Urbanization impacts on epiphytic nitrogen and metal cycling in *Acer macrophyllum* stands in Western Washington, USA

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Abstract

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Dr. Thomas H. DeLuca

Bryophytes have been used extensively in Europe to assess the impacts of air pollution and atmospheric deposition; however there have been no studies conducted in Washington State to examine how pollution associated with urbanization is impacting canopy and forest floor bryophyte communities. This study investigated in N_2 fixation rates and metal concentrations in bryophytes across *Acer macrophyllum* Pursh stands in western Washington. We used an intensive, vertically stratified sampling approach in *Acer macrophyllum* canopies in the Hoh Rainforest on the Olympic Peninsula, WA and in Seattle, WA to collect 214 samples of *Isothecium stoloniferum (Brid.).* An extensive, ground-based sampling approach was used across an urban-to-wildland gradient to collect 59 *Kindbergia praelonga (Hedw.) Ochyra* samples. Intensive samples were collected four times (April, July, and October of 2016; and in January

2017) and extensive samples three times (April, July, and October 2016) to assess seasonal differences in N_2 fixation rates and metal concentrations across sampling locations.

Nitrogen fixation rates in canopy *I. stoloniferum* samples was found to be dependent on sampling season and location. Average N_2 fixation rates reached peak levels in spring 2016 for both peninsula and urban samples (588.34 and 179.02 µmol m^{-2} day^{-1}, respectively) and were lowest in fall 2016 (18.47 and 5.29 µmol m^{-2} day^{-1}). In total, *I. stoloniferum* in canopies of *A. macrophyllum* was found to yield 1.13 kg N ha^{-1} yr^{-1} in the Hoh Rainforest and only 0.009 kg N ha^{-1} yr^{-1} in the Seattle urban area.

For the heavy metal analysis, elevated concentrations of Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn were found in both intensive and extensive bryophyte samples collected in Seattle across all collection seasons. The elevated concentrations of heavy metals observed in *I. stoloniferum* samples collected from Seattle may be related to the low the N_2 fixation rates observed in the same samples.

The results from this study highlight that canopy and ground floor bryophytes has the potential to be used as a low-cost screening tool to evaluate N and heavy metal pollution in urban and rural areas.

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Chapter 1

Introduction

The Pacific Northwest (PNW) is considered to be one of the cleanest air quality regions in the United States (NADP/NTN, 2015), owing primarily to a small industrial base, an extensive hydroelectric system, and a region-wide lack of coal-fired power plants. However, despite this history and continued reductions in point source and per capita vehicular emissions, recent population increases and associated transportation growth is threatening to reverse the region's history of high air quality (Geiser & Neitlich, 2007). In the Puget Sound Region, the population is expected to grow by roughly 1.5 million people over the next 25 years, which will in turn increase demand for travel throughout the region by 40% (Puget-Sound-Regional-Council, 2014). Seattle traffic, increasingly known for rampant transportation gridlock, was recently ranked as the 10th most congested city in North America (INRIX, 2016).

This region-wide expansion in the transportation sector has introduced a new set of atmospheric pollutants to forests of the PNW. Vehicle emissions associated with exhaust, lubricants, and tire and brake attrition represent a major form of pollution in busy urban centers in the PNW. Increasing urban vehicle operation and increasing traffic on major transportation corridors throughout the region (e.g., I-5 and I-90) can adversely affect forest ecosystem health, and there is growing evidence that traffic-related emissions, such as through tire and brake attrition, can impact ecosystems (Maltby et al., 1995; Pearson et al., 2000; Bermejo-Orguna, 2014). As personal and commercial vehicle traffic continue to rise, the region will experience an increase in atmospheric deposition associated with nitrogen oxide (NOx) emissions from gasoline and diesel vehicles. Further, transportation activities can greatly increase heavy metal emissions and subsequent deposition in heavily used transportation corridors (Maltby et al., 1995; Pearson et al., 2000; Bakirdere & Yaman, 2008). Due to the limited use of coal throughout the region, metal deposition has generally been of little concern; however zinc (Zn), copper (Cu) and titanium (Ti) deposition has been increasing primarily due to tire and brake attrition (Thorpe & Harrison, 2008; Apeagyei et al., 2011).

These transportation pollutants have the potential to negatively affect forest ecosystem health by altering canopy epiphytes and their associated foodwebs (Creamer et al., 2008, Zvereva & Kozlov, 2011). Despite the iconic image of PNW moss-covered trees, the impact of transportation-related pollutants on PNW canopy epiphytes, and their impacts on ecosystem function, has been poorly examined.

Canopy dwelling epiphytes (i.e. lichens, bryophytes, algae) play important roles in biogeochemical cycles worldwide (Cornelissen et al., 2007) and are of particular importance in both deciduous and coniferous forests of the PNW (Binkley & Graham, 1981; McCune et al., 1997; Peterson & McCune, 2003; Antoine & McCune, 2004; Pypker et al., 2006). In the PNW, canopy epiphytes represent a substantial component of the forest ecosystem biomass, with standing epiphyte loads of 900-6,870 kg ha^{-1} on some regional tree species (Pike et al., 1977 and Nadkarni, 1984, respectively). Tree canopies are complex biogeochemical components of an ecosystem, serving as key sites for the accumulation and processing of nutrients and other chemical elements (Klopatek et al., 2006).

Despite their fundamental role in ecosystem function and nutrient cycling, epiphytes are exceptionally sensitive to both direct and indirect effects of anthropogenic stress. Bryophytes are widely used as biomonitors to assess the impacts of air pollution and atmospheric deposition (Ruhling & Tyler, 1973; Steinnes, 1989; Berg et al., 1995; Pott & Turpin, 1996; Reimann et al., 2006; Zechmeister et al., 2005; Harmens et al., 2008; Aničić et al., 2009; Ares et al., 2010; Donovan et al., 2016). As bryophytes lack roots, they absorb nutrients and water from atmospheric deposition (Bates, 1992) rather than from the soil. Bryophyte leaves are only one cell-layer thick and do not have a protective epidermis. As a result, they demonstrate ion exchange properties, all of which allows for the accumulation of water, organic compounds and inorganic ions that are deposited on their surfaces (Gjengedal & Steinnes, 1990; Tyler, 1990; Aboal et al., 2011; Gonzalez & Pokrovsky, 2013).

Exposure to nitrogen (N) based pollutants is known to negatively influence bryophyte growth, impacting epiphyte community structure (Davies et al., 2007; Bermejo-Orduna, 2014). Moss shoot growth has been found to be is sensitive to NO_2 emissions from vehicles (Bell, 1992). Cyanobacteria, facultative autotrophs, associated with bryophytes have the ability to fix substantial quantities of N in environments that experience little N deposition (DeLuca et al., 2002). Multiples studies have demonstrated the sensitivity of forest floor N_2 fixing bryophytes to N deposition (DeLuca et al., 2008; Zackrisson et al., 2009; Gundale et al., 2011) and N_2 fixation has been shown to cease in areas with increasing N deposition (DeLuca et al., 2008, Gundale et al., 2011).

Heavy metal deposition associated with industry and urbanization are also known to negatively influence bryophyte communities (Pearson et al. 2000; Aničić et al., 2009; Zvereya & Kozloy, 2011). As bryophytes exhibit high cation exchange capacity and can generate acidity through proton release from exchange sites (Spearing, 1972), the bioaccumulation of heavy metals in bryophyte tissue may inhibit microbial populations and thus limited N_2 fixation capacity (Tyler, 1990; Pearson et al., 2000; Basiliko & Yavitt 2001). Bengtsson et al. (1982) found a reduction in the photosynthetic tissue responsible for growth in *Hylocomium splendens* samples collected from sites with elevated Cu and Zn concentrations.

To date, no comprehensive studies have examined N_2 fixation in canopy bryophytes in forests of western Washington or how increasing N and metal deposition in PNW forest ecosystems may influence canopy bryophyte communities and N_2 fixation rates.

Objectives

While there has been scattered interest in epiphyte ecology and its connection to forest health (McCune, 2000; Lindo & Gonzalez, 2010), there have been no recent studies that assess the influence of transportation sector pollutants on forest canopy bryophytes in the PNW. The overarching goal of the proposed research is to evaluate whether N and metal deposition associated with urbanization is creating a serious threat to canopy epiphyte and forest floor bryophyte communities throughout Western Washington. This thesis is broken down into three primary objectives that are presented in two data chapters:

1. Evaluate N_2 fixation rates in canopy bryophytes in *A. macrophyllum* stands in the Hoh Rainforest on the Olympic Peninsula and in the urban center of Seattle, WA (Chapter II)
2. Assess how urbanization affects canopy bryophyte N_2 fixation rates in this region (Chapter II).
3. Assess whether transportation sourced metals contribute to shifts in canopy epiphyte and forest floor bryophyte biodiversity and metal retention across an urban-to-wildland gradient in Western WA (Chapter III)

References:

Aboal, J. R., Pérez-Llamazares, A., Carballeira, A., Giordano, S., & Fernández, J. A., 2011. Should moss samples used as biomonitors of atmospheric contamination be washed?. Atmospheric environment, 45(37), 6837-6840.

Aničić, M., Tomašević, M., Tasić, M., Rajšić, S., Popović, A., Frontasyeva, M.V., Lierhagen, S. and Steinnes, E., 2009. Monitoring of trace element atmospheric deposition using dry and wet moss bags: accumulation capacity versus exposure time. Journal of hazardous materials, 171(1), 182-188.

Antoine, M. E., & McCune, B., 2004. Contrasting fundamental and realized ecological niches with epiphytic lichen transplants in an old-growth Pseudotsuga forest. The Bryologist, 107(2), 163-172.

Apeagyei E., Bank M.S. & Spengler J.D., 2011. Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts. Atmospheric Environment 45, 2310-2323.

Ares, Á., Fernández, J. Á., Aboal, J. R., & Carballeira, A., 2011. Study of the air quality in industrial areas of Santa Cruz de Tenerife (Spain) by active biomonitoring with Pseudoscleropodium purum. Ecotoxicology and environmental safety, 74(3), 533-541.

Bakirdere S. & Yaman M., 2008. Determination of lead, cadmium and copper in roadside soil and plants in Elazig, Turkey. Environmental Monitoring and Assessment 136, 401-410.

Basiliko, N., & Yavitt, J. B., 2001. Influence of Ni, Co, Fe, and Na additions on methane production in Sphagnum-dominated Northern American peatlands. Biogeochemistry, 52(2), 133-153.

Bates, J. W., 1992. Mineral nutrient acquisition and retention by bryophytes. Journal of Bryology, 17(2), 223-240.

Bell, S., Ashenden, T. W., & Rafarel, C. R., 1992. Effects of rural roadside levels of nitrogen dioxide on Polytrichum formosum Hedw. Environmental pollution, 76(1), 11-14.

Bengtson, C., Folkeson, L., & Göransson, A., 1982. Growth reduction and branching frequency in Hylocomium splendens near a foundry emitting copper and zinc. Lindbergia, 129-138.

Berg, T., Røyset, O., & Steinnes, E., 1995. Moss (Hylocomium splendens) used as biomonitor of atmospheric trace element deposition: estimation of uptake efficiencies. Atmospheric Environment, 29(3), 353-360.

Bermejo-Orduna R., et al., 2014. Biomonitoring of traffic-related nitrogen pollution using Letharia vulpine Hue in the Sierra Nevada, California. Science of The Total Environment 490 205-212.

Binkley D. & Graham R.L., 1981. Biomass, production, and nutrient cycling of mosses in an old-growth Douglas-Fir forest. Ecology 62, 1387-1389.

Cornelissen JHC, Lang SI, Soudzilovskaia NA, During HJ, 2007. Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. Annu Rev Bot 99:987–1001

Creamer R.E., Rimmer D.L. & Black H.I.J., 2008. Do elevated soil concentrations of metals affect the diversity and activity of soil invertebrates in the long-term? Soil Use and Management, 24, 37-46.

Davies, L., Bates, J. W., Bell, J. N. B., James, P. W., & Purvis, O. W., 2007. Diversity and sensitivity of epiphytes to oxides of nitrogen in London. Environmental Pollution, 146(2), 299-310.

Donovan, G. H., Jovan, S. E., Gatziolis, D., Burstyn, I., Michael, Y. L., Amacher, M. C., & Monleon, V. J., 2016. Using an epiphytic moss to identify previously unknown sources of atmospheric cadmium pollution. Science of the Total Environment, 559, 84-93.

DeLuca, T. H., Zackrisson, O., Nilsson, M. C., & Sellstedt, A., 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature, 419(6910), 917-920.

DeLuca, T. H., Zackrisson, O., Gundale, M. J., & Nilsson, M. C., 2008. Ecosystem feedbacks and nitrogen fixation in boreal forests. Science, 320(5880), 1181-1181.

Geiser, L. H., & Neitlich, P. N., 2007. Air pollution and climate gradients in western Oregon and Washington indicated by epiphytic macrolichens. Environmental Pollution, 145(1), 203-218.

Gjengedal, E., & Steinnes, E., 1990. Uptake of metal ions in moss from artificial precipitation. Environmental Monitoring and Assessment, 14(1), 77-87.

González, A. G., & Pokrovsky, O. S., 2014. Metal adsorption on mosses: toward a universal adsorption model. Journal of colloid and interface science, 415, 169-178.

Gundale, M. J., Deluca, T. H., & Nordin, A., 2011. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. Global Change Biology, 17(8), 2743-2753.

Harmens, H., Norris, D. A., Koerber, G. R., Buse, A., Steinnes, E., & Rühling, Å., 2008. Temporal trends (1990–2000) in the concentration of cadmium, lead and mercury in mosses across Europe. Environmental Pollution, 151(2), 368-376.

INRIX, 2016. Traffic Scorecard.

Klopatek, J. M., Barry, M. J., & Johnson, D. W., 2006. Potential canopy interception of nitrogen in the Pacific Northwest, USA. Forest ecology and management, 234(1), 344-354.

Lindo, Z., & Gonzalez, A., 2010. The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems, 13(4), 612-627.

Maltby L., Boxall A.B.A., Forrow D.M., Calow P. & Betton C.I., 1995. The effects of motorway runoff on freshwater ecosystems. 1 Field study. . Environmental Toxicology and Chemistry 14, 1079-1092.

McCune B., Amsberry K.A., Camacho F.J., Clery S., Cole C., Emerson C., Felder G., French P., Greene D., Harris R., Hutten M., Larson B., Lesko M., Majors S., Markwell T., Parker G.G., Pendergrass K., Peterson E.B., Peterson E.T., Platt J., Proctor J., Rambo T., Rosso A., Shaw D., Turner R. & Widmer M., 1997. Vertical profile of epiphytes in a Pacific Northwest old-growth forest. Northwest Science 71, 145-152.

McCune, B. 2000. Lichen communities as indicators of forest health. The Bryologist 103:353–356.

Nadkarni, N. M., 1984. Biomass and mineral capital of epiphytes in an Acer macrophyllum community of a temperate moist coniferous forest, Olympic Peninsula, Washington State. Canadian Journal of Botany, 62(11), 2223-2228.

NADP/NTN, 2015. National Atmospheric Deposition Program (NRSP-3)/ National Trends Network. Program Office, Illinois State Water Survery, Champaign, IL. See animated isopleth maps. <http://nadp.sws.uiuc.edu>.

Pearson J., Wells D.M., Seller K.J., Bennett A., Soarse A., Woodall, J., & Ingrouille J., 2000. Traffic exposure increase natural 15N and heavy metal concentrations in moss. . New Phytologist 147, 317-326.

Peterson E.B. & McCune B., 2003. The importance of hotspots for lichen diversity in forests of western Oregon. Bryologist 106, 246-256.

Pike, L. H., Rydell, R. A., & Denison, W. C., 1977. A 400-year-old Douglas fir tree and its epiphytes: biomass, surface area, and their distributions. Canadian Journal of Forest Research, 7(4), 680-699.

Pott, U., & Turpin, D. H., 1996. Changes in atmospheric trace element deposition in the Fraser Valley, BC, Canada from 1960 to 1993 measured by moss monitoring with Isothecium stoloniferum. Canadian Journal of Botany, 74(8), 1345-1353.

Puget Sound Regional Council, 2014. Transportation 2040: toward a sustainable transportation sesystem (Update). PSR Council, Seattle, WA.

Pypker T.G., Unsworth M.H. & Bond B.J., 2006. The role of epiphytes in rainfall interception by forests in the Pacific Northwest. I. Laboratory measurements of water storage. . Canadian Journal of Forest Resources 36, 809-811.

Reimann, C., Arnoldussen, A., Boyd, R., Finne, T. E., Nordgulen, Ø., Volden, T., & Englmaier, P., 2006. The influence of a city on element contents of a terrestrial moss (Hylocomium splendens). Science of the Total Environment, 369(1), 419-432.

Rühling, Å., & Tyler, G., 1973. Heavy metal deposition in Scandinavia. Water, Air, & Soil Pollution, 2(4), 445-455.

Spearing, A. M., 1972. Cation-exchange capacity and galacturonic acid content of several species of Sphagnum in Sandy Ridge Bog, central New York State. Bryologist, 154-158.

Steinnes, E., 1989. Biomonitors of air pollution by heavy metals. In Control and fate of atmospheric trace metals (pp. 321-338). Springer Netherlands.

Thorpe A. & Harrison R.M., 2008. Sources and properties of non-exhaust particulate matter from road traffic: A review. Science of The Total Environment, 400, 270-282.

Tyler, G., 1990. Bryophytes and heavy metals: a literature review. Botanical journal of the Linnean Society, 104(1-3), 231-253.

Zackrisson, O., DeLuca, T. H., Gentili, F., Sellstedt, A., & Jäderlund, A., 2009. Nitrogen fixation in mixed Hylocomium splendens moss communities. Oecologia, 160(2), 309.

Zechmeister, H. G., Hohenwallner, D., Riss, A., & Hanus-Illnar, A., 2005. Estimation of element deposition derived from road traffic sources by using mosses. Environmental Pollution, 138(2), 238-249.

Zvereva E. & Kozlov M., 2011. Impacts of Industrial Polluters on Bryophytes: a Meta-analysis of Observational Studies. *Water Air Soil Pollut*, 218, 573-586.

Chapter 2:
Epiphytic nitrogen fixation in *Acer macrophyllum* canopies in Western Washington, U.S.A.

Abstract

Old-growth forests in the Pacific Northwest are unique in their capacity to host a variety of epiphytes on their branches and trunk. Given the high epiphytic biomass associated with *Acer macrophyllum* trees, we evaluated how seasonal weather changes and urbanization, as proxied through metal deposition, affect canopy epiphytic N_2 fixation in the Hoh Rainforest of the Olympic Peninsula and in urban parks and forests in Seattle. We used an intensive sampling approach to collect *Isothecium stoloniferum (Brid.)* samples from both the Hoh Rainforest and Seattle during four seasons from April 2016 through January 2017. Levels of N_2 fixation were found to be the highest during the April 2016 sampling period. Permutational multivariate analysis of variance (perMANOVA) revealed a significant effect of sampling location and season. Elevated levels of heavy metals were observed in *I. stoloniferum* samples collected in the urban boundaries of Seattle, suggesting that N_2 fixation is sensitive to the bioaccumulation of heavy metals. In total, *I. stoloniferum* in canopies of *A. macrophyllum* was found to yield 1.13 kg N ha^{-1} yr^{-1} in the Hoh Rainforest and only 0.009 kg N ha^{-1} yr^{-1} in the Seattle urban area. Our results suggest that epiphytic N_2 fixation in *A. macrophyllum* stands on the Olympic Peninsula may contribute nitrogen in secondary successional maple forests.

Keywords: nitrogen fixation; bryophytes; heavy metals; Pacific Northwest

Introduction

Canopy dwelling epiphytes play an important role in biogeochemical cycles in deciduous and coniferous forests of the Pacific Northwest (PNW) (Pike et al., 1977; Binkley & Graham, 1981; Pypker et al., 2006; Cornelissen et al., 2007; Haristoy et al., 2014); however, few studies have evaluated these epiphytic mosses for their capacity to fix N. Dense, extensive epiphytic moss mats establish on the trunk and branches of big leaf maple (*Acer macrophyllum* Pursh) trees as a result of high rainfall rates in the region, the calcium rich bark, and neutral pH of maple throughfall (Kenkel & Bradfield, 1986). On the Olympic Peninsula of Western Washington state (USA), *A. macrophyllum* canopies generally have an estimated standing epiphytic load of 6,870 kg ha^{-1} (Nadkarni, 1984). These epiphytic mats, which contain a variety of lichen and bryophyte species, contribute to a number of important ecosystem functions including the processing of nutrients and water (Proctor, 2001; Klopatek et al., 2006; Pypker et al., 2006).

