Quantifying nitrogen-fixation in Acer macrophyllum canopy bryophytes in the Pacific Northwest, USA

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Research Article

Keywords: Nitrogen fixation, Bryophytes, Epiphytic moss, Temperate rainforest, Pacific Northwest

Posted Date: December 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2351539/v1

Version of Record: A version of this preprint was published at Plant and Soil on June 5th, 2023. See the published version at https://doi.org/10.1007/s11104-023-06082-8.

Abstract

Purpose **:** Old-growth forests in the Pacific Northwest host a variety of epiphytes on their branches and stem. Given the common and often large epiphytic biomass associated with *Acer macrophyllum* (Pursh) in this region, we evaluated how seasonal weather changes and urbanization (metal and nitrogen deposition), affect canopy epiphytic N_2 fixation in the Hoh Rainforest of the Olympic Peninsula and in urban parks and forests in Seattle.

Methods **:** We collected *Isothecium stoloniferum* (Brid.) samples from both the Hoh Rainforest and Seattle at four periods from April 2016 through January 2017. Moss-associated N_2 fixation rates were measured in the laboratory using the acetylene reduction assay and trace metal concentrations in the moss were analyzed using NO_3 + H_2O_2 digestion.

Results : We found levels of N_2 fixation were highest during the spring sampling period. Elevated levels of heavy metals were observed in *I. stoloniferum* samples collected in the urban canopies in Seattle, suggesting N_2 fixation is sensitive to the bioaccumulation of heavy metals. In *A. macrophyllum* canopies, *I. stoloniferum* 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 Seattle.

Conclusions : These results highlight a rarely explored source of biological N_2-fixation in temperate rainforests and suggest that epiphytic N_2-fixation may contribute bio-available nitrogen in secondary successional *A. macrophyllum* stands.

Introduction

There is an increasing interest in the role that epiphytic mosses play in forest ecosystem function and specifically N cycling (Gotsch et al., 2016; Shi et al., 2017; Van Langenhove et al., 2021); however, to date there has been limited effort to directly study this important component of temperate wet forest ecosystems. In the Pacific Northwest (PNW) region of North America, canopy dwelling epiphytes are thought to play an important role in biogeochemical cycles in late successional deciduous and coniferous forests (Pike et al., 1977; Binkley and Graham 1981; Pypker et al. 2006; Cornelissen et al. 2007; Haristoy et al. 2014), but to date there has been little effort to quantify N_2 fixation in these dense moss epiphytic communities. Bigleaf maple (*Acer macrophyllum* Pursh), a common hardwood species of this region, has a calcium rich bark and neutral pH that encourages the establishment of thick epiphytic mats on the stem and branches (Kenkel & Bradfield, 1986). On the Olympic Peninsula, epiphytic biomass in A. *macrophyllum* canopies have been estimated at 6,870 kg moss ha^{-1} (Nadkarni, 1984). These epiphytic mats, which contain a variety of lichen and bryophyte species, serve as sites for the accumulation and processing of nutrients and water (Proctor, 2001; Klopatek et al. 2006; Pypker et al., 2006).

Unlike vascular plants, bryophytes lack roots and primarily obtain their nutrients from atmospheric deposition or from capillary action from host plant branches (Bates, 1992). Bryophytes possess ion exchange properties, which allow for 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 forest floor bryophytes and cyanobacteria in boreal forests have been reported to contribute significant amount of nitrogen (N) to these ecosystems, especially 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. Cyanobacteria associated with canopy bryophytes have the potential to function as an important N source in N-limited PNW forests supporting 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 negatively influence bryophyte growth (Bengtsson et al., 1982; Pearson et al., 2000; Scott et al. 2018). Forest floor N_2 fixing bryophytes have been found to be sensitive to N deposition (Zackrisson et al., 2004; DeLuca et al., 2008; Ackermann et al., 2012) and N_2 fixation has been shown to cease in areas with increasing N deposition (Gundale et al. 2011; Zackrisson et al 2009). To date, there has not been a comprehensive study to evaluate N_2 fixation rates in epiphytic bryophytes of *A. macrophyllum* canopies. We hypothesized that 1) there will be measurable N_2 fixation in canopy bryophytes of *A. macrophyllum*; 2) N_2 fixation will be higher in samples collected from the Olympic Peninsula relative to urban sites; 3) urban sites will have higher concentrations of heavy metals in canopy bryophyte tissue.

Materials & Methods

2.1 Study Area

Moss samples were collected using a 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. *Acer macrophyllum* was chosen as it is widely distributed across western WA and hosts abundant growth of bryophytes. The study was conducted in one stand located in the Hoh Rainforest on Hoh River Trust land adjacent to Olympic National Park (47.8208 °N, -124.2002 °W) and three stands located in Seattle City Parks (Seward Park: 47.5551 °N, -122.2507 °W; Interlaken Park: 47.6358 °N, -122.3072 °W; Ravenna Park: 47.6732 °N, -122.3081 °W) (Fig. 1).

In the Hoh Rainforest, the dominant epiphytic moss species in *A. macrophyllum* canopies were *Isothecium stoloniferum (*Brid.*), Antitrichia curtipendula (*Hedw.*)*, and *Hylocomium splendens (Hedw.).* In the city of Seattle, the dominant epiphytic moss species in *A. macrophyllum* canopies were *I. stoloniferum (Brid.), Homalothecium fulgescens (Mitten ex Müller Hal.)*, and *Orthotrichum lyellii* Hook. & Taylor. *Isothecium stoloniferum* was chosen as the species of interest to compare N_2 fixation rates across all locations, because it is present at all sites (although total biomass and it has been used previously for biomonitoring studies conducted in British Columbia, Canada (Pott & Turpin, 1998; Raymond & Pott, 2003; Raymond et al., 2010). *Antitrichia curtipendula* and *H. splendens* samples were also collected from the Hoh Rainforest site to better understand canopy wide fixation rates in non-urban environments (Fig. 2).

