Characterization of the chemical environment of phylogenetically diverse Alaskan mosses: does *Sphagnum* harbor a unique metabolome?

Megan N. Nickerson[1] Malak M. Tfaily[1] Jana M. U'Ren[1]

[1] University of Arizona, Department of Environmental Science, Tucson, AZ, 85721, USA

Abstract

The study of boreal and arctic ecosystems is of growing importance as they represent a significant global carbon sink and are especially threatened by climate change. Mosses often dominate higher latitude ecosystems as the number of vascular plants declines. In high latitude peatlands, the high abundance of complex compounds (e.g., polyphenols) present in mosses such as *Sphagnum* are hypothesized to slow the rate of decomposition of plant litter. However, previous chemical studies focused on one *Sphagnum* species in a single geographic location. Here, we are using untargeted metabolomics to characterize the chemical composition of multiple species of Sphagnum, as well as other co-occurring moss species (*Pleurozium schreberi, Aulacomnium palustre, Hylocomium splendens*). Living and decomposing tissues of 2-3 moss species were collected from four boreal and tundra locations near Fairbanks, Alaska. Metabolites were extracted using Folch extraction and characterized using tandem mass spectrometry coupled with liquid chromatography (LC MS/MS) on an Orbitrap Exploris Mass Spectrometer. On-going analysis will compare the impact of moss species, geographic location, and tissue age on metabolome diversity and composition.

Research Question

What is the impact of moss genus, geographic location, and tissue age (living vs. decomposing) on metabolome diversity and composition?

Methods

- Living and decomposing tissue samples of each moss species were collected from four geographic locations near Fairbanks, AK (Bonanza Creek, Delta Junction, Healy, and Poker Flats; Fig 1). Sites differed in types of vegetation present ranging from boreal spruce forests (BZS, DEJU, PRR) to tundra (HEAL) with additional variation within sites.
- Living and decomposing tissue of each moss was separated and placed into 15 mL conical tubes and frozen at -20°C within a few hours of collection.
- Frozen tissue was lyophilized and ground in liquid Nitrogen prior to metabolite extraction through the Folch extraction method, which separates polar and non-polar metabolites (Folch et al. 1957).
- Liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed on an Orbitrap Exploris Mass Spectrometer.
- Raw data was visualized using MZmine3. Features identified and compared to reference databases on Compound Discoverer. MetaboAnalyst was used to visualize differences due to host, site, and tissue (Pang et al. 2021).

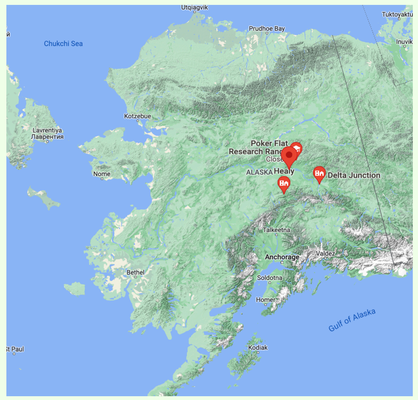

Fig. 1. Map of sampling sites in Alaska. Mosses were collected in four boreal locations near Fairbanks, AK.

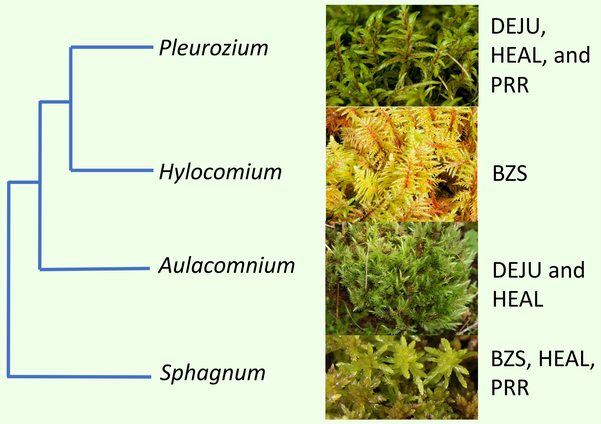

Fig. 2. Phylogenetic relationships and morphological differences among four moss genera. Branch lengths are not to scale. Text to the right depicts which sites each moss was collected from.