Unlike vascular plants, epiphytes lack roots and obtain their nutrients from the atmosphere (Bates, 1992). Bryophytes leaves demonstrate ion exchange properties, which facilitate the absorption of organic compounds and inorganic ions that are deposited on their surfaces (Gjengedal & Steinnes, 1990; Tyler et al., 1990; Gonzalez & Pokrovsky, 2013). Symbiotic associations between cyanobacteria and forest floor bryophytes can contribute significant input of Nitrogen (N) in late successional boreal forests (DeLuca et al. 2002, Zackrisson et al., 2004). Lindo and Whiteley (2011) confirmed the presence of cyanobacteria in canopy bryophyte species associated with old growth Sitka spruce (*Picea sitchensis* (Bong) Carr.) trees in British Columbia, Canada and reported significant rates of N_2 fixation in these mats. Cyanobacteria associated with canopy bryophytes have the potential to function as an important N source in N-limited PNW forests, which supports forest productivity by supplying stand-level N in mid to late succession.

Despite their fundamental role in ecosystem function and nutrient cycling, bryophytes are extremely sensitive to both direct and indirect effects of anthropogenic stress. Bryophytes have been widely used in Europe to assess the impacts of air pollution and atmospheric deposition (Berg et al., 1995; Berg & Steinnes, 1997; Reimann et al., 2000; Harmens et al., 2008). Heavy metal deposition and exposure to N based pollutants are known to negatively influence bryophyte growth (Bengtsson et al., 1982; Pearson et al., 2000). Previous studies have highlighted the sensitivity of forest floor N_2 fixing bryophytes to N deposition (DeLuca et al., 2008; Ackermann et al., 2012), and rates of N_2 fixation has been shown to decrease or even cease in areas with increasing N deposition (Gundale et al., 2011).

To date, no comprehensive studies have examined epiphytic N_2 fixation in bigleaf maple, *Acer macrophyllum,* or assessed how changes in N and metal deposition associated with the increasing population and transportation growth in Western Washington could influence N_2 fixation in *A. macrophyllum* bryophyte communities. *Acer macrophyllum* is a prominent component of the rural and urban forest in Western Washington, which lies at the heart of the native range of *A. macrophyllum*. In our observations, *A. macrophyllum* is perhaps the only woody plant species that consistently supports epiphytes in heavily urbanized areas in Seattle, even though epiphytes are an iconic feature of rural western Washington forests that are found on a number of woody different plant species, including *A. macrophyllum*. Thus, we chose *A. macrophyllum* due to both its wide distribution across Western WA (urban and rural) and its support of bryophytes. We hypothesized that 1) canopy bryophytes harbor N_2 fixing bacteria; 2) N_2 fixation will be higher in samples collected from the somewhat pristine Olympic Peninsula compared to urban sites in the Puget Sound region; 3) N_2 fixation will vary by season across sampling locations; and 4) urban areas will be exposed to higher concentrations of heavy metals in canopy bryophyte tissue.

Materials & Methods

2.1 Study Area

We collected moss samples using an intensive, vertically stratified sampling approach in *A. macrophyllum* canopies in the Hoh Rainforest on the Olympic Peninsula, WA and in the urban center of Seattle, WA. We collected samples from one stand located on Hoh River Trust land in the Hoh Rainforest (47.82°N, 124.20°W) and from three stands located in Seattle City Parks: Seward Park (47.55°N, 122.25°W), Interlaken Park (47.63°N, 122.31°W), and Ravenna Park (47.67°N, 122.30°W).

In the Hoh Rainforest stand, understory vegetation was dominated by sword fern (*Polystichum munitum* (Kaulf.) C. Presl), redwood sorrel (*Oxalis oregano* Nutt.) and vine maple (*Acer circinatum* Pursh). In the Seattle stands, understory vegetation was dominated by sword fern, salah (*Gaultheria shallon Pursh*), Oregon grape (*Mahonia aquifolium (Pursh)* Nutt.), and stinging nettle (*Urtica dioica L.*). The dominant epiphytic moss species in *A. macrophyllum* canopies in the Hoh Rainforest stand were *Isothecium stoloniferum (*Brid.*), Antitrichia curtipendula (*Hedw.*),* and *Hylocomium splendens (Hedw.).* The dominant epiphytic moss species in *A. macrophyllum* canopies in Seattle were *I. stoloniferum (Brid.), Homalothecium lutescens (Hedw.), and Orthotrichum lyelli* Hook. & Taylor*.* We chose *I. stoloniferum* as the epiphyte species in our study as it could readily be found across all stands, and because it has been used previously in biomonitoring studies from the Pacific Northwest region (Pott & Turpin, 1998; Raymond & Pott, 2003; Raymond et al., 2010).

2.2 Sample Collection

We collected replicate *I. stoloniferum* core samples every three months beginning in April 2016 to January 2017. Samples from extracted from a total of 18 *A. macrophyllum* trees: nine from the Hoh Rainforest, WA and nine from city parks in Seattle, WA, USA (three per park). We selected individual trees at stands based on safety criteria and sampling considerations. For example, selected trees had to have a least three accessible branches below a solid anchor point to allow us to obtain samples from three stratified heights throughout the crown: low (6-10 m), mid (12-15 m) and upper (15-18 m). We used the Single Rope Technique (SRT) (Coffey & Anderson, 2012) in which a rope wrench and a friction hitch was used to gain access to the canopy. We deployed throughfall collectors in the form of ionic resin (UNIBEST Ag Manager, Walla Walla, WA) lysimeters (c.f., DeLuca et al. 2008) in each tree and at each sampling height. Lysimeters were deployed in January 2016, and removed in January 2017. The lysimeters were used to evaluate wet NO_3^--N and NH_4^+-N deposition throughfall rates (Susfalk & Johnson, 2002; Kopatek et al., 2006; DeLuca et al., 2008). We also deployed HOBO data loggers (Onset Computer Corps., Bourne, MA, USA) to record hourly temperature at the three sampling heights throughout the canopy in a total of six trees (three from the Hoh Rainforest and in one tree per city park in Seattle).

In the nine trees from the Hoh Rainforest, we also collected replicate *A. curtipendula* and *H. splendens* cores every three months beginning in April 2016 to January 2017. These cores allowed for the comparison of difference in fixation rates across dominant canopy moss species from the Hoh Rainforest.

A black parachute cord was tied to the climbing line to facilitate sampling in the same tree during seasonal sampling. Installing the parachute cord saved time and prevented any potential damage to the tree that might occur through repeating the rigging process.

2.3 Laboratory Analysis:

i. Nitrogen Fixation Analysis

N_2 fixation rates were estimated using the acetylene reduction (AR) method (Schöllhorn and Burris, 1967). Three replicate moss samples were collected per sample position within each tree and placed into 20 mL glass vials and then sealed with a rubber septum. Ten-percent of the headspace was removed using a syringe and replaced with reagent grade acetylene. The tubes were allowed to incubate at 18°C for 24 hours. Ethylene generated by bacterial reduction in the headspace was analyzed using a Thermo Scientific Trace Gas Chromatograph equipped with a flame ionization detector and Porapak Q column. Controls consisted of moss without acetylene gas, and blank samples contained acetylene gas without moss. Data are presented as the average amount of acetylene reduced to ethylene in µmol m^{-2} d^{-1} across the three replicates per sample.

To determine the amount of N_2 fixed per unit area, 20 replicate samples of the three moss species for were taken with a 20.27cm^2 PVC tube core. For *I. stoloniferum*, 20 cores were collected from the Hoh Rainforest and 20 from Seattle *A. macrophyllum* canopies. Hoh Rainforest and Seattle *I. stoloniferum* were found to have an average of 0.42 shoots cm^{-2} and 0.88 shoots cm^{-2}, respectively. The other two species of interest in the Hoh Rainforest, A. *curtipendula* and *H. splendens,* were found to have an average of 0.16 shoots cm^{-2} and 0.07 shoots cm^{-2}, respectively.

To quantify the amount dry-weight moss biomass per unit area for *I. stoloniferum* and *A. curtipendula,* $10cm^2$ core were collected along with average moss depth per sampling branch. Hoh Rainforest and Seattle *I. stoloniferum* were found to have an average of 0.43 g cm^{-2} and 0.05 g cm^{-2}, respectively. *Antitrichia curtipendula* samples from the Hoh Rainforest were found to have an average of 0.80 g cm^{-2}.

ii. Trace Metal Concentrations:

After samples had been analyzed for N_2 fixation, they were decapped and allowed to vent for 24 hours. Next, they were dried for 24 hours at 40 °C and ground to a fine powder with the facilitation of liquid N. The HNO_3 + H_2O_2 digestion method was used to prepare the dried and ground samples for analysis. Four mL of concentrated reagent-grade HNO_3 were added to samples in 50 mL borosilicate tubes. The tubes were covered with plastic watch glasses and sat overnight at ambient temperature in a fume hood (approximately 18°C) to allow some initial oxidation of the samples by the HNO_3. Samples were digested at 95 °C for 90 minutes in borosilicate tubes heated evenly in a 36-tube graphite block digester. Samples were allowed to cool, after which 4 mL of reagent-grade 30% H_2O_2 were added to each tube followed by a 30-minute digestion at 95 °C and time to cool. Next, an additional 4 mL of H_2O_2 were added to each sample and heated again at 95 °C. After cooling, deionized water was added to each tube to the 20-mL mark. To remove any undigested particulates not dissolved in the HNO_3 and H_2O_2, samples were filtered through 0.45-µm membrane syringe filters.

Digests were analyzed for a suite of metals (Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn) using inductively coupled plasma optical emission spectrometry (ICP-OES). Instrument performance tests were carried out daily to ensure proper calibration. After calibration, quality control samples consisting of blanks, low and high concentration check standards were analyzed.

iii. Resin Analyses for NO_3^--N and NH_4^+-N:

The ionic resins from canopy lysimeters were collected in January 2017. Resin capsules were extracted by placing the ionic resin capsules in three successive 10 mL aliquots of 0.5M HCl. Extractable NO_3^--N and NH_4^+-N were analyzed using microplate colorimetric technique using the salicylate- nitroprusside method for NH_4^+ (Mulvaney, 1996) and the vanadium method for NO_3^- (Miranda et al., 2001).

2.4 Statistical Analysis:

Data on N_2 fixation, represented as µmol m^{-2} d^{-1}, were transformed using a $\log_{10}(y + 1)$ to normalize the distribution. A two-way permutational multivariate analysis of variance (perMANOVA) was carried out to test if N_2 fixation rates and moss metal concentrations were significantly different across location types and sampling seasons for *I. stoloniferum* samples. The subsequent test of multivariate homogeneity of groups (DISPER) was used to calculate significant multivariate dispersions in variables among the location types for *I. stoloniferum*. Euclidean distance was chose for the two-way perMANOVA, and significance of the two-way perMANOVA R-value was determined via 1000 random permutations. For this study, effects with probabilities of $p < 0.05$ were assumed to be significant.

Principle component analysis (PCA) was selected as the appropriate ordination method where dissimilarity was calculated as Euclidean distance (Legendre & Legendre 1998). PCA was performed on the data matrix 'location type x chemical parameters' for *I. stoloniferum* for urban and peninsula sampling sites. Thirteen parameters (µmol m^{-2} d^{-1}, Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn) were introduced as the analysis variables. PCA was performed to assess the dominant patterns in moss acetylene reduction rates and metal concentrations across locations. The loadings of variables to each PC was examined by converting eigenvector coefficients to structure correlations.

We used an Analysis of variance (ANOVA) to test the main effects of species (e.g., *I. stoloniferum, A. curtipendula,* and *H. splendens*), sampling time, and canopy height on N_2 fixation rates. All statistical analyses were conducted in the R Statistical Environment (R Core Team, 2013).

Results

Nitrogenase activity, measured by using AR, was highest in *I. stoloniferum* samples collected in spring for both peninsula and urban sampling sites (Figure 1). Of the in *I. stoloniferum* samples collected in spring, five peninsula samples and one urban sample nitrogenase activity exceeded 1,000 µmol m^{-2} day^{-1}. The shoot density and biomass estimates per unit area were used to scale up the acetylene reduction rates to landscape levels N_2 fixation. By using a season-weighted average, we estimate a canopy N_2 fixation rate (given a molar ratio of 3:1 acetylene reduced to N_2) of 1.13 kg N ha^{-1} yr^{-1} for *I. stoloniferum* and 0.63 kg N ha^{-1} yr^{-1} *A. curtipendula* in the Hoh Rainforest. N_2 fixation rates for Seattle canopy *I. stoloniferum* are estimated at 0.009 kg N ha^{-1} yr^{-1}.

Canopy collection height did not have a significant effect on N_2 fixation observed across the three species (*I. stoloniferum, A. curtipendula,* and *H. splendens)* collected from A. macrophyllum canopies on the Olympic Peninsula (p=0.11). The ANOVA results across the three moss species found significant differences across species ($F_{2,208} = 56.70$, $p<0.001$) and across sampling seasons($F_{3,208} = 84.98$, $p<0.001$). A significant interaction between species and sampling season was observed ($F_{6,208} = 4.72$, $p<0.001$).

In the Hoh Rainforest, the highest average nitrogenase activity measured was observed in. *H. splendens* (2,388.18 µmol m^{-2} day^{-1}); followed by *I. stoloniferum* (588.33 µmol m^{-2} day^{-1}) and *A. curtipendula* (309.21 µmol m^{-2} day^{-1}) in April 2016 (Figure 2). N_2 fixation rates in dropped in July with the highest average rates observed in *H. splendens* (373.01 µmol m^{-2} day^{-1}); followed by *A. curtipendula* (121.44 µmol m^{-2} day^{-1}) and by *I. stoloniferum* (96.71 µmol m^{-2} day^{-1}). The lowest average nitrogenase activity rates were found in October 2016 for *A. curtipendula* and *I. stoloniferum* (49.36 and 18.47 µmol m^{-2} day^{-1}, respectively) and January 2017 for *H. splendens* (135.27 µmol m^{-2} day^{-1}).

For the canopy *I. stoloniferum* samples, N_2 fixation and metal concentrations were significantly different across peninsula and urban locations ($F_{1,206} = 256.34$, $p<0.001$) and across sampling seasons ($F_{3,206} = 29.69$, $p<0.001$) (Table 3a). There was a significant interaction found between location type and sampling season ($F_{3,206} = 4.00$, $p<0.001$). This would suggest that N_2 fixation rates and metal concentration amount found in the moss tissue is dependent on the season it was collected. Considering the location type term, the DISPER results show no significant differences in between-site variation ($F_{1,212}$=1.00, p= 0.324) and pairwise comparison also showed no significant differences among location types.

Significant proportions of the variability were explained by their first two PCs (Figure 3). Structure correlation coefficients between each variable and the first two PCs for urban *I. stoloniferum* canopy samples are presented in Table 1. The total amount of variance explained by the first two PCs were (86.27%): PC1 (60.57%) and PC2 (25.07%). Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn moss tissue concentrations are highly correlated with PC1; Cd concentrations and µmol m^{-2} day^{-1} are highly correlated with PC2. Vector arrows for the metal concentrations point towards the urban sampling points, suggesting that the metal concentration tends to be higher in urban *I. stoloniferum* canopy samples compared to peninsula *I. stoloniferum* canopy samples. The µmol m^{-2} day^{-1} arrow points towards to the peninsula sampling points, suggesting that biological N_2 fixation rates are higher in peninsula *I. stoloniferum* canopy samples compared to urban *I. stoloniferum* canopy samples.

Deposition of NO_3^--N and NH_4^+-N in peninsula and urban A. macrophyllum canopies as estimated using resin-based throughfall collectors are reported in Figure 4. Canopy height did not have a significant influence on resin-sorbed NO_3^--N and NH_4^+-N (p=0.11 and p=0.51, respectively), therefore an average deposition rate was used per location. Higher rates of wet NH_4^+-N deposition were observed in the Hoh Rainforest canopy (p<0.1) compared to urban canopies in Seattle, WA. No significant differences were observed across peninsula and urban canopies for annual wet NO_3^--N deposition rates.

Discussion

Measurable rates of N_2 fixation were recorded in moss communities of A. *macrophyllum* canopies across the sampling area in Western WA. Rates of N_2 fixation in canopy mosses were found to be dependent on sampling season and location. Average N_2 fixation rates for *I. stoloniferum* reached peak levels in spring 2016 for both peninsula and urban samples, 588.34 and 179.02 µmol m^{-2} day^{-1}, respectively. Rates dropped off during summer 2016 (96.71 and 53.99 µmol m^{-2} day^{-1}) and reached the lowest average levels in fall 2016 (18.47 and 5.29 µmol m^{-2} day^{-1}) for peninsula and urban samples, respectively. There was a slight increase in N_2 fixation rates in winter 2017 in both sampling locations (28.18 and 20.72 µmol m^{-2} day^{-1}).

Abiotic factors such as temperature and light have been studied extensively and are known to influence N_2 fixation rates in ground floor mosses (Alexander et al., 1973; Basilier et al., 1978; Davey, 1983; Meeks, 1998; Zielke et al., 2002; Gentili et al., 2005). Several studies have found that temperature is a stronger driver for N_2 fixation than light (Chapin, 1991; Lennihan et al., 1994; Liengen & Olsen, 1997; Solheim et al., 2002; Gundale et al., 2009). Gentili et al. (2005) found that N_2 fixation in feather mosses reached a peak between 13°C -22 °C. Temperature data was recorded at three heights within the canopy of sample trees, but no significant difference in temperature across canopy height was observed (p=0.75). Figure 5 shows seasonal changes in canopy temperature across the Hoh Rainforest and Seattle sampling sites. An average daily temperature on the date of collection was compared to N_2 fixation rates across all four sampling seasons and locations (Figure 6). The highest average N_2 fixation rates were observed under temperatures greater than 17°C, which occurred in April 2016. The lowest N_2 fixation rates, observed during October 2016, did not correspond to the lowest average daily temperature. It is possible that total precipitation during the month of October, in addition to cooler daily temperatures, influenced N_2 fixation rates in canopy *I. stoloniferum* samples. There was a sharp increase in total precipitation (cm) in October (Figure 7) for both the Hoh Rainforest and Seattle locations (50.05cm and 25.53cm, respectively). Several studies have observed a positive relationship between rainfall frequency and N_2 fixation rates in arctic and boreal ecosystems (Zielke et al., 2002; Gundale et al., 2009; Jackson et al., 2011; Gundale et al., 2012; Rousk et al., 2014). It is possible that the hydrological status in the samples collected in October 2016 exceeded the optimum moisture requirement to facilitate N_2 fixation.

Lindo and Whiteley (2011) provided the first study to confirm the presence of N_2 fixation in canopy moss communities in the temperate rainforest on Vancouver Island, British Columbia, Canada. Canopy fixation rates associated old-growth Sitka spruce (*Picea sitchensis* (Bong) Carr) stands are estimated at 0.5 kg N ha^{-1} yr^{-1}. Annual kg N ha^{-1} yr^{-1} associated with canopy mosses in this study is intermediate compared to forest floor levels reported in late successional forests in the boreal (2.1 kg N ha^{-1} yr^{-1}) (DeLuca et al., 2002). The lower annual N_2 fixation rates in Seattle is likely a result of the limited biomass and higher heavy metal concentrations in urban *A. macrophyllum* stands compared to the Hoh Rainforest. However, the values reported in this study may be a conservative estimate of stand level N-inputs as it does not take not take branch density on each tree into consideration.

This study did not identify all potential cyanobacteria genera that may be living epiphytically on canopy bryophytes in *A. macrophyllum* trees. In the boreal, four cyanobacteria species (*Nostoc*, *Stigonema*, *Calothrix*, and *Cylindrospermum*) are known colonizers on forest floor *P. schreberi* (DeLuca et al., 2007; Ininbergs et al., 2011). Lindo and Whiteley (2011) found a linear relationship between density of *Scytonema* cells on moss and N_2 fixation in the canopy of Sitka spruce (*Picea sitchensis* (Bong) Carr.) trees. As *Scytonema* are predominately aerially dispersed (Komarek et al., 2003), it is possible *Scytonema* would be the dominant genera in *A. macrophyllum* canopies. Future research is needed to identify the diversity of cyanobacteria living epiphytically on canopy bryophytes in old growth *A. macrophyllum* canopies.

