**ECOSYSTEMS**

# Host Identity as a Driver of Moss-Associated $N_2$ Fixation Rates in Alaska

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## ABSTRACT

Moss-associated $N_2$ fixation provides a substantial but heterogeneous input of new N to nutrient-limited ecosystems at high latitudes. In spite of the broad diversity of mosses found in boreal and Arctic ecosystems, the extent to which host moss identity drives variation in $N_2$ fixation rates remains largely undetermined. We used $^{15}N_2$ incubations to quantify the fixation rates associated with 34 moss species from 24 sites ranging from 60° to 68° N in Alaska, USA. Remarkably, all sampled moss genera fixed $N_2$, including well-studied feather and peat mosses and genera such as *Tomentypnum*, *Dicranum*, and *Polytrichum*. The total moss-associated $N_2$ fixation rates ranged from almost zero to 3.2 mg N m$^{-2}$ d$^{-1}$, with an average of 0.8 mg N m$^{-2}$ d$^{-1}$, based on abundance-weighted averages of all mosses summed for each site. Random forest models indicated that moss taxonomic family was a better predictor of rate variation across Alaska than any of the measured environmental factors, including site, pH, tree density, and mean annual precipitation and temperature. Consistent with this finding, mixed models showed that trends in $N_2$ fixation rates among moss genera were consistent across biomes. We also found "hotspots" of high fixation rates in one-fourth of sampled sites. Our results demonstrated the importance of moss identity in influencing $N_2$ fixation rates. This in turn indicates the potential utility of moss identity when making ecosystem N input predictions and exploring other sources of process rate variation.

**Key words:** $N_2$ fixation; Alaska; Bryophytes; Boreal forest; Arctic tundra; Hotspots.

## HIGHLIGHTS

- Host moss identity is a key driver of associated $N_2$ fixation rates
- Significant, consistent $N_2$ fixation rate variation was observed between moss genera

- Measuring all present mosses revealed hotspots of $N_2$ fixation

# INTRODUCTION

High latitude ecosystems, such as Arctic tundra and boreal forest, are globally important carbon (C) reservoirs that are often nitrogen (N) limited for vascular plants (Shaver and Jonasson 1999; Lebauer and Treseder 2008; Tarnocai and others 2009; Hugelius and others 2014). The largest source of new N in these ecosystems comes from microbial $N_2$ fixers that live as epiphytes on boreal and Arctic mosses (Alexander and Schell 1973; Basilier 1979; DeLuca and others 2002; Lindo and others 2013; Vile and others 2014). The $N_2$-fixing microbes that are associated with mosses are generally considered to be autotrophic cyanobacteria, although some evidence does exist for a material exchange between host moss and symbiont as well as for the presence of heterotrophic $N_2$ fixers (Vile and others 2014; Warshan and others 2017). Rates of moss-associated $N_2$ fixation are connected to ecosystem nutrition, disturbance response, and C budget (Cornelissen and others 2007). Current evidence indicates that moss community structure and $N_2$ fixation rates will be affected directly or indirectly by warming temperatures (Gundale and others 2012; Turetsky and others 2012; Deane-Coe and others 2015; Carrell and others 2019). Given this, making accurate predictions to changes in N inputs and its downstream effects on plant communities and C cycling must also rely on knowledge of interspecific variation in $N_2$ fixation (Hobbie 1995; Chapin 2003). Mosses are often undifferentiated from each other (or very coarsely differentiated) in vegetation models, but $N_2$ fixation rates could be an important classification trait, particularly since microbial symbionts can be considered an extension of plant phenotype (Turetsky and others 2012; Wullschleger and others 2015; St. Martin and Mallik 2017). The importance of moss-associated $N_2$ fixation rates in regulating C balance is clear (Lindo and others 2013), and exploring the role of host identity in $N_2$ fixation can complement and improve biogeochemical predictions as climate changes.

Although $N_2$ fixation associated with mosses is presumably as important in boreal and Arctic Alaska as it is in other high-latitude ecosystems, very few studies have been published on moss-associated $N_2$ fixation rates in Alaska (Alexander and Schell 1973; Holland-Moritz and others 2018; Jean and others 2018). Angiosperms with symbiotic $N_2$ fixers in Alaska, such as *Alnus* spp., fix $N_2$ at locally high rates, but mosses are ubiquitous in the understory of the boreal forest and tundra ecosystems (Hobbie and others 2005; Mitchell and Ruess 2009; Turetsky and others 2010). Cyanolichens such as *Peltigera* spp. also fix $N_2$ at high rates per unit biomass, but are less abundant on the landscape (Weiss and others 2005). Alaska is relatively pristine, largely underlain by permafrost, and expected to respond differently to climate change than similar ecosystems in Europe (Van Wijk and others 2004; Holland and others 2005; Pastick and others 2015; Gisnås and others 2017). The majority of reported $N_2$ fixation rates associated with mosses focus on northern Europe and common mosses such as *Sphagnum* spp., *Hylocomium splendens,* and *Pleurozium schreberi*. Feather mosses are often abundant in upland forest areas, where *Hylocomium splendens* and *Pleurozium schreberi* are co-dominant, but other mosses (*Aulacomnium turgidum, Aulacomnium palustre, Tomentypnum nitens*, and so on) can have patchy but high local abundances throughout Alaska (Vanderpuye and others 2002; Walker and others 2003; Turetsky and others 2010). In Siberia, *P. schreberi* is less abundant than *H. splendens, T. nitens,* and *Aulacomnium turgidum*, which often co-dominate (Suzuki and others 2007; Minke and others 2009; Boike and others 2013). Functional traits of mosses have been identified as influential on rates of $N_2$ fixation, and there is a large diversity in growth form and habitat preferences among the mosses listed above (Darell and Cronberg 2011; Elumeeva and others 2011; Jonsson and others 2014). Further, most studies have utilized $^{15}N_2$ calibrated or uncalibrated acetylene reduction assays to measure $N_2$ fixation, though recent evidence suggests that conversion factors for this method may be inconsistent temporally, spatially, or across moss species (Saiz and others 2019). Expanding the scope of $N_2$ fixation measurements to include more mosses and different geographic areas while utilizing $^{15}N_2$ uptake can improve the current state of knowledge about this process.

Numerous biotic and abiotic variables have been shown to affect rates of moss-associated $N_2$ fixation, but often experiments that focus on sources of environmental variation (such as temperature, moisture, N deposition, or phosphorus (P) availability) will test their hypotheses with only one or two species of host mosses (Rousk and others 2013). Several studies report that the study location (and its associated biotic and abiotic factors) appears to be less important to microbial community composition and *nifH* gene expression than the host species in question, indicating a specificity

between N$_2$ fixer communities and host mosses (Ininbergs and others 2011; Bragina and others 2012; Holland-Moritz and others 2018; Jean and others 2020). Bay and others (2013) have shown that mosses likely chemo-attract cyanobacteria and induce hormogonia formation, which may be a source of specificity in host-microbe association. Mosses also have individual and community traits that may influence rates of N$_2$ fixation, such as community water retention or shade tolerance, and occupy specific micro-niches suitable to their growth (Mills and Macdonald 2004; Elumeeva and others 2011; Jonsson and others 2014). Differing microbial assemblages will react inconsistently to the same abiotic conditions (Gundale and others 2012; Leppänen and others 2015), as, for example, cyanobacteria have different temperature optima than other N$_2$-fixing bacteria (Gentili and others 2005). There may also be seasonal variation in N$_2$ fixation rates both within and between species, making it potentially difficult to disentangle these microbial community composition effects (Bay and others 2013; Lett and Michelsen 2014; Rousk and Michelsen 2017). Other studies indicate that site and abiotic factors are just as important, or more important, than host species identity in their effects on N$_2$ fixation (Gavazov and others 2010; Arróniz-Crespo and others 2014). Nitrogen availability has consistently been shown to drive N$_2$ fixation rates (DeLuca and others 2007; Ackermann and others 2012; Bay and others 2013; Gundale and others 2013b). Moisture, light availability, and micronutrient availability have also been shown to positively affect rates of N$_2$ fixation in general (Gundale and others 2012; Rousk and others 2013, 2017). Vascular plant assemblage can have indirect effects on N$_2$ fixation rates (for example, through canopy light penetration), which itself is affected by moisture and permafrost thaw depth (Yang and others 2013; Jonsson and others 2014). While environmental factors clearly influence rates of N$_2$ fixation, their impacts can be complex and likely interact with host identity.

