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Original Articles

The potential use of elemental content of saxicolous lichens as bioindicators of nitrogen deposition in the central and southern California mountains

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ABSTRACT

High elevation and arid wilderness areas generally lack epiphytic lichen species used for air quality biomonitoring. We assessed the potential of saxicolous lichens to serve as bioindicators of nitrogen (N) and sulfur (S) deposition by examining relationships among deposition, environmental variables, and elemental concentrations in the saxicolous lichens *Umbilicaria phaea* (n = 51), *Rhizoplaca melanophthalma* (n = 20), and the epiphytic lichen *Letharia vulpina* (n = 16) in the southern Sierra Nevada (SN) and San Bernardino Mountains (SBM) of California, USA. Lichen samples were collected between 2013 and 2019 in 31 plots between 180 and 3330 m elevation. These samples were analyzed for concentrations of 23 elements, including N and S. Signal-to-noise ratios were high comparing the variation among plots to within plots and within samples for N and S concentrations of the *Umbilicaria phaea* samples. While *Umbilicaria phaea* N and S concentrations were correlated with modeled N and S deposition estimates, *Rhizoplaca melanophthalma* and *Letharia vulpina* N and S concentrations were not. Subsequent principal components analysis and cluster analysis of the *Umbilicaria phaea* dataset revealed differences in elemental profiles between the SN and SBM, with the SBM samples having higher concentrations of most elements, including heavy metals. Additionally, the first two principal components explained most of the variation (61 %) in elemental content and had strong relationships to latitude, longitude, N and S deposition, and precipitation. We conclude that the elemental profile of *Umbilicaria* could be used to assess N and S deposition for areas in the western USA that lack suitable epiphytic lichen species.

1. Introduction

Air resource managers need a cost-effective way to estimate nitrogen (N) deposition in remote high elevation Class 1 wilderness areas, which includes over 890,000 ha above 1980 m in elevation in California in the USA (Jovan et al., 2021). Class 1 Wilderness areas fall under Category 1b of the protected area management categories developed by the International Union for Conservation of Nature (IUCN). Like legislation in other countries, the USA has the Clean Air Act that mandates that land managers monitor and protect Class I wilderness areas from visibility-impairing pollution (i.e. smog), which includes NO_x, SO_x and other pollutants (Jovan et al., 2021). While sulfur (S) deposition is generally lower than N deposition in California, detecting S is still desirable. *Letharia vulpina* is a lichen species commonly used as a bioindicator of N deposition in established lichen biomonitoring protocols in the western USA (Jovan & Carlberg, 2007; Root et al., 2013). However, much of the high elevation wilderness in the southern half of California has little to no epiphytic (tree-dwelling) species, including *Letharia vulpina.* Deposition monitoring equipment is both impractical and costly in remote places such as wilderness areas, resulting in gaps in understanding of deposition in these areas. Prior research has proposed certain saxicolous (rock-dwelling) lichens as possible target species for biomonitoring in this region (Jovan et al., 2021). If saxicolous lichen species are identified as reliable bioindicators of N deposition, then it will be possible to expand collection of deposition-related biomonitoring data to high elevation wilderness areas.

Somewhat like epiphytic lichens, saxicolous lichens survive on nutrients and water from atmospheric sources but with additional nutrients derived from rock substrates (Ahmadjian & Hale, 1973). Lichens lack specialized tissues to filter pollutants, causing them to accumulate pollutants proportionally to local atmospheric deposition. In addition to N and S, several other elements (mostly metals, Supplementary Table 2) can be measured in lichen samples as a percentage or parts per million (ppm); the resulting elemental profile may provide additional context

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for understanding the local deposition regime. For epiphytic lichens, relationships of their elemental concentrations to deposition have been demonstrated in many studies (Geiser, 2004; Jovan & Carlberg, 2007; Massimi et al., 2019; McClenahen et al., 2012; Riga-Karandinos & Karandinos, 1998). Although saxicolous lichens have not been critically evaluated as pollution biomonitors in this region, they often occur in places where epiphytic lichens do not, presenting the possibility to help to fill geographic gaps in lichen biomonitoring data.

Saxicolous lichens generally have higher concentrations of N, S, and crustal elements such as calcium (Ca), magnesium (Mg), iron (Fe), silicon (Si), potassium (K), and aluminum (Al) than epiphytic lichens due to the influence of their substrate rock and soils (Ahmadjian & Hale, 1973; Fenn et al., 2007; Rhoades, 1999), which could confound the elemental signal of air pollution. In the western USA however, saxicolous lichen N and S concentrations indicated N and S deposition (Fenn et al., 2007) while certain elemental concentrations (antimony, palladium, copper (Cu), cadmium (Cd), tin and zinc (Zn)) related to anthropogenic sources, while others related to rock substrate (Zschau et al. 2003). In central Mexico similar findings were reported, with lead (Pb), Zn, vanadium, and Cu being indicative of anthropogenic sources (Puy-Alquiza et al., 2017). All of these studies used *Xanthoparmelia* species, which start to drop out at higher elevations in California (Jovan et al., 2021); furthermore, they are difficult to identify to species. *Rhizoplaca melanophthalma* is another saxicolous species that had concentrations of heavy metals that decreased with distance from the pollutant source (Dillman, 1996), but it has not yet been used to relate concentrations of N and S to deposition. Selecting new target saxicolous species could allow for improved monitoring of N and S deposition in remote ecosystems that have few to no epiphytic lichens, in particular high elevation ecosystems.

Elemental analysis results could help prioritize where to sample lichen communities or place air pollution monitors. Above treeline in alpine areas in the USA, the best air quality information available comes from emissions-based models such as the Community Multiscale Air Quality (CMAQ; Byun & Schere, 2006) model from the Environmental Protection Agency and the Total Deposition model (TDEP; Schwede & Lear, 2014) from the National Atmospheric Deposition Program Total Deposition Science Committee. CMAQ combines meteorological, chemical transport and emissions models to estimate N and S deposition nationwide. TDEP is a hybrid of CMAQ that incorporates national monitoring network empirical data into the model (Schwede & Lear, 2014). Several monitoring campaigns have shown local inaccuracies of these models in complex mountainous terrain (Hutten, 2015; Jovan et al., 2021). Low model resolution (e.g. 12 × 12 km grid) obscures important details such as N and S deposition hotspots (Bytnerowicz et al., 2015). Saxicolous lichen biomonitors could create a tool for higher resolution evaluation in areas that lack target epiphytic lichen species for elemental analysis.

Higher resolution evaluation of N and S deposition is important because excess deposition of these elements has been shown to alter plant and lichen community composition (Clark et al., 2019), influence lake water chemistry (Shaw et al., 2014), and acidify soils (Breiner et al., 2007; Duan et al., 2016; Josipovic et al., 2011). Excess N and S deposition also has negative impacts on human health. Places that have chronically elevated levels of NO_2 have consistently higher rates of asthma in humans (Cisneros et al., 2021). SO_2 pollution has been associated with increased rates of human mortality, especially due to respiratory related illnesses (Stieb et al., 2003).

A major source of N and S pollution is the combustion of fossil fuels, which also results in emissions of many other elements, including heavy metals, to the atmosphere (Nriagu, 1992). Sulfur deposition is low in California and the West (Bytnerowicz et al., 2016) compared to the eastern USA, in great part because a large amount of energy production in the western USA is renewable (New data shows nearly two-thirds of California's, 2019) and most power plants in California burn natural gas, which results in lower S and metals emissions than coal (Häsänen et al., 1986). In California the San Joaquín Valley is highly agricultural and is a large, diffuse source of ammonia (NH_3) emissions from fertilizer and livestock waste (Bytnerowicz et al., 2016), as well as nitrogen oxides (NO_x) from combustion of fossil fuels and unregulated agricultural soils emissions (Almaraz et al., 2018). Agricultural applications of S to fulfill nutrient requirements and as a pesticide can be large for certain crops (Hinckley et al., 2020). The Santa Ana Valley and adjacent Los Angeles basin, south of the San Joaquín Valley, are in one of the most densely populated regions of the country, where agricultural air pollution is minimal and vehicular and industrial emissions dominate (Breiner et al., 2007).