In boreal regions, biological N_2 fixation associated with *Pleurozium schreberi* (Brid.) Mitt. is a primary source of ecosystem N at 1.7 kg N ha^{-1} yr^{-1} (DeLuca et al., 2002). However it remains unclear how N_2 fixed through cyanobacteria associations enters the soil for plant uptake, but it appears to be a tortuous pathway (DeLuca et al., unpublished data). As mosses are efficient in retaining nutrients (Oechel and Van Cleve, 1986; Aldous, 2002), the rate at which fixed N is transferred to the soil and plants is expected to be slow (Rousk et al., 2014). In *A. macrophyllum* canopies, fixed N may enter the environment by accumulating in canopy soil. Studies conducted in *A. macrophyllum* canopies along the Queets River, located on the Olympic Peninsula, found a C/N ratio of 22 for canopy soils and NH_4^+ to be the dominant form of extractable N (Haristoy et al., 2014). This would suggest that *A. macrophyllum* canopy soils have a higher potential for mineralization and thus increased N availability for plants, host tree adventitious roots, and microorganisms (Nadkarni, 1994; Brady and Weil 2000; Lindo & Winchester, 2007; Berg & McClaugherty, 2008). Another potential pathway for fixed N from canopy bryophytes is being deposited to the forest floor as epiphytic litterfall. As epiphytic litterfall decomposes on the forest floor it can be available for terrestrially root plants (Nadkarni & Matelson, 1992). Tejo et al. (2015) found a total of 495 kg ha^{-1} yr^{-1} input from bryophyte litterfall in the Queets River Watershed. It is possible that the input of nutrients from bryophyte litterfall is providing of a much-needed source of N in N-limited PNW forests.

Nitrogen deposition is known to negativity influence bryophyte growth and N_2 fixation rates (Davies et al, 2007; DeLuca et al., 2008; Gundale et al., 2011). Despite being considered one of the cleanest air quality regions in the U.S., the PNW has experienced an increase in total wet inorganic-N deposition since 1985 (NADP/NTN, 2015). Several studies have observed shifts in lichen community composition as a result of the adverse effects of increasing N pollution throughout the region (Fenn et al., 2003; Geiser & Neitlich, 2007). Canopy ionic resin lysimeters captured wet inorganic-N across the four studies sites in 2016 and were compared to bulk wet inorganic-N levels measured at the four NADP/NTN monitoring stations across Western WA (Table2). There was a significant increase in NH_4^+-N deposition measured in 2016 compared to 2015 at the NADP WA-14 Hoh Rainforest monitoring site (Figure 8). Elevated rates of NH_4^+-N deposition were also measured in the canopy lysimeters. The elevated rates of NH_4^+-N deposition in 2016 may be attributed to higher rainfall inputs in spring as well as the influence of wildfire ash inputs on the Western side of the Olympic peninsula in the summer of 2015. NH_4^+ deposition has been shown to be more impactful than NO_3^- at decreasing biodiversity of sensitive species (Erisman et al., 2007), which is of particular concern for canopy epiphytes. The transition of the dominant N species from NO_3^- to NH_4^+ deposition in the PNW (Li et al., 2016) fit current projections for ammonia (NH_3^+) emission growth and increasing influences from wildfire in the western U.S. (Ellis et al., 2013). The increase in emissions throughout the region would suggest NH_4^+ deposition will continue to rise and have the potential to negatively impact canopy epiphyte ecosystem interactions.

As bryophytes lack roots, they primarily absorb dissolved nutrients and particulate pollutants from the atmosphere (Aboal et al., 2011) although some nutrients are thought to be collected via fungal associates (Carleton & Read, 1991) and capillary movement of nutrients upward from the humus layer (Ayres et al., 2006). They exhibit high cation exchange capacity and can generate acidity through proton release from exchange sites (Spearing, 1972), which may inhibit microbial populations and thus limited N_2 fixation capacity (Tyler, 1990; Pearson et al., 2000; Basiliko & Yavitt, 2001). In this study, *I. stoloniferum* metal tissue concentrations were found to be higher in the Seattle canopies (Table 3) and N_2 fixation rates were significantly higher across all seasons in the Hoh Rainforest canopies in spite of slightly higher rates of N deposition in this area. Nitrogen deposition rates are low enough across this region to not directly inhibit moss N_2 fixation (Gundale et al., 2011). This would suggest that N_2 fixation is not only a function of the seasonal weather influences discussed above, but perhaps strongly influenced by metal concentrations in the moss tissue. The expected lower level of metal deposition in the Hoh Rainforest samples is likely due to lower population density and lower daily traffic counts. As the Puget Sound Region is expected to experience significant population and transportation growth by 2040 (Puget Sound Regional Council, 2014), it is likely urban canopies will continue to experience higher rates of metal deposition and decreasing rates of biological N_2 fixation.

Conclusion

The forest canopies of the world play an important role in the regulation and cycling of nutrients. Increasing atmospheric pollution associated population increases and transportation growth in the PNW threatens to uncouple the intricate ecosystem services canopy epiphytes provide. Our study is the first to assess the effects of urbanization on seasonal N_2 fixation associated with canopy bryophytes. The results suggest that N_2 fixation associated with canopy bryophytes are sensitive to seasonal changes in temperature and precipitation, and the bioaccumulation of anthropogenic heavy metals in bryophyte tissue. Nitrogen deposition did not appear to have a noted impact on N_2 fixation across location types. Changes in temperature and precipitation patterns associated with climate change will likely affect ecosystem N_2 fixation inputs through the bryophyte-cyanobacteria pathway. In order to better understand the ecological impacts of climate change on canopy bryophytes an increase in the number of sampling sites across Western WA and an increase sampling frequency across seasons would be appropriate.

Tables:

Table 1: Structure correlation coefficients between measured parameters and the first two principal components (PC1 and PC2) for I. stoloniferum for average N_2 fixation rates and metal concentrations across urban and peninsula A. macrophyllum canopies.

Principle Component	**PC1**	**PC2**
(% Variance explained, significance value)	**60.57%, p<0.001**	**25.07%, p<0.05**
umol m^{-2} d^{-1}		0.899
Cd		0.978
Cr	-0.894	
Cu	-0.967	
Fe	-0.918	
K	-0.948	
Mg	-0.903	
Mn	-0.999	
Ni	-0.887	
Pb	-0.894	
Sr	-0.766	
Ti	-0.839	
Zn	-0.983	

Table 2: Comparison of wet inorganic-N deposition across four sampling sites instrumented with canopy resin lysimeters and four National Atmospheric Deposition Program National Trend Network sites in Washington State, USA.

Location	Type	NO_3^--N	NH_4^+-N
Hoh Rainforest	Resin Lysimeter	1.92	2.61
Interlaken Park	Resin Lysimeter	2.28	1.03
Ravenna Park	Resin Lysimeter	1.87	0.57
Seward Park	Resin Lysimeter	2.83	1.14
NADP WA-14 Station (Olympic NP)	Rainfall Collector	2.97	3.24
NADP WA-19 Station (North Cascades NP)	Rainfall Collector	3.36	1.2
NADP WA-99 Station (Mt. Rainier NP)	Rainfall Collector	2.81	1.47
NADP WA-98 Station (Columbia River Gorge)	Rainfall Collector	2.55	1.37

Table 3: Element descriptive statistics in mg kg^{-1} for (a) Hoh Rainforest metal concentrations; and (b) Seattle metal concentrations from canopy I. stoloniferum samples.

(a)

Element	Minimum	Maximum	Mean	Median	Standard Deviation	Fisher-Pearson Skewness Coefficient	# Samples below detection limit
Cd	BD	3.44	0.17	0.07	0.39	0.76	41
Cr	0.08	1.93	0.65	0.49	0.41	1.12	0
Cu	0.52	11.97	3.52	3.21	1.98	0.47	0
Fe	33.79	316.27	95.71	86.89	46.80	0.56	0
K	2373.22	10170.00	5408.02	5108.82	1277.03	0.70	0
Mg	1253.71	3721.43	1969.96	1902.66	450.61	0.44	0
Mn	16.10	373.83	93.61	76.66	67.72	0.75	0
Ni	BD	4.28	1.42	1.35	0.87	0.26	11
Pb	BD	5.79	0.86	0.68	0.80	0.69	5
Sr	11.42	149.17	51.19	46.08	26.59	0.57	0
Ti	1.39	16.99	5.43	4.94	2.82	0.51	0
Zn	17.62	162.67	44.47	38.45	22.67	0.79	0

(b)

Element	Minimum	Maximum	Mean	Median	Standard Deviation	Fisher-Pearson Skewness Coefficient	# Samples below detection limit
Cd	BD	0.5	0.17	0.18	0.13	-0.23	17
Cr	0.35	5.91	2.49	2.32	1.09	0.46	0
Cu	1.78	21.71	12.05	11.48	3.90	0.44	0
Fe	78.11	1396.46	594.70	565.81	281.98	0.31	0
K	2289.71	12482.35	8243.29	8155.06	1999.65	0.13	0
Mg	809.14	4415.38	2390.33	2317.81	624.51	0.35	0
Mn	9.62	290.46	110.40	100.45	54.14	0.55	0
Ni	0.34	98.91	4.04	2.73	9.43	0.42	0
Pb	0.62	64.06	12.34	9.41	11.11	0.79	0
Sr	24.15	300.00	122.89	116.57	49.05	0.39	0
Ti	3.58	60.51	27.09	25.59	13.32	0.34	0
Zn	15.42	191.82	79.46	76.12	30.58	0.33	0

Figures:

Figure 1: Nitrogen fixation rates as measured by acetylene reduction for *I. stoloniferum* samples collected across Olympic peninsula and Seattle urban A. macropyllum canopies in western WA.

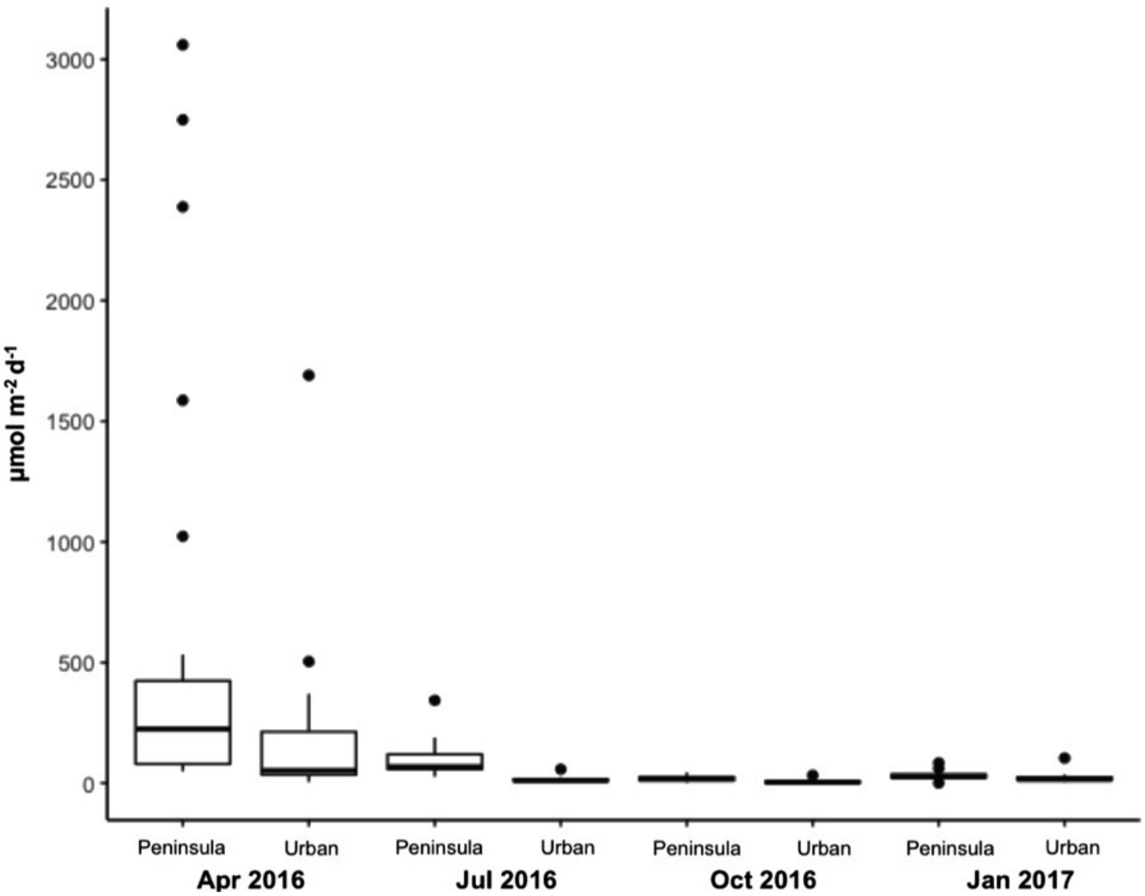
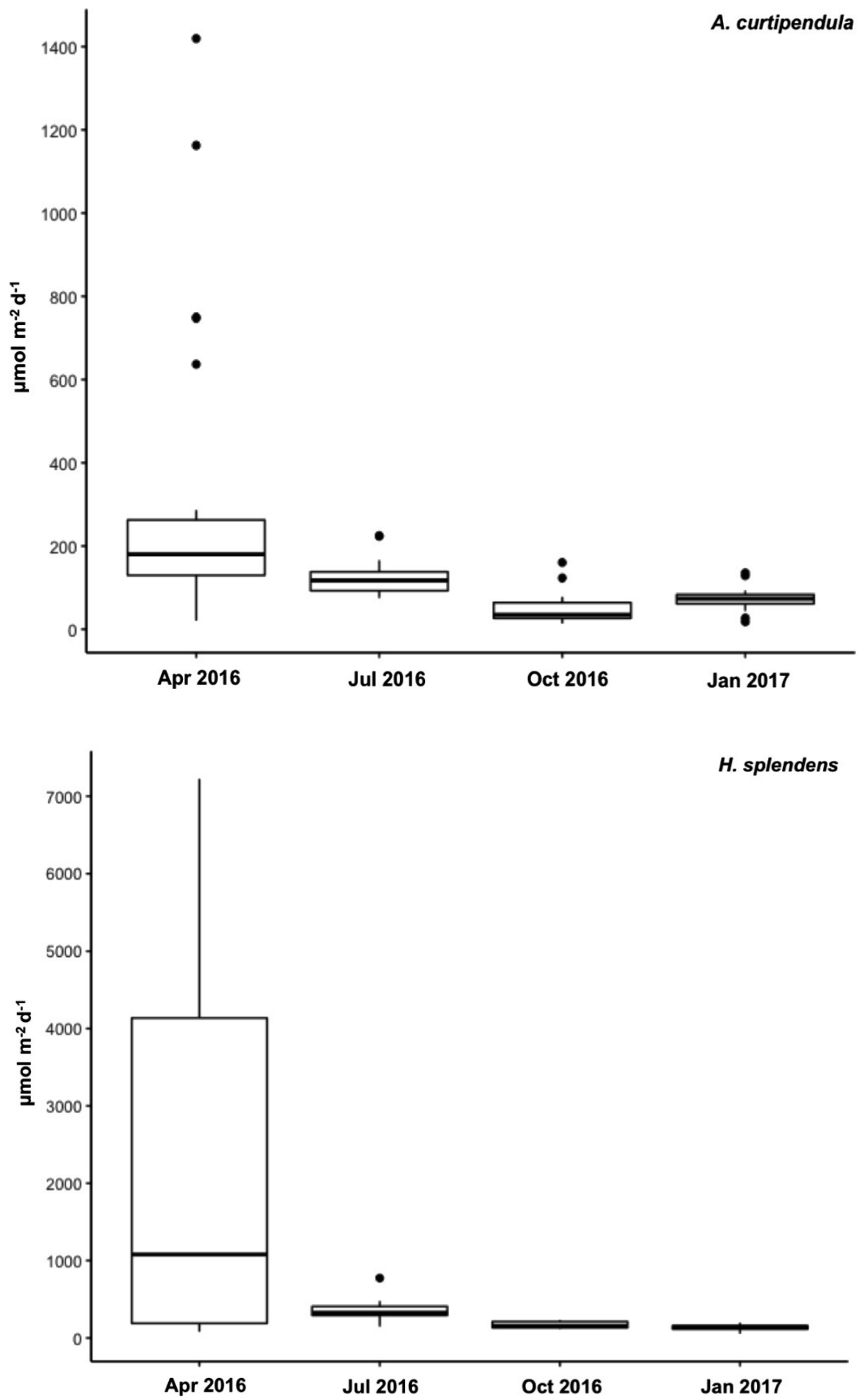


Figure 2: Seasonal N_2 fixation rates across one A. macrophyllum stand on the Olympic Peninsula for (a) *A. curtipendula*, (b) *H. splendens*, and (c) *I. stoloniferum*.



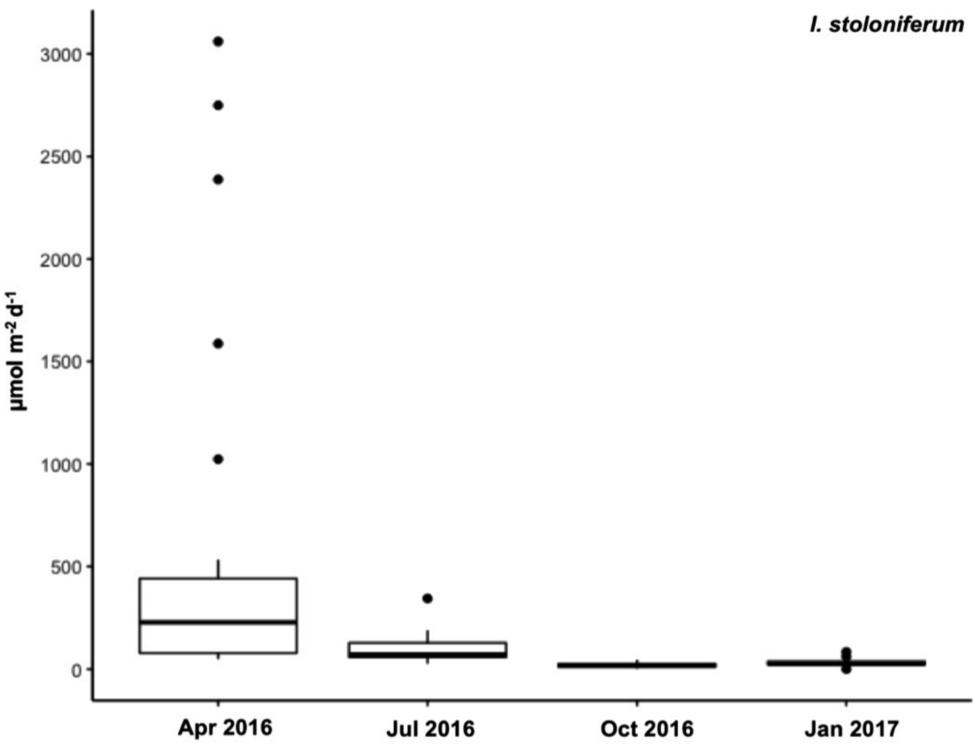


Figure 3: Principle component analysis (PCA) ordination of seasonal *I. stoloniferum* samples collected from the Olympic Peninsula and Seattle urban and rural forests in western WA.

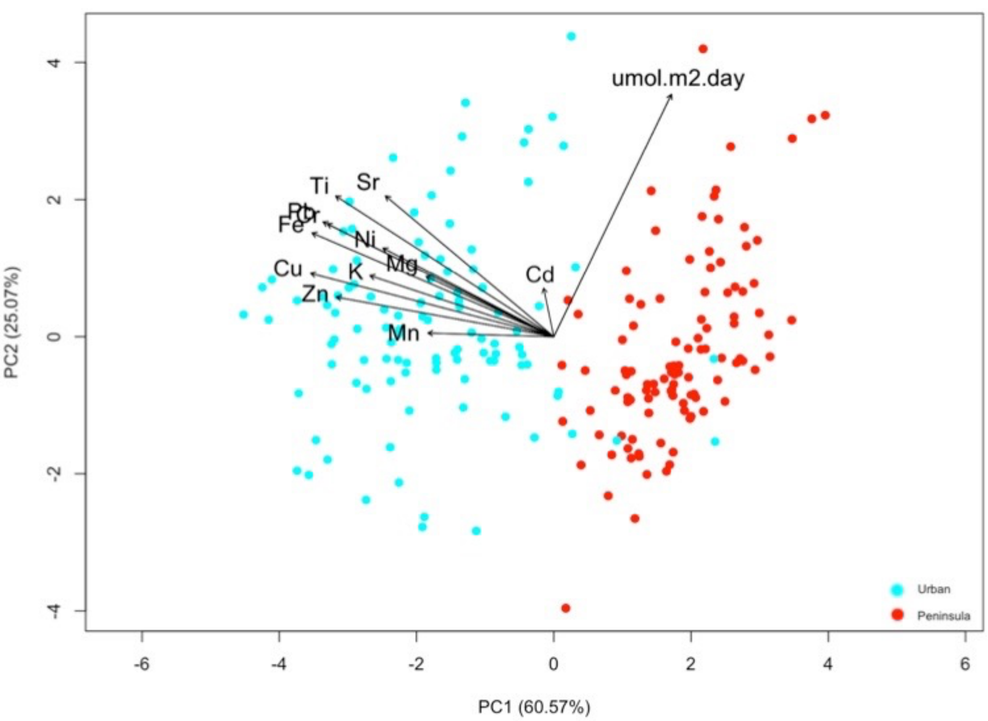


Figure 4: Average NO_3^--N and NH_4^+-N wet deposition rates collected using resin lysimeters instrumented for 12-months in *A. macrophyllum* canopies in the Hoh Rainforest, Olympic Peninsula and three city parks in Seattle, WA.