Results

- Using reverse phase (RP) LC-MS/MS we identified a total of 110 features in positive ion mode and 289 features in negative ion mode using HILIC LC-MS/MS.
- Hierarchical clustering of features identified in (+) RP mode revealed significant separation in the metabolic profiles of *Sphagnum* compared to other boreal mosses (Fig. 3A).
- Ordination with PCA demonstrates that 38.8% of the variation in metabolic profiles is explained by host differences (PC1) (Fig. 3B).
- Site appeared to also drive differences in the metabolic profile of *Sphagnum:* species collected from site 3 appeared to be more different than those collected from sites 1 and 4 (data not shown).
- The status of the plant (living vs. dead) appeared to have less of an effect on the metabolic profile compared to species type and location (Fig. 3C).

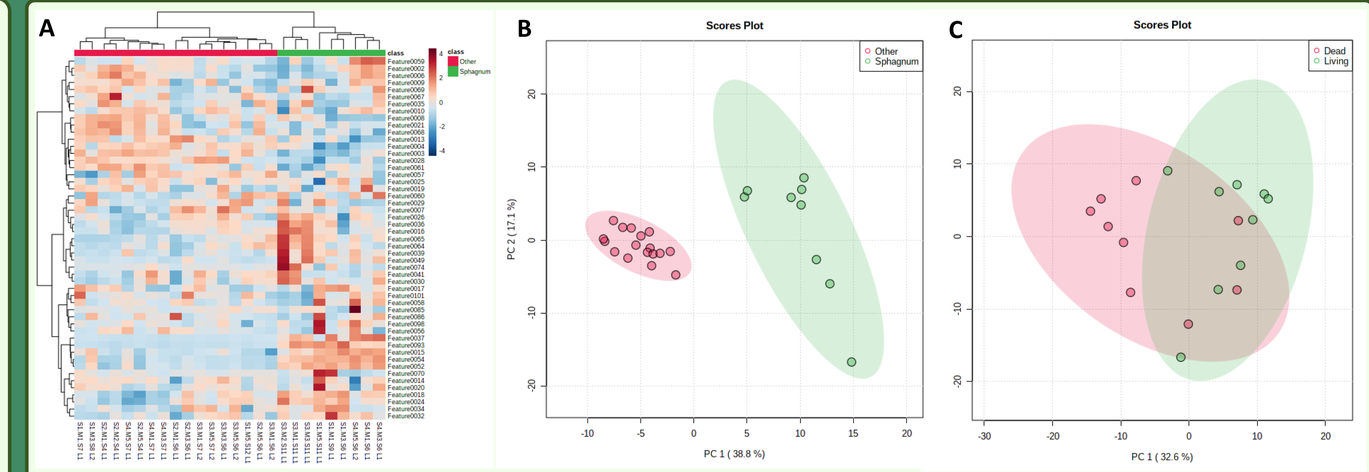

Fig. 3. (A) Heatmap and hierarchical clustering of MS2 features identified in reverse phase (RP) LC/MS-MS. Colors indicate differential abundance of features (see legend). **(B)** PCA 2D Scores Plot comparing living *Sphagnum* samples from all sites to other living moss samples from all sites sampled. **(C)** PCA 2D Scores Plot comparing living and dead *Sphagnum* samples from all sites where *Sphagnum* was collected.

Discussion

These data represent preliminary analyses to elucidate differences in the metabolome between *Sphagnum* and the other co-occurring mosses. Multivariate analyses demonstrate the metabolome of *Sphagnum* is distinct from other genera of mosses collected. We determined the metabolome of living *Sphagnum* samples varies among sites, although this may be the result of intraspecific differences. Consistent with the slow rate of *Sphagnum* decomposition, our analyses found fewer differences in metabolome between living and dead tissues of *Sphagnum*. A greater difference was observed between living and dead samples of co-occurring mosses (data not shown). A number of the compounds found to be upregulated in *Sphagnum* relative to co-occurring mosses were found to be phenols which is consistent with previous work (Mellegård et al 2009).

Future Directions

Additional statistical analyses will quantify the relative impact of host, site, and tissue type. Future analyses will also compare metabolome data to microbiome data generated from subsets of the same tissue. Currently, microbiome characterization is being carried out through Illumina amplicon sequencing. Future *in vitro* analysis will assess the growth of *Sphagnum*-associated fungal symbionts in the presence of moss associated metabolites, providing insights into the role of fungi to decompose *Sphagnum* and other boreal mosses. Given the quantity of carbon sequestered in this ecosystem, understanding decomposition rates (i.e., carbon release) is of growing importance.

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