To address the following research goals, we assembled and analyzed saxicolous lichen datasets in California, including existing data from the southern Sierra Nevada and San Bernardino mountains, as well as additional data collected for this study. These datasets were collected within a few years of each other, and plots represent a range of climatic environments, with substantial variation in local atmospheric N and S deposition. Our primary objectives were to:

Describe N and S concentrations of the lichens and assess sources of error in the *Umbilicaria phaea* dataset.
Evaluate relationships between N and S concentrations across lichen species and with modeled N and S deposition estimates from CMAQ and TDEP.
Evaluate the elemental profile of *Umbilicaria phaea*, the most widespread and abundant saxicolous macrolichen in this region, in relationship to environmental variables, including modeled N and S deposition. In particular, does elemental composition, including 23 additional metals and other elements, differ between the Sierra Nevada and the San Bernardino mountains?

2. Methods

2.1. Study area

We studied elemental content of lichens in two mountain ranges: the southern Sierra Nevada (SN) and the San Bernardino (SBM; Fig. 1). The SN study area falls along a N deposition and elevational gradient that extends northeastward from the greater Fresno area of the San Joaquín Valley with relatively high N deposition into the high elevation mountains of the Sierra National Forest that experience relatively low N deposition. The SN has extensive alpine wilderness areas and is relatively close to the highly polluted San Joaquín Valley. Past lichen work suggests that adjacent National Parks (Yosemite and Sequoia Kings-Canyon) are significantly impacted by air quality in the SN (McCune et al., 2007), which have large swaths of land above the treeline that cannot be evaluated with typical epiphytic lichen biomonitoring methods. The SN has been shown to have elevated levels of N deposition, but empirical measurements of N deposition have mostly been taken on the periphery of the many Class I wilderness areas in the SN (Bytnerowicz et al., 2019).

The SBM study area is the northern portion of the San Bernardino National Forest where N deposition is relatively low compared to the greater San Bernardino area in the urban Santa Ana Valley to the west. Prior lichen work in this region, including in the SBM, has shown a loss of pollution-sensitive epiphytic lichen species over time and an increase in abundance and diversity of N pollution tolerant species (Jovan et al., 2012; Riddell et al., 2011). N deposition in this landscape is dominated by dry deposition, which is highly variable (Tulloss & Cadenasso, 2015), demonstrating that large-scale modeled deposition estimates oversimplify actual deposition patterns (Jovan et al., 2021).

Average precipitation across the SN plots is 933 mm annually, which is substantially higher than the 654 mm annually across the SBM plots according to the PRISM climate model (Daly et al., 2000) discussed in more detail in section 2.6, with a portion of that precipitation being snow in the winter months at the higher elevation plots and most

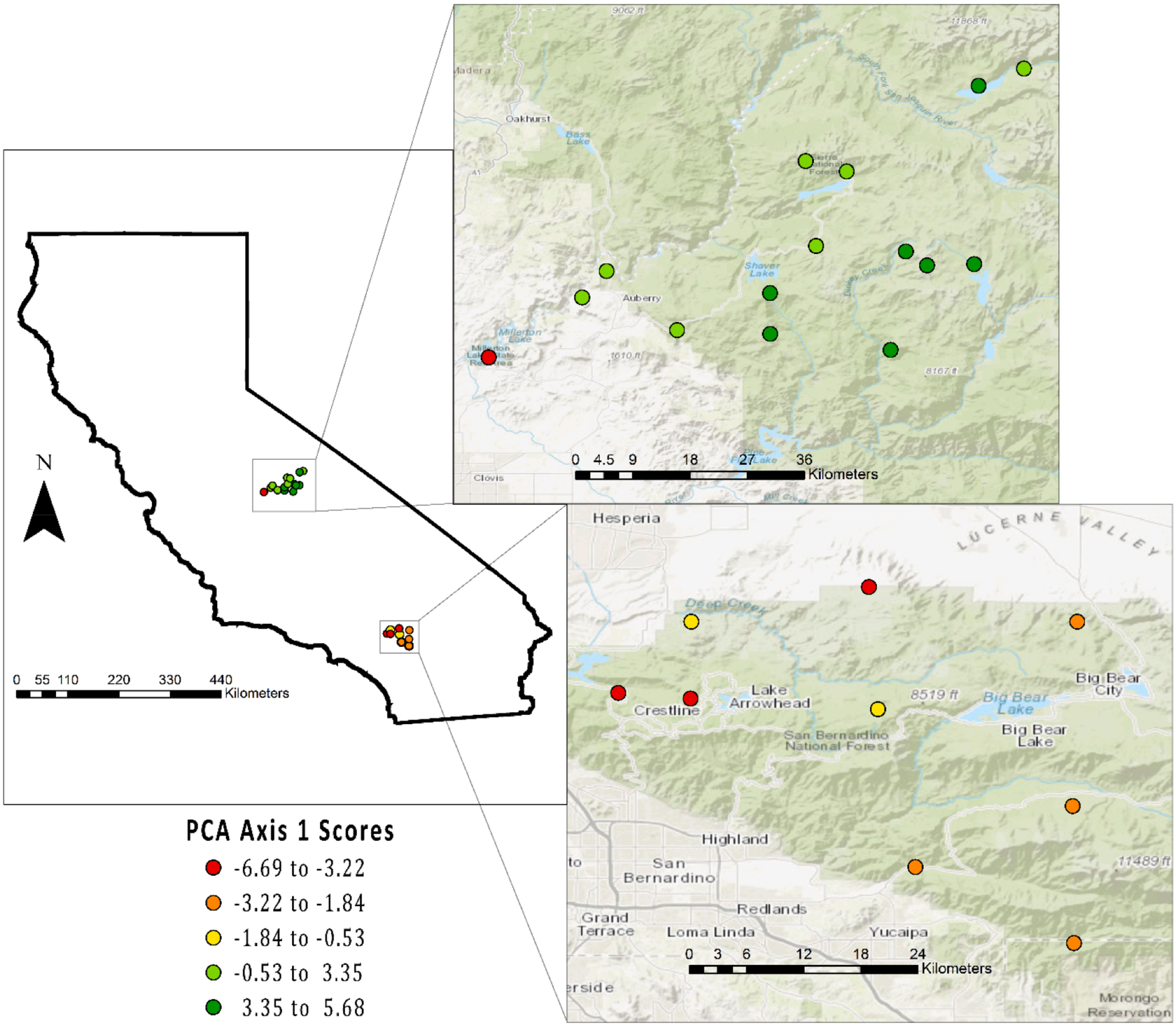

Fig. 1. Plot locations in the Sierra Nevada (top) and San Bernardino (bottom) Mountains; map at left shows them within the boundary of the state of California, USA. Plot symbols are color coded by principal component analysis (PCA) Axis 1 scores representing the strongest gradient in elemental content of *Umbilicaria phaea*. Low scores indicate higher N and S deposition, while high scores indicate lower N and S deposition.

precipitation occurring in the winter months. Mean annual temperature at the SBM plots is 2 °C hotter than the SN plots. N deposition is predominantly dry in both landscapes, according to the deposition models, but the SBM more drastically so. Mean N deposition is almost three times higher in the SBM (14 kg N/ha/yr) compared to the SN (5 kg N/ha/yr) according to the CMAQ model, with S deposition being almost twice as high (1.2 vs 0.7 kg S/ha/yr). The rock composition (mostly granite) of both regions is low in N (Morford et al., 2016). While S deposition has been thought of as having little ecological effect in California (Bytnerowicz et al., 2016; Fenn et al., 2010), recent work has shown effects on some tree species in the state (Fenn et al., 2020), thus we also assess the modeled S deposition in relationship to the S concentrations of the lichen samples.

2.2. Target species selections

We chose *Umbilicaria phaea* and *Rhizoplaca melanophthalma* as our target saxicolous species for collection because they were more abundant in the study areas compared to other saxicolous macrolichens. Also, because they are widely distributed across the western USA (Jovan et al., 2021) our findings might be more broadly useful. We included elemental analysis data for an epiphytic species, *Letharia vulpina,* that we collected whenever it occurred with the saxicolous lichens. This comparison strengthens the connection between our results and the large preexisting *Letharia vulpina* dataset for the western USA (U. S. Forest Service, 2020). This dataset has been used in a variety of air assessments since 1989 (Jovan et al., 2021; McMurray et al., 2013; Root et al., 2013).

