Evaluating *Letharia vulpina* transplants for bioindication of nitrogen deposition

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Abstract. The epiphytic lichen *Letharia vulpina* has been commonly sampled in-situ for nitrogen (N) deposition biomonitoring studies but has never before been transplanted for this purpose. In the high-elevation wilderness areas of southern California *Letharia vulpina* is generally uncommon, making in-situ sampling difficult. In this study, we compared thallus N accumulation between in-situ *Letharia vulpina* reference samples from the relatively low N deposition environment of the northern Sierra Nevada mountains and *Letharia vulpina* transplants that were deployed at nine plots of varying climatic and N deposition regimes in the southern Sierra Nevada mountains for 12 months. Survival of transplants was low (33%) and only occurred at the plots within the current range of *Letharia vulpina*. Transplant N concentrations became higher than those of the reference samples, while transplants that died had a net loss of N. Transplants that survived had strong relationships of N concentrations to N deposition and approached N concentrations of in-situ *Letharia vulpina* at the same plots. At the same time, reference plot N concentrations in a relatively clean environment increased substantially from early summer 2020 to 2021, presumably in response to extended exposure to smoke from huge wildfires in summer and fall of 2020.

Keywords. Bioindicator, Federal Class 1 areas, nitrogen deposition, Sierra Nevada mountains, transplants, smoke.

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Transplanting epiphytic lichens and measuring bioaccumulated pollutants in their thalli is an experimental approach to studying deposition of both heavy metals (Loppi et al. 2019; Paoli et al. 2019) and pollutants that contain nitrogen (N; Branquinho et al. 2010; Frati et al. 2006, 2007, 2011; Søchting 1995). Transplanted lichens are typically moved from a "clean" area to a "polluted" area to see if there is change in thallus pollutant concentrations (e.g., metals, N, sulfur, etc.) over time (Boonpragob et al. 1989; Cecconi et al. 2021; Zambrano et al. 1999). This also allows for greater experimental control due to a shared starting location and option to more easily control for climate and other environmental variables (Branquinho et al. 2010). Most lichen transplant experiments involve moving transplants to climatically similar environments or have a short deployment timespan to ensure survival of the transplants. This is done because dead or dying lichens may not accumulate deposited pollutants in the same way as living lichens (Purvis et al. 2005) and to reduce variability from climate factors such as temperature and precipitation. But what about transplanting lichens into environments well outside of their climatic "comfort zones," for example, in places where a transplanted lichen species does not occur naturally? Because species distributions are expected to have some mismatch with a changing climate, transplanted lichens may actually thrive in areas where they are naturally absent (e.g., Antoine & McCune 2004). If transplants outside a species regular habitat are successful, this expands the area of potential application of lichen transplants to evaluate

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air quality. We tested this idea with the wolf lichen *Letharia vulpina*.

Letharia vulpina (henceforth "*Letharia*") is a fluorescent chartreuse green fruticose epiphytic lichen that is abundant at montane sites in parts of California and elsewhere in western North America. In-situ sampling of *Letharia* to estimate throughfall N deposition via thallus N concentrations has been common in the western U.S.A. (Bermejo-Orduna et al. 2014; Hutten 2015; Jovan & Carlberg 2007; Root et al. 2013). The method has been applied by land managers in this region (Geiser 2004; Jovan et al. 2021). *Letharia* and other epiphytic lichens represent atmospheric deposition because their survival depends on acquiring water and nutrients solely from atmospheric sources. This, in addition to lacking tissues capable of filtering out pollutants, causes them to accumulate some pollutants proportionally to the local atmospheric deposition. The abundance of *Letharia* from 1220 to 2500 m in elevation in the Sierra Nevada mountains of California makes it quite easy to collect large quantities for transplants in a sustainable manner, with low impacts on local population. Furthermore, *Letharia* has been successfully transplanted in growth experiments (Antoine & McCune 2004; Bidussi & Glauslaa 2015) and in a limited way for air quality monitoring (Pearson 1994). To our knowledge, *Letharia* has never before been transplanted in an attempt to relate N concentrations with N deposition, unlike other species that have been commonly transplanted to monitor deposition.