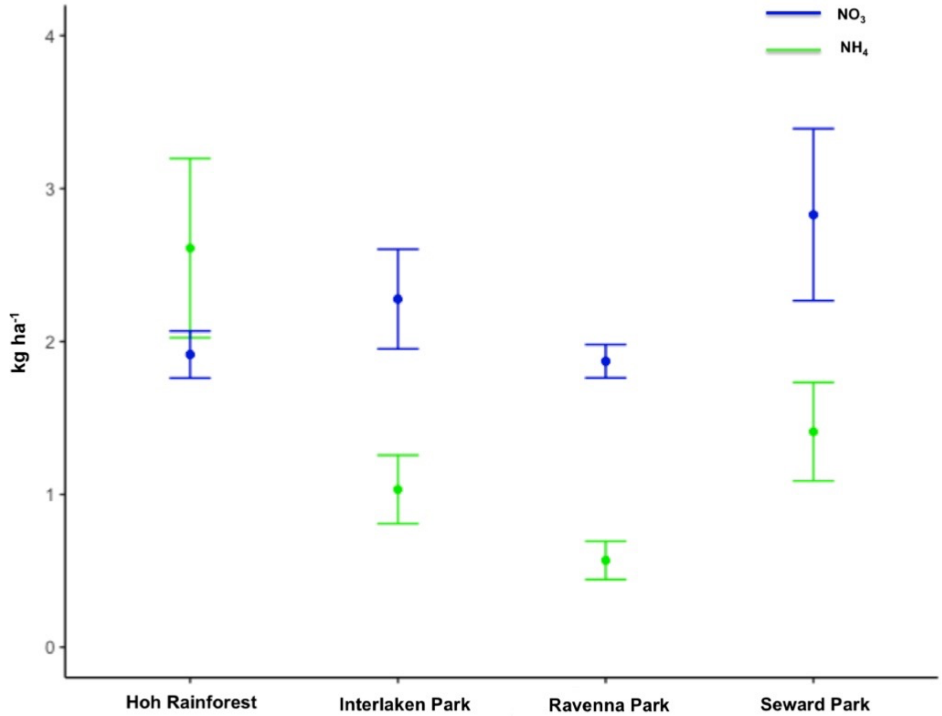


Figure 5: Average daily temperature data gathered using HOBO monitors installed in A. macrophyllum canopies in the Hoh Rainforest and Seattle, WA for the 2016-2017 sampling period.

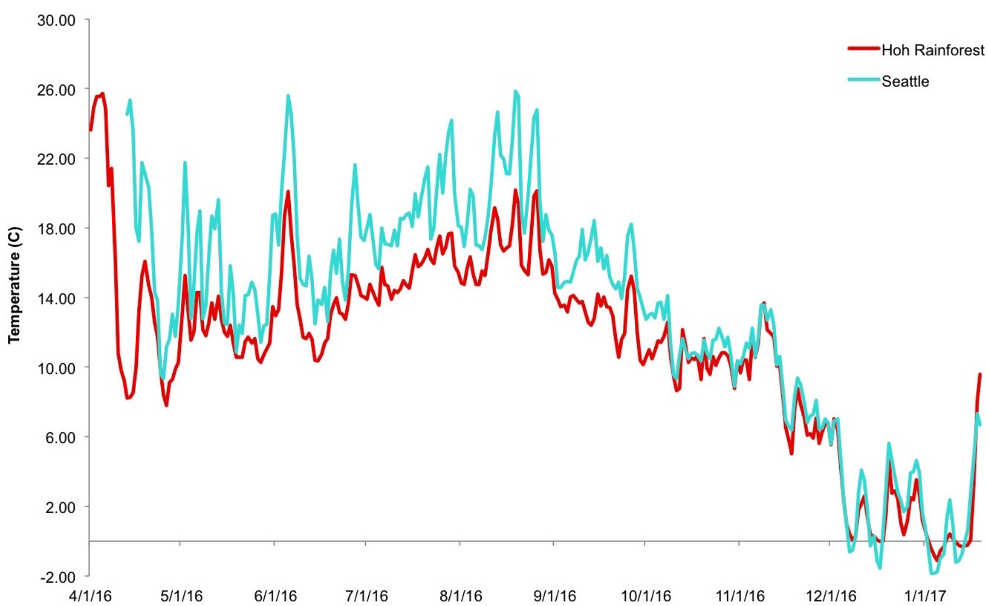
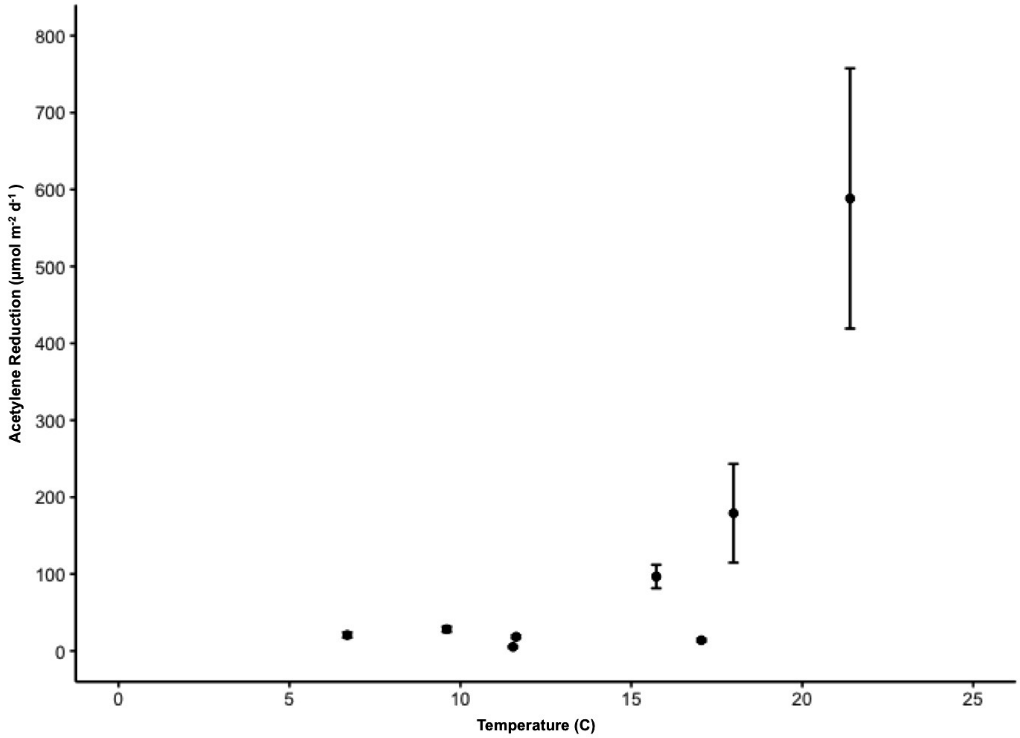


Figure 6: Relationship between the seasonal mean (+/- SE) acetylene reduction rates across Hoh Rainforest and Seattle *I. stoloniferum* samples and temperature.



References:

Aboal, J. R., Pérez-Llamazares, A., Carballeira, A., Giordano, S., & Fernández, J. A., 2011. Should moss samples used as biomonitors of atmospheric contamination be washed?. Atmospheric environment, 45(37), 6837-6840.

Ackermann, K., Zackrisson, O., Rousk, J., Jones, D. L., & DeLuca, T. H., 2012. N2 fixation in feather mosses is a sensitive indicator of N deposition in boreal forests. Ecosystems, 15(6), 986-998.

Aldous, A. R., 2002. Nitrogen translocation in Sphagnum mosses: effects of atmospheric nitrogen deposition. New Phytologist, 156(2), 241-253.

Alexander, V., & Schell, D. M., 1973. Seasonal and spatial variation of nitrogen fixation in the Barrow, Alaska, tundra. Arctic and Alpine Research, 77-88.

Ayres, E., Van der Wal, R., Sommerkorn, M., & Bardgett, R. D., 2006. Direct uptake of soil nitrogen by mosses. Biology Letters, 2(2), 286-288.

Basilier, K., Granhall, U., & Stenström, T. A. (1978). Nitrogen fixation in wet minerotrophic moss communities of a subarctic mire. Oikos, 236-246.

Basiliko, N., & Yavitt, J. B., 2001. Influence of Ni, Co, Fe, and Na additions on methane production in Sphagnum-dominated Northern American peatlands. Biogeochemistry, 52(2), 133-153.

Bates, J. W., 1992. Mineral nutrient acquisition and retention by bryophytes. Journal of Bryology, 17(2), 223-240.

Bengtson, C., Folkeson, L., & Göransson, A., 1982. Growth reduction and branching frequency in Hylocomium splendens near a foundry emitting copper and zinc. Lindbergia, 129-138.

Berg, B., & McClaugherty, C., 2008. Plant litter. Decomposition, humus formation, carbon sequestration. 2nd Ed Springer.

Berg, T., Røyset, O., & Steinnes, E., 1995. Moss (Hylocomium splendens) used as biomonitor of atmospheric trace element deposition: estimation of uptake efficiencies. Atmospheric Environment, 29(3), 353-360.

Berg, T., & Steinnes, E., 1997. Recent trends in atmospheric deposition of trace elements in Norway as evident from the 1995 moss survey. Science of the Total Environment, 208(3), 197-206.

Binkley, D., & Graham, R. L., 1981. Biomass, production, and nutrient cycling of mosses in an old-growth Douglas-fir forest. Ecology, 62(5), 1387-1389.

Brady, N. C., & Weil, R. R., 2000. Elements of the nature and properties of soils (pp. 463-471). Upper Saddle River, NJ, USA:: Prentice Hall.

Carleton, T. J., & Read, D. J., 1991. Ectomycorrhizas and nutrient transfer in conifer–feather moss ecosystems. Canadian Journal of Botany, 69(4), 778-785.

Chapin, F. S., 1991. Integrated responses of plants to stress. BioScience, 41(1), 29-36.

Cornelissen, J. H. C., S. I. Land, N. A. Soudzilovskaia, & H. J. During, 2007. Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. Annals of Botany 99: 987-1001.

Davey, A., & Marchant, H. J., 1983. Seasonal variation in nitrogen fixation by Nostoc commune Vaucher at the Vestfold Hills, Antarctica. Phycologia, 22(4), 377-385.

DeLuca, T. H., Zackrisson, O., Nilsson, M. C., & Sellstedt, A., 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature, 419(6910), 917-920.

DeLuca, T. H., Zackrisson, O., Gundale, M. J., & Nilsson, M. C., 2008. Ecosystem feedbacks and nitrogen fixation in boreal forests. Science, 320(5880), 1181-1181.

Ellis, R.A., Jacob, D.J., Sulprizio, M.P., Zhang, L., Holmes, C.D., Schichtel, B.A., Blett, T., Porter, E., Pardo, L.H. and Lynch, J.A., 2013. Present and future nitrogen deposition to national parks in the United States: critical load exceedances. Atmospheric Chemistry and Physics, 13(17), 9083-9095.

Erisman, J. W., Bleeker, A., Galloway, J., & Sutton, M. S., 2007. Reduced nitrogen in ecology and the environment. Environmental Pollution, 150(1), 140-149.

Fenn, M.E., Baron, J.S., Allen, E.B., Rueth, H.M., Nydick, K.R., Geiser, L., Bowman, W.D., Sickman, J.O., Meixner, T., Johnson, D.W. and Neitlich, P., 2003. Ecological effects of nitrogen deposition in the western United States. BioScience, 53(4), 404-420.

Geiser, L. H., & Neitlich, P. N., 2007. Air pollution and climate gradients in western Oregon and Washington indicated by epiphytic macrolichens. Environmental Pollution, 145(1), 203-218.

Gentili, F., Nilsson, M. C., Zackrisson, O., DeLuca, T. H., & Sellstedt, A., 2005. Physiological and molecular diversity of feather moss associative N2-fixing cyanobacteria. Journal of experimental botany, 56(422), 3121-3127.

Gjengedal, E., & Steinnes, E., 1990. Uptake of metal ions in moss from artificial precipitation. Environmental Monitoring and Assessment, 14(1), 77-87.

González, A. G., & Pokrovsky, O. S., 2014. Metal adsorption on mosses: toward a universal adsorption model. Journal of colloid and interface science, 415, 169-178.

Gundale, M. J., Gustafsson, H., & Nilsson, M. C., 2009. The sensitivity of nitrogen fixation by a feathermoss–cyanobacteria association to litter and moisture variability in young and old boreal forests. Canadian Journal of Forest Research, 39(12), 2542-2549.

Gundale, M. J., Deluca, T. H., & Nordin, A., 2011. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. Global Change Biology, 17(8), 2743-2753.

Gundale, M. J., Wardle, D. A., & Nilsson, M. C., 2012. The effect of altered macroclimate on N-fixation by boreal feather mosses. Biology letters, rsbl20120429.

Haristoy, C. T., Zabowski, D., & Nadkarni, N., 2014. Canopy soils of Sitka spruce and bigleaf maple in the Queets River Watershed, Washington. Soil Science Society of America Journal, 78(S1), S118-S124.

Harmens, H., Norris, D. A., Koerber, G. R., Buse, A., Steinnes, E., & Rühling, Å., 2008. Temporal trends (1990–2000) in the concentration of cadmium, lead and mercury in mosses across Europe. Environmental Pollution, 151(2), 368-376.

Ininbergs, K., Bay, G., Rasmussen, U., Wardle, D. A., & Nilsson, M. C., 2011. Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. New Phytologist, 192(2), 507-517.

Jackson, B. G., Martin, P., Nilsson, M. C., & Wardle, D. A., 2011. Response of feather moss associated N2 fixation and litter decomposition to variations in simulated rainfall intensity and frequency. Oikos, 120(4), 570-581.

Kenkel, N. C., & Bradfield, G. E., 1986. Epiphytic vegetation on Acer macrophyllum: a multivariate study of species-habitat relationships. Plant Ecology, 68(1), 43-53.

Klopatek, J. M., Barry, M. J., & Johnson, D. W., 2006. Potential canopy interception of nitrogen in the Pacific Northwest, USA. Forest ecology and management, 234(1), 344-354.

Komárek, J., Kling, H., & Komárková, J., 2003. Filamentous cyanobacteria. Freshwater Algae of North America, 4, 117-196.

Legendre, P. & Legendre, L., 1998. Numerical Ecology, Elsevier.

Lennihan, R., Chapin, D. M., & Dickson, L. G., 1994. Nitrogen fixation and photosynthesis in high arctic forms of Nostoc commune. Canadian Journal of Botany, 72(7), 940-945.

Li, Y., Schichtel, B.A., Walker, J.T., Schwede, D.B., Chen, X., Lehmann, C.M., Puchalski, M.A., Gay, D.A. and Collett, J.L., 2016. Increasing importance of deposition of reduced nitrogen in the United States. Proceedings of the National Academy of Sciences, 201525736.

Liengen, T., & Olsen, R. A., 1997. Seasonal and site-specific variations in nitrogen fixation in a high arctic area, Ny-Ålesund, Spitsbergen. Canadian Journal of Microbiology, 43(8), 759-769.

Lindo, Z., & Winchester, N. N., 2007. Oribatid mite communities and foliar litter decomposition in canopy suspended soils and forest floor habitats of western redcedar forests, Vancouver Island, Canada. Soil Biology and Biochemistry, 39(11), 2957-2966.

Lindo, Z., & Whiteley, J. A., 2011. Old trees contribute bio-available nitrogen through canopy bryophytes. Plant and soil, 342(1-2), 141-148.

Meeks, J. C., 1998. Symbiosis between Nitrogen-Fixing Cyanobacteria and Plants The establishment of symbiosis causes dramatic morphological and physiological changes in the cyanobacterium. BioScience, 48(4), 266-276.

K. M., Espey, M. G., & Wink, D. A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide, 5(1), 62-71.

Mulvaney, R. L., 1996. Nitrogen—inorganic forms. Methods of soil analysis part 3—Chemical methods, (methodsofsoilan3), 1123-1184.

Nadkarni, N. M., 1984. Biomass and mineral capital of epiphytes in an Acer macrophyllum community of a temperate moist coniferous forest, Olympic Peninsula, Washington State. Canadian Journal of Botany, 62(11), 2223-2228.

Nadkarni, N. M., & Matelson, T. J., 1992. Biomass and Nutrient Dynamics of Fine Litter of Terrestrially Rooted Material in an Neotropical Montane Forest, Costa Rica. Biotropica, 113-120.

NADP/NTN, 2015. National Atmospheric Deposition Program (NRSP-3)/ National Trends Network. Program Office, Illinois State Water Survery, Champaign, IL. See animated isopleth maps. <http://nadp.sws.uiuc.edu>.

Oechel, W. C., & Van Cleve, K., 1986. The role of bryophytes in nutrient cycling in the taiga. In Forest ecosystems in the Alaskan taiga (pp. 121-137). Springer New York.

Pearson, J., Wells, D. M., Seller, K. J., Bennett, A., Soares, A., Woodall, J., & Ingrouille, M. J., 2000. Traffic exposure increases natural 15N and heavy metal concentrations in mosses. New phytologist, 147(2), 317-326.

Pike, L. H., Rydell, R. A., & Denison, W. C., 1977. A 400-year-old Douglas fir tree and its epiphytes: biomass, surface area, and their distributions. Canadian Journal of Forest Research, 7(4), 680-699.

Pott, U., & Turpin, D. H., 1998. Assessment of atmospheric heavy metals by moss monitoring with Isothecium stoloniferum Brid. in the Fraser Valley, BC, Canada. Water, Air, & Soil Pollution, 101(1), 25-44.

Proctor, M., 2001. Patterns of desiccation tolerance and recovery in bryophytes. Plant Growth Regulation, 35(2), 147-156.

Puget Sound Regional Council, 2014. Transportation 2040: toward a sustainable transportation sesystem (Update). PSR Council, Seattle, WA.

Pypker, T. G., Unsworth, M. H., & Bond, B. J., 2006. The role of epiphytes in rainfall interception by forests in the Pacific Northwest. II. Field measurements at the branch and canopy scale. Canadian Journal of Forest Research, 36(4), 819-832.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raymond, B.A. & U. Pott. 2003. Atmospheric contaminants in biota of forested watersheds near Vancouver, BC: Environment Canada. Georgia Basin Ecosystem Initiative.

Raymond, B. A., & Bassingthwaighte, T., 2010. Measuring nitrogen and sulphur deposition in the Georgia Basin, British Columbia, using lichens and moss. Journal of Limnology, 69(1s), 22-32.

Reimann, C., Niskavaara, H., Kashulina, G., Filzmoser, P., Boyd, R., Volden, T., Tomilina, O. and Bogatyrev, I., 2001. Critical remarks on the use of terrestrial moss (Hylocomium splendens and Pleurozium schreberi) for monitoring of airborne pollution. Environmental Pollution, 113(1), 41-57.

Rousk, K., Jones, D. L., & DeLuca, T. H., 2014. Exposure to nitrogen does not eliminate N2 fixation in the feather moss Pleurozium schreberi (Brid.) Mitt. Plant and soil, 374(1-2), 513-521.

Schöllhorn, R., & Burris, R. H., 1967. Acetylene as a competitive inhibitor of N-2 fixation. Proceedings of the National Academy of Sciences, 58(1), 213-216.

Solheim, B., Johanson, U., Callaghan, T. V., Lee, J. A., Gwynn-Jones, D., & Björn, L. O., 2002. The nitrogen fixation potential of arctic cryptogram species is influenced by enhanced UV-B radiation. Oecologia, 133(1), 90-93.

Spearing, A. M., 1972. Cation-exchange capacity and galacturonic acid content of several species of Sphagnum in Sandy Ridge Bog, central New York State. Bryologist, 154-158.

Susfalk, R. B., & Johnson, D. W., 2002. Ion exchange resin based soil solution lysimeters and snowmelt solution collectors. Communications in Soil Science and Plant Analysis, 33(7-8), 1261-1275.