In addition to the challenge of identifying the primary drivers of N$_2$ fixation rate variation, the presence of biogeochemical ''hotspots'' can further increase the difficulty of scaling N inputs to plant communities or ecosystems (Reed and others 2011). After Reed and others (2010), a hotspot is defined as a rate of N$_2$ fixation that exceeds the median rate by more than three standard errors (SE). For other aspects of the N cycle, identifying where and why hotspots and hot moments occur was identified as critical for improving models (Groffman and others 2009). Determining the

geographic or temporal abundance of hotspots, as well as gaining insight into the causes of hotspots, can facilitate their inclusion in models (Reed and others 2011). To our knowledge, hotspots have not been explicitly explored in moss-associated N$_2$ fixation, although past research indicates that increasing microbial diversity is tied to higher N$_2$ fixation rates and the occurrence of hotspots in tropical free-living N$_2$ fixers (Reed and others 2010).

Our objective in this study was to evaluate the relative importance of host moss identity in driving landscape-level variation in associated N$_2$ fixation rates and, more specifically, to test for significant differences in N$_2$ fixation rates among mosses. We used $^{15}$N$_2$ incubation assays to determine the fixation rates associated with a total of 34 moss species across three broad geographic regions in Alaska. We used an exploratory random forest approach to determine variable importance in predicting N$_2$ fixation rates. We hypothesized that host moss genus would be a significant source of variation in N$_2$ fixation rates across a geographic region. To test for differences in N$_2$ fixation rates between mosses, we used mixed models with moss genus as a fixed effect. We also assessed the occurrence of hotspots of N$_2$ fixation and what may contribute to their presence. In this context, evaluating the role of moss identity in predicting trends in associated N$_2$ fixation across a latitudinal gradient can provide valuable insights into the sources of process variation and the occurrence of hotspots. Through these goals, we highlighted possible tools and challenges for producing more accurate regional estimates of N$_2$ fixation rates. The diverse array of host mosses included in our research, along with a corresponding suite of environmental data from a range of ecosystems, allowed us to uniquely identify the importance of host moss identity in contributing to variation in N$_2$ fixation rates.

## MATERIALS AND METHODS

### Sites

During late June 2016, ten sites were sampled near Fairbanks, AK and 4 sites sampled near Toolik Field Station, AK, in boreal forest or alpine tundra and Arctic tundra, respectively. The following year, in June 2017, an additional ten sites were sampled in the Anchorage, AK area, including *Tsuga* spp. dominated stands on the Kenai peninsula, *Picea* spp. areas near the University of Alaska Anchorage, and alpine tundra (Tables 1, 2). Sites were selected based on the absence of obvious disturbance, their

**Table 1.** Location and Description of Sites

| Site No. | Region | Location | Site Description |
|---|---|---|---|
| 1 | Fairbanks | N 64° 76.759′ W 148° 29.651′ | *P. mariana* upland |
| 2 | Fairbanks | N 64° 77.113′ W 148° 27.303′ | *P. mariana* upland |
| 3 | Fairbanks | N 64° 76.823′ W 148° 29.586′ | *P. mariana* and *P. glauca* upland |
| 4 | Fairbanks | N 64° 70.662′ W 148° 30.995′ | Mixed deciduous/conifer upland |
| 5 | Fairbanks | N 64° 70.377′ W 148° 29.731′ | *P. mariana* wetland |
| 6 | Fairbanks | N 64° 70.213′ W 148° 29.165′ | Open canopy *Sphagnum* wetland |
| 7 | Fairbanks | N 64° 86.718′ W 147° 85.897′ | *P. mariana* tussock |
| 8 | Fairbanks | N 64° 95.692′ W 148° 36.926′ | Alpine heath tundra |
| 9 | Fairbanks | N 64° 88.142′ W 148° 39.093′ | *B. neoalaskana* upland |
| 10 | Fairbanks | N 64° 88.324′ W 148° 39.555′ | *P. mariana* upland |
| 11 | Toolik | N 68° 64.132′ W 149° 58.541′ | Heath tundra |
| 12 | Toolik | N 68° 63.869′ W 149° 56.812′ | *B. nana* shrub tundra |
| 13 | Toolik | N 68° 63.902′ W 149° 56.761′ | Moist acidic tussock tundra |
| 14 | Toolik | N 68° 63.404′ W 149° 63.964′ | Moist non-acidic tussock tundra |
| 15 | Anchorage | N 61° 11.720′ W 149° 48.396′ | *P. mariana* wetland |
| 16 | Anchorage | N 61° 11.853′ W 149° 48.584′ | *P. mariana* upland |
| 17 | Anchorage | N 61° 09.397′ W 149° 47.879′ | Mixed deciduous/conifer upland |
| 18 | Anchorage | N 60° 59.802′ W 149° 05.236′ | *T. mertensiana* forest |
| 19 | Anchorage | N 60° 57.968′ W 149° 06.812′ | Mixed conifer upland |
| 20 | Anchorage | N 60° 57.954′ W 149° 06.798′ | Open canopy *Sphagnum* wetland |
| 21 | Anchorage | N 61° 08.384′ W 149° 46.458′ | *P. mariana* upland |
| 22 | Anchorage | N 61° 09.996′ W 149° 47.046′ | *P. mariana* upland |
| 23 | Anchorage | N 61° 11.772′ W 149° 48.710′ | *P. mariana* upland |
| 24 | Anchorage | N 61° 13.410′ W 149° 25.498′ | *B. nana* open canopy alpine |

accessibility, and the presence of moss. At each site, a 30-m transect was established, with replicate measurements of variables of interest along the transect at 5-m intervals ($n = 6$ per transect) referred to hereafter as subplots.

## Site-Level Data Collection

A $0.5 \times 0.5$-m frame was placed at every 5-m increment subplot along the 30-m transect to visually assess percent cover. Percent cover of vascular plants and bryophytes was agreed upon by two investigators. Thaw depth, the depth from the surface of the green moss to permafrost, was measured by inserting a metal probe into the ground thrice at each subplot. A note was made if permafrost was either deeper than 1 m or unmeasurable due to rocky soils. Organic layer depth was recorded after digging a small pit and having two researchers agree on the depth from the surface to the top of the mineral soil layer. Soil pH was measured at each subplot along the transect with a Milwaukee Instruments Professional Portable pH probe and a 2:1 water/soil slurry. Gravimetric water content was assessed by removing a $5 \times 5 \times 5$ cm plug of moss at each subplot along the transect,

placing it in an airtight plastic bag, transporting to the laboratory, and immediately recording a field wet and, after 48 h in a 60° drying oven, dry weight. Water content was calculated as (field wet weight-dry weight)/dry weight. To assess exchangeable ammonium and nitrate, an index of N availability and plugs of moss were collected at each subplot along the transect and then extracted with 50 mL 1 M KCl under vacuum power through Büchner funnels and pre-leached Whatman 1 filter papers following 1 h of manual agitation of the sample/KCl slurry. Extracts were frozen and transported to Northern Arizona University, where ammonium ($NH_4^+$-N) and nitrate ($NO_3^-$-N) concentrations were analyzed colorimetrically on a SmartChem 200 Discrete Analyzer (Unity Scientific, Milford, MA USA) following the salicylate method and the cadmium coil reduction method, respectively. Inorganic N is expressed as μg N g dry moss$^{-1}$ based on volume of extract and dry weight of extracted sample. Tree density was measured by taking the diameter at breast height (DBH) or basal diameter (BD) if a tree was shorter than breast height for every living tree within 1 m on either side of the transect.