Directly measuring N deposition in remote, high-elevation wilderness areas well outside of the current elevational range of *Letharia* is expensive. Much of the wilderness above 2500 m in the southern half of California has little to no *Letharia* overall, although it can sometimes be locally abundant, which precludes using the lichen biomonitoring protocol based on abundant in-situ *Letharia* (Jovan et al. 2021). Furthermore, there are no other species that are as abundant as *Letharia* over a widespread geographic area. If *Letharia* transplants could survive for a year at elevations well above their in-situ range and subsequently reflect the N deposition regime of the transplant destination, then they could possibly be used to estimate N deposition, which would increase the area where biomonitoring could be conducted and allow for more experimental control.

Our objectives were to:

1. Evaluate the suitability of *Letharia* transplants as indicators of atmospheric N deposition.
2. Assess variability of N concentrations of the different sampling levels.
3. Estimate annual variation in N concentration of in situ *Letharia* based on the source area for transplants based on repeated sampling.
4. Compare the N concentrations of the reference plot *Letharia* to those of the transplants and assess how survival may have affected N.
5. Compare transplant N concentrations with measurements of N deposition and in-situ *Letharia* N concentrations.

Methods

Transplant study area. Transplant material was collected from a remote area ("reference plot") in the northern Sierra Nevada. After constructing the transplants, they were deployed in the southern Sierra Nevada (**Fig. 1**). Our nine transplant deployment plots fell along a N deposition (15 to 2 kg N/ha/year) and elevational gradient (512 to 3494 m) in the southern Sierra Nevada mountains on the Sierra and Inyo National Forests (**Fig. 1**). These plots were selected to co-locate our transplants with passively sampled bulk deposition measurements that were made in 2012 (Bytnerowicz et al. 2019), which are the latest available measurements. Mean annual precipitation increases along this gradient from 589 to 1258 mm (**Table 1**) and mean annual temperature decreases from 15.9°C to –0.1°C as one moves up in elevation. Two of the plots, Wahoo Lake and Piute Pass, are located within the Class 1 John Muir Wilderness Area. The Clean Air Act of 1973 requires monitoring for pollutants in Class 1 Wilderness areas that impair visibility, including NOx, SOx, and other compounds (Jovan et al. 2021). The nearby San Joaquín Valley is a major source of N emissions from large urban population centers and expanses of agricultural land (Almaraz et al. 2018; Bytnerowicz et al. 2019). The 2020 Creek Fire occurred in this area in September through December 2020. It burned 153,738 hectares, including the transplants at the Mountain Rest plot.

