Decreasing N_2-fixation in *Lobaria oregana* is likely caused by anthropogenic nitrogen emissions

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Abstract

Nitrogen (N)-fixation is the dominant natural source of N in Pacific Northwest ecosystems, but low N_2-fixation rates result in N limitations to net primary productivity. However, anthropogenic emissions of N have increased globally due to agriculture and burning of fossil fuels. Long term N deposition has adverse effects such as eutrophication, soil acidification, and loss of biodiversity. In Pacific coastal ecosystems, epiphytic lichen communities are a dominant source of N_2-fixation, but exceedance of the critical load of N from deposition may decrease N_2-fixation by epiphytic lichens. Here, we test the hypothesis that atmospheric N deposition from anthropogenic sources is decreasing N_2-fixation by the cyanolichen, *Lobaria oregana* (Tuck.) Müll. Arg. We tested the hypothesis by measuring the N stable isotope composition ($\delta^{15}N$) of herbarium lichen specimens from 1899 to current day. A $\delta^{15}N$ of 0 ‰ indicates N_2-fixation, negative values represent agricultural emissions while positive values indicate N originating from fossil fuel emissions. Lichen $\delta^{15}N$ suggests N_2-fixation was the dominant source of N until the 1970's. A large increase in $\delta^{15}N$ in the 1970's corresponds to the completion of I-5 and rapid development along the I-5 corridor. Lichen $\delta^{15}N$ values that correspond to fossil fuel emissions have steadily increased to present, but large spatial variation exists due to localized sources. This suggests that over the short term, anthropogenic N deposition is causing a decrease in N_2-fixation by *Lobaria oregana*. The long term consequences are a likely shift from N sensitive, to N tolerant species.

Background

One of the most vital nutrients required by flora and fauna of the Pacific Northwest is nitrogen. Nitrogen often limits net ecosystem productivity, and while it is abundant in the atmosphere in the form of N_2, 99% of this nitrogen is not available for 99% of plants or animals until it has been converted into a reactive form by N_2-fixation (Galloway et al. 2003).

The primary organisms capable of N_2-fixation in mature temperate forests of the Pacific Northwest are epiphytic cyanolichens (Figures 1 & 2). These lichens consist of a fungus that lives in symbiosis with a cyanobacteria that is capable of N_2-fixation.

Figure 1. Wind River Canopy Crane located in the Gifford Pinchot National Forest in western Washington. *Lobaria oregana* (Tuck.) Müll. Arg. is located in the "light transition zone", at approximately 13 to 37 m in canopy height (McCune et al. 1997). (1)

Figure 2. *Lobaria oregana* is a dominant lichen in forest canopies and is capable of assimilating atmospheric nitrogen and converting it into organic forms via N_2-fixation. (2)