Umbilicaria phaea Tuck. (henceforth "*Umbilicaria*") is a foliose saxicolous lichen that has abundant black apothecia with concentric fissures and a brown umbilicate thallus (Fig. 2). It is common up to middle elevations in the study areas, diminishing at higher elevations, and dropping out above 3170 m (McCune & Geiser, 2009). Globally it is found in western North and South America (Consortium of North American Lichen Herbaria, 2022).

Rhizoplaca melanophthalma (DC.) Leuckert & Poelt (henceforth "*Rhizoplaca*") is a foliose saxicolous lichen that has a light to moderately greenish-yellow umbilicate thallus with abundant olive to blackish apothecia (McCune & Geiser, 2009). It is common at most elevations below 4200 m in California, but less so than *Umbilicaria* (Jovan et al., 2021), and is found on every continent except Australia (Consortium of North American Lichen Herbaria, 2022). *Letharia vulpina* (L.) Hue (henceforth "*Letharia*") is a chartreuse green fruticose epiphytic lichen that lacks apothecia. It is most common at middle elevations (900 to 2500 m) in the mountains of California. Worldwide it is found in western North America and western Eurasia (Consortium of North American Lichen Herbaria, 2022).

Fig. 2. Photographs of *Rhizoplaca*, *Letharia*, and *Umbilicaria* from left to right. Photo credit: Adrienne Kovasi.

2.3. Field sampling protocol

Lichens were sampled using the U. S. Forest Service standard tissue collection approach (Geiser, 2004) with modifications for smaller tissue amounts (minimum 1 g) and the inclusion of saxicolous species. All lichens were collected using powder free nitrile gloves and SpeciPAK saran bags manufactured by Ampac. Six or more different rocks for saxicolous species and woody substrata for epiphytes were sampled up to a few hundred meters away from the center of the plot. This large area was necessary to find enough individual lichen thalli to be spatially representative of each plot and to reach, and ideally surpass, the minimum weight threshold of 1 g. The center of the plot was defined as the point where latitude and longitude were recorded or the central point of the U. S. Forest Service Forest Inventory and Analysis plot (FIA; Bechtold & Patterson, 2005). Lichens were removed from the rock using multiple tools allowing for collection of whole specimens. To reduce the unlikely possibility of contaminating samples from the collection knife, we used a ceramic scalpel for the 2019 round of sampling.

Availability of the target lichen species (*Umbilicaria, Rhizoplaca*, and *Letharia*) at plots was inconsistent, which led to gaps in the data, as described in the "Sample allocation" section. We define a "sample" as one SpeciPAK bag containing multiple thalli of one species of lichen. A minimum of one sample was collected for each target lichen species present at a plot. When larger quantities of target species were present, repeat samples were collected to assess within plot variability of lichen elemental concentrations (Table 1). During processing, large samples that didn't have a repeat sample at the plot level, or "plot repeat", were split into two halves, which we will refer to as "splits".

2.4. Sample allocation

In total we have *Umbilicaria* (n = 51), *Rhizoplaca* (n = 20), and *Letharia* (n = 16) from the SN and SBM, where n is the number of samples (Table 2). These samples were collected between 2013 and 2019. The SBM *Umbilicaria* and *Rhizoplaca* elemental analysis data came from nine plots sampled by Kovasi et al., (unpublished) in 2014. No *Letharia* was collected, as it was not present. The SN had existing *Rhizoplaca* and *Letharia* samples that were collected in 2013 at four plots by Cisneros et al., (unpublished). We visited the remaining 22 plots in 2019 to collect *Umbilicaria*, *Rhizoplaca,* and *Letharia* for this study.

Table 1
Sampling hierarchy, arranged from largest to smallest scale.

Scale	Size (approximate land area and/or lichen mass)	Definition
Region	1000 km^2	SN or SBM
Plot	3000 m^2, <10 g	Up to a few hundred meters from plot center
Plot repeat	3000 m^2, <10 g	Repeat sample at the same plot
Plot split	3000 m^2, <5 g	50/50 splits of plot level samples
Thalli	0.1 to 1 g	Lichen individual

2.5. Laboratory methods

The 2019 SN samples were prepared for elemental analysis as follows. Lichens were sorted and cleaned while wearing powder free nitrile gloves using equipment cleaned with 70 % ethanol. Debris, diseased portions of the thalli, and other lichen species were removed. Lichens were not washed to avoid leaching of elements and removal of informative particulate material (Upreti et al., 2015). After cleaning, a subset of samples that didn't have a plot repeat were divided in half into "splits" to measure within sample variability. Splits are not included in the Table 2 numbers.

Lichens were dried for 24 h at 70 °C, ground in an IKA tube mill grinder with metal blades, and then microwave digested (CEM Mars 6). Metal blades could be a source of Cr, Ni, Mo, and Mn but we did not have a way to quantify potential contamination. After digestion, samples were analyzed for a suite of 25 elements (Supplementary Table 2) on a Thermo-Fisher iCAPTM 7600 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) and a Leco 628 Series C/H/N Analyzer. Certified reference lichen materials (IAEA-336 *Evernia;* Heller-Zeisler et al., 1999) were analyzed concurrently with the lichen samples.

The Kovasi et al. (2014, unpublished) samples were processed using similar equipment and methodology, with some differences in the suite of elements measured. Splits were not created because that was not part of the sampling design for the study that collected these data.

The Cisneros et al. (2013, unpublished) samples were processed differently, being washed via sonication in 1 l of distilled water for one min then air dried. While this cleaning method is quite different, these samples represented a small portion of the dataset overall and we had no methodology to relate lichen elemental concentrations to cleaning methods. Again, splits were not created. The samples were then analyzed with similar methodology using an Elementar combustion analyzer for N and S, and a Thermo-Fisher iCap 7600 Duo ICP-OES Analyzer. Unfortunately, IAEA-336 *Evernia* was not analyzed concurrently for the Cisneros and older Kovasi data sets. Results were blank corrected and compared to the machine detection minima to ensure that that all results were above detection limits.

2.6. Data structure and analysis

All data manipulations and analyses were done in R Studio v. 1.4.1106 (R Studio Team, 2021) and PC-ORD v. 7.07 (McCune & Mefford, 2018). Modeled total N and S deposition estimates came from 3 yr rolling averages of the TDEP v. 2018.02 and CMAQ v. 5.0.2 (Bash et al.,

Table 2
Plot and species sampling. Many plots had collections of multiple species so the plot numbers in this table do not sum to 31 plots. While most data from US Forest Service lichen inventories may be downloaded from http://gis.nacse.org/lichenair, these datasets are not yet publicly available.

Region	*Letharia*		*Rhizoplaca*		*Umbilicaria*		Source	Year
	Plots	Samples	Plots	Samples	Plots	Samples		
SBM	0	0	5	8	9	25	Kovasi et al. unpublished	2014
SN	3	7	3	7	0	0	Cisneros et al. unpublished	2013
SN	6	7	4	4	15	26	this study	2019

2013) models. Thirty-year climate normals from 1991 to 2020 were extracted from the Parameter-elevation Regressions on Independent Slopes Model (PRISM; Daly et al., 2000). Climate, CMAQ, and TDEP values were assigned to plot coordinates in ArcMap 5.1 (Environmental Systems Research Institute, 2018). Elemental composition of granite (substrate for saxicolous lichens collected) in the study area was derived from Ague & Brimhall (1988).