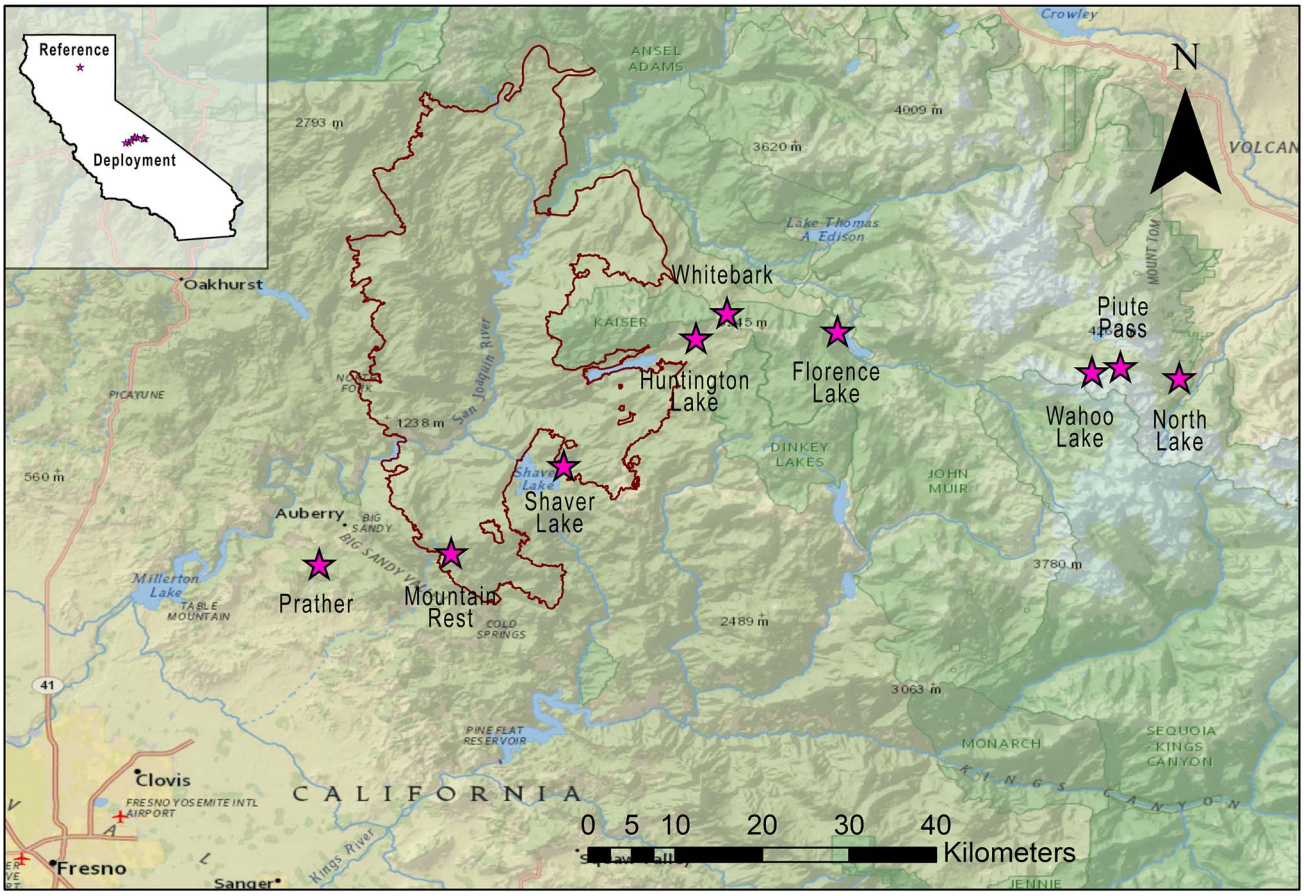

Figure 1. Topographic map of transplant reference and deployment plots in the southern Sierra Nevada. Solid stars represent plot locations and red line shows the perimeter of the Creek Fire. Inset map shows reference and deployment areas on an outline of California. Online pdf in color.

Letharia transplant source. On June 15, 2020, we collected a large bulk sample of *Letharia* for transplant pendants from the Lassen National Forest in the northern Sierra Nevada mountains. We chose this area because N deposition was relatively low here compared to the southern Sierra Nevada according to deposition modeling (Byun & Schere 2006) and it had abundant *Letharia*. At this reference plot, annual N deposition is 3.4 kg/ha/year, precipitation is 1122 mm annually, and monthly average

Table 1. Site characteristics and transplant survival in Sierra Nevada plots. Mean annual precipitation, annual minimum and maximum mean temperatures are 30-year climate normals for the period 1991–2020 from the PRISM model. CMAQ N is estimated annual N deposition in kg/ha/yr based on the CMAQ model. After the reference plot, plots are in order of location from west to east.

Plot	Elevation (m)	Precipitation (mm)	Min Temp °C	Max Temp °C	Survival	CMAQ N
Reference	1737	1122	1.2	15.7	–	3.4
Prather	512	589	8	24.1	dead	6.3
Mountain Rest	1192	914	9	20.3	dead	6.3
Shaver Lake	1756	962	3.7	16.8	survived	4.8
Huntington Lake	2558	1170	−1.1	12.9	survived	3.1
Whitebark	2917	1258	−1.4	11.5	dead	3.1
Florence Lake	2263	640	−1.9	14.2	survived	2.4
Wahoo Lake	3436	925	−6.1	7	dead	2.2
Piute Pass	3494	901	−5.3	6.7	dead	2.2
North Lake	2867	410	−2.9	10.6	dead	2.2

temperature ranges from 1.2°C to 15.7°C (**Table 1**), commonly with snow and freezing temperatures during the winter. We collected medium-sized *Letharia* thalli from conifers >30 m from the road, placed them in a polyethylene bin that had been wiped with 99% isopropyl alcohol, rinsed with water, and then rinsed a second time with distilled water. To gather baseline data on in-situ *Letharia* N concentrations, we collected five samples, or "plot repeats," of five target sized thalli apiece to simulate median-sized transplants. We repeated this sampling on July 12, 2021, to assess annual variability in *Letharia* N concentrations at the reference plot.

Transplant creation methods. Transplant pendants were created by gluing loops of monofilament fishing line to the bases of the individual lichens (Antoine & McCune 2004; McCune et al. 1996). We cut 13–15 cm lengths of fishing line and tied them into approximately 455 loops to make the equivalent number of pendants. We glued the *Letharia* pieces to the fishing line loops by squeezing a pea-sized amount of clear 100% silicone sealant onto wax paper, then placing the base of the *Letharia* into one side of the sealant, then the knot of the fishing line loop into the other side (**Fig. 2**). After drying for 24 hours the pendants were placed in Styrofoam egg cartons previously cleaned by rinsing with tap water, then distilled water. These egg cartons protected the pendants and helped prevent tangling in transport. We wore powder-free nitrile gloves throughout this process. We stored pendants in a cool, dry location until deployment.