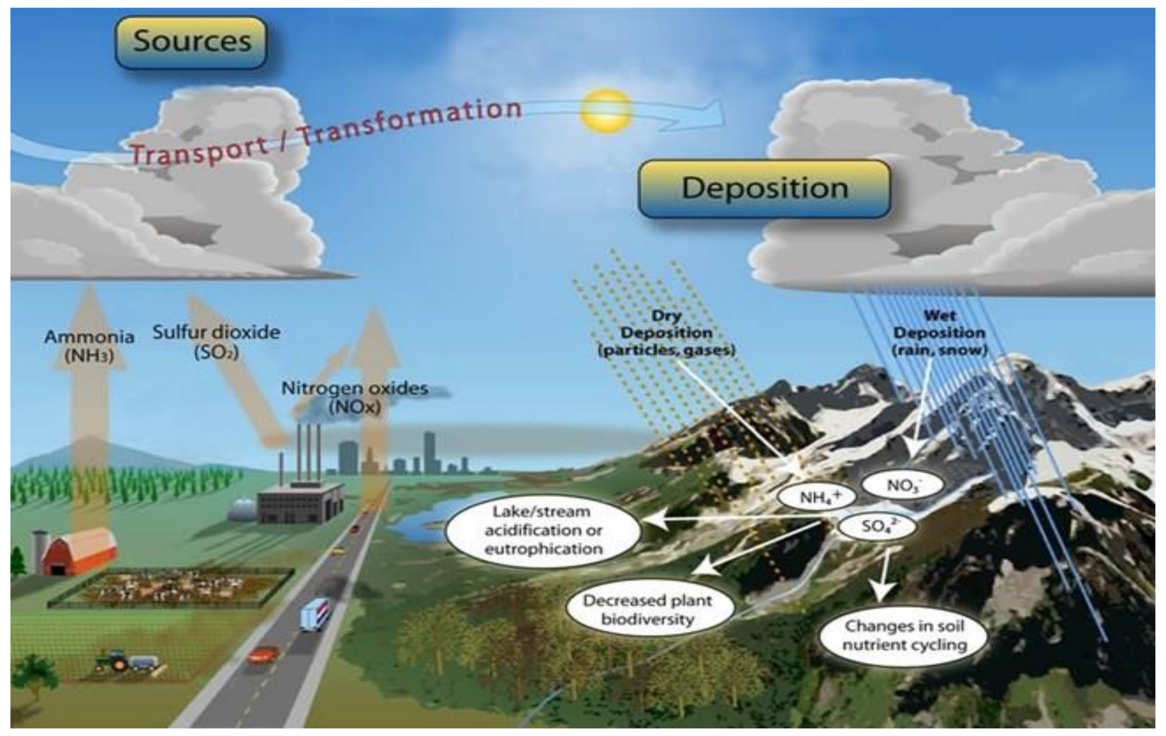

Figure 3. Agriculture and burning of fossil fuels releases reactive N into the environment. It is deposited back into terrestrial and aquatic ecosystems as atmospheric deposition. (NPS, 2014)

Population growth, industrialization, and agriculture have increased anthropogenic emissions of N in the Pacific Northwest, increasing atmospheric deposition of reactive N and causing loss of biodiversity and changes in ecosystem nutrient and energy cycling (Figure 3).

This study was conducted in order to give us a clearer understanding of how anthropogenic emitted N is affecting the protected lands and parks of Washington and Oregon. It is predicted that as sources of anthropogenic N are deposited into ecosystems, naturally fixed N will decrease in response and therefore alter species composition. Since the biomass of lichens in temperate forests is extremely large, conservation of these species in response to climate change is becoming increasingly important in order to maintain healthy forest ecosystems.

Hypothesis

I hypothesize that anthropogenic N deposition will negatively impact N_2-fixation by canopy lichens in temperate forests by causing a shift in N input into forest ecosystems from natural N_2-fixation, to anthropogenic N deposition.

Approach

In order to test my hypothesis, I measured the relative abundance of stable isotopes of nitrogen (^{15}N and ^{14}N) of *L. oregana* specimens that spanned from pre-industrial (before 1900) to present day. Since N_2-fixation and atmospheric deposition each have unique nitrogen isotope compositions ($\delta^{15}N$), they provide distinct "signatures" that allow us to understand the origin of N, and ultimately determine if atmospheric deposition is affecting N_2-fixation. Each sample was categorized as being either *coastal* (Coast Range ecoregion), *urban* (Puget Lowland and Willamette Valley ecoregions), or *inland* (Cascades and North Cascades ecoregions) in order to test if different locations were affected more or less by anthropogenic N deposition. This will give us a better understanding of which ecosystems are most affected by N deposition.