**Table 2.** Mean Site Characteristics for All Sampled Sites, Including Mean Annual Temperature (MAT) in °C (4 Year Average), Mean Annual Precipitation (MAP) in mm (4 Year Average), Altitude in m, Depth of the Organic Layer, From the Surface to the Boundary of the Mineral Horizon (in cm, BPF = Below Permafrost, or Organic Layer Extended to the Active Layer Boundary), pH, Tree Density (trees m$^{-2}$), Active Layer Depth (in cm, Where NA Means No Permafrost Was Found in the Top Meter of Soil), Total Extractable Inorganic N (TIN, µg N g moss$^{-1}$, ± SE), and Sampling Date (dd/mm/yy)

| Site No. | MAT | MAP | Altitude | Org. layer depth | pH | Tree density | Active layer depth | TIN | Sampling Date |
|---|---|---|---|---|---|---|---|---|---|
| **1** | − 0.477 | 462.50 | 425 | 26 | 5.02 | 47.0 | 41 | 16.2 ± 3.6 | 06/21/16 |
| **2** | − 0.473 | 472.00 | 405 | 36 | 4.80 | 31.3 | 56 | 2.5 ± 0.3 | 06/22/16 |
| **3** | − 0.477 | 462.50 | 425 | 28 | 5.09 | 51.3 | 39 | 4.8 ± 0.6 | 06/22/16 |
| **4** | − 0.569 | 439.50 | 125 | 14 | 5.65 | 50.8 | 57 | 2.1 ± 0.4 | 06/23/16 |
| **5** | − 0.598 | 428.50 | 119 | BPF | 4.55 | 11.6 | 29 | 9.6 ± 1.1 | 06/23/16 |
| **6** | − 0.592 | 424.00 | 119 | BPF | 5.77 | NA | 41 | 10.1±1.0 | 06/23/16 |
| **7** | − 0.288 | 419.50 | 163 | BPF | 5.16 | 18.1 | 44 | 12.4±3.8 | 06/23/16 |
| **8** | 0.088 | 703.25 | 790 | 14 | 4.90 | NA | 35 | 17.2±3.3 | 06/24/16 |
| **9** | − 0.050 | 538.50 | 240 | 9 | 5.58 | 80.6 | 60 | 7.3 ± 0.8 | 06/24/16 |
| **10** | − 0.015 | 547.75 | 305 | 22 | 5.07 | 24.7 | 31 | 7.4 ± 0.5 | 06/24/16 |
| *11* | − 6.415 | 352.25 | 735 | 10 | 4.95 | NA | 24 | 4.3 ± 0.6 | 06/27/16 |
| *12* | − 6.460 | 361.50 | 765 | 16 | 5.76 | NA | 29 | 12.1±1.0 | 06/28/16 |
| *13* | − 6.460 | 361.50 | 765 | BPF | 5.31 | NA | 20 | 6.9 ± 1.2 | 06/28/16 |
| *14* | − 6.413 | 351.25 | 728 | 14 | 6.20 | NA | 24 | 10.1 ± 1.3 | 06/28/16 |
| *15* | 3.535 | 425.75 | 60 | BPF | 4.61 | 9.7 | 29 | 11.3 ± 1.5 | 06/27/17 |
| *16* | 3.544 | 426.00 | 60 | 33 | 4.93 | 62.4 | 61 | 9.7 ± 0.4 | 06/27/17 |
| *17* | 3.383 | 415.25 | 84 | 15 | 5.19 | 59.0 | NA | 14.2 ± 1.0 | 06/28/17 |
| *18* | − 0.454 | 1982.25 | 206 | 20 | 4.88 | 81.3 | NA | 29.8 ± 3.2 | 06/28/17 |
| *19* | 1.556 | 1677.75 | 87 | 18 | 5.02 | 68.7 | 42 | 17.5 ± 3.6 | 06/29/17 |
| *20* | 1.571 | 1676.25 | 87 | >100 | 5.05 | NA | NA | 52.6 ± 3.3 | 06/29/17 |
| *21* | 3.150 | 413.25 | 145 | 7 | 4.91 | 43.0 | NA | 20.2 ± 1.9 | 06/30/17 |
| *22* | 3.410 | 417.75 | 86 | 33 | 5.41 | 54.6 | 71 | 15.5 ± 1.4 | 06/30/17 |
| *23* | 3.554 | 425.50 | 60 | 22 | 5.18 | 77.5 | 40 | 10.1 ± 0.4 | 06/30/17 |
| *24* | 2.579 | 576.00 | 763 | 27 | 4.78 | NA | NA | 14.6 ± 4.2 | 07/01/17 |

*Bold, italic, and bolditalic font in site column corresponds to geographic area: Bold is Fairbanks, italic is Toolik, and Bolditalic is Anchorage.*

## N$_2$ Fixation Measurements

At each site, all moss samples were identified to the genus or species level. Common mosses (appearing in six or more patches) were collected six times per site (one per subplot), while rare mosses (appearing in fewer than six patches) were sampled in all distinct subplots in which the species was present. Bulk density of common moss species was measured by recording the dry weight of three 5×5×5 cm plugs of monospecific moss material per site. For each N$_2$ fixation measurement sample, roughly 40 moss ramets were collected. After collection, moss samples were returned to the laboratory and several moss ramets were removed as a voucher sample for identification at the University of Florida. Subsequently, the sample was divided into two subsamples, each containing ten ramets of moss, each of approximately 5 cm of length including green and senesced tissue. One subsample was immediately placed in a drying oven for 48 h at 60° C and then shipped to Northern Arizona University to be measured for the natural abundance (NA) of $^{15}$N. The second subsample was wetted with distilled water and placed in an airtight 60-ml polypropylene syringe. The syringe was filled with 10 ml of ambient air before 10 ml of 98 at% enriched $^{15}$N$_2$ gas was added for a final airspace volume of 20 ml and a 50% enriched headspace (Sigma-Aldrich Inc., lot no. MBBB3807V and MBBB9003V). Samples were incubated for 24 h in a common garden centrally located within each sampling area (Fairbanks, Anchorage, and Toolik). Previous studies have shown no significant difference in measured fixation rate from incubations *in situ* or incubations that occur in a similar but distinct environment (DeLuca and others 2007). Three syringes of the same volume containing a Thermochron iButton (Model DS1921G-F5#, Embedded Data Systems, USA) were deployed simultaneously to record temperature every 10

minutes throughout the duration of the incubation. A temperature mean, minimum, and maximum was calculated for each incubation period based on iButton measurements. Following incubation, moss samples were removed from the syringes, bagged, dried as described above, and sent to Northern Arizona University for analysis.

## Laboratory Analysis and Rate Calculations

Both NA and incubated moss samples were finely ground. Six mg of each sample was rolled into tin capsules and run on a Costech ECS4010 elemental analyzer coupled to a Thermo Scientific Delta V Advantage Isotope ratio mass spectrometer to obtain $\delta^{15}N$ values. Fixation rates were calculated using the atom percent enrichment (APE) of each sample compared with its paired NA sample and then scaling isotopic uptake by the sample weight and air/tracer ratio to calculate total ($^{15}N + ^{14}N$) $N_2$ fixation (Jean and others 2018). Rates are expressed on a per mass basis as µg N g moss$^{-1}$ day$^{-1}$. To scale rates to mg N m$^{-2}$ d$^{-1}$, each genus was given an average bulk density based on measurements made at the study sites (values, in g moss cm$^{-2}$, were $0.146 \pm 0.008$, $0.067 \pm 0.013$, $0.066 \pm 0.005$, $0.037 \pm 0.003$, $0.043 \pm 0.004$, $0.027 \pm 0.004$, $0.046 \pm 0.001$, and $0.028 \pm 0.002$ for *Polytrichum*, *Dicranum*, *Aulacomnium*, *Pleurozium*, *Hylocomium*, and *Rhytidiadelphus*, *Ptilium*, *Tomentypnum*, and *Sphagnum*, respectively) as described above or, for *Polytrichum spp.* only, from the literature (Fenton 1980). A site-level average percent cover was calculated for each genus, and that number was multiplied by the area-based $N_2$ fixation rate derived from the bulk density and the mass-based fixation rate. For each site, the average areal fixation rates of all mosses were summed together to estimate total mg N m$^{-2}$ d$^{-1}$ for each site, based on measured $N_2$ fixation rates and percent cover of mosses present at the site.

## Sample Distribution

Across all locations, $N_2$ fixation rates were measured for 580 samples. If samples did not have a paired NA sample, as was the case for about 60 samples, an average based on that host species within the site was used as reference. The average NA and enriched $\delta^{15}N$ were $-3.07 \pm 0.07‰$ (mean ± SE) and $62.86 \pm 4.24‰$, respectively. The range of NA values was $-7.64$ to $5.31‰$, and enriched samples ranged from $-6.12$ to $675.40‰$. Samples with less than 2‰ difference between NA

and enriched samples were assumed to have a fixation rate of 0 based on the sensitivity of the isotope ratio mass spectrometer.