Transplant deployment and retrieval protocol. We collected transplants on June 15, 2020 from the reference plot and deployed them between June 25, 2020 and July 2, 2020. We originally intended to retrieve transplants after 3 and 12 months, with half collected at the end of each period. We chose 3 months to favor transplant survival rates. We chose the second period of twelve months to allow a full year of local exposure and a stronger test of transplant survival. Unfortunately, due to the 2020 Creek Fire and subsequent snow limiting access, we retrieved all transplants after 12 months.

Transplants were hung in two trees in each of the nine transplant plots. Each tree received 24–29 pendants hung from one to two branches. Pendants were attached to tree branches using 30 cm zip ties while wearing nitrile gloves. Where practical the pendants were placed high in the trees using a ladder, mostly to prevent people from disturbing them. They were retrieved between July 4, 2021 and August 4, 2021 by cutting the zip ties with wire cutters. Pendants were collected into SpeciPAK saran bags manufactured by Ampac, with fishing line loops still attached. To estimate variability within plots, the pendants from each tree were divided into two roughly equal sets, resulting in four plot repeats per plot for a total of 36 transplant sample units.

Figure 2. Photograph of deployed transplants. Online pdf in color.

In-situ sampling protocol. At the three plots where *Letharia* was present in-situ, we collected at least 10 g using SpeciPAK bags following the standard USFS lichen tissue collection approach (Geiser 2004). Collections were made from six or more locations up to 30 m away from the plot center, to spatially represent each plot. Plot repeats were collected for in-situ *Letharia,* just like the transplants, to assess within plot variability. All collections were made while lichens were dry, so there was no need to follow additional drying procedures. We collected in-

situ samples at the same time the transplants were retrieved. Two plot repeats were collected at the Shaver Lake and Florence Lake plots. Only one sample was collected at the Huntington Lake plot from only two trees due to poor availability of *Letharia,* as this plot was at 2558 m, above the elevational band where *Letharia* is abundant.

Laboratory methods. Prior to elemental analysis lichens were processed while wearing powder free nitrile gloves using equipment cleaned with 99% ethanol. Samples were not washed, as it is likely that washing leaches N from the lichen thalli (Garty & Garty-Spitz 2015). The fishing line loops, diseased portions of the thalli, debris, and other lichen species were removed. The largest sample from each of the nine plots was split in half to measure within-sample variation. During cleaning we evaluated the condition of the transplants. We found no obvious gradient in condition, so we assigned individuals to only two classes: alive or dead, determined by color change and thallus fragmentation. A dead or dying lichen is brittle and easily fragments. Healthy *Letharia* is chartreuse but becomes distinctly yellower when the algal symbiont within the lichen dies. All dead transplants were yellow and very fragmented.

Samples were oven dried for 24 hours at 70°C, ground with an IKA tube mill grinder and then microwave digested (CEM Mars 6). Total N was measured using combustion analysis (Leco 628 Series C/H/N Analyzer) with blank-corrected results expressed as a percentage. Certified reference material (Rice flour 502-907 lot 1000) was analyzed concurrently with the lichen samples to assess the accuracy and precision of the combustion analysis, which fell within the expected ranges. Five duplicate samples, or "lab duplicates," were run once lichen samples were ground and digested to measure variability from the combustion analysis.

Statistical analysis. Summary statistics, simple linear regressions, Welch's two-sample t-tests, and analyses of variance (ANOVA) were performed using R Studio v. 1.4.1106 (R Studio Team 2021). To assess variability in *Letharia* transplant N concentrations at different scales we analyzed several sources of error: between plots, within plots, within sample, and error from the combustion analysis lab. Plot repeats provided the basis to estimate within-plot error, 50/50 splits of individual samples allowed estimation of within-sample error, and lab duplicates assessed error from the combustion analysis. ANOVAs were performed to obtain the mean square error (MSE) for each of the four levels: plot, plot repeat, split, and lab duplicate. Signal-to-noise ratios were calculated using ratios of the MSEs across levels (McCune et al. 1997), with "signal" referring to among-plot variance. For significance tests we combined plot repeats, splits and lab duplicates by averaging to fulfill the statistical assumption of independence. To compare N content of transplants with N deposition estimate we obtained replicated passive air pollution sampler data for NH_3 and NO_2 from Bytnerowicz et al. (2019) as they sampled deposition in 2012 at the same plots where we deployed the transplants. These data did not have measurements at the source area in Lassen, so for additional comparisons we extracted three-year rolling averages of modeled total N deposition estimates from 2009 to 2012 from the Community Multiscale Air Quality model v. 5.0.2 (CMAQ; Bash et al. 2013; Byun & Schere 2006) at a resolution of 12 × 12 km. CMAQ total N deposition was correlated with both NH_3 and NO_2 measurements ($r^2 = 0.83$ and 0.82 respectively).