To evaluate variability in elemental contents of lichens at various scales we analyzed several sources of error in the N and S concentrations of the larger *Umbilicaria* dataset: between plots, within plots, and within sample. Plot repeats provided a basis to estimate within-plot error, and splits of individual samples to analyze within-sample error. Analyses of variance were performed to obtain the mean square error (MSE) for each of the three levels: plot, plot repeat, and split. Signal-to-noise ratios were calculated using ratios of the MSEs across levels (McCune et al., 1997), with “signal” referring to among-plot differences. Upon determining that the data fulfilled the statistical assumptions of normality, equal variance, and independence, we used simple linear regression to evaluate relationships between N and S concentrations of *Umbilicaria*, *Rhizoplaca*, and *Letharia* and modeled N and S deposition estimates at a given plot. For regressions, plot repeats and splits were averaged to give each plot a single N and S value and fulfill the independence assumption.

To evaluate the *Umbilicaria* elemental profile, we ordinated samples in elemental space to assess dimensionality of the covariation among elements and to relate that covariation to environmental variables. We defined the elemental profile as concentrations of the 23 elements used in the following analysis. We had 51 samples, which included plot repeats and splits. The main data matrix consisted of the 51 samples × 23 elements after removal of elements that weren’t represented in both datasets or had drastically different values between the two datasets due to the differences in accuracy of lab equipment used for measurement (selenium, vanadium, carbon, mercury, and titanium). Unfortunately, carbon was not included because it was not measured in the SBM samples. Elements with skewness close to a value of one or greater were log transformed, avoiding excess weight given to extreme high values and resulting in reduced average skewness of 0.5. Elements often have linear correlations with one another in living beings such as plants and lichens (Zschau et al., 2003), and as these data had approximately linear relationships after log transformation, Principal Components Analysis (PCA; Pearson, 1901) was selected to assess the strongest covariation among the *Umbilicaria* elemental concentrations. The form of the cross products matrix was correlation coefficients. An outlier analysis was performed, and no samples had an average distance from other samples that was greater than two standard deviations from the grand mean. A randomization test for significance of correlation structure was performed using Rnd-Lambda in PC-ORD (Peres-Neto et al., 2005). The environmental data matrix consisted of 14 variables for each of the 51 samples and was used to calculate linear correlations of axis scores with the environmental variables (Supplementary Table 3).

To evaluate differences between the SN and SBM we first relativized the main matrix by standard deviations within each element, and then performed a two-way hierarchical cluster analysis using Euclidean distance and Ward’s linkage method to create dendrograms grouping plots and grouping elements, displayed simultaneously. The dendrograms were scaled by Wishart’s objective function expressed as percent of information remaining (McCune & Mefford, 2018). Differences between SN and SBM in the means for each of the 23 elements were evaluated with Student’s t-tests with alpha = 0.05.

Table 3
Sampling error of *Umbilicaria* partitioned into sources of variability. MSE is mean squared error derived from analysis of variance tables for various subsets of the data. Signal-to-noise ratios are F ratios constructed from MSE estimates from various analyses.

Element	Source	MSE	Signal-to-noise
N	Plot	0.219	Plot to plot repeat = 25 Plot to split = 53
	Plot repeat	0.009	Plot repeat to split = 2.1
	Split	0.004	
S	Plot	0.014	Plot to plot repeat = 67.7 Plot to split = 212.8
	Plot repeat	0.0002	Plot repeat to split = 3.1
	Split	0.0001	

We assessed differences in the elemental composition of granite for the SN and SBM by comparing them to those of the *Umbilicaria* using dominance curves in PC-ORD, which plotted log elemental concentrations by ranked concentration. We obtained the granite elemental data from Ague & Brimhall (1988), which included four types of granite that they characterized with 15 different elements and minerals that overlapped with the elements in our *Umbilicaria* samples. We reduced the mineral concentrations down to elemental concentrations by multiplying mineral concentrations by ratios of the elements of interest over the total elemental composition of the mineral multiplied by the appropriate atomic weights. We then calculated the means of the 15 elements for each of the four granite types. Ague & Brimhall (1988) mapped the four granite types across the SN and SBM, so we approximated the quantities present of each type in both of the study areas. The SN and SBM were composed of different, but relatively evenly divided granite types, so we simply averaged the types present to create the overall means for the SN and SBM.

3. Results

3.1. Sources of error

Reliable biomonitoring requires that the environmental signal of interest be large compared to sources of error. Ideally, variation among plots should be much larger than variation between repeated sampling of the same plots (plot repeats). Within sample (split) variation should be even lower than plot repeat variation. We found high signal-to-noise ratios for plots for N and S concentrations in *Umbilicaria* (Table 3).

The ratio of MSE of plots to MSE of plot repeats measures the variation among plots compared to the variation in the sample collection process. The ratio of MSE of plots to MSE of field replicates for N concentration was 25.0 while the ratio was 66.5 for S concentration, confirming that there is much higher variance between plots than between plot repeats. The highest ratios, as expected were MSE-plots:MSE-splits (52.9 for N and 212.8 for S; Table 3). The error from plot repeats was only slightly higher than that for splits (Table 3). The *Rhizoplaca* and *Letharia* datasets had too few plot repeats and splits for us to adequately assess variability as in *Umbilicaria*.

3.2. N and S content

Several metrics can used to compare variability of elemental concentrations between SN and SBM, such as comparing means, minima, maxima, standard deviations (SD), and coefficients of variation (CV). *Umbilicaria* had higher N than *Rhizoplaca* and *Letharia*. The SBM *Umbilicaria* N and S datasets had higher minimum, maximum and mean values than the SN *Umbilicaria* N and S datasets (Table 4). Lichen S concentrations had higher CVs than N concentrations. *Umbilicaria* N had relatively similar SDs and CVs across sites, unlike S, which showed higher variability in the SN. *Rhizoplaca* mean N and S concentrations were slightly higher in the SBM than in the SN, with higher SDs for N in the SBM and higher SDs for S concentration in the SN. *Rhizoplaca* had lower concentrations of N than *Umbilicaria,* but not of S. *Letharia* had lower minimum and maximum values of N than the saxicolous lichens, but similar minimum and maximum values to the SN *Rhizoplaca* S concentrations. SDs of *Letharia* N and S were on the higher end relative to the SN saxicolous lichens. There were no *Letharia* samples in the SBM, hence comparisons to SN samples were not possible.

3.3. Relationships between thallus N, S, and deposition estimates

Umbilicaria was the only species whose elemental concentrations had significant relationships to modeled deposition (Supplementary Table 6). CMAQ N estimates had a loose positive linear relationship with *Umbilicaria* N concentrations ($R^2 = 0.34$, $p = 0.003$; Fig. 3). The TDEP N deposition estimates were not significantly correlated with N concentrations ($R^2 = 0.13$, $p = 0.08$). The positive correlation between CMAQ S deposition estimates and S concentrations in *Umbilicaria* was strong ($R^2 = 0.70$, $p < 0.001$), while TDEP S estimates had a negative correlation with S content ($R^2 = 0.21$, $p = 0.02$). Scatterplots were checked visually for outliers as the source of this negative relationship, and none were identified.

Rhizoplaca N and S concentrations had weak, non-significant relationships with CMAQ (Fig. 4) and TDEP N and S estimates. *Letharia* N concentrations were weakly related to CMAQ N (Fig. 4, $R^2 = 0.39$, $p = 0.07$) and TDEP ($R^2 = 0.30$, $p = 0.26$). *Letharia* S concentration showed a weak relationship with TDEP S ($R^2 = 0.19$, $p = 0.24$), and no relationship with CMAQ S ($R^2 = 0.00$, $p = 0.98$). No outliers were found. Additionally, TDEP S and CMAQ S were not related ($R^2 = 0.08$, $p = 0.16$).

3.4. Covariation in N and S content among species

No significant positive relationships were found between species for either N and S concentrations, but the analysis was hampered by small sample sizes caused by the sparse pattern of co-occurrences and one bivariate outlier. *Rhizoplaca* had lower mean N concentrations and similar mean S to the *Umbilicaria* that it was collected with at six plots. When collected at the same plots ($n = 6$), *Umbilicaria* had higher mean N concentration and lower mean S concentration than *Letharia*, with mediocre regression results for N ($R^2 = 0.18$, $p = 0.4$) and S ($R^2 = 0.4$, $p = 0.25$).