2.2 Sample Collection

Using a 50.26cm^2 PVC tube core, replicate cores of moss samples were collected from a total of eighteen *A. macrophyllum* trees: nine from the Hoh Rainforest, WA and nine from city parks in Seattle, WA, USA over the course of four seasons (April (Spring), July (Summer), and October (Fall) 2016; and January (Winter) 2017). The selected trees had to have at least three accessible branches below a solid anchor point to allow us to obtain samples from three stratified heights throughout the crown: low (6-10m), mid (12-15m) and upper (15-18m). For each season at each tree (n = 18), three replicate moss cores were composited at each of the three canopy sampling locations. Details of tree selection and climbing techniques are available in Bidwell et al. (2019). Study trees were instrumented with ionic resin lysimeters (UNIBEST Ag Manager) between January 2016 and January 2017 to evaluate wet NO_3^--N and NH_4^+-N deposition throughfall rates (Susfalk and Johnson 2002; Kopatek et al. 2006; DeLuca et al. 2008), and HOBO data loggers (Onset Computer Corps., Bourne, MA, USA) to record hourly temperatures within the canopy.

2.3 Laboratory Analysis:

i. N_2 Fixation Analysis

Rates of N_2 fixation were estimated using the acetylene reduction (AR) method (Schöllhorn and Burris 1967). Three replicates per sample were placed into 20 mL glass vials and sealed with a rubber septum. Ten percent of the headspace was removed using a syringe and replaced with reagent grade acetylene. The tubes were incubated for 24 hours under greenhouse conditions (18°C) with ambient light to reflect natural day length. Ethylene generated 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 were represented 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, the theoretical conversion ratio of acetylene reduced to N_2 reduced of 3:1 was used as recently verified for *Pleurozium schreberi* and *H. splendans* (DeLuca et al. 2002; Zackrisson et al. 2009) Further, 20 samples from each of the three mosses were taken with a 20.27cm^2 PVC tube core. For *I. stoloniferum*, 20 cores were collected from *A. macrophyllum* canopies in the Hoh Rainforest and 20 from Seattle. 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.

ii. Trace Metal Concentrations:

After samples had been analyzed for N_2 fixation, caps were removed and tubes 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 nitrogen. The $HNO_3 + H_2O_2$ digestion method, described in Bidwell et al. (2019), was used to prepare the dried and ground samples for trace metal analysis. 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 and functioning of the ICP-OES. After calibration, quality control samples consisting of reagent and method blanks, low and high concentration standards, as well as certified standards SRM-1575 and SRM-1547 were analyzed.

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

Ionic resins were collected from canopy lysimeters in January 2017 during the final sampling round. Resin capsules were extracted with 30mL of 0.5M HCl using a 10 mL sequential extraction according to Ackermann et al. (2012). 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:

Response variables were tested to determine if they met the assumptions of Analysis of Variance (ANOVA). When needed, data were transformed using $\log_{10} + 1$ to normalize the distribution. ANOVA was used to test the main effects of sampling location (peninsula and urban), season, and canopy height, and their interactions on AR for *I. stoloniferum* samples collected from *A. macrophyllum*. To contrast N_2 fixation rates across the three moss species collected on the Olympic Peninsula (*I. stoloniferum, A. curtipendula*, and *H. splendens*), ANOVA was used to test the main effects of species, season and canopy height, and their interactions. Post-hoc testing (Tukey HSD) was carried out if significant interaction effects were found.

A two-way permutational multivariate analysis of variance (perMANOVA), based upon Euclidean distance, was conducted to test if AR 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*. Significance of the perMANOVA R-value was determined based upon 1000 random permutations when using a = 0.05.

Principle component analysis (PCA) was used as an ordination method where dissimilarity was calculated as Euclidean distance (Legendre & Legendre 1998). PCA was performed on a data matrix comprising the sampling location (urban or peninsula) and chemical variables for *I. stoloniferum*. Thirteen analytes were used in the PCA (µmol m^{-2} d^{-1}, Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn). PCA was performed to assess the dominant patterns in moss acetylene reduction rates and metal concentrations by sampling location. The loadings of variables to each PC was examined by converting eigenvector coefficients to structure correlations. All statistical analyses were conducted in the R Statistical Environment (RStudio Team, 2020).

Results

N_2 fixation rates in *I. stoloniferum* were highest in spring relative to all other seasons in both the Hoh Rainforest and Seattle (Fig. 3). Of the *I. stoloniferum* samples collected in spring, five Hoh rainforest samples and one Seattle sample AR exceeded 1,000 µmol m^{-2} day^{-1}. Canopy sampling height was not significant for *I. stoloniferum* AR rates; however, AR rates did significantly differ between Hoh Rainforest and Seattle locations ($F_{1,190}$ = 95.17, p < 0.001), and among sampling periods ($F_{3,190}$ = 86.96, p < 0.001). A significant interaction between location and sampling season was observed ($F_{3,190}$ = 7.30, p < 0.001), suggesting AR rates found in *I. stoloniferum* samples are dependent on the season in which they were collected. Both *A. curtipendula*, and *H. splendens* followed a similar season AR pattern observed for *I. stoloniferium* samples (Fig. 4). Canopy collection height had no significant effect on AR observed across the three moss species collected from the *A. macrophyllum* canopies in the Hoh Rainforest on the Olympic Peninsula. Individual moss species expressed significantly different AR rates ($F_{2,207}$ = 55.78, p < 0.001) and responded differently to seasonal change resulting in a significant interaction between species and sampling period ($F_{6,207}$ = 4.72, p < 0.001).