Results and Discussion

Transplant survival. All *Letharia* transplants died at six out of nine plots (**Table 1**) and no plots had partial survival. The transplants at Mountain Rest died due to being singed by low intensity fire (2020 Creek Fire) in the area. At each plot a few transplants detached from the nylon loops, with a total of 4% of pendants detaching. We saw no higher detachment at windier ridgetop plots like Whitebark and Piute Pass. Even the singed transplants at Mountain Rest had lower than average detachment.

Weather could have negatively influenced transplant survival. Transplants died below 1220 m or above 2500 m elevation, the current range of *Letharia* in the southern Sierra Nevada. *Letharia* is virtually absent in-situ from the plots where the transplants died, except in very sheltered locations at the bases of trees and shrubs and even this is somewhat rare. Exact causes of death were unknown, but probably differed between the high and low elevation transplants. Although deployment of transplants for less than a year (Branquinho et al. 2010; Frati et al. 2006, 2007,

Table 2. Mean squared error (MSE) of total nitrogen content of transplants at different levels of the sampling design.

Source	MSE	Signal:noise
plot	0.219	plot:plot repeat = 5.5 plot:split = 12.2 plot:lab duplicate = 73.3
plot repeat	0.040	plot repeat:split = 2.2
split	0.018	split:lab dupe = 6.1
lab duplicate	0.003	

2011) would have reduced mortality, it would also represent less well the depositional environment, and we wished to expose transplants to a full annual cycle of deposition to include any seasonal variation.

Ecotypic and species differences may have negatively affected survival rates of transplants in this study as well. What we have been calling "*Letharia vulpina*" looks like one species morphologically but could include two species, the distinction requiring DNA sequencing (Altermann et al. 2016; Kroken & Taylor 2001). *Letharia vulpina* and the other species, *L. lupina* Alterm., Leavitt & Goward, both occur in California, but *L. lupina* is much more common throughout the Sierra Nevada (Altermann et al. 2016). It is possible that we transplanted a mixture of both species, which might differ in survival rates when transplanted to the southern Sierra Nevada. However, if *Letharia* transplants were mixed species, and the species differed in environmental tolerances, we would expect partial mortality at some sites. Because we did not observe this, we conclude that the transplant material was physiologically rather uniform. We were aware of the two species prior to our study but considered it impractical to sequence each transplant. Past use of *Letharia* for biomonitoring work in California has also overlooked the different species.

N concentration variability. Variability was higher among plots than between plot repeats, splits, and lab duplicates of the transplants (**Table 2**). The ratio of mean squared error (MSE) of plots to MSE of plot repeats measures the variation among plots compared to the variation in the sample collection process. This ratio for N concentration was 5.5, showing much higher variation among plots than between plot repeats. The ratio of MSE of plot repeats to MSE of split samples measures the variation in the field sampling compared to the variation within the sample itself. We found a lower ratio of 2.1 for N, indicating that the variation introduced from the field sampling process is only slightly higher than the variation within samples. The ratio of MSE of splits to MSE of lab duplicates measures the variation inherent in the sample itself compared to the variation introduced from the laboratory combustion analysis. This ratio was the highest of all at 6.1, showing much higher variation within a sample than the variation introduced by the combustion analysis. Variability at the plot and plot repeat levels for in-situ *Letharia* samples (Jovan et al. 2021) was similar to that for our transplants.

Table 3. Nitrogen concentrations as percent of dry weight for both sampling years at the reference (divided by sampling year) and transplant plots with plot repeats, splits and lab duplicates averaged. The coefficient of variation (CV) is the standard deviation (SD) divided by the mean. Plot names are bolded where all the transplants died. Survival rates do not apply to in-situ samples from the reference plot.