The N scatterplot (Fig. 4B) shows that *Umbilicaria* and *Letharia* have more structure that covaries more predictably, while the S scatterplot shows less structured covariation of *Letharia* and *Rhizoplaca* (Fig. 4A).

3.5. Relationships between elemental profile and environmental variables

Umbilicaria's elemental profile had strong relationships to environmental variables and significant differences between the SN and SBM. The first two axes of the PCA had higher eigenvalues than expected by chance (999 randomizations, $p = 0.001$). Axis 1 of the PCA described 45.3 % of the variation in the elemental community data and axis 2 described 15.6 % of the variation, totaling 60.9 %. The ordination was rotated to maximize the strongest environmental relationships on Axis 1, which corresponded with latitude, CMAQ N deposition, TDEP N, CMAQ S, and longitude ($R^2 > 0.3$, Fig. 5B). The variables with the strongest correlations to Axis 1 were latitude and CMAQ N (both $R^2 = 0.69$), with longitude ($R^2 = 0.60$) and CMAQ S ($R^2 = 0.58$) being the next strongest. Axis 2 had only a weak relationship to precipitation ($R^2 = 0.21$).

Positive scores on Axis 1 related to more northerly latitudes, lower CMAQ/TDEP N and CMAQ S deposition, and higher precipitation, while negative scores on Axis 1 were related to more easterly longitudes, higher CMAQ/TDEP N and CMAQ S deposition, and lower precipitation. Most of the SN plots had positive Axis 1 scores, except for the most westerly SN plot (Fig. 1). That plot, and corresponding *Umbilicaria* samples, is also the one that grouped with the SBM samples in the ordination biplots (Fig. 5B). The SBM plots all had negative Axis 1 scores.

Axis 1 scores were negatively correlated with most elements ($R^2 > 0.3$; Supplementary Table 3; Fig. 5A). Axis 1 had the strongest relationships ($R^2 > 0.48$) with Al, cadmium (Cd), cobalt (Co), Fe, Mg, N, nickel (Ni), S, strontium (Sr), and zinc (Zn), with all decreasing in concentration along Axis 1. Axis 2 was most strongly related ($R^2 > 0.3$) with phosphorus (P), K, Si, and chromium (Cr), with only K exceeding $R^2 > 0.45$ (Supplementary Table 1). Cr and Si are the only two elements that decreased in concentration along Axis 2.

Two-way cluster analysis contrasted the SN and SBM and allowed for better visualization of groupings of elements and samples by concentration (Fig. 6). If the elemental dendrogram is pruned at 25 % information remaining, then three clusters of elements are formed. The first cluster was composed of N, P, K, molybdenum (Mo), Cu, and Zn. The second cluster was Si all by itself. Mg, S, Cd, Pb, Na, Sr, Ba, Fe, Co, Al, Ni, Cr, manganese (Mn), boron (B), Ca, arsenic (As) formed the third cluster; all are either heavy metals or abundant crustal elements (Fig. 6). The two highest-level clusters of samples almost perfectly separate the SN from the SBM, except for two samples (600ft–41 and 600ft–42, Fig. 6) in the SN that fell within the SBM cluster. This is not surprising because this was the most polluted SN plot according to the CMAQ and TDEP models. These are also the only SN samples that had negative Axis 1 scores, mentioned above. The two-cluster level retained about 37 % of the total information. Taking elements individually, 21 of 23 of the elements differed between regions (*t* tests), with Cr and Mo the exceptions

Table 4
Concentrations of N and S as percent of dry weight in all samples of target species in the two regions of southern California.

Element	Species	Region	min	mean	max	SD	CV
N	*Umbilicaria*	Sierra Nevada	1.70	2.16	2.67	0.28	0.13
		San Bernardino	1.87	2.42	2.83	0.25	0.10
	Rhizoplaca	Sierra Nevada	1.29	1.6	2.1	0.22	0.14
		San Bernardino	1.11	1.65	2.17	0.36	0.22
	Letharia	Sierra Nevada	1.2	1.61	2.16	0.31	0.19
S	*Umbilicaria*	Sierra Nevada	0.03	0.08	0.17	0.04	0.50
		San Bernardino	0.15	0.21	0.26	0.02	0.10
	Rhizoplaca	Sierra Nevada	0.04	0.16	0.38	0.11	0.69
		San Bernardino	0.12	0.20	0.28	0.06	0.30
	Letharia	Sierra Nevada	0.05	0.20	0.32	0.10	0.50

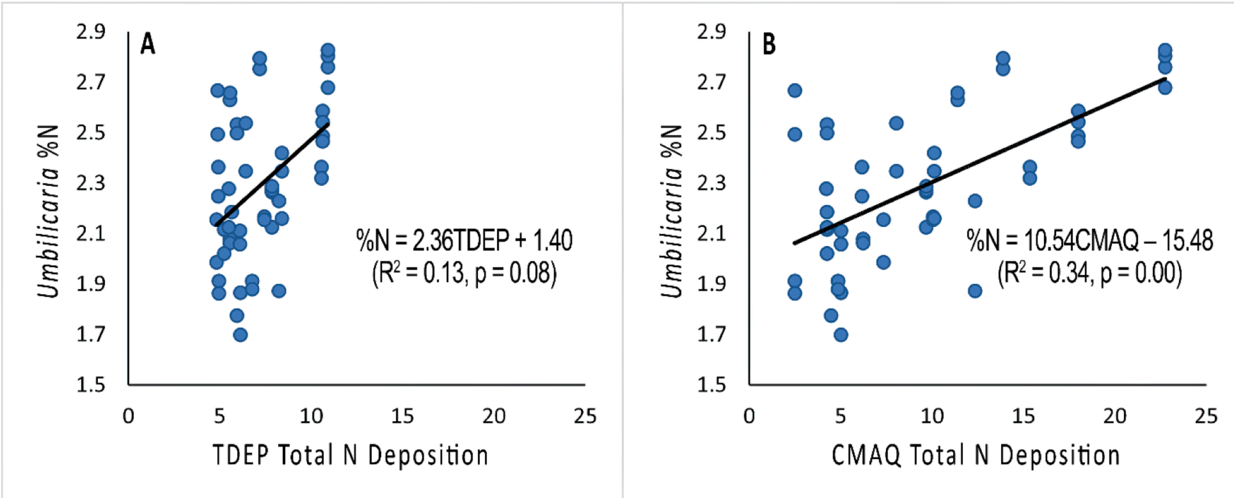

Fig. 3. Simple linear regression models of *Umbilicaria* N concentration and modeled N estimates. Plot repeats and splits are plotted individually to show variance. CMAQ (right) and TDEP (left) N deposition estimates in kg/ha/year. Linear equations shown in scatter plots. Results for other species are compared in Fig. 4. Equations for all other relationships can be found in Supplementary Table 6.

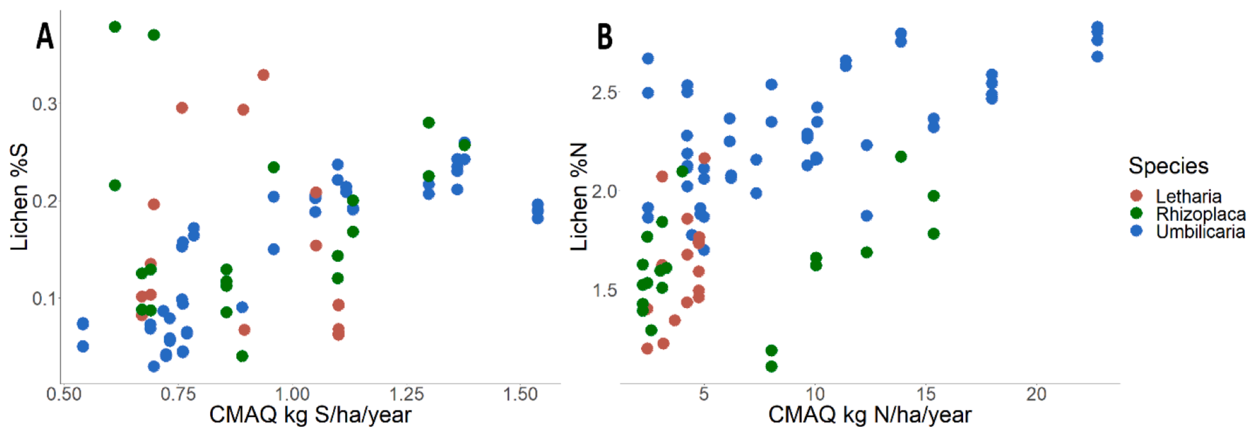

Fig. 4. Lichen S (left) and N (right) concentrations (%) related to CMAQ deposition by lichen species at all plots (Plot repeats and splits were not averaged to show variance).