## Statistical Analyses

All analyses were conducted in R 3.4.1 (R Core Development Team 2019) using the packages lme4 version 1.1–14 (Bates and others 2015), emmeans version 1.3.0 (Lenth 2016), randomForest version 4.6–14 (Liaw and Wiener 2002), VSURF version 1.0.4 (Genuer and others 2015), vegan version 2.5–5 (Oksanen and others 2019), and car version 3.0–0 (Fox and Weisberg 2011).

To explore the importance of the environmental and taxonomic variables in explaining variation in fixation rates, we applied a random forest algorithm to each of the three geographic sampling areas: Anchorage, Fairbanks, and Toolik Field Station. Random forests are a flexible and unbiased approach that can create an informative and parsimonious model through a variable selection process while incorporating both continuous and categorical variables (Cutler and others 2007). We opted to use random forest models over other approaches, such as variance partitioning or structural equation models, to more effectively deal with non-normally distributed data and for the ease of inclusion of categorical variables. For the random forest, collected mosses were divided into families based on the classification by Goffinet and Buck (2019) (see Table 3). Family was selected as the unit for analysis to capture taxonomic diversity and some trait cohesion while not overfitting the model by including a variable with many categories (that is, moss genus), thus risking the inflation of variable importance or $R^2$ values. For each location, we used the VSURF variable selection package to identify variables that were most important at the threshold, interpretation, and prediction step. All variables from the "threshold" step were included in the random forest model. Each VSURF model started with the following variables to predict $N_2$ fixation rates (µg N g moss$^{-1}$ day$^{-1}$) : tree density (trees m$^{-2}$, not included in Toolik model as no trees were present at that location, as a relative index of light penetration), temperature minimum, maximum, and average during incubation (°C), gravimetric water content, altitude (m), pH, $NH_4^+$-N, $NO_3^-$-N, and total extractable inorganic N (µg N g dry moss$^{-1}$), permafrost depth category (Shallow: 0–25 cm from surface, Moderate: 26–49 cm from surface, Deep: 50–100 cm from surface, or no permafrost above 1 m), organic layer depth category (Shallow: 0–25 cm from soil surface, Moderate: 26–

**Table 3.** Fixation Rate and Taxonomic Family of All Measured Moss Species

| Family | Species measured | Mean fixation rate ($\mu$g N g moss$^{-1}$ day$^{-1}$, $\pm$SE) | $n$ |
|---|---|---|---|
| Amblystegiaceae | *Tomentypnum nitens*[*] (Hedw.) Loeske | 14.58 ± 2.97 | 8 |
| | *Sanionia uncinata* (Hedw.) Loeske | 2.23 ± 1.82 | 5 |
| Aulacomniaceae | *Aulacomnium turgidum* (Wahlenb.) Schwgr. | 2.11 ± 0.73 | 18 |
| | *Aulacomnium palustre* (Hedw.) Schwgr. | 2.30 ± 0.42 | 48 |
| | *Aulacomnium acuminatum* (Lindb. & Arnell) Kindb. | 3.88 ± 0.35 | 3 |
| Dicranaceae | *Dicranum acutifolium* (Lindb. & Arnell) C. Gens. ex Weinm | 0.99 ± 0.40 | 14 |
| | *Dicranum elongatum* Schwgr. | 1.46 ± 0.44 | 10 |
| | *Dicranum fragifolium* Lindb. | 0.08 ± 0.05 | 5 |
| | *Dicranum fuscescens* Turner | 0.36 ± 0.19 | 4 |
| | *Dicranum montanum* Hedw. | No measured fixation | 2 |
| | *Dicranum polysetum* Swartz | 0.22 ± 0.14 | 21 |
| | *Dicranum scoparium* Hedw. | 1.93 ± 0.63 | 9 |
| | *Dicranum undulatum* Brid. | 0.34 ± 0.25 | 7 |
| Grimmiaceae | *Racomitrium lanuginosum* (Hedw.) Brid. | 11.69 ± 3.83 | 6 |
| | *Niphotrichum canescens* (Hedw.) Brid. | 2.62 ± 1.28 | 3 |
| Hylocomiaceae | *Pleurozium schreberi* (Brid.) Mitt. | 0.79 ± 0.18 | 90 |
| | *Hylocomium splendens* (Hedw.) Schimp. | 3.60 ± 0.43 | 99 |
| | *Rhytidiadelphus triquetrus* Hedw. | 0.09 ± 0.04 | 4 |
| Hypnaceae | *Hypnum lindbergii* Mitt. | 4.24 ± 1.60 | 5 |
| | *Ptilium crista-castrensis* (Hedw.) DeNot | 2.28 ± 0.56 | 37 |
| Polytrichaceae | *Polytrichum juniperum* Hedw. | 0.27 ± 0.05 | 2 |
| | *Polytrichum strictum* Brid. | 0.82 ± 0.28 | 25 |
| | *Polytrichum commune* Hedw. | 0.03 ± 0.02 | 33 |

**Table 3.** continued

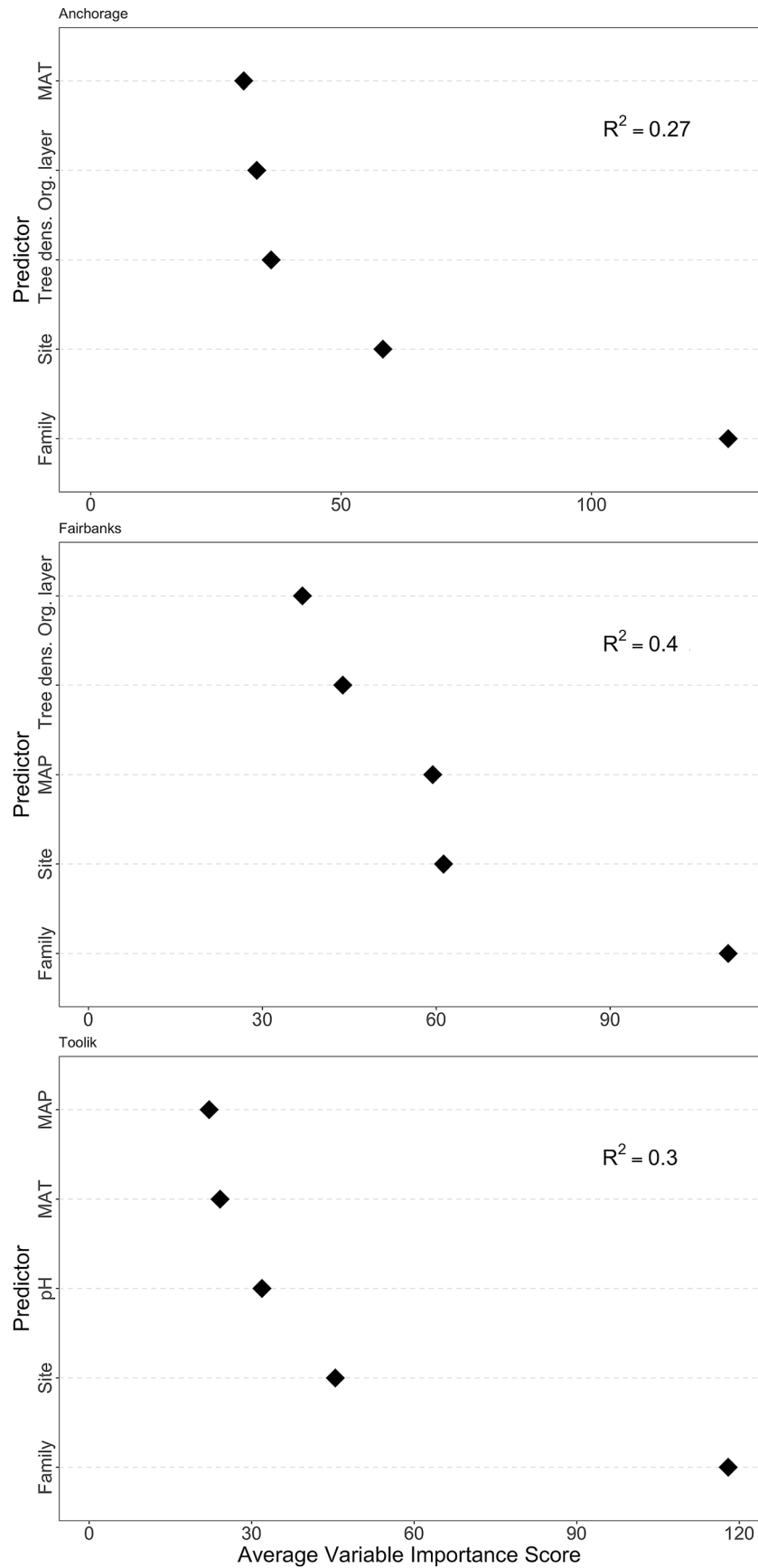| Family | Species measured | Mean fixation rate ($\mu$g N g moss$^{-1}$ day$^{-1}$, $\pm$SE) | $n$ |
|---|---|---|---|
| Sphagnaceae | *Sphagnum alaskense* Andrus & Janssens | 7.17 | 1 |
| | *Sphagnum angustifolium* (Ehrh.) Hedw. | 6.33 $\pm$ 0.98 | 15 |
| | *Sphagnum arcticum* Flatberg & Frisvoll | 1.85 $\pm$ 0.66 | 2 |
| | *Sphagnum capillifolium* (Ehrh.) Hedw. | 2.91 | 1 |
| | *Sphagnum fimbriatum* Wilson | 10.80 $\pm$ 5.00 | 2 |
| | *Sphagnum fuscum* (Schimp.) H. Klinggr | 5.35 $\pm$ 2.41 | 7 |
| | *Sphagnum girgensohnii* Russow | 5.20 $\pm$ 1.04 | 21 |
| | *Sphagnum magellanicum* Brid. | 2.51 $\pm$ 0.78 | 9 |
| | *Sphagnum russowii* Warnst. | 5.91 $\pm$ 0.95 | 28 |
| | *Sphagnum squarrosum* Crome | 6.28 $\pm$ 2.55 | 5 |