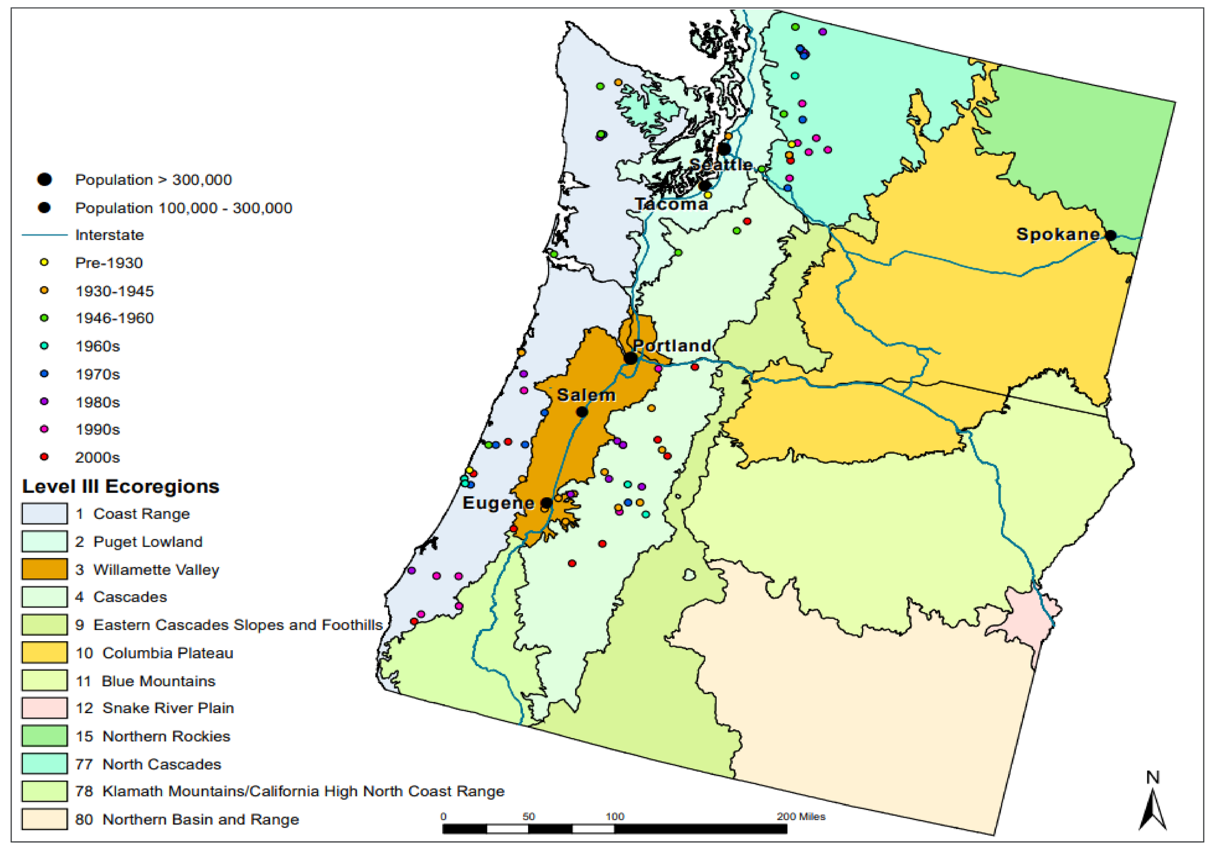

Figure 4. Specimens of *L. oregana* were sampled at regional herbaria to represent a spatial and chronological distribution.