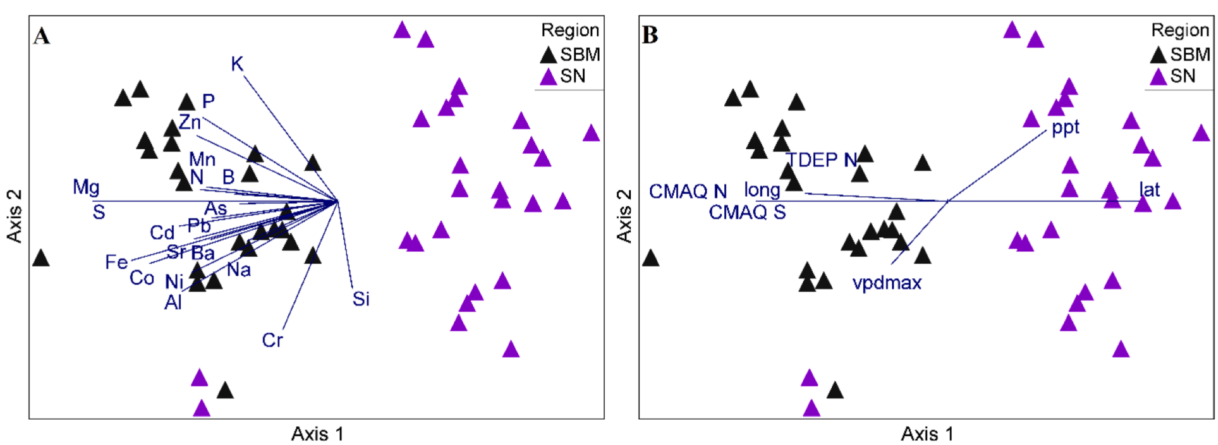

Fig. 5. Biplots overlaid on PCA ordination of samples in *Umbilicaria* elemental space. Purple triangles represent samples in the SN and black triangles representing plots in the SBM. (A) Elemental biplot overlaid on PCA ordination of samples in *Umbilicaria* elemental space. Elemental vectors are shown for variables with $R^2 > 0.3$; abbreviations in Supplementary Table 2. (B) Environmental vectors are shown for variables with $R^2 > 0.3$; ppt = mean annual precipitation; lat = latitude; long = longitude; vpdmax = maximum vapor pressure deficit; CMAQ N = CMAQ modeled total N deposition; CMAQ S = CMAQ modeled total S deposition; and TDEP N = TDEP modeled total N deposition (Supplementary Table 4). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

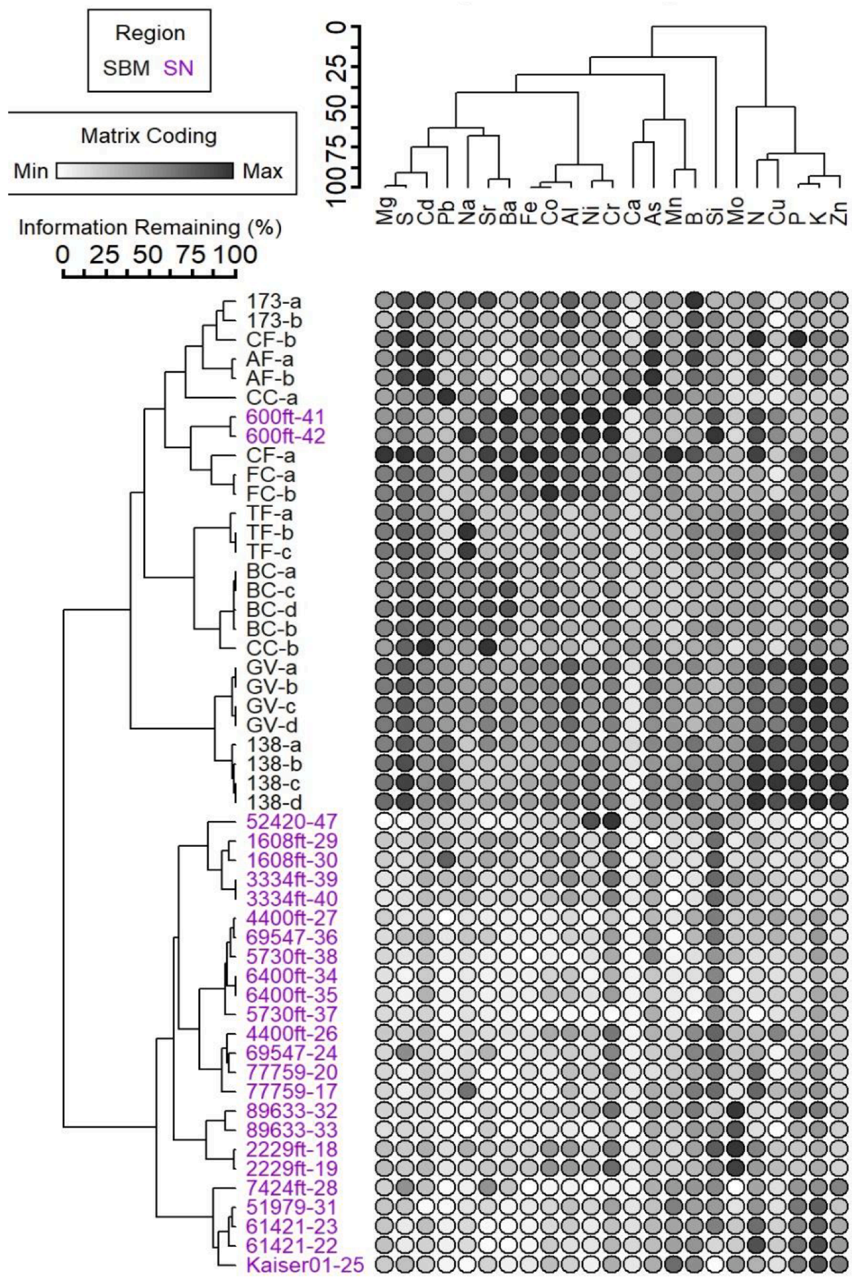

Fig. 6. Two-way cluster analysis of samples (rows) and elements (columns), based on elemental content of *Umbilicaria*. Elements in the heatmap are shaded proportional to standard deviations from the element means, with darker shades indicating higher values. Sample numbers are color coded by region (SBM black vs SN magenta). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Supplementary Table 5). Cr and Mo are common elements in steel (Ashby & Jone, 1986), and could represent contaminants from the IKA tube mill grinder used in sample processing, although this is unlikely because these elements did not increase in the same direction in ordination space (Fig. 5). While we did not adjust p-values for multiple comparisons, 21 p-values were less than our alpha = 0.05, compared to the fewer than two we would expect based on chance alone.

The cluster analysis shows the distinct groupings of the SN and SBM samples, but there was also structure among the elemental concentrations in the heatmap within the cluster that is composed primarily of SBM plots, along with higher values overall as previously mentioned (Fig. 6). In particular the samples from the plots GV a-d and 183 a-d (Fig. 6) had similar elemental profiles that were distinct from the rest of the SBM cluster. Mg, S, and Cd have similarly high standard deviations from the element means that stood out within the majority SBM cluster as well. The SN had lower values overall.

The relationship between log transformed parts per million (ppm) and ranked concentration of elements differed between SN and SBM for both *Umbilicaria* and granite (Fig. 7), but the major difference was whether the subject was *Umbilicaria* or granite. Elemental concentrations were lower in *Umbilicaria* than in granite and had a different order of ranked concentration. In *Umbilicaria*, K, P, and Ca had the highest concentrations, while Si, Al, K, and Fe had the highest concentrations in granite. Al, Fe, Mg, Ca, Mn, P, Ni, Cr, Cu, Zn, and Ba all had higher concentrations in the SN granite, while Si, Na, K, and Cr had higher concentrations in the SBM granite.