Tejo, C. F., Zabowski, D., & Nadkarni, N. M., 2015. Total and epiphytic litter under the canopy of Acer macrophyllum in an old-growth temperate rainforest, Washington State, USA. Canadian Journal of Forest Research, 45(11), 1654-1661.

Tyler, G., 1990. Bryophytes and heavy metals: a literature review. Botanical journal of the Linnean Society, 104(1-3), 231-253.

Zackrisson, O., DeLuca, T. H., Nilsson, M. C., Sellstedt, A., & Berglund, L. M., 2004. Nitrogen fixation increases with successional age in boreal forests. Ecology, 85(12), 3327-3334.

Zackrisson, O., DeLuca, T. H., Gentili, F., Sellstedt, A., & Jäderlund, A., 2009. Nitrogen fixation in mixed Hylocomium splendens moss communities. Oecologia, 160(2), 309.

Zielke, M., Ekker, A. S., Olsen, R. A., Spjelkavik, S., & Solheim, B., 2002. The influence of abiotic factors on biological nitrogen fixation in different types of vegetation in the High Arctic, Svalbard. Arctic, Antarctic, and Alpine Research, 293-299.

Chapter 3:
Bioaccumulation of trace metal deposition in mosses in Western Washington, USA

Abstract

Heavy metal pollution across Western Washington, USA was assessed in 2016 and 2017 using *Isothecium stoloniferum (Brid.)* and *Kindbergia praelonga (Hedw.) Ochyra*. We used an intensive, vertically stratified sampling approach in *Acer macrophyllum* canopies in the Hoh Rainforest on the Olympic Peninsula, WA and in Seattle, WA to collect 214 samples of *I. stoloniferum*. An extensive, ground-based sampling approach was used across an urban-to-wildland gradient to collect 59 *K. praelonga* samples. Intensive samples were collected four times (April, July, and October of 2016 and in January 2017) and extensive samples three times (April, July, and October 2016) to assess seasonal differences in metal concentrations across sampling locations. A total of 273 moss samples were analyzed for Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn. Elevated concentrations of all of these elements were found in both intensive and extensive samples collected in Seattle across all collection seasons. Permutational multivariate analysis of variance (perMANOVA) revealed a significant effect of sampling location and seasonal for both intensive and extensive samples. Metal deposition in and around Seattle appears to be primarily derived from the transportation sector.

Keywords: air quality; biomonitoring; bryophytes; heavy metals; traffic pollution

Introduction

Elevated metal deposition within in urban areas of the Pacific Northwest have been recorded in Portland and Seattle; however, until recently, few studies have used mosses in this region as indicators of metal pollution. There is a long history of using moss (Ruhling & Tyler, 1968; Pott & Turpin, 1996; Reimann et al., 2001; Aboal et al., 2010; Ćujić et al, 2014; Donovan et al., 2016) and lichen (McCune, 2000; Geiser & Neitlich, 2007) as bioindicators of air quality and atmospheric pollution. Unlike vascular plants, moss lack roots and absorb most nutrients from the atmospheric (Bates, 1992). Moss leaves are only one cell-layer thick, lack a protective epidermis and demonstrate ion exchange properties, all of which allows for the absorption of water, organic compounds and inorganic ions that are deposited on their surfaces (Gjengedal & Steinnes, 1990; Tyler et al., 1990; Aboal et al., 2011; Gonzalez & Pokrovsky, 2013).

Hylocomium splendens and *Pleurozium schrbeberi* are two moss species that have been successfully used as bioindicators of pollution deposition in northern Europe (Berg et al, 1995; Berg & Steinnes, 1997; Reimann et al., 2000). These studies show a highly significant positive correlation between moss tissue metal concentrations and wet deposition values. The growth rate for *H. splendens* is highly constant allowing the moss to be used to assess changes in metal concentrations over time (Berg et al., 1995). In most other bioindicator species, the time period represented for pollutant accumulation is unknown, whereas for mosses it consistently ranges from several months to a maximum of three years. Generally, the top two-thirds of the shoot are used in analyses of bioaccumulation, which approximately represents up to three years of growth (Bargagli, et al., 2002; Schintu et al., 2005; Gatziolis et al., 2016). Because there is little recycling of metals from senescent tissue (Brown & Bates, 1990), the concentration of metals in the upper two-thirds of the moss shoots can be used to infer metal deposition over a three year or less time period.

The Pacific Northwest (PNW) is considered to have high air quality among the United States (NADP/NTN, 2015), in part due to a lack of coal-fired power plants and a small industrial base. However, rapid increases in total population and increased transportation growth threatens to decrease air quality within the region (Geiser & Neitlich, 2007). In the Puget Sound Region (PSR) alone, the population is expected to grow by roughly one million people from 2010 to 2040, which will in turn increase demand for travel throughout the region by 25% (Puget Sound Regional Council, 2014). As evidence of this increase, Seattle highways and roads were recently ranked the 10th most congested urban areas in the United States (INRIX, 2016).

This region-wide expansion in the transportation sector has introduced a new set of atmospheric pollutants to western Washington. Brake and tire attrition, as well as lubricant degradation produce high rates of metal deposition in highly congested areas (Garg et al., 2000; Apeagyei et al., 2011; Hulskotte et al., 2014). Brake bands and tires contain heavy metals, including chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb), strontium (Sr), titanium (Ti), and zinc (Zn), that are released with disintegration and can lead to adverse human health and wildlife effects (U.S. Government, 2001; Perrenoud et al., 2010; Denier van der Gon et al., 2013). These transportation pollutants as well as long-range atmospheric transports originating from Asia (Jaffe et al., 1999) have the potential to negatively impact ecosystem health by altering bryophyte communities (Davies et al., 2007; Aničić et al., 2009; Zvereva and Kozlov, 2011).

To date there have been no extensive or intensive assessment of metal deposition using moss as a bioaccumulator of metals in Washington State. We sought to quantify the extent and intensity of metal accumulation in urban and rural areas in western Washington using mosses as bioaccumulator of atmospheric heavy metal deposition. We hypothesized that: 1) moss tissue metal concentrations will be higher in samples collected from urban sites compared to suburban and rural peninsula sites; and 2) urban sites will experience higher annual average wet deposition rates of heavy metals.

Materials & Methods

2.1 Study Area & Design

We collected moss samples for metal analyses by using two approaches. One involved the use of an intensive, quantitative, vertically stratified sampling in *A. macrophyllum* canopies in the Hoh Rainforest on the Olympic Peninsula, WA, USA and in the urban center of Seattle, WA. The second involved an extensive, broad ranging sampling across an urban-to-wildland gradient in Western WA. We chose *A. macrophyllum* because it is widely distributed across the urban-to-wildland gradient and hosts abundant growth of a variety of moss species on its branches and trunk. The two sampling approaches are detailed below:

i. Intensive Approach:

   We collected samples from one stand located on Hoh River Trust land in the Hoh Rainforest (47.82°N, 124.20°W) and from three stands located in Seattle City Parks: Seward Park (47.55°N, 122.25°W), Interlaken Park (47.63°N, 122.31°W), and Ravenna Park (47.67°N, 122.30°W). Replicate moss samples were collected from a total of 18 *A. macrophyllum* trees: nine from the Hoh Rainforest, Olympic Peninsula, WA, USA, and nine from three city parks in Seattle, WA, USA over the course of four seasons (April, July and October 2016, and January 2017). Trees were selected based on several safety criteria and sampling needs. The samples trees had to have a least three accessible branches below a solid anchor point in order that researchers could obtain samples from three different heights throughout the crown. Trees were ascended using the Single Rope Technique (SRT) (Coffey & Anderson, 2012) employing the Singing Tree Rope Wrench and a friction hitch to gain access to the canopy. The 18 trees were instrumented with ionic resin lysimeters (UNIBEST Ag Manager, Walla Walla, WA) and installed over the 12-month sampling period (January 2016 – January 2017) at the three sampling heights within the canopy to evaluate wet deposition throughfall rates (Susfalk and Johnson 2002; Kopatek et al. 2006; DeLuca et al. 2008). As moss metal concentrations are not reliable integrators of metal deposition rates (Aboal et al. 2010), the lysimeters provide an estimate of annual wet deposition levels.

The dominant epiphytic moss species in *A. macrophyllum* canopies in the Hoh Rainforest includes: *Isothecium stoloniferum (Brid.), Antitrichia curtipendula (Hedw.),* and *Hylocomium splendens (Hedw.).* The dominant epiphytic moss species in *A. macrophyllum* canopies in Seattle includes: *I. stoloniferum (Brid.), Homalothecium lutescens (Hedw.), and Orthotrichum lycelli* Hook & Taylor*.* *Isothecium stoloniferum* was chosen as the target species for the intensive design as it was the only species we found available to sample across both locations and has been used previously for biomonitoring studies conducted in the Georgia Basin, British Columbia, Canada (Pott & Turpin, 1998; Raymond & Pott, 2003; Raymond et al., 2010).

As samples were collected once each season (April, July, and October 2016; and January 2017), a black parachute cord was tied to the climbing line to replace it when the line was pulled out of the tree. Installing the parachute cord saved time and prevented any potential damage to the tree that might occur through repeating the rigging process.

ii. Extensive Approach:

To extend the spatial coverage of the intensive sampling approach, we also sampled epiphytes using ground-based sampling techniques across an urban-to-wildland gradient in western Washington. Replicate field samples were collected from a total of twenty primarily *A. macrophyllum* stands across western Washington: five sites from the western side of the Olympic Peninsula, five suburban to rural sites in the Cascades along Interstate 90 heading towards Snoqualmie Pass, and 10 urban sites in Seattle, WA (Table 1). Samples of *Kindbergia praelonga (Hedw.) Ochyra*, a common feather moss found throughout western Washington, were collected from the trunk of *A. macrophyllum* approximately 1m above the ground. Extensive samples were collected within five days of the intensive sample collections for April, July and October 2016.

2.2 Laboratory Analysis:

iv. Trace Metal Concentrations:

Prior to analysis, all debris and necrotic tissue were removed with sterilized plastic forceps from the base of moss samples keeping only the upper two-thirds of the shoots (Gatziolis et al., 2016). We did not wash the moss samples prior to drying because previous studies have shown that is ineffective in removing particulates (Aboal et al., 2011) and that some elements such as Cd and Zn may increase in concentration after washing (Fernández et al., 2010).

Moss samples were dried for 24 hours at 40 °C and ground to a fine powder while immersed in liquid nitrogen. The HNO_3 + H_2O_2 digestion method was used to prepare the dried and ground samples for analysis. Four mL of concentrated reagent-grade HNO_3 were added to 0.2-0.3 g subsamples in 50 mL graduated glass digestion tubes. The tubes were covered with plastic watch glasses and left overnight at ambient temperature in a fume hood (approximately 18°C) to allow some initial oxidation of the samples by the HNO_3. Samples were digested at 95°C for 90-minute in borosilicate tubes heated evenly in a 36-tube graphite block digester. Samples were allowed to cool, following which 4 mL of reagent-grade 30% H_2O_2 were added to each tube followed by a 30-minute digestion at 95 °C and allowed to cool. Next, an additional 4 mL of H_2O_2 were added to each sample and heated again at 95°C. After cooling, deionized water was added to each tube to the 20-mL mark. To remove any undigested particulates not dissolved in the HNO_3 and H_2O_2, the samples were filtered through 0.45-µm membrane syringe filters. Digests were analyzed for a suite of metals (Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn) using inductively coupled plasma optical emission spectrometry (ICP-OES). Instrument performance tests were carried out daily to ensure proper calibration.

After calibration, quality control samples consisting of blanks, low and high concentration check standards were analyzed.

v. Trace Metal Wet Deposition:

The ionic resin from the canopy lysimeters was collected in January 2017. Elements adsorbed in the resin capsules were extracted with 30 ml of 0.5M HCl and analyzed for Cd, Cr, Cu, Fe, Ni, Pb, Sr, Ti, and Zn by ICP-OES.

vi. Lead and Strontium Isotopic Analysis:

Utilizing the HNO_3 + H_2O_2 digestion method described above, we analyzed 10 moss samples collected in January 2017 from the intensive sites for Pb and Sr isotopes. Five samples were collected from the Hoh Rainforest site on the Olympic Peninsula, WA, five samples from Seattle, WA (Ravenna, Interlaken, and Seward Parks) and run alongside aliquots of an internal laboratory standard (UW BCR-1). All reagents used for isotopic analysis were purified by a double sub-boiling distillation in Teflon.

a. Lead separation and analysis:

Immediately following the HNO_3 + H_2O_2 digestion method, samples were dried down at 93-107 C on a hot plate overnight and redissolved in 2.5mL of 6N HCl. Samples were dried down once again and dissolved in 1N HBr. Pb separation was achieved using 0.3mL anion-exchange resin (BioRad AG 1-X8, 100-200 mesh) that were rinsed with 6N HCl, DI water, and 1N HBr. Next, the 1N HBr samples were loaded onto the column and the liquid sample collected in a 30mL Teflon beaker was saved for Sr isotopic analyses. To elute Pb off the resin, 6N HCl was added to each column. Immediately following this step, the samples were placed on a hotplate to dry down completely for 2-3 hours at 190 C. The columns were cleaned a second time and the cooled samples, dissolved in 1N HBr, were run through the resin columns to achieve complete Pb purification.

The samples were dried down once more, fluxed in 2mL 2% HNO_3 and spiked with thallium with a known $^{203}Tl/^{205}Tl$ ratio prior to being analyzed for Pb isotopes (^{208}Pb / ^{204}Pb, $^{207}Pb/^{204}Pb$, $^{206}Pb/^{204}Pb$) on the Multicollector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS). Prior to isotopic analyses, a small aliquot of the sample solution was measured for concentration in order to adjust sample intensity to within 10% of the intensity of the Pb isotope standard (SRM 981) (Kayzar et al., 2014). Tl spiked aliquots of SRM 981 provided the mass fractionation relationship between Tl and Pb isotopes used for correction of the sample unknowns. The Pb isotope standard was run after every three samples to ensure proper calibration.

b. Strontium separation and analysis:

Strontium separation was achieved using 0.3 mL of a Sr-specific crown ether resin (Eichrom Sr-SPEC resin). The Sr-bearing elutions from the previous Pb column procedure were collected and dried down at 107 C and then redissolved in 0.4 mL of 4N HNO_3. This solution was loaded onto the Eichrom resin column and rinsed with 3.2 mL of 4N HNO_3 to elute Ca, Na, Rb, and Ba. Strontium was eluted with 3 mL of 0.05N HNO_3. Samples were dried and redissolved in 3 mL of 2% HNO_3. Concentrations were adjusted to within 10% of the bracketing NIST-987 Sr standard. The procedure for MC-ICP-MS Sr isotope analysis follows that of Brach-Papa et al. (2009). The IAPSO seawater standard was run after every three samples to verify analytical accuracy.

2.3 Statistical Analysis:

Descriptive statistics (minimum, maximum, mean, median, standard deviation, and Fisher-Pearson Skewness coefficient) were calculated for each element across intensive and extensive moss samples. A Pearson product-moment correlation test was used to determine the relationship between moss metal concentrations and average daily traffic counts across the intensive sampling locations. Daily traffic counts were gathered from WSDOT traffic counters near the four sampling locations.

Data on metal concentrations, represented as mg kg^{-1}, were transformed using a $\log_{10}(y + 1)$ to normalize the distribution. We used a two-way permutational multivariate analysis of variance (perMANOVA) to test if moss metal concentrations were significantly different across location types and sampling seasons for both intensive and extensive sites. The subsequent test of multivariate homogeneity of groups (DISPER) was used to calculate significant multivariate dispersions in variables among the location types for intensive and extensive moss samples. Euclidean distance was chosen for the two-way perMANOVA, and significance of the two-way perMANOVA R-value was determined via 1000 random permutations. For this study, effects with probabilities of $p < 0.05$ were assumed to be significant.

Principle component analysis (PCA) was selected as the appropriate ordination method where dissimilarity was calculated as Euclidean distance (Legendre & Legendre 1998). PCA was performed on the data matrix 'location type x metal concentration parameters' for both intensive (*I. stoloniferum* for urban and peninsula) and extensive (*K. praelonga* for urban, suburban, and peninsula) sampling sites. Twelve metal concentration parameters (Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn) were introduced as the analysis variables. PCA was performed to assess the dominant patterns in moss metal concentrations across locations. The loadings of variables to each PC was examined by converting eigenvector coefficients to structure correlations.

Linear regression models were used to determine the relationship between metal concentrations in *I. stoloniferum* tissue with average annual wet deposition measured using resin lysimeters across intensive sampling sites. All statistical analyses were conducted in the R Statistical Environment (R Core Team, 2013).

Results

For intensive (*I. stoloniferum* at peninsula and urban sites) and extensive (*K. praelonga* at peninsula, suburban, and urban sites) moss samples, descriptive statistics are shown in table 2. Fisher-Pearson skewness coefficient was calculated to identify elements with extremely high concentrations (Shepard, 1968). Within the intensive sites, urban *I. stoloniferum* samples exhibited higher metal concentrations compared to peninsula *I. stoloniferum* samples. For the extensive sites, *K. praelonga* metal concentrations were generally highest in the urban sites and lowest in the peninsula sites with suburban metal concentrations falling in the middle range as hypothesized. Both intensive and extensive samples were relatively free of exceptionally high concentrations, resulting in smaller skewness coefficients. The Pearson correlation was used to reveal any potential correlations between elemental concentrations and daily traffic counts across the intensive sites. Correlation coefficient values greater than 0.22 indicate significant correlation at $P \leq 0.001$ (Table 3). Cadmium concentrations showed no correlation either with other elements or with daily traffic rates.

For the intensive canopy samples, metal concentrations were significantly different in comparing between peninsula and urban *I. stoloniferum* samples ($F_{1,206} = 326.41$, $p<0.001$) and across sampling seasons ($F_{3,206} = 5.25$, $p<0.01$). Canopy height is not correlated with metal concentrations ($p = 0.67$) and therefore was not included in the perMANOVA. There was a significant interaction between location type and sampling season ($F_{3,206} = 2.67$, $p \leq 0.05$). This would suggest that metal concentration in the moss tissue is dependent on the season it was collected. Considering the location type term, the DISPER results show no significant differences in between-site variation ($F_{1,212}=1.35$, $p= 0.245$) and pairwise comparison also showed no significant differences among location types.

For the extensive moss samples, metal concentrations were significantly different across the three location types ($F_{2,50}= 33.33$, $p<0.001$) and across sampling seasons ($F_{2,50} = 12.5$, $p<0.001$). There was a significant interaction between location type and season ($F_{4, 50}= 2.7$, $p<0.01$), which suggests a seasonal effect on metal concentrations across the sampling locations. Considering the location type term, the DISPER results showed no significant differences in between-site variation ($F_{2, 56} =1.14$, $p=0.326$) and pairwise comparison also showed no significant differences among location types.

Significant proportions of the variability in the 'location type x metal concentration parameters' for both intensive and extensive samples were explained by their first two PCs. Structure correlation coefficients between each variable and the first two PCs for intensive and extensive samples are presented in Table 4. The total amount of variance explained by the first two PCs in PCA 1 were (85.26%): PC1 (78.2%) and PC2 (7.06%) (Figure 1). In PCA 1, representing the intensive sampling data, Cr, Cu, Fe, K, Mg, Ni, Pb, and Ti moss tissue concentrations are highly correlated with PC1; Cd, Mn, Sr, and Zn concentrations are highly correlated with PC2. Vector arrows point towards the urban sampling points, suggesting that the metal concentration tends to be higher in urban *I. stoloniferum* canopy samples compared to peninsula *I. stoloniferum* canopy samples (Figure 1).

The total amount of variance explained by the first two PCs in PCA 2 were (80.57%): PC1 (70.29%) and PC2 (10.26%) (Figure 2). In PCA 2, representing the extensive sampling data, Cr, Cu, Fe, Pb, Ti, and Zn were negatively and heavily loaded to PC1; Cd, K, Mg, Mn, Ni, and Sr were positively and heavily loaded to PC2. As hypothesized, PCA 2 revealed that the three location types (peninsula, suburban, and urban) are environmentally distinct from each other. Vector arrows point towards the urban sampling points, suggesting metal concentration tends to be greater in urban *K. praelonga* samples than in suburban or peninsula *K. praelonga* samples (Figure2).

