalli, and eutroph/oligotroph functional group indices (Jovan and Riddell –*in review*; McCune and Geiser 2009).

Eutroph and oligotroph lichen species designated from the literature were largely backed by species indicator scores derived from % N *L. vulpina*. We found a difference in distribution of eutrophic and oligotrophic species throughout our study area. Percent eutroph richness increased and % oligotroph richness decreased with N deposition. Both indices were also related to climate gradients. Eutrophs were widely found throughout the study area. Species from the genera *Physcia*, *Xanthomendoza,* and *Xanthoria* were associated with N deposition, low precipitation, and extreme temperatures. We recommend including *M. subolivacea* and *X. Montana* in future eutroph indices. Oligotrophs were typically restricted to moist temperate regions. The exception was *M. exasperata* which was found only in the Wind River Range and associated with dry cold environments. Future use of oligotroph and eutroph indices for N indication should include considerations of climate.

No site characteristics were significant in the model, likely swamped by climate and pollution gradients, due to the large spatial extent of the study. Site variables such as

stand age and tree species are often significant at smaller spatial scales (Will-Wolf et al. 2006). Climate variables Tmax, Tmin, and RH were the most important variables to community composition. A signal from N pollution existed even after climate was accounted for.

N deposition was estimated from <0.5 to 4.26 kg N ha^{-1} $year^{-1}$. These forests are considered N–limited environments and slight changes can alter lichen communities. We found clear evidence of degraded communities at 4.0 kg N ha^{-1} $year^{-1}$ which indicates CLOs of < 4.0 kg N ha^{-1} $year^{-1}$ are needed to protect communities from chronic levels of N deposition. The two most notable areas of N deposition were around Bozeman, MT (Gallatin NF) and Pinedale, WY (Bridger-Teton NF).

The Clean Air Act requires the Forest Service to protect national ambient air quality standards (NAAQS), which include nitrogen, in Class I and II Wilderness Areas. We have shown that epiphytic macrolichens can be used as a tool to assess N deposition patterns and climate patterns over large spatial scales. Areas where elevated N deposition is estimated can then be further assessed. This application of modeling should also be tried with other pollutants of concern in the study area.

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CHAPTER FOUR

CLIMATE PATTERNS AS INDICATED BY EPIPHYTIC LICHEN COMMUNITIES: A FOREST INVENTORY AND ANALYSIS INDICATOR MODEL FOR THE NW INTERIOR MOUNTAINS, USA

Contribution of Authors and Co-Authors

Manuscript in Chapter Three

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Abstract

The Forest Inventory and Analysis (FIA) program is a nation-wide branch of the United States Forest Service (USFS) which collects data on forest attributes. Part of the FIA mission is to assess and track changes in forest health due to changes in climate and air pollution. Epiphytic macrolichen communities are one of the attributes used to monitor forest health. We used nonmetric multi-dimensional scaling (NMDS) to model variation among lichen communities across the NW Interior Mountain states of central Idaho, Montana, and western Wyoming. Generalized additive models (GAMs) were used to assess the importance of environmental gradients related to lichen community composition. One-hundred and twelve different epiphytic macrolichen species were collected throughout the study area. Strong patterns existed between temperature, moisture, and lichen community composition. Species richness decreased with temperature extremes. Marked differences between the distribution of eutroph (N-tolerant) and oligotroph (N-sensitive) lichen species were shown, which related only partially to climate. We did not have direct air quality measurements to further investigate the relationship between eutrophic and oligotrophic species with nitrogen deposition. Indicator species from weighted averages of species mean abundances found species associated with temperature extremes and drier environments were eutrophic, while species associated with wet, humid, and moderate temperatures tended to be oligotrophic. Revisited or new plots can be added to the model to assess changes in lichen communities over time or to predict associated climate conditions. The model created can adequately predict values for important environmental variables along ordination gradients.

Keywords: FIA; lichen indicator; epiphyte, climate change, monitoring; modeling; EPA ecoregion; Montana; Idaho; Wyoming

Introduction

The Forest Inventory and Analysis (FIA) program of The United States Forest Service (USFS) collects data on forest attributes along a nation-wide grid with at least one plot for every 6,000 acres of forest (private and public). Part of the FIA mission is to assess current and changing conditions in forest health, including response to changes in climatic conditions (FIA P3 Field Guide 2011). To address this goal, a subset of FIA

plots are sampled for additional forest heath attributes (every 96,000 acres); included is the lichen indicator, which documents epiphytic macrolichen community composition and abundance.

Lichens are poikilohydric organisms that rely on liquid water or water vapor to hydrate and become metabolically active (Lange et al. 1986). Each lichen species has an optimal range of moisture, temperature, light, and nutrient availability for maximum growth (Sillett et al. 2000). Lichens lack regulatory structures such as a cuticle or stomata, which makes them sensitive to changes in climate, forest conditions, and air pollution (Geiser 2007; Jovan and McCune 2005, 2004; van Herk et al 2003). Because of their sensitivity, lichen communities can be used to analyze gradients in climate, forest conditions, and air pollution. Communities can then be tracked over time to monitor changing conditions (Geiser and Neitlich 2007; Jovan and McCune 2004, 2005; Mitchell et al. 2005; Neitlich et al. 2003). In addition to monitoring utility, lichen communities are important to nutrient cycling, food webs, provision of habitat, and diversity (McCune et al. 2007).

Previous lichen work documenting epiphytic macrolichen richness for Idaho and Wyoming found lichen species richness decreased with elevation (Neitlich et al. 1999, 2003). This pattern was interpreted as a response to moisture and temperature gradients (Neitlich et al. 1999, 2003). Unfortunately, no climate data were available at the time of these studies. Another study of lichen communities in the northern Rocky Mountains (Montana and Wyoming) found temperature extremes and relative humidity were the most important variables associated with patterns in lichen community composition

(Grenon et al –*in prep*). However, this model had relatively few plots (n=68) unevenly distributed over a large geographic area.

Over the last century, average temperatures in the NW Interior Mountains have increased by 0.74 °C (1.33 °F), while precipitation has decreased between 0.79 and 2.84 cm (EPA 1997; NOAA National Climatic Data Centers Climate, Services, and Monitoring Division—web accessed March 2012). Predicted decrease in precipitation or the transition of precipitation from snow to rain will result in less snow water equivalent (SWE) stored in the snowpack and thus lower stream flows in the summer. Rising summer temperatures in the NW Interior Mountains has been linked to treeline encroachment on alpine vegetation (Hessel and Baker 1997), increased water stress—due to decreased SWE, and more intense fire seasons (EPA 1997).

The objectives of this study were to: 1) gain knowledge of epiphytic macrolichen richness and diversity across the NW Interior Mountains, 2) identify the important forest and climate variables associated with gradients in lichen community composition, 3) assess whether lichen indices or individuals could be used as indicators of climate conditions, and 4) evaluate the prediction ability of climate values for new plots added to the model. Though we included numerous variables in our modeling efforts, the scale of our study is large and likely to favor climate over forest characteristic gradients (Will-Wolf et al. 2006).

We used nonmetric multi-dimensional scaling (NMDS), an unconstrained ordination technique, to model variability among lichen communities in the NW Interior Mountains. Generalized additive models (GAMs) were used to fit environmental

gradients to the ordination coordinates to assess importance along gradients in lichen community composition. Plots were associated with a value or 'score' along the fitted gradients. These scores can be used to track changes in revisited plots over time. The fitted GAMs can be used to predict environmental conditions of new plots. To test prediction ability, new plots were added to the ordination; climate scores were predicted and then evaluated against actual known scores. Lichen species were also assessed for individual indicator value along environmental gradients by using weighted averages from species abundance codes.

This project is part of the FIA forest health monitoring framework. We created a baseline model that documents lichen diversity in the NW Interior Mountains and can be used to track changes in lichen communities related to climate over time. This model is a working tool with practical applications for both management and conservation purposes.

Methods

Study Area and FIA Plots

The Environmental Protection Agency (EPA) has classified North America into unique areas based on similarly functioning ecosystems called ecoregions (Omernik 1995; 2004). There are four ecoregion 'levels' (I – IV) which increase with the level of detail of habitat divisions. FIA plots for this study were located in The Canadian Rockies (CRE; n=29), Middle Rockies (MRE; n=186), and Idaho Batholith (IBE; n=105) level III ecoregions (Figure 4.1).

Most FIA plots in the study area fall on public land in mountainous terrain, though some plots exist on private land. The summers tend to be dry and the winters cold to severe. The majority of the precipitation falls as snow especially at higher elevations. Precipitation across plots ranged from 32 to 177 cm and elevation ranged from 764 to 3109 m. Common tree species include *Pinus contorta*, *P. ponderosa*, *P. albicaulis*, *Pseudotsuga menziesii*, *Picea engelmannii*, *Abies lasiocarpa* and *Populus tremuloides*. Plots were stratified across elevation, slope, aspect, and climate gradients. All plots included in this model were sampled between 2002 and 2011.

Two types of FIA plots were included in this data set; on-frame and off-frame plots. On-frame plots are permanently installed along a nation-wide grid following standard FIA protocols (FIA P2 Field Guide 2007). Off-frame plots for lichen surveys consist of a temporary 0.378 ha circular plot (FIA P3 Field Guide 2011). FIA on-frame plot data can be downloaded online from: http://fia.fs.fed.us/tools-data/default.asp.

Lichen Data

On each plot certified FIA crew members collected lichens from woody substrates above 0.5m. Sampling occurred for a minimum of 45 minutes and a maximum of 2 hours (FIA P3 Field Guide 2011). Each species was placed into a separate packet and sent to a certified lichenologist for identification. Taxonomic identification followed McCune and Geiser (2009) and McCune and Goward (1995). For each collected species a corresponding abundance rating was recorded, where 1 = rare (≤ 3 thalli), 2 = uncommon (4 – 10 thalli), 3 = common (> 10 thalli, and species occupy less than half the available substrate), and 4 = abundant [>10 thalli, and species occupy over half the available

substrate] (FIA P3 Field Guide 2011). In forests of Montana, McCune and Lesica (1992) found that accuracy with visual abundance ratings for epiphytic lichens was comparable to methods using continuous coverage.

