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# Using Epiphytic Lichens to Monitor Nitrogen Deposition Near Natural Gas Drilling Operations in the Wind River Range, WY, USA

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**Abstract** Rapid expansion of natural gas drilling in Sublette County, WY (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 concentrations in lichen thalli were highest at plots closest to drilling operations (<30 km). N concentrations in lichens decreased exponentially with distance from drilling activity. Highest TF N deposition, 4.1 $kgha^{-1}year^{-1}$, coincided with clear evidence of damage to lichen thalli. This deposition value is above estimated preindustrial deposition conditions (0.9 $kgN\ ha^{-1}year^{-1}$) and regional critical loads (a deposition value below which ecosystem harm is prevented) of N deposition for sensitive ecosystem components. N concentrations in *Usnea 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 *Letharia vulpina* were associated with estimated background conditions of 0.9 $kgN\ ha^{-1}year^{-1}$. 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

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## 1 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 of North America, even slight increases in N deposition can alter ecosystem processes.

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), nitrogen dioxide ($NO_2$), 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 ecosystem characteristics and the level, duration, and type of N deposition (Baron et al. 2011; Bobbink et al. 2010; Fenn et al. 2003). Eutrophication can lead to stimulation of plant and algal growth, and increased competition within biotic communities — favoring invasive species and decreasing occurrence of sensitive species (Bobbink et al. 2010; Baron 2006; Baron et al. 2011; Beem et al. 2010; Howarth 2008). 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 $kgN ha^{-1} year^{-1}$ (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 $kgN ha^{-1} year^{-1}$ (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-h 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 (activity on private land is not included) in Sublette County. As of December 2011, there were 4,184 active PGWs and POWs (BLM, Pinedale office *per. comm.*).

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.

Currently there are only a few deposition monitoring programs active on the west side of the WRR (within national forest boundaries). The National Atmospheric Deposition Program (NADP) has measured an average annual wet N deposition (2005–2010) of 0.9 and 1.19 $kg ha^{-1}$ at two sites (data available for download at http://nadp.sws.uiuc.edu/NTN/ntnData.aspx, accessed January 2012). 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 $kg ha^{-1}$. 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). Long-term lake monitoring by the BTNF (Svalberg and Porwoll 2002) has measured increased ammonium and nitrate concentrations in high alpine lakes between 1984 and 2010 (Grenon et al. 2010). Annually averaged N deposition from two bulk precipitation sites (co-located with two alpine lake monitoring 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 two monitors (only one has a long-term record) which measure ambient aerosols and tracks trends in visibility. An increase in ammonium nitrate (between 1992 and 2007) during winter months was found at the BRID1 IMPROVE site (Grenon et al. 2010).

The high-surface area profiles of forest canopies intercept airflow and trap ambient pollution (Fowler et al. 1999). During dry spells, atmospheric pollutants accumulate on tree boles, branches, and leaves. When precipitation events occur, accrued dry deposition is delivered to the forest floor in solution as throughfall deposition (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. We know of no other study which has measured Nr deposition of canopy TF within close proximity of a large energy development operation.

Epiphytic lichens live 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 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. 2012; Munzi et al. 2010; Riddell et al. 2008; van Herk et al. 2003; van Dobben and 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 (kgha$^{-1}$ year$^{-1}$), providing an economical tool to continue monitoring and further identify areas of elevated Nr deposition in the WRR.
- Quantify Nr deposition from ammonium, nitrate, and total dissolved inorganic Nr (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 TF 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.

## 2 Methods

### 2.1 Study Area

The 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. 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 (Fig. 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*.

### 2.2 Study Design

In the summer of 2010, nine temporary 0.378 hectare circular plots were established in the WRR just outside the Bridger Wilderness (Fig. 1). At each plot, passive ion exchange resin-filled collectors (IER) were installed to measure throughfall (TF) and bulk deposition. In addition, lichen thalli from *L. vulpina* and *U. 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 (Forest Inventory and Analysis Phase 3 Field Guide, version 5.1 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,

