Title: Elucidating Sphagnum microbiome genetic interactions for improved growth at elevated temperatures

Description:

We propose a collaborative project with JGI to determine how Sphagnum peatmoss and microbial genetic variation interact to influence community function at elevated temperatures. As part of a DOE Early Career award, we have developed synthetic community and microbiome transfer approaches where communities can be constructed onto germ-free Sphagnum using laboratory strains or native microbiomes. An exciting result from this newly funded project is that Sphagnum grows better at elevated temperatures when inoculated with a warming adapted microbiome relative to those inoculated with an ambient microbiome or no microbes at all. We seek to engage JGI and the international peatland community conducting experimental warming to identify plant genetic variation and genotype by microbiome combinations that influence community outcomes to elevated temperatures. This joint effort will leverage long-term peatland experimental warming sites, including the DOE supported SPRUCE, two European sites (The Alps, 10 years of experimental warming and Northern Sweden, 24 years) and a recently initiated warming site in Patagonia. Because of the community interest and impact that these developing resources would have to DOE BER science and the plant microbiome community in general, we request 10.4 Tb of total sequence. A description of the sequencing request is below:

To support the identification of core components of plant and microbial genetic variation (including virus diversity) mediating community membership and metabolic potential at peatland field sites exposed to warming, we request:
1. 32 high-quality Sphagnum metagenomes. Using four high density TB flowcells with 150 bp paired-end illuma reads, we request one sample per lane. Previous results with JGI (Schmutz group) has shown that this is sufficient to get enough depth for Sphagnum genome resequencing and assembly of relatively abundant plant associated microbes. Together this represents 4 Tb of sequence.
2. 32 metatranscriptomes from the same samples as above, at 50M reads per sample of 150 bp paired-end illuma reads. Together this represents about 1 Tb of sequence.
3. 8 single-cell individual genome sequences from identified microeukaryotes. MDA (amplification) performed at ORNL and sequenced at JGI. Estimate 1 Tb for 8 samples. Note that this is a stretch task and will be done with considerable input and optimization from JGI staff including Dr. Tanja Woyke.

To support the identification of plant genes responsible for conferring enhanced growth from a 'warming adapted' microbiome.
1. Resequencing of 192 individuals of a newly established S. magellanicum pedigree for QTL analysis. Prior JGI results with S. fallax show that 96 individuals were assembled at about 15x coverage from 1 Tb of sequence. 192 individuals will require 2 Tb sequence.
2. LC-MS metabolic profiles of the 192 individuals (without microbiome) from the pedigree for metabolic phenotyping.
3. 64 Metatranscriptomes from synthetic communities and microbiome transfer studies. We request 50M reads per sample of 150 bp paired-end illuma reads. Together this represents about 2 Tb of sequence

4. 400 heterotrophic isolates from Sphagnum tissue collected from the SPRUCE site to support synthetic communities. We request de novo genome sequencing of 400 bacterial isolates to draft quality of ~ 10Mb genomes at 100x coverage for total of 400 Gb of sequencing.

Justification:

Sphagnum (peatmoss) arguably has a greater impact on global carbon fluxes, and therefore climate, than any other single genus of plants[1-5]. Sphagnum-dominated peatlands develop where net production exceeds decomposition, and over extended time have accumulated tens of meters of partially decomposed plant material that globally store roughly 400 gigatons of carbon. It is estimated that approximately 25% of terrestrial carbon is stored in peatlands, which occupy only about 3% of the earth's land surface[1]. Sphagnum fitness and productivity is largely due to the unique interactions with its associated microbiome and environment[6-7]. For example, much of the plant host nitrogen comes from N2 fixing (diazotrophic) bacteria[8]. Further, much of the deep peat methane produced in bogs is oxidized by the Sphagnum - microbiome to CO2 that is then used by the plant as a substrate for photosynthesis[9-10]. These plant - microbiome traits cascade to influence ecosystem level C and N cycling[7]. However, a basic understanding of mechanistic processes driving these plant - microbe interactions is unknown. Without such insight, there is tremendous uncertainty regarding how changing environmental factors will influence plant - microbiome, and thereby carbon and nitrogen cycling.
Our results from 16S rRNA and nifH community composition profiling from living Sphagnum at the DOE supported SPRUCE site (https://mnspruce.ornl.gov) show that cyanobacteria, specifically members of the Nostocaceae family, increased with warming treatment as diversity decreased[11]. To gain insight into the functional consequences of a such a 'temperature adapted' microbiome on Sphagnum performance we recently initiated microbiome transfer studies, where field grown Sphagnum microbiomes are isolated from temperature specific manipulations and then transferred to germ-free laboratory Sphagnum. Our results show that Sphagnum grows better at a novel temperature when it is matched with a microbiome adapted to that temperature. For example, Sphagnum grows better at elevated temperature when inoculated with a warming adapted microbiome relative to those inoculated with an ambient microbiome or no microbes at all. Although these are unpublished results from a newly funded project, the experiment has been independently replicated three times with reproducible trends and outcomes.
The resources developed as part of this collaborative effort will inform both field and laboratory derived questions. At the field scale we will address questions regarding the conservation of microbiome taxa and predicted metabolic potential to warming across diverse sites. Are there signs of selection on specific plant or microbial genes? Is the transcriptional response specific to plant host? At the laboratory scale, development of QTL populations, sequenced strains and characterized microbiome transfers will allow us to address more fundamental questions. For example, is there host genetic control of the microbiome and how does this interaction change with temperature? Are the plant genes involved in microbial interactions specific to lineage or conserved throughout the Sphagnum genus? Can we use genomic information to select genotypic combinations of plants and microbes for specific environmental conditions?

References:
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6. Kostka J et al. 2017 New Phytologist 211: 57-64
7. Lindo Z et al. 2013 Global Change Biology 19: 2022-2035.
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Utilization:

Understanding the core components of plant and microbial genetic variation mediating community assembly and function represents a grand challenge facing biological and environmental sciences. Furthermore, the finding from us and others[12] that a microbiome preadapted to a novel environment can increase plant fitness is promising given changing environmental conditions, but remains elusive without a fundamental mechanistic understanding. Therefore, the data and resources generated as part of this proposal will be of broad interest to not just peatland biologists, but the larger plant microbiome community.

The development of a sequenced QTL pedigree will be distributed to colleagues in Sweden (Evolutionary Biology Centre, Uppsala University, Sweden) and Duke University. The ability to keep individuals within culture plates allows one to keep a 'common garden' of sequenced individual for high-throughput phenotype characterization for genotype-to-phenotype studies. Such resources are extremely rare for plants in general, and especially rare for plants controlling major ecosystem functions.

The combination of metagenomics and metatranscriptomics is also especially suitable for Sphagnum. Most plant metatranscriptomes are over 97% plant reads and very little microbial reads. Because Sphagnum have the majority of cells as dead microbe filled hyaline cells, about 40% of metatranscriptome reads are microbial, this provides an unprecedented opportunity to the plant microbiome community to investigate interacting plant-microbe gene networks. We therefore foresee these recourses as useful pilot datasets for computational tool development by the bioinformatic community including KBase.

The metatranscriptomes and metagenomes will also be used to mine virus characteristic for the Sphagnum microbiome. We hypothesize that warming will increase virus load and act as a major determinant in community structure. We recently demonstrated the utility of the Sphagnum system for virus research through a recent collaboration with the PIs and the Schmutz group, and a manuscript is now in review from this work led by the Wilhelm group.

Furthermore, we are active participants of the KBase cyberinfrastructure that emphasizes predictive biology through open-source and open-development. Code and methods in the form of "narratives" are now available for Sphagnum and diazotroph metabolic models to share and encourage open-access modeling development with the community. Our knowledge of the infrastructure will ensure that all development adheres to the data model, ensuring that all KBase functionalities for microbe, community, and plant modeling services are accessible to the scientific community.