By using a season-weighted average and a scaling approach based on moss biomass and canopy density, we estimated that *I. stoloniferum* yields a 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 the Hoh Rainforest canopy and 0.009 kg N ha^{-1} yr^{-1} for the Seattle canopy. N_2 fixation rates for *A. curtipendula* in the Hoh Rainforest are estimated at 0.63 kg N ha^{-1} yr^{-1}.

Nitrogen fixation and trace metal concentrations were significantly different between peninsula and urban locations ($F_{1,212}$ = 249.72, p < 0.001), and among sampling seasons ($F_{3,212}$ = 29.06, p < 0.001) for the canopy *I. stoloniferum* samples. There was a significant interaction between sampling location and season ($F_{3,206}$ = 4.00, p < 0.001), suggesting that N_2 fixation rates and metal concentrations in moss tissue depends on the location and season it was collected. Considering the location type term, the DISPER results showed no significant differences in between-site variation ($F_{1,211}$=1.00, p = 0.324) and pairwise comparisons also showed no significant differences by location. The first two dimensions of the PCA explained ~ 85.8% of the variation, with the first dimension explaining ~ 60.7% (Fig. 5). Within this first dimension, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn were positively and heavily loaded (loading scores 0.765 to 0.999). For the second dimension, which accounted for ~ 25.2% of the variance, $\mu mol\ m^{-2}\ day^{-1}$ and Cd were positively and heavily loaded (loading scores 0.899 to 0.984). Metal concentration vectors indicated that *I. stoloniferum* in urban canopy samples were high in metal concentration compared to those collected from the peninsula.

Total NO_3^--N and NH_4^+-N (kg ha^{-1}) accumulated in resin lysimeters for 1 year in Hoh Rainforest and Seattle *A. macrophyllum* canopies were not significantly influenced by canopy height ($p = 0.11$ and $p = 0.51$, respectively); therefore, an average deposition rate was used for each sampling location. Higher rates of wet NH_4^+-N deposition were observed in the Hoh Rainforest canopy compared to urban canopies in Seattle (Table 1). However, no significant differences were observed across peninsula and urban canopies for annual wet NO_3^--N deposition rates.

Table 1

Comparison of wet inorganic-N deposition (kg ha^{-1}) across the four sampling locations instrumented with canopy resin lysimeters, and four National Atmospheric Deposition Program – National Trends Network sites in Washington State, USA.

Location	Latitude (°N), Longitude (°W)	Collection Type	NO_3^- - N (kg ha^{-1})	NH_4^+ - N (kg ha^{-1})
Hoh Rainforest	47.8208, -124.2002	Canopy Resin Lysimeter	1.92	2.61
Interlaken Park	47.6358, -122.3072	Canopy Resin Lysimeter	2.28	1.03
Ravenna Park	47.6723, -122.3081	Canopy Resin Lysimeter	1.87	0.57
Seward Park	47.5551, -122.2507	Canopy Resin Lysimeter	2.83	1.14
NADP WA-14 Station (Olympic NP – Hoh Ranger Station)	47.8597, -123.9325	Rainfall Collector	2.97	3.24
NADP WA-19 Station (North Cascades NP – Marblemount Ranger Station)	48.5403, -121.4460	Rainfall Collector	3.36	1.20
NADP WA-99 Station (Mt Rainier NP – Tahoma Woods)	46.7582, -122.1243	Rainfall Collector	2.81	1.47
NADP WA-98 Station (Columbia River Gorge)	45.5694, -122.2100	Rainfall Collector	2.55	1.37

Temperature data was recorded at three heights within the canopy of sample trees, and no significant difference in temperature across canopy height was found ($p = 0.75$). For both Hoh Rainforest and Seattle sites, the highest average AR rates were observed under temperatures greater than 17°C, which occurred in April 2016 (Fig. 6).

Discussion

This is the first study to quantify N_2 fixation rates in canopy mosses found in *A. macrophyllum* stands in the PNW and add to the list of ecosystem services provided by the iconic moss draped forests of the PNW. These results document and quantify biological N_2 fixation in epiphytic mosses in *A. macrophyllum* canopies and their N contribution to the forest ecosystem. N_2 fixation rates were found to vary by season and were notably greater in rural settings compared to urban environments. N_2 fixation in rural settings were found to average 1.6 kg N2 ha-1 which is similar to the rates described for epiphytic mosses in tropical rainforests (Van Langenhove et al. 2022).

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 & Marchant, 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 and Olsen, 1997; Solheim et al., 2002; Gundale et al., 2009). Gentili et al. (2005) found that N_2 fixation in feathers moss, *P. schreberi*, reached its peak between 13–22°C. The lowest AR rates, observed during October 2016, did not correspond to the lowest average daily temperature. It is possible that precipitation during the month of October, in addition to cooler daily temperatures, influenced AR rates in canopy *I. stoloniferum* samples. Total precipitation in the PNW in summer is generally low, but increases in fall. In October 2016, total precipitation in Forks, WA (closest NOAA weather station located to the Hoh Rainforest) and Seattle was 59.99 cm and 26.16 cm, respectively (NOWData). Previous studies have observed a positive relationship between rainfall frequency and N_2 fixation rates in boreal ecosystems (DeLuca et al., 2002; Zielke et al., 2002; Gundale et al., 2009; Gundale et al., 2012; Rousk et al., 2014). In this case there was no moisture limitation in October, but perhaps samples collected in October 2016 exceeded the optimum moisture requirement to facilitate N_2 fixation (see Rousk et al. 2017). However, the moisture dependence of N_2 fixation in lichens can differ between habitats, likely as a result of adaptations, and a completely hydrated status may lead to glucose efflux, depleting the energy reserves necessary for N_2 fixation (Kershaw 1985).