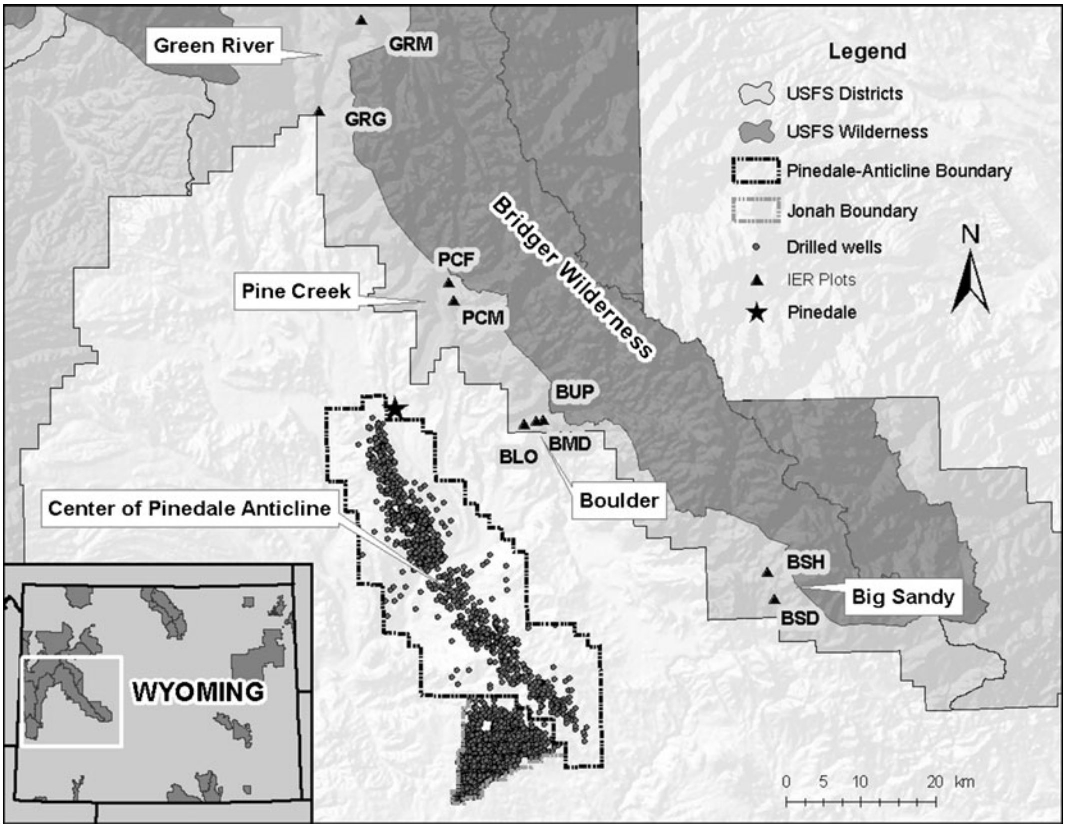


**Fig. 1** The Wind River Range, plot locations and nearby oil and gas wells from the Pinedale Anticline and Jonah project areas. Boundary lines for project areas show potential expansion of drilling activity. Center of the Pinedale Anticline approximates where distance measures were taken from. Green River, Pine Creek, Boulder, and Big Sandy represent the four drainages in which this study took place. Three-letter acronyms for plots: *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

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] (Fig. 1).

## 2.3 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, Fig. 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** Installation of ion exchange resin TF collectors near Boulder Lake (site—BLO). Snow tubes were attached to the collector funnels to allow for collection of snow in the winter season

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 1.9×1.9 cm squares) was placed over the snow tube opening to keep birds and small mammals from fatally 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 (Fig. 2).

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 IRN150 Rohm and Haas) captured positive and negative ions as they passed through. IER tubes were left on the collectors for 1 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 ($PO_4^{3-}$). Measured phosphate was used to indicate contamination via bird excrement. Sulfate ($SO_4^{2-}$) was also measured, but is not included in this report.

At each TF plot, 2–4 separate samples of *L. vulpina* and *U. 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 six substrates to ensure that a representative sample of the population was collected. *L.vulpina* and *U. lapponica*, both common lichens throughout the study area, have fruiticose (hair-like) growth forms where new-growth stems from the tips (thus each sample contains multiple years of thalli growth). 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° resolution). Linear distance between each plot and the center of the Pinedale Anticline (PA) natural gas field (Fig. 1) was measured using ArcGIS (ArcGIS 9.3.1; ESRI 2009).

### 2.4 Laboratory 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 subsampled. 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 h (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 laboratory. Contaminated samples from bird droppings were identified by high ammonium coupled with high phosphate ion concentrations and were deleted from further analysis.