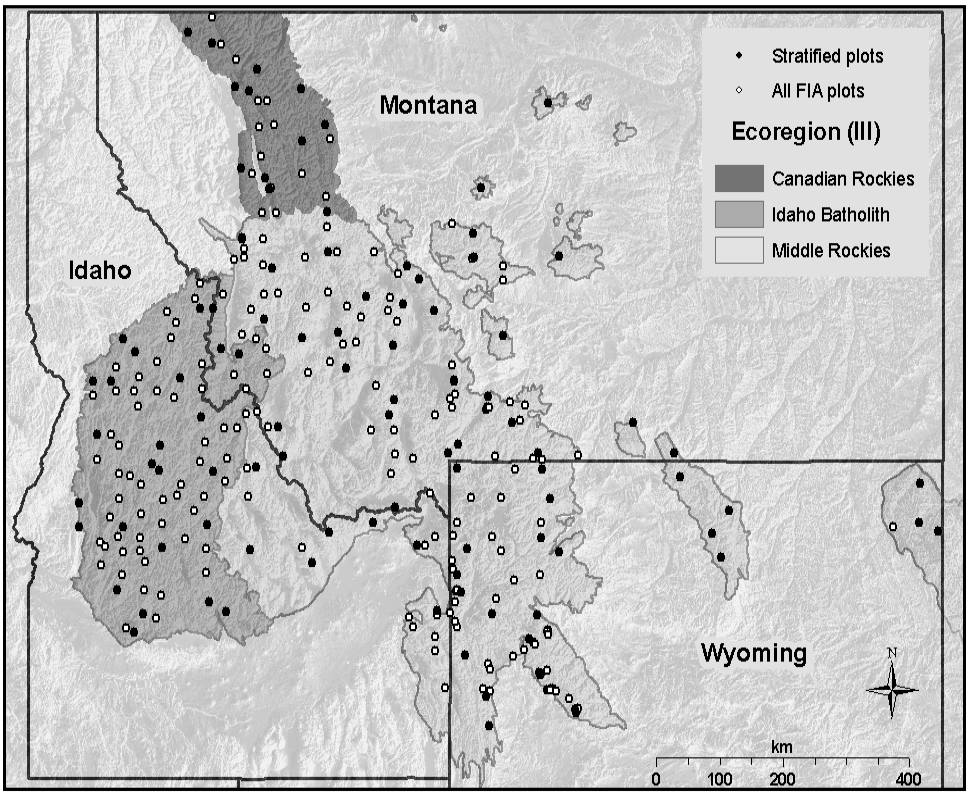


Fig. 4.1 FIA plot distribution across three ecoregions in the NW Interior Mountains. Ecoregions are shaded in different colors. Black dots represent FIA plots included in the stratified model. White dots represent FIA plots withheld from the original model to reduce spatial clustering effects.

Environmental and Climate Data

Site data collected by FIA crew members included: latitude, longitude, elevation, aspect, slope, percent basal area of live trees, percent basal area of hardwood trees,

elevation, tree size class, percent gap disturbance, shrub percent, and stand age (FIA P2 Field Guide 2007). A heat load index was calculated from McCune and Keon (2002):

eq.2.3.1

$$Heat\ load\ index = \frac{1 - \cos(\theta - 45)}{2}$$

Where θ = aspect in degrees.

Climate variables were extracted from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) (Daly and Taylor 2000) via gridded data layers in ArcGIS (ArcGIS 9.3.1; ERSI 2009). PRISM employs point data and a digital elevation model (800m resolution) in algorithms to give continuous spatial information on climate data (4km resolution). Climate data for this study included: annual averages of precipitation, minimum temperature (Tmin), maximum temperature (Tmax), dew point (dew), relative humidity (RH), relative humidity from maximum temperatures (RHmax) and relative humidity from minimum temperatures (RHmin). Climate variables represent yearly averages from 1971-2000 except for dew point and RH (1999-2009).

Eutroph and Oligotroph Indices

Sensitivity and response to N pollution differs among lichen species. As a result lichens are often placed into three categories—eutrophs, mesotrophs, and oligotrophs (Geiser and Neitlich 2007; Jovan 2008; Jovan and Riddell –*in review*). Eutrophs (also called nitrophytes, nitrophiles) tend to flourish in N–enriched environments, mesotrophs show little change, and oligotrophs (also called acidophytes, acidophiles) typically decline or vanish with increasing N. Patterns in eutroph and oligotroph species are also

associated with climate conditions (Geiser and Neitlich 2007; Grenon et al –*in prep*; Jovan and McCune 2004). We created two indices, the percent of eutroph and oligotroph richness per plot. Species designations (Table 4.1) were based on published classifications from the Western United States (Geiser et al. 2010; The United States Forest Service National Lichen & Air Quality Database and Clearinghouse: http://gis.nacse.org/lichenair/ web accessed- Feb. 2012; Grenon et al. –*in prep*; Jovan 2008; Jovan and Ridell—*in review*; McCune et al. 2010; Neitlich et al. 1997).

Table 4.1 Eutroph and oligotroph indexed species.

category	***Lichen species***
Eutroph	*Candelaria pacifica, M. subolivacea, Parmelia saxatilis, Phaeophyscia nigricans, P. orbicularis, Physconia americana, P. enteroxantha, P. isidiigera, P. perisidiosa, Physcia adscendens, P. aipolia, P. biziana, P. dimidata, P. dubia, P. stellaris, P. tenella, Xanthomendoza fallax, X. fulva, X. galericulata, X. montana, X. oregana, Xanthoria hasseana, X. parietina, X. polycarpa, X. tenella*
Oligotroph	*Ahtiana sphaerosporella, Alectoria imshaugii, A. sarmentosa, Bryoria fremontii, B. fucellata, B. pseudofuscescens, B.simplicior, B. trichodes, Cetraria merrillii, C orbata, C. platyphylla, Esslingeriana idahoensis, Hypogymnia apinata, H. enteromorpha, H. metaphysodes, H. occidentalis, Leptogium cellulosum, L. saturninum, Lobaria pulmonaria, Melanohalea exasperata, Nodobryoria abbreviata, N. oregana, Parmeliopsis hyperopta, Platismatia glauca, P.stenophylla, Usnea filipendula, U. scabrata*

Statistical Procedures

Continuous variables were evaluated for linear relationships with Pearson's correlation coefficient. We calculated species richness per plot (α-diversity) and across the whole study area [γ- diversity] (Whittaker 1972). Diversity measures were also calculated for each ecoregion. All statistical analyses were completed with R.2.9.2 (R Development Core Team 2011).

Latitude and longitude are intertwined with climate gradients in the northern Rocky Mountains (Grenon et al. –*in prep*; McCune et al. 2010). In order to model out bias due to geographic clustering, we stratified plots across latitude and longitude. The complete dataset included 320 plots and the stratified subset contained 101 plots.

Nonmetric Multidimensional Scaling (NMDS; Kruskal 1964) is a non-parametric, unconstrained ordination technique commonly used to detect gradients in community composition (McCune and Grace 2002, Minchin 1987). A matrix of dissimilarities from the lichen communities was created using the Bray-Curtis (Bray and Curtis 1957) dissimilarity index. This matrix was subjected to NMDS (package = 'vegan'; Oksanen et al. 2011), and the coordinates of points in the NMDS were plotted to portray communities in multidimensional space. Ordination plot coordinates for each dimension are denoted as NMDS 1, NMDS 2, and NMDS 3.

Through an iterative process, NMDS seeks to minimize "stress", which is the square root of the sum of the squared differences between the ranked distance measures in the dissimilarity matrix and the ranked distance measure in the ordination space divided by the differences in the ordination squared (Kruskal and Wish 1978). We compared ordination solutions from 400 runs of random starts to maximize the probability of finding the best global solution (McCune and Grace 2002). Final ordination selection was based on the solution with lowest stress (reported as a ratio) and highest correlation between matrix dissimilarities and ordination distances (package = 'labdsv', Roberts 2010).

Generalized additive models (GAMs) (Hastie and Tibshirani 1986; package = 'mgcv', Wood 2011) of site and climate variables were fit to the ordination coordinates to identify gradient importance (package = 'labdsv', Roberts 2010). GAMs have the unique ability to fit flexible surfaces or lines to represent complex (nonlinear) relationships through the use of smoothing algorithms (Hastie and Tibshirani 1986, Hastie and Tibshirani 1987; Wood 2011). We used individual smoothing splines to allow flexible fits of the environmental variables along community gradients (the axes of the ordination).

GAMs are a data-driven compromise between parsimony and goodness - of -fit with a goal to minimize residual deviance and maximize deviance explained (D^2), selecting the appropriate model degrees of freedom (df) of the smoothing with a penalty function.

eq.2.5.1

$$D^2 = \frac{null\ deviance - residual\ deviance}{null\ deviance}$$

D^2 = Deviance explained. The specific GAM function we used also estimates adjusted r^2 which incorporates penalties for actual df used. To avoid over fitting variables we used a gamma of 1.4 (unless otherwise noted) which increases the penalty for df used (Wood 2006). Models were fit with Gaussian or Poisson distributions as appropriate.

All plots were assigned a predicted value or 'score' along individual fitted GAM surfaces for each climate or pollution gradient of interest (package = 'vegan'; Oksanen et al. 2011). Lichen species were identified as indicators for climatic and pollution

conditions based on weighted averages of plot values using species abundances in the plots as weights (package = 'vegan', Oksanen et al. 2011).

Variable Predictions for New Plots

The 229 plots withheld from the stratified model were added into the ordination without changing the configuration (customized R function = addpoints; Roberts 2012). The fitted GAMs for Climate and elevation variables from the stratified model were used to predict values for the new plots. Accuracy of predicted values was evaluated against real values with Pearson's correlations and linear models (adjusted r^2).