*Based on Vanderpoorten and others (2001). Means of rate measurements across all geographic areas.*

45 cm from soil surface, Deep: below 46 cm from surface, or under permafrost), mean annual temperature (MAT in °C, 4-year average from 2014–2017 extracted from Climate NA), mean annual precipitation (MAP in mm, 4-year average from 2014–2017 extracted from Climate NA), site of collection, and moss family (Table 3)(Wang and others 2016). We then executed six averaged runs of a random forest with set randomization for all interpretation variables in each location, with mtree set at 10,000. We also ran one random forest model in the same fashion including all data and with broad geographic location (Toolik, Fairbanks, or Anchorage) as a predictor.

To test for genus-level differences in N$_2$ fixation rate on a per-unit mass basis, we used a linear mixed effects model with host moss genus as a fixed effect and subplot nested in site as a random effect for each of the three geographic areas sampled (Toolik, Fairbanks, and Anchorage, with site being one of the 24 locations summarized in Table 1 and 2). Genus was selected as a fixed effect due to the cohesion of traits such as growth form, anatomy, cell wall thickness, and rate of water loss within a genus (Elumeeva and others 2011). Site was planned as a random effect in order to be able to collect target mosses across the spectrum of natural variation in habitats in which these genera

occur. Due to the natural survey style of collection, only six genera had a large enough sample size to include in the model. Those genera (*Aulacomnium, Dicranum, Pleurozium, Hylocomium, Polytrichum,* and *Sphagnum*) represent a large spectrum of anatomical diversity found in mosses of Alaska. The log + 1 mass-basis N$_2$ fixation rate ($\mu$g N g moss$^{-1}$ day$^{-1}$) was used as the response variable. Post hoc analyses for all models were performed using the estimated marginal means to assign significance based on $\alpha = 0.01$. Except one random forest model for exploratory purposes, the geographic areas were analyzed separately due to the difference in sampling times, both in terms of year and progress of the growing season, to avoid confounding seasonal differences with variation from environment or identity. Site as a random effect can act as a proxy for some environmental variation (for example, inter-site variation in altitude, MAT, and MAP) but cannot fully account for other sources of environmental variation that can change on a very fine scale (between 5 m subplot intervals), such as tree density, organic layer depth, and moisture.

To test the effect of moss diversity on N$_2$ fixation rates, the number of moss genera present at each site (richness, $S$) was calculated. To account not just for presence, but for moss cover and dominance, the Simpson's diversity index $D_1$ (Simpson 1949)

Anchorage

$R^2 = 0.27$

Fairbanks

$R^2 = 0.4$

Toolik

$R^2 = 0.3$

Average Variable Importance Score

◀ **Figure 1.** Variable importance scores of the top five predictors averaged across six random forest iterations for each sampling location (Anchorage, Fairbanks, and Toolik). The calculated $R^2$ for all models is based on a model run which contained variables identified at the "Threshold" step as identified through the VSURF variable selection tool, which eliminates all irrelevant variables. Initial models included MAT (°C, average from 2014–2017), MAP (mm, average from 2014–2017), permafrost category (see Methods), Organic layer depth category (see Methods), moss taxonomic family (see Table 2), pH, altitude (*m*), gravimetric water content, temperature minimum, maximum, and average during 24 h incubation (°C), tree density (trees $m^{-2}$), extractable $NH_4^+$-N, $NO_3^-$-N, and total inorganic nitrogen (µg N g dry $moss^{-1}$), and site of collection. The response variable was $N_2$ fixation rate (µg N g $moss^{-1}$ $day^{-1}$). Model root mean square error (RMSE) is 2.89, 0.63, and 5.0, for Anchorage, Fairbanks, and Toolik, respectively. Please note scale changes in *x*-axis.

was also calculated for each site. These diversity indices were then regressed against the site-level summed $N_2$ fixation rates described above, and, if appropriate, analyzed using a linear model. Other site-level characteristics, such as MAT, MAP, N availability, gravimetric water content, and pH, were also regressed against the site-level summed fixation rates to explore the occurrence of hotspots.

# RESULTS

Across all three locations (Anchorage, Fairbanks, and Toolik), moss taxonomic identity consistently was the top ranked predictor of measured $N_2$ fixation rates (Figure 1). In other words, the identity of the moss was a better predictor of $N_2$ fixation rates than any of the measured environmental variables or the site of collection. For all three geographic areas, site of collection was the second-ranked variable and was consistently included at the "interpretation" step of the random forest variable selection tool. In both Fairbanks and Anchorage, tree density was ranked in the top five predictors, though there was no clear linear trend relating $N_2$ fixation rates and tree density in post hoc testing. Additionally, the depth of the organic layer was an important predictor in both Fairbanks and Anchorage, where post hoc analysis revealed that shallow organic layers were associated with lower $N_2$ fixation rates. Toolik was the only geographic area for which pH was an important predictor of $N_2$ fixation rate (Figure 1), and all three locations included either MAP, MAT, or both as important predictors. In the random forest model that in-

**Table 4.** Random Forest Results From a Model that Included All Data From Across Alaska

| Ranking | Predictor | Average variable importance score |
|---|---|---|
| 1 | Family | 183.0 |
| 2 | Site | 101.6 |
| 3 | MAP | 45.3 |
| 4 | MAT | 35.0 |
| 5 | Organic layer depth | 33.5 |

*Geographic area was the eleventh-ranked variable. Model $R^2 = 0.34$, model RMSE = 0.58.*

cluded all data, family was still the top ranked predictor, followed by site. The geographic area (Toolik, Fairbanks, or Anchorage) was not a highly ranked predictor (Table 4).

When accounting for subplot nested in site as a random factor, host moss genus had a significant effect on $N_2$ fixation rate at all three geographic sampling regions (for Anchorage, Fairbanks, and Toolik, $p < 0.001$, Figure 2). Further, means comparisons indicated relatively consistent differences among host moss genera across the three geographic sampling locations. *Polytrichum* spp. and *Dicranum* spp. had fixation rates that were, at all locations, significantly lower than those of *Sphagnum spp.* or *Hylocomium* (Figure 2). *Aulacomnium* spp. was significantly lower than *Hylocomium* only at Toolik, whereas *Pleurozium* was never significantly higher than *Dicranum* spp. (Figure 2). Toolik typically had higher fixation rates, both overall and within moss genera, than the other two latitudinal sampling locations (Figure 2). Site as a random effect accounted for 6.7, 24, and 17% of variance explained in the model for Toolik, Fairbanks, and Anchorage, respectively. For the same models, subplot nested in site as a random effect explained very little variation: less than 10% at Toolik and less than 3% in Anchorage and Fairbanks.

