Scientific Innovation Through Integration

Implementation of a spatial metabolomics approach for exploring interactions within a tripartite plantfungus-cyanobacterium system

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Peatlands and their global influence





DOE SPRUCE site



Large-scale peatbog warming and CO₂ manipulation studies

Peatlands: 3% of the Earth's land surface Store ~25% of terrestrial C

Sphagnum (moss) is the foundational species of this ecosystem



What is the role of the members? How will this interaction be impacted by climate perturbations?

Peatland-related model system of interest



Yang et al., J. Bacteriol. **2012**, 194, 6023

- Inoculated on N-free media agar
- Excised regions and explored the metabolic exchanges within the tripartite using a multimodal MSI approach



Multimodal MSI methodology employed



Improved MALDI sample preparation using an automated sprayer



Yang et al., J. Bacteriol. 2012, 194, 6023

Anderton et al., JASMS 2016, 27, 556-559

MALDI-FTICR MSI



Correlative LESA-FT-MSI

LESA sampling configuration, post-MALDI MSI



1394 sample-related ions detected 104 ion correlating with MALDI-MSI list *+33 more sample-related peaks detectable in MALDI-MSI*

LESA MS_n increases confidence in molecular identification



Detection of inversion of growth factors



Providing spatial confirmation to detected metabolites

Disaccharide (m/z 365.1052)





Detection of distinctive sugars originating from different species

Lactose or Isomaltose 1-4 or 1-6 glycosidic linkage, need MS₃ to differentiate

Kojibiose

High spatial resolution FTICR-SIMS



beam desorption processes

Revealing molecules invisible to MALDI

high Iow

FTICR-SIMS

Highest lateral resolution, minimal sample preparation

Higher lateral resolution, no signal

Summary

- Multimodal MSI provided insight into metabolic exchanges within the peat moss ecosystems
 - Illuminating mechanisms related to linkages in carbon and nitrogen cycling (e.g., sugar and amino acid metabolism)
 - Provided evidence of mode of action of members in simplified community
- LESA-MS_n proved to be a powerful complementary MSI modality, providing an orthogonal analysis method capable of providing increased confidence in molecular identification <u>and</u> spatial distribution.