4. Discussion

We found that of the two saxicolous lichens, *Umbilicaria* N and S

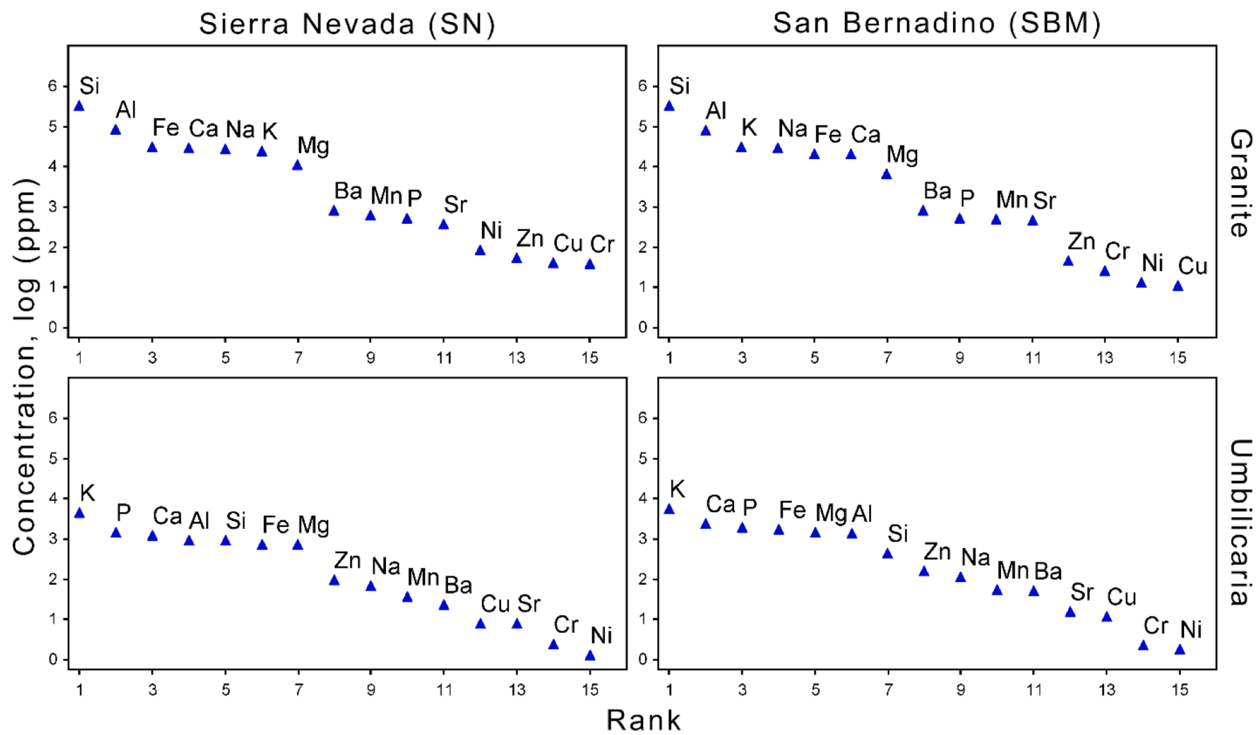

Fig. 7. Dominance curves consisting of log transformed parts per million (ppm) of granite and *Umbilicaria* elements (labeled) plotted by ranked concentration.

concentrations had strong relationships to modeled deposition while concentrations in *Rhizoplaca* did not. N and S concentrations were not significantly correlated among any of the three lichen species, but the correlations of the saxicolous lichens' N and S concentrations with *Letharia* concentrations were strong enough to recommend further exploration with a larger sample size in future studies to seek statistical significance and possibly calibrate elemental concentrations between the species. The *Umbilicaria* elemental profile showed even stronger relationships to modeled deposition than the N and S concentrations alone, further indicating the potential usefulness of *Umbilicaria* as a target biomonitoring species.

4.1. Sources of error

For N and S concentrations in *Umbilicaria* sampling error was small relative to the differences between plots, based on signal-to-noise ratios from repeat sampling and split samples. Thus, our plot sampling methodology provided sufficiently consistent estimates to be useful. Within-plot errors were similar to those of the commonly sampled *Letharia* (Jovan et al., 2021). Our sample weights were lower for *Umbilicaria* (2.6 g, on average) due to scarcity than typical sample weights for epiphytic lichens (15 g on average), which we thought might increase sampling error. Given our high signal-to-noise ratio findings, however, we believe that our lower sample weights adequately represent local environmental conditions. While we did not assess variability in *Rhizoplaca* because of small sample sizes, others have found low variability of N concentration in plot repeats of that species (Jovan et al., 2021).

4.2. N and S content

Saxicolous lichens generally had 10 to 20 % higher concentrations of N than epiphytic *Letharia* at co-located plots, with *Umbilicaria* having higher N than *Rhizoplaca*. Differences in habitat could partially explain this. *Letharia* N concentrations were strongly correlated with throughfall N deposition (Root et al., 2013). The saxicolous lichens we sampled experienced both throughfall and bulk deposition (no filtering by the forest canopy; Fenn & Poth, 2004) because they were collected both in the open and under forest canopy. Throughfall inorganic N and S deposition can underestimate total atmospheric deposition due to volatilization, plant and microbial uptake, and dry fallout of particles (Fenn et al., 2000). Past work also found that saxicolous lichens had elevated levels of N compared to epiphytic lichens, which was attributed to fine soil and dust accumulation in crevices of the thalli that was not removed in the cleaning process (Fenn et al., 2007), but this doesn't explain the difference between *Umbilicaria* and *Rhizoplaca*. Different epiphytic lichen species have been shown to uptake N differently in controlled experiments (Gaio-Oliveira et al., 2005), so it is possible that *Umbilicaria* and *Rhizoplaca* accumulate N differently. S concentrations did not vary between saxicolous lichens and *Letharia* as N concentrations did.

Rhizoplaca may be a nitrophile given its observed growth on rock outcrops that serve as bird perches in the western USA (McCune pers. obs.) as well as Antarctica (Pandey & Upreti, 2000). This means that many of the locations supporting *Rhizoplaca* populations would likely be higher in N due to the presence of bird droppings, thus making the relationship with modeled deposition less meaningful. *Rhizoplaca* also has a very wrinkled growth form, which could increase variability in how it accumulates N and S relative to accumulation in the flatter and smoother *Umbilicaria*.

Umbilicaria had higher N and S concentrations in the SBM than the SN, suggesting higher N and S deposition in the former, while concentrations in *Rhizoplaca* did not differ between regions. We didn't have *Letharia* from our plots in SBM to compare to the SN, but Jovan et al. (2021) described *Letharia* from the entire Los Angeles basin as having higher minimum and maximum N concentrations than our SN samples, similar to the *Umbilicaria* results for the region.

4.3. Relationships between thallus N, S, and deposition estimates

Umbilicaria and *Letharia* N concentrations had significant positive relationships with CMAQ modeled N deposition, but not TDEP. The relatively weak relationship between *Letharia* N concentrations and modeled N estimates in the SN was surprising given strong relationships with throughfall N deposition for this species in the western USA

(Bermejo-Orduna et al., 2014; McMurray et al., 2013; Root et al., 2013). Strangely, *Umbilicaria* S concentrations had negative correlations with TDEP S. TDEP and CMAQ S were not related, so the two models represent different quantities. Schwede & Lear (2014) found that TDEP overestimated S deposition compared to CMAQ, at sites mostly in the western USA, likely due to few deposition monitoring stations whose data are used in the TDEP model, but not in CMAQ.

While it seems possible to calibrate *Umbilicaria* S concentrations against CMAQ S, the relationship between N concentration and CMAQ N has considerable scatter. We expect some scatter because there are other factors that could affect this relationship. Lichen sampling error and inherent variability among lichen samples are two factors that introduce a small amount of variability into the relationship. Another factor is that the CMAQ model's deposition estimates also show scatter when related to empirical measurements of deposition (Byun & Schere, 2006), and actual deposition is what matters to the lichen. The multivariate approach using the *Umbilicaria* elemental profile appeared to reduce the scatter in the relationship with CMAQ deposition estimates and retain valuable information about other elements. Nevertheless, we discuss the N and S concentrations apart from the elemental profile, as these have been commonly investigated in past biomonitoring research (Geiser, 2004; Jovan & Carlberg, 2007; Root et al., 2013, 2021).

4.4. Covariation in N and S content between species

Umbilicaria remains relatively common up until about 2800 m in elevation in our study areas. *Letharia* becomes uncommon at about 2200 m, well below treeline at about 2700–3000 m. Using *Umbilicaria* should allow for extended sampling into high-elevation wilderness areas across the western USA. Additionally, other species of *Umbilicaria* occur to 4000 m or more elsewhere worldwide and have had their elemental profile studied (Bargagli et al., 1999). We found that N and S concentrations did not correlate significantly between any of our three lichen species, possibly due to low sample size. Being unable to correlate *Umbilicaria* and *Rhizoplaca* N and S concentrations with each other is unfortunate given that *Rhizoplaca* is much more common in alpine and subalpine areas than *Umbilicaria* in our study areas. Relating the two would be critical for sampling at the highest elevations and/or adding other species of *Umbilicaria* as target sample species in the Sierra Nevada. It is possible that more co-located samples would strengthen correlations as other studies have found inter-species correlations using epiphytic lichens (Root et al., 2013, 2021; Will-Wolf et al., 2017).

4.5. Relationships between elemental profile and environmental variables

The *Umbilicaria* elemental profile was strongly related to modeled N and S deposition. It also showed strong relationships to precipitation, latitude, and longitude as did previous *Letharia* elemental work in California (Jovan & Carlberg, 2007). Variation in lichen elemental content within the region is produced by climatic and environmental differences (e.g., precipitation and soils), and degree and types of pollutants. Furthermore, those are likely to interact, given that climate affects the delivery of pollutants. In our study area, correlations between principal components of elemental content and CMAQ N and S estimates were almost double that of precipitation, suggesting strong non-climatic influences on elemental content. Similar to the *Umbilicaria* N and S concentration results, the *Umbilicaria* elemental profile had a stronger relationship to CMAQ N and S than to TDEP N and S estimates.

Overall, the SBM samples had higher elemental concentrations than the SN samples. A possible climatic reason for the observed difference in elemental concentrations between locations could be lower precipitation in the SBM, leading to less leaching from the lichens (Geiser et al., 2021). Nitrogen accumulates in lichens during the dry season and then partially leaches out in the wet season (Boonpragob et al., 1989), along with As, Cd, Cr, Cu, Fe, Pb, and Zn (Paoli et al., 2018). While this might explain some of the difference in elemental concentrations between the SN and SBM, the relatively strong correlations of elemental profiles with N and S depositional differences suggest that N and S emissions are related to emissions of other elements (Nriagu, 1992). According to CMAQ modeled deposition, N and S are higher in the SBM versus the SN. The urban Santa Ana Valley adjacent to the SBM is a large source of vehicle related N emissions due to high population density. Vehicle-related particulates (emissions and wear and tear) contain Zn, Cu, As, Pb, and Cd (Bozlaker et al., 2014; Sternbeck et al., 2002); all were positively correlated with *Umbilicaria* elemental profile Axis 1. Enhanced levels of As, Pb, and Cu in lichens have been associated with road emissions from vehicle exhaust, tire and brake wear (Bermejo-Orduna et al., 2014; Zechmeister et al., 2005; Zschau et al., 2003). While natural gas power plants emit small amounts of metals compared to other fossil fuels (Häsänen et al., 1986), there are far more natural gas power plants in the Santa Ana Valley and Los Angeles basin than in the more agricultural San Joaquín Valley (*California Electric Infrastructure* App, 2017) near the SN, that this could be considered a small but meaningful local source of metals due to the quantity of natural gas emissions. While granite composition differed between the SN and SBM, these differences did not correspond with differences in crustal elemental composition between the *Umbilicaria* samples, making it unlikely that the substrate granite was the source of the higher crustal element concentrations in the SBM.

Other explanations for positive correlations between concentrations of other elements and N deposition are also possible. Urbanization, which is related to N emissions, has also been related to increased dust deposition, rich in crustal elements (Reheis & Kihl, 1995). Deposition high in nitrate could increase deposition acidity and promote weathering, but this is unlikely as granite is quite resistant to weathering (Shaw et al., 2014). Some *N*-tolerant lichen species increase their growth rates with increased inputs of N (Palmqvist & Dahlman, 2006) and organic acids produced by lichen leachate are a well-known component of rock weathering (Chen et al., 2000). This means that excess N could increase lichen growth and thus organic acid leachate volume to dissolve even highly resistant granite at higher rates contributing to higher elemental content within the lichens. No research has been conducted on differences in lichen growth rates between the SN and SBM.

Diesel-fueled ships at the ports of Long Beach and Los Angeles have relatively high emissions of S (Bates et al., 1992; Cai et al., 2021) that might penetrate the SBM. Naturally occurring S emissions from the ocean could also be making their way inland to the SBM. However, the Pacific Ocean is approximately 110 km from the SBM, which is far enough to cast doubt on this being a major source of S in our study area. Diesel emissions have higher levels of metals than gasoline (Coufalík et al., 2019), so it is possible that diesel-fueled vehicles contribute metals to the atmosphere given the large number of vehicles in urban areas.

While we were most concerned with N and S, monitoring for Pb is also required by the Clean Air Act in the USA and by other legislation in other countries. According to lichen enhancement levels derived in Rhoades (1999), several plots in the SBM had enhanced Pb levels (Rhoades, 1999), while only one plot in the SN did and it was not the plot that experiences the highest N deposition. It is possible that because this plot is near a defunct galena (Pb) mine (The Diggings, 2021) , so Pb-rich dust from mining waste could be the source. *Umbilicaria virginis* (a different species than the *Umbilicaria* we studied) from the San Gabriel Mountains west of the SBM sampled in the late 1980′s contained elevated levels of Pb (Rhoades, 1999). Our SBM mean levels of Pb were higher than these levels. This is surprising given that the *U. virginis* samples were collected during the time when vehicles emitted more Pb than they do currently because of widespread use of leaded gasoline.

Although most of the SN plots had lower deposition than those in the SBM, the SN experiences elevated levels of N pollution from the San Joaquín Valley, as well as long range transport of polluted air masses (Bytnerowicz et al., 2016). These have contributed to ecological effects such as changes in lichen community composition and injury to pines via ozone formation (Arbaugh et al., 1998; Bytnerowicz et al., 2019;

McCune et al., 2007).

5. Conclusion

Even though signal-to-noise ratios were high for N and S concentrations of *Umbilicaria* and significant correlations with modeled N and S deposition estimates were found, relationships between N and S deposition and the elemental profile yielded stronger correlations using multivariate methods. The *Umbilicaria* elemental profile differed significantly between the SN and SBM samples by all measures employed, with the SBM samples having higher concentrations of most elements. This method of analyzing the elemental profile of *Umbilicaria* to assess deposition could be evaluated in other areas of the western USA that lack suitable epiphytic lichen species. While simpler methods that look at concentrations of a few key elements are often favored, our method allowed us to consider all the elemental data to gain key insights about how N and S deposition may be affecting the *Umbilicaria* elemental profile. More work needs to be done assessing the biomonitoring capabilities of *Rhizoplaca*, which would allow us to expand our saxicolous lichen sampling habitats up into the highest elevations. As both *Rhizoplaca* and *Umbilicaria* (along with other species in these genera) occur globally, these methods could be used in other locations beyond the western USA to gain more insight about the efficacy of saxicolous lichens as biomonitors of N and S deposition.

CRediT authorship contribution statement

Adrienne Kovasi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Bruce McCune:** Conceptualization, Supervision, Writing – review & editing. **Sarah Jovan:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109541.

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