No significant differences in wet deposition values collected in the resin lysimeters were observed across the canopy heights, resulting in the use of an average annual precipitation-weighted wet deposition value per tree (Chance et al., 2015; Wetherbee et al., 2010). The moss values used are the arithmetic mean of metal concentrations per tree from the January 2017 collection. Linear regression analyses were only carried out on moss samples collected from the January sampling round as they were exposed to the full 12-months of wet deposition captured by the canopy lysimeters. Plots of metal concentrations in the intensive *I. stoloniferum* moss vs. wet atmospheric deposition of the same metal are shown in Figure 3. Of the nine metals, Cr, Cu, Fe, Ni, Pb, Ti, and Zn showed significant correlation ($p<0.05$) between moss tissue concentrations and annual average wet deposition rates. No correlation was observed for Cd and Sr between annual average wet deposition rates and moss tissue concentrations ($r^2= 3.8e^{-05}$, $p= 0.983$; $r^2=0.085$, $p=0.238$ respectively).

Table 5 lists the trace element concentrations and isotopic composition for the ten *I. stoloniferum* samples analyzed in this study. In general, elemental concentrations for Cu, Pb, Sr, and Zn were elevated in the Seattle samples compared to those from the Hoh Rainforest. Trace metal concentrations are also expressed in term of metal/metal ratios for Cu/Pb and Zn/Pb (Figure 4). Samples from Seattle contain lower ratios of Cu/Pb and Zn/Pb ratios and less radiogenic Pb isotope values ($^{206}Pb/^{207}Pb$ = 1.1751-1.1859). Samples from the Hoh Rainforest are characterized by higher ratios of Cu/Pb and Zn/Pb ratios and more radiogenic Pb isotope values ($^{206}Pb/^{207}Pb$ generally >1.1849). Measured Pb isotope compositions (Figure 5) show that natural geologic samples (Chan et al., 2012) are more radiogenic than Seattle or West Coast industrial sources (Jones et al., 2000; Bollhöfer & Rosman, 2001). Samples from the Hoh Rainforest contain higher $^{86}Sr/^{87}Sr$ (0.7051 – 0.7067) and lower Sr concentrations compared to Seattle samples (0.7047- 0.7052) (Figure 6).

Discussion

Metal deposition and accumulation in *I. stoloniferum* and *K. praelonga* were higher in urban sites in Seattle than in suburban and remote sites. For the intensive sites, the metal concentrations are significantly correlated with heavier daily traffic rates, with the exception of Cd (Table 3). The traffic density is highest in Seattle with a citywide average daily traffic volume of 1,010,000 vehicles (WSDOT, 2015) in areas where metal concentrations are among the highest recorded. The expected lower metal concentrations on the western side of the Olympic Peninsula are correlated with lower daily traffic counts of 1,300 (WSDOT, 2015) on few nearby highways. As the PSR is expected to experience significant population growth by 2040, increasing pressure from construction and the transportation sector is likely lead to higher levels of heavy metal pollutants in the region.

Apeagyei et al (2011) sampled 115 different tires and found that the most abundant element is Zn (17,720 ppm), followed Ca>W>K>Fe>Ti>Cr>Mo>Cu>V>Sr>Zr>Pb. In our study we observed a significant correlation ($p<0.001$) between higher daily traffic rates with Cr, Cu, Fe, K, Pb, Sr, Ti, and Zn suggesting a common pollution source (Table 2). Fugitive dust from tire attrition is a potentially substantial source of Zn entering the environment (Councell et al., 2004). The higher levels of Zn found in the intensive and extensive urban sites may be attributed to tire attrition from major roadways such as Interstate 5 and WA State Route 99.

Although Cu is a plant-essential micronutrient, it is of particular concern in the PNW as it can be hazardous to wildlife in high concentrations in the environment. This is a particular problem in road runoff where Cu concentrations ranging from 1-30 μg/L have been shown to produce sublethal neurotoxicity in salmon (Linbo et al., 2006; Sandahl et al., 2004; Sandahl et al.

2007; Baldwin et al., 2003). In an attempt to regulate sources of Cu entering waterways by surface runoff and dust deposition, Washington State passed the Better Brakes Law (Chapter 173-901 WAC) in 2010. The law will be fully implemented in 2025, but until then brake pads will still be a source of Cu pollution in Washington State. We observe a significant correlation between Cu concentrations in intensive *I. stoloniferum* samples with daily traffic rates ($p<0.001$), as well as a significant ($p<0.01$) relationship between annual average Cu deposition rates and moss metal concentrations. These correlations suggest that Cu released as fugitive dust from brake pads along major roadways, such as Interstate 5 and Washington State Route 99, may account for the higher levels of Cu we found in the intensive and extensive urban moss samples.

Lead is a more obvious trace metal of environmental concern given that it is not an essential nutrient and it is toxic to mammals in relatively low concentrations. A multitude of studies have examined the impact of Pb pollution on human and wildlife health (Lanphear et al. 2005; Nevin, 2000; Needleman & Gatsonis, 1990; Holcombe et al, 1976; Spehar et al., 1978). It is clear that *I. stoloniferum* collected from Seattle have slightly less radiogenic isotopic composition, which is consistent with the anthropogenic Pb composition of other West Coast cities (Figure 5). In this study, Pb was strongly correlated with average daily traffic counts ($p<0.001$) for the intensive moss samples (Table 3). This would suggest that the source of Pb most likely originates from brake pad and tire abrasion (Apeagyei et al., 2010). Hallock (2010) found that Pb is likely to be transported as particle-bound fugitive dust rather than in the dissolved phase as Pb has a high affinity for particulate matter. Existing studies suggest a significant relationship between bulk deposition levels and the concentrations of Pb in moss (Ross, 1990; Berg et al., 1995; Thoni et al., 1996; Berg & Steinnes, 1997; Schintu et al, 2005; Fowler et al., 2006), which is supported by the findings in our study ($p<0.05$). Emissions from Asia reach the western U.S. within 10 days (Jaffe et al., 1999) and Chinese aerosol is enriched in

^{208}Pb (Bollhöfer & Rosman, 2001; Ewing et al., 2010). The Pb isotopic compositions for Hoh Rainforest samples indicate potential trans-Pacific contamination by Asian emissions.

Approximately 1.0 Mg yr^{-1} of Cd is released from anthropogenic sources in Puget Sound, which includes leaching from roofing materials, release from agricultural fertilizer, air emissions for industrial facilities, and road-related sources such as tire and brake pad wear (Ecology & King County, 2011 As all of the sampling sites in this study are located in either in the Hoh Rainforest or in city parks, they are unlikely to experience Cd leachate from roofing materials. Spectrum Glass was the only industrial facility in the Puget Sound region reporting fugitive air releases of cadmium in the last 10 years, but they ceased production in July 2016. We did not observe significantly high levels of Cd in either intensive or extensive samples; therefore we do not believe emissions from Spectrum Glass were a source of Cd in the urban moss samples. As Cd did not show significant correlation with daily traffic rates (Table 3), it is unlikely that tire and brake wear are the source of cadmium detected in *I. stoloniferum* and *K. praelonga* samples across the sampling locations.

Elevated levels of Sr were observed in urban areas in *I. stoloniferum* and *K. praelonga* samples (122.89 mg kg^{-1} and 101.48 mg kg^{-1}, respectively) compared to rural samples (51.19 mg kg^{-1} and 50.40 mg kg^{-1}, respectively). Strontium is an alkaline earth element with an average concentration in the Earth's crust of 370ppm and 240ppm in soil minerals (Sposito, 1989). The $^{87}Sr/^{86}Sr$ isotope ratio has been used extensively to measure atmospheric inputs of Sr to soils and vegetation (Graustein & Armstrong, 1983; Graustein, 1989; Miller et al., 1993; Capo and Chadwick, 1999). The relationship in Figure 7 between $^{86}Sr/^{87}Sr$ and Sr (mg kg^{-1}) indicates Hoh Rainforest samples may be influenced by seawater input given the proximity to the Pacific

Ocean. However, it is not clear what is causing the higher levels of Sr associated with urban samples is. One possible source for the elevated Sr, given its high correlation with daily traffic rates (Table 3), may be from diesel soot associated with traffic along major corridors in Seattle.

A comparison of mean element concentrations in mosses obtained from this study with recent moss monitoring studies in the PNW region is presented in Table 6. The Portland study used *Orthotrichum lyellii* to monitor pollution patterns, whereas this study and that conducted in British Columbia (B.C.) used *I. stoloniferum* (Gatziolis et al., 2016; Potts & Turpin, 1998). While interspecies variation may affect the comparison of metals levels to some extent, it is unlikely to affect the general trend of higher metal levels in urban vs. rural environments. Mean metal concentrations in the urban *I. stoloniferum* samples gathered in this study were higher than values previously reported in the PNW, with the exception of Cd in the Portland study, and Cd and Pb in the B.C. study. The higher levels of Cd and Pb found in the Canadian samples collected in 1993 may be attributed to legislative policies that did not remove leaded gasoline from the Canadian market until December 1990. Several studies that have attempted to address the time period represented by metals in moss tissue (Boquete et al., 2013; Fernadez et al., 2013), none of which have used *I. stoloniferum, K. praelonga, or O. lyellii.* The metal range captured in these three studies likely represents a maximum of a three-year exposure period (Gatziolis et al., 2016) as only the top two-thirds of the moss shoot was analyzed. The sources of the Cd levels reported in the Portland study were identified as industrial atmospheric deposition originating from two stained-glass manufacturers (Donovan et al., 2016). With the exception of Cd, both Ni and Pb levels found in the urban *I. stoloniferum* from this study were 1.42 and 1.74 higher, respectively, than the *O. lyellii* levels reported in Portland. It is possible that the combustion of diesel fuels is a main source of Ni pollution in the canopy environment (National Research

Council, 1975). This study would support that suggestion due to the significant correlation between daily traffic rates and Ni concentrations from the intensive sampling (Table 3).

Conclusion

In the PNW, lichen has been widely used as biomonitors to assess the impacts of air quality and regional atmospheric deposition (Berg et al., 1995; McCune, 2000; Geiser & Neitlich, 2007). Results from this study suggest moss has the potential to be used as a low-cost biomonitor to evaluate heavy metal pollution in urban and rural areas. The values found in this study cannot be treated as an absolute measure of metal deposition, but rather used as a comparative index of metal deposition and a screening tool to assess areas of metal pollution.

In the PSR, the public is increasingly concerned with the effect of the growing rate of urbanization on regional air quality. The moss metal concentration data presented in this paper helps elucidate the impacts of the transportation sector on ecosystem health throughout the PSR. It seems appropriate to expand the existing lichen monitoring network to include moss sampling and better establish regional distribution patterns of transportation-based pollutants. As the transport and fate of heavy metals in the environment is complex, particularly in urban environments where natural biogeochemical pathways have been highly altered, calibration of moss values with instrument measurements for both wet and dry deposition is needed.

Tables:

Table 1: GPS coordinate locations for Extensive sampling sites.

Site Name	Coordinates	Location Type
Coppermine Bottom	47.65544, -124.19863	Peninsula
Upper Clearwater	47.67858, -124.11877	Peninsula
South Fork Hoh	47.80391, -123.99083	Peninsula
Matheny Creek	47.56717, -124.07724	Peninsula
Hard Rain	47.81609, -124.13964	Peninsula
South Fork Snoqualmie River	47.43216, -121.63053	Suburban
Little Si Trailhead	47.48722, -121.75411	Suburban
Highpoint Trailhead	47.52827, -121.97495	Suburban
Nook Trailhead	47.52632, -121.99917	Suburban
Poo Poo Point	47.49861, -122.01954	Suburban
Carkeek Park	47.71050, -122.37593	Urban
Colman Park	47.56415, -122.30041	Urban
Cheasty Boulevard	47.56415, -122.30041	Urban
Lincoln Park	47.53018, -122.39373	Urban
Llandover Woods	47.73273, -122.36494	Urban
Longfellow Creek	47.55338, -122.36676	Urban
Matthews Beach	47.69681, -122.27505	Urban
Schmitz Park	47.57731, -122.40210	Urban
Thornton Creek Park #1	47.72445, -122.31963	Urban
Woodland Park	47.67082, -122.34583	Urban

Table 2: Element descriptive statistics (a) Peninsula intensive metal concentrations I. stoloniferum; (b) Urban intensive metal concentrations I. stoloniferum; (c) Peninsula extensive metal concentrations in K. praelonga; (d) Suburban extensive metal concentrations in K. praelonga; and (e) Urban extensive metal concentrations in K. praelonga.

(a)

Element	Minimum	Maximum	Mean	Median	Standard Deviation	Fisher-Pearson Skewness Coefficient	# Samples below detection limit
Cd	BD	3.44	0.17	0.07	0.39	0.76	41
Cr	0.08	1.93	0.65	0.49	0.41	1.14	0
Cu	0.52	11.97	3.52	3.21	1.98	0.47	0
Fe	33.79	316.27	95.71	86.89	46.80	0.56	0
K	2373.22	10170.00	5408.02	5108.82	1277.03	0.70	0
Mg	1253.71	3721.43	1969.96	1902.66	450.61	0.44	0
Mn	16.10	373.83	93.61	76.66	67.72	0.75	0
Ni	BD	4.28	1.42	1.35	0.87	0.26	11
Pb	BD	5.79	0.86	0.68	0.80	0.69	5
Sr	11.42	149.17	51.19	46.08	26.59	0.57	0
Ti	1.39	16.99	5.43	4.94	2.82	0.51	0
Zn	17.62	162.67	44.47	38.45	22.67	0.79	0

(b)

Element	Minimum	Maximum	Mean	Median	Standard Deviation	Fisher-Pearson Skewness Coefficient	# Samples below detection limit
Cd	BD	0.5	0.17	0.18	0.13	-0.23	17
Cr	0.35	5.91	2.49	2.32	1.09	0.46	0
Cu	1.78	21.71	12.05	11.48	3.90	0.44	0
Fe	78.11	1396.46	594.70	565.81	281.98	0.31	0
K	2289.71	12482.35	8243.29	8155.06	1999.65	0.13	0
Mg	809.14	4415.38	2390.33	2317.81	624.51	0.35	0
Mn	9.62	290.46	110.40	100.45	54.14	0.55	0
Ni	0.34	98.91	4.04	2.73	9.43	0.42	0
Pb	0.62	64.06	12.34	9.41	11.11	0.79	0
Sr	24.15	300.00	122.89	116.57	49.05	0.39	0
Ti	3.58	60.51	27.09	25.59	13.32	0.34	0
Zn	15.42	191.82	79.46	76.12	30.58	0.33	0

(c)

Element	Minimum	Maximum	Mean	Median	Standard Deviation	Fisher-Pearson Skewness Coefficient	# Samples below detection limit
Cd	BD	0.31	0.09	0.08	0.09	0.23	5
Cr	BD	2.21	0.55	0.50	0.58	0.25	4
Cu	1.82	6.81	3.77	3.96	1.29	-0.43	0
Fe	20.54	994.40	243.09	154.84	257.67	1.03	0
K	3393.80	11492.86	6076.00	5011.11	2456.17	1.30	0
Mg	1282.50	2830.63	1775.76	1721.82	382.44	0.42	0
Mn	41.01	278.77	119.30	104.28	65.60	0.69	0
Ni	BD	5.20	1.77	1.56	1.62	0.39	5
Pb	BD	1.89	0.85	0.75	0.53	0.59	1
Sr	23.64	102.86	50.40	45.25	19.35	0.80	0
Ti	1.57	50.13	9.69	5.64	12.39	0.98	0
Zn	21.18	90.82	43.64	36.75	21.59	0.96	0

(d)

Element	Minimum	Maximum	Mean	Median	Standard Deviation	Fisher-Pearson Skewness Coefficient	# Samples below detection limit
Cd	BD	0.27	0.11	0.13	0.09	-0.54	5
Cr	0.24	3.09	1.64	1.34	0.99	0.91	0
Cu	4.69	10.88	7.78	7.85	1.92	-0.11	0
Fe	162.85	1096.67	612.04	502.74	334.98	0.98	0
K	4560.69	9355.56	6134.55	5620.00	1366.00	1.13	0
Mg	1175.10	1935.20	1509.25	1581.85	219.71	-0.99	0
Mn	41.25	286.15	93.92	85.93	58.24	0.41	0
Ni	BD	4.36	1.64	1.45	1.47	0.38	5
Pb	1.20	9.90	3.98	3.06	2.58	1.07	0
Sr	18.92	68.16	34.82	34.72	12.66	0.02	0
Ti	7.87	62.32	29.96	25.25	17.65	0.80	0
Zn	33.29	108.92	65.93	68.54	24.92	-0.31	0

(e)

Element	Minimum	Maximum	Mean	Median	Standard Deviation	Fisher-Pearson Skewness Coefficient	# Samples below detection limit
Cd	BD	1.48	0.28	0.19	0.32	0.89	2
Cr	0.61	6.69	2.89	2.90	1.67	-0.01	0
Cu	6.79	21.70	11.58	10.79	3.58	0.66	0
Fe	212.86	2029.25	940.98	1007.10	503.15	-0.39	0
K	4791.04	13525.93	8159.48	7985.71	1882.45	0.28	0
Mg	1495.40	2917.87	2079.71	2020.74	307.07	0.58	0
Mn	73.52	411.81	154.77	141.43	69.16	0.58	0
Ni	0.00	5.95	2.71	2.94	2.06	-0.34	8
Pb	1.22	35.47	8.12	6.99	6.77	0.50	0
Sr	34.46	232.35	101.48	96.29	46.36	0.34	0
Ti	10.06	99.46	50.87	54.57	27.45	-0.40	0
Zn	37.17	196.50	83.17	69.33	43.38	0.96	0

Table 3: Pearson's correlation coefficients between annual average daily traffic counts and metal concentrations in I. stoloniferum samples collected the intensive sampling sites. * = p<0.05, ** = p<0.01, *** = p<0.001.

	Daily traffic rates	Cd	Cr	Cu	Fe	K	Mg	Mn	Ni	Pb	Sr	Ti	Zn
Daily traffic rates	•												
Cd	-0.01	•											
Cr	0.75***	0	•										
Cu	0.81***	0.01	0.9***	•									
Fe	0.78***	0.01	0.97***	0.9***	•								
K	0.65***	0	0.64***	0.75***	0.64***	•							
Mg	0.36***	-0.05	0.45***	0.55***	0.42***	0.71***	•						
Mn	0.14*	0.24	0.33***	0.38***	0.31***	0.23***	0.25***	•					
Ni	0.19**	0.01	0.22**	0.2**	0.18**	0.06	0.08	0.08	•				
Pb	0.59***	0.07	0.82***	0.78***	0.83***	0.51***	0.34***	0.3***	0.28***	•			
Sr	0.67***	0.03	0.75***	0.79***	0.76***	0.7***	0.57***	0.22**	0.16*	0.73***	•		
Ti	0.75***	0.02	0.94***	0.86***	0.98***	0.61***	0.38***	0.29***	0.18*	0.81***	0.72***	•	
Zn	0.55***	0.08	0.7***	0.82***	0.69***	0.62***	0.53***	0.54***	0.22***	0.73***	0.7***	0.66***	•

Table 4: Structure correlation coefficients between measured parameters and the first two principal components (PC1 and PC2) for (a) intensive metal concentrations I. stoloniferum and (b) extensive metal concentrations in K. praelonga.

(a)

Principle Component (% variance explained, significance value)	**PC1 78.2%, p<0.01**	**PC2 7.06%, p<0.01**
Cd		-0.99725
Cr	0.99623	
Cu	0.90504	
Fe	0.97718	
K	0.97946	
Mg	0.70275	-0.71144
Mn		-0.98723
Ni	0.8548	
Pb	0.96123	
Sr		0.77069
Ti	0.97175	
Zn		-0.84258

(b)

Principle Component (% variance explained, significance value)	**PC1 70.29%, p<0.001**	**PC2 10.28%, p<0.001**
Cd		0.88191
Cr	-0.99995	
Cu	-0.99244	
Fe	-0.90389	
K		0.98481
Mg		0.97284
Mn		0.90725
Ni		0.93047
Pb	-0.99206	
Sr		0.97521
Ti	-0.87398	
Zn	-0.77781	

Table 5: Isotopic composition and elemental concentrations at Hoh Rainforest and Seattle sites.