### 2.5 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 was used to test if N concentrations in *U. lapponica* could be used to predict N concentrations in *L. vulpina* (R Development Core Team 2011). To increase sample size for this regression, plots from the surrounding area were included. Linear regression was also used to approximate the relationships between lichen N

**Table 1** Coefficient of variation (CV) for within and between plot measurements of lichen thalli

| | 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 |

*Usnea*, *U. lapponica*; *Letharia*, *L. vulpina*

concentrations and TF deposition $kg\,ha^{-1}\,year^{-1}$ for $NO_3^-$-N, $NH_4^+$-N and DIN.

## 3 Results

### 3.1 Variation Among Samples

Laboratory accuracy was acceptable with an average deviation of 0.03 %N for material of known value with a slight bias for underestimation (<0.001 %). Laboratory precision tested with reference materials had a mean CV and SE of 0.023 and 0.009 for N. Repeat measurements 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, within plot N concentrations between the two species were different and could not be averaged ($n$=12, $p\leq0.001$). The variation in *U. lapponica* N concentrations between plots was almost seven times the variation measured within plots (Table 1).

The variation of TF DIN measurements between plots was twice the variation measured within plots (Table 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 deposition sample data in any analyses because contamination from bird droppings resulted in insufficient replication of valid samples.

### 3.2 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 related to paired samples of *L. vulpina* [$R^2$=0.83, $p\leq0.001$, $n$=

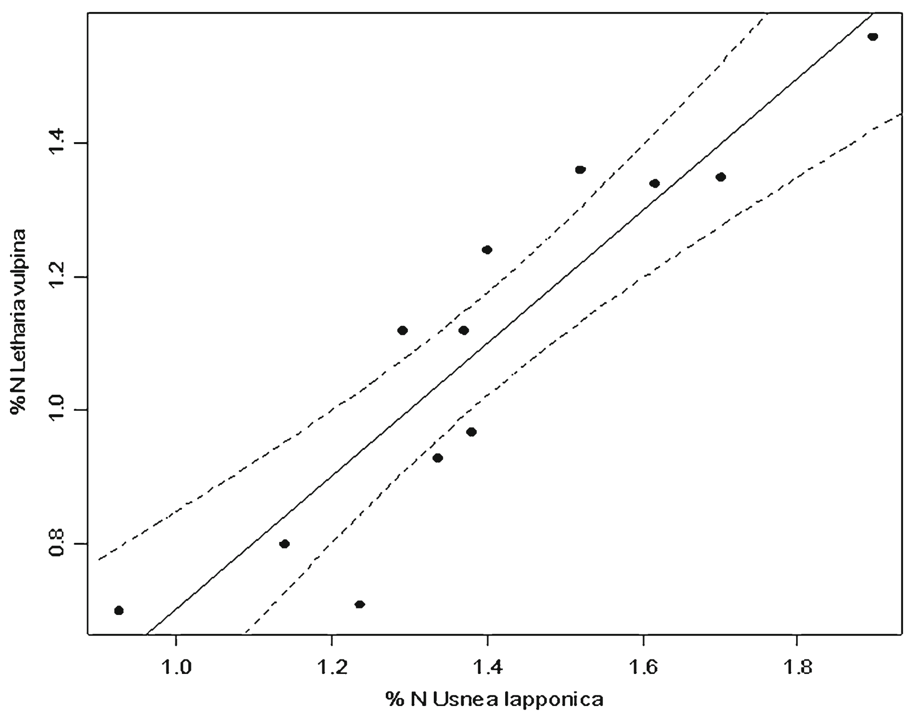


**Fig. 3** % N (dry weight) found in *Usnea lapponica* vs. %N (dry weight) found in *Letharia vulpina*, shown as simple linear regression with 95 % confidence bands (*dotted lines*; $r^2$=0.83, $p\leq$ 0.001). In order to increase sample size, plots from WY without IER monitors were included in the model

**Table 2** Measures of throughfall N deposition (kgNha$^{-1}$year$^{-1}$) and lichen N concentrations (% dry weight) in the Wind River Range

| 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 |

*Elev* elevation, *Dist*. distance to the middle of the PA gas field, *DIN* dissolved inorganic nitrogen, *SE* standard error, *Usn*. *U. lapponica*, *Let*. = *L. vulpina*

12] (Fig. 3). 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). A negative relationship was found between N concentration in *U. lapponica* and distance from the middle of the PA oil and gas development ($R^2$=0.76 after log transformation; Fig. 4).