Results

Lichen Diversity

A total of 112 different species of epiphytic macrolichens (γ diversity) was collected over 320 FIA plots in CRE, IBE, and MRE. Lichen species richness within plots ranged from 1 to 33 with a mean richness (α diversity) of 11.93. Of the 112 species, 60 occurred fewer than 10 times and 22 were found once (Appendix B). Seven species were collected on over half the plots; *Letharia vulpina, Bryoria fuscescens, Parmeliopsis ambigua, Usnea lapponica, Melanohalea exasperatula,* and *Hypogymnia imshaugii* (Appendix B). Twenty-eight lichen species were found only in the Middle Rockies ecoregion and 8 lichen species were ecoregion-specific to the Idaho batholith.

The stratified dataset (101 plots) contained 96 species, 86% of the species included in the whole dataset. Common lichen species were the same between the two datasets. Sixty-five species were collected less than 10 times and 29 occurred once

(Appendix B). Lichen species richness within the stratified dataset ranged from 1 to 33, with a mean richness of 11.63.

On average, plots in the CRE had higher species richness than in the IBE or MRE (Table 4.2). Ecoregion α diversity for the stratified dataset mirrored that of the larger dataset (Table 4. 2). Differences in α and γ diversity for total, eutrophic, and oligotrophic species existed between ecoregions (Table 4.2). However, lichen communities exhibited a large amount of ecoregion overlap in ordination space (Figure 4.2) with no outlier plots found in the dissimilarity matrix ((package = 'labdsv', Roberts 2010).

Table 4.2 Mean richness of total, oligotrophic and eutrophic lichen species among ecoregions: Canadian Rockies (CRE), Idaho Batholith (IBE), and Middle Rockies (MRE). SD = standard deviation. The eutroph and oligotroph indices include 25 and 27 species respectively.

Variable	***320 plots***			***101 stratified plots***		
	CRE	**IBE**	**MRE**	**CRE**	**IBE**	**MRE**
Total richness (γ)	66	73	92	55	58	72
Species richness (α)	18.6	11.4	11.2	18.6	11.1	10.6
SD of species richness (α)	8.9	6.6	6.3	9.6	7.6	5.3
Eutroph richness	0.97	0.79	2.3	0.6	0.9	2.2
Oligotroph richness	7.3	3.8	1.5	7	3.8	1.0
% of eutroph richness	4.3	8.24	20.9	2.0	12.7	20.7
% of oligotroph richness	36.9	27.4	10.1	33.4	26.3	7.4
Number of plots	29	105	186	11	26	64

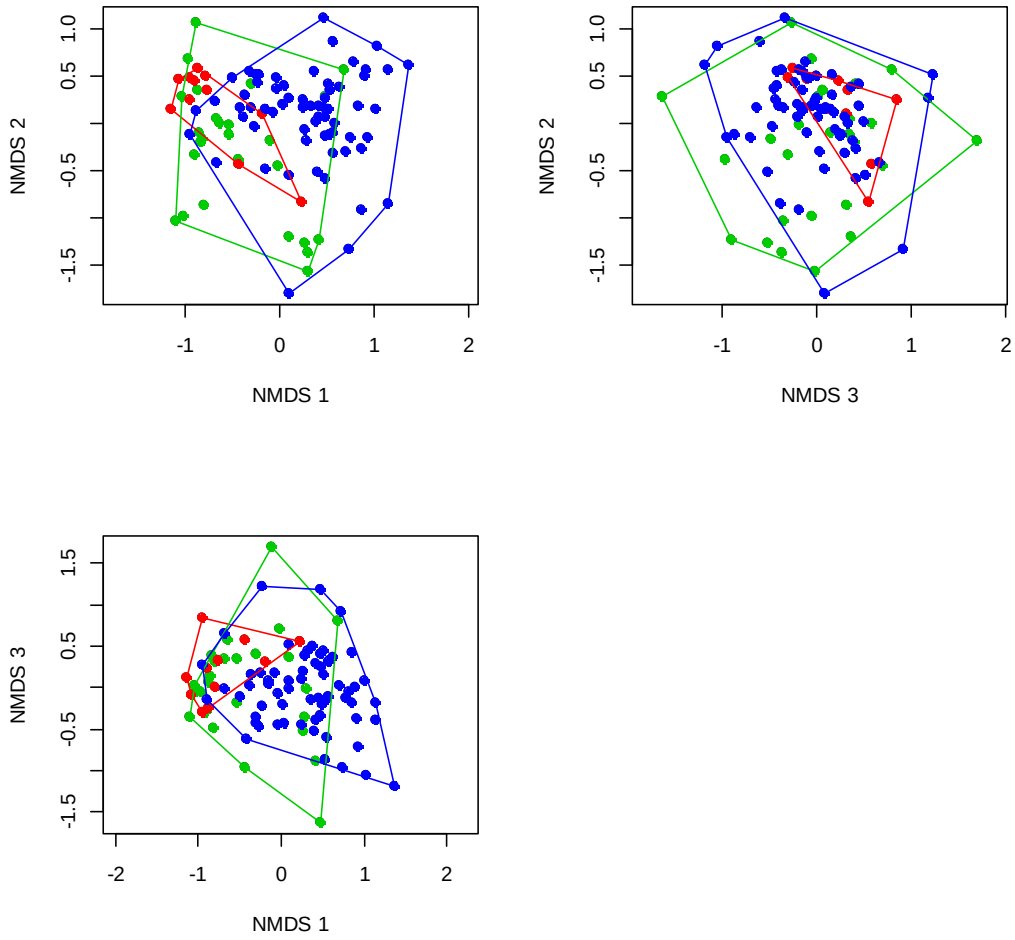


Fig. 4.2 Ecoregion overlap in ordination space along the different axes (NMDS 1,2,3). Red = Canadian Rockies, Green = Idaho batholith, and Blue = Middle Rockies.

Variable Correlations and Distributions

Climate gradients were correlated with geography and elevation. RH, precipitation, and temperature increased with latitude and decreased with longitude (Table 4.3). RH and temperature also decreased with elevation. Precipitation, however, was not correlated with elevation (Table 4.3).

Table 4.3 Correlations among climate variables from 101 stratified plots. Latitude (Lat), longitude (Lon), elevation (Elev), precipitation (Precip), maximum temperature (Tmax), minimum temperature (Tmin), relative humidity (RH), relative humidity from maximum temperatures (RHmax), and dew point (Dew). Correlations stronger than 0.5 are in bold.

	Lat	*Lon*	*Elev*	*Precip*	*Tmax*	*Tmin*	*Dew*	*RH*
Lat	1.00	--	--	--	--	--	--	--
Lon	-0.25	1.00	--	--	--	--	--	--
Elev	**-0.54**	0.30	1.00	--	--	--	--	--
Precip	0.25	-0.31	0.18	1.00	--	--	--	--
Tmax	-0.03	-0.14	**-0.76**	**-0.53**	1.00	--	--	--
Tmin	0.34	-0.16	**-0.76**	-0.17	**0.66**	1.00	--	--
Dew	0.31	-0.37	**-0.76**	-0.06	**0.63**	**0.81**	1.00	--
RH	0.33	-0.48	-0.38	0.36	0.09	0.37	**0.74**	1.00
RH max	0.44	-0.34	-0.31	0.40	-0.08	0.40	**0.64**	**0.92**

No significant differences in site characteristics were found between ecoregions. There were, however mean climatic differences among ecoregions (Figure 4.3). On average, MRE plots were higher in elevation and lower in precipitation than the other ecoregions. The MRE also displayed the widest range in temperature, as was expected given the geographic and elevational spread of this ecoregion. The CRE was characterized by moderate temperatures and high precipitation, RH, and species richness (Figure 4.3).