The N_2 fixation rates observed for *H. splendens* collected from the Hoh Rainforest during the April sampling season exceeded levels previous recorded in Northern Sweden (Zachrisson et al., 2009). However, in the PNW, *H. splendens* has a patchy distribution in *A. macrophyllum* canopies, which would limit its contribution to stand-level N.

We did not identify or quantify cyanobacterial communities associated with canopy bryophytes in *A. macrophyllum*. Previous studies have identified four cyanobacteria species (*Nostoc*, *Stigonema*, *Calothrix*, and *Cylindrospermum*) as known colonizers on *Pleurozium schreberi* (Brid.) Mitt. (DeLuca et al., 2007; Ininbergs et al., 2011). Lindo and Whiteley (2011) found a linear relationship between density of *Scytonema* cells and N_2 fixation in the canopy of Sitka spruce (*Picea sitchensis* (Bong) Carr.). As *Scytonema* spp. are predominately aerially dispersed (Komarek et al., 2003), it is possible that *Scytonema* would be the dominant genus in *A. macrophyllum* canopies. Future research would be required to evaluate the diversity of cyanobacteria living epiphytically on canopy bryophytes in old growth *A. macrophyllum* canopies.

In boreal regions where there is limited presence of leguminous or actinorhizal N_2 fixing plants, biological N_2 fixation associated with *P. schreberi* represents a primary source of ecosystem N at 1–3 kg N ha^{-1} yr^{-1} (DeLuca et al., 2002; Zackrisson et al. 2009). However, it remains unclear how N fixed through cyanobacteria associations enters the soil for plant uptake. Because mosses are efficient in retaining nutrients (Oechel and Van Cleve, 1986; Aldous, 2002), the rate at which fixed N is transferred to soil and plants is expected to be slow (Rousk et al., 2014; DeLuca et al 2022). In *A. macrophyllum* canopies, fixed N may enter the environment by accumulating in canopy soil. In a study conducted in *A. macrophyllum* canopies along the Queets River, located on the Olympic Peninsula, Haristoy et al. (2014) reported a C/N ratio of 22 for canopy soils, and NH_4^+ to be the dominant form of extractable N. This suggests 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 and Winchester, 2007; Berg and McClaugherty, 2008). Another potential pathway for fixed N from canopy bryophytes is being deposited to the forest floor as epiphytic litterfall and throughfall. As epiphytic litterfall decomposes on the forest floor it can be available for terrestrially root plants (Nadkarni and 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 source of N in PNW forests where N is a primary limiting nutrient (Oren et al. 2001; DeLuca et al., 2022)

Given that bryophytes exhibit a high cation exchange capacity in their surface tissue and generate acidity through proton release from exchange sites (Spearing, 1972), this effect may inhibit microbial populations and thus limit 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 and N_2 fixation rates were significantly higher across all seasons in the Hoh Rainforest canopies. This would suggest that N_2 fixation is not only a function of the seasonal weather influences, but also strongly influenced by metal concentrations absorbed and adsorbed on the moss tissue (Scott et al., 2018). The expected lower levels of metals in the Hoh Rainforest is likely due to lower industrial activity and a lower human population density. Because the Puget Sound Region is expected to continue to increase in human population and transportation networks (Puget Sound Regional Council, 2020), it is likely urban canopies will continue to experience increasing rates of metal deposition and decreasing rates of biological N_2 fixation compared to canopies on the relatively pristine Olympic Peninsula.

Conclusion

Few studies have evaluated N_2 fixation in canopy bryophytes. This study represents the first report on bryophyte N_2 fixation in *A. macrophyllum* canopies of the PNW and is the first to assess the effects of urbanization on seasonal N_2 fixation associated with canopy bryophytes. N_2 fixation in canopy bryophytes was found to be highly sensitive to urban pollution, likely due to the bioaccumulation of anthropogenic heavy metals in bryophyte tissue. Mosses have been found to be highly sensitive to seasonal influences with the greatest N_2 fixation observed during wet spring conditions. Predicted changes in temperature and precipitation patterns associated with climate change will affect N_2 fixation inputs into the environment through bryophyte-cyanobacteria pathways. These results help improve our understanding of ecological function of bryophyte communities that cover the limbs of trees throughout maritime and temperate rainforest landscapes.

Declarations

ACKNOWLEDGEMENTS

This work was supported by the USDA National Institute of Food and Agriculture, McIntire-Stennis Cooperative Forestry Program (Project #1006427 to THD and PCT). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA. The authors would like to thank Sean Callahan, Jake Betzen, and Constance Lin for their hard work in the field, and Si Gao for her help in the lab. This research was conducted under Hoh River Trust general use permit, and the City of Seattle Department of Parks and Recreation special use permit. This research was conducted by ALB in partial fulfillment of the requirements for the M.S. degree from the University of Washington.