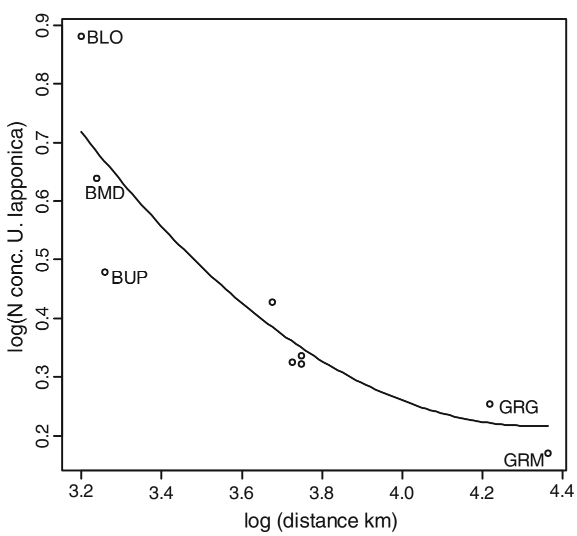


**Fig. 4** Log transformation of both nitrogen concentrations in *U. lapponica* and distance from the center of the Pinedale Anticline natural project area. $R^2$=0.76, $p$=0.006. Curve=7.54−3.38$x$+0.39$x^2$. *GRM* Green River Moose Creek, *GRG* Green River Gypsum Creek, *BLO* Boulder low, *BMD* Boulder middle, *BUP* Boulder up. The three points in the center of the graph are not co-located

## 3.3 Throughfall Measurements

Annual DIN in TF samplers ranged from 0.78 to 4.13 (kgNha$^{-1}$), respectively. The plots with the highest

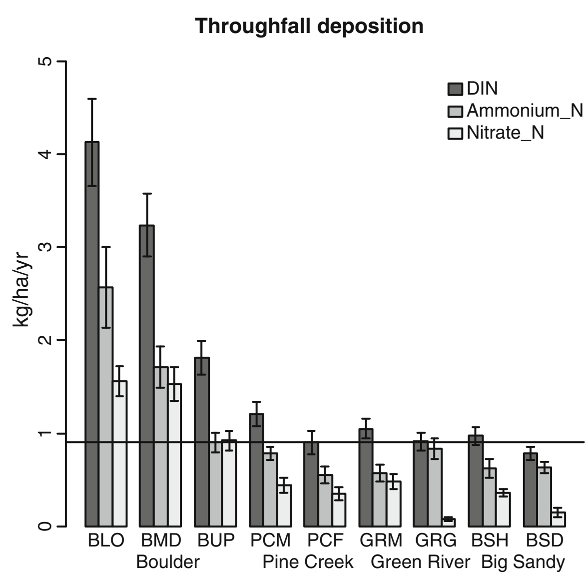


**Fig. 5** Annual throughfall N deposition from four drainages in the Wind River Range. DIN refers to total dissolved inorganic N (ammonium N plus nitrate N). The horizontal line represents background levels of N deposition in the Northern Rockies as estimated by Sverdrup et al. (2012)

measured annual $NO_3^-$-N and $NH_4^+$-N were located in the Boulder drainage. A near 5-fold increase between $NH_4^+$-N measurements and an 11-fold increase in $NO_3^-$-N was found between the Boulder drainage and other drainages (Fig. 5). The plot with the lowest measured DIN deposition was located in the Big Sandy drainage (Fig. 5; Table 2).

### 3.4 Lichen N vs. Throughfall N Deposition

Throughfall DIN deposition could be predicted from concentrations of N in both *U. lapponica* and *L. vulpina* ($R^2$=0.91 and $R^2$=0.78, respectively; Fig. 6, Table 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 (Fig. 6, Table 3). TF DIN decreased with distance from the middle of the PA oil and gas project areas ($R^2$=0.76, $p$=0.006 after log transformation). $NO_3^-$-N was more strongly correlated than $NH_4^+$-N with distance to project areas ($r$=−0.74 and −0.69, respectively, after log transformations).

No relationship was found between sulfate or percent 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 more plots contained *U. lapponica* ($n$=9) than *L. vulpina* ($n$=5).