Plot	Plot Mean	Min	Max	SD	CV	Survival
2020 Reference	0.85	0.71	0.98	0.11	0.13	n.a.
2021 Reference	1.06	0.98	1.12	0.05	0.05	n.a.
Prather	1.33	1.13	1.52	0.16	0.12	dead
Mountain Rest	1.10	0.95	1.47	0.19	0.17	dead
Shaver Lake	1.73	1.56	2.09	0.21	0.12	alive
Huntington Lake	1.52	1.42	1.61	0.08	0.05	alive
Whitebark	1.20	0.97	1.38	0.19	0.16	dead
Florence Lake	1.35	1.25	1.53	0.10	0.07	alive
Wahoo Lake	0.98	0.83	1.04	0.09	0.09	dead
Piute Pass	1.01	0.90	1.13	0.10	0.10	dead
North Lake	1.01	0.89	1.15	0.09	0.09	dead

Reference plot changes. The mean in-situ *Letharia* N concentration at the reference plot differed significantly between 2020 (0.85%) and 2021 (1.06%; **Table 3**; Welch's t-test, $p = 0.006$). The difference suggests an effect from the heavy smoke from wildfires between our 2020 and 2021 sampling. Mid to late summer of 2020 (after the initial sampling) was unprecedented in terms of acreage burned from wildfire in California. Smoke contains NH_3 and NO_2 (Burley et al. 2016) and this area was inundated with smoke during much of the summer and fall of 2020. Additionally, the 2021 sampling date was a month later than the 2020 sampling, June vs. July. We expected both heavy smoke and later sampling to increase N concentration in *Letharia*.