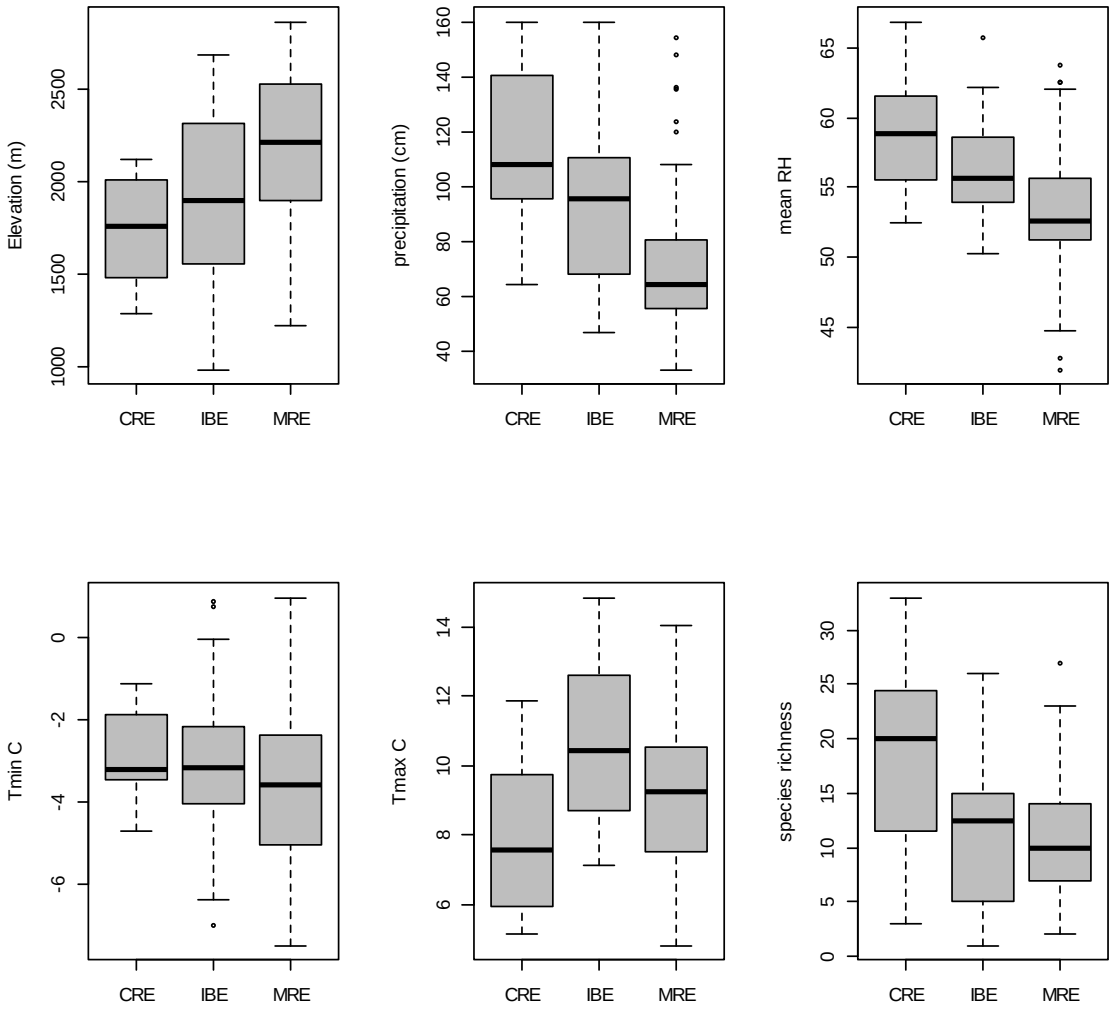


Fig.4.3 Climate variables by ecoregion for stratified FIA plots. CRE = Canadian Rockies, IBE = Idaho Batholith, and MRE = Middle Rockies.

NMDS Community Analysis
and Fitted GAM Surfaces

We found a 3-dimensional solution with a stress of 14.7 was a good approximation of the full dimensional space (r = 0.911). The dissimilarity matrix used in

the ordination included all lichen species in our dataset. The removal of rare species did not enhance our understanding of the model.

Latitude, longitude, and elevation were strongly linked to gradients in lichen communities (Table 4.4). Figure 4.4 displays the geographic distribution of plots in ordination space. Tmin and Tmax were the most significant climate variables in our model. However, Tmin was strongly correlated to Tmax and nearly all other climate variables. Precipitation was also found to be important and was independent of Tmin (Figure 4.5). When Tmin and precipitation were included in a GAM the significance of other variables decreased considerably (Table 4.4). It should be noted that percent eutroph and oligotroph richness patterns were only partially explained by climate (Table 4.4).

Table 4.4 Deviance explained (D^2) and adjusted r^2 values for variables fit to the ordination. r^2A = r^2 values for variables fit independently and r^2B = variables fit after Tmin and precipitation were accounted for in a GAM.

Variable	D^2 (%)	r^2A	r^2B
Latitude	41.0	0.39	0.23
Longitude	47.2	0.46	0.38
Elevation	60.3	0.58	0.26
Dew	35.4	0.33	0.00
Precipitation	25.7	0.23	---
Tmin	39.1	0.37	---
Tmax	39.6	0.37	0.11
RH	21.3	0.18	0.01
% eutroph richness	81.3	0.80	0.55
% oligotroph richness	85.1	0.83	0.48
Species richness	80.6	0.79	0.57

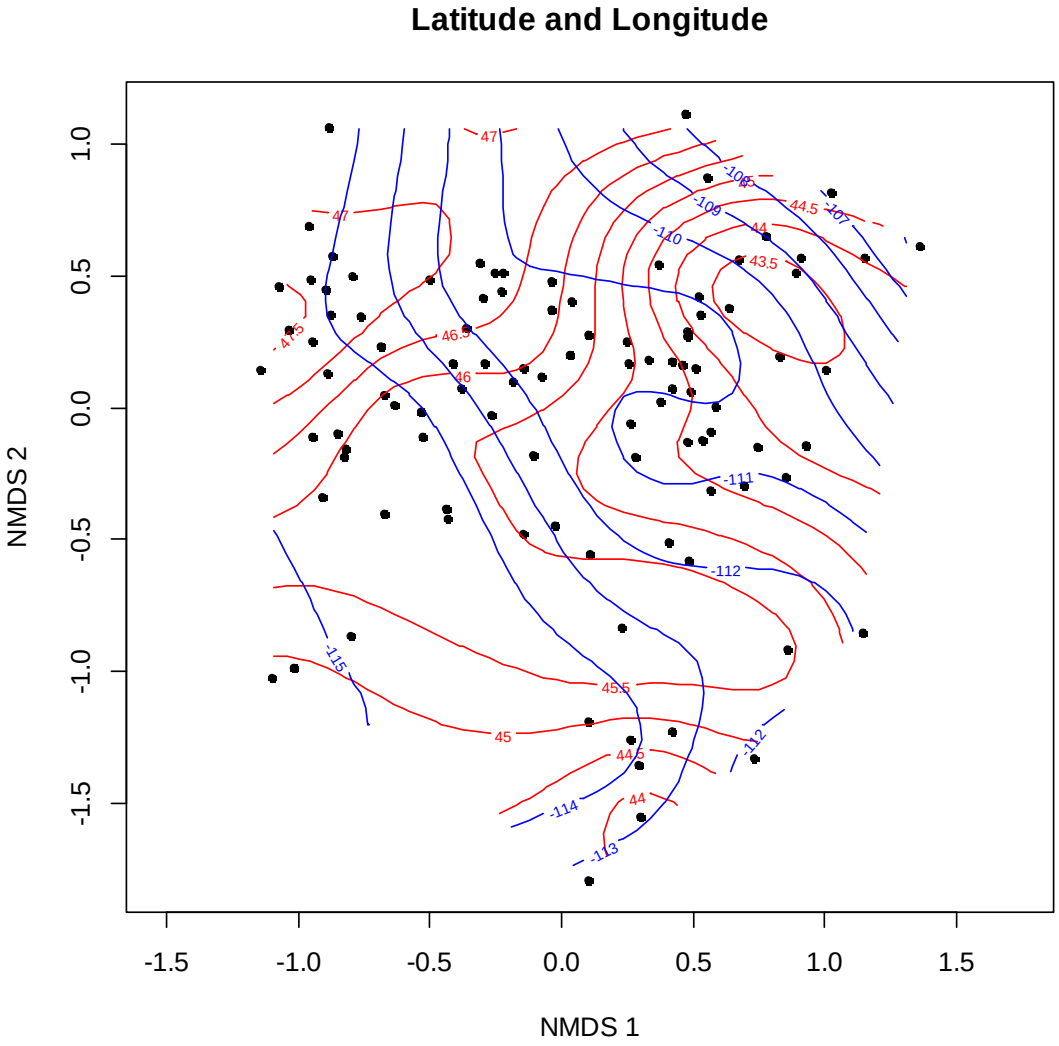


Fig. 4.4 NMDS ordination (axes 1 and 2) with fitted GAM surfaces of geographic coordinates: red lines = Latitude and blue lines = Longitude. Latitude was also correlated with elevation.

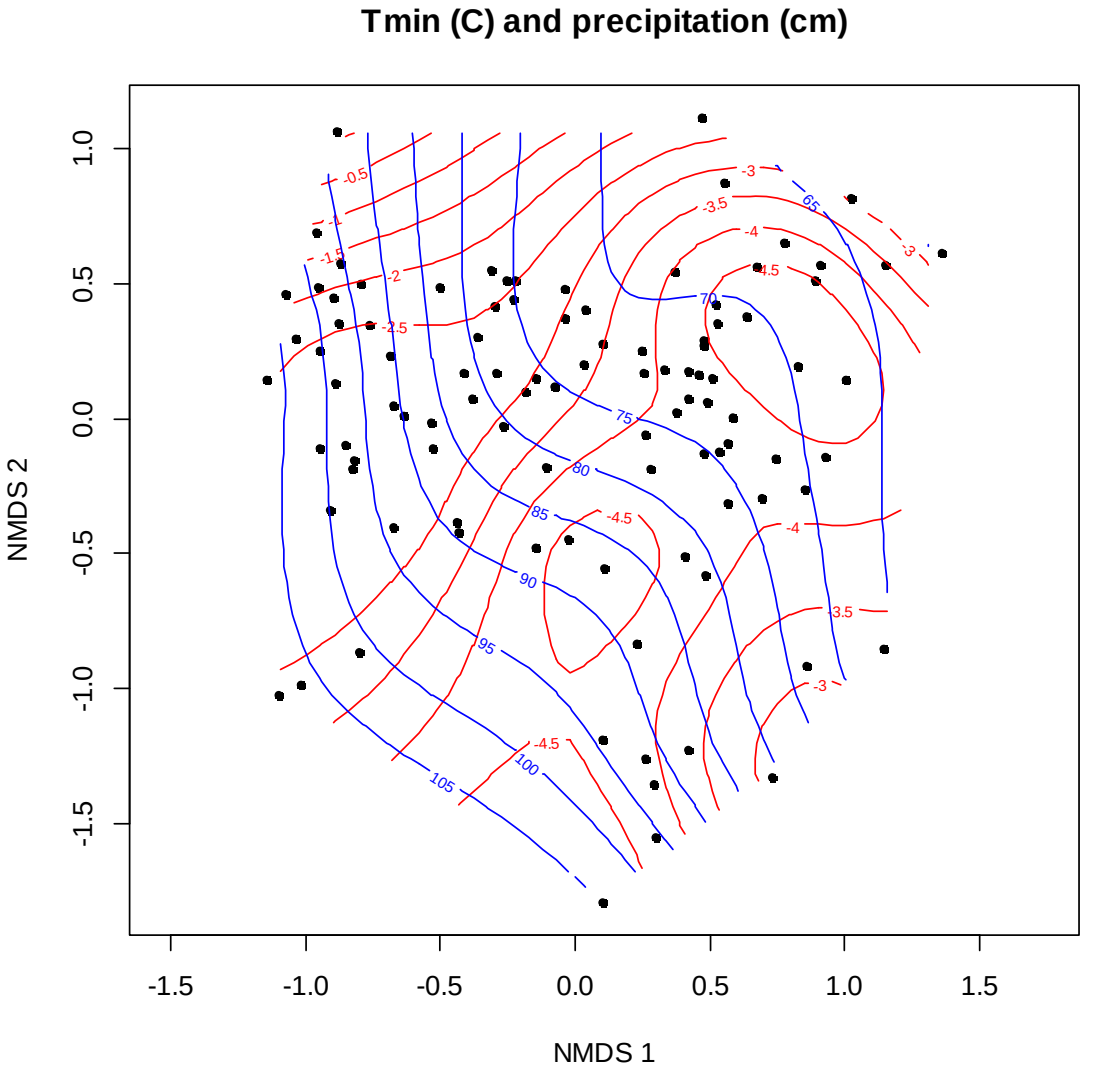


Fig. 4.5 NMDS ordination (axes 1 and 2) with two fitted GAM surfaces: blue lines = precipitation (cm) and red lines = Tmin (°C). Tmin was also highly correlated with dew, Tmax, and elevation.