Sample	Elemental Concentration ($mg\ kg^{-1}$)				Isotopic Composition					
	Cu	Pb	Sr	Zn	206Pb/204Pb	207Pb/204Pb	208Pb/204Pb	206Pb/207Pb	208Pb/207Pb	87Sr/86Sr
Hoh Rainforest										
HRT3-M	2.14	0.48	53.4	31.95	18.6965	15.6597	38.3131	1.1939	2.0508	0.7052
HRT5-L	2.83	0.52	51.42	37.61	18.5447	15.6504	38.3434	1.1849	2.066	0.7058
HRT6-L	5.81	0.79	47.38	55.45	18.2967	15.618	38.182	1.1715	2.0868	0.7068
HRT7-T	3.43	0.34	38.1	48.22	18.616	15.6521	38.3293	1.1894	2.0589	0.7062
HRT8-L	2.2	0.36	86.2	33.13	18.6073	15.6473	38.3133	1.1892	2.059	0.7059
Seattle										
INT1-T	11.11	16.4	135.1	77.45	18.523	15.6192	38.1316	1.1859	2.0586	0.7052
INT2-L	15.55	34.63	191.63	122.03	18.3654	15.6031	38.0268	1.177	2.0706	0.7053
RAV2-T	9.86	10.76	87.82	54.89	18.3343	15.6022	37.9992	1.1751	2.0726	0.705
SEW3-M	9.31	5.77	94.18	54.33	18.4321	15.6127	38.0886	1.1806	2.0664	0.705
SEW3-T	9.92	2.85	105.68	59.48	18.505	15.6272	38.1558	1.1842	2.0619	0.7047

Table 6: Comparison of mean element concentrations (mg/kg) in mosses obtained in this study with those collected in British Columbia, Canada and Portland, OR, USA. The Hoh Rainforest and Bridal Veil Falls represent rural sites, whereas Seattle, Vancouver, and Portland represent urban sites. a= I. stoloniferum, b= O. lyelli

	Washington, USA[a]		British Columbia, Canada[a]		Oregon, USA[b]
	Hoh Rainforest, Olympic Peninsula	Seattle	Bridal Veil Falls Provincial Park	Vancouver	Portland
Cd	0.17	0.17	0.17	0.42	0.31
Cr	0.65	2.49	1.3	0.9	2.38
Mn	93.61	110.4	35	184	87.65
Ni	1.42	4.04	1.1	3	2.83
Pb	0.86	12.34	2.5	20.06	7.09
Zn	44.47	79.46	19.3	43.2	71.94

Figures:

Figure 1: Principle component analysis (PCA) ordination of Intensive *I. stoloniferum* samples

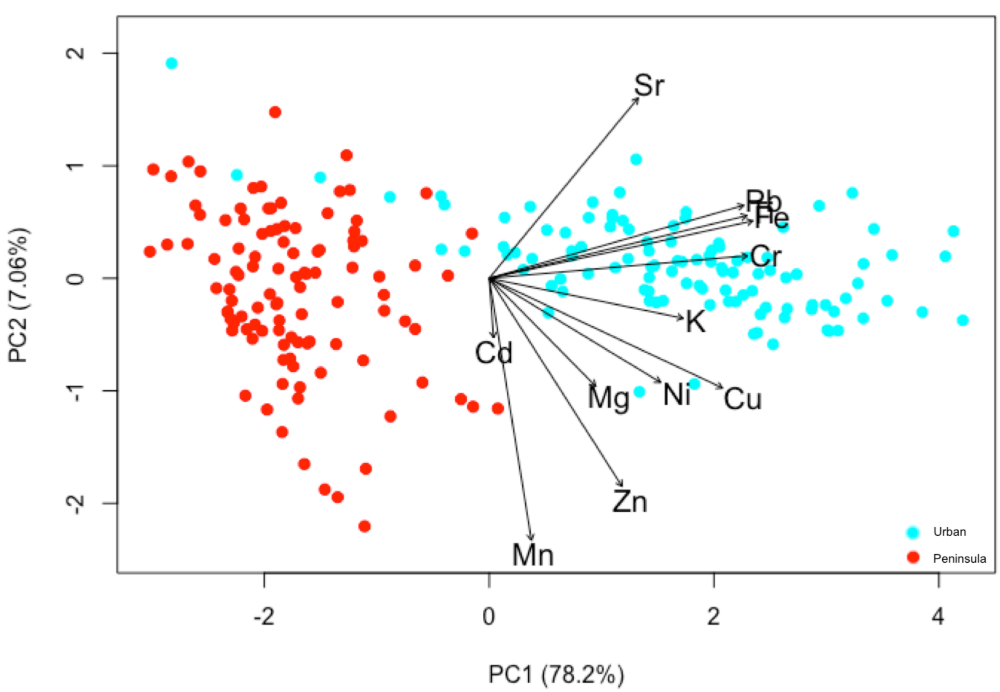


Figure 2: Principle component analysis (PCA) ordination of Intensive *K. praelonga* samples.

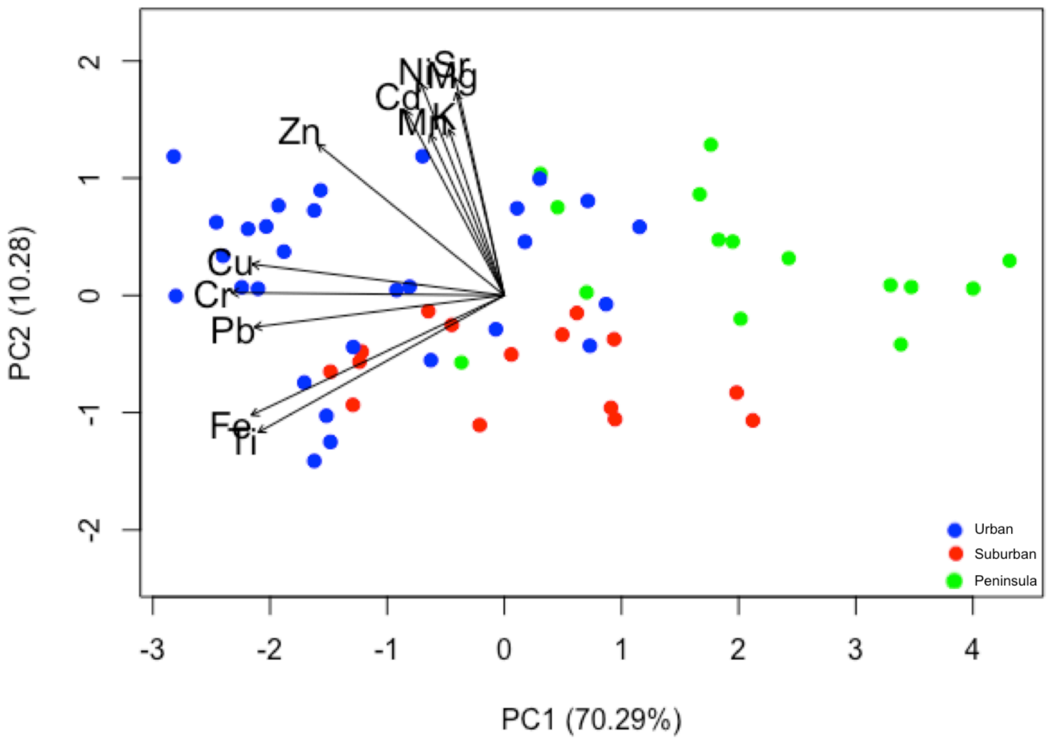
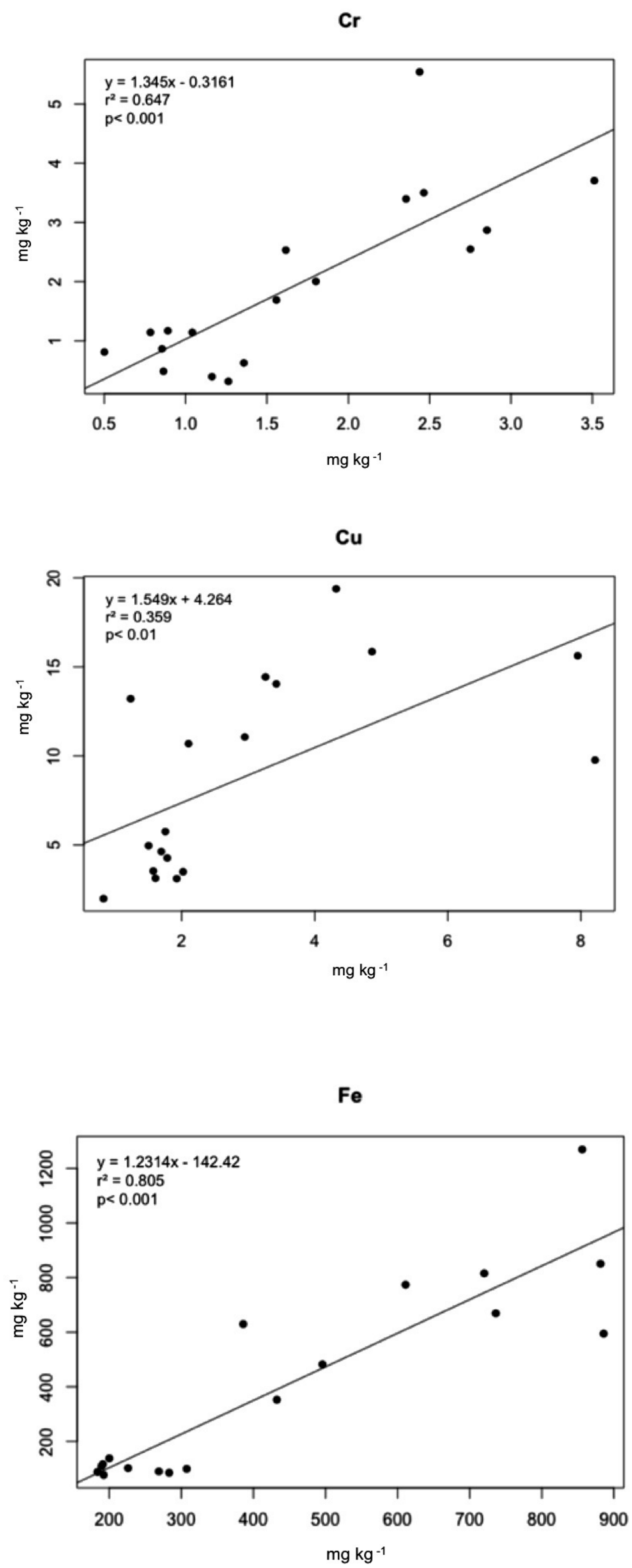
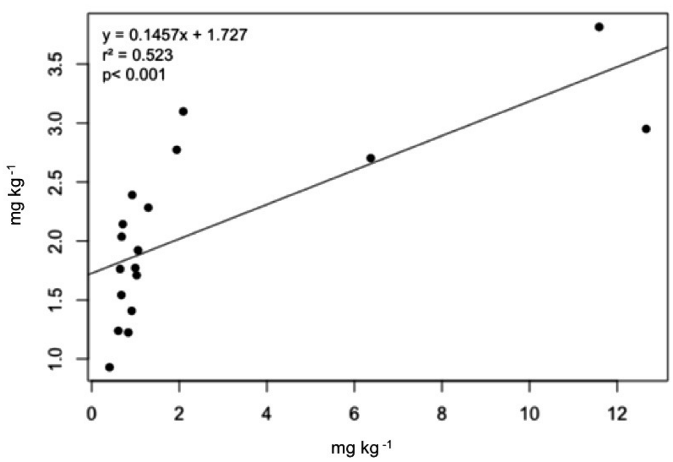


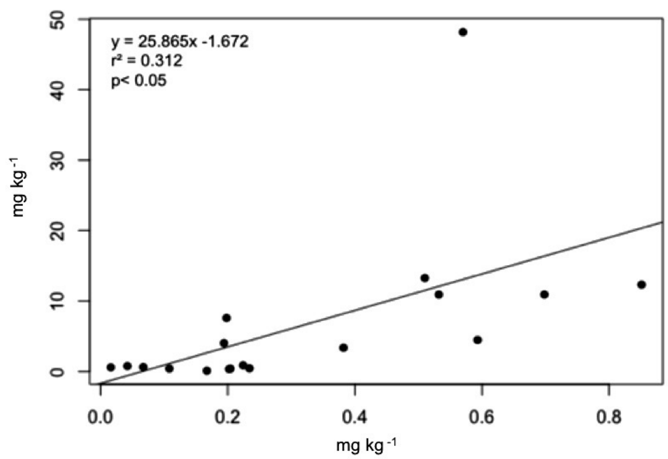
Figure 3: Relationship between trace element concentrations in I. *stoloniferum* tissue (mg kg^{-1}) and annual atmospheric wet deposition (mg kg^{-1}).



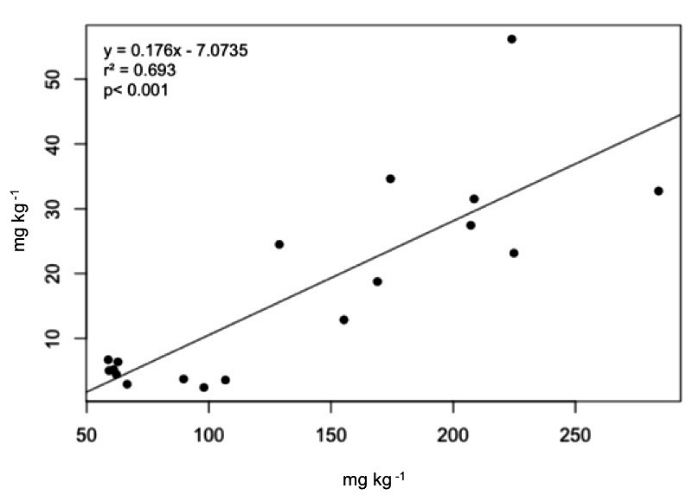
Ni


y = 0.1457x + 1.727
r² = 0.523
p< 0.001
mg kg⁻¹
mg kg⁻¹

Pb


y = 25.865x -1.672
r² = 0.312
p< 0.05
mg kg⁻¹
mg kg⁻¹

Ti


y = 0.176x - 7.0735
r² = 0.693
p< 0.001
mg kg⁻¹
mg kg⁻¹

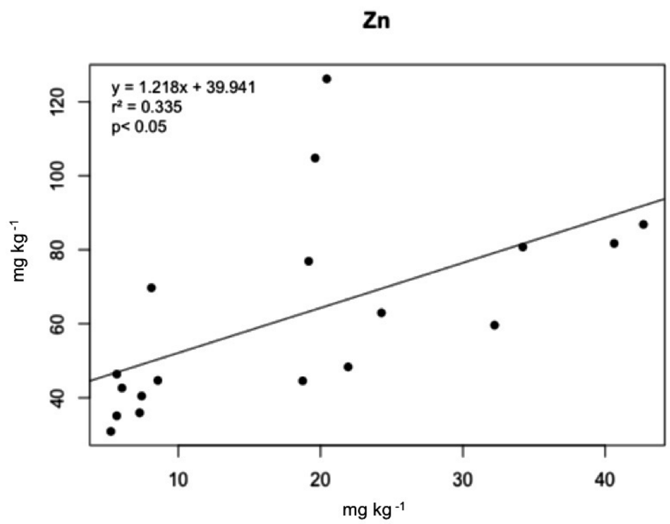


Figure 4: Illustration of the variation between Zn/Pb versus Cu/Pb ratios from samples analyzed for isotopic composition.

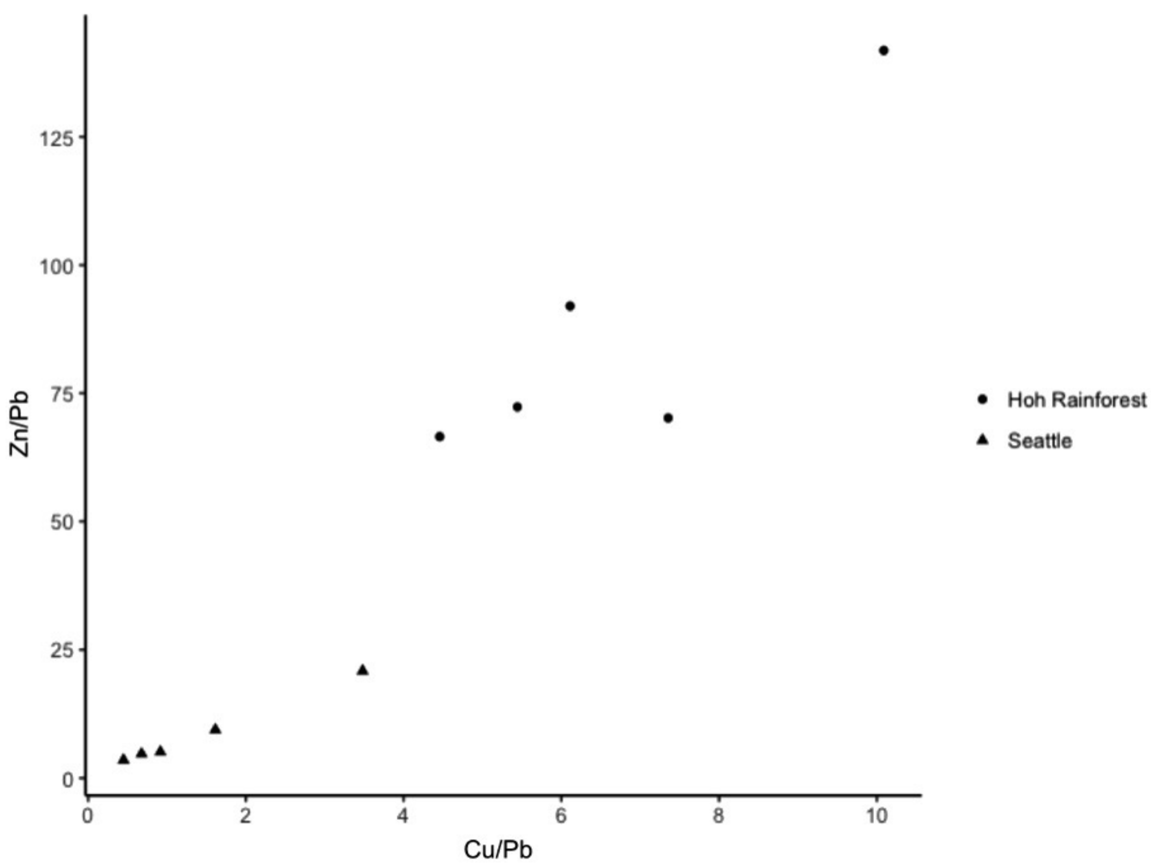


Figure 5: Plot of $^{208}Pb/^{206}Pb$ vs. $^{206}Pb/^{207}Pb$ for samples collected from the Hoh Rainforest and Seattle. Also shown are the compositions for samples representative of cities in Western U.S., Western Canada, and Asia (Bollhöfer & Rosman, 2001), Chinese loess (Jones et al., 2000), Asian industries (Tan et al., 2006), and geologic influences from Washington State, USA (Chan et al., 2012). The red line represents the North American array and the blue line represents the Asian array of isotopic ratios.