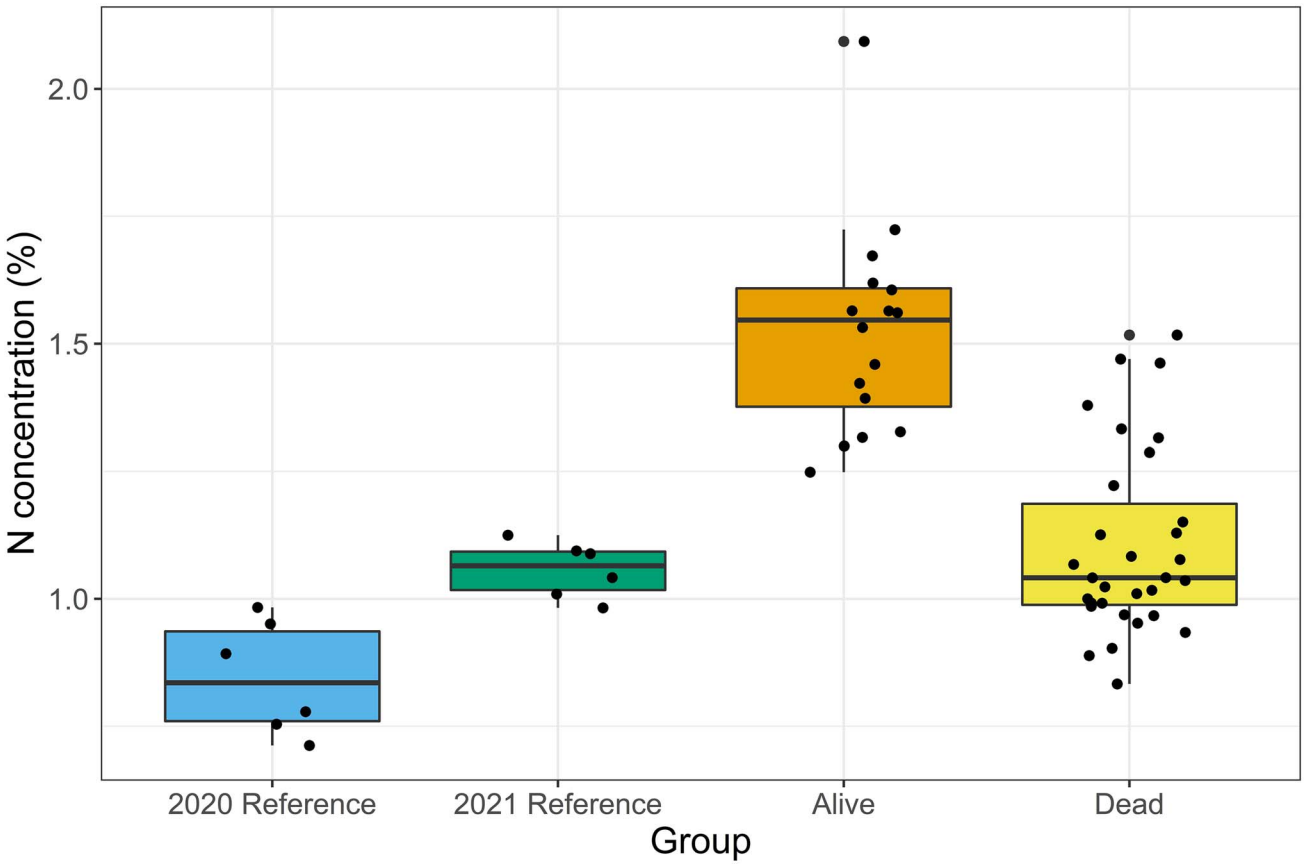

Figure 3. Boxplots of the reference and transplant groups N concentrations showing the distribution of the sample values overlaid over the boxplots. Boxes represent the 25th to the 75th percentiles; whiskers indicate 1.5× the interquartile range. Outlying points beyond the end of the whiskers are plotted individually. Individual dots represent plot repeats, splits, and lab duplicates and have been jittered horizontally. Online pdf in color.

It is possible that the additional month of dry deposition before sampling could have contributed to raising the *Letharia* N concentration. In support of this idea, Boonpragob et al., (1989) found that other lichen species accumulate N mostly in the dry season, and that this was the primary period of N accumulation in the studied lichens (Boonpragob & Nash 1990). Additionally, certain lichen species can also accumulate N quite quickly (Branquinho et al. 2010); therefore, a month between the sampling times at the reference plot could make a big difference in terms of N concentrations. Conversely Ra et al. (2005) found that lichen species sampled at the same sites both in June and then December did not significantly differ in N concentrations.

Transplants vs. reference plot. Mean N concentration was not significantly higher in the transplants (mean = 1.24%) than the in-situ *Letharia* from the reference plot (0.95%, $p = 0.13$; **Fig. 3**; **Table 3**). We calculated the mean reference N concentration from both the 2020 and 2021 samples to best represent N concentrations of the in-situ *Letharia* over the full year of deposition and climate conditions at the plot. The minimum N concentration of the reference and transplant groups (plot repeats, splits and lab duplicates were averaged) were similar around 0.8%, but the maximum of the transplant group exceeded 2%, while the maximum for the reference plot was below 1.25%. Using all the samples (plot repeats, splits, and lab duplicates) the coefficient of variation (CV) of the reference group was 0.14, while the CV was 0.22 for the transplant group. This is to be expected considering that the reference samples were from a single plot, while the transplant samples were from nine different plots that experienced a wide range of environmental conditions.

The mean N concentration of the living transplants (1.52%) was significantly higher than in the dead transplants (1.10%) (Welch's t-test, p = 0.038).

Table 4. Comparison of in-situ and living transplant N concentrations for *Letharia*. Plot repeats, splits, and lab duplicates were averaged to obtain these statistics.

Site	NH_3 (μg/liter)	NO_2 (ppb)	In-situ N%	Transplant N%	In-situ: transplant ratio
Florence Lake	2.1	1	1.25	1.35	0.9
Huntington Lake	3.1	1.1	1.56	1.52	1.0
Shaver Lake	3.50	1.6	1.95	1.73	1.1

N has been shown to decrease over time in dead mosses even when they were exposed to high levels of N deposition, likely due to decay and lack of active uptake (Vingiani et al. 2004). Immersion experiments have also shown that only living, not dead, lichens actively uptake ammonia from solution (Lang et al. 1976). Living lichen material has also been shown to accumulate more N from deposition than dead lichen material in controlled experiments where lichen transplants were purposefully killed (Adamo et al. 2007). However, Purvis et al. (2005) found much higher N content in dead lichens sampled in-situ from the same sites as live lichens, although the lichens in their study were of a different species than *Letharia*.

We expect that the N content of dead transplanted lichens would depend on the net effect of losses from leaching after death, gains from active uptake before death, and gains from passive deposition both before and after death. None of these were specifically measured, but combined, they produced a net gain in N in dead transplants, but less than the gains for healthy transplants.