AUTHORS CONTRIBUTIONS

Amanda L. Bidwell: Conceptualization, Data Curation, Analysis, Methodology, Visualization, Writing (Original draft preparation, Reviewing & Editing), Funding Acquisition. Patrick C. Tobin: Conceptualization, Methodology, Writing (Original draft preparation, Reviewing & Editing), Funding Acquisition. Thomas H. DeLuca: Conceptualization, Methodology, Writing (Original draft preparation, Reviewing & Editing), Supervision, Project Administration, Funding Acquisition.

DATA ACCESSABILITY

The datasets generated during and analyzed during the current study are available at DRYAD entry DOI

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Figures

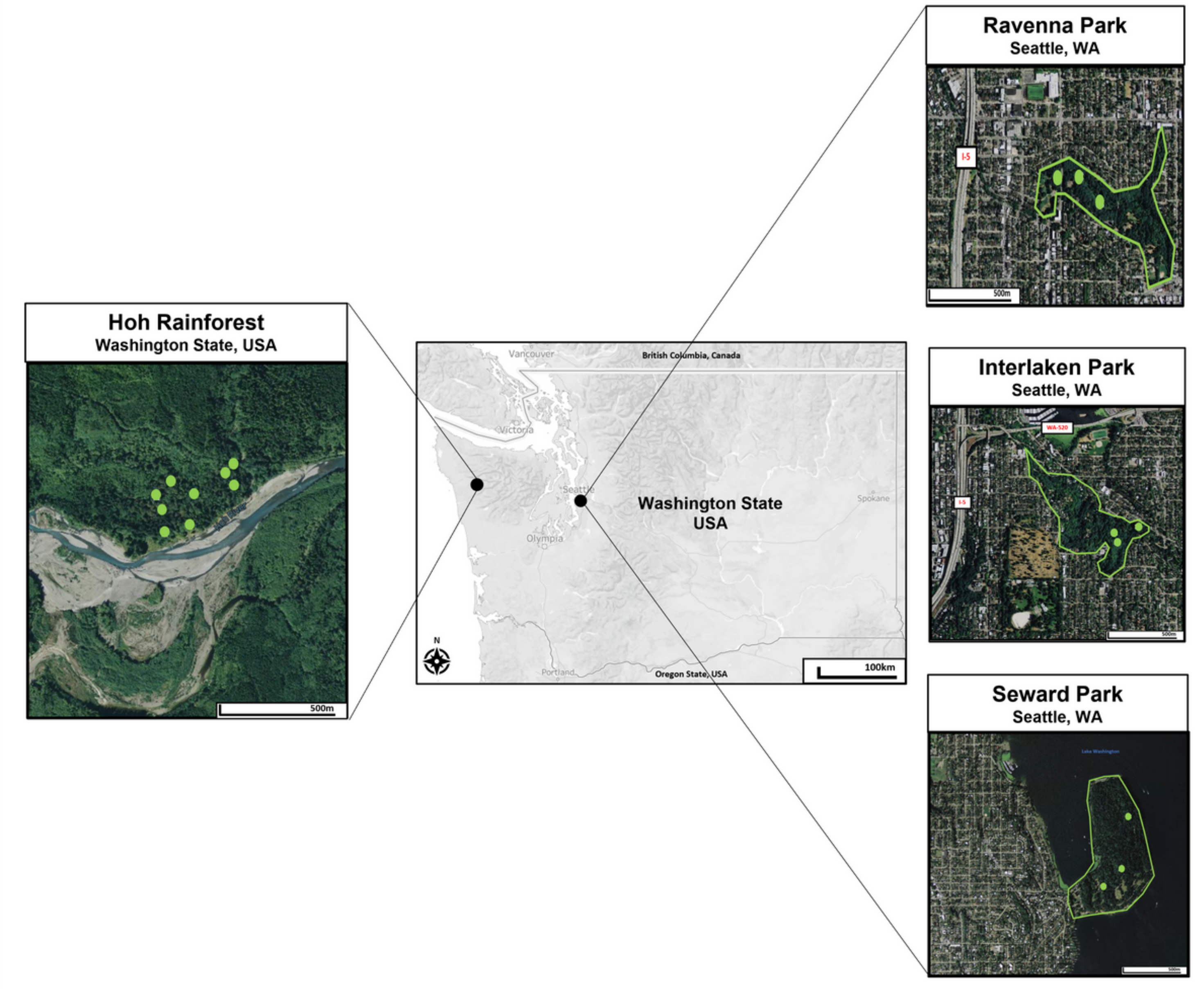

Figure 1

Location of sampling sites for epiphytic mosses in canopies of *Acer macrophyllum* in the Hoh Rainforest on the Olympic Peninsula, and in three distinct urban parks in the City of Seattle.

Figure 2

A comparison of canopy moss distribution from the Hoh Rainforest (left) and Seattle (right) sites. The photo on the left is from the mid canopy sampling location for Tree 3 at Hoh Rainforest site. The photo on the right is from the mid canopy sampling location for Tree 2 at the Ravenna Park site in Seattle.