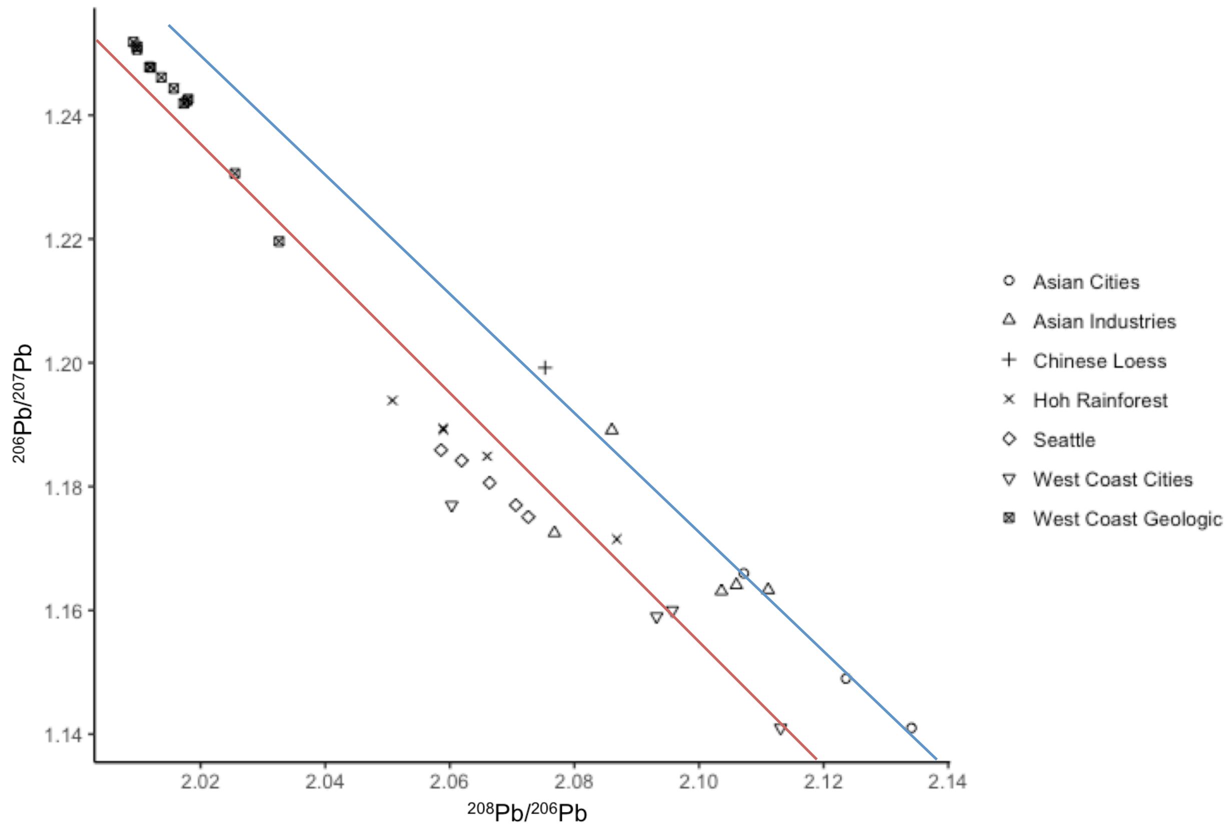
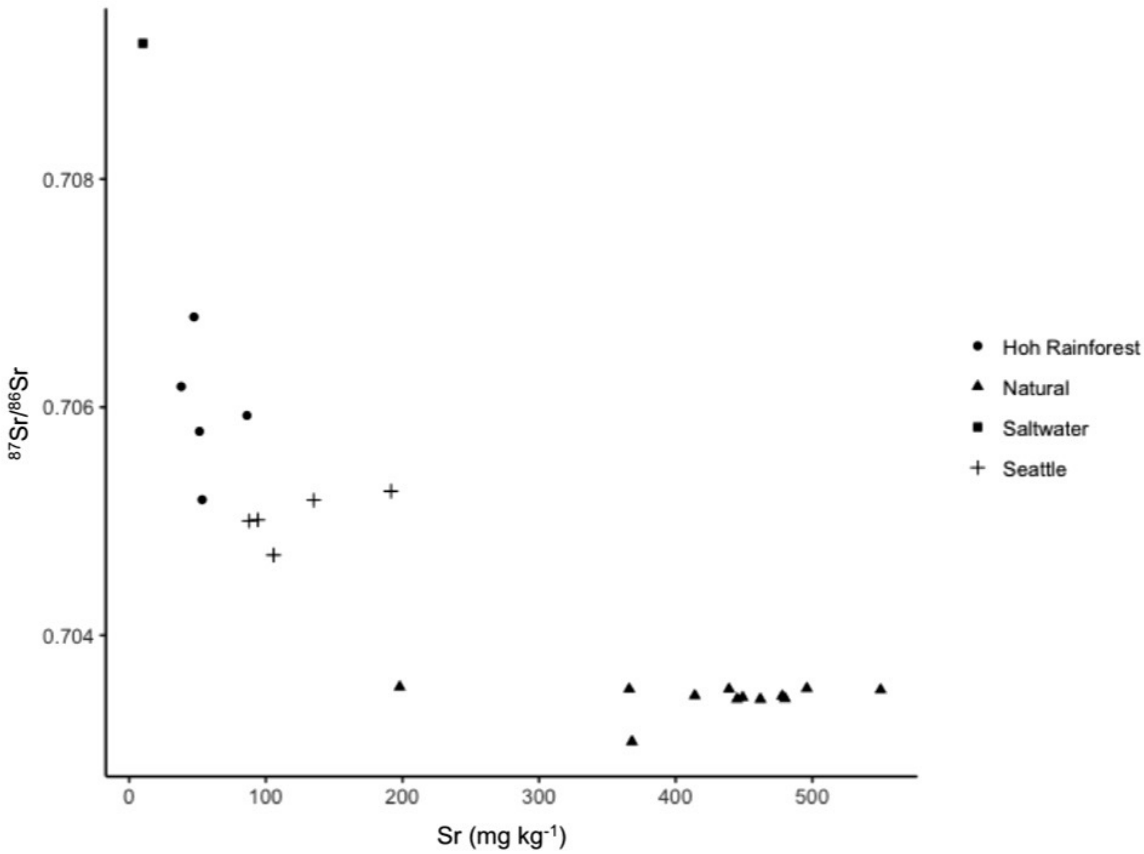


Figure 6: Plot of $^{87}Sr/^{86}Sr$ vs. Sr concentrations (mg kg^{-1}) for samples collected from the Hoh Rainforest and Seattle. Also shown are the compositions for samples representative of saltwater influences (IAPSO certified standard) and geologic influences from Washington State, USA (Chan et al., 2012).



References:

Aboal, J. R., Fernández, J. A., Boquete, T., & Carballeira, A., 2010. Is it possible to estimate atmospheric deposition of heavy metals by analysis of terrestrial mosses?. Science of the Total Environment, 408(24), 6291-6297.

Aboal, J. R., Pérez-Llamazares, A., Carballeira, A., Giordano, S., & Fernández, J. A., 2011. Should moss samples used as biomonitors of atmospheric contamination be washed?. Atmospheric environment, 45(37), 6837-6840.

Aničić, M., Tasić, M., Frontasyeva, M.V., Tomašević, M., Rajšić, S., Strelkova, L.P., Popović, A. and Steinnes, E., 2009. Active biomonitoring with wet and dry moss: a case study in an urban area. Environmental chemistry letters, 7(1), 55-60.

Apeagyei, E., Bank, M. S., & Spengler, J. D., 2011. Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts. Atmospheric Environment, 45(13), 2310-2323.

Baldwin, D.H., J.F. Sandahl, J.S. Labenia, and N.L. Scholz, 2003. Sublethal effects of copper on coho salmon: Impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. Environmental Toxicology and Chemistry 22:2266-2274.

Bargagli, R., Monaci, F., Borghini, F., Bravi, F., & Agnorelli, C., 2002. Mosses and lichens as biomonitors of trace metals. A comparison study on Hypnum cupressiforme and Parmelia caperata in a former mining district in Italy. Environmental pollution, 116(2), 279-287.

Bates, J. W., 1992. Mineral nutrient acquisition and retention by bryophytes. Journal of Bryology, 17(2), 223-240.

Berg, T., Røyset, O., & Steinnes, E., 1995. Moss (Hylocomium splendens) used as biomonitor of atmospheric trace element deposition: estimation of uptake efficiencies. Atmospheric Environment, 29(3), 353-360.

Berg, T., & Steinnes, E., 1997. Recent trends in atmospheric deposition of trace elements in Norway as evident from the 1995 moss survey. Science of the Total Environment, 208(3), 197-206.

Better Brakes Rule (Chapter 173-901 WAC)

Bollhöfer, A., & Rosman, K. J. R., 2001. Isotopic source signatures for atmospheric lead: the Northern Hemisphere. Geochimica et Cosmochimica Acta, 65(11), 1727-1740.

Boquete, M. T., Fernández, J. Á., Carballeira, A., & Aboal, J. R., 2013. Assessing the tolerance of the terrestrial moss Pseudoscleropodium purum to high levels of atmospheric heavy metals: a reciprocal transplant study. Science of the Total Environment, 461, 552-559.

Brach-Papa, C., Van Bocxstaele, M., Ponzevera, E., & Quétel, C. R., 2009. Fit for purpose validated method for the determination of the strontium isotopic signature in mineral water samples by multi-collector inductively coupled plasma mass spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy, 64(3), 229-234.

Brown, D. H., & Bates, J. W., 1990. Bryophytes and nutrient cycling. Botanical Journal of the Linnean Society, 104(1-3), 129-147.

Capo, R. C., & Chadwick, O. A., 1999. Sources of strontium and calcium in desert soil and calcrete. Earth and Planetary Science Letters, 170(1), 61-72.

Chan, C. F., Tepper, J. H., & Nelson, B. K., 2012. Petrology of the Grays River volcanics, southwest Washington: Plume-influenced slab window magmatism in the Cascadia forearc. Geological Society of America Bulletin, 124(7-8), 1324-1338.

Chance, R., Jickells, T. D., & Baker, A. R., 2015. Atmospheric trace metal concentrations, solubility and deposition fluxes in remote marine air over the south-east Atlantic. Marine Chemistry, 177, 45-56.

Coffey, D. & Andersen, T., 2012. Best Practices for SRT in Arboriculture. Tree Care Industry Association, Londonerry, New Hampshire.

Councell, T. B., Duckenfield, K. U., Landa, E. R., & Callender, E., 2004. Tire-wear particles as a source of zinc to the environment. Environmental science & technology, 38(15), 4206-4214.

Ćujić, M., Dragović, S., Sabovljević, M., Slavković-Beškoski, L., Kilibarda, M., Savović, J., & Onjia, A., 2014. Use of mosses as biomonitors of major, minor and trace element deposition around the largest thermal power plant in Serbia. CLEAN–Soil, Air, Water, 42(1), 5-11.

Davies, L., Bates, J. W., Bell, J. N. B., James, P. W., & Purvis, O. W., 2007. Diversity and sensitivity of epiphytes to oxides of nitrogen in London. Environmental Pollution, 146(2), 299-310.

DeLuca, T. H., Zackrisson, O., Gundale, M. J., & Nilsson, M. C., 2008. Ecosystem feedbacks and nitrogen fixation in boreal forests. Science, 320(5880), 1181-1181.

Denier van der Gon, H. A., Gerlofs-Nijland, M. E., Gehrig, R., Gustafsson, M., Janssen, N., Harrison, R. M., ... & Krijgsheld, K., 2013. The policy relevance of wear emissions from road transport, now and in the future—an international workshop report and consensus statement. Journal of the Air & Waste Management Association, 63(2), 136-149.

Donovan, G. H., Jovan, S. E., Gatziolis, D., Burstyn, I., Michael, Y. L., Amacher, M. C., & Monleon, V. J., 2016. Using an epiphytic moss to identify previously unknown sources of atmospheric cadmium pollution. Science of the Total Environment, 559, 84-93.

Ecology & King County, 2011. Control of Toxic Chemicals in Puget Sound: Assessment of Selected Toxic Chemicals in the Puget Sound Basin, 2007-2011. Washington State Department of

Ecology, Olympia, WA and King County Department of Natural Resources, Seattle, WA. Ecology Publication No. 11-03-055. www.ecy.wa.gov/biblio/1103055.html

Ewing, S. A., Christensen, J. N., Brown, S. T., Vancuren, R. A., Cliff, S. S., & Depaolo, D. J. (2010). Pb isotopes as an indicator of the Asian contribution to particulate air pollution in urban California. Environmental science & technology, 44(23), 8911-8916.

Fernández, J. A., Aboal, J. R., & Carballeira, A., 2010. Testing differences in methods of preparing moss samples. Effect of washing on Pseudoscleropodium purum. Environmental monitoring and assessment, 163(1), 669-684.

Fernández, J. Á., Pérez-Llamazares, A., Carballeira, A., & Aboal, J. R., 2013. Temporal variability of metal uptake in different cell compartments in mosses. Water, Air, & Soil Pollution, 224(3), 1481.

Fowler, D., A. G. McDonald, A. Crossley, E. Nemitz, D. Leaver, J. N. Cape, R. I. Smith et al., 2006. UK heavy metal monitoring network.

Garg, B. D., Cadle, S. H., Mulawa, P. A., Groblicki, P. J., Laroo, C., & Parr, G. A., 2000. Brake wear particulate matter emissions. Environmental Science & Technology, 34(21), 4463-4469.

Gatziolis, D., Jovan, S., Donovan, G., Amacher, M., & Monleon, V., 2016. Elemental atmospheric pollution assessment via moss-based measurements in Portland, Oregon.

Geiser, L. H., & Neitlich, P. N., 2007. Air pollution and climate gradients in western Oregon and Washington indicated by epiphytic macrolichens. Environmental Pollution, 145(1), 203-218.

Gjengedal, E., & Steinnes, E., 1990. Uptake of metal ions in moss from artificial precipitation. Environmental Monitoring and Assessment, 14(1), 77-87.

González, A. G., & Pokrovsky, O. S., 2014. Metal adsorption on mosses: toward a universal adsorption model. Journal of colloid and interface science, 415, 169-178.

Graustein, W. C., 1989. 87Sr/86Sr ratios measure the sources and flow of strontium in terrestrial ecosystems. Stable isotopes in ecological research. Springer New York., pp. 491-512.

Graustein, W. C., & Armstrong, R. L., 1983. The use of strontium-87/strontium-86 ratios to measure atmospheric transport into forested watersheds. Science, 219(4582), 289-292.

Hallock, D., 2010. River and Stream Water Quality Monitoring Report – Water Year 2009. Washington State Department of Ecology, Olympia, WA. Publication No. 10-03-046. www.ecy.wa.gov/biblio/1003046.html

Harmens, H., Norris, D.A., Sharps, K., Mills, G., Alber, R., Aleksiayenak, Y., Blum, O., Cucu-Man, S.M., Dam, M., De Temmerman, L. and Ene, A., 2015. Heavy metal and nitrogen concentrations in mosses are declining across Europe whilst some “hotspots” remain in 2010. Environmental Pollution, 200, 93-104.

Holcombe, G. W., Benoit, D. A., Leonard, E. N., & McKim, J. M., 1976. Long-term effects of lead exposure on three generations of brook trout (Salvelinus fontinalis). Journal of the Fisheries Board of Canada, 33(8), 1731-1741.

Hulskotte, J. H. J., Roskam, G. D., & Van Der Gon, H. D., 2014. Elemental composition of current automotive braking materials and derived air emission factors. Atmospheric Environment, 99, 436-445.

INRIX, 2016. Traffic Scorecard.

Jaffe, D., Anderson, T., Covert, D., Kotchenruther, R., Trost, B., Danielson, J., Simpson, W., Berntsen, T., Karlsdottir, S., Blake, D. & Harris, J., 1999. Transport of Asian air pollution to North America. Geophysical Research Letters, 26(6), 711-714.

Jones, C.E., Halliday, A.N., Rea, D.K., & Owen, R.M., 2000. Eolian inputs of lead to the North Pacific. Geochimica et Comochimica Acta, 64(8), 1405-1416.

Kayzar, T. M., Nelson, B. K., Bachmann, O., Bauer, A. M., & Izbekov, P. E., 2014. Deciphering petrogenic processes using Pb isotope ratios from time-series samples at Bezymianny and Klyuchevskoy volcanoes, Central Kamchatka Depression. Contributions to Mineralogy and Petrology, 168(4), 1-28

Klopatek, J. M., Barry, M. J., & Johnson, D. W., 2006. Potential canopy interception of nitrogen in the Pacific Northwest, USA. Forest ecology and management, 234(1), 344-354.

Lanphear, B.P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D.C., Canfield, R.L., Dietrich, K.N., Bornschein, R., Greene, T. and Rothenberg, S.J., 2005. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environmental health perspectives, 894-899.

Legendre, P. & Legendre, L., 1998. Numerical Ecology, Elsevier.

Linbo, A. O., C.M. Stehr, J.P. Incardona, and N.L. Scholz, 2006. Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fishes. Environ. Toxicol. Chem. 25: 597-603.

McCune, B., 2000. Lichen communities as indicators of forest health. The Bryologist, 103(2), 353-356.

Miller, E. K., Blum, J. D., & Friedland, A. J., 1993. Exchangeable-cation loss and weathering rates using Sr isotopes. Nature, 362, 1.

NADP/NTN, 2015. National Atmospheric Deposition Program (NRSP-3)/ National Trends Network. Program Office, Illinois State Water Survery, Champaign, IL. See animated isopleth maps. <http://nadp.sws.uiuc.edu>.

National Research Council, 1975. Committee on Medical and Biological Effects of Environmental Pollutants Nickel, National Academy of Science, Washington, DC, USA.

Needleman, H. L., & Gatsonis, C. A., 1990. Low-level lead exposure and the IQ of children: a meta-analysis of modern studies. Jama, 263(5), 673-678.

Nevin, R., 2000. How lead exposure relates to temporal changes in IQ, violent crime, and unwed pregnancy. Environmental Research, 83(1), 1-22.

Perrenoud, A., Gasser, M., Rothen-Rutishauser, B., Gehr, P., and Riediker, M., 2010. Characterization of Nanoparticles Resulting from Different Braking Behaviours. Int. J. Biomed. Nanosci. Nanotechnol., 1:17–33.

Pott, U., & Turpin, D. H., 1998. Assessment of atmospheric heavy metals by moss monitoring with Isothecium stoloniferum Brid. in the Fraser Valley, BC, Canada. Water, Air, & Soil Pollution, 101(1), 25-44.

Puget Sound Clean Air Agency, 2015. 2015 Air Quality Data Summary Report. Seattle, WA.

Puget Sound Regional Council, 2014. Transportation 2040: toward a sustainable transportation sesystem (Update). PSR Council, Seattle, WA.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raymond, B.A. & U. Pott. 2003. Atmospheric contaminants in biota of forested watersheds near Vancouver, BC: Environment Canada. Georgia Basin Ecosystem Initiative.

Raymond, B. A., & Bassingthwaighte, T., 2010. Measuring nitrogen and sulphur deposition in the Georgia Basin, British Columbia, using lichens and moss. Journal of Limnology, 69(1s), 22-32.

Reimann, C., Niskavaara, H., Kashulina, G., Filzmoser, P., Boyd, R., Volden, T., Tomilina, O. and Bogatyrev, I., 2001. Critical remarks on the use of terrestrial moss (Hylocomium splendens and Pleurozium schreberi) for monitoring of airborne pollution. Environmental Pollution, 113(1), 41-57.

Ross, H. B., 1990. On the use of mosses (Hylocomium splendens and Pleurozium schreberi) for estimating atmospheric trace metal deposition. Water, Air, & Soil Pollution, 50(1), 63-76.

Ruhling, A., & Tyler, G., 1968. An ecological approach to lead problem. Botaniska Notiser, 121(3), 21.

Sandahl, J. F., Baldwin, D. H., Jenkins, J. J., & Scholz, N. L., 2004. Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (Oncorhynchus kisutch) exposed to copper, chlorpyrifos, or esfenvalerate. Canadian Journal of Fisheries and Aquatic Sciences, 61(3), 404-413.

Sandahl, J. F., Baldwin, D. H., Jenkins, J. J., & Scholz, N. L., 2007. A sensory system at the interface between urban stormwater runoff and salmon survival. Environmental science & technology, 41(8), 2998-3004.

Schintu, M., Cogoni, A., Durante, L., Cantaluppi, C., & Contu, A., 2005. Moss (Bryum radiculosum) as a bioindicator of trace metal deposition around an industrialised area in Sardinia (Italy). Chemosphere, 60(5), 610-618.

Shepard, D., 1968. A two-dimensional interpolation function for irregularly-spaced data. In Proceedings of the 1968 23rd ACM national conference (pp. 517-524). ACM.

Spehar, R. L., Anderson, R. L., & Fiandt, J. T., 1978. Toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. Environmental Pollution (1970), 15(3), 195-208.

Sposito, G., 1989. The chemistry of soils. Oxford Unvi. Press, New York, 277 pp.

Susfalk, R. B., & Johnson, D. W., 2002. Ion exchange resin based soil solution lysimeters and snowmelt solution collectors. Communications in Soil Science and Plant Analysis, 33(7-8), 1261-1275.

Tan, M.G., Zhang, G.L., Li, X.L., Zhang, Y.X., Yue, W.S., Chen, J.M., Wang, Y.S., Li, A.G., Li, Y., Zhang, Y.M. and Shan, Z.C., 2006. Comprehensive study of lead pollution in Shanghai by multiple techniques. Analytical chemistry, 78(23), 8044-8050.

Thöni, L., Schnyder, N., & Krieg, F., 1996. Comparison of metal concentrations in three species of mosses and metal freights in bulk precipitations. Fresenius' journal of analytical chemistry, 354(5-6), 703-708.

Wetherbee, G. A., Shaw, M. J., Latysh, N. E., Lehmann, C. M., & Rothert, J. E., 2010. Comparison of precipitation chemistry measurements obtained by the Canadian Air and Precipitation Monitoring Network and National Atmospheric Deposition Program for the period 1995–2004. Environmental monitoring and assessment, 164(1), 111-132.

WSDOT, 2015. Traffic Report.

U.S. Government, 2001. Control of emissions of hazardous air pollutants from mobile sources; final rule. Federal Register 40, CFR parts 80 and 86, US Government Printing Office, Washington, DC (2001)

Zvereva, E. L., & Kozlov, M. V., 2011. Impacts of industrial polluters on bryophytes: a meta-analysis of observational studies. Water, Air, & Soil Pollution, 218(1-4), 573-586.