Comparing transplant N to estimates of deposition. The three points comparing N concentrations of living transplants with in-situ *Letharia* fell on a straight line ($r^2 = 1.0$, presumably in part by chance), but with slope of 1.8 rather than one. It appears that the transplants accumulated enough N over the course of the year to reach relatively similar concentrations to those of the N concentrations of the in-situ *Letharia*. It is possible that this accumulation occurred more quickly than 12 months as other work has found this to occur in a matter of months (Gaio-Oliveira et al. 2005; Olsen et al. 2010). Only the Shaver Lake, Huntington Lake, and Florence Lake plots, had enough in-situ *Letharia* present to collect, which highlights the limitations in in-situ sampling of *Letharia* in this area.

Additionally, the living transplants related strongly with NH_3 and NO_2 concentrations from passive monitors (**Table 4**; **Fig. 4**). The positive relationship between the passively sampled NH_3 and NO_2 concentrations and the N concentrations of the dead transplants was destroyed when dead transplants were included. This supports lichen biomonitoring protocols that prohibit collection of litterfall lichens because they may be dead or dying (Jovan et al., 2021), thus weakening the relationship between thallus N concentrations and N deposition.

Conclusions

We found that *Letharia vulpina* transplants for bioindication of nitrogen deposition might be useful in

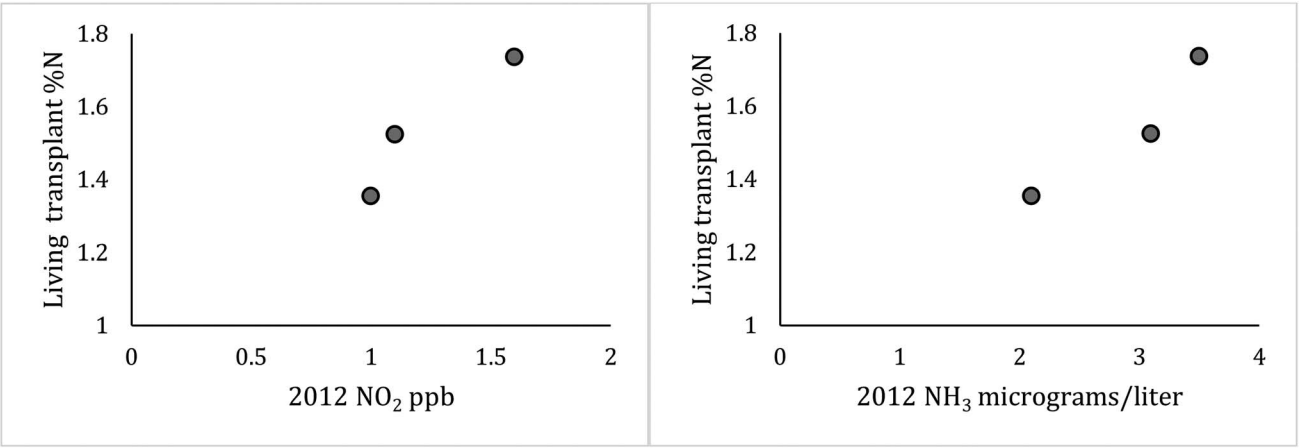

Figure 4. Averaged living transplant N concentrations (%N) plotted against NO_2 and NH_3 measured at the plots in 2012.

some applications, but that a one-year deployment outside its current range is likely to fail because of mortality of the transplants and subsequent mortality-related losses of N. Furthermore, N concentrations of *Letharia* may be increased by persistent wildfire smoke.

Letharia transplants increased in N concentration one year after deploying from a relatively clean area into the higher N deposition environment of southern Sierras. Living transplants reached similar concentrations of N as in-situ *Letharia* and had strong relationships with NO_2 and NH_3 deposition.

All transplants that were deployed at plots outside of the natural range of *Letharia* died. Dead transplants had lower N concentrations than transplants that survived, supporting other studies that showed lichens lose N from their thalli after death. While we presume that climatic factors resulted in *Letharia* mortality, we have no information on specific causes of mortality. Nor do we know at what season mortality occurred. We recommend further studies to determine the timing and specific causes of mortality at both higher and lower elevations than the normal distributional range of *Letharia*.

For future study transplants could be deployed for only two to three summer months in hopes of increasing survival rates at plots outside of the natural range of *Letharia*. We do not know, however, whether summer weather induced mortality.

Another limitation of transplants in comparison to in-situ lichen sampling is that a repeat visit is required. Twelve months is a more predictable time period in terms of fieldwork because it allows for both visits to take place in early season, before likelihood of wildfire is high. As we found out, planning for mid-summer to mid-fall visits is risky in California because of the distinct possibility of wildfire-related forest closures preventing completion of the experiment in the same field season.

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