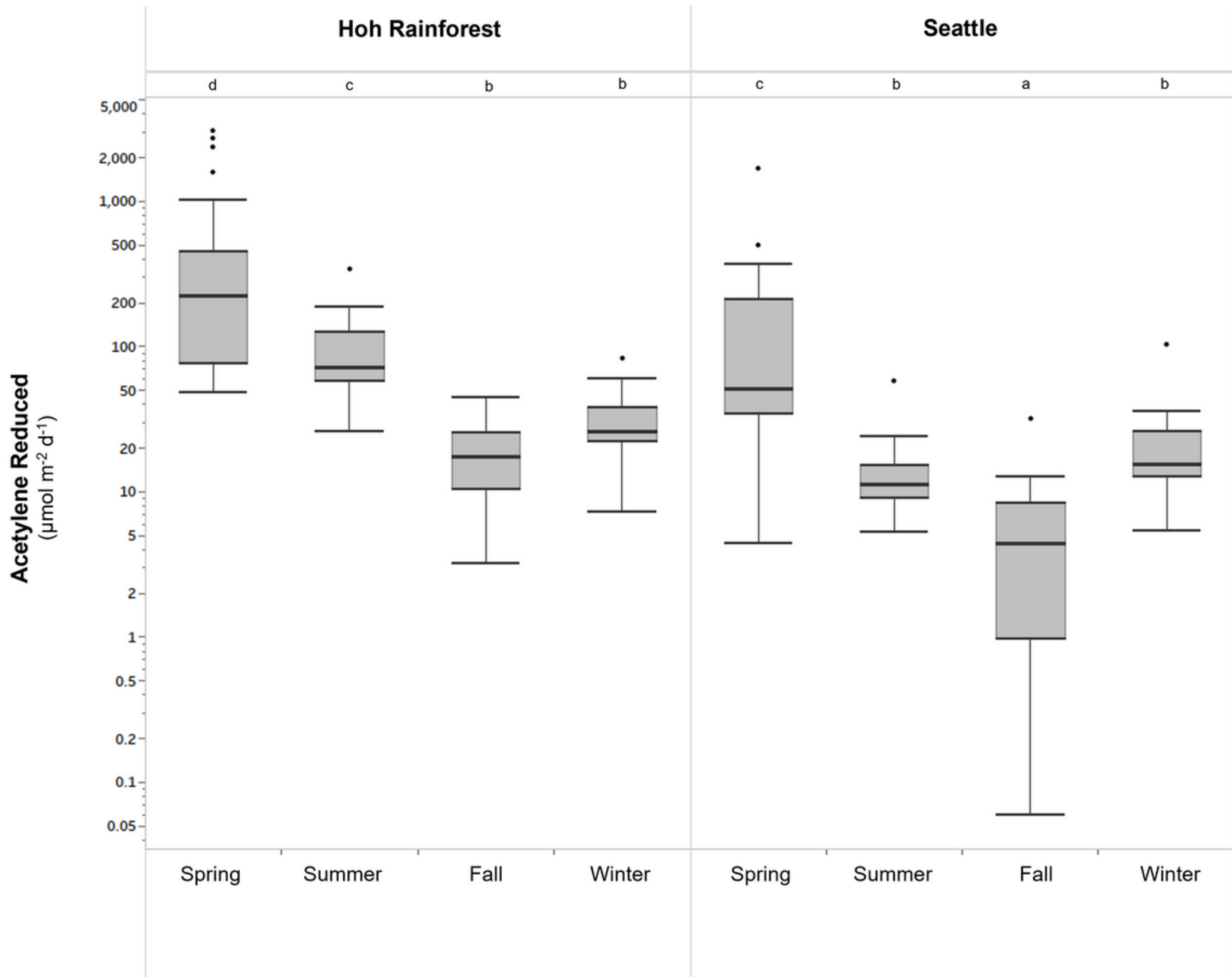

Figure 3

Nitrogenase activity measured in *I. stoloniferum* collected from *A. macropyllum* canopies from the Hoh Rainforest on the Olympic Peninsula and Seattle urban parks using the acetylene reduction method and reported as µmol acetylene reduced m^{-2} day^{-1} (note log scale). Significant differences (a $\underline{=}$ 0.05) in N_2 fixation across locations and season are indicated by different letters shown above individual boxplots based upon Tukey's HSD test.