Eutroph and Oligotroph Indices

Percent oligotroph richness increased with precipitation, RH, and dew point—measures of available water (Table 4.5). Geographically, percent oligotroph richness increased with latitude and decreased with longitude. Percent eutroph and oligotroph

richness were polarized along lichen community gradients to the point where little overlap occurred in ordination space (Figure 4.6).

Table 4.5 Correlations between selected variables and total species richness, percent of total richness as oligotrophs (%), and percent of total richness as eutrophs (%) found per plot. maximum temperature (Tmax), minimum temperature (Tmin), relative humidity (RH), relative humidity from maximum temperature (RHmax), and dew point (Dew). Correlations stronger than 0.5 are in bold.

Variable	**Pearson's correlations (r)**		
	Sp. rich	**Oligotroph (%)**	**Eutroph (%)**
Latitude	0.48	0.47	-0.42
Longitude	-0.19	**-0.51**	0.33
Elevation	**-0.59**	-0.40	-0.02
Precipitation	0.01	0.40	-0.39
Tmin	0.33	0.23	0.13
Tmax	0.44	0.11	0.28
RH max	0.30	0.36	-0.13
RH	0.29	0.41	-0.12
Dew point	0.39	0.31	0.10
Species richness	1.00	**0.51**	-0.18
Oligotroph (%)	**0.51**	1.00	**-0.52**
Eutroph (%)	-0.19	**-0.52**	1.00

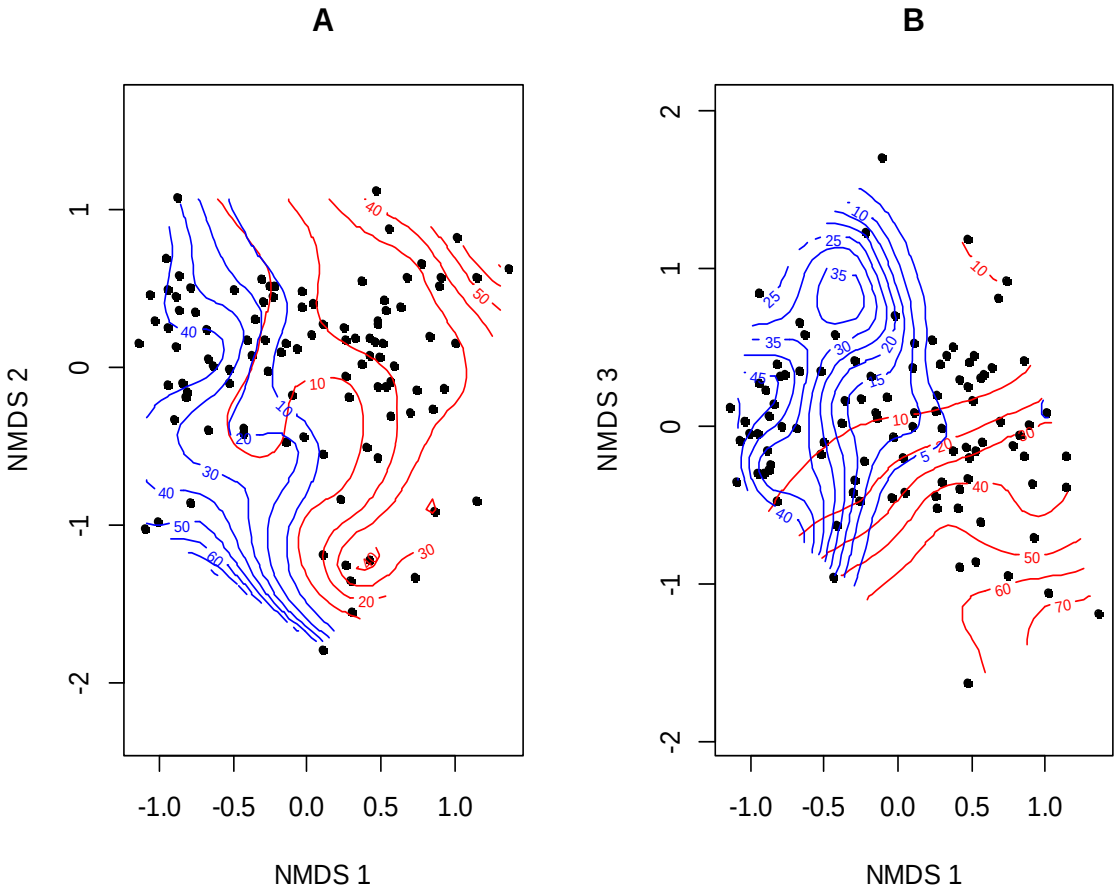


Fig. 4.6 Two NMDS ordinations with fitted GAM surfaces (Poisson distributions): blue lines = % oligotroph richness and red lines = % eutroph richness. Numbers represent percent classes. NMDS A shows axes 1 and 2 for consistency with other graphs (gamma=10). NMDS B shows axes 1 and 3 which were found to be the most important axes for eutroph and oligotroph distribution (gamma = 4).

Individual Species Associated with Climate

Indicator species associated with low precipitation and temperature extremes largely overlapped with species in the eutroph index (Table 4.6). The most common species were the genera *Physcia*, *Xanthomendoza*, and *Xanthoria*. Species associated with high RH and precipitation coincided with species from the oligotroph index (Table

4.6). The whole dataset (n = 320) was used in this analysis to maximize species information.

Six species associated with temperature extremes and low precipitation were found only in the MRE: *Bryoria glabra, B. furcellata, Melanohalea exasperata, Phaeophyscia orbicularis, Physcia biziana,* and *P. tenella.* We identified two species associated with hardwood presence, *Xanthoria oregana* and *Physcia biziana*. However, further investigation is warranted because only 4 stands of pure hardwoods and 14 hardwood dominant stands existed in the dataset.

Table 4.6 Weighted averages of lichen species associated with climate gradients (package = 'vegan' Oksanen et al. 2011). ***** = oligotroph species and †= eutroph species. Rare species which occurred < 4 times were not considered.

Variable	***Indicator species***
Precipitation >95 cm	*Ahtiana sphaerosporella*, Alectoria imshaugii*, A.sarmentosa*, Bryoria capillaris, B. pseudofuscescens*, Hypogymnia enteromorpha*, H. occidentalis*, Lobaria pulmonaria*, Nodobryoria oregana*, Parmeliopsis hyperopta*, Usnea scabrata**
Precipitation < 65 cm	*Bryoria furcellata*, Melanohalea exasperata, Phaeophyscia orbicularis†, Physcia aipolia†, P. biziana†, P. caesia†, P. dimidata†, P. stellaris†, Usnea hirta, Xanthomendoza fallax†, X. montana†*
Tmax > 11 °C	*Cetraria Canadensis, Esslingeriana idahoensis*, Evernia prunastrii, Phaeophyscia orbicularis†, Physcia aipolia†, P. biziana†, P. caesia†, P. stellaris†, Xanthomendoza montana†, Xanthoria candelaria†*
Tmin < (- 3.75) °C	*Bryoria lanestris, B. glabra, Hypogymnia austeroides, Melanohalea elegantula, M. exasperata*, M. subelegantula, Physcia adscendens†, P. biziana†, P. tenella†, Xanthomendoza fallax†*
RH max >36	*Alectoria imshaugii*, A.sarmentosa*, Bryoria capillaris, Esslingeriana idahoensis*, Hypogymnia enteromorpha*, Lobaria pulmonaria*, Nodobryoria oregana*, Platismatia glauca*, Usnea filipendula*, U. scabrata**

Prediction Ability for New Plots

How well each variable was fit originally (by a GAM) in ordination space was related to the predictive accuracy for that variable (Table 4.7). Eutroph richness is shown to exemplify how well the model could predict a variable strongly associated with lichen community composition. Predicted values from variables that were marginally significant (e.g. precipitation) should be used as a relative measure rather than an absolute predicted value.

Table 4.7 Correlation (r) and linear model (r^2) between real values and predicted values for selected climate variables. n= 229 plots withheld from the stratified model. Original r^2 represent how well the variable performed in the stratified ordination model (n=101).

Variable	***r***	***r²***	***Original r²***
Elevation	0.71	0.50	0.58
Tmin	0.45	0.20	0.36
Tmax	0.42	0.17	0.39
Precipitation	0.45	0.20	0.23
Dew point	0.52	0.27	0.33
Eutroph richness	0.87	0.75	0.80

Discussion

Lichen Communities

FIA crew members found 112 different species of epiphytic macrolichens throughout the CRE, IBE, and MRE. Though species richness was consistently higher in the CRE, more species were found in the MRE. The CRE is known to host diverse lichen flora (DeBolt and McCune 1993; McCune 1982). Lower γ diversity in the CRE is likely due to the lower number of plots sampled and smaller geographic size compared to the MRE and IBE.