References:
12. Lau & Lennon 2011 New Phytologist 192: 215-224

Community interest:

As part of the Sphagnum genome JGI CSP, we established an international advisory committee consisting of experts in peatland ecology (Merritt Turestsky, University of Guelph, Canada), adaptive genomics (John Willis, Duke University), moss genomics (Stefan Rensing, University of Marburg, Germany), and a large-scale DOE Next-Generation Ecosystem Experiments (NGEE) project representative (Stan Wullschleger). We will ask this committee to participate in the proposed project to ensure not only well-advised research, but immediate extension of developed resources for the greater community. For example, we are generating QTL populations (nontransgenic) that are an unprecedented resource for the peatland ecology and moss community for conducting genotype to phenotype studies in common gardens. This group could help extend this resource as transplants across various peatlands on a global scale. In addition, we have co-organized two workshops funded by the National Evolutionary Synthesis Center (NESCent) in 2015 and the New Phytologist Trust in 2016 to organize ecologists, bioinformaticians, physiologists, molecular biologists, and quantitative geneticists on how best use these emerging genomic resources. Insights from these workshops were recently published and authored by a community of 27 scientists spanning multiple countries, disciplines and institutions. The workshop has sparked a community-wide interest in utilizing novel sequencing techniques and genomic data to address new and old questions using Sphagnum. In fact, Sphagnum is among the more interesting emerging model systems as it includes farming (for horticulture/bioenergy), global carbon cycling modelling and speciation processes. Such projects will directly benefit from resources generated in this effort as it can lay the foundation for plant - microbe biodesign applications, and provide insight into how molecular genomic information can inform multi-scale processes from cell to ecosystems levels.

DOE mission:

Both Arctic tundra and high-latitude peatland ecosystems have provided vast sinks in terrestrial carbon. The DOE Office of Biological and Environmental Research currently sponsors two next-generation research activities that aim to better quantify the chemical, physical, and biological underpinnings of ecosystem responses to a changing climate (The NGEE-Arctic, http://ngee.ornl.gov; and SPRUCE, http://mnspruce.ornl.gov/). A pivotal question for these projects is, will these ecosystems continue to provide immense sinks in terrestrial carbon or transition to carbon sources with changing climatic conditions? The proposed project will use field samples, microbiome transfer studies and synthetic communities as experimental systems that are amenable to sophisticated genomics sciences technologies to define the genetic and metabolic basis of host plant and microbial genetic variation driving community function. These data are critical if we are to understand and predict what controls the fate of peatland productivity and the potential consequences of disrupted species interactions (e.g., what happens if there is a shift to non-diazotroph microbial associates?). Furthermore, the ability to harness molecular genomics resources and technologies to define more accurate representations in carbon and nitrogen interactions at the cellular and organismal levels can provide a major opportunity in constraining key uncertainties in Earth System Model simulations on the climate

sciences side of DOE BER. By carefully designing our studies in manipulative systems that are field corroborated and tractable to large DOE ecosystem studies in an open-source and open-development infrastructure, we will be well positioned to make great strides in advancing fundamental systems biology to nitrogen and carbon cycle processes in critical peatland ecosystems. Furthermore, the requested JGI resources will benefit ongoing research funded through the DOE Early Career research program, PMI SFA, and SPRUCE. The development of an experimentally tractable model system with key organisms for C cycling, and the application of omics-enabled techniques for the quantitative analysis of plant and microbial community structure and function directly addresses fundamental knowledge gaps identified by the DOE BER Genomic Sciences program.

Sample preparation:

We do not anticipate problems delivering high quality Sphagnum microbiome DNA, RNA or metabolic samples. We have already provided JGI with quality Sphagnum DNA for 16 species and a 184-member S. fallax pedigree using a modified CTAB approach. We have also provided JGI with high quality RNA for the S. magellanicum and S. fallax draft genome gene annotation using a modified CTAB approach coupled with a Spectrum Total Plant RNA extraction kit (Sigma). This approach continuously yields 260/280 values of 2-2.2 and RIN values above 6. We have also provided JGI (Trent Northen group) with samples for metabolite and exometabolite analysis for a Sphagnum-microbe cross-feeding study. The exometabolites were filtered for large molecules and desalted using methanol. After lyophilizing, samples were sent to JGI with satisfactory results. We have provided samples for Sphagnum metagenomes and metranscriptomes to the Schmutz group at HudsonAlpha with no problems in sample amount or quality.
We acknowledge that the single cell protist amplification and sequencing may be challenging and thus regard this as a stretch goal. We have been in contract with Drs. Tringe and Woyke and look forward to troubleshooting this procedure further with them. We note that any advance in linking protist ID to genome will greatly advance our ability to interpret metagenome and metatransriptome results including virus characteristics.