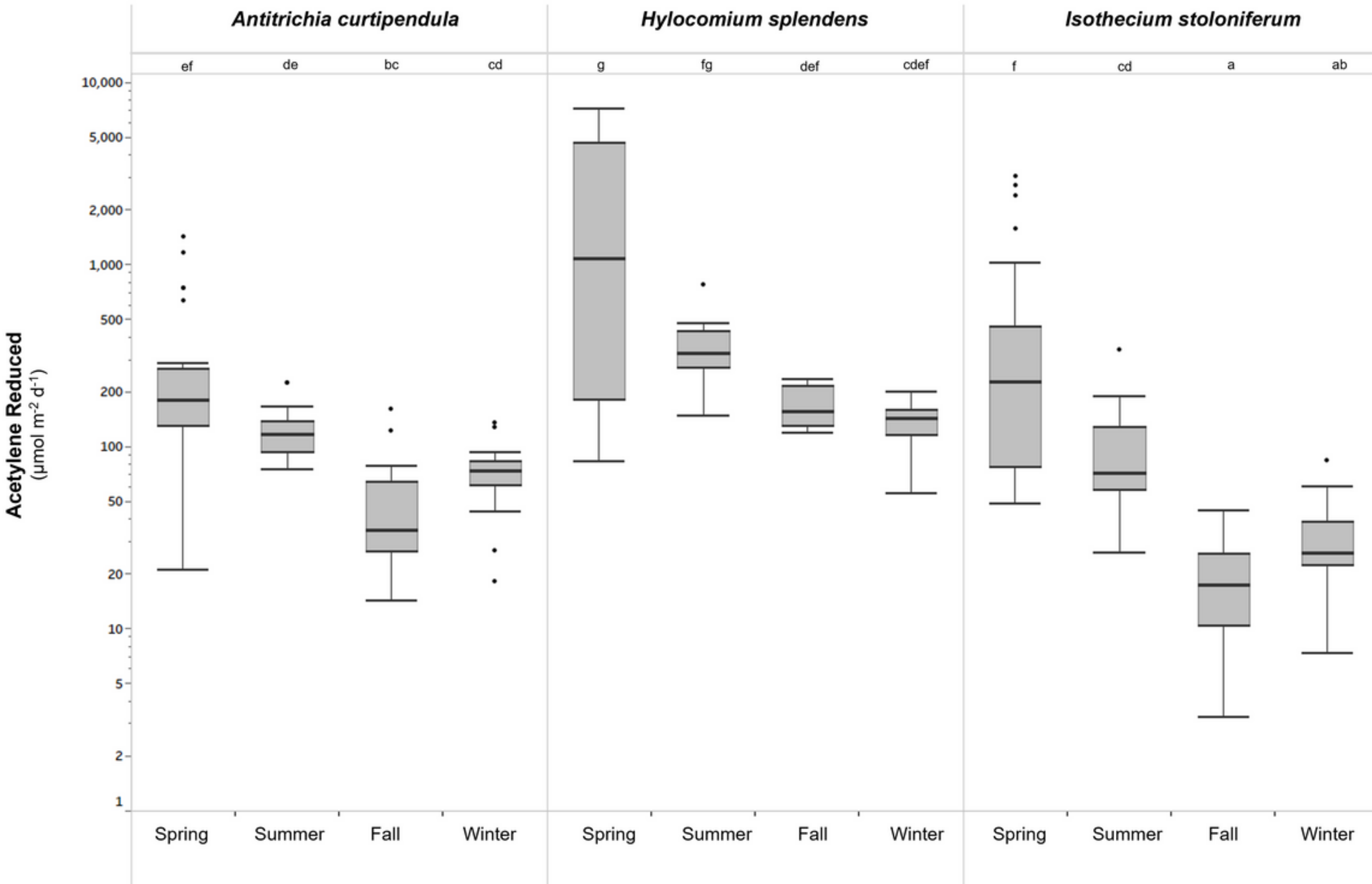

Figure 4

Seasonal estimates of nitrogenase activity as measured using acetylene reduction across a single *Acer macrophyllum* stand in the Hoh Rainforest on the Olympic Peninsula for the individual moss species: *Antitrichia curtipendula* (n=107), *Hylocomium splendens* (n=31), and *Isothecium stoloniferum* (n=105) and reported as μmol acetylene reduced m^{-2} day^{-1} (note log scale). Significant differences (a = 0.05) in nitrogenase activity across locations and season are indicated by different letters shown above individual boxplots based upon Tukey's HSD test.