For each sampled site (n = 24), the average areal $N_2$ fixation rate by host moss genus was summed within each site by adding the averages of all present families (Figure 3). The abundance-weighted $N_2$ fixation rates for individual sites ranged from less than 0.01 to 3.16 mg N $m^{-2}$ $d^{-1}$, with a mean of $1.04 \pm 0.19$ mg N $m^{-2}$ $d^{-1}$. The mosses with the largest contributions to $N_2$ fixation across all sites were *Sphagnum* spp. and *H. splendens*, but *T. nitens* and *Aulacomnium* spp. were locally important at some sites (for example, Sites 6, 7, 13, and 14, Figure 3).
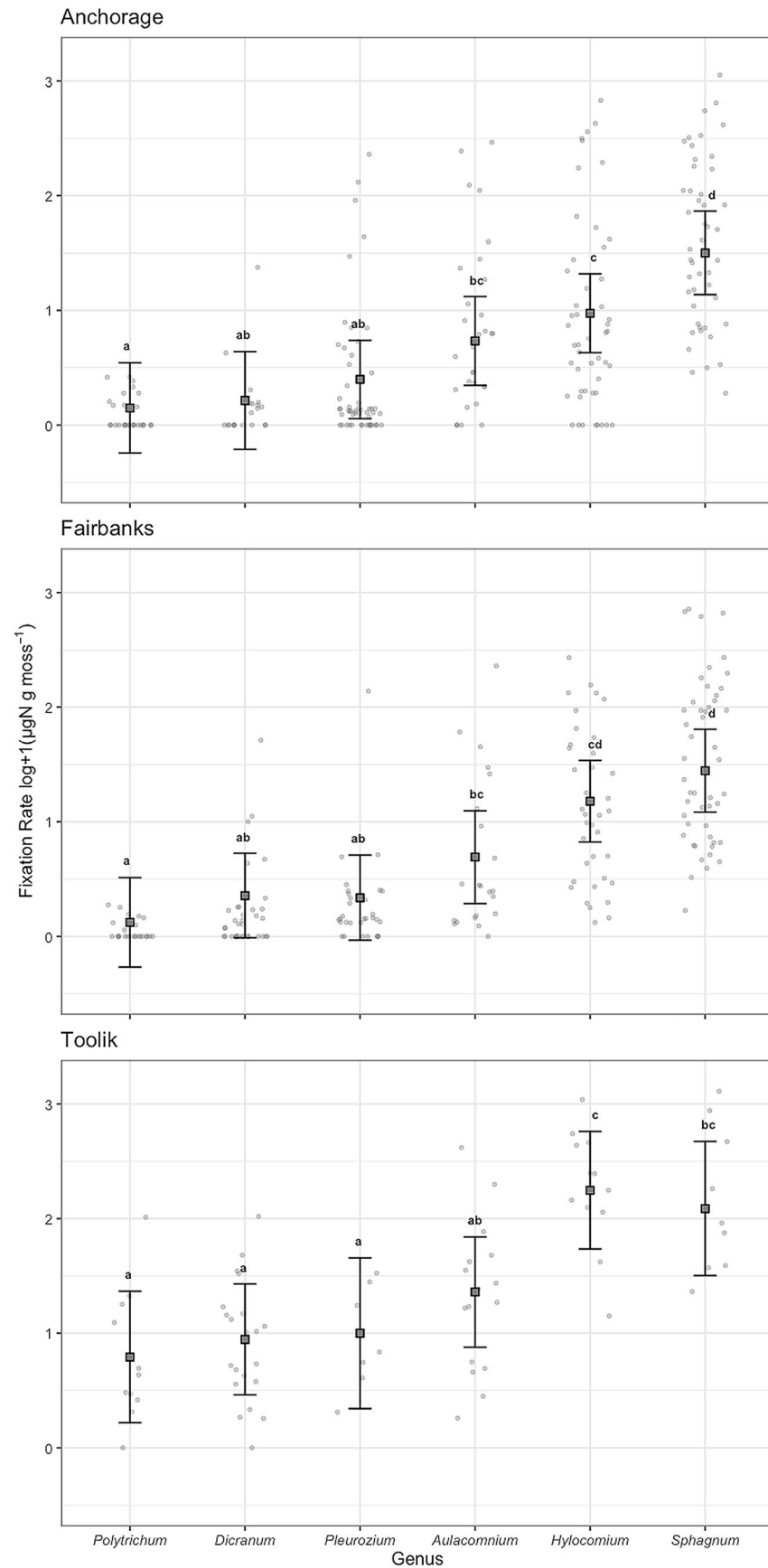
◄**Figure 2.** Model results for N₂ fixation rates for each moss genus with subplot nested in site as a random factor in each region (Anchorage, Fairbanks, and Toolik in separate panels). Significant differences are represented by letters above each bar, which were based on post hoc-estimated marginal means pairwise comparisons at α = 0.01, wherein data were log-transformed to meet model assumptions. Boxes are group means, bold bars are the 95% confidence interval, and gray points are raw data points.

Six of the 24 sampled sites were hotspots of $N_2$ fixation [three SE's over the median rate (Reed and others [2010])]. Three of those sites occurred near Anchorage, two occurred near Toolik, and one near Fairbanks (Sites 7, 12, 14, 22, 23, and 24; median N fixation rate = 0.86 mg N m⁻² d⁻¹, SE = 0.19 mg N m⁻² d⁻¹). As this definition classified a full quarter of our sites as hotspots, we have added an additional tier of sites that exceeded the median $N_2$ fixation rate by more than 10 SE's, highlighting
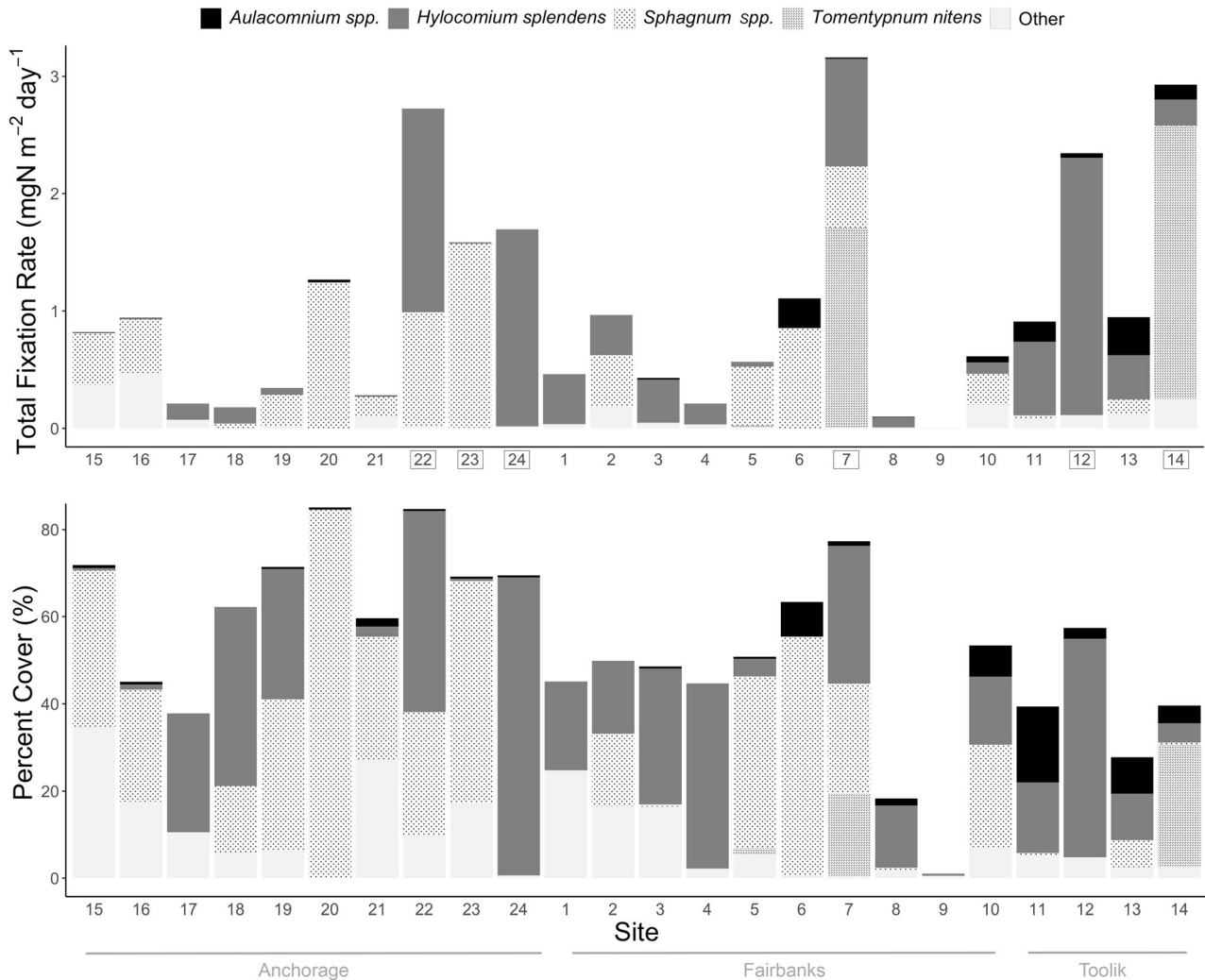