Geographically, the northwestern sections of the IBE and MRE were more species rich than the southern portions. The strong correlation between latitude and species richness is likely related to a general increase in elevation in the southern half of the study area. Many lichen studies report a strong negative correlation between elevation and species richness (Jovan and McCune 2004; McCune et al. 2010; Neitlich et al. 1999, 2003). Higher elevations are naturally reduced in available habitat and typically experience climate extremes (Körner 2007).

FIA plots only include epiphytic macrolichens which represent a fraction of total lichen species. One study in central MT documented merely 34% of 168 total lichen species as epiphytic (Salix 2004).

Climate

Each lichen species has an optimal range of moisture, temperature, light, and nutrient availability for maximum growth (Sillett et al. 2000). Warm temperatures between 15 °C and 20 °C have been found to maximize mycobiont growth (Hale 1983). We found warmer temperatures were associated with an increase in species richness. Minimum temperature was strongly correlated with elevation and dew point and thus, the associated decrease in species richness cannot fully be discerned between these variables.

Paterson (1983) found available moisture to be the most important factor determining lichen growth. Moisture from liquid or vapor water rehydrates desiccated lichen thalli and activates metabolism (Lange et al. 1986). While some species (mainly those with a cyanobacteria photobiont) need liquid water to metabolically reactivate, other species (those with a green algae photobiont) can reach this state through water

vapor alone (Lange et al. 1986). Dew point and precipitation, both measures of liquid water, were important in the model. RH however, was not. Interestingly, a smaller model nested within our study area found the opposite result—RH was more important than precipitation (Grenon et al. –*in prep*). The differing results could be contributed to the increased number of high precipitation plots at low elevations in our model which creates favorable climate conditions for certain lichen species. Overall, species richness did not increase with precipitation most likely because of a confounding effect from elevation where the majority of precipitation falls as snow.

Over the last two decades, climate has changed in the NW Interior Mountains with fewer cold days in the winter and a rise in summertime temperatures (National Climatic Data Center, web accessed March 2012). Warming winter temperatures may increase climatic suitability for certain lichen species. However, if warming temperatures lead to increases in freeze thaw cycles, lichens can be damaged or killed through repeated ice encapsulation—which traps gases and creates an anoxic environment (Bjerke 2011). Warming temperatures in the summer coupled with decreasing available liquid and vapor water (drought conditions) would likely shift lichen communities towards extirpation of moisture sensitive species and proliferation of drought and temperature tolerant species.

Eutroph and Oligotroph Indices and Individual Species Affiliations

Eutrophic lichens tend to be xerophytic, photophilic, and temperature tolerant in nature (Davies et al. 2007; Grenon et al. – *in prep*; Jovan and McCune 2004; Neitlich et al. 2003). We also found individual lichen species associated with low precipitation and

temperature extremes were predominantly eutrophs. Common genera included *Physcia*, *Melanohalea, Xanthomendoza*, and *Xanthoria*. *Physcia* species were strikingly diverse in harsh climatic conditions, including both temperature extremes. Surprisingly, our eutroph index as a whole was weakly correlated to Tmax and only moderately so to precipitation. This result may be influenced by an interaction between precipitation and temperature. Eutrophs were largely absent from warm wet environments. Percent eutroph richness was negatively correlated with oligotroph richness; we interpret this to be a result of different habitat requirements and thalli adaptations to temperature and moisture regimes.

Lichen species associated with low rainfall and minimum temperatures often exhibit tolerance to air pollution (Davies and others 2007). Air pollution, especially from nitrogen, can cause shifts in lichen communities (Geiser and Neitlich 2007; Jovan and McCune 2005; van Herk et al. 1999)—from oligotrophic to eutrophic over relatively short time frames [< 8 years] (Larsen Vilsholm et al. 2009). The importance of eutroph and oligotroph indices in our model was only partly explained by climate, and therefore we cannot rule out an effect of air pollution in our study area. Unfortunately, we did not have pollution data to test the relationship between air quality and eutroph/oligotroph indices. A similar study in the northern Rocky Mountains found eutrophic species correlated to a nitrogen gradient (Grenon et al. -*in prep*). However, the nitrogen gradient was based on % N in lichen thalli. It is unclear how much nitrogen content within lichen thalli varied with time spent metabolizing. Warm wet conditions (negatively associated

with eutroph richness) favor metabolic activity (Hale 1983; Lange et al. 1986; Paterson et al. 1983) and thus the use of N reserves for growth.

Oligotrophs tend to be larger than eutrophs and have higher surface area exposed to the environment. We found oligotrophs exhibited opposite response patterns of eutrophs. Numerous oligotrophs were restricted to areas associated with high precipitation and moderate temperatures. Temperature and moisture conditions should be considered, or held constant, when assessing oligotroph responses to other variables such as air quality. Individual species (from weighted averages) associated with high precipitation and relative humidity greatly overlapped with species from the oligotroph index.

New Plots

Significant correlations were found between predicted values and real values for the climate and elevation variables tested. The linear model adj. r^2 values related to the original performance of the fitted variable (adj. r^2). Our model was able to successfully predict values for environmental variables as long as the variable was fairly important along ordination gradients. In the case of moderately important variables, predicted values should be used with caution or more as a relative than an absolute measure. The tolerance of prediction error depends on the nature of the study and the question being asked.

Modeling Considerations

It is important we acknowledge that because FIA plots exist on private land exact plot locations are classified information. Though the 'fuzzed' locations in our dataset fall within a mile of actual plot locations, it is entirely possible the climate data used were not fully representative of plot conditions. Also, the majority of climate data used were averaged from 1971-2000, whereas our gradient model is based on lichen community data from 2002-2011.

Another consideration with FIA data involves the error associated with using different lichen observers (McCune and Lesica 1992). FIA crew members are checked routinely by lichen experts in part of a QA process which ensures the majority of lichens on a plot are found (FIA P3 Field Guide 2011). We were not able to check for individual observer bias across the study area. We assume the majority of common lichens were found most of the time.

Lichen communities respond to many environmental attributes aside from climate. Variables to consider in future modeling efforts could include species dispersal limitations, pollution deposition values, potential evapotranspiration (PET), and stratified plots of hardwood stands vs. conifer stands. Hardwood and conifer forests are known to host differing lichen communities (Rogers and Ryel 2008). We were unable to assess the difference between these two stand types due to low sample size of hardwood dominated plots.

The scale of a study is an important consideration when assessing different environmental attributes (Will-Wolf et al. 2006). We left the study area intact because we

were assessing climatic response. The study area could certainly be partitioned into smaller sections to reduce the climatic effect and allow other variables such as forest structure to be assessed. Study areas at the forest or individual tree level have found factors such as tree circumference, light, and stand age important (Lesica et al. 1991; Nascimbene et al. 2009; Rogers et al. 2008; Sillett et al. 2000). Whereas lichen studies across broad areas tend to isolate climate and air quality signals (Geiser and Neitlich 2007; Jovan and McCune 2004, 2005; Will-Wolf et al. 2006).

We recommend increased sampling of off-frame plots in polluted areas to help separate an air quality signal from a climate signal. According to FIA procedures, most plots should be revisited in 8 to 10 years. Revisited plots can be entered into this model and assessed for changes. Any spatially broad or directional shifts in lichen communities would likely reflect shifts in climate or air pollution regimes.

Conclusions

Lichen commutities were diverse across the NW Interior Mountains (112 species found). Species richness was highest in the northern sections of the IBE, MRE, and in the CRE. The decrease in species richness with southern latitudes was due inpart to climate gradients associated with increasing elevation.

Species richness increased with precipitation only in areas of moderate temperatures. Species richness decreased when precipitation was confounded with high elevations, which also related to low temperatures and humidity.

Patterns in individual lichen species associated with climate largely overlapped with the species and patterns from the eutroph and oligotroph indicies. Eutrophs were

related to dry environments and temperature extremes. The genera *Physcia* was impressively diverse in harsh conditions. *Melanohalea, Xanthoria* and *Xanthomendoza* were also associated with low precipitation and temperature extremes. The pattern of eutrophs across our study area was only partially related to climate. Eutrophs are also known to thrive in areas with increased nitrogen deposition; unfortunately we did not have pollution data to test this response.

Oligotroph species exhibited different patterns of distribution than eutrophs. Percent oligotroph richness was associated with measures of liquid water and moderate temperatures. Oligotroph richness was strongly affiliated with the NW section of our study area.

If climate change continues to increase temperatures in the NW Interior Mountains, suitable habitat for species sensitive to cold temperatures could increase. Warming temperatures in the summer coupled with decreasing available liquid and vapor water (drought conditions) would likely shift lichen communities towards extirpation of moisture sensitive species and proliferation of drought and temperature tolerant species.

Our model was able to successfully predict values for environmental variables as long as the variable was important along ordination gradients. In the case of marginally significant variables, predicted values should be used with caution or more as a relative than an absolute measure.

If reliable pollution or other environmental information becomes available it can be analyzed with the current model. New or revisited plots can be added to the current model to track changes in lichen community composition overtime. Large directional

shifts may reflect changes in climatic conditions. This model can be used as a tool to address questions in management and conservation related to individual lichen species, lichen communities, forest health, and climate conditions.

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CHAPTER FIVE

CONCLUSIONS

Forested ecosystems in the NW Interior Mountains of the United States are jeopardized by degrading air quality and changes in climate regimes. Monitoring current conditions, tracking changes, and predicting ecosystem responses to changes in climate and air pollution can be an expensive and challenging task through instrumentation alone. This thesis explored the utility of lichens as an economical and practical means to assess nitrogen deposition, climate, and thus forest health across three sections of the NW Interior Mountains. Three biomonitoring techniques (McCune and Geiser 2009) were employed: analysis of elemental concentrations in lichen thalli (Fenn et al. 2008; Fenn et al 2007; Geiser and Neitlich 2007); analysis of lichen community composition (Geiser and Neitlich 2007; Joavn and McCune 2004, 2005; McCune et al 2010); and eutroph/oligotroph functional group indices (Jovan and Riddell –*in review*).