Results

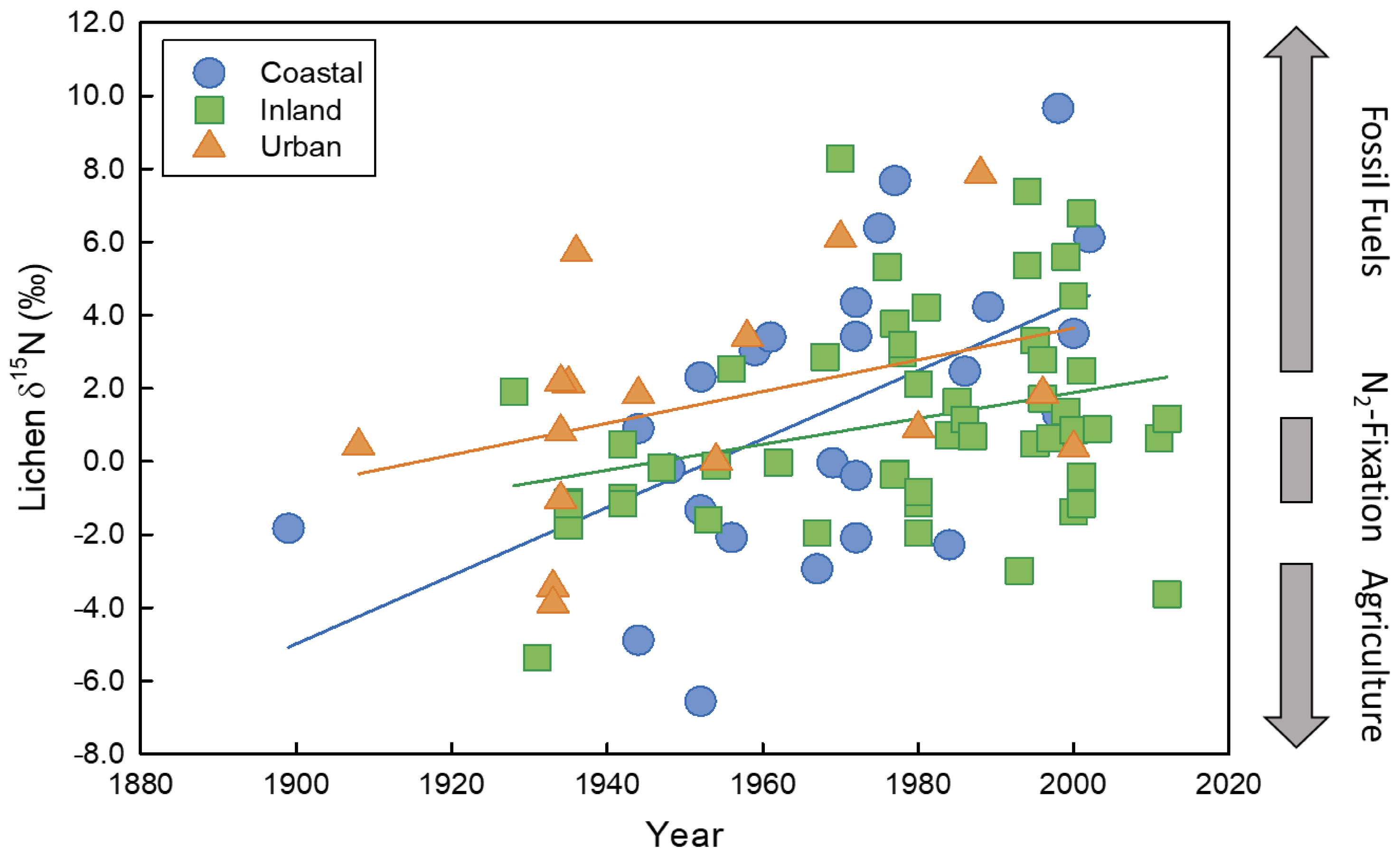

Figure 5. Lichen $\delta^{15}N$ of herbarium specimens (n=93) from pre-industrial to present day. Specimens originated from coastal (n=25), urban (n=16), and inland (n=52) regions of western Oregon and Washington. Data was analyzed using a PROC GLM in SAS® with *year* as a continuous variable and *location* as a categorical variable with three levels (coast, urban, and inland). There was a significant effect of year on $\delta^{15}N$, with $\delta^{15}N$ increasing over time ($F = 16.45$, $p < 0.0001$). There was not an effect of location or year*location interaction on $\delta^{15}N$ ($F = 1.70$, $p = 0.1881$; $F = 1.72$, $p = 0.1853$).