**Figure 3.** Total $N_2$ fixation rates (top) and percent cover (bottom) where each bar represents one site. Sites in Anchorage are on the left (Sites 15–24), Fairbanks sites in the middle (Sites 1–10), and Toolik sites (Sites 11–14) furthest to the right. All genera not in legend are represented by ''Other''; see Table 3 for full list. Sites that are hotspots of $N_2$ fixation have a box around the site number in top panel.
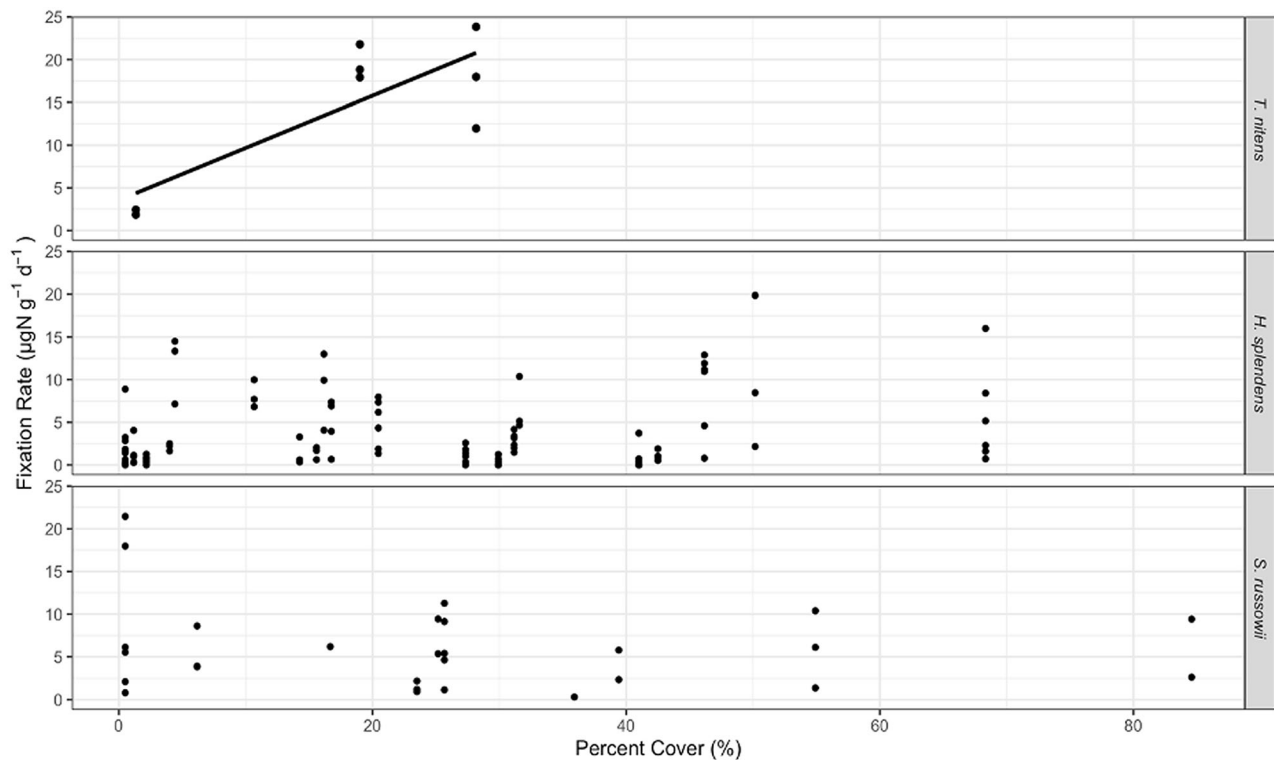
**Figure 4.** Linear model results for three host moss genera (*T. nitens, H. splendens, and S. russowii*) where the independent variable is percent cover and the dependent variable is N₂ fixation rate (in µg N g moss$^{-1}$ day$^{-1}$). Only *T. nitens* had a significant positive relationship, where $p = 0.004$ and $R^2 = 0.73$.

sites 7 and 14 as particularly active hotspots. We found no significant relationships between site-level N₂ fixation rate and moss richness (Figure S1), Simpson's Diversity Index (Figure S1), or any environmental variable (MAT, MAP, and so on) at the site level, but did see significant correlation between percent cover and N₂ fixation rate in *T. nitens* (Figure 4). There were non-significant positive trends between N₂ fixation rate and pH and fixation rate and gravimetric water content.

## DISCUSSION

From the earliest attempts to quantify moss-associated N₂ fixation in Alaska, a large range of N₂ fixation rates have been observed in bryophyte-associated microbial communities while the main drivers of that variation have remained largely undetermined (Alexander and Schell 1973). Host species identity has been an intriguing avenue for explaining the high amounts of variation seen in this process and may be a valuable tool as moss communities and associated N₂ fixation rates shift with climate (Gavazov and others 2010; Turetsky and others 2012; Bay and others 2013; Warshan and others 2017; Holland-Moritz and others 2018;

Carrell and others 2019). Here, we found host moss family to be the most important predictor of N₂ fixation rate across a broad geographic range (Figure 1, Table 4). We also found consistent and significant differences in N₂ fixation rate among moss genera (Figure 2). Site of collection was an important source of variation, but our analyses consistently found moss identity differences to be significant despite that variation. These findings indicate that host moss identity can play an important role in both predicting landscape-scale N inputs from moss-associated N₂ fixation and in further exploration of drivers of process rate variation.

Moss-associated N₂ fixation was nearly ubiquitous among collected mosses. Almost all potential host moss species (34 out of 35) collected in this survey had measurable rates of N₂ fixation (Table 3). By direct comparison of an incubated sample to a paired natural abundance sample, we were able to quantify even very low rates of N₂ fixation. Our results are in agreement with a previous study which used an isotopic approach to measuring moss-associated N₂ fixation (Gavazov and others 2010), indicating that the use of $^{15}$N may be particularly valuable for measuring low rates of N₂

fixation that may be missed when using acetylene reduction assays. When making larger-scale calculations of N inputs, the ubiquity of measurable rates of $N_2$ fixation associated with mosses underscores the importance of including a diversity of host mosses when measuring or predicting $N_2$ fixation rates.

Diversity in free-living $N_2$ fixer communities in other ecosystems, such as tropical forests, has been shown to be associated with higher total rates of $N_2$ fixation (Reed and others 2010, 2011). While past studies have focused directly on the positive relationship between microbial diversity and fixation rates, host moss diversity could reflect microbial diversity based on the specificity between host identity and microbial community and thus also be positively correlated with $N_2$ fixation rates. Though we saw no relationship between diversity and function, it is notable that the two largest $N_2$ fixation hotspots had high moss diversity, indicating that diversity alone does not appear to cause hotspots but is a potential feature of hotspots (see Figure S1, Figure 3). Both active hotspots included *T. nitens*, which fixed $N_2$ at rates disproportionate to its cover (Figure 3). Additionally, we found a strong positive relationship between percent cover and $N_2$ fixation rate in *T. nitens*, a relationship that was absent in other high-fixing species such as *H. splendens* and *S. russowii* (Figure 4). Percent cover of all mosses was not associated with hotspots in a straightforward manner; all hotspots other than Site 14 had greater than 50% moss cover, but other sites had high moss cover without a correspondingly high total $N_2$ fixation rate (Figure 3).