The first study took place in the Wind River Range (WRR) where rapid expansion of natural gas drilling in close proximity to the forest has caused warranted concern over nitrogen deposition. Correlations were found between percent N (% N) concentrations in *Usnea lapponica* and *Letharia vulpina* thalli and N deposition measured by canopy throughfall monitors. More samples are needed to solidify the relationship with *L. vulpina*, but *U. lapponica* can be used as a cost effective means (~ $22.00/ sample for N lab analysis) to expand N monitoring in the WRR with special focus on the Class I

Bridger Wilderness. Another notable finding was % N in *Usnea lapponica* exponentially decreased with distance from oil and gas development.

As part of this study, N deposition was quantified and spatially assessed. Nitrogen deposition in the Boulder drainage was elevated (> 4.0 kg N ha^{-1} $year^{-1}$) above background conditions (0.9 kg N ha^{-1} $year^{-1}$; Sverdrup et al. 2012), with deposition measurements 2 to 5 times higher than all other drainages sampled. It is likely portions of the Class I Bridger Wilderness in the Boulder drainage are affected by current rates of N deposition. A critical load was not established; however clear evidence of degraded lichen communities were found above 4.0 kg N ha^{-1} $year^{-1}$, including necrotic and bleached thalli and deformed and stunted growth. Thus chronic levels of N deposition less than 4.0 kg ha^{-1} $year^{-1}$ are liable to negatively affect lichen communities.

The next study expanded north from the WRR along the Rocky Mountains. Eighty-eight different macrolichen species were found. The correlation between throughfall N deposition and % N in *U. lapponica* (from study 1) was used to estimate N deposition along the Rocky Mountains. The extrapolation is acknowledged but justified based on the similar forest structure and climate conditions along the northern Rocky Mountains. N deposition was estimated from <0.5 to 4.26 kg N ha^{-1} $year^{-1}$. The two most notable areas of N deposition were around Bozeman, MT (Gallatin NF) and Pinedale, WY (Bridger-Teton NF).

An N pollution signal (using % N in lichen thalli) was isolated from the climate signal. The most important variables related to lichen community composition were latitude, longitude, and elevation. The strength of the signals was likely due in part to

dense clustering of plots around the WRR. Temperature (Tmin and Tmax) and relative humidity were the most important climate variables and when added to the model, greatly reduced the signal of latitude, longitude, and elevation.

Distribution of eutrophic and oligotrophic species differed throughout the northern Rocky Mountains. Percent eutroph richness increased while percent oligotroph richness decreased with N deposition. It is hard to say whether this is a response to N since both indices were markedly related to climate gradients. Eutrophs were widely found throughout the study area. Species from the genera *Physcia*, *Xanthomendoza,* and *Xanthoria* were found to be associated with N deposition, low precipitation, and extreme temperatures. Oligotrophs were generally restricted to moist temperate regions. The exception was *M. exasperata* which was found only in the Wind River Range and associated with dry cold environments. Future use of oligotroph and eutroph indices for N indication should include considerations of climate affinities and species distributions.

The final study increased longitudinally to include much of the NW Interior Mountains. One-hundred and twelve epiphytic macrolichen species were recorded from this study area. To exclude clustering effects observed in study 2, plots were stratified across longitude and latitude. Geographically extending longitude expanded climate gradients. Temperature (also correlated with dew point and elevation) was the most important climate variable related to patterns in lichen community composition. In contrast to the northern Rocky Mountains study, a precipitation gradient was more important than relative humidity. However, species richness increased with precipitation only in areas of moderate temperatures. Species richness decreased when precipitation

was confounded with high elevations where snow dominates the precipitation regime. High elevations were also correlated with low temperatures and low humidity. The NW section of this study area was on average the most species rich, though species rich pockets were found throughout the northern half of the NW Interior Mountains. Eutroph/oligotroph indices and indicator species patterns associated with climatic variables matched findings from the northern Rocky Mountains. The pattern of percent eutroph richness was only partially related to climate. Unfortunately, no nitrogen deposition data was available for this project.

Increasing temperatures expected from climate change may actually increase habitat availability and hospitable conditions for lichen species in our study area, especially if the number of cold days is reduced. Lange and Green (2005) suggest that the ability of lichens to adapt to changes in climate and other habitat conditions has greatly been underestimated. However, if increase temperatures occur coupled with drought conditions (both decreases in precipitation and water vapor) a decline or shift in lichen communities either directly—extirpation of sensitive moisture dependent lichens—or indirectly though loss of habitat would be expected. Drought-stressed forests are more susceptible to wildland fires, disease, and bark beetle infestations (Dale et al. 2001).

The three studies in this thesis have shown multiple approaches to successfully utilize lichens as biomonitors. The models created can adequately predict values for environmental variables as long as the variable is important along ordination gradients. In the case of marginally significant variables, predicted values should be used with

caution or as a relative rather than an absolute measure. New or revisited plots can be added to the current models to track changes in lichen community composition overtime. Large directional shifts may reflect changes in climatic or air quality conditions. These models can be used to address management and conservation questions related to individual lichen species, lichen communities, forest health, diversity, air quality, and climate conditions.

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APPENDICES

APPENDIX A

EPIPHYTIC MACROLICHEN SPECIES FROM SURVEYED PLOTS FOR CHAPTER 3

Epiphytic macrolichen species and their Forest Inventory and Analysis (FIA) codes, number of plots detected, frequency (% freq.) of occurrence, and mean abundance from 68 plots in the northern Rocky Mountains.

Species name	***FIA code***	***Detected***	***% Freq.***	***abundance***
Ahtiana sphaerosporella	Ahtsph	4	4.5	1.75
Alectoria imshaugii	Aleims	7	8.0	1.86
Alectoria sarmentosa	Alesar	17	19.3	1.71
Bryoria capillaris	Brycap	4	4.5	3.00
Bryoria fremontii	Bryfre	19	21.6	2.95
Bryoria furcellata	Bryfur	5	5.7	2.60
Bryoria fuscescens	Bryfus	57	64.8	2.98
Bryoria glabra	Brygla	17	19.3	2.71
Bryoria implexa	Bryimp	1	1.1	3.00
Bryoria lanestris	Brylan	10	11.4	2.50
Bryoria pseudofuscescens	Brypse	13	14.8	2.46
Bryoria simplicior	Brysim	5	5.7	2.00
Bryoria trichodes	Brytri	1	1.1	2.00
Candelaria pacifica	Cndpac	37	42.0	2.54
Cetraria canadensis	Cetcan	4	4.5	1.25
Cetraria chlorophylla	Cetchl	31	35.2	2.26
Cetraria merrillii	Cetmer	10	11.4	1.30
Cetraria orbata	Cetorb	9	10.2	1.22
Cetraria pinastri	Cetpin	16	18.2	2.00
Cetraria platyphylla	Cetpla	10	11.4	2.50
Cladonia chlorophea	Clachl	2	2.3	2.50
Cladonia coniocrcraea	Clacon	2	2.3	1.00
Cladonia fimbriata	Clafim	2	2.3	1.00
Cladonia ochrchlora	Claoch	2	2.3	1.50
Cladonia pyxidata	Clapyx	1	1.1	1.00
Esslingeriana idahoensis	Essida	4	4.5	2.75
Evernia mesomorpha	Evemes	2	2.3	1.50
Evernia prunastri	Evepru	2	2.3	1.50
Hypogymnia appinata	Hypapi	1	1.1	3.00
Hypogymnia austeroides	Hypaus	24	27.3	1.83
Hypogymnia imshaugii	Hypims	36	40.9	2.61
Hypogymnia metaphysodes	Hypmet	7	8.0	2.43
Hypogymnia occidentalis	Hypocc	3	3.4	2.00
Hypogymnia physodes	Hypphy	28	31.8	2.64
Hypogymnia tubulosa	Hyptub	24	27.3	2.17
Imshaugia aleurites	Imsale	2	2.3	1.50
Leptogium saturnium	Lepsat	1	1.1	1.00

Letharia columbiana	Letcol	34	38.6	2.26
Letharia vulpina	Letvul	66	75.0	2.91
Melanelia disjuncta	Meldis	1	1.1	1.00
Melanohalea elegantula	Melele	53	60.2	3.25
Melanohalea exasperata	Melexa	9	10.2	3.56
Melanohalea exasperatula	Melexl	36	40.9	2.83
Melanelixia fuliginosa	Melful	1	1.1	3.00
Melanohalea subelegantula	Melsel	9	10.2	2.67
Melanohalea subolivacea	Melsol	41	46.6	2.68
Melanelixia subaurifera	Melsub	1	1.1	1.00
Nephroma helveticum	Nephel	1	1.1	1.00
Nephroma parile	Neppar	2	2.3	1.00
Nodobryoria abbreviata	Nodabb	24	27.3	2.29
Nodobryoria oregana	Nodore	9	10.2	2.00
Parmelia hygrophila	Parhyg	21	23.9	2.29
Parmelia saxatilis	Parsax	2	2.3	1.00
Parmelia sulcata	Parsul	43	48.9	2.21
Parmeliopsis ambigua	Popamb	61	69.3	2.84
Parmeliopsis hyperopta	Pophyp	14	15.9	1.64
Peltigera collina	Pelcol	1	1.1	1.00
Phaeophyscia nigricans	Phanig	1	1.1	1.00
Phaeophyscia orbicularis	Phaorb	4	4.5	1.25
Physcia adscendens	Phyads	50	56.8	2.74
Physcia aipolia	Phyaip	5	5.7	1.80
Physcia biziana	Phybiz	4	4.5	1.50
Physcia caesia	Phycae	8	9.1	1.13
Physcia dimidata	Phydim	6	6.8	2.00
Physcia dubia	Phydub	1	1.1	1.00
Physcia stellaris	Physte	1	1.1	2.00
Physcia tenella	Phyten	9	10.2	1.89
Physciella chloantha	Pclchl	1	1.1	2.00
Physconia americana	Phoame	1	1.1	2.00
Physconia enteroxantha	Phoent	1	1.1	2.00
Physconia isidiigera	Phoisi	1	1.1	3.00
Physconia perisidiosa	Phoper	1	1.1	2.00
Platismatia glauca	Plagla	20	22.7	2.25
Ramalina farinacea	Ramfar	1	1.1	1.00
Usnea cavernosa	Usncav	1	1.1	3.00
Usnea filipendula	Usnfil	1	1.1	2.00
Usnea hirta	Usnhir	23	26.1	2.35
Usnea lapponica	Usnlap	63	71.6	3.24