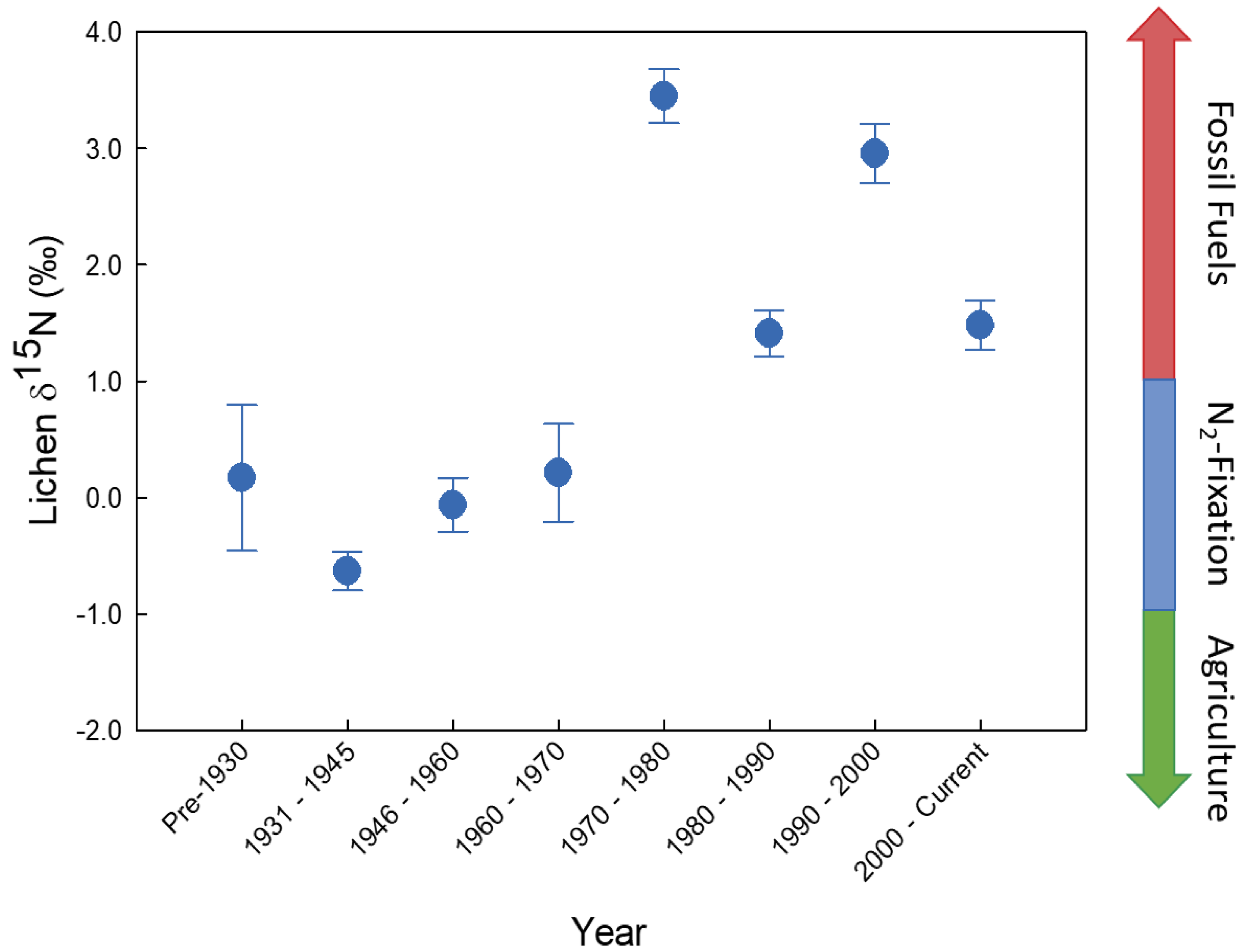

Figure 6. Mean lichen $\delta^{15}N$ (‰) (± S.E.) for herbarium specimens within 8 distinct time periods. Increasing values across time represent a shift from N_2-fixation towards fossil fuel emissions. Since N_2- fixation and atmospheric deposition each have a unique ratio of nitrogen isotopes, they provide distinct "signatures" that will be easy to compare. A $\delta^{15}N$ of 0 ‰ indicates N_2-fixation, while positive values indicate N input from fossil fuels and negative values indicate agricultural sources (Hoffman et al. 2019).

Stable Isotopes

$$\delta^{15}N = (R_x / R_s - 1) \cdot 1000$$

Lichen stable isotope composition is expressed as the molar ratio (R) of the heavier to lighter N isotope ($^{15}N/^{14}N$). The R of each sample (R_x) is normalized relative to an international standard (R_s) and expressed as the deviation from unity. Values are expressed as parts per thousand or per mil (‰) by multiplying by 1000 because differences in R between samples are small.

The nitrogen (N) content and stable isotope composition of each lichen sample was measured using an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA, USA) coupled to a stable isotope ratio mass spectrometer (Delta Plus XP, Thermofinnigan, Bremen) at the WSU Stable Isotope Core Facility.

Conclusions

- Data from herbarium specimens supports our hypothesis; an increase in $\delta^{15}N$ in the 1970's corresponds to the completion of I-5 and rapid development along the I-5 corridor.
- Over the short term, anthropogenic N deposition is causing a decrease in N_2-fixation by *L. oregana*. The long term consequences are a likely shift from N sensitive, to N tolerant species. N tolerant species often will not be affected by additional inputs of N into the ecosystem, while N sensitive species are likely to be heavily influenced.

Literature Cited

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Photo Credit

(1) https://phys.org/news/2014-01-image-reality-leaf-photos-lab.html

(2) https://alchetron.com/Lobaria-oregana

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