The data presented here are based on snapshot measurements from only one point in the growing season at each sampled location. Fixation rates are known to vary over the course of the growing season, perhaps in relation to N demand during reproduction or in response to environmental changes (Lett and Michelsen 2014; Warshan and others 2016; Rousk and Michelsen 2017). Our sampling sites cover a wide range of naturally occurring differences in environmental conditions (see Table 2), but we cannot account for seasonal variation arising from phenology. Therefore, it is possible that our observed hotspots could also represent hot moments for *T. nitens*. Past measurements of *T. nitens* show a peak in $N_2$ fixation rates in mid-June to early July (Rousk and Michelsen 2017). Subplot nested in site was not a large source of variation within our models, but even subplot cannot fully capture diversity in microclimate conditions on a sub-5 m scale. Although some of these microclimate conditions can

arise due to traits of the mosses and their community structure and function, moss identity can only capture the combination of these traits as opposed to a single driver (Eviner 2004; Rixen and Mulder 2005; Gornall and others 2007).

There are other possible explanations for the high observed $N_2$ fixation rates within *T. nitens*, none of which were experimentally addressed here. Non-acidic tundra surfaces which often contain *T. nitens* communities, such as in Site 14, have higher $P$ availability when compared to other tundra types (Hobbie and Gough 2002). However, previous research indicates that high latitude moss-associated $N_2$ fixation is rarely limited by $P$ (Zackrisson and others 2004; Rousk and others 2017). Fixation rates were not disproportionately higher in all mosses at Site 14, despite the commingling growth of species in this location. Relative to its colony density, *T. nitens* retains moisture more effectively than similarly structured species (Elumeeva and others 2011). This indicates perhaps a dual advantage for *T. nitens*, as it maximizes moisture while still allowing for light and air penetration into the colony structure. Other mosses, such as *P. commune* or *Sphagnum* spp., could also have anatomical features that affect their respective conditions for promoting or decreasing rates of $N_2$ fixation. *P. commune* contains transport cells that may allow it to obtain more water and nutrients from its substrate (Brodribb and others 2020), thus decreasing the demand for N obtained via fixation. In our study, *Polytrichum* spp. tended to have higher tissue N. *Sphagnum* mosses are known to exert control over their environment through specialized hyaline cells for holding water (van Breemen 1995) which can create a moist microenvironment that is suited for optimization of rates of $N_2$ fixation. For *T. nitens*, as for other mosses, traits such as growth habitat and morphological features require further exploration to parse their role in driving rates of $N_2$ fixation, particularly since a combination of traits that constitute identity rather than a single trait may be important drivers of biogeochemical processes (Eviner 2004).

Random forest models also indicated the importance of certain environmental factors. The ranked predictors of $N_2$ fixation rate varied between the three latitudinal sampling sites (Toolik, Fairbanks, and Anchorage), where Arctic tundra was distinct from the other two locations. Site emerged consistently as a top predictor, perhaps as a distillation of a matrix of environmental variables that can influence process rates. Tree density was a large driver in Fairbanks and Anchorage ecosystems. Given the importance of light for phototrophic N

fixers, such as *Nostoc*, canopy structure, and its attendant light penetration, is a logical driver of process rates (Gentili and others 2005; Gundale and others 2012). Additionally, litter inputs from the canopy could be affecting moss community structure and/or N$_2$ fixation rates (Rousk and Michelsen 2017; Jean and others 2020). Organic layer depths, another important predictor for N$_2$ fixation rates in Anchorage and Fairbanks, can affect soil temperature and moisture, which may in turn affect N$_2$ fixation rates indirectly through, for example, the surrounding vascular plant assemblage (Kasischke and Johnstone 2005; Gundale and others 2012; Jonsson and others 2014). At Toolik Field Station, the importance of pH may be related to the relatively higher pH communities that contain *T. nitens* and its associated high N$_2$ fixation rates (Hobbie and others 2005). Past studies have produced strong evidence for N availability downregulating N$_2$ fixation (Rousk and others 2013). Extractable inorganic N was not a good predictor of N$_2$ fixation rates in our study. Nitrogen depositions rates are generally low across Alaska, but mosses may also be utilizing soil N or resorption N from senescent materials. Taken together, this means that extractable inorganic N from the mosses may not be the best index for N availability (Aldous 2002; Hember 2018; Liu and others 2019). It is also important to note that inter-site differences in canopy structure and organic layer depth tended to be greater than those found in TIN or, within a geographic area, MAT.

Gravimetric water content and incubation temperature were generally not important predictors of N$_2$ fixation rates in our study, but MAT and MAP were often ranked highly in the random forest. Past studies have shown a positive effect of increased moisture on N$_2$ fixation rates (Rousk and others 2013). Despite this, the lack of a direct effect of water content may be because only one sampled site (Site 8) was below the threshold identified by Zielke and others (2005) of 60% water content and N$_2$ fixation rates at this site were quite low. We did observe a non-significant positive trend between site-level N$_2$ fixation and gravimetric water content. In our study, N$_2$ fixation seemed more affected by long-term precipitation averages instead of the conditions on the day of sampling. The range of average temperatures in our incubations was 13.7–20.7°C, far below the threshold of where we would expect to see warm temperature-related inhibition of N$_2$ fixation (Gundale and others 2012). Although there may have been some temporary suppression of N$_2$ fixation in association with high temperature maximums inside of syr-

inges during incubation, temperature maximum was still not a strong predictor of rates. Again, the long-term temperature trend was more important for N$_2$ fixation variation. It is notable that temperature and moisture conditions may also be important in determining the distribution of host mosses, which in the longer term could alter landscape level N$_2$ fixation patterns (Deane-Coe and others 2015).

The rates we obtained fall within the previous scope of rates of moss-associated N$_2$ fixation both in Europe and North America. We observed higher N$_2$ fixation rates for *S. fuscum, T. nitens, A. palustre, P. schreberi,* and *H. splendens* than Gavazov and others (2010) despite the use of isotopic measurement, though there was some agreement of trends between mosses. The consistently lower rates associated with *P. schreberi* were surprising given the abundance of higher rates in the literature, but some papers do show a similar result particularly in comparison with *H. splendens* (Gentili and others 2005; Bay and others 2013; Gundale and others 2013a; Leppänen and others 2013; Jean and others 2020). Rousk and Michelsen (2017) saw a similar mean rate of N$_2$ fixation for *T. nitens* as reported here. Looking at rates of N$_2$ fixation associated with cyanolichens in these ecosystems provides further contextualization for mosses. In some high-latitude ecotypes, cyanolichens account for the majority of fixed N$_2$ (Rousk and others 2015). By a per mass basis, cyanolichens from Toolik fixed an order of magnitude more N$_2$; however, their percent cover at Site 14 was also less than 4%, leading to a probable lower overall N source (Weiss and others 2005).

## CONCLUSIONS

Given the strength of moss identity as a predictor of N$_2$ fixation rates, the consistency of patterns between moss genera across broad geographic and environmental variation, and the importance of certain species in determining the presence of hotspots, we conclude that moss identity could be a valuable tool to increase the precision of regional-scale predictions of landscape N$_2$ fixation rates. Existing moss abundance datasets or advanced remote sensing techniques could be leveraged to make these predictions. Such landscape-level models could be further augmented by exploring the occurrence of hotspots on both spatial and temporal scales and incorporating that knowledge with information on moss community composition. Elucidating the relationships between N$_2$ fixation and host identity, as well as exploring the

mechanisms underlying that specificity, can better inform how N dynamics in these valuable and vulnerable ecosystems will be affected by ongoing climate change.

## ACKNOWLEDGEMENTS

## DATA AVAILABILITY

The data was published by the Arctic Data Center. The link to the publicly available dataset is https://arcticdata.io/catalog/view/doi:10.18739/A2QV3C475.

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