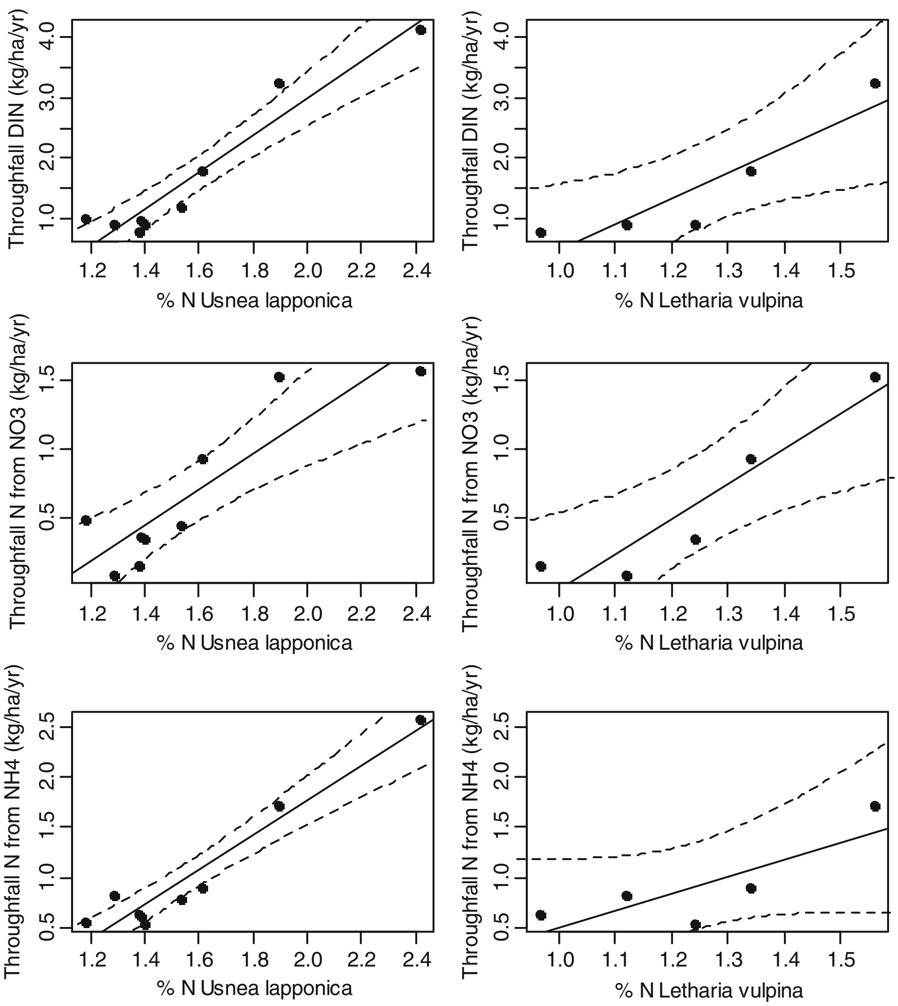


**Fig. 6** Simple linear regressions of %N in lichens vs. throughfall (*TF*) dissolved inorganic N, TF $NO_3^-$-N, and TF $NH_4^+$-N deposition ($kg\,ha^{-1}\,year^{-1}$). *Dotted lines* represent 95 % confidence bands

**Table 3** Regression models and approximated equations

| 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*)* | $y=0.84x+0.48$ | 0.83 | 12 | <0.001 |

*=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

## 4 Discussion

### 4.1 TF DIN Deposition

Four of the nine plots measured TF DIN deposition less than 1.0 $kg ha^{-1} year^{-1}$, reflecting pre-industrial background conditions (0.9 $kgN\ ha^{-1} year^{-1}$). 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 12 % and 17 % of the total wet N deposition in spring and summer seasons, respectively (Beem et al. 2010).

Though, 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 et al. 2011), the WRR is a pristine N-limited environment and is likely to respond to small increases in Nr deposition (Baron et al. 2011; Bobbink et al. 2010). Nr deposition in the Boulder drainage was elevated above all other drainages in the study area (1.8–4.13 $kgN\ ha^{-1} year^{-1}$). 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 decreased with distance to oil and gas project areas (the Boulder drainage is topographically closest in proximity to the project areas).

### 4.2 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). 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 Wyoming Department of Environmental Quality (WYDEQ) monitoring stations centrally located within Sublette County indicate up to 50 % of the wind flow comes from the NW and NNW. Although 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.

The Jim Bridger coal-fired power plant, less than 100 miles south of Pinedale, WY, is a large stationary source of NOx. According to WY DEQ Title V emission inventories the Jim Bridger plant emitted 16,371 tons of $NO_x$ in 2010 (http://deq.state.wy.us/aqd/Actual%20Emissions.asp). 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 (UTDEQ 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; 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 $NH_4^+$, $NH_4NO_3$, and ammonium sulfate ($(NH_4)_2SO_4$) (Howarth et al. 2002; Twigg et al. 2011; van Herk et al. 2003).

### 4.3 Reduced vs. Oxidized Nr Deposition

Oxidized forms of N ($NO_x$, $HNO_3$) are associated with fossil fuel emissions, whereas reduced forms ($NH_y$) typically come from agriculture (fertilizer application and animal waste), fertilizer production (Fenn et al. 2003; Howarth 2008; Bobbink et al. 2010) and due to over-reduction of N oxides by three-way catalytic converters in newer model vehicles (Bishop 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 Nr deposition 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 ($NH_4^+$, $NO_x$) 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 four and ten 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 $NO_x$ trapped by inversions in the Boulder drainage could form $HNO_3$, a molecule with a high deposition velocity. Unfortunately, no ambient measurements of $HNO_3$ exist within the Boulder drainage.

### 4.4 Critical Loads

The CLO for N deposition varies among different ecosystem components (Pardo et al. 2011). In alpine lakes of 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. 2003; Rueth and Baron 2002; Saros et al. 2010; Williams and Tonnessen 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 United States Forest Service National Lichen and Air Quality Database, http://gis.nacse.org/lichenair/index.php?page=cleansite).

No CLOs have been ascribed to epiphytic macrolichen communities in the northern Rocky Mountains. In montane forests of Oregon and Washington, USA, CLO estimates, sensitive to precipitation regimes, range from 2.3 to 9.5 $kgN\ ha^{-1} year^{-1}$ (Geiser et al. 2010). A parallel study implemented in mixed conifer forests of California, USA found a CLO of 3.1 $kgN\ ha^{-1} year^{-1}$ related to a known threshold value for N concentration in *L. vulpina* (1.03 %) and to the early stages of decline in sensitive lichen species (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 et al. 1989; Geiser et al. 2010; Jovan and Carlberg 2007). To exemplify differences in ecosystems, if the highest N concentration value for *L. vulpina* (1.56) from the BMD plot is used in the California mixed conifer forest model (Fenn et al. 2008), a deposition level of 7.76 kgN $ha^{-1}year^{-1}$ (±2.4) is estimated. This deposition amount is well above the actual measured N deposition at this site (3.24 kg$ha^{-1}year^{-1}$ [±0.34]).

Though further studies and manipulative experiments are needed to help solidify CLO estimations, we observed clear evidence of degraded lichen communities subjected to 4.0 kgN $ha^{-1}year^{-1}$, including necrotic and bleached thalli and deformed and stunted growth. Therefore, chronic levels of N deposition under 4.0 kgN $ha^{-1}year^{-1}$ are likely to have negative impacts on lichen communities. The degree to which nitrogen deposition affects lichen communities 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.

### 4.5 Lichen Thresholds

A strong linear relationship 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 near-background conditions are associated with 1.35 % and 1.12 %N in *U. lapponica* and *L. vulpina*, respectively. Both concentration estimates are above designated thresholds for the Pacific NW region of the USA (Oregon and Washington). 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, 2008). This further exemplifies the need for region-specific studies. The Pacific NW receives greater amounts of precipitation per year than the interior west; it is likely that soluble elements such as N 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). In wetter climates, lichens spend more time in metabolic activity, which may also affect N concentrations.

## 5 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 all other drainages was measured. Lichen communities in the Boulder drainage were degraded when Nr inputs were above 4.0 kgN $ha^{-1}year^{-1}$. 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 $NO_x$ in the Boulder drainage could help us better understand sources of Nr deposition.

Percent N concentrations in *U. lapponica* and *L. 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 laboratory analysis of N concentrations) to expand N monitoring in the WRR with special focus on the Class I Bridger Wilderness. N concentrations of 1.35 % in *U. lapponica* and 1.12 % 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 expected to continue with the ongoing extraction of natural gas. It is important to continue monitoring N deposition in the WRR.

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