Usnea rigida	Usnrig	1	1.1	1.00
Usnea scabrata	Usnsca	1	1.1	3.00
Xanthoria candelaria	Xancan	6	6.8	2.17
Xanthomendoza fallax	Xanfal	6	6.8	2.00
Xanthomendoza fulva	Xanful	32	36.4	2.53
Xanthomendoza galericulata	Xangal	1	1.1	2.00
Xanthomendoza montana	Xanmon	15	17.0	2.47
Xanthomendoza oregana	Xanore	12	13.6	2.00
Xanthoria parietina	Xanpar	2	2.3	2.00
Xanthoria polycarpa	Xanpol	14	15.9	2.07

**Parmelia sulcata* includes the recently described species *Parmelia barrenoae* (Hodkinson et al. 2010)

APPENDIX B

EPIPHYTIC MACROLICHEN SPECIES FROM SURVEYED PLOTS FOR CHAPTER 4

Epiphytic macrolichen species from FIA plots in the NW Interior Mountains. Forest Inventory and Analysis (FIA) codes, number of plots detected, frequency of occurance, and mean abundance from the whole dataset (n=320) and detection number form the stratified dataset.

Species	FIA code	Detected 320 plots	Frequency	Mean Abundance	Detected 101 plots
Ahtiana sphaerosporella	Ahtsph	12	3.73	2.16	3
Alectoria imshaugii	Aleims	30	9.32	2.33	9
Alectoria sarmentosa	Alesar	71	22.05	2.42	17
Bryoria capillaris	Brycap	25	7.76	2.92	9
Bryoria fremontii	Bryfre	78	24.22	2.97	20
Bryoria furcellata	Bryfur	6	1.86	2.67	2
Bryoria fuscescens	Bryfus	242	75.63	2.86	73
Bryoria glabra	Brygla	17	5.28	2.70	6
Bryoria implexa	Bryimp	1	<0.5	3.00	0
Bryoria lanestris	Brylan	13	4.04	2.62	6
Bryoria pseudofuscescens	Brypse	63	19.57	2.89	11
Bryoria simplicior	Brysim	20	6.21	2.55	3
Bryoria trichodes	Brytri	1	<0.5	2.00	0
Candelaria pacifica	Cndpac	76	23.75	2.50	23
Cetraria canadensis	Cetcan	21	6.56	1.95	7
Cetraria chlorophylla	Cetchl	85	26.40	2.47	26
Cetraria fendleri	Cetfen	3	0.93	3.00	3
Cetraria merrillii	Cetmer	54	16.77	2.13	12
Cetraria orbata	Cetorb	62	19.25	2.42	15
Cetraria pallidula	Cetpal	1	<0.5	1.00	1
Cetraria pinastri	Cetpin	28	8.70	1.96	11
Cetraria platyphylla	Cetpla	55	17.08	2.64	20
Cladonia carneola	Clacar	3	0.93	2.67	2
Cladonia chlorophea	Clachl	9	2.80	2.67	5
Cladonia coniocrcraea	Clacon	2	0.62	1.00	0
Cladonia cornuta	Clacor	1	0.31	1.00	0
Cladonia fimbriata	Clafim	11	3.42	1.82	1
Cladonia norvegica	Clanor	1	<0.5	2.00	1
Cladonia ochrchlora	Claoch	20	6.21	2.25	8
Cladonia pyxidata	Clapyx	1	<0.5	1.00	1
Cladonia squamosa	Clasqu	1	<0.5	3.00	0
Cladonia sulphurina	Clasul	6	1.86	2.00	2
Cladonia umbricola	Claumb	1	<0.5	2.00	1
Collema nigrescens	Colnig	1	<0.5	3.00	1

Esslingeriana idahoensis	Essida	12	3.73	2.33	6
Evernia mesomorpha	Evemes	2	0.62	1.50	1
Evernia prunastri	Evepru	14	4.38	1.57	4
Flavopunctelia soredica	Fpusor	2	0.62	1.50	1
Hypogymnia appinata	Hypapi	1	<0.5	3.00	0
Hypogymnia austeroides	Hypaus	37	11.56	1.92	14
Hypogymnia enteromorpha	Hypent	6	1.86	2.67	2
Hypogymnia imshaugii	Hypims	185	57.45	2.74	48
Hypogymnia inactiva	Hypina	1	<0.5	3.00	0
Hypogymnia metaphysodes	Hypmet	35	10.87	2.77	15
Hypogymnia occidentalis	Hypocc	36	11.18	2.81	6
Hypogymnia physodes	Hypphy	92	28.75	2.88	31
Hypogymnia tubulosa	Hyptub	71	22.05	2.56	22
Imshaugia aleurites	Imsale	2	0.62	1.50	1
Imshaugia placorodia	Imspla	2	0.62	2.50	1
Leptogium cellulosum	Lepcel	1	<0.5	3.00	1
Leptogium saturnium	Lepsat	2	0.62	1.00	1
Letharia columbiana	Letcol	126	39.13	2.73	37
Letharia vulpina	Letvul	270	84.38	2.81	85
Lobaria pulmonaria	Lobpul	4	1.24	2.50	2
Melanelia disjuncta	Meldis	1	<0.5	1.00	0
Melanohalea elegantula	Melele	143	44.41	2.94	44
Melanohalea exasperata	Melexa	9	2.80	3.56	2
Melanohalea exasperatula	Melexl	189	58.70	2.80	56
Melanelixia fuliginosa	Melful	2	0.62	3.00	1
Melanohalea multispora	Melmul	4	1.25	2.50	0
Melanohalea subelegantula	Melsel	36	11.18	2.78	7
Melanohalea subolivacea	Melsol	134	41.61	2.81	41
Melanelia sorediata	Melsor	1	0.31	3.00	0
Melanelixia subaurifera	Melsub	2	0.62	1.50	1
Nephroma helveticum	Nephel	2	0.62	2.00	1
Nephroma parile	Neppar	2	0.62	1.00	2
Nephroma resupinatum	Nepres	2	0.62	3.00	2
Nodobryoria abbreviata	Nodabb	137	42.81	2.64	35
Nodobryoria oregana	Nodore	24	7.45	2.54	12
Parmelia hygrophila	Parhyg	49	15.22	2.41	19
Parmelia saxatilis	Parsax	3	0.93	1.67	0
Parmelia sulcata	Parsul	142	44.38	2.56	45
Parmeliopsis ambigua	Popamb	223	69.25	2.67	66
Parmeliopsis hyperopta	Pophyp	87	27.02	2.43	26
Peltigera collina	Pelcol	1	<0.5	1.00	1

Phaeophyscia hirsuta	Phahir	1	<0.5	3.00	1
Phaeophyscia nigricans	Phanig	3	0.93	2.33	2
Phaeophyscia orbicularis	Phaorb	7	2.17	1.86	3
Physcia adscendens	Phyads	94	29.19	2.67	31
Physcia aipolia	Phyaip	7	2.17	1.86	3
Physcia biziana	Phybiz	5	1.55	1.80	1
Physcia caesia	Phycae	11	3.42	1.55	4
Physcia callosa	Phycal	1	<0.5	3.00	1
Physcia dimidata	Phydim	11	3.42	2.27	4
Physcia dubia	Phydub	3	0.94	1.67	1
Physcia stellaris	Physte	8	2.50	2.13	3
Physcia tenella	Phyten	9	2.80	1.89	4
Physciella chloantha	Pclchl	3	0.93	2.67	1
Physconia americana	Phoame	1	<0.5	2.00	0
Physconia enteroxantha	Phoent	3	0.94	2.00	1
Physconia isidiigera	Phoisi	3	0.93	2.33	1
Physconia perisidiosa	Phoper	2	0.62	2.50	1
Platismatia glauca	Plagla	65	20.19	2.39	17
Platismatia stenophylla	Plaste	1	<0.5	3.00	1
Ramalina farinacea	Ramfar	2	0.62	1.50	1
Usnea cavernosa	Usncav	1	<0.5	3.00	0
Usnea filipendula	Usnfil	6	1.86	2.50	1
Usnea hirta	Usnhir	53	16.46	2.40	20
Usnea lapponica	Usnlap	198	61.88	2.80	68
Usnea rigida	Usnrig	1	<0.5	1.00	0
Usnea scabrata	Usnsca	11	3.42	2.55	4
Usnea substerilis	Usnsst	8	2.48	2.50	3
Xanthoria candelaria	Xancan	20	6.21	2.00	5
Xanthomendoza fallax	Xanfal	12	3.73	2.17	3
Xanthomendoza fulva	Xanful	83	25.94	2.52	27
Xanthomendoza galericulata	Xangal	3	0.93	2.00	1
Xanthomendoza hasseana	Xanhas	2	0.62	3.00	1
Xanthomendoza montana	Xanmon	29	9.01	2.48	9
Xanthomendoza oregana	Xanore	15	4.66	2.20	6
Xanthoria parietina	Xanpar	2	0.62	2.00	0
Xanthoria polycarpa	Xanpol	29	9.01	2.38	8
Xanthoria tenax	Xanten	1	<0.5	3.00	0

**Parmelia sulcata* includes the recently described species *Parmelia barrenoae* (Hodkinson et al. 2010)