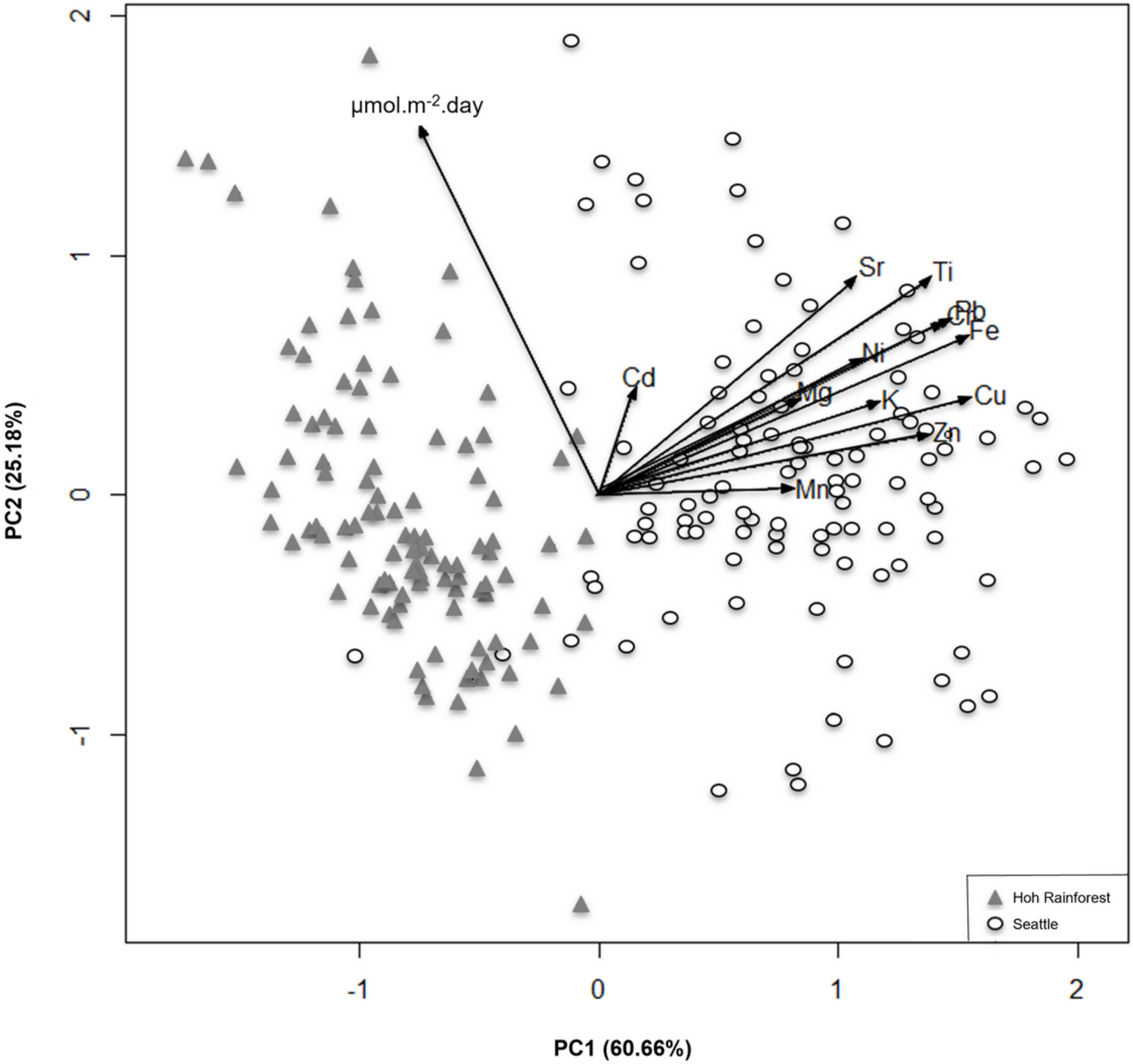

Figure 5

Principal component analysis (PCA) ordination of *I. stoloniferum* samples collected from the Hoh Rainforest on the Olympic Peninsula (gray triangles) and Seattle urban parks (open circles) in western WA. Nitrogenase activity is represented as $\mu mol.m^{-2}.day$.

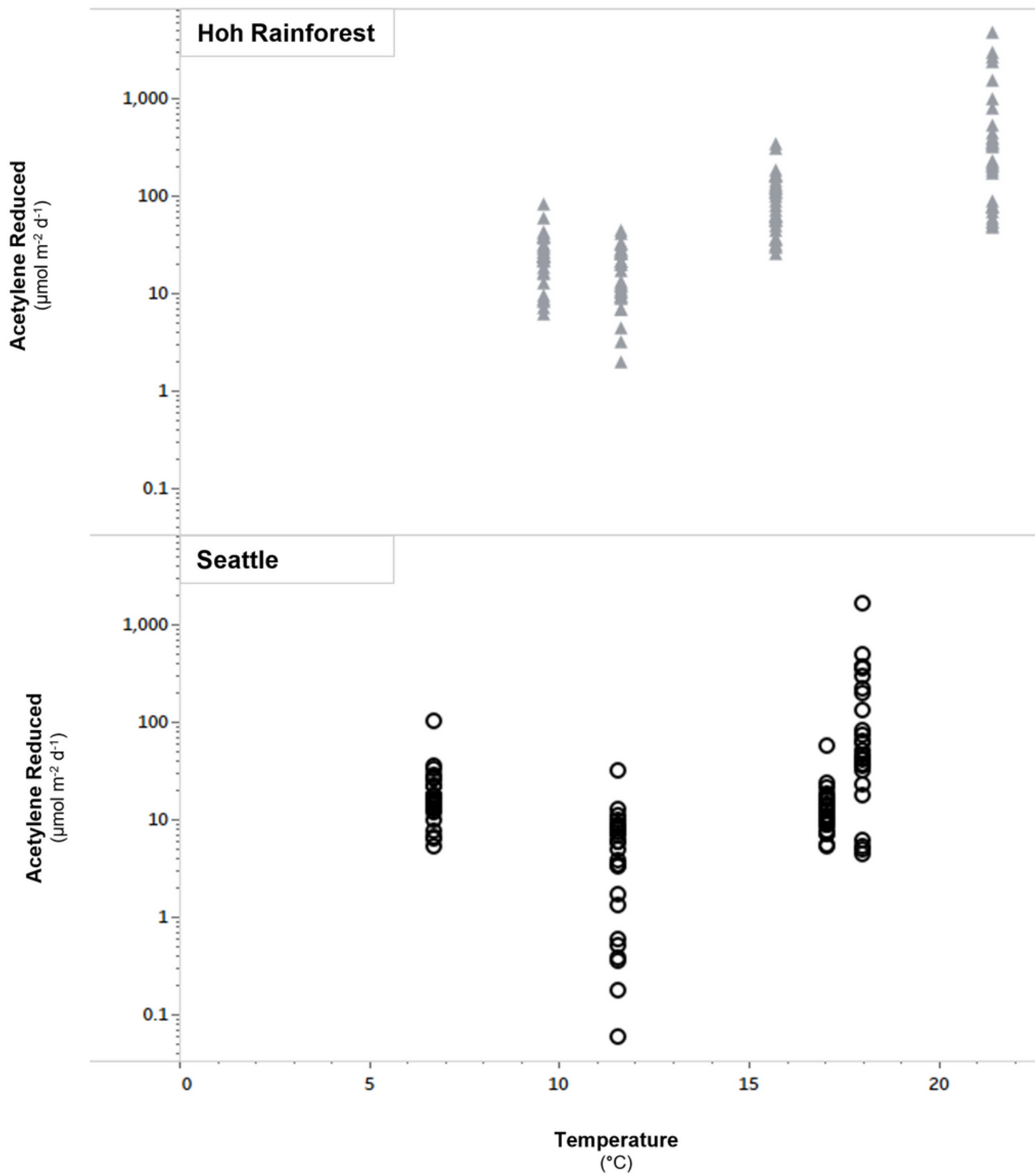

Figure 6

Relationship between seasonal nitrogenase activity represented as µmol acetylene reduced m^{-2} day^{-1} (note log scale) and ambient temperature at the point of sample collection for *I. stoloniferum* samples collected from the Hoh Rainforest (gray triangles) and Seattle urban parks